The role of arachidonic acid 5-lipoxygenase promoter genotype in montelukast responsiveness in wheezing preschool children

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ABSTRACT

The role of arachidonic acid 5-lipoxygenase promoter genotype in montelukast responsiveness in wheezing preschool children

Dr Chinedu Eze Chukwuemeka Nwokoro

INTRODUCTION:

Wheeze is a cause of significant morbidity in the young. The effectiveness of intermittent montelukast for wheeze in preschool children is unclear. Previous work has been equivocal. Variation in copy number of the Sp1-binding motif in the arachidonate 5-lipoxygenase (ALOX5) gene promoter influences montelukast efficacy in asthmatic adults and this polymorphism may also identify a responsive subgroup within the preschool population. This work sought to ascertain the effectiveness of montelukast in preschool wheezing children, to explore the influence of ALOX5 promoter genotype on this effect, and to investigate the mechanisms involved by exploration of the role of related biomarkers and genes in preschool wheeze and montelukast response. In addition it explored parental experience of preschool wheeze and genetically stratified clinical trials.

METHODS:

A multi-centre, parallel group, double blinded, randomised, placebo-controlled trial was conducted in 41 secondary care sites and 21 primary care sites in England and Scotland. Children aged 10 months to 5 years with two or more recent wheeze episodes but no other significant respiratory vulnerabilities were recruited, stratified by ALOX5 promoter genotype (either 5/5 (wild type) or [5/x + x/y] where x or y ≠ 5), and randomised (1:1) to receive either parent-initiated montelukast 4mg oral granules or identical placebo administered once daily for 10 days from the onset of every viral cold or wheeze episode over 12 months. The primary outcome measure was need for unscheduled medical attendance for wheezing. ALOX5 promoter and related genotypes were identified by analysis of salivary DNA. Primary outcome data came from treatment diaries, scheduled phone calls and caregiver records. Analysis was by intention to treat. Urine was collected for eicosanoid biomarker analysis using high performance liquid chromatography tandem mass spectrometry. Parental attitudes were obtained via qualitative structured face-to-face interview.

RESULTS:

Main trial

1358 children were randomised to receive montelukast (n=669) or placebo (n=677). Consent was withdrawn for 12 (1%) children. Primary outcome data were available for 1308 (96%)

children. There was no difference in unscheduled medical attendances for wheezing episodes between children in the montelukast and placebo groups (mean 2·0 [SD 2·6] vs 2·3 [2·7]; incidence rate ratio [IRR] 0·88, 95% CI 0·77-1·01; p=0·06). Compared with placebo, unscheduled medical attendances for wheezing episodes were reduced in children given montelukast in the 5/5 stratum (2·0 [2·7] vs 2·4 [3·0]; IRR 0·80, 95% CI 0·68-0·95; P=0·01), but not in those in the [5/x + x/y] stratum (2·0 [2·5] vs 2·0 [2·3]; 1·03, 0·83-1·29; p=0·79, $P_{interaction}$=0·08). There was one serious adverse event, a skin reaction in a child allocated to placebo.

Urine eicosanoids

Urinary LTE_4 was higher in subjects with two variant ALOX5 alleles (x/y) compared with those with one or more wild type (5/5 or 5/x) allele. There was an increase in urinary leukotriene E_4 ($uLTE_4$) during preschool wheeze exacerbation, while baseline urinary tetranor PgD-M (the primary prostaglandin D_2 metabolite) was elevated in preschool wheezing children compared with controls.

Eicosanoid pathway polymorphisms

Polymorphisms in eicosanoid pathway genes SLCO2B1 and LTB4R2 had some (non-robust) association with montelukast response and warrant further study.

Qualitative study

Parents expressed varying understanding of and motivations for participation in the clinical trial, with some suggestion of an ethnically divergent response.

DISCUSSION AND CONCLUSION:

There is no clear benefit of intermittent montelukast in young children with wheeze but the data suggest that the 5/5 ALOX5 promoter genotype *might* identify a montelukast-responsive subgroup. However, the direction of this possible genotype stratum effect is contrary to that hypothesized, the study lacked power to confirm its validity, and the observed ALOX5 genotype:$uLTE_4$ association was not supportive. A repeat trial solely recruiting subjects with the apparently more responsive (5/5) genotype is required to confirm this putative effect. There is also a role for studies with alternative stratification criteria.

FUNDING AND REGISTRATION:

This study was funded by the NIHR Efficacy and Mechanism Evaluation programme (08/43/03) and registered at the US NIH ClinicalTrials.gov database (NCT01142505).

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LIST OF ABBREVIATIONS & DEFINITION OF TERMS

ADP	Adenosine Diphosphate
ADRB2	$Beta_2$ Adrenoceptor gene
AE	Adverse Event
AERD	Aspirin Exacerbated Respiratory Disease
ALSPAC	Avon Longitudinal Study of Parents and Children
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
AOCS	American Oil Chemists Society
API	Asthma Predictive Index
AR	Adverse Reaction
ALOX5	Arachidonate 5-lipoxygenase (5-LO, 5-LOX)
BAL	Bronchoalveolar lavage
β-Arr	Beta-Arrestin
BNF	British National Formulary
CHR	Chromosome
CI	Confidence Interval, Chief Investigator
CLP	Coactosin-like Protein
COX	Cyclooxygenase (PGHS, PTGS, Prostaglandin H_2 Synthase)
CRTH2	Chemoattractant Receptor Homologue expressed on Th2 cells (DP2, PgD_2 receptor)
CRF	Case Report Form
(Cys-)LTX_n	(Cysteinyl) Leukotriene X_n
(Cys-)LTRn	(Cysteinyl) Leukotriene Receptor Type n
DC	Diary Card
DMSO	Dimethyl sulfoxide, $(CH_3)_2SO$
dNTP	deoxyNucleoside Triphosphate
DSMC	Data Safety and Monitoring Committee (DSMB, DMB, DMC)
EBC	Exhaled Breath Condensate
ECP	Eosinophilic Cationic Protein
ED	Emergency Department
EDTA	EthyleneDiamineTetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
EPn	Prostaglandin E_2 receptor Type n
EPX	Eosinophilic Protein X
ERS	European Respiratory Society
EU	European Union

EVW	Episodic Viral Wheeze
FCER2	Low Affinity IgE receptor (CD23)
FeNO	Fractional Exhaled Nitric Oxide
FDR	False Discovery Rate
FLAP	5-Lipoxygenase Activating Protein (ALOX5AP)
GC-MS	Gas Chromatography-Mass Spectrometry
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GPRX	G Protein-Coupled Receptor Type X (GPCR-X)
GSH	Glutathione
GWAS	Genome Wide Association Studies
HCl	Hydrochloric Acid
12-HHT	12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid
5-HETE	5-hydroxyeicosatetraenoic acid
5-HPETE	5-hydroperoxyeicosatetraenoic acid
HPLC-tMS	High Performance Liquid Chromatography-tandem Mass Spectrometry
HR	Hazard Ratio, Health Records
HRU	Health Resource Utilisation
IB	Investigator Brochure
ICS	Inhaled Corticosteroids
IMP	Investigational Medicinal Product
IQR	Interquartile Range
IS	Induced Sputum
ISAAC	International Study of Asthma and Allergies in Children
IRR	Incidence Rate Ratio
ITT	Intention To Treat
LABA	Long-Acting Beta Agonist
LAMA	Long-Acting Muscarinic Agonist
LF	Lung Function
LTC4S	Leukotriene C_4 Synthase
LTB4Rn	Leukotriene B4 receptor Type n (BLTn)
MAPK	Mitogen Activating Protein Kinase
MCRN	Medicines for Children Research Network
MD	Medical Doctor/Doctor of Medicine (Higher Degree)
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	messenger Ribonucleic Acid
MSD	MSD™ (Merck, Sharp and Dohme™)
MTW	Multiple Trigger Wheeze

NIEHS	National Institute of Environmental Health Sciences (USA)
NO	Nitric Oxide
NS	Not statistically Significant
NSA	Non-Substantial Amendment
NSAE	Non-Serious Adverse Event
NSAID	Nonsteroidal Anti-Inflammatory Drug
OCS	Oral Corticosteroids
OR	Odds Ratio
OXGR1	Oxoglutarate Receptor Type 1 (GPR99, Cys-LTR_E)
PC	Phone call questionnaire
PCR	Polymerase Chain Reaction
PCT	Primary Care Trusts (since replaced by Clinical Commissioning Groups)
PCTU	Pragmatic Clinical Trials Unit
PEAK	Prevention of Early Asthma in Kids Study
PgX_n	Prostaglandin X_n
PI	Principal Investigator
PIC	Patient Identification Centre
PIS	Patient Information Sheet
PPARγ	Peroxisome proliferator-activated receptor gamma
PS	Parent Study
QS	Qualitative Study
RCT	Randomised Control Trial
REC	Research Ethics Committee
ROC	Receiver-Operator Characteristic (curve)
SABA	Short-Acting Beta Agonist
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SD	Standard Deviation
SE(M)	Standard Error (of the Mean)
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedures
Sp1	Specificity Protein 1 transcription factor
SPC	Summary of Product Characteristics
SPT	Skin Prick Test
SRS(-A)	Slow Reacting Substance (of Anaphylaxis)
SSAR	Suspected Serious Adverse Reaction
STRA	Severe Therapy Resistant Asthma
SUSAR	Suspected Unexpected Serious Adverse Reaction

TCRS	Tucson Children's Respiratory Study
TMF	Trial Management File
TSC	Trial Steering Committee
TX	Thromboxane
(u)LTX_n	(urinary) Leukotriene X_n
(U)RTI	(Upper) Respiratory Tract Infection
USMA	Unscheduled Medical Attendance
UTR	UnTranslated Region (of mRNA)
VNTR	Variable Number Tandem Repeat
WAIT	Wheeze And Intermittent Treatment trial

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From the University of Cambridge, my *alma mater* and the institution that will confer my doctorate should I prove worthy, I owe a debt of thanks for guidance and faith through several years to Steve Hladky, Mike Waring, Jim Ajioka, Chris Allen, Chris Verity, Donna McShane, Richard Iles, Rob Ross Russell, and above all to Wilf Kelsall - whose wise and generous advice patiently and deftly steered me from the brink of career change toward successful completion of specialist training (via this wonderful opportunity), and who opened his home to me to bookend what would prove to be my final fenland sojourn.

Amongst these lofty mentions I must also look to those who tolerated and toiled alongside me at stages of this journey. Like so many Peregrines, Meriadocs and Aragorns to my oft-bewildered Frodo, this motley Fellowship sustained and succoured me through dark and challenging times, fingernails dirtied and sweat beading as they subordinated their needs to my own. Thanks then must go to Bea Howell, Hafiza Khatun, Nanna Christiansen, Mandy Wan, Jeanette Hansen, Rossa Brugha, Abi Whitehouse, Chris Griffiths, Ilya Kantsedikas and finally to Cassie Brady, the 'work wife' who kept me true to task as long as she could before ultimately returning to the Shire.

There are those who travelled with me by design, and those whose purposes were more accidentally aligned with mine. Neither Louise Houson of MSD nor Elaine Godfrey of the MHRA had reason to assist me to the extent that they did but perhaps they did more than any others to prevent terminal derailment of the study at critical junctures.

The former Justice Secretary recently claimed that we "have had enough of experts", but in producing this work I must acknowledge the contributions of those who lent their considerable expertise to make it possible. Steve Turner and Hitesh Pandya are experienced, motivated and motivating clinician-triallists, Robert Walton is an expert on

personalized medicine, Tom Vulliamy, Lee Koh, and Iain Dickson guided and performed the genotyping that underpinned the stratification process, while Marek Sanak oversaw the urinary eicosanoid estimation. Statistical support from Sandra Eldridge, Gordon Forbes, Valerie Kuan and Clare Robinson (née Rutterford) was invaluable, and Marie-Claire Rickard guided me safely through a changing regulatory landscape with humour and patience. Qualitative researchers Clive Seale and Virginia MacNeill showed me the patients' perspective on the clinical and academic world I sometimes take for granted, teaching me lessons that I only fully understand now, as a father; and so along with them I thank the children and parents who consented to lend me their trust and their time in pursuit of this work.

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My final and most sincere appreciation goes to my family. To my parents, who suffered for me, rearing me to see things through and to back myself in the face of universal doubt; to my siblings, Nnamdi, Precious and Rachel, who schooled me in resilience, and who hold an essential and unflattering mirror to my flaws; to my daughters, Freya and Lucy, watching you grow I understand Elton's assertion that: "time on my hands should be time spent with you", and the lack of such is my chief incentive to draw a line under this magnum opus; finally I thank Clare, my beautiful, long-suffering wife and the love of my life; darling I'm yours again, let's go and have some fun.

Formal statement of contributorship

This thesis represents the culmination of several years of my own work. However, a project of this magnitude requires a team of disparate and overlapping talents to succeed. Main personnel are named in Table 0-1, with key contributions and institutional support detailed below.

JG was the chief investigator, secured funding, planned and provided overall supervision of the study, and assisted with first and final drafts of the main peer-reviewed manuscript. I (CN) supervised and managed the study, built and maintained the trial management website, wrote monthly recruitment and motivational bulletins, liaised with ethics, funding and regulatory boards, secured a study extension, assessed adverse reactions, recruited subjects, collected biological samples, conducted the urinary analysis and guided the main trial and pharmacogenetics analysis. I wrote the main (with JG), review, urinary (with JG and Abigail Whitehouse) and qualitative manuscripts (with JG, CS and VM) as well as the report for the funding body.

HP, ST contributed to study planning and to the main manuscript and also recruited subjects. RB managed the study in the later stages, contributed to the analysis, and to the main and urinary manuscripts. CS, VM conducted the qualitative study and wrote the qualitative manuscript (with CN). CJG and DP facilitated subject recruitment, contributed to study planning and to the main manuscript. TV contributed to study planning, supervised genotype analysis, and contributed to the main manuscript. ID contributed to study planning, genotype analysis, and the main manuscript, LK contributed to genotype analysis with ID and TV. JH, RW contributed to study planning, advised on genotype analysis, and contributed to the main manuscript. MS performed the urinary leukotriene analysis and contributed to the main and urinary manuscripts. HK performed urine cotinine measurement.

CR supported the data monitoring and safety committee, wrote the final statistical analysis plan, and did the statistical analysis assisted by GF. SE contributed to study planning and supervised the statistical analysis. VK performed the urinary eicosanoid statistical analysis and contributed to the manuscript.

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mitigate the effect of a global shortage of montelukast 4mg oral granules, their assistance prevented the failure of the study.

TABLE 0-1 - PRINCIPAL STUDY PERSONNEL

Title	Name	Affiliation
Chief Investigator	Professor Jonathan Grigg	Queen Mary, University of London
Research Fellow, Coordinating Principal Investigator	Dr Chinedu Nwokoro	
Principal investigator (main) (See 8.1.1 for other Local Principal Investigators)	Dr Hitesh Pandya	University Hospitals Leicester
	Dr Steve Turner	Royal Aberdeen Children's Hospital
	Professor Chris Griffiths	Queen Mary, University of London
	Dr Rossa Brugha	
Qualitative Research Team	Professor Clive Seale	Queen Mary, University of London
	Dr Virginia MacNeill	
Statistics Team	Dr Sandra Eldridge	Queen Mary, University of London
	Dr Clare Rutterford	
	Dr Gordon Forbes	
	Dr Valerie Kuan	
Independent Trial Steering Committee	Professor Warren Lenney	University Hospitals, N. Staffordshire
	Professor David Price	University of Aberdeen
	Dr Jay Panickar	Royal Manchester Children's Hospital
	Dr Hussain Mulla	University Hospitals Leicester
	Dr Edward Simmonds	Walsgrave General Hospital
	Professor Robert Walton	Queen Mary, University of London
	Professor John Holloway	University of Southampton
Data Monitoring and Safety Committee	Professor Andy Bush (Chair)	Royal Brompton Hospital
	Paul Lambert (Statistician)	University of Leicester
	Ian Jarrold	British Lung Foundation
Sponsor	Mr Gerry Leonard	Queen Mary, University of London
Project Management and Trial Monitoring	Ms Suzi Miranbeg	Queen Mary, University of London
	Miss Cassie Brady	
	Ms Amy Hoon	
Research Nurses	Mrs Teresa McNally	University Hospitals Leicester
	Ms Belinda Howell	Queen Mary, University of London
	Ms Donna Nelson	Royal Aberdeen Childrens' Hospital
Pharmacy Team	Mrs Nanna Christiansen	Royal London Children's Hospital, Barts Health NHS Trust
	Ms Rupal Patel	
	Ms Jeanette Hansen	
	Ms Judith Bwire	University Hospitals Leicester
	Ms Julia Subedi	Royal Aberdeen Childrens' Hospital
	Ms Mandy Wan	Medicines for Children Research Network
Laboratory investigator	Dr Tom Vulliamy	Queen Mary, University of London
	Mr Iain Dickson	
	Ms Lee Koh	
	Professor Marek Sanak	Jagiellonian University, Krakow
	Dr Helen King	King's College, London
Data Management	Miss Hafiza Khatun Miss Sandy Smith	Queen Mary, University of London

SCIENTIFIC SUMMARY

Background

Wheeze in preschool children is a common and important cause of morbidity, with an associated social and economic burden through strain on health services and parental resources. Current evidence does not support the use of oral corticosteroids in this population, due to a lack of efficacy in reducing hospital stay, and demonstrable treatment-associated morbidity when used to excess. The majority of children wheeze only with colds, with little or no symptoms in the interim. There is an appetite for a treatment that can be administered effectively during symptomatic episodes but can be discontinued when children are well.

The cysteinyl leukotrienes are inflammatory mediators derived from arachidonic acid that have potent bronchodilator effects. Previous work has shown a transient increase in leukotriene production (measured as urinary Leukotriene E_4, $uLTE_4$) in preschool children during acute wheezing episodes, implicating them as a probable mediator for episodic wheeze in this population.

Montelukast is the only leukotriene receptor antagonist licensed for use in children. It is a competitive inhibitor of the cysteinyl leukotriene receptor binding site and prevents the downstream bronchoconstrictor and pro-inflammatory effects of the cysteinyl leukotrienes. Moreover it is safe and orally available, with a half-life, formulation and posology suitable for all ages. Previous work has suggested a role for intermittent therapy in the management of acute childhood wheeze but the effects have been modest. Analysis of adult trials suggests that variation in copy number of a CG-rich Sp1-binding motif (wild type is 5 copies) in the promoter region of the arachidonate 5-lipoxygenase (ALOX5) gene may influence response to montelukast, presumably by altering baseline or exacerbation-related leukotriene production.

Objectives

This work aims to assess the efficacy of parent-initiated intermittent montelukast for reduction of unscheduled medical attendances (and other secondary outcomes where possible) for preschool wheeze and to explore the role of ALOX5 promoter genotype in montelukast efficacy. In addition I will examine the role of urinary biomarkers and selected eicosanoid pathway polymorphisms in both preschool wheezing disease and montelukast response. Secondary outcomes to be assessed include respiratory morbidity and mortality,

concomitant medication usage, adverse events, health economic effects, urinary biomarker levels, and qualitative outcomes related to wheeze.

Methods

I hypothesized that overall montelukast would be moderately effective, but that a subgroup of children with a variant (non-5 repeat) allele on one or both chromosomes would have a greater response to montelukast, manifest by decreased need for unscheduled medical attention compared to their peers when treated with montelukast. This would be expected to associate with elevated leukotriene activity either at baseline or during wheezing exacerbation.

To test this hypothesis children were recruited from primary and secondary care settings. Eligible children were aged 10 months to 5 years, had had 2 or more previous episodes of wheeze, with one occurring within the previous 3 months, and had no associated significant respiratory morbidity. Younger infants and older children were excluded so as not to confuse the pathology studied with viral bronchiolitis or so-called classical asthma. At enrolment children provided salivary DNA and were stratified by ALOX5 promoter genotype with one stratum comprising those with 5 copies of the ALOX5 promoter polymorphism on each allele (wild type), and the other comprising all those with one or more non-5 repeat allele (variant). The two strata were subsequently independently randomised in a 1:1 ratio (randomly permuted blocks of 10) to receive parent-initiated montelukast oral granules or identical placebo every day for 10 days from the start of a cold or wheezing episode. Need for unscheduled medical attention (USMA) over a period of 12 months was assessed as the primary outcome. Outcome data were collected via a treatment diary completed with every course of investigational medicinal product, and via a bimonthly investigator phone call which additionally screened for adverse events.

Urinary LTE_4 was measured at baseline and during exacerbation (where possible), to provide pathophysiologic corroboration of any associations observed. Urine was collected fresh into a universal container and placed on ice before being transferred within 48 hours to a -70°C freezer. Urine samples were then batch analysed using high performance liquid chromatography tandem mass spectrometry (HPLC-tMS) for a panel of eicosanoid mediators, with results indexed to urinary creatinine to account for dilution, and also for cotinine concentration (by ELISA) as a marker of tobacco smoke exposure. Salivary DNA was also analysed for a selection of eicosanoid pathway SNPs.

A subset of recruits underwent semi-structured qualitative interviews conducted by an experienced qualitative researcher, with an interpreter where required. Questions addressed background information about the child and family as well as parental experiences and attitudes to their role in the trial. Interviews were audio-recorded, transcribed and imported into Nvivo9™ (a qualitative data analysis program) for analysis.

Results

Primary Outcome

1358 subjects were recruited, with 1308 (96%) having data available on which to assess the primary outcome. Analysis was by intention to treat. Overall montelukast did not outperform placebo in intermittent usage for preschool wheeze (IRR = 0.88, P = 0.06). Children treated with montelukast had marginally reduced use of rescue oral corticosteroids (IRR 0.75, P = 0.03), a recognised severity marker, but the study was not adequately powered to robustly detect such a change.

Analysis by genotype suggested an improved montelukast effect (contrary to that hypothesized, but in keeping with certain earlier work) in the wild type (5/5) stratum (IRR = 0.80, P = 0.01). When subject to more detailed scrutiny this observation was not statistically robust with a p-value for interaction of only 0.08. There was no effect seen when the primary outcome was analysed by use of inhaled corticosteroids, wheezing phenotype, or alternative genotype grouping (x/y vs [5/x and 5/5].

Urinary eicosanoids

LTE_4 appeared higher in subjects with two variant (non-5 repeat) alleles [x/y] ($P<0.05$). This was not consistent with the direction of association predicted by the possible improved montelukast effect in the 5/5 population. $uLTE_4$ was elevated during wheezing exacerbations. Tetranor PgD-M was elevated in preschool wheeze prone children compared with non-wheezing controls but did not increase during exacerbations.

Exploratory Genetics

No robust associations between eicosanoid pathway SNPs and clinical or laboratory outcomes were identified, although two polymorphisms (in SLCO2B1 and LTB4R2) warrant further investigation.

Qualitative Results

Bangladeshi families were relatively reluctant to participate in the qualitative study, despite strong engagement with the parent study. Anxiety related to wheezing was a common primary motive for trial enrolment. Parents viewed the trial as a route to improved treatment. Verbal delivery of trial information appeared more effective than study literature, especially for Bangladeshi families, with low parental literacy and high levels of trust in medical professionals potential contributors to this effect. All ethnic groups displayed a poor understanding and/or retention of essential study concepts such as randomisation and genetic testing.

Conclusions

This study does not support the routine use of intermittent montelukast in preschool wheezing children. It does not speak to the value of continuous montelukast in this population, nor does it preclude the consideration of short-term therapeutic trials on an individual patient basis in this context. The suggested superior montelukast response in the 5/5 stratum is of interest but is not robust insofar as the test of interaction does not meet statistical significance and the finding contradicts both the *a priori* hypothesis and the urinary LTE_4 data.

Future Research

The effect seen in the 5/5 stratum should be prospectively evaluated in a study population comprising children with only wild type (5/5) alleles. Should this study be negative it remains possible that a montelukast responsive subgroup exists. Future trials should be stratified by $uLTE_4$ increment, or should target children with a high increment as more likely to respond. The role of PgD_2 in preschool wheeze should be investigated, with a view to trials of novel orally available inhibitors of PgD_2 in this population.

1 INTRODUCTION

1.1 Preschool wheeze

Wheeze describes an expiratory sound produced by airway narrowing of intraluminal, intrinsic or extrinsic aetiology. For example, wheeze may occur due to the infective secretions of pneumonia or bronchiectasis, the interstitial oedema of congestive cardiac failure, or from the external pressure of a vascular malformation or thoracic lymphadenopathy, as well as from intrinsic bronchoconstriction. The term preschool wheeze is specific to bronchoconstrictive wheeze occurring in children aged between 1 and 5-6 years of age and is accepted to encompass a wide range of imperfectly defined entities. Infants are excluded in order to avoid confusion with acute bronchiolitis(1), recognised as a distinct entity despite some overlap, while older children are generally recognised as having greater phenotypic and pathophysiologic similarities with adult 'classical' asthmatics.

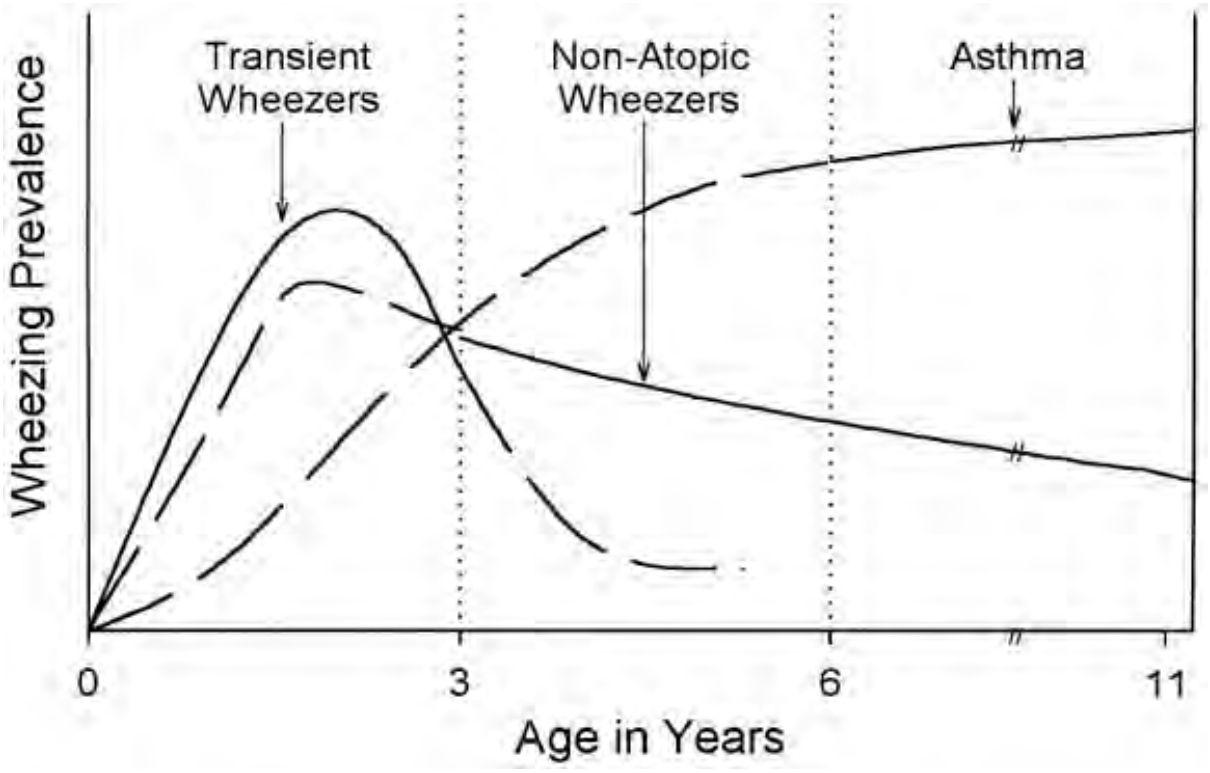
A quarter of preschool children between 1 and 5 years of age will develop at least one attack of wheeze(2). The majority of affected children have several attacks of wheeze triggered by viral colds, with minimal or no symptoms between attacks(3). A minority of preschool children will also wheeze between colds (multiple trigger wheeze). Preschool wheeze is a major clinical problem, with significant costs to primary and secondary care(4,5).

Accurate classification of wheeze is key to facilitate appropriate prognostication and therapy, as well as to define populations and target substrates for research.

1.1.1 Epidemiological classification

Longitudinal study of large birth cohorts can identify wheeze patterns based on the evolution of symptoms. The Tucson Children's Respiratory Study (TCRS) described four distinct preschool wheezing classes termed 'never wheezers', 'transient early wheezers', 'late onset (non-atopic) wheezers' and 'persistent (asthmatic/atopic type) wheezers' (6,7) defined according to wheeze onset and persistence. These groups were re-evaluated in the subsequent ALSPAC(8) and Southampton(9) cohorts with consequent derivation of a related six-class model. These classes map loosely to clinical phenomena: persistent wheezers are more prone to have a maternal history of asthma, be atopic, and to have serum eosinophilia, while non-atopic wheeze is not associated with family history of asthma, tends to follow an early childhood lower respiratory tract infection, and has delayed resolution compared to transient early wheeze, which also has no atopic association and associates with antenatal tobacco smoke exposure and low lung function from birth. While both four and six class models have potential use as epidemiologic descriptors, patients can only ever be classified retrospectively, and thus the classification has no utility in individual clinical decision-making(10).

FIGURE 1-1 - TEMPORAL PATTERNS OF WHEEZE



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1.1.2 Phenotypic classification

In 2008 the European Respiratory Society (ERS) Task Force on preschool wheeze recognised and described 2 main clinical patterns of preschool wheeze; episodic viral wheeze (EVW) which affects the majority of wheezing children, and multiple trigger wheeze (MTW) which affects the minority(11). Episodic viral wheeze is defined as wheezing during discrete time periods, often in association with clinical evidence of a viral cold, with absence of wheeze between episodes. Conversely, multiple trigger wheezing shows discrete (viral) exacerbations, but also symptoms between episodes. The Task Force recommended montelukast as first line preventer therapy for episodic viral wheeze, while inhaled corticosteroids (ICS) were recommended for multiple trigger wheeze. This recommendation was based in part on observations from the PEAK Study(12), which showed that preschool children at high risk for asthma (positive modified Asthma Predictive Index (mAPI), Figure 1-2) had more symptom-free days and reduced exacerbations on ICS when compared to placebo. The Asthma Predictive Index has been through serial iterations since its origins in the TCRS, but in essence comprises an assessment, before the age of three, of the presence of parental history of doctor-diagnosed asthma or eczema (the major criteria) or peripheral eosinophilia, multiple trigger wheeze or doctor-diagnosed allergic rhinitis (the minor criteria). 1 major criterion or 2 minor criteria in the context of frequent wheezing at a young age suggest increased likelihood of asthma persistence beyond the age of 6

years(13). The mAPI adds aeroallergen sensitisation to the major criteria, and replaces rhinitis with food allergen sensitisation in the minor(14).

While these classes are acknowledged to be imperfect (due to phenotypic instability and lack of clear pathophysiological and therapeutic response correlates) they remain the most useful schemata for describing wheeze in this age group(10,15).

FIGURE 1-2 - THE MODIFIED ASTHMA PREDICTIVE INDEX

The child must have a history of 4 or more wheezing episodes with at least one physician diagnosis.

mAPI: Major criteria	Original API: Major criteria
• Parental history of asthma	• Parental history of asthma
• Physician-diagnosed atopic dermatitis	• Physician-diagnosed atopic dermatitis
• **Allergic sensitization to ≥1 aeroallergen**	
mAPI: Minor criteria	**Original API: Minor criteria**
• **Allergic sensitization to milk, egg, or peanuts**	• **Physician-diagnosed allergic rhinitis**
• Wheezing unrelated to colds	• Wheezing unrelated to colds
• Blood eosinophils ≥4%	• Blood eosinophils ≥4%

*Differences in indices are in bold.

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1.1.3 Pathophysiologic classification

Asthma and wheezing disease are associated with atopy, with atopic features such as eczema, hayfever and serum eosinophilia forming part of the asthma predictive index (API) which predicts asthma persistence into later childhood(6). Hypothesising that atopic sensitisation represents a group of latent endotypes with differing clinical significance, Lazic *et al*. used a machine learning approach to generate a five class model of atopic sensitisation based on serial skin prick tests and specific IgE assays to common allergens in children recruited to the Manchester Asthma and Allergy Study birth cohort. Falling within the "Multiple Early" sensitisation class was associated with asthma, lower lung function, airway reactivity and hospital attendance with wheeze(16). The associations were significantly stronger than those related to the presence of conventional atopic sensitisation. The validity of this model was subsequently confirmed in the Isle of Wight cohort(17).

While it is safe to perform endobronchial biopsy in preschool children(18), it is neither practical nor acceptable in most healthcare settings to use this method to assess airway histology as a matter of routine. As such there is limited data describing airway histology in this population, and even less supporting a role for airway histology in prognostication or choice of treatment.

Older children with established severe asthma can be shown to have similar histopathology to adults, with reticular basement membrane and increased airway smooth muscle thickening

(remodelling) and airway eosinophilia prominent(19). A small but important study by O'Reilly *et al*. suggests that airway smooth muscle thickness (but not reticular basement membrane thickness or mucosal eosinophilic infiltration) predicts school age asthma(20). Previous work has shown that reticular basement membrane thickness, mucosal mast cell infiltration and reduced lung function at one year of age associate with respiratory morbidity in early childhood(21), however these changes did not predict asthma at 8 years of age in this group(22).

Analysis of airway fluid, via bronchoalveolar lavage (BAL), and increasingly via sputum induction, yields additional information pertinent to wheezing phenotypes. Airway fluid supernatant analysis can inform microbiological(23), cytological(24), inflammatory mediator(25) and even transcriptomic(26) correlates of wheezing illness. BAL shares some of the limitations of endobronchial biopsy when considered as a technique for routine practice, and induction of sputum (IS) can be more difficult to perform in this age group (although concerns regarding hypertonic saline-induced bronchoconstriction seem exaggerated). Recently IS techniques have been established to provide decent cellular yields in preschool children and even toddlers(23).

While analysis of induced sputum and peripheral blood cellularity(27) are attractive, relatively non-invasive methods to gauge pulmonary inflammation, they are not conducive to frequent use in the very young. Furthermore, it is doubtful that IS accurately reflects lower airway cellularity(23), and in any case attempts to use BAL cytology to decide or drive therapy have not been fruitful in children as compared with adults(28,29), perhaps due to lack of longitudinal stability in lower airway cellular phenotype (30), and thus neither method has found widespread usage outside the research and/or highly specialised setting. Measurement of Fractional Exhaled Nitric Oxide (FeNO) is another potential proxy for eosinophilic airway inflammation, however two recent systematic reviews showed no convincing benefit in guiding treatment in school-aged children(31,32). Although Sonnappa *et al*. note a correlation between prior airway remodelling and elevated FeNO in older pre-schoolers(33), the technique is generally unsuitable for younger children(34) and offers nothing in addition to the techniques previously discussed in this age group.

Markers of eosinophilic inflammation such as serum eosinophilic cationic protein (ECP) and urinary/serum eosinophilic protein X (EPX), both released by eosinophil degranulation, and Immunoglobulin E (IgE, which triggers basophil and mast cell degranulation in response to allergen) are implicated in wheezing disease. ECP has some utility in predicting persistence of asthma symptoms in later childhood(35), but neither has a role in determining acute treatment response(36), and neither has found widespread utility outside of the research

setting due to a combination of acceptability, availability and utility. A recent systematic review demonstrated a role for serum IgE testing in several imperfect models for predicting development of school age asthma(37), but to date there is no proven role for serum IgE in guiding treatment in the preschool age group.

Airway inflammation can also be assessed through measurement of inflammatory mediators in the supernatant from lower airway samples. As well as a global elevation in BAL cell counts, Krawiec *et al*. report increased eicosanoid mediators in BAL fluid derived from wheezing preschool children compared with normal controls(24). Eicosanoids, specifically the cysteinyl leukotrienes, are implicated in preschool wheeze through symptomatic association(38), therapeutic modification(39) and biological plausibility(40), and can be measured via a number of methods, including BAL(24), IS(23,25), urine(38,41,42) and exhaled breath condensate (EBC)(43). The existence of a safe, orally available, antileukotriene agent licensed for preschool children has driven interest in assessing the degree and determinants of any therapeutic response.

1.1.4 Classification by therapeutic response phenotype

Atopic asthma in older children and adults can be classified according to the degree of treatment responsiveness. Severe Therapy Resistant Asthma (STRA - which can be defined as persistent symptoms despite correctly delivered treatment with high dose inhaled steroids, long-acting beta agonist and leukotriene receptor antagonist and optimisation of non-pharmacological factors) is the focus of particular attention as the association of peripheral eosinophilia (as part of the API) with asthma persistence to some extent provides mechanistic support for the use of ICS in the treatment of preschool wheeze. In preschool children there is, as yet, no accurately defined therapeutic response phenotype, nor is there a standout candidate for an effective acute, preventative, or disease-modifying therapy. Current nationally-endorsed regimens which focus on as-required inhaled $beta_2$ agonist, inhaled steroid, and regular leukotriene receptor antagonist (LTRA) extrapolate from adult research, and are based on consensus, rather than convincing evidence(10,11). This lack of evidence likely reflects substantial heterogeneity in preschool wheeze aetiology, with attendant within-class variation in therapeutic response, rendering a ‘one size fits all’ or even ‘one size fits most’ treatment recommendation elusive. The corollary of this is that some children receive treatment with no proven longterm benefit but with clear evidence of a potential health risk. The 2006 study by Guilbert *et al*. showed reduced respiratory morbidity with inhaled fluticasone in an API-selected cohort at high risk for subsequent asthma, but at the cost of a small but sustained reduction in height, and with no longterm effect on symptom persistence once treatment was discontinued(12). Higher dose intermittent (symptomatic) ICS treatment reduced recourse to rescue oral corticosteroids (OCS) but again at the cost of

reduced linear growth(44), while others have demonstrated adrenal suppression with regular ICS use in children(45). Reliably effective preschool wheeze treatment is likely to remain remote while scientifically and clinically robust pathophysiological phenotypes (permitting targeted therapy) remain to be established. Gaillard *et al*. suggest therapeutic trials stratified by the presence or absence of peripheral blood eosinophilia(27), however the same group found that frequent preschool wheeze exacerbations were not in fact associated with elevated serum eosinophils during attacks(46), calling this approach into question. A newer approach involves pharmacogenetic analysis as a tool to predict treatment response.

1.2 Pharmacogenetics of asthma treatment

While medication compliance, inhaler technique, misdiagnosis, environmental factors and comorbidity influence treatment response in asthma and preschool wheeze, there is growing evidence that variation in therapeutic efficacy (as distinct from disease severity) may be genetically determined(47). This is important because non-response (or even paradoxical deterioration) may be seen as reflecting inadequate dosing, poor compliance or perhaps poor treatment choice, depending on perspective, and may adversely affect the doctor-patient relationship. The main classes of therapeutic agent in preschool wheeze are $beta_2$ agonists, anticholinergics, corticosteroids and leukotriene receptor antagonists.

1.2.1 $Beta_2$ agonists

Inhalation of short-acting $beta_2$ agonist (SABA) forms the cornerstone of acute treatment of childhood wheezing disorders beyond infancy. Examples include salbutamol and terbutaline, effect onset is within 15 minutes and duration of action can be up to 6 hrs. They stimulate $beta_2$ adrenoceptors to cause smooth muscle relaxation and consequent bronchodilation, with potential adverse effects including hypokalaemia, tachycardia, tremor, myocardial dysfunction, arrhythmia and lactic acidosis. Despite widespread use a 2009 Cochrane review by Chavasse, Seddon *et al*. found no clear evidence of benefit in the under 2s(48), and administration at home can be limited by patient compliance.

Heterogeneity in response to $beta_2$ agonists is well-recognised, with some responding poorly, or not at all, while others may even experience clinical deterioration(49). This may be due to a paradoxical bronchospasm to the active agent, or perhaps an intolerance of the propellant in certain formulations. A 1997 analysis of the Tucson cohort identified a single nucleotide polymorphism at amino acid locus 16 (Arg16Gly) of the beta-2 adrenoceptor gene which associated with response to a single dose of inhaled salbutamol(50). Arg16 homozygotes and Arg16Gly heterozygotes were (5.3x and 2.3x respectively) more likely to increase FEV-1 (>15.3% predicted) compared to Gly16 homozygotes; a similar finding was observed in the CAMP cohort(51), while Drysdale found greatest bronchodilator reversibility in Gly16

homozygotes(52), and Choudhry *et al*. found contradictory results in different ethnic groups(53). Regular longterm (rather than intermittent or one-off) use of SABA has different effects, with Arg16 positive subjects having reduced lung function and increased exacerbation frequency compared to Gly16 during treatment in some studies(54,55), while Gly16 associates with poorer outcomes with regular salbutamol in others. These apparent contradictions speak to the complexity of ADRB2 pharmacogenetics, however a simplistic explanation of effects at this locus postulates that Arg16 confers a baseline higher beta-2 adrenoceptor density (perhaps Gly16 ADRB2 is more susceptible to downregulation in response to low level endogenous beta agonist[1]), explaining the increased initial response, but that there is an associated increased propensity to downregulation in response to repeated frequent stimulation when compared with Gly16, leading to enhanced tachyphylaxis and poorer outcomes. This would go some way to explain why regular salbutamol use is associated with asthma mortality(56) and morbidity(57,58), and why longterm frequent salbutamol usage (and thus chronic $beta_2$ adrenoceptor overstimulation) may drive (rather than purely reflect) poor asthma control.

The polarity of the downregulatory response seen in vivo is at odds with earlier in vitro findings. Green *et al*. predicted that Gly16 ADRB2 would show greater agonist-induced downregulation than Arg16, based on cell culture studies. They also predicted that Gln27→Glu would impart resistance to downregulation, but only in the presence of Arg16(59,60). This discrepancy *may* result from the doses of beta agonist (isoproterenol) used in the cell studies, as compared to the effective dose from real world usage, or it may reflect other genetic or environmental influences such as concomitant glucocorticoid therapy. De Paiva *et al*. observed divergent allele frequencies between asthmatic (Arg16 = 0.53, Gln27 = 0.67) and non-asthmatic (Arg16 = 0.27, Gln27 = 0.33) subjects(61), with each allele occurring at a level where a significant pharmacogenetic effect would have real clinical implications. The 2007 review by Ortega *et al*. provides a useful exploration of $beta_2$-agonist pharmacogenetics at these loci(62), while his 2015 update puts them in the context of other asthma therapies(47).

A 2014 review by Walker and DeFea examines the role of alternative (non-G-protein coupled) $beta_2$-adrenoceptor signalling, postulating a strong case for a pro-inflammatory effect mediated via a beta-arrestin (β-Arr) dependent pathway(63). They do not identify a therapeutic approach, but later work by the same group demonstrates abrogation of established airway hyperresponsiveness in a β-Arr_2 -/- murine asthma model, providing hope

[1] Gly16 associates with 'nocturnal asthma', a phenotype characterised by nocturnal downregulation of $beta_2$ adrenoceptors, perhaps in response to circadian variation in endogenous catecholamine(228).

for identification of a human molecular or genetic analogue selectively targeting the G-protein pathway(64).

Long acting $beta_2$ agonists (LABA) such as salmeterol are recognised as second line preventer therapy in childhood asthma(65). A minority of patients have been shown not to benefit from this medication class as add-on therapy. The substitution of Arginine for Glycine at position 16 (rs1042713, Arg-16) of the ADRB2 $beta_2$-adrenoceptor gene is associated with enhanced downregulation and uncoupling of $beta_2$-receptors and has been shown to predict this reduced responsiveness(66). Furthermore it has been suggested that this polymorphism might guide choice of add-on therapy in older children(67).

LABA, when used in the absence of inhaled steroids, are implicated in abrupt, severe asthma exacerbations and asthma-related deaths(68,69). This association may be due to downregulation of adrenoceptors coupled with maintenance of a degree of airway dilatation, such that without suppression of airway inflammation (and upregulation of ADBR2) by concomitant inhaled steroids, subjects are at risk of exacerbation and poor response to reliever medication. It has been suggested that this excess mortality persists even when inhaled steroids are co-administered(69), but a recent large trial in older children does not support this and combined ICS/LABA remain part of most national guidelines(70).

1.2.2 Anticholinergics

Inhaled anticholinergics such as ipratropium bromide are widely used in the management of acute preschool wheeze, acting to block muscarinic acetylcholine receptors and reduce smooth muscle contraction and mucus hypersecretion. Onset and duration of action are comparable to the short acting $beta_2$ agonists, and the synergistic mechanism of action lends itself to combination preparations although these are not commonly used in the UK outside of the emergency room, where so-called ‘burst therapy’ includes frequent co-nebulization of salbutamol, ipratropium bromide and occasionally magnesium(65). There is evidence of both efficacy and synergy with $beta_2$ agonist(71), although in isolation they appear to be less effective than $beta_2$ agonists(72). There is some evidence of a genetically-determined anticholinergic responsive phenotype(73), but the study population was small, and the documented efficacy and wide therapeutic window of this drug class makes further pursuit of this avenue unattractive. The long-acting antimuscarinic agents (LAMA) are not licensed in the preschool age group and are not discussed here.

1.2.3 Corticosteroids

The role of corticosteroids in the treatment of acute preschool wheezing disease is a matter of debate. Steroids act to suppress airway inflammation by altering the balance of expression of anti- and pro-inflammatory genes, inhibiting inflammatory cells and upregulating $beta_2$-adrenoceptor expression and function. Endogenous cortisol passively crosses cell membranes to bind with high affinity to cytoplasmic glucocorticoid receptors (GCR) to form an activated complex (cortisol-GCR) that translocates rapidly into the nucleus, inhibiting inflammation through three molecular mechanisms:

- It dimerizes and then binds to glucocorticoid response elements (specific DNA sequences) to modify nuclear gene expression, thereby increasing or decreasing gene transcription (known as transactivation – a direct genomic effect);
- It blocks the activity of nuclear factor (NF)-κB, a transcription factor present in an inactivated state that can itself rapidly *transactivate* inflammatory pathway genes, stimulating transcription of cytokines, chemokines, cell adhesion molecules, and associated receptors (this inhibitory effect on NF-κB is known as transrepression – an indirect genomic effect);
- It activates glucocorticoid signalling through membrane-associated receptors and second messengers (non-genomic effect).

The results of these indirect genomic actions are manifold, with inhibitory impacts on:

- Eicosanoid production - via induction of lipocortin-1 and consequent inhibition of phospholipase A2 (PLA_2) synthesis, thus reducing arachidonic acid liberation from the cell membrane.
- Inflammatory protein transcription and PLA_2-α activity (indirectly) – via induction of the mitogen-activated protein kinase (MAPK) phosphatase 1 that dephosphorylates and inactivates members of MAPK cascades.
- Expression of cyclooxygenase 2 and thus prostaglandin synthesis – via antagonism of NF-kB.

The main non-genomic mechanisms of cortisol action result in augmented NO synthesis via activation of NO synthase with subsequent vasodilation, and also increased noradrenergic activation in the airway vasculature, with associated reduction in airway blood flow(74) and oedema which in turn reduces wheeze. These mechanisms may operate independent of eosinophilic airway inflammation, and may therefore be more broadly relevant to preschool wheeze than classical steroid mechanisms. Glucocorticoids also affect $beta_2$ adrenoceptor function directly by transiently increasing ADRB2 mRNA (indicating increased gene expression) and increasing cyclic AMP response to the non-selective $beta_2$ agonist isoproterenol (indicating increased functional response)(75).

Acute preschool wheeze can be treated with oral or inhaled steroids. Studies of oral prednisolone have provided no evidence of efficacy in episodic viral wheeze(36,76), the commonest pattern in the preschool age group; despite this (and in the face of significant potential adverse effects) it persists in national guidelines(65). There is, however, increasing evidence supporting inhaled steroids in preschoolers, with a preference for intermittent dosing in episodic viral wheezers, and daily dosing in persistent wheezers(77), instability of preschool wheezing phenotypes notwithstanding(78). While this strategy is more attractive than the high dose inhaled steroids proposed in Ducharme's 2000 Cochrane Review(44), parents and perhaps even clinicians may remain squeamish about the potential effects on linear growth contingent on frequent steroid use(79). A recent review of asthma pharmacogenetics encompassing adult and child studies focused on inhaled corticosteroid and leukotriene modifier response genes(80). The FCER2 gene encodes a low affinity IgE receptor (CD23), activation of which inhibits T-cell regulated IgE-mediated immune reactions (a CD23 knockout mouse has exaggerated IgE responses and airway hyperactivity(81,82)); of interest, the rs28364072 (T2206C) SNP in this gene has repeatedly associated with poor childhood response to inhaled corticosteroids(80), with increased exacerbation risk observed in one study(83), reduced spirometric response in another(84), and increased asthma-related hospital visits in a third(85). Mechanistic support for the significance of this SNP is found in the observations from this work that CC homozygosity at this locus associates with increased exacerbation frequency at baseline, increased serum IgE, and reduced expression of FCER2. The relatively high minor allele frequency at this locus makes it a plausible pharmacogenetic predictor of steroid response and worthy of prospective study to this end in an adequately powered genetically stratified randomised controlled trial(83). While other candidate genes exist, to date there are no established genetic markers of steroid response in acute or chronic childhood asthma regardless of age.

1.2.4 Leukotriene modifiers

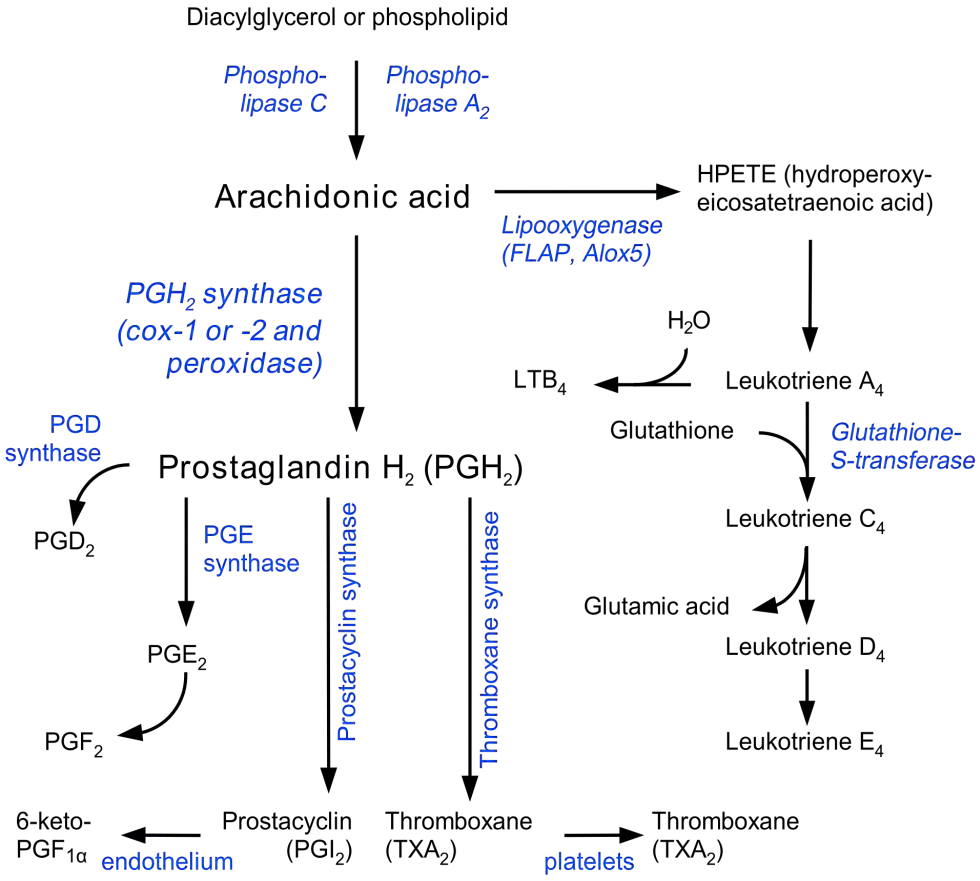
Leukotriene modifiers are divided into those that act as antagonists at the LTR1 receptor binding site (montelukast, pranlukast, zafirlukast) and those that disrupt leukotriene synthesis by inhibiting function of 5-lipoxygenase (zileuton). The only one in either class that is licensed in the preschool age group is montelukast. There follows an exploration of the existing evidence around the role of montelukast in preschool wheeze.

1.3 The Eicosanoids - structure, biochemistry and function

1.3.1 Arachidonic acid metabolism

Leukotrienes ("leuko" from their predominantly white blood cell source, and "triene" - for the three conjugated double bonds that form part of their structure) are products of the 5-lipoxygenase pathway of arachidonic acid metabolism. Arachidonic acid is cleaved from the C-2 position of membrane-bound phospholipid by the action of phospholipase A2 (although it can be generated from diacylglycerol by diacylglycerol lipase) in response to cell stimulation by mechanical, immunoallergic, toxic or infective triggers. Subsequently arachidonic acid is metabolised via two primary pathways, the cyclooxygenase pathway to create thromboxanes and prostaglandins, and the 5-lipoxygenase pathway to create the leukotrienes. The term "eicosanoid" is properly used to describe oxidation products of essential fatty acids, but is routinely extended to include the arachidonic acid metabolites (arachidonic acid is not a true essential fatty acid), and it is used to refer to this group here. The thromboxanes are potent vasoconstrictors and are known to stimulate platelet activation and aggregation; they are associated with pulmonary hypertension and inflammation in various disease states. The prostaglandins have multiple autocrine and paracrine roles and are implicated in pulmonary inflammation and bronchoconstriction.

FIGURE 1-3 - EICOSANOID METABOLISM



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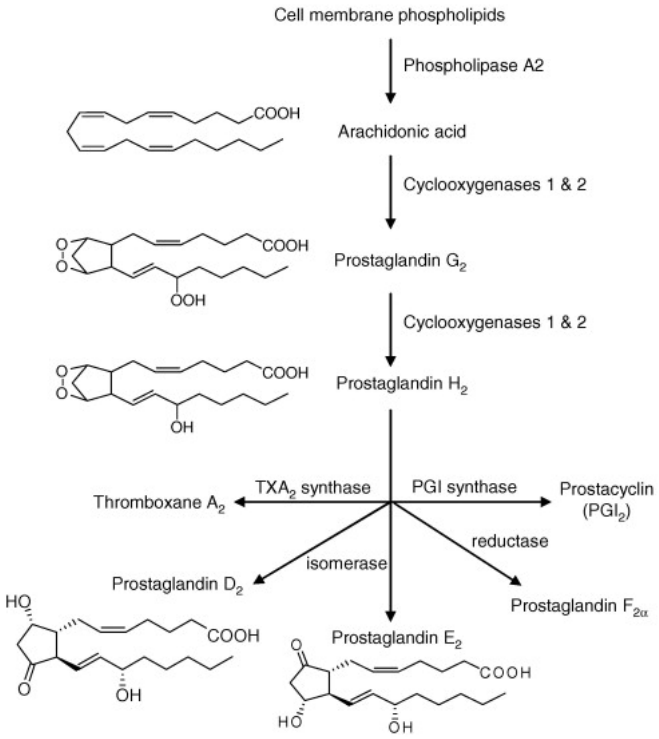
1.3.2 The prostanoids

1.3.2.1 Prostanoid synthesis

The prostaglandins (Pg, named for their presumed origin when first isolated in seminal fluid in 1935)(86) are part of the prostanoid class of eicosanoids and result from the metabolism of arachidonic acid via the cyclooxygenase (COX) pathway.

Briefly, liberated arachidonic acid is metabolised via the COX activity of a Pg Endoperoxide H synthase (PgHS, a dimeric membrane enzyme with distinct catalytically active sites) to the unstable Prostaglandin G_2, which is immediately reduced to Prostaglandin H_2 (PgH_2) by the peroxidase activity of the PgHS. PgH_2 is a substrate for specific isomerases that catalyse the formation of Prostaglandin D_2 (PgD_2, by PgD synthases - predominantly in activated mast cells), Prostaglandin E2 (PgE_2, by PgE synthases), Prostacyclin (PgI_2, by Prostacyclin synthase), Prostaglandin $F_{2\alpha}$ ($PgF_{2\alpha}$, by PgF synthases) and Thromboxane A_2 (TXA_2, by TX synthase).

FIGURE 1-4 - PROSTANOID SYNTHESIS



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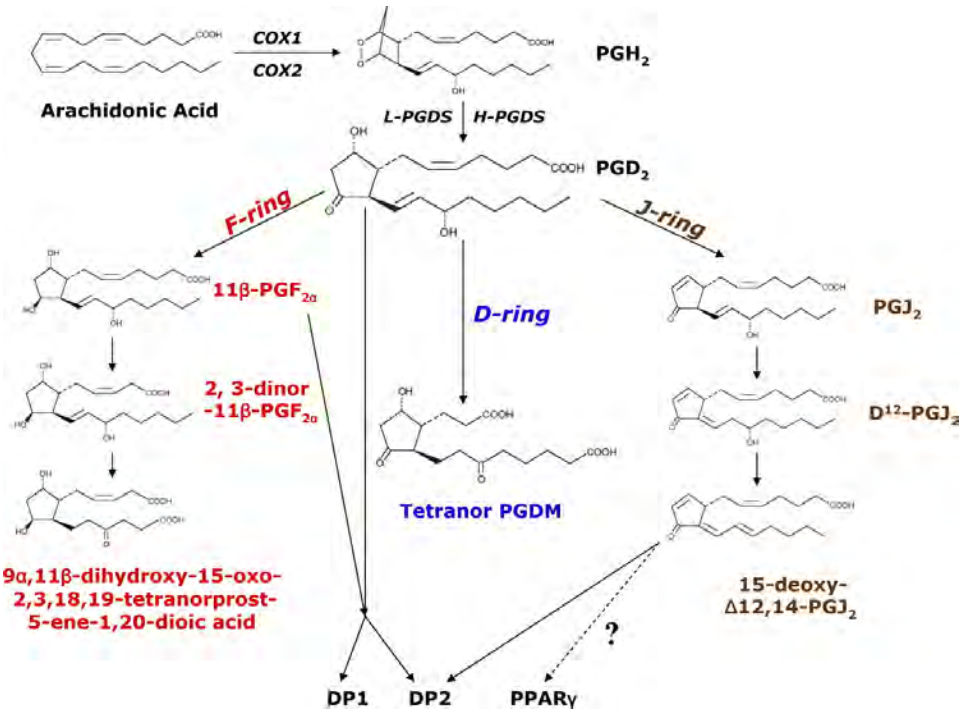
The COX moiety of PgHS has two distinct isoforms: COX-1 is ubiquitously and constitutively expressed, generating prostanoids with primarily homeostatic functions, while COX-2 is inducible in response to cytokine stimulation. Both isoforms are NSAID sensitive, but the binding site differs such that COX-1 is irreversibly inhibited by aspirin while COX-2 is modified to favour (predominantly anti-inflammatory) lipoxin rather than (predominantly pro-inflammatory) prostanoid production. This difference was the basis for the development of gastroprotective COX-2 specific inhibitors, some of which have since been withdrawn due to increased cardiovascular morbidity (attributable to loss of prostacyclin-mediated inhibition of thromboxane A_2)(88).

1.3.2.2 Prostanoid function and metabolism

PgD_2

Mast cell-derived prostaglandin D_2 is predominantly pro-wheeze. It interacts with receptor $CRTH_2$/DP2 to promote T_h2-lymphocyte, eosinophil and basophil chemotaxis(89), and also with DP1 where it is generally pro-inflammatory and can trigger reflex cough(90); at high levels it also causes bronchoconstriction via an interaction with the thromboxane receptor TP, and perhaps via a vasodilatory effect via DP1. It rapidly (plasma $T_{½}$ is about 6 seconds) undergoes NAD^+-linked oxidation to 13,14-dihydro-15-keto PgD_2 via the action of 15-hydroxy PgD_2 dehydrogenase (PgDH) or alternatively to 9α, 11β-PgF_2 and tetranor PgD-M (11, 15-Dioxo-9-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid), both of which are available in the urine(91). Barnes *et al*. have recently demonstrated reduced bronchoconstriction in asthmatics using an orally active $CRTH_2$-antagonist, lending clinical credence to the postulated role of PgD_2 in asthma(92). PgD_2 has not previously been studied in preschool wheeze.

FIGURE 1-5 - PROSTAGLANDIN D_2 METABOLISM



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PgE_2

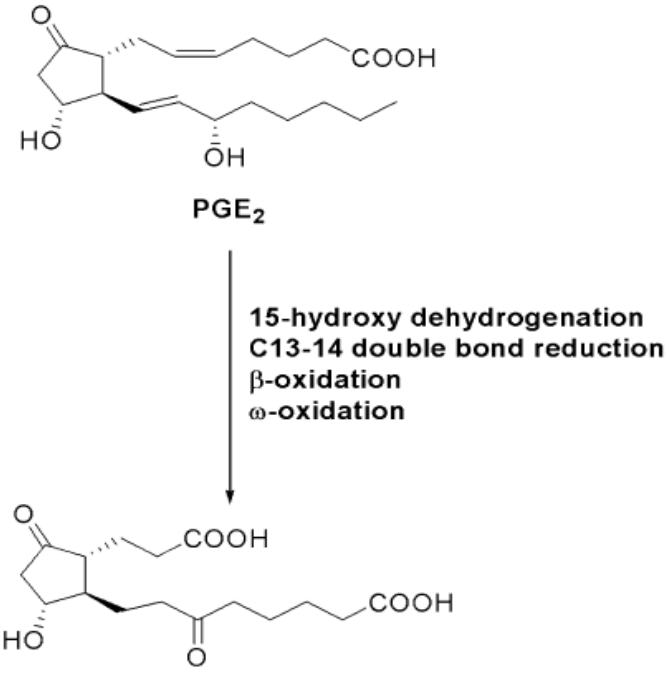
Prostaglandin E_2 is generally thought to be pro-inflammatory but has mixed pro- and anti-inflammatory and bronchodilatory effects in the lungs. Cytokine-driven upregulation of COX-2 (PTGS2) results in increased PgE_2 production, and this is seen in asthmatic, as well as COPD-affected airways (the latter in a dose-dependent fashion). There exists, therefore, a temptation to pursue blanket PgE_2/COX-2 antagonism as a therapeutic strategy, but this is

tempered by the acknowledged complexity of COX-2 induction and inhibition (such that COX-2 may actually be suppressed in certain Th_2 driven inflammatory conditions) and by the fact that COX-2 cannot be targeted without direct effects on other prostanoids such as PgD_2 and knock-on effects on other eicosanoid pathway products; there is also a growing recognition of the multiple pathways of PgE_2 effect mediated by its four receptors EP1-4. Mechanisms via which PgE_2 may promote wheezing disease include beta adrenoceptor desensitisation in human airway smooth muscle with consequent reduced bronchodilator efficacy and worsening of asthma control. This PgE_2-mediated tachyphylaxis can be induced by bacterial and viral infection, perhaps via formation of heterodimeric complexes between PgE_2 receptors and ADRB2(93). PgE_2 is also linked to mucus hypersecretion and airway remodelling, and is implicated in airway cough reflexes via EP3(94).

However, PgE_2 is also implicated in anti-asthma processes. It has long been known that PgE_2 can cause airway smooth muscle relaxation(95), but it is also implicated in bronchoprotection in other ways. Torres *et al*. describe a probable EP2-receptor-mediated mast cell inhibition, manifest in human and murine models, with putative beneficial effects on remodelling, inflammation and immunomodulation(96). Additionally, Aspirin-exacerbated Respiratory Disease (AERD) is mediated via inhibition of COX-1, which in susceptible individuals diverts arachidonate substrate from prostanoid (including PgE_2 and TXA_2) synthesis, stimulating an avalanche of cys-LT-driven mast cell and eosinophil activity with associated bronchoconstriction and airway inflammation, and this effect is negated by exogenous PgE_2 (97,98).

Prostaglandin E_2 is rapidly inactivated on passage through the lung where 15-hydroxy prostaglandin dehydrogenase (15-OH-PgDH) oxidizes it to 15-keto PgE_2, and it is thence metabolised to 15-hydroxy PgE_2, which is then further catabolised by beta and omega oxidation in the kidney and excreted in the urine as several shorter metabolites. The major stable urinary metabolite of PgE_2 is thus tetranor PgE-M (11α-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostan-1,20-dioic acid), which reliably reflects systemic PgE_2 generation.

FIGURE 1-6 - PROSTAGLANDIN E_2 METABOLISM



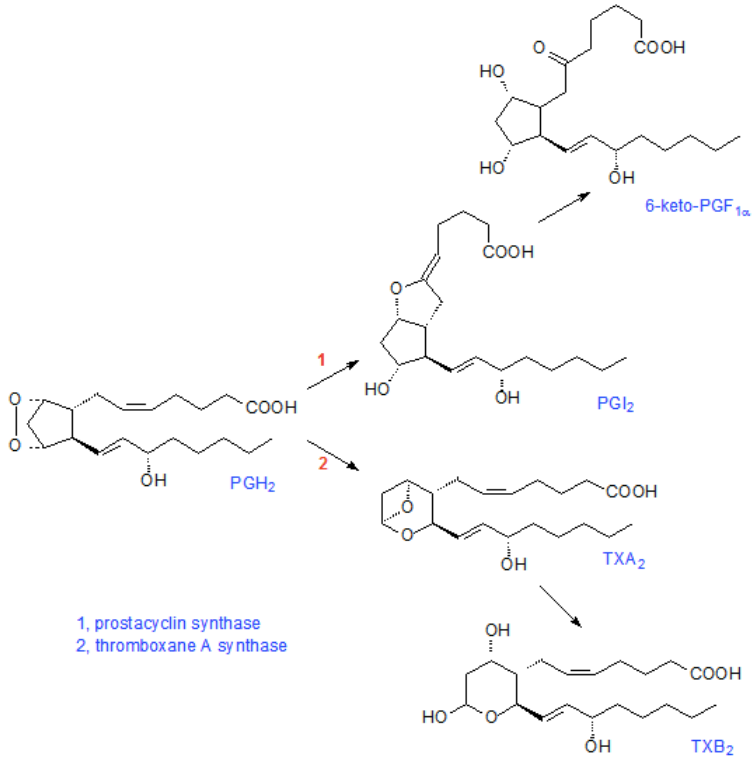
11α-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M)

Reproduced with permission from Murphey *et al*. Analytical Biochemistry. 2004 (230)

TXA_2 and PgI_2

The release of arachidonic acid by phospholipase A_2 and subsequent conversion to PgG_2 and then PgH_2 by the cyclooxygenase enzymes has been described elsewhere. Thromboxane A_2 (TXA_2) is produced by the action of thromboxane synthase in platelets and macrophages, while prostacyclin (PgI_2) is produced by endothelium-derived prostacyclin synthase. Both these prostanoids act locally via G-protein coupled receptors: PgI_2 predominates in endothelium and vascular smooth muscle via the I prostanoid receptor (IP), while TXA_2 has effect in platelets and lung via the thromboxane receptor (TP). Both TXA_2 and PgI_2 incorporate unstable ether moieties and are rapidly hydrolysed to (inert) TXB_2 and 6-keto-$PgF_{1\alpha}$ respectively, with consequent short half-life and local action.

FIGURE 1-7 - THROMBOXANE AND PROSTACYCLIN METABOLISM



Reproduced from A Lipid Primer (AOCS) - William Christie(231)

TXA_2 was identified in 1975 as a short-lived, locally-acting platelet aggregating and vasoconstrictor agent. It was subsequently found to be a potent bronchoconstrictor. The resultant early interest in both TP-antagonism and thromboxane synthase inhibition yielded equivocal results, with some suggestion of an ethnically divergent effect(99). Prostacyclin (PgI_2) was identified as Prostaglandin X by Vane's group in a paper published the subsequent year(100). Primarily a potent inhibitor of platelet aggregation, it has putative impacts on asthma pathology via immunomodulatory effects including inhibition of fibroblast and smooth muscle cells, Th_2-lymphocytes, eosinophils, neutrophils, dendritic cell and macrophages, and upregulation of Th_{17} cells(97). There is no specific evidence implicating either TXA_2 or PgI_2 in the pathophysiology of preschool wheeze.

1.3.3 The cysteinyl leukotrienes (cys-LTs)

1.3.3.1 Discovery

The cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) are distinguished from LTB_4 by the presence of a cysteine moiety within their structure. First identified in 1938 in the lung perfusate of guinea pig lungs exposed to cobra venom, they were noted to produce a slow-onset, sustained smooth muscle contraction(101). Further work by this group differentiated the time course of this activity from that of histamine, but the lack of a histamine antagonist prevented independent study of the 'slow-reacting (muscle-stimulating) substance' (SRS) in

isolation. In 1960 Brocklehurst demonstrated that explanted lung fragments from allergic asthmatic subjects released SRS under allergen challenge, naming it SRS-A, the 'Slow-Reacting Substance of Anaphylaxis'(102). This finding, in conjunction with the earlier work by Kellaway *et al*. focused attention on SRS-A as a putative bronchoconstrictive mediator in allergic asthma culminating in the identification of the cysteinyl leukotrienes by Samuelsson *et al*. in 1979(103,104).

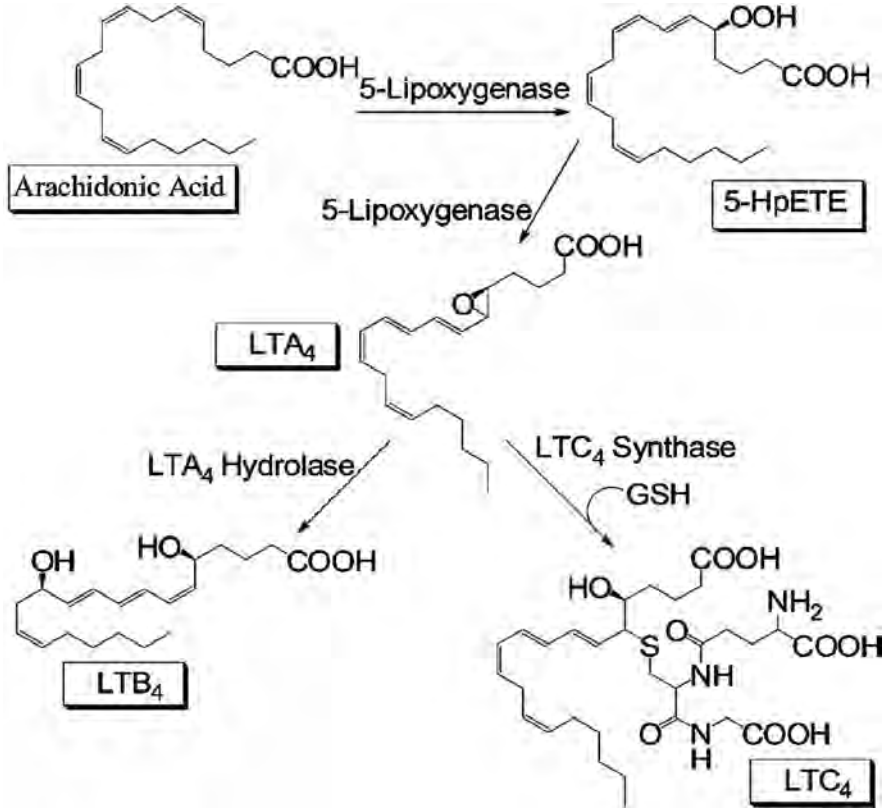
1.3.3.2 Biosynthesis

The cys-LTs are primarily generated intracellularly by activated eosinophils, basophils, mast cells, macrophages and myeloid dendritic cells. Arachidonic acid is oxidised at the C-5 position by arachidonic acid 5-lipoxygenase (ALOX-5) in conjunction with the helper molecules 5-lipoxygenase activating protein (FLAP) and coactosin-like protein (CLP) via 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which spontaneously reduces to 5-hydroxyeicosatetraenoic acid (5-HETE) and thence to the unstable epoxide intermediate Leukotriene A_4 (LTA_4). LTA_4 is subsequently conjugated with reduced glutathione to the parent cys-LT Leukotriene C_4 (LTC_4) by Leukotriene C_4 synthase (LTC_4S). LTA_4 may also be hydrolysed to leukotriene B_4 (LTB_4, a neutrophil stimulant and chemoattractant) by Leukotriene A_4 hydrolase (LTA_4H) within neutrophils and monocytes. LTB_4 is not one of the cys-LTs and is not discussed further. LTC_4 is then actively exported from the cell where it may directly stimulate cys-LT receptors (cys-LTRs) or undergo enzymatic conversion (by sequential amino acids hydrolysis) to Leukotriene D_4 and thence to the highly stable Leukotriene E_4 (LTE_4), which is excreted in urine and is the final product of leukotriene metabolism.

Transcellular Biosynthesis

Cells lacking adequate ALOX5 but expressing LTC_4S (such as platelets and endothelial cells) can generate cys-LTs via a transcellular mechanism by accepting and converting extracellular LTA_4 (produced and exported by ALOX5 positive cells, predominantly leukocytes) to LTC_4 and thence to its bioactive metabolites (LTD_4, E_4) as above, and may form an additional source of cys-LTs in certain conditions such as aspirin-exacerbated respiratory disease (AERD)(105).

FIGURE 1-8 - LEUKOTRIENE SYNTHESIS



Reproduced with permission from Murphy and Gijon(105)

1.3.3.3 Function

The cysteinyl leukotrienes were marked as potent *in vitro* human bronchoconstrictors soon after their isolation (104) and subsequent human and animal data has confirmed this. Drazen *et al.* showed elevation of urinary LTE_4 in subjects with salbutamol-responsive acute airway obstruction compared to non-responders or normal controls(106), and inhaled LTE_4 causes airway narrowing with far greater potency than histamine(107). People with AERD have constitutively high urinary excretion of LTE_4, with significant further elevation and bronchoconstriction in response to NSAID therapy(108). Antileukotriene agents attenuate this AERD effect(109,110), and multiple studies demonstrate their influence on short and longterm parameters of asthma severity(111,112) in the aspirin-tolerant majority. Those with AERD also have greater sensitivity to exogenous LTE_4 than non-aspirin sensitive subjects(107), suggesting divergent receptor number, type or function.

1.3.3.4 Cysteinyl leukotriene receptors

Cys-LTs have long been known to act on at least two G-protein coupled receptors Cys-LTR1 and Cys-LTR2, with circumstantial evidence for at least one further(113). LTC_4 and LTD_4 (but not LTE_4) stimulate peripheral bronchoconstriction in guinea pig lung, however, LTE_4 elicits guinea pig tracheal ring constriction with 10 times greater potency than that observed

with LTC_4 and LTD_4. Drazen *et al*. also induced a large drop in pulmonary compliance (but no effect on resistance) in anaesthetised guinea pigs with intravenous LTC_4 and D4, but a rise in resistance (as well as compliance changes) with LTE_4. LTE_4 (but not the other cys-LTs) was also shown to prime guinea pig trachea to histamine-induced contraction, an effect that was negated by cyclooxygenase-blockade with indomethacin. These findings were again consistent with a greater role for LTE_4 in the proximal airways than the periphery, via a COX-generated Thromboxane A_2. Together they indicate the existence of three distinct cys-LT receptors.

Human studies support this prediction, insofar as inhalation of LTC_4 and LTD_4 elicit bronchoconstriction with 1000 times the potency exhibited by histamine in both asthmatics and non-asthmatics, while LTE_4 causes a far more modest effect. LTE_4 is only 40 times as potent as histamine in non-asthmatics, increasing to 400 times as potent in asthmatics, with aspirin-exacerbated subjects a further 16 times more sensitive to LTE_4 than aspirin-tolerant subjects. There was no difference in dose-response between asthmatics and non-asthmatics for LTC_4- and LTD_4-induced airway obstruction, and AERD subjects were no different to their aspirin-tolerant counterparts in response to LTC_4 and histamine(113). The inference is that a specific phenotype of LTE_4-sensitive asthma (mediated by a novel receptor) exists separately to other phenotypes, and that high levels of this putative LTE_4-specific receptor may mediate AERD. Human in vivo mechanistic support for this hypothesis stemmed from the observation that inhalation of equipotent doses of LTE_4 and LTD_4 induced eosinophil and mast cell accumulation in bronchial mucosa and sputum with LTE_4 but not with LTD_4, and also that LTE_4 increased sensitivity to histamine-induced bronchoconstriction, as observed in Drazen's guinea pig trachea preparation; as with Drazen this effect was negated by indomethacin COX-blockade, implicating increased COX product synthesis as an effector mechanism for some LTE_4 effects.

Established cys-LTRs

Cys-LTR1 and Cys-LTR2 are G-protein coupled receptors arising from distinct chromosomes (Xq13-Xq21 and 13q14 respectively), they are found in structural cells (e.g. airway smooth muscle, nasal mucosal interstitium, bronchial fibroblasts) and cells of the innate (mast cells, macrophages, eosinophils, basophils, dendritic cells) and adaptive immune systems (B- and T-lymphocytes), consistent with the roles of their ligands in immunity, inflammation and airway responses. Cys-LTR1 binds LTD_4 with higher affinity than LTC_4, while Cys-LTR2 binds both equally; neither shows significant affinity for LTE_4, nor does LTE_4 appear to stimulate signalling effects in cells expressing Cys-LTR1 or 2 alone. Cys-LTR1 blockade or knockout eliminates LTD_4 signal response, despite the presence of active Cys-LTR2, while Cys-LTR2 blockade increases Cys-LTR1 activity. This reflects the role of Cys-LTR2 as a

homeostatic check on Cys-LTR1 activity, via the formation of a heterodimer between the two proteins. Thus constitutively low levels of Cys-LTR2 with normal cys-LT production may result in paradoxically increased vulnerability to LTD_4:Cys-LTR1 mediated effects such as bronchoconstriction. Cys-LTR1 and 2 polymorphisms associate with atopic disease; in the genetically homogenous, highly atopic and genetically isolated population of Tristan de Cunha, variant forms of Cys-LTR1 and 2 predominate in asthmatic and atopic subjects(114–116).

Novel cys-LTRs

GPR17 (the gene is located on chromosome 2 and codes for a G-protein-coupled receptor) has been considered and largely dismissed as a putative LTE_4 receptor, as sequential studies have not supported the early suggestion that it may be a cys-LT target, except perhaps in the nervous system(117). Latterly GPR17 has gained attention for a possible regulatory interaction with Cys-LTR1 analogous to that described in Cys-LTR2 (113,118).

The gene encoding GPR99 (now also known as the 2-oxoglutarate receptor - OXGR1, cysteinyl leukotriene receptor 3/E - Cys-LTR3/Cys-LTE) was identified in 2001, and went through sequential appraisals of its function and specificity before the gene product was identified as a G-protein receptor and potential primary target for Leukotriene E_4(117). Wild type mice exhibit an ear swelling vascular permeability response to intradermal injection of each of the three cys-LTs, but while the LTE_4 response persists in Cys-LTR1 and 2 knockout mice (Cys-LTR1/2 -/-), the LTC_4 and D4 responses are diminished(119). In triple knockout (Cys-LTR1/2/GPR99 -/-) mice the vascular permeability response to all three ligands is abolished, whereas single knockout (GPR99 -/-) mice have a substantially reduced LTE_4 response but no change in response to LTC_4 and D4, indicating a clear preference for GPR99 over the established cys-LT receptors(120). Further evidence for a role of GPR99 in LTE_4-mediated asthma symptoms comes from the observation that both intranasal alternaria and intranasal LTE_4 stimulate mast cell-associated epithelial mucin production in wild type mice, and that this effect is lost in mast cell, LTC_4S or GPR99 deficient mice(121). The protective effect of Cys-LTR1 antagonist montelukast in (primarily-LTE_4 mediated) AERD suggests that it may have some efficacy as an inhibitor of GPR99, but development of specific antagonists is a promising avenue for investigation.

Another protein implicated in LTE_4 effects is the purinergic $P2Y_{12}$ receptor ($P2Y_{12}R$). $P2Y_{12}R$ is a G-protein coupled purinergic (ADP is the primary ligand) receptor found predominantly in platelets, and inhibited by antiplatelet agents such as clopidogrel. Early modelling suggested it may be a receptor for LTE_4, but subsequent work has indicated that it is a co-receptor for

LTE_4, necessary for LTE_4 activity but acting by binding a primary receptor, now thought to be GPR99(122,123).

1.4 Antileukotriene therapies

Montelukast is licensed for use from 6 months of age(124) and functions as a competitive antagonist at the Cys-LTR1 receptor, acting in bronchial epithelium and airway smooth muscle to reduce the bronchoconstrictive effect of endogenous LTC_4, LTD_4 and to a lesser extent LTE_4(124).

1.4.1 Montelukast in preschool wheeze

Montelukast is a promising therapy for both clinical phenotypes of preschool wheeze. This beneficial effect of inhibition of cys-LT in preschool wheeze was suggested by a previous study of urinary cysteinyl leukotrienes, where levels of urinary LTE_4 were elevated during acute attacks of preschool wheeze, then fell into the normal range on convalescence(38). A study relevant to multi-trigger preschool wheeze is a RCT of 689 young children where regular oral montelukast given over a 12 week period reduced the rate of wheeze exacerbations by 30%(125). For episodic (viral) preschool wheeze Bisgaard *et al*(126) reported that regular daily use of oral montelukast over 12 months reduced the rate of preschool wheezing episodes by 32% compared with placebo. The Preempt study recruited a heterogeneous group of children aged between 2 and 14 years with intermittent asthma into a 12-month randomised placebo-controlled trial of oral montelukast. Trial medication was started at the onset of a viral upper respiratory tract infection and continued for a minimum of 7 days, or until symptoms had resolved for 48 hours. The montelukast-treated group had 162 unscheduled health-care resource utilisations for wheeze compared to 288 in the placebo group, and symptoms were significantly reduced by 14% in the montelukast treated group(39). Subsequent data, including a number of robust reviews, have since shifted the balance of favour away from montelukast(77,127–129), but since the data available at the time suggested that intermittent therapy *may* be effective in preschool wheeze, the aim of the WAIT trial was to assess whether parent-initiated montelukast therapy would be efficacious in this condition.

1.4.2 Genetics of montelukast response and study rationale

The beneficial effect of montelukast, such as it is, is clinically relatively modest(39). The overall modest benefit is thought due to marked heterogeneity of montelukast response; i.e. some children respond very well while others do not respond at all. One explanation for this marked heterogeneity in response is variation in genes encoding components of the LT pathway(130,131). The first step in cys-LT production is the release of membrane bound

arachidonic acid by phospholipase A_2, followed by conversion to Leukotriene A_4 (via 5(S)-HETE) by arachidonic acid 5-lipoxygenase (ALOX5; other names for ALOX5 being 5-LO, and Leukotriene A_4 synthase) in association with 5-LO-activating protein (FLAP; encoded by the ALOX5AP gene), and/or coactosin-like protein (CLP; encoded by COTL1)(132,133). This appears to be a rate-determining step in cys-LT production. A polymorphism in the promoter region of the ALOX5 gene results in a variation in the number of CG-rich Sp1 transcription factor-binding motifs which alters transcription factor binding, and influences ALOX5 gene expression(134). Five Sp1-binding repeats in the ALOX5 promoter is classified as the wild type, while other numbers of repeats represent variant or “mutant" genotypes. Lima *et al*(130) found that adults carrying a variant number of repeats on one allele [x/y or 5/x] (where x or y $\neq$ 5) have a 73% reduction in the risk of having an asthma attack if taking montelukast, compared with homozygotes for the 5-repeat (5/5; wild type) allele. We therefore hypothesized that overall, parent-initiated montelukast therapy in preschool wheeze would be clinically moderately effective, but that there would be a highly responsive subgroup of children defined by ALOX5 promoter polymorphism status (i.e. carrying a variant number of repeats on at least one allele). Sayers *et al.* suggest that 30% of UK children carry a variant allele, making this polymorphism a plausible driver for montelukast response heterogeneity(135). In this trial we therefore included a stratification step for ALOX5 promoter polymorphism status, to ensure that an equal number of children with the variant and wild type number of Sp1-binding repeats in the ALOX5 promoter received placebo and active medication.

While there are other genetic candidates to explain variability in montelukast response, including exonic(130) or epigenetic(136) modifications in ALOX5, and variations in the genes for FLAP and CLP (132,133) amongst other pathway proteins(137,138), the ALOX5 promoter polymorphism is the only one with clinical trial evidence of influence on montelukast efficacy (with respect to wheeze exacerbations(130)). Telleria(139) found a contradictory polarity of effect on montelukast efficacy to Lima(130), but the larger sample and effect sizes described by Lima hold greater sway. Given the acknowledged complexity of the cys-LT metabolic pathway we additionally performed an exploratory investigation of a panel of genes with potential to influence eicosanoid airway inflammation and wheezing outcomes.

Leukotriene E_4 has previously been discussed as an independent mediator in atopic and wheezing disease, but it is, in addition, recognised as the final common stable stage of cys-LT metabolism and is readily measurable in urine(42) and exhaled breath condensate(43). Previous work by our group has indicated that, in atopic children, urinary (u)LTE_4 increases during acute preschool wheeze (38), while Cai *et al*. demonstrate that elevated uLTE_4 levels can predict likelihood of response to montelukast in moderate adult asthmatics(140).

Rabinovitch *et al*. suggest increased likelihood of asthma exacerbation in older, tobacco smoke-exposed children with high $uLTE_4$ but not those with low $uLTE_4$(141), and also indicate that the ratio between $uLTE_4$ and FeNO (a marker of eosinophilic inflammation) can predict montelukast response(142). Drazen *et al*. noted elevated $uLTE_4$ during exacerbation in montelukast-responsive adult subjects compared with non-responders and normal controls(106). Interestingly, a recent study pertinent to this age group clearly indicates an increase in $uLTE_4$ during wheezing exacerbation compared with remission in both atopic and non-atopic preschool children, but that atopic subjects have higher resting and exacerbating $uLTE_4$ than non-atopic children, and also that in remission non-atopic subjects had similar levels to healthy controls(41). Mougey *et al*. demonstrated an association between variant (x/y, where $x/y \neq 5$) ALOX5 promoter polymorphism, elevated uLTE4 and reduced FEV1; in addition, there was a trend to reduced asthma control and subjects were more likely to be prescribed montelukast(143). Therefore we hypothesized $uLTE_4$ would relate to ALOX5 promoter genotype, montelukast response or both.

In light of the previously noted implication of other arachidonic acid products in wheezing disease(92–97) and cys-LT metabolism we also explored urinary levels of other eicosanoids in our subjects and healthy controls. Normal values of urinary eicosanoids may help to identify wheezing subgroups responsive to specific anti-eicosanoid therapies. In healthy individuals a fall in $uLTE_4$ with age(144) and an increase during exacerbation of atopic eczema(145) has previously been reported, but the effect of these factors on other urinary eicosanoid metabolites is unknown. We therefore sought to describe the effect of age and atopic status on a range of urinary eicosanoids in healthy children.

Finally, the use of genetic information to inform treatment decisions is relatively novel(47) and certainly not commonplace; additionally, South Asian communities such as our local predominantly Bangladeshi population are notoriously difficult to engage in clinical trials(146,147), as well as having relatively high rates of admission to hospital for asthma(148). We therefore incorporated a small, qualitative study within the main trial to explore some of these issues, with a view to suggesting improvements in study design and clinical approach for the future.

1.5 Hypotheses

1.5.1 Main hypothesis

Intermittent montelukast is effective in preschool wheeze

1.5.2 Secondary hypotheses

- Subjects with at least one non-5-repeat ALOX5 promoter allele will show superior montelukast efficacy.
- Subjects with at least one non-5-repeat ALOX5 promoter allele will have elevated baseline or exacerbation leukotriene activity and this will relate to montelukast efficacy in this group.

1.6 Aims

- The chief aim of this work was to assess the efficacy of parent-initiated intermittent montelukast for reduction of unscheduled medical attendances for preschool wheeze, and to describe the role of ALOX5 promoter genotype in the polarity and magnitude of any effect observed.
- Secondary aims were quantification of the role of intermittent montelukast on respiratory morbidity, health service usage and economic outcomes, concomitant medication usage and adverse events.
- The pharmacogenetics and pathophysiological mechanisms of preschool wheeze remain opaque, thus an additional aim sought to explore the role of selected eicosanoid pathway genes and mediators in preschool wheeze and montelukast response.
- The final aim was to gain insight into the parent and child experience of and attitudes towards preschool wheeze, parent-initiated therapy, and participation in a genetically stratified interventional trial.

1.7 Objectives

- To conduct and report a genotype-stratified double blind placebo-controlled randomised controlled clinical trial of montelukast efficacy in preschool wheeze.
- To describe genetic and biomarker mechanistic correlates of trial outcomes and preschool wheeze phenotypes.
- To conduct a qualitative study of parental attitudes to preschool wheeze and clinical trial concepts.

In the next chapter I describe the methods employed by my study team and collaborators to address the questions outlined above.

2 METHODS

2.1 Overall study design

2.1.1 Approvals and funding

Ethical approval was obtained from the UK NHS South East Multicentre Research Ethics Committee (Ref: 09/H1102/110, Appendix 8.3.2). Regulatory approval was obtained from the UK Medicines and Healthcare Products Regulatory Agency (Ref: 21313/0024/01-0001, Appendix 8.3.3). The study was registered with the European Clinical Trial Database (EudraCT Ref: 2009-015626-11) and the US National Library of Medicine ClinicalTrials.gov database (Ref: NCT01142505). Funding was from the UK National Institute for Healthcare Research Efficacy and Mechanisms Evaluation programme (NIHR-EME, Ref: 08/43/03, Appendix 8.3.6) and the sponsor was Queen Mary University of London (Appendix 8.3.4).

2.1.2 Study overview

This was a double blind randomised placebo controlled trial of intermittent montelukast therapy. The study population comprised preschool children (10 months to 5 years inclusive) with two or more previous episodes of wheeze. Target accrual was 1300 patients (2.10.2). Eligibility criteria were as stated in section 2.2. An overview is provided in Figure 2-2. Patients were recruited in secondary care. They were stratified according to ALOX5 promoter genotype and then randomised within their strata to receive either intermittent montelukast or placebo for 10 days from the start of a viral cold or wheezing episode, with need for unscheduled medical attention monitored over a 12 month follow-up period.

2.2 Participants

2.2.1 Eligibility criteria

Patients were eligible for the study if they fulfilled the following criteria:

- age ≥ 10 months and ≤ 5 years on the day of the first dose of IMP.
- two or more attacks of parent-reported wheeze.
- at least one attack with wheeze validated by a clinician (nursing or medical)
- the most recent attack within the last 3 months.
- contactable by telephone and able to attend one face-to-face review
- parent or guardian able to give written informed consent for their child to participate in the study.

2.2.2 Exclusion criteria

The following characteristics rendered patients ineligible for the study:

- any other chronic respiratory condition diagnosed by a clinician including structural airway abnormality (e.g. floppy larynx) and cystic fibrosis
- any chronic condition that increases vulnerability to respiratory tract infection such as severe developmental delay with feeding difficulty or sickle cell disease
- history of neonatal chronic lung disease
- current continuous oral montelukast therapy
- in a trial using an IMP in the previous 3 months prior to recruitment.

2.2.3 Selection of study population

As indicated previously wheezing is common in otherwise healthy preschool children, while safe effective treatment options are limited. We therefore sought to conduct a pragmatic trial with the widest possible useful application. Thus participants were not limited in terms of wheeze severity or concomitant medications, notwithstanding the prohibition of regular montelukast. We did not include children below 10 months and above 5 years of age so as to exclude children with classical bronchiolitic or asthmatic phenotypes, where treatment strategies differ.

2.3 Recruitment and patient journey

2.3.1 Recruitment setting

Participants were identified in primary care (Patient Identification Centres only, Appendix 8.1.2) and secondary care centres. Recruitment was planned to encompass only three secondary care centres (The Royal London Hospital, University Hospital Leicester, and The Royal Aberdeen Children's Hospital) but increased to 41 secondary care centres in England and Scotland (see Appendix 8.1) in response to suboptimal observed recruitment rates.

2.3.2 Invitation of potential study participants to attend screening visit

Members of the child's usual GP care team or the hospital paediatric team (as appropriate) identified potentially eligible children based on age and history of wheeze from reviewing surgery and emergency department records. The parent/guardian was then approached in person or via a posted invitation letter and or information sheet, to ask if they would like to be contacted about the study by a member of the (hospital-based) research team. Individuals who agreed to be contacted about the study were then contacted by a research nurse or research assistant, who briefly described the study to them, and asked them if they would like to read a parent information sheet (PIS, Appendix 8.4.1) if not already given. The research nurse or research assistant then provided a PIS to parents who expressed an interest in the study; those who subsequently confirmed their interest in participation were offered a screening appointment at a study site. A second invitation letter was posted to

individuals who did not respond to the first invitation letter. All public documentation underwent ethical review.

2.3.3 T-2 screening visit (-2 weeks)

At the screening visit an investigator, or a suitably trained person delegated by the investigator (a research nurse or a research assistant who had attended a UK regulations GCP training course) gave an adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. The eligibility of children to participate in the study was assessed according to the criteria documented in section 2.2. The investigator then obtained written informed consent (Appendix 8.4.2) from the parent or guardian prior to participation in the study. A period of at least 24 hours or an overnight stay in hospital (for patients recruited during an acute admission) was required for consideration by the parent or guardian before they gave consent to enter the study. During the consent process it was made clear that parents or guardians were completely free to refuse to enter the study or to withdraw at any time during the study, without citing a reason. The parents of all eligible children were asked to complete baseline assessments of their child's wheeze status including recording of baseline demographic and clinical data and details of concomitant medications (Appendix 8.5, Figure 8-1). They also underwent measurement of weight and height, provided a salivary sample for genotyping (Appendix 8.8.1) and gave a urine sample for leukotriene analysis (detail in Appendix 8.8.2). A follow-up appointment (T0) was arranged for the issue of the IMP.

2.3.4 Stratification (-1 week)

Saliva samples were posted to the Blizard Institute, Queen Mary University of London, where DNA was extracted and children assigned to either ALOX5 promoter polymorphism "5/5", or "[5/x and x/y]" strata, depending on the number of copies of the ALOX5 promoter polymorphism they had on each allele. Extracted DNA was stored at -70°C for later batch analysis of ≈150 polymorphisms in ≈20 genes encoding components of the LT biosynthetic pathway and the LT receptors. The study pharmacist then randomised subjects within their strata, and the corresponding box of active or placebo medication was dispensed for issue at the T0 visit (Figure 2-1 and Figure 2-2).

2.3.5 Method of assigning patients to treatment groups - randomisation

Nova Laboratories Ltd (Novalabs, Leicester) prepared the IMP for this trial. Preparation was intended to comprise six monthly batches tailored to recruitment rate, with an expectation that 1300 boxes of 50 sachets containing active montelukast and 1300 containing placebo would be produced at a minimum. However, a national shortage of montelukast necessitated a production of boxes containing between 20 and 50 sachets so as to maintain supply and not compromise recruitment and subject retention. The change in box size

received approval from the MHRA prior to implementation. Boxes were allocated randomisation numbers in blocks of ten using a computer-generated random sequence. Novalabs was responsible for generation of the random number sequence and labelling of boxes. Boxes bearing randomisation numbers were initially delivered to the pharmacy at participating sites. Subsequently, the expansion of site numbers prompted a move to central randomisation and distribution of IMP (from the sponsor pharmacy to participating sites). Novalabs produced additional boxes of IMP for those children whose IMP supply was lost, reached expiry, or was exhausted such that they required additional boxes during the one year follow-up period. Clinicians remained blinded to allocation throughout.

Randomisation was stratified according to ALOX5 promoter polymorphism status yielding two genotype groups:

Group I Children with the [5/5] ALOX5 promoter polymorphism genotype.

Group II Children with [5/x or x/y] ALOX5 promoter polymorphism genotype; where x or y ≠ 5 Sp1-binding repeats. (Groups were referred to as Stratum A or B).

Children in each of these two genotype groups (strata) were assigned consecutive randomisation numbers from randomised permuted blocks of 10 representing the randomisation numbers on the IMP boxes. Within each block equal numbers of children were randomly allocated to placebo and active treatment. When all numbers from the first block had been assigned a new block of randomisation numbers was allocated to that stratum, until a total of 1300 children in the two strata combined had been assigned a randomisation number (Figure 2-1).

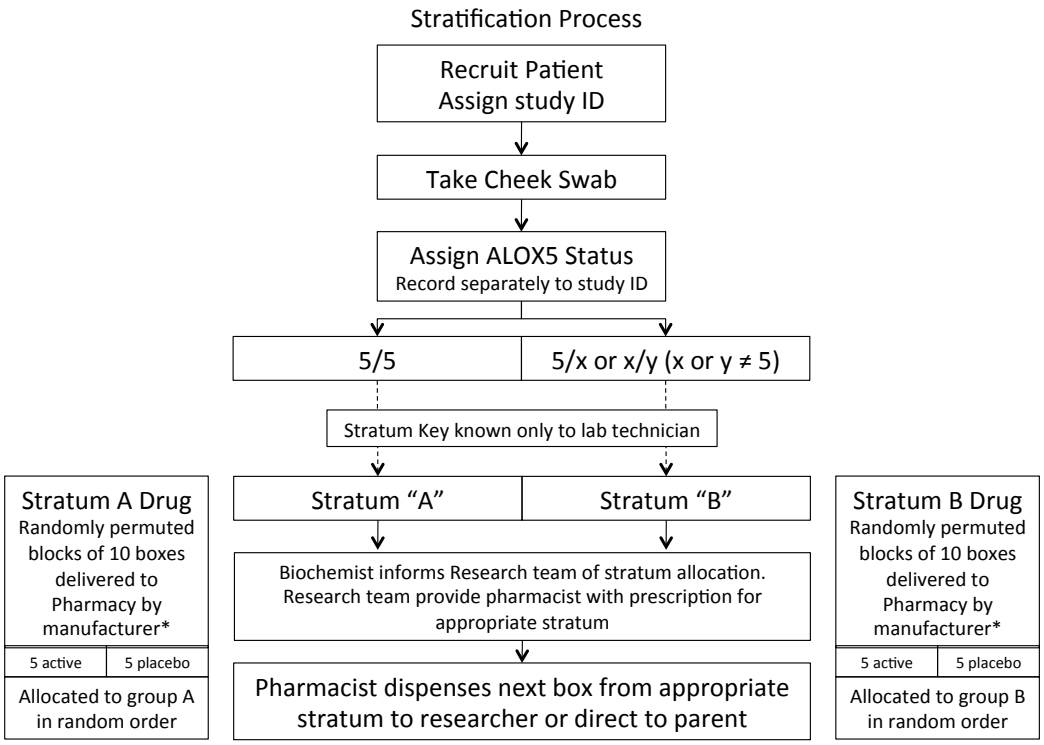
2.3.6 Blinding

Novalabs produced a corresponding randomisation code denoting whether a given IMP box contained active medication or placebo. This was kept sealed and held only by the clinical trials pharmacist and a member of the Independent Data and Safety Monitoring Committee (DSMC), in this way all other clinical investigators and participants remained blinded to treatment allocation. Both investigators and patients were also blinded to genotype stratum.

2.3.7 T0 visit (0 months)

The research nurse or research assistant met with parents, confirmed eligibility, and issued parents a box containing IMP sachets. Parents were taught how to use the IMP. They were also provided with one study diary card (Appendix, Figure 8-4) and one freepost return envelope (addressed to the Sponsor organisation) per 10 sachets. Parents were asked to return completed diary cards and empty sachets at completion of a course of IMP. Each diary card recorded clinical and IMP usage data for the 10 days of the IMP course.

FIGURE 2-1 - STRATIFICATION AND RANDOMISATION SCHEMATIC



*Randomisation key provided by manufacturer, held in sealed envelope by Pharmacist and Data Monitoring Committee

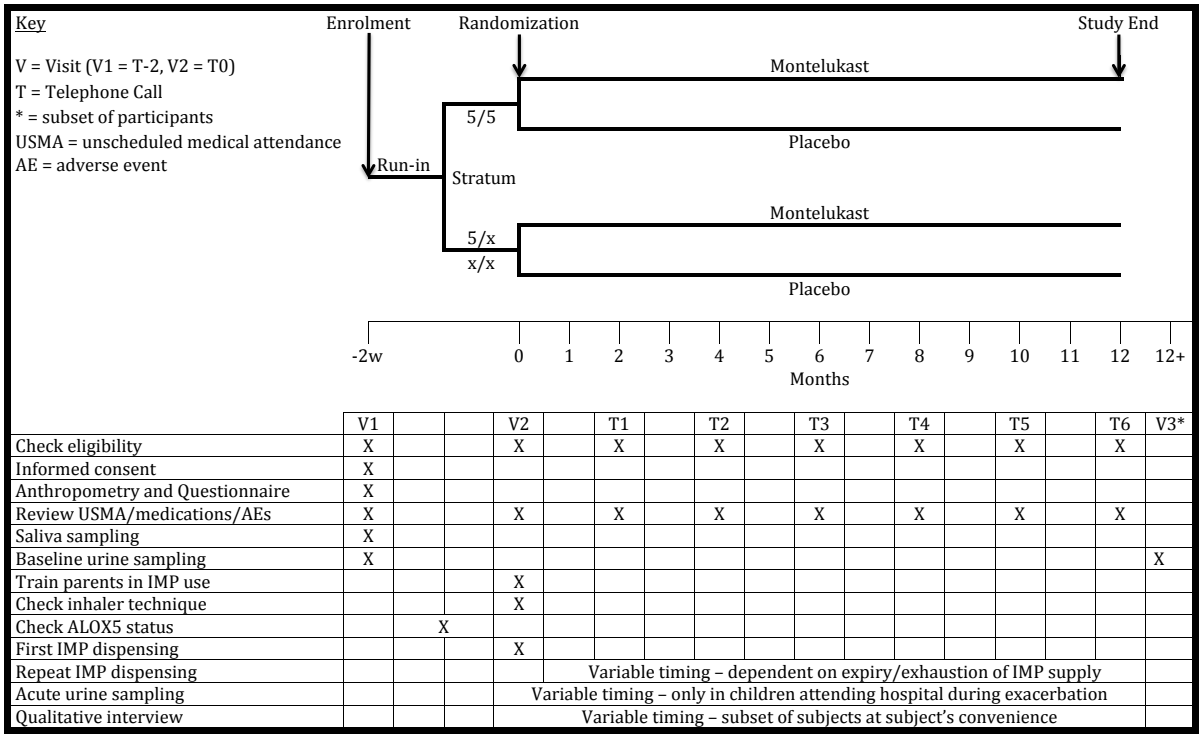
2.3.8 T2-T12 phone calls (2,4,6,8,10,12 months)

At approximately two monthly intervals following the T0 visit a research nurse or research assistant telephoned the subject's carer to check whether they had initiated the IMP, the numbers of days the IMP had been used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Any adverse events experienced were also recorded.

2.3.9 Qualitative interview visit (variable timing)

In a subgroup of families recruited at the sponsor site qualitative interviews were conducted. The aim of these was to establish attitudes towards genetic testing to guide personalised therapy, acceptability of parent-initiated therapy for preschool wheeze, the expected advantages and disadvantages of using the IMP, and their views on the consent process and parent information sheet. Interviews included either or both parents, and where possible were conducted at the parental home. Interviewing, transcribing, and analysis of interviews were done by a researcher skilled in qualitative research, where necessary in the presence of a translator.

FIGURE 2-2 - SCHEMATIC CHART OF PROTOCOL



	V1			V2		T1		T2		T3		T4		T5		T6	V3*
Check eligibility	X			X		X		X		X		X		X		X	
Informed consent	X																
Anthropometry and Questionnaire	X																
Review USMA/medications/AEs	X			X		X		X		X		X		X		X	
Saliva sampling	X																
Baseline urine sampling	X																X
Train parents in IMP use				X													
Check inhaler technique				X													
Check ALOX5 status		X															
First IMP dispensing				X													
Repeat IMP dispensing					Variable timing – dependent on expiry/exhaustion of IMP supply												
Acute urine sampling				Variable timing – only in children attending hospital during exacerbation													
Qualitative interview				Variable timing – subset of subjects at subject's convenience													

2.3.10 Withdrawal of patients from therapy or assessment

Patients were free to withdraw from the study at any time without giving a reason. Patients were advised that if they requested to withdraw from the study, at any time during the trial, then this would have no negative consequences. The investigator could also withdraw patients from the trial if they deemed it appropriate for safety or ethical reasons or if it was considered to be to be detrimental to the wellbeing of the patient. Where possible, patients who withdrew or were withdrawn underwent a final telephone or face-to-face evaluation. Those participants who withdrew and provided permission to use their data were included in the analysis up to the point of withdrawal. Full documentation was made of any withdrawals that occurred during the study in the CRF. The Investigator documented the date and time of the withdrawal and results of any assessments made at this time. If the patient withdrew because of an adverse event (AE) or a serious adverse event (SAE) then details were forwarded to the Ethics committee as required and to the Sponsor, who forwarded details to the regulatory authorities as appropriate.

2.4 Interventions

2.4.1 Active drug

Trade name: Singulair Granules

Composition: 4mg Montelukast sodium (which is equivalent to 4mg montelukast) granules with mannitol excipient

ATC code: R03DC03

Pharmaceutical form:	Granules
Dosage regimen:	One sachet to be given once a day at the start of a cold or wheezy episode, and continued for 10 days.
Route of administration:	Oral
Manufacturer	Merck Sharpe and Dohme Ltd (purchased on the open market)

2.4.2 Placebo

Trade name:	Mannitol EP (Pearlitol SD 200)
Composition:	Mannitol Granules
ATC code:	Not applicable; drug master file lodged with the European Pharmacopoeia commission
Pharmaceutical form:	Granules
Dosage regimen:	One sachet to be given once a day at the start of a cold or wheezy episode, and continued for 10 days.
Route of administration:	Oral
Manufacturer:	Roquette Pharma

2.4.3 Administration of investigational medicinal product

Subsequent to stratification children were randomised within their strata to receive either montelukast or identical placebo. All study treatment was dispensed from study pharmacy either directly to the patient carer or to the study investigator or designated member of staff for distribution to the carer. IMP was administered unsupervised by patient carers in their usual place of residence. IMP was presented as white granules administered either directly into the child's mouth, or mixed with a spoonful of cold or room temperature soft food (e.g., apple sauce, ice cream, carrots and rice). The IMP was used according to the primary manufacturer's instructions. Specifically, parents were advised not to open the sachet containing the granules until ready to use. After opening the sachet, the full dose of granules was administered within 15 minutes. If mixed with food, the granules must not be stored for future use. The granules were not to be dissolved in liquid for administration however liquids could be taken subsequent to administration. The granules could be administered without regard to the timing of food ingestion. The dose was one 4mg sachet per day, started when the child had evidence of a viral cold or had wheeze, and stopped after 10 days. Children were permitted to commence a second course of IMP should the wheeze not resolve within 10 days. If a child vomited after the administration of the IMP no additional dose was given, and parents recorded this on the diary card.

2.4.4 Selection of doses in the study

Montelukast is an established medication in this patient population with an accepted dosing of 4mg daily. The granule formulation was selected to achieve the broadest tolerability

across the preschool age group. IMP was commenced at the first sign of a cold and continued to 10 days to give the best chance of covering the entire duration of any virus-induced LTE_4 over-production. There was no variation of dosing strategy or posology between patients.

2.4.5 Prior and concomitant therapy

Subjects were eligible for the study as long as they were not taking regular montelukast. No limitations were placed on concomitant medications, however these were recorded on the CRFs at study entry and during follow-up.

2.5 Other assessments

2.5.1 Safety assessments

Montelukast is an established drug with a good safety profile. Safety assessments were limited to standard adverse event reporting, with patterns monitored by the DSMC.

2.5.2 Weight

Weight in light clothing was measured with weighing scales and recorded in kilograms.

2.5.3 Height

Height without shoes was measured using a stadiometer (calibration of which was the responsibility of the recruiting hospital).

2.5.4 Salivary DNA sampling

Saliva for DNA was collected using the Oragene-infant sponge system. The sponge tips were cut into an Oragene DNA kit to preserve the DNA and prevent bacterial growth. This method yields high-quality DNA and eliminates the need for traditional cheek scraping methods.

2.5.5 Urine sampling

A spontaneously voided urine sample was obtained from children using an age-appropriate method into a sterile receptacle. A first urine sample was obtained when patients were well and a second during an acute wheezing illness where possible. On receipt samples were separated into 1ml aliquots, placed on ice and then frozen to -70^{o}C within 24 hours of collection for subsequent batch analysis. Our group has previously demonstrated stability of urine eicosanoids for two years at -70^{o}C(8.8.2). At appropriate intervals samples were courier-transported on dry ice to the Jagiellonian Institute, Krakow and to Kings College Hospital, London for batch eicosanoid and cotinine estimation respectively.

As well as the main study participants, children up to 15 years of age with no history of asthma or wheeze (controls) were recruited in the paediatric outpatient department of the sponsor site. Controls were: i) siblings of children attending the outpatient clinics of the Royal London Children's Hospital, and ii) children attending a separate paediatric allergy clinic at the Royal London Children's Hospital. Children attending the allergy clinic were eligible if there was no other medical condition besides food allergy. Atopic status was determined by parental report along with review of skin prick test data where available. Urine was analysed for eicosanoid profile and cotinine. This additional sampling was approved by the local research ethics committee and conducted with written informed consent from parents/carers. Urine sampling of control patients was approved by the UK National Health Service Multicentre Research Ethics Committee (Ref: 09/H1102/110, Appendix, 8.3.2), and required written informed consent from the parent or guardian.

2.5.6 Symptom diary

An A6 booklet form symptom diary (Appendix 8.5, Figure 8-4) was designed and professionally printed. Parents were asked to complete one questionnaire for every 10-day course of IMP they commenced, and then send the diary back with the empty sachets in a pre-printed postage paid envelope. In this way we attempted to assess IMP and protocol compliance. Questions included the presence or absence wheeze or viral cold symptoms, use of salbutamol, absence from education, childcare or work, IMP administration and tolerance, and medical review. There was a section for free text comment, which parents could use to record anything, from perception of efficacy to suspected adverse events.

2.5.7 Telephone questionnaire

A brief, loosely scripted telephone questionnaire was administered to each recruited subject on an alternate monthly basis. Questions concerned unscheduled healthcare usage, adverse events, IMP usage, school and work absence, and concomitant medications. The questionnaire also incorporated prompts to use the IMP appropriately, to complete and return diary cards, and to report adverse events. Details of unscheduled healthcare utilisations were used to verify primary outcome data.

2.5.8 Qualitative interview

2.5.8.1 Parent study procedures

Teams of children's research nurses and secondary care paediatricians recruited preschool children with a history of wheezing following hospital attendance for wheeze or after receiving information from their primary care physician. For hospital attendees, recruitment occurred immediately prior to or shortly after discharge from hospital. Families received a trial information pack and subsequently discussed the study with the research team. Written

and real-time verbal Bengali[2] translation was available as required. Amenable parents then gave written informed consent (to paediatrician, research nurse or both) after which a mouth swab (for leukotriene pathway genes) and urine sample (for leukotriene levels) were collected. Parents agreed to administer a 10-day course of oral medication (randomly allocated to montelukast or placebo) at the onset of a cold or wheezing symptoms, and to complete a daily diary record for the same period. They also received progress calls from the clinical research team at regular intervals and were encouraged to phone if they had any queries or concerns. Children were followed up for 1 year and the need for unscheduled respiratory medical attendance assessed. The qualitative study (QS) was based at the East London host centre only.

2.5.8.2 Participant recruitment

The parent study (PS) was conducted as previously described in 41 recruiting centres nationwide. The qualitative study (QS) was based at the East London host centre of this multicentre trial and involved an audio-recorded semi-structured interview with parents of enrolled children. All participants in the trial and the QS received written information about the study at recruitment. At the time of the QS one hundred and thirty-nine host centre families had given formal written consent for their child to be enrolled in the trial and 85 of these parents had given written consent to a qualitative interview at the same time (Table 5-1). The initial plan was to sample purposively from the 85 consenting parents, aiming for maximum variation(149) in terms of ethnicity, gender and other variables and then theoretically, according to iterative analysis of initial interviews.

2.5.8.3 Data generation

Individual interviews were considered the most appropriate method for data collection as this ensured confidentiality. A semi-structured interview guide (Box 2-1) for the interviews was developed following a literature review and discussions between the qualitative study team(150–153). An experienced non-clinical qualitative researcher with an interest in the development of healthcare services in partnership with the patient population conducted interviews. Interviews took place in the family home and lasted 25-60 min. Preschool children were present in many instances. Interviews were conducted in English except one, which was supported by a Bengali interpreter.

[2] Bangladeshis are a large minority in East London and primary carers often do not speak English - Table 5-3.

BOX 2-1 - TOPICS INCLUDED IN SEMI-STRUCTURED INTERVIEW GUIDE

1. Family and child background information
   - Child's history of wheeze
   - Treatment and diagnosis
   - Impact on child/parents/family
2. Parents experiences of joining the trial
   - Motivations
   - Consent and research governance processes
   - Attitudes towards the collection of DNA and genetically guided therapy
3. Parent's attitudes to and experiences of giving the trial drug to their child.

2.5.8.4 Data analysis

Interviews were audio-recorded and professionally transcribed verbatim. Transcripts and field notes were imported into NVivo9 (QSR International Pty Ltd., Melbourne, Victoria, Australia), a qualitative data analysis program. From this we developed a coding framework that drew on the research questions, previous research about patient experiences of taking part in clinical trials and themes that emerged in the course of the analysis. The data were systematically coded and analysed using a modified grounded theory approach(154) incorporating the constant comparison technique to elicit key themes and explore deviant cases(155,156).

2.6 Laboratory measurements

2.6.1 Genotyping of ALOX5 promoter polymorphism

2.6.1.1 DNA extraction and amplification

ALOX5 polymorphism status was determined at the Blizard Institute Laboratory within 1 week of sampling. DNA was extracted according to a local SOP and the manufacturer's instructions (DNAgenotek). Approximately 20ng of genomic DNA was added to a reaction mix containing 1x PCR buffer and 0.5U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Waltham, MA, USA) with 2.5mM $MgCl_2$, 5% DMSO, 0.2µM dNTPs containing a 3:1 ratio of dGTP to 7-deaza-dGTP and 0.2µM of each primer in a final volume of 20µl. The primer sequences were 5'FAM-AGGAACAGACACCTCGCTGAGGAGAG-3' and 5'GAGCAGCGAGCGCCGGGAGCCTCGGC3'. Cycling conditions were 95^{o}C for 6 min followed by 35 cycles of 95^{o}C for 15s, 62^{o}C for 23s and 72^{o}C for 30s followed by a final extension at 72^{o}C for 5 minutes.

2.6.1.2 Genotyping

Products of the PCR were diluted 1:5 in water and 1µl of this dilution was added to 9µl Hi-Di™ formamide (Applied Biosystems) + 0.3µl ROX500 size standard (Applied Biosystems) and analysed by capillary electrophoresis on a 3130xl Genetic Analyser (Applied Biosystems). Fragments of 256-292bp were obtained depending on the copy number (2-8) of the repeat sequence and were visualized using GeneMapper® v4.0 or Peak Scanner™ v1.0 software (Applied Biosystems). Genotypes were called manually from duplicate amplifications. Samples with known genotype (previously verified by DNA sequence analysis) were included in each run.

Alleles were called according to the number of simple repeats. Samples with the most common genotype (homozygous 5/5) were allocated to stratum A. Samples with any other genotype (5/x or x/y, where x or y is any allele other than 5) were allocated to stratum B. Stratum-genotype key was known only to laboratory staff who generated stratum reports which were subsequently posted on the study website (2.11.1.1) to direct IMP prescription.

2.6.1.3 Genotyping validation

Random DNA samples were obtained from within the paediatrics department and used to test the ALOX5 genotyping process and to look for different genotypes prior to sequencing.

As described previously the simple sequence length polymorphism in the promoter region of ALOX5 was amplified using PCR. PCR products were run on a 1.6% agarose gel to check for successful amplification. These products were then transferred to a microplate in a mix of formamide and the size standard 500 ROX™ (Applied Biosystems), before being run on the 3130xl capillary sequencer. Depending on the alleles present, PCR fragments of varying size were obtained corresponding to the number of copies of the Sp1-binding motif. The GeneMapper software was used to visualize these size fragments and to assign genotypes.
DNA sequencing was performed on DNA samples with interesting genotypes in addition to a homozygous wild type sample. The sequencing process involved amplifying the promoter region of ALOX5 in the same way as detailed above, except with a non-fluorescent forward primer. Excess primers and dNTPs were removed from the PCR product using an ExoSAP-IT® PCR cleanup kit (Applied Biosciences). The ExoSAP-IT product was then used in a sequencing PCR which consisted of 0.5µl of Big Dye®, 2µl of both forward primer and buffer and 4.5µl of water with the following thermal profile: 25 cycles of 96°C for 10s, 50°C for 5s and 60°C for 4mins. These products were transferred to a microplate where another cleanup was performed which consisted of a 30 minute incubation on ice with 125mM EDTA and 100% ethanol followed by high speed centrifuging and removal of the supernatant. This was

repeated with just 70% ethanol before resuspending the pellet in 10µl formamide and loading onto the 3130xl capillary sequencer.

14 DNA samples were used to validate the process. All samples were successfully amplified, demonstrated by running the PCR products on a 1.6% agarose gel. The PCR products were then run on the 3130xl capillary sequencer. Figure 2-3 shows some example electropherograms produced by this process, while Table 2-1 lists the various fragment sizes from each sample.

The homozygous wild type genotype consisting of 5 Sp1-binding repeats in the promoter region is demonstrated by a single peak in the electropherograms. These fragments were found to have a size of ≈266 base pairs. The most common heterozygous genotype had two peaks at ≈261 and ≈266 base pairs. The smaller allele is approximately 1 Sp1-binding repeat smaller than the 5-repeat peak. These two peaks therefore correspond to the 4/5 genotype. A third genotype was found with peaks at 256 and 266. The difference between these is approximately two Sp1-binding repeats, therefore this would imply a genotype of 3/5.

3 samples, numbered W/001, 2535 and 2551 were sequenced to verify the genotypes shown in Table 2-1. The sequencing results for these samples are shown in Figure 2-3, showing deletions of 1 and 2 Sp1-binding repeats in the 4/5 and 3/5 genotypes respectively.

These data demonstrate the effectiveness of the method employed to genotype the ALOX5 promoter polymorphism. The three most common genotypes(135) were successfully amplified and analysed by the 3130xl capillary sequencer with relevant software. Peak sizes of around 266, 261 and 256 base pairs were shown to correspond to 5, 4 and 3 repeats of the Sp1 transcription binding factor motif respectively. These genotypes were then successfully sequenced to confirm the correct assignment of genotype from the fragment analyses. This fragment analysis method was used for stratification of trial candidates according to genotype.

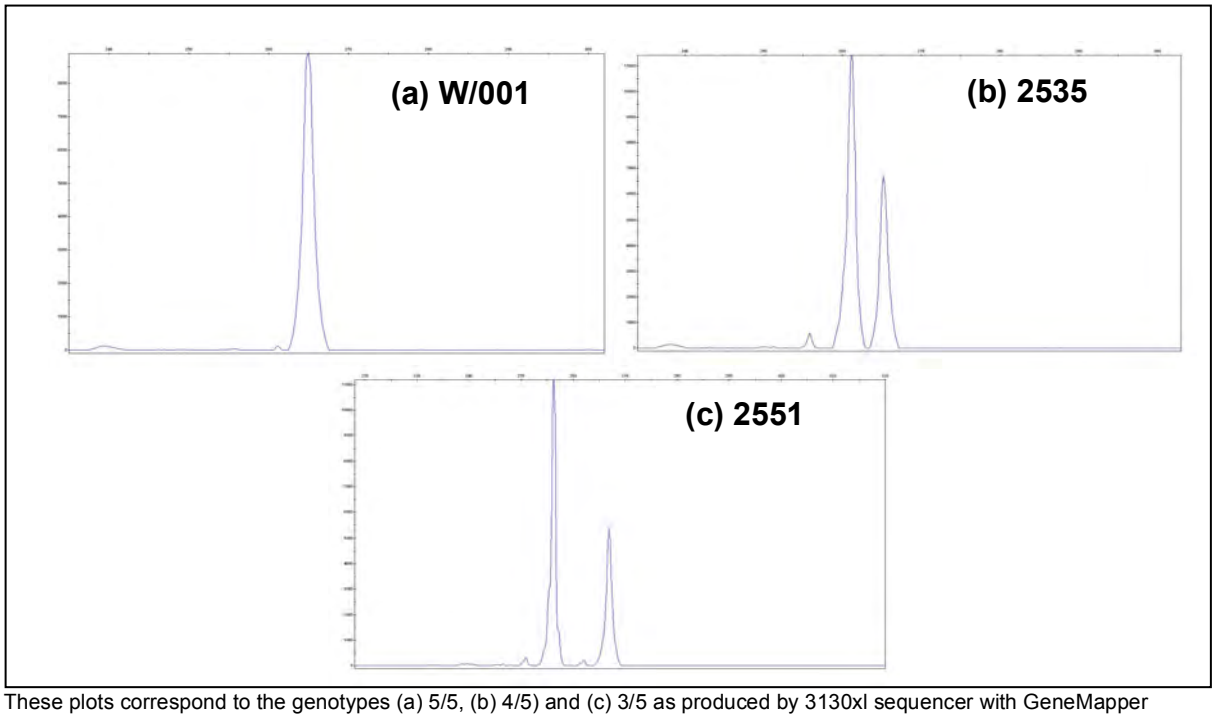
On one occasion inadequate fragment amplification (see 3.2.2) resulted in inaccurate stratification (a variant allele [5/6] was called as wild type [5/5]), while on another a clerical error led to a variant allele (correctly identified as [4/5]) being stratified as wild type [5/5]). These rare errors were identified during laboratory quality assurance processes (6.9.4) but only after medication had been dispensed.

TABLE 2-1 - RESULTS FROM TEST GENOTYPING RUN

Sample No	Peak Size (base pairs)		Genotype
2535	261	266	4/5
2546	261	266	4/5
2547	266	-	5/5
2551	256	266	3/5
2555	266	-	5/5
2557	261	266	4/5
2563	261	266	4/5
2572	265	-	5/5
2573	261	265	4/5
2578	261	266	4/5
2584	266	-	5/5
2588	267	-	5/5
2593	262	266	4/5
W/001	265	-	5/5

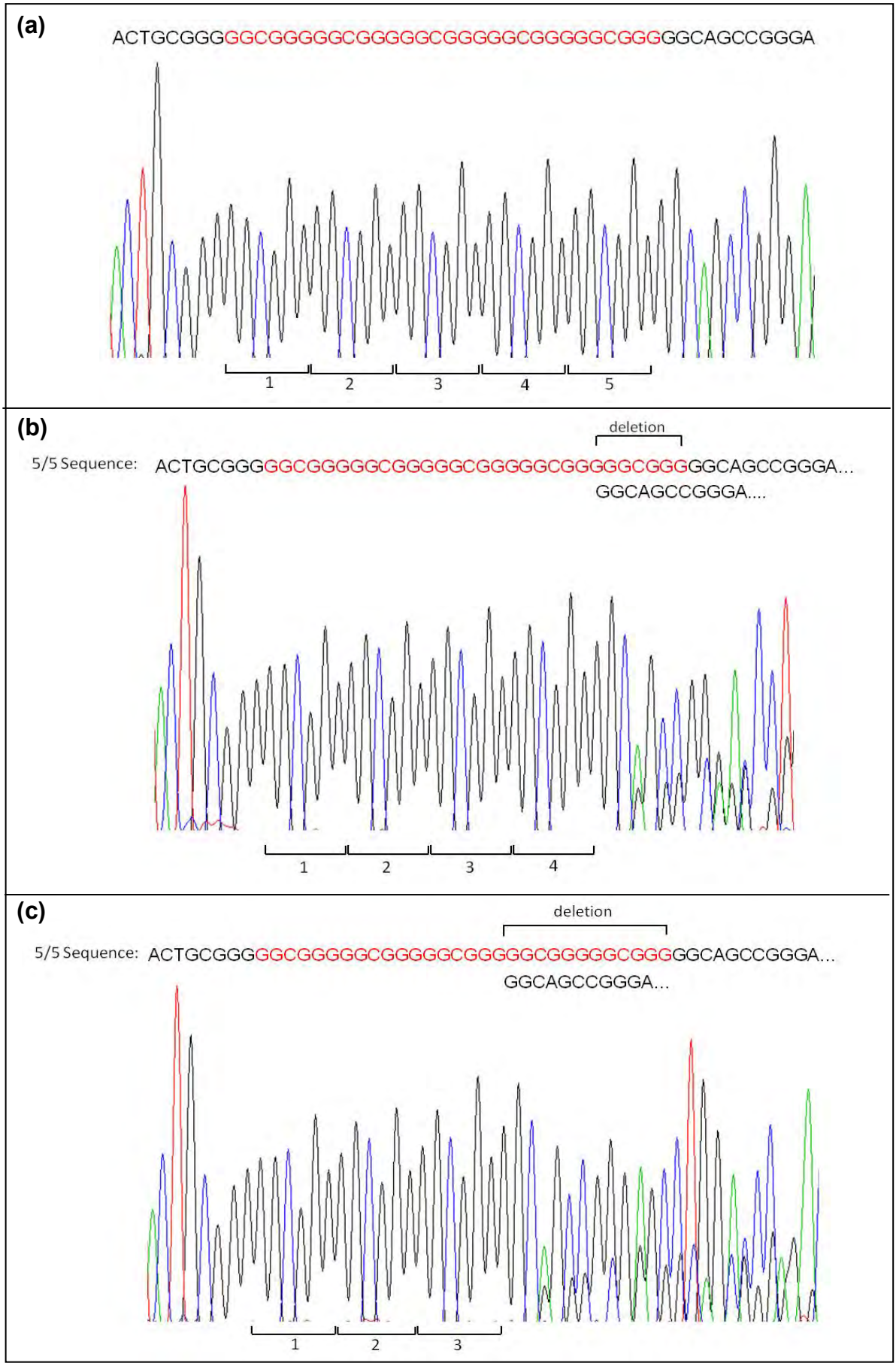
Peak sizes correspond to the size of the ALOX5 amplicon generated by PCR (also shown on the x-axes of Figure 2-3).

FIGURE 2-3 - EXAMPLE ELECTROPHEROGRAM OUTPUTS



These plots correspond to the genotypes (a) 5/5, (b) 4/5) and (c) 3/5 as produced by 3130xl sequencer with GeneMapper

FIGURE 2-4 - EXAMPLE SEQUENCING RESULTS



Sequencing results from samples W/001 (a), 2535 (b) and 2551 (c), showing the 5/5, 4/5 and 3/5 genotypes respectively. The GGCGGG repeat is shown in red.

2.6.2 Exploratory SNP analysis

We additionally assessed 143 polymorphisms in several genes encoding components of the leukotriene and eicosanoid biosynthetic pathway (ALOX5, ALOX5AP, LTC4S, CYSLTR1, CYSLTR2, PLA2G4A, LTA4H, LTB4R1, LTB4R2, CYP3A4, CYPC9, ADRB2, NR3C1, SLCO2B1, PTGDR, TBXA2R, PTGS1, PTGS2, PTGER2, PTGER3, PTGIR and MRP1 - Appendix, 8.9). These included all SNPs located in promoter regions, exons and intron-exon boundaries and the SNPs within the ALOX5AP haplotypes (referred to as Hap A and Hap B). Choice of SNPs was based on initial review of the literature, with a particular reference to the work of Lima *et al*.(130), Klotsman(138), Duroudier(157) and Tantisira(158,159). These were used to build a core list of SNPs with broad coverage of the leukotriene biosynthetic pathway, but which also included other asthma and eicosanoid-related genes. The list was then augmented by inclusion of tag SNPs for each of the genes; these were identified by searching the (now archived) International HapMap project database using the CEU (western European ancestry) population (using alternative populations did not yield different results). R^2 (>0.8) and minimum allele frequency (5%) cut offs were kept at HapMap default settings.

Additional tagSNPs were selected using the LDselect algorithm on the basis of linkage disequilibrium patterns across the genes using data from previous studies in cardiovascular disease and asthma(130,138,157,160–164) as well as resequencing data available from the Seattle SNPs(165) and NIEHS SNPs(166) databases.

SNP genotyping was carried out using the KASPar™ competitive allele-specific PCR method (KBiosciences, Hitchin, UK)(167).

2.6.3 Urinary eicosanoid quantification

Urinary eicosanoid estimation was performed at the Jagiellonian Institute in Krakow, under the supervision of Professor Marek Sanak. LTE_4, 13,14-dihydro-15-keto-PgE_2, 13,14-dihydro-15-keto-tetranor-PgE_2, 13,14-dihydro-15-keto-PgD_2, 13,14-dihydro-15-keto-tetranor-PgD_2, tetranor-PgE-M, tetranor-PgD-M and 15-deoxy-γ-12,14-PgJ_2 were measured using High Performance Liquid Chromatography-tandem mass spectrometry (HPLC-tMS)(42), while 9α,11β-PgF_2 was measured using derivatisation and gas chromatography mass spectrometry (GC-MS). To allow for variable hydration status values were expressed in pg/mg of creatinine.

2.6.3.1 Organic phase extraction

Organic phase extraction method was common to both spectrometry techniques. Samples were passively thawed on ice or in the fridge (4°C, 3-5 hrs) in batches of up to 20 samples. Aliquot pH was adjusted to 3.5 with 1N HCl (30 - 80µL), pH was checked using a standard

narrow range pH stick. Subsequently, 10µL of an internal deuterated standards mix (in methanol) was added; the standards (with quantities) were: LTE_4-d_3 (2ng), tetranor-PgE-M-d_6 (10ng), tetranor-PgD-M-d_6 (10ng), 13,14-dihydro-15-keto-PgE_2-d_4 (1ng), 13,14-dihydro-15-keto-PgD_2-d_4 (1ng), 13,14-dihydro-15-keto-tetranor-PgE_2-d_4 (1ng), 13,14-dehydro,15-keto-tetranor-PgD_2-d_4 (1ng), 9α,11β-PgF_2-d_4 (1ng), 15-deoxy-γ-12,14-PgJ_2-d_4 (1ng). If a uric acid precipitate was present samples were spun for 10 minutes at 10000g at 4°C (microcentrifuge) and the resulting supernatant transferred to a fresh 10ml conical sample tube and mixed with 1mL tertiary-butylmethyl-ether (TBM), vortexed for 2 minutes, and spun again at 10000g as previously. The upper organic phase was again collected to a fresh tube, and then repeat extracted with another 1mL TBM, followed by combination of the organic phases. The pooled sample was then dried at room temperature under nitrogen flow (1 L/min) for 30 min. It was then dissolved in 60µL methanol and immediately analysed by either HPLC-tMS or GC-MS.

2.6.3.2 High performance liquid chromatography - tandem mass spectrometry

Methanol dissolved aliquots (10µL) were injected onto a reverse phase column (Zorbax Eclipse XDB C-18 - Agilent Technologies, Santa Clara, CA, USA), stabilised thermally at 37°C and a gradient consisting of two mobile phases: A - acetonitrile/water/acetic acid (20/80/0.0001) and B - acetonitrile/isopropanol/acetic acid (55/45/0.0001) was used to elute LTE_4 and other eicosanoid compounds with the flow rate 0.11 mL/min using HPLC equipped with an autosampler (Shimadzu Sil-2-AC - Shimadzu Scientific Instruments, Kyoto, Japan). The mobile phase binary linear gradient was 1 min 8% B, 9.5 min 8-95% B, 0.5 min 95% B, 0.5 min 95-100% B, 2 min 100% B. Leukotriene E_4 and other compounds were measured using multiple reaction monitoring mode (MRM) tandem mass spectrometry (Qtrap 4000, Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ion source negative ionization mode, using batch profile for urinary eicosanoids.

2.6.3.3 Gas chromatography - mass spectrometry

Organic phase aliquots (2µL) were prepared by a 3-step derivatisation to pentafluorobenzyl and trimethylsilyl esters and methoxyoxime which modified carboxyl and hydroxyl groups of the compounds, with subsequent purification by a thin-layer chromatography (TLC) and methanol elution from the TLC silica. A single quadrupole mass spectrometer (Engine 5989B series II - Hewlett Packard, Palo Alto, CA, USA) in gas chromatography negative-ion chemical ionization mode (GC-NICI-MS) with a 15m capillary column was used for quantification, using a urinary prostanoids protocol.

2.6.3.4 Data analysis

Ion pairs (internal standard and analyte) for HPLC-tMS measurements were as follows:

- LTE_4-d_3 441-336[3] and LTE_4 438-333
- tetranor-PgE-M-d_6 333-315 and tetranor-PgE-M 327-309
- tetranor-PgD-M-d_6 333-315 and tetranor-PgD-M 327-309[4]
- 13,14-dihydro-15-keto-PgE_2-d_4 355-337 and 13,14-dihydro-15-keto-PgE_2 351-333
- 13,14-dihydro-15-keto-PgD_2-d_4 355-337 and 13,14-dihydro-15-keto-PgD_2 351-333
- 13,14-dihydro-15-keto-tetranor-PgE_2-d_4 301-283 and 13,14-dihydro-15-keto-tetranor-PgE_2 297-279
- 13,14-dihydro-15-keto-tetranor-PgD_2-d_4 301-283 and 13,14-dihydro-15-keto-tetranor-PgD_2 297-279
- 15-deoxy,delta-12,14-PgJ_2-d_4 319-275 and 15-deoxy,delta-12,14-PgJ_2 315-271

Ion pairs (internal standard and analyte) for GC-MS measurements were as follows:

- 9α,11β-PgF_2-d_4 573 and 9α,11β-PgF_2 569

The area under the peak (AUP) for the eicosanoid analyte and corresponding internal standard (IS) were integrated. The formula: [analyte] = [IS] x ($AUP_{analyte}/AUP_{IS}$) was used to calculate eicosanoid values, which were then divided by urinary creatinine concentration in order to express them in pg/mg creatinine. All solvents were HPLC grade and purchased from Avantor™ (formerly Mallinckrodt Baker), Phillipsburg, NJ, USA, while other chemicals were from Sigma-Aldrich Co., St. Louis, MO, USA.

2.6.4 Urinary cotinine quantification

Determination of urinary cotinine concentrations was performed at King's College, London using a commercial microplate enzyme immunoassay (Cozart Forensic Microplate EIA for cotinine, product no. M155B1) from Concateno (Abingdon, UK). Exposure to environmental tobacco smoke was determined as a creatinine corrected cotinine value greater than 30 ng/mg(168,169).

2.6.5 Urinary creatinine quantification

At King's College urinary creatinine concentrations were determined using a commercially available kit (Cayman Chemical Company, Ann Arbor, MI, USA). At the Jagiellonian Institute

[3] These numbers represent the Mass/Charge ratio of the ion (M/Z) which identifies the mass spectrum peak.
[4] PgE_2 and PgD_2 metabolites have the same molecular mass but have different retention time during HPLC.

creatinine was measured from a separate aliquot, thawed on ice or in the fridge (4°C, 3-5 hrs) to provide a minimum volume of 200μL, and assessed using a standard protocol and the Vitros® 350 Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ, USA).

2.7 Appropriateness of measurements

The primary outcome measure (need for unscheduled medical attendance) is one that is of importance to patient/carers, clinicians, and policy makers and is deemed more robust to local variations in treatment practices than other measures. It has previously been used in similar studies(39) in this population and is measurable without undue patient inconvenience.

Urine LTE_4 reflects leukotriene metabolism and has been correlated with asthma severity and bronchoconstriction(124). A significant correlation with montelukast efficacy would provide both a non-invasive and inexpensive marker to guide treatment choice. The complexity of the eicosanoid pathway (combined with emergence of new therapeutic agents) supports exploratory assessment of related metabolites and pathway genes.

The anthropometric and urine measurements are of minimal inconvenience while the Oragene™ saliva kit is high yield and well tolerated.

2.8 Data quality assurance

Data from source material and CRFs was entered into a secure electronic database managed by a clinical trials unit data manager. Prior to analysis the coordinating Principal Investigator randomly checked 10% of records against source data with good concordance. All available data can be provided on request.

2.9 Study outcomes and definitions

2.9.1 Primary outcome

The primary outcome was need for unscheduled medical attention and was defined as the number of times a child attends for an unscheduled medical opinion (to GP, emergency department or both) for respiratory problems over a 12 month period, as recorded on diary cards (DC), bimonthly phone call questionnaire (PC) and as confirmed from primary and secondary care health records (HR).

2.9.2 Clinical secondary outcomes

The following outcomes were assessed as indicated via diary card, bimonthly phone call questionnaire, and health records.

2.9.2.1 Respiratory morbidity

- Number of admissions to hospital over the 12 month trial period (DC/PC/HR).
- Duration of admissions to hospital over the 12 month trial period (DC/PC/HR)
- Time to first attack of wheeze - This was defined as the number of days from the date of IMP receipt (T0) to the first date on which a wheeze exacerbation attains severity to require unscheduled medical attendance or IMP usage (DC/PC/HR).
- Number of unscheduled GP consultations for wheeze (DC/PC/HR)

2.9.2.2 Health service use

- Unscheduled GP consultation with exacerbation of wheeze, expressed as time from the date of IMP receipt (T0) to first GP attendance and annual GP attendance rate (DC/PC/HR)
- A&E attendance with wheeze exacerbation, expressed as time from the date of IMP receipt (T0) to first A&E attendance and annual A&E attendance rate (DC/PC/HR)
- Hospital admission with wheeze exacerbation, as time from the date of IMP receipt (T0) to first admission and annual rate of admissions (DC/PC/HR)
- Total duration of hospital admissions for exacerbation of wheeze (HR)

2.9.2.3 Adverse events (AE)[5]

- Severe adverse events
- Withdrawal from the trial
- Mortality due to exacerbation of asthma
- Mortality due to respiratory infection
- All-cause mortality

2.9.2.4 Medication use

- Use of oral corticosteroids (OCS), expressed as number of courses[6] taken per year, and proportion of children receiving at least one course of OCS during the trial

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[5]An AE is any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An SAE is an AE risking or causing death, or causes hospitalisation, delayed discharge, or significant/persisting disability/incapacity.

[6] A course is a discrete administration of OCS lasting ≥ 1 day and separated from another by ≥ 3 days.

- Use of inhaled reliever medication (salbutamol), expressed as mean usage per wheeze episode as recorded in diary card by parent/guardian.

2.9.3 Laboratory secondary outcomes

2.9.3.1 Inflammatory outcomes

- Association between urinary cysteinyl leukotriene level and:
    - ALOX5 status
    - Other polymorphisms of leukotriene genes
    - Previous history of viral-triggered episodic and multi-trigger wheeze
    - Responsiveness to montelukast
    - Acute history of wheeze
    - Other urinary eicosanoids
    - Urinary cotinine

2.9.3.2 Genetic outcomes

- Differential responsiveness to montelukast for the primary outcome in the stratum with wild type ALOX5 promoter polymorphism [5/5], compared with the stratum with the ALOX5 [5/x or x/y] genotype.
- Differential responsiveness to montelukast for the primary outcome resulting from other polymorphisms in genes influencing leukotriene synthesis, leukotriene metabolism and leukotriene activity.

2.9.4 Qualitative secondary outcomes

- Parental attitudes towards genetic testing in order to personalise therapy
- Parental acceptability of parent-initiated therapy for preschool wheeze
- Parental experience of using the trial medication
- Parent experience of preschool wheeze
- Difficulties/advantages of the parent-initiated approach
- Views on parent information sheet

2.10 Statistical methods

2.10.1 Statistical analysis plan

The statistical analysis plan is available as Appendix 8.7. Analysis was on an intention to treat basis.

2.10.2 Determination of sample size

This trial was powered to detect a clinically significant difference in the number of attacks of wheeze between intervention and control arms. We also had power to detect large differential responsiveness (in terms of the primary outcome) to montelukast in the stratum with ALOX5 promoter polymorphism [5/5], compared with the stratum with the ALOX5 [5/x and x/y] genotype.

Prior to the start of the trial, data on mean (0.76) and standard deviation (1.22) of number of attacks came from data from the UK General Practitioner Research Database(170) on courses of oral steroids (a proxy for number of episodes). These data followed an overdispersed Poisson distribution. To take account of this we used Markov chain Monte Carlo simulation in WinBUGs™(171) to estimate sample sizes required: To detect a 33% drop in attack rate requiring medical attention, with a power of 90% and at a significance level of 5%, and a 6% loss to follow up, required 1050 children in total. A 33% drop in attack rates equates to an attack rate of 0.51 for the treatment group. The clinical significance of these changes is that approximately four children will need to be treated to prevent one clinically severe attack. A sample size of 1200 also gave just over 80% power at the 5% significance level to detect an interaction between treatment and genotype if the effect is a 60% reduction in the [5/x plus x/y] and a 20% reduction in the [5/5] stratum. Assuming a 6% dropout, 1300 children needed to be recruited.

2.10.3 Analysis of primary endpoints

Initial analyses were performed according to intention-to-treat for all participants with outcome data. Per protocol efficacy analyses were also performed, excluding data collected after discontinuation of IMP for those participants who discontinued IMP. Poisson regression with a random effect representing individuals was used to account for overdispersion. Fixed effects represent the stratification factor (ALOX5 promoter) and treatment centre. The incident rate ratio (relative risk) and 95% confidence interval was calculated. Analysis was conducted in Stata version 12™. To test for a differential effect by stratum an interaction term between stratum and treatment was fitted to this model as described in 2.10.5.

2.10.4 Analysis of secondary endpoints

A Poisson regression analysis with a random effect for individuals to allow for over-dispersion was applied to determine the influence of treatment allocation on number of days with parent-reported wheeze, number of hospital attendances, number of admissions to hospital. An incident rate ratio for each factor is presented with 95% confidence intervals.

Time to first attack of wheeze was analysed using a log-rank test with adjustment for clustering and (where hazards are proportional) Cox's proportional hazards models adjusting for clustering. In a Cox model, stratum and centre are included as covariates.

Other continuous variables were analysed with analysis of covariance. Dichotomous variables were analysed with logistic regression analysis. Adverse events were analysed with descriptive statistics.

2.10.5 Genetic analysis

To assess the difference in responsiveness to montelukast in the two ALOX5 strata an interaction term was fitted to test for the interaction between montelukast and stratum in the main model, for each treatment arm. We also report the associations between genotype and clinical phenotype, urinary leukotriene level, and clinical outcome.

2.10.6 Analysis of urinary eicosanoids

Urinary eicosanoid values were $\log_{10}$ transformed because of their non-normal distribution. Pearson's correlation coefficients were calculated to determine correlations between urinary eicosanoids. Linear regression was performed to investigate the association between each urinary eicosanoid level (response variable) and demographic factors/disease phenotypes (predictor variables): age, sex, ethnicity, baseline unscheduled medical attendances in the past 12 months, cotinine, body mass index (BMI) z-score, preterm birth, low birth weight, food allergy, itchy rash, eczema, in utero tobacco, in household tobacco, maternal asthma, paternal asthma, multitrigger wheeze, hospital admission for wheeze in the past year, oral steroid use in the past year and maintenance inhaled corticosteroid use. We used a false discovery rate (FDR) corrected p-value threshold of 0.00029 to account for the 171 (nine urinary eicosanoids against 19 variables) tests.

2.10.7 Protocol changes during the study

No scientifically significant protocol changes occurred during the study. The ethics committee and DSMC approved all amendments unless the sponsor deemed them to be minor amendments. A list of changes is included in Appendix 8.3.5. No changes in planned analysis (Appendix, 8.7) occurred after the database was locked.

2.10.8 Study duration

The study was intended to recruit for 24 months. Slower than predicted early recruitment necessitated an increase in recruitment period to 26 months and an expansion of recruitment sites (from 3 to 41). This extension was approved by the research ethics committee, the regulatory authority and also by the funding body. Thus recruitment spanned October 2010

to December 2012 and follow-up was completed in December 2013 with data cleaning, verification and database lock completed by January 2014.

2.11 Study management

The WAIT study was at the time the largest paediatric asthma trial in UK history, and was delivered with a core project staff of one medical research fellow, two research nurses, one of whom doubled as trial coordinator, a Bengali-speaking research assistant, a paediatric pharmacy technician, and one laboratory technician all under the supervision of the Chief Investigator. Together this team coordinated, managed and motivated 41 hospital sites (each with a local Principal Investigator, a Pharmacist, and a Research Nurse and Research and Development Team), 21 primary care patient identification centres, more than 1300 study subjects (each with their individual consent, compliance, and adverse event profile), and several thousand IMP dispensings (occurring in the context of an unexpected and profound world shortage of montelukast oral granule supply). Despite this recruitment was completed within budget, only two months off target, with subject retention in excess of 90%, and the study has passed three regulatory inspections. Key to this success was a core team primed and able to function with cohesion and agility in the face of challenge, and which, in the absence of early support from an industry or in-house pragmatic clinical trials unit, designed and implemented robust solutions to problems as they arose.

2.11.1 Trial website and email bulletins

In order to maintain a core set of trial documentation, disseminate stratum reports, keep the wider national team both motivated and appraised of progress, challenges and study requirements, two avenues were exploited.

2.11.1.1 Trial website

A password protected study website[7] was designed by the research fellow, using the Google sites™ platform. Staff from each site had access to the main site and also restricted access to a subsite for their individual recruitment centre. The website was branded with the study logo, and hosted:

- whole study and individual site recruitment progress charts, plotted against time, with comparison to predicted rates.
- an interactive UK map of recruitment sites, highlighting the lead recruiter.
- a per cent recruitment progress pie chart, prominent on the home page.
- a stratum report page, which would be updated weekly with the latest genotyping stratum report for download for each site.

[7] https://sites.google.com/site/waittrial/ (accessed 31st October 2017)

- a study documentation page, with a downloadable trial management file pack, complete with amendments information.

Screenshots from the website are included below (Figure 2-5 to 2-10).

FIGURE 2-5 - WEBSITE HOMEPAGE

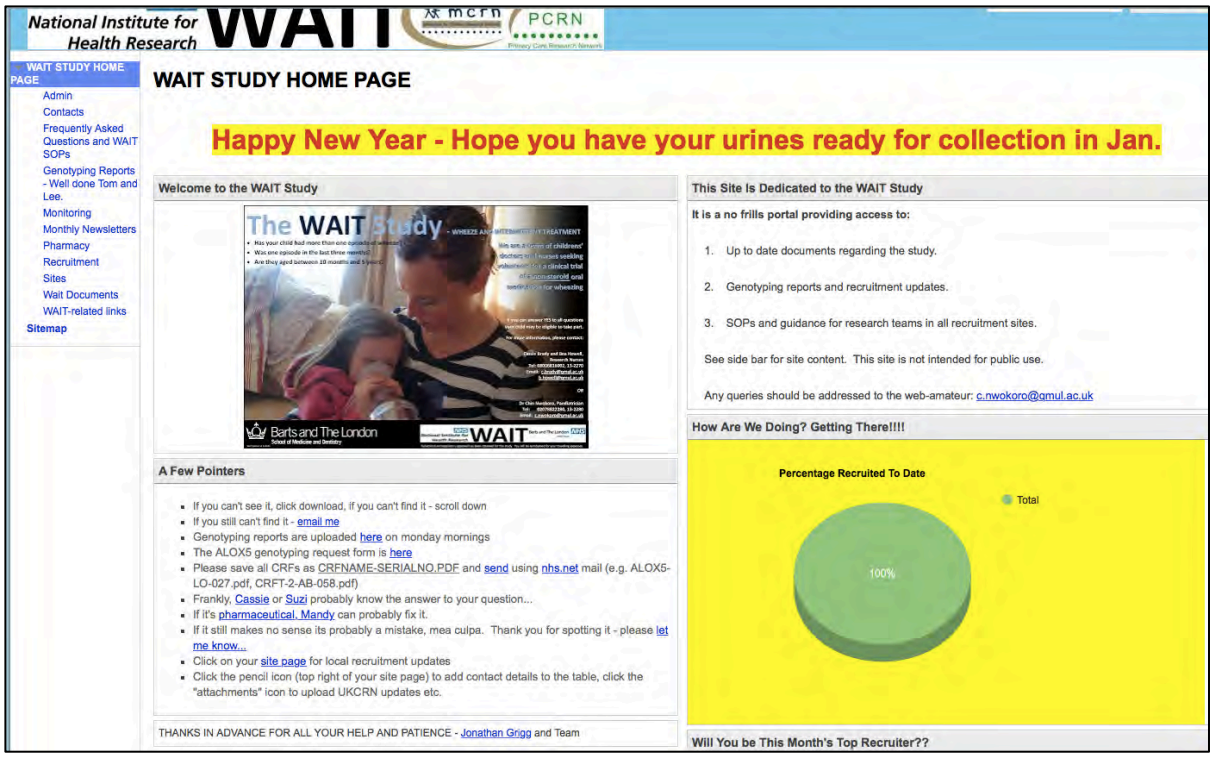


FIGURE 2-6 - INTERACTIVE RECRUITING SITE MAP

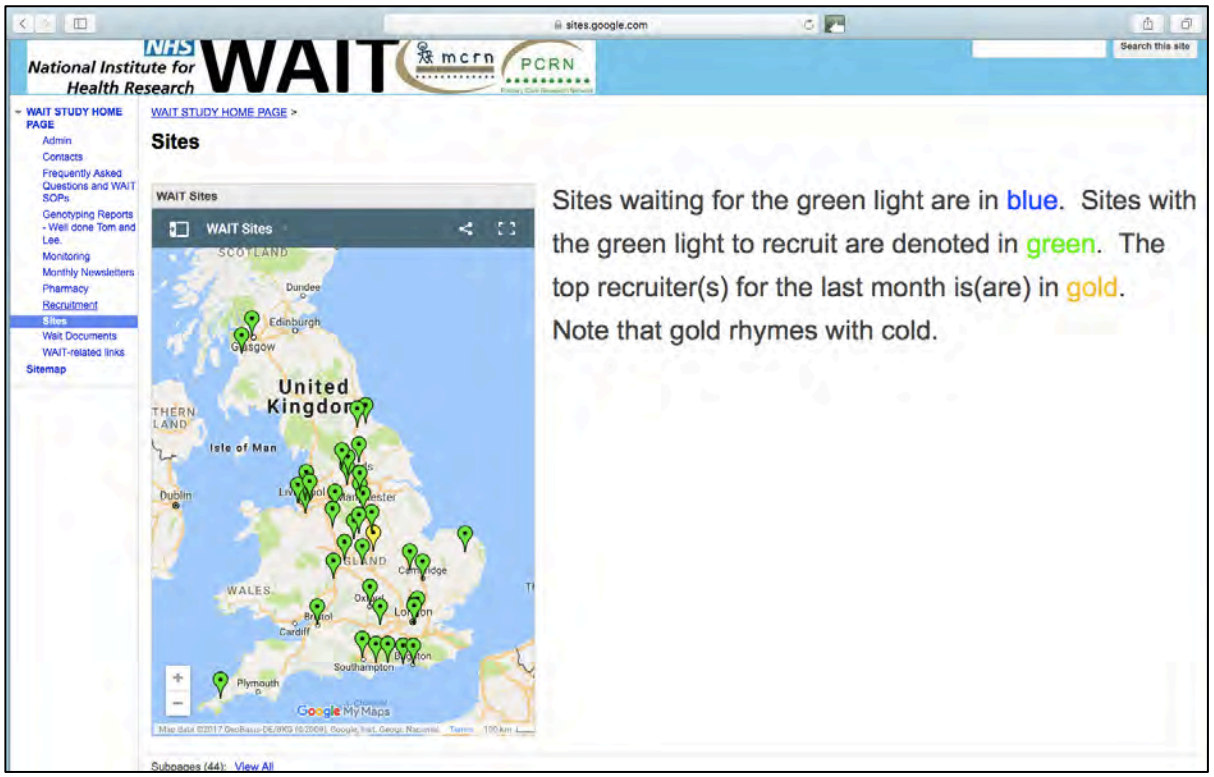


FIGURE 2-7 - LIVE RECRUITMENT TARGET PIE CHART

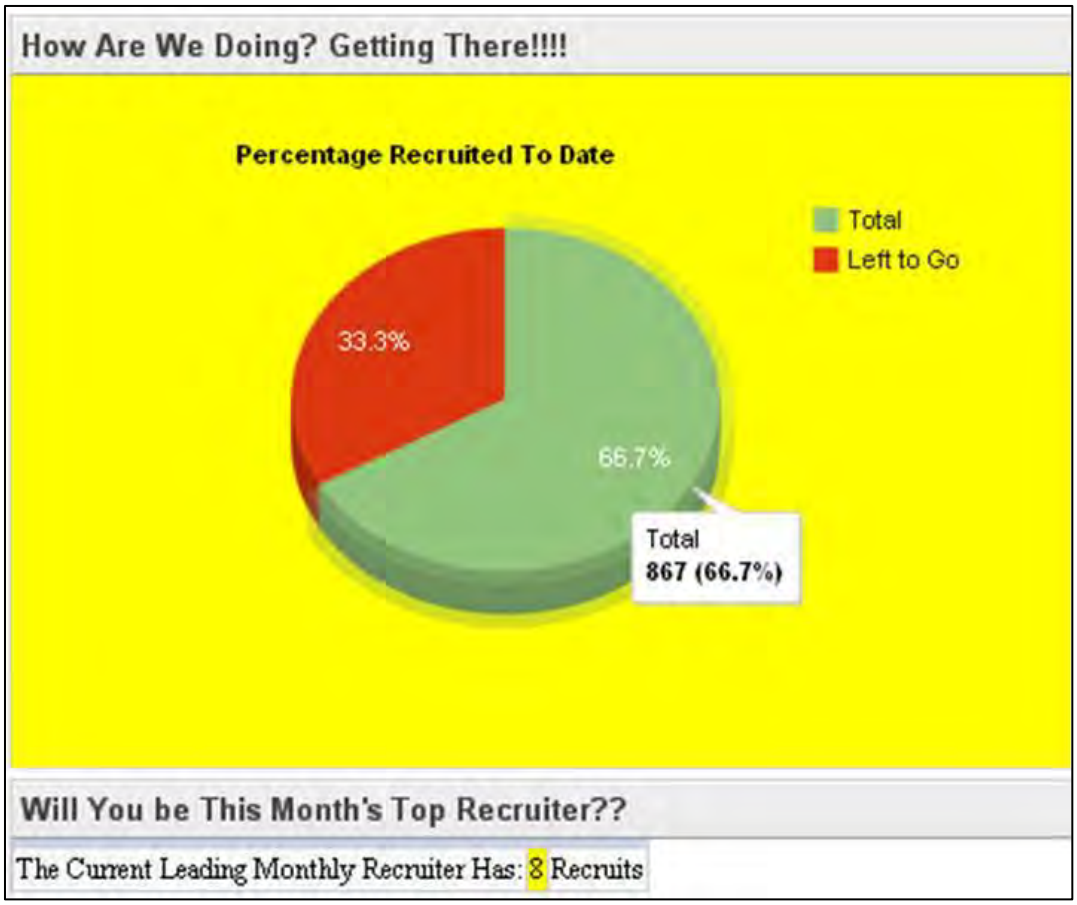


FIGURE 2-8 - WEEKLY ELECTRONIC STRATIFICATION REPORT

StratumDate_231112b.xls — Open with Microsoft

	A	B	C	D	E	F
1	Serial No	Stratum	Comments			
2	AB0178	A				
3	AB0179	A				
4	AB0180	A				
5	BI0029	A				
6	BI0030	A				
7	BR0047	B				
8	BR0048	A				
9	BR0049	A				
10	CO0026	B				
11	DD0005	B				
12	DD0008	A				
13	HB0016	A				
14	KL0007	A				
15	LE0238	A				
16	LE0239	B				
17	LE0240	A				
18	NO0055	A				
19	OX0045	B				
20	RC0006	A				
21	RC0008	A				
22	RO0030	A				

FIGURE 2-9 - LIVE RECRUITMENT AND SCREENING STATUS

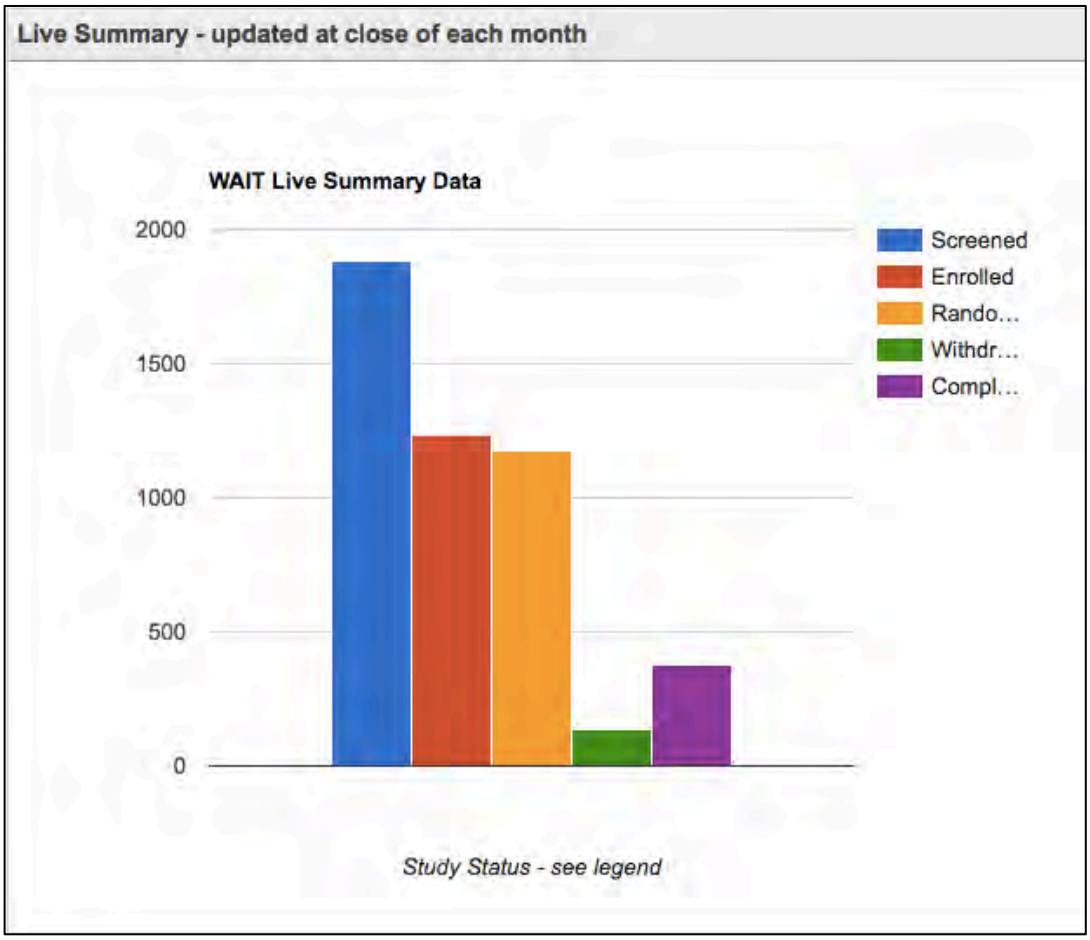
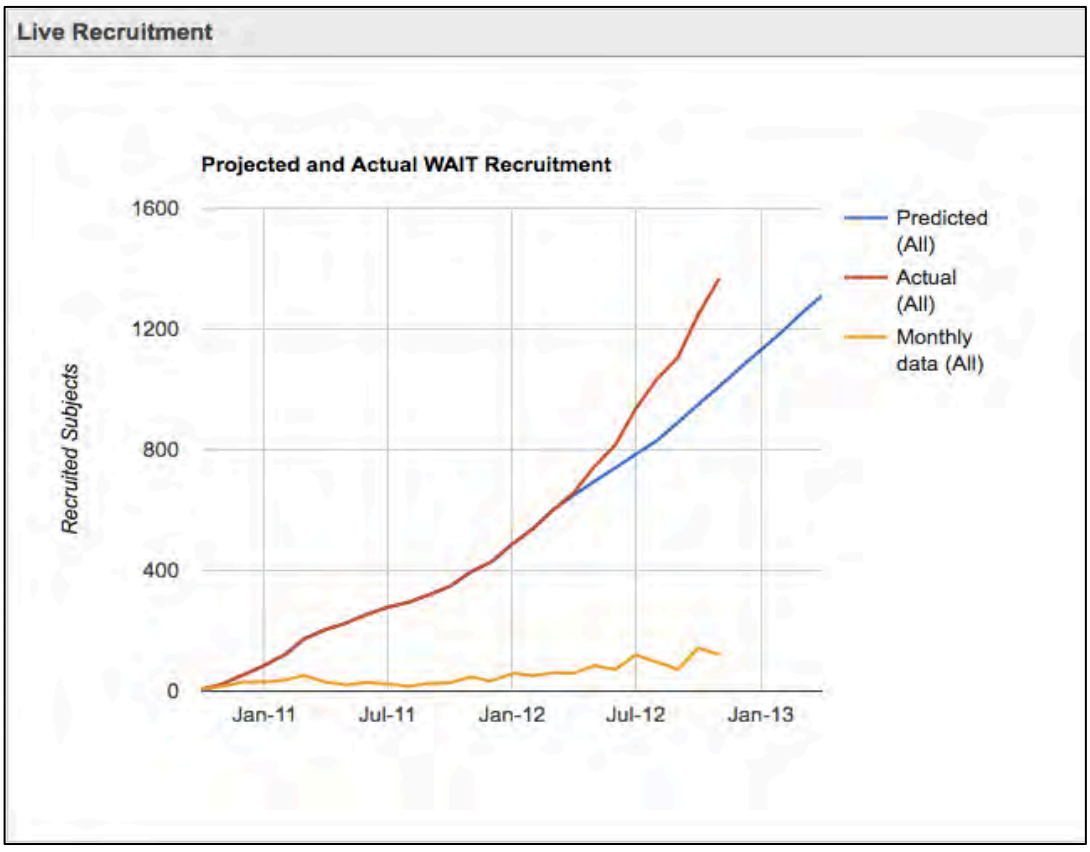


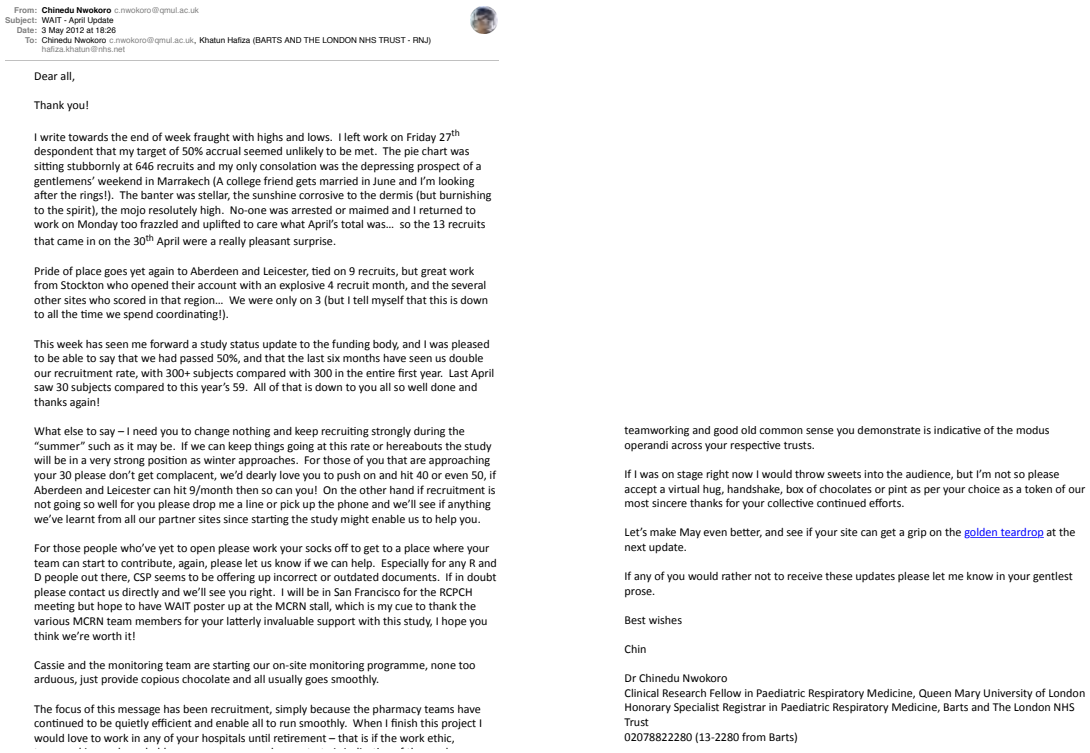
FIGURE 2-10 - GRAPH OF PROJECTED AGAINST ACTUAL RECRUITMENT



2.11.1.2 Trial email bulletins

Emails intended to be both motivational and informative were sent on an approximately monthly basis to the approximately 160 members of the national study team. This was in recognition of the fact that not all team members would regularly access the website, and that information may need to be communicated in a more directed/persuasive/engaging/personal style than would be possible through the website. These enabled individuals to seek rapid clarification in a safe and timely fashion, and were critical to maintaining collective calm through clear communication of contingency plans during the destabilizing IMP shortage. An example is included below (Figure 2-11).

FIGURE 2-11 - SAMPLE MONTHLY UPDATE EMAIL



From: **Chinedu Nwokoro** c.nwokoro@qmul.ac.uk
Subject: WAIT - April Update
Date: 3 May 2012 at 18:26
To: Chinedu Nwokoro c.nwokoro@qmul.ac.uk, Khatun Hafiza (BARTS AND THE LONDON NHS TRUST - RNJ) hafiza.khatun@nhs.net

Dear all,

Thank you!

I write towards the end of week fraught with highs and lows. I left work on Friday 27th despondent that my target of 50% accrual seemed unlikely to be met. The pie chart was sitting stubbornly at 646 recruits and my only consolation was the depressing prospect of a gentlemens' weekend in Marrakech (A college friend gets married in June and I'm looking after the rings!). The banter was stellar, the sunshine corrosive to the dermis (but burnishing to the spirit), the mojo resolutely high. No-one was arrested or maimed and I returned to work on Monday too frazzled and uplifted to care what April's total was… so the 13 recruits that came in on the 30th April were a really pleasant surprise.

Pride of place goes yet again to Aberdeen and Leicester, tied on 9 recruits, but great work from Stockton who opened their account with an explosive 4 recruit month, and the several other sites who scored in that region… We were only on 3 (but I tell myself that this is down to all the time we spend coordinating!).

This week has seen me forward a study status update to the funding body, and I was pleased to be able to say that we had passed 50%, and that the last six months have seen us double our recruitment rate, with 300+ subjects compared with 300 in the entire first year. Last April saw 30 subjects compared to this year's 59. All of that is down to you all so well done and thanks again!

What else to say – I need you to change nothing and keep recruiting strongly during the "summer" such as it may be. If we can keep things going at this rate or hereabouts the study will be in a very strong position as winter approaches. For those of you that are approaching your 30 please don't get complacent, we'd dearly love you to push on and hit 40 or even 50, if Aberdeen and Leicester can hit 9/month then so can you! On the other hand if recruitment is not going so well for you please drop me a line or pick up the phone and we'll see if anything we've learnt from all our partner sites since starting the study might enable us to help you.

For those people who've yet to open please work your socks off to get to a place where your team can start to contribute, again, please let us know if we can help. Especially for any R and D people out there, CSP seems to be offering up incorrect or outdated documents. If in doubt please contact us directly and we'll see you right. I will be in San Francisco for the RCPCH meeting but hope to have WAIT poster up at the MCRN stall, which is my cue to thank the various MCRN team members for your latterly invaluable support with this study, I hope you think we're worth it!

Cassie and the monitoring team are starting our on-site monitoring programme, none too arduous, just provide copious chocolate and all usually goes smoothly.

The focus of this message has been recruitment, simply because the pharmacy teams have continued to be quietly efficient and enable all to run smoothly. When I finish this project I would love to work in any of your hospitals until retirement – that is if the work ethic,

teamworking and good old common sense you demonstrate is indicative of the modus operandi across your respective trusts.

If I was on stage right now I would throw sweets into the audience, but I'm not so please accept a virtual hug, handshake, box of chocolates or pint as per your choice as a token of our most sincere thanks for your collective continued efforts.

Let's make May even better, and see if your site can get a grip on the golden teardrop at the next update.

If any of you would rather not to receive these updates please let me know in your gentlest prose.

Best wishes

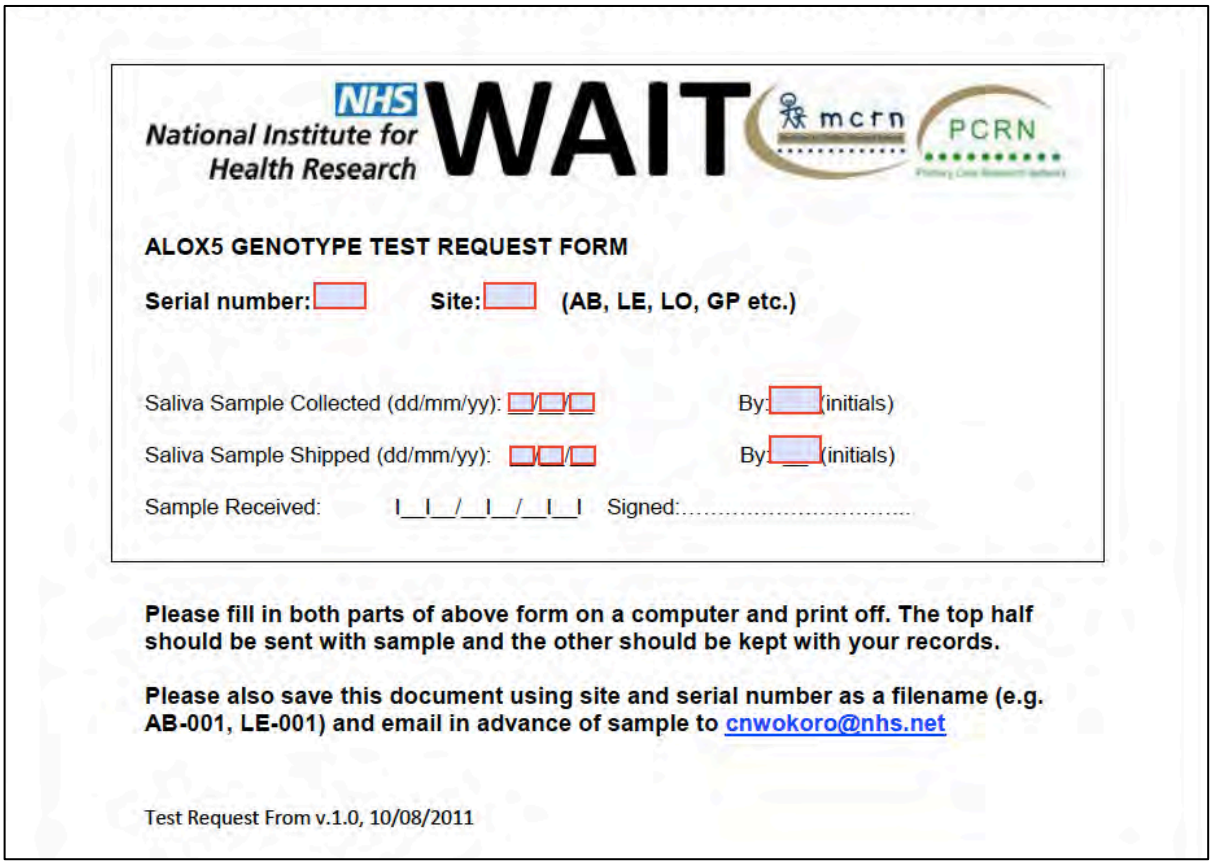
Chin

Dr Chinedu Nwokoro
Clinical Research Fellow in Paediatric Respiratory Medicine, Queen Mary University of London
Honorary Specialist Registrar in Paediatric Respiratory Medicine, Barts and The London NHS Trust
02078822280 (13-2280 from Barts)

2.11.1.3 Electronic genotyping request forms

DNA samples in the DNA Genotek kit were sent by secure recorded mail in preprinted postage paid polythene envelopes. On recruiting a subject, local research teams completed a pdf form (Figure 2-12) which was automatically emailed to the sponsor team as recruitment notification, without including any patient identifiable data (ethics approval conditions precluded electronic transmission of consent forms). Receipt of the emailed form was accepted as preliminary proof of recruitment and the website was updated with the recruitment log completed on receipt of the sample. A paper copy of the pdf form was placed in the polythene envelope with the DNA sample to enable laboratory staff to reconcile notification emails.

FIGURE 2-12 - ELECTRONIC GENOTYPING REQUEST FORM



NHS National Institute for Health Research WAIT mcrn PCRN

ALOX5 GENOTYPE TEST REQUEST FORM

Serial number: **Site:** **(AB, LE, LO, GP etc.)**

Saliva Sample Collected (dd/mm/yy): __/__/__ By: (initials)

Saliva Sample Shipped (dd/mm/yy): __/__/__ By: (initials)

Sample Received: __/__/__ Signed:.....

Please fill in both parts of above form on a computer and print off. The top half should be sent with sample and the other should be kept with your records.

Please also save this document using site and serial number as a filename (e.g. AB-001, LE-001) and email in advance of sample to cnwokoro@nhs.net

Test Request From v.1.0, 10/08/2011

2.11.2 Medication dispensing

The study began with only 3 recruiting sites, in London, Leicester and Aberdeen, however slower than expected recruitment necessitated expansion, eventually to 41 recruiting sites. The three-site model lent itself to local dispensing, with three pharmacies holding IMP supply which was replenished from the IMP manufacturer. As the number of sites increased this system became unwieldy and inefficient, in part due to difficulties matching site supply to local recruitment rate, and also due to the complex logistics of transferring IMP safely around the country. Additional layers of complexity were applied when resupply (when patients had run out of IMP medication) became a factor, and then in early 2011 the trial faced a global shortage of montelukast oral granules which prevented the IMP manufacturer (Novalabs) from manufacturing more IMP. Faced with not only a halt to recruitment but also the probable forced withdrawal of existing recruits due to a dearth of IMP supply we implemented three steps to mitigate the shortage and prevent collapse of the trial:

- We sought and received permission from the Medicines and Healthcare Products Regulatory Agency (MHRA) to repack the standard 50 sachet IMP boxes into boxes of 20 and 30 doses each.
- We switched to central postal IMP distribution (with relaxation of temperature monitoring), with a pharmacy process that was designed in conjunction with the Medicines for Children Research Network (MCRN) and the MHRA

pharmacist to maintain IMP integrity and me*et al*l ethical and safety obligations while reducing waste, inefficiency, and study expense.

- We appealed directly to MSD™, the primary manufacturer of montelukast oral granules, convincing them to secure a supply of IMP raw material from mainland Europe in order to support the trial to completion, even as our hospital pharmacy was unable to source supply for clinical usage.

In this way we maintained all recruited subjects within the trial and did not need to halt either recruitment or opening of new sites as had been feared.

2.11.3 Study management overview

Central to the success of the trial was the promotion of a culture of innovation, challenge and open communication within the core project management team, and this was allied to a willingness and ability to strike up and cultivate productive, personal working relationships with key personnel in the Regulatory Authority, Strategic Research Network, Local Research and Development department and our counterparts in industry and in other recruitment sites. With the knowhow gleaned through this process subsequent trials would be designed to be less reliant on outside assistance, however the emphasis on strong internal and external relationships would be maintained as the greatest insurance against unforeseen challenge. The success of this approach was recognized in an invited lecture entitled: 'WAIT - working together to deliver a large paediatric trial' which featured in the MCRN Parallel Session: "*The Future of Paediatric Research Across Europe - Improving Opportunities for Children"* at the 2013 Royal College of Paediatrics and Child Health Annual Conference.

3 RESULTS - MAIN STUDY

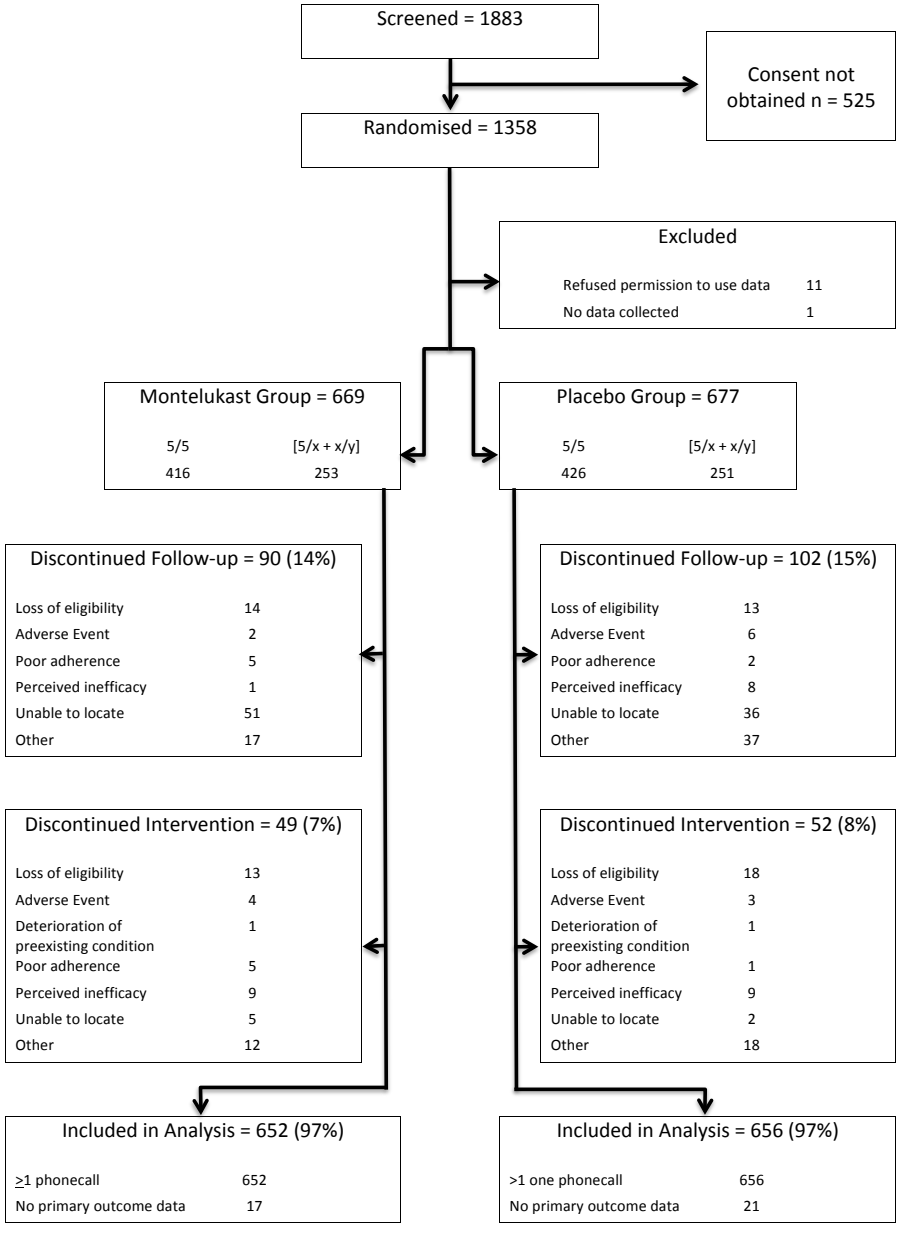
3.1 Overview

In this chapter I describe the results of the main trial, including recruitment and retention, safety data, baseline demographics and primary and secondary outcomes. Mechanistic and qualitative data are discussed in subsequent sections.

3.2 Recruitment and retention

1366 subjects were recruited, however 8 subjects withdrew prior to randomisation, and 12 subjects subsequently withdrew permission for use of their data, leaving 1346. Of these, 97% in each treatment arm completed at least one bimonthly telephone call and were thus eligible for inclusion in the primary analysis as per Figure 3-1 and Table 3-1.

FIGURE 3-1 - CONSORT DIAGRAM



3.3 Available data sets

Recruited subject retention (Table 3-1 and Table 3-2) was relatively good and thus sample size remained adequate to provide statistical power to assess the primary outcome. All analyses were performed on the intention to treat (ITT) population (or available case population where outcome data was not available for analysis) unless otherwise stated. These populations are indicated in Table 3-2.

TABLE 3-1 - DISPOSITION OF RECRUITED SUBJECTS

	Montelukast	Placebo	Total
Enrolled	669	677	1358
Permitted use of data	Unknown split	Unknown split	1346
Received at least 1 phone call	652 (97%)	656 (97%)	1308 (96%)
Completed 12m follow-up	579 (87%)	575 (85%)	1154 (85%)
Withdrawn:	90 (13%)	102 (15%)	192 (14%)
Lost to follow up	51 (8%)	36 (5%)	87 (6%)
Adverse event	4 (0.6%)	3 (0.4%)	7 (0.5%)
Death	0 (0%)	0 (0%)	0 (0%)
Other	37 (6%)	60 (9%)	97 (7%)

TABLE 3-2 - NUMBERS (%) OF INDIVIDUALS WITHDRAWING FROM STUDY BY MONTH

	Montelukast		Placebo	
ITT Population	n=669	(50%)	n=677	(50%)
Timing of last contact				
T0 (no data)	17	(3%)	21	(3%)
Withdrew before T1	16		16	
T1 (month 2)	21	(3%)	20	(3%)
T2 (month 4)	15	(2%)	12	(2%)
T3 (month 6)	12	(2%)	19	(3%)
T4 (month 8)	13	(2%)	15	(2%)
T5 (month 10)	12	(2%)	15	(2%)
T6 (month 12)	579	(87%)	575	(85%)
Per protocol population	579		575	

3.4 Protocol deviations

There were 31 reported protocol deviations throughout the study. Very few necessitated withdrawal from the trial, none exposed a participant to risk of harm, none appeared systematic or particular to an individual site, and none had potential to compromise study validity. Most were addressed by a gentle reminder of the study requirements to the parent or carer. Table 3-3 gives details of study protocol deviations.

TABLE 3-3 - PROTOCOL DEVIATIONS

Deviation	BR	BD	BI	CA	CO	DE	WH	CH	PO	NO	RO	HG	ST
Entry criteria	0	0	0	0	1	0	0	0	0	0	0	0	0
Withdrawal criteria	0	0	0	0	0	0	0	0	0	0	0	0	0
Concomitant medication	0	0	0	0	0	0	0	0	0	0	2	0	0
Incorrect dosing regimen	1	0	2	0	2	0	1	0	2	1	4	1	3
Expired medication	1	1	0	0	0	0	0	0	0	0	0	0	0
Incorrect administration	0	0	0	2	0	2	0	0	0	0	0	1	2
Lost samples	0	0	0	0	0	0	2	0	0	0	0	0	0

BR=Bristol, BD=Bradford, BI=Birmingham, CA=Cambridge, CO=Coventry, DE=Derby, WH=Whiston, CH=Countess of Chester, PO=Portsmouth, NO=Nottingham, RO=Royal Berkshire, HG=Harrogate, ST=University Hospitals of North Staffordshire

3.5 Demographic and other baseline characteristics

Subjects appeared well-matched between genotype strata and treatment groups (Table 3-4). Anthropometrically children were on the 75th-91st body mass index centile, and ethnic make-up (76% white) was comparable to Bisgaard (71%), and Valovirta (75%), as was gender (65% male, Bisgaard - 64%, Valovirta - 60%), while measures of atopy cannot be compared due to inconsistent terminology or methods of assessment(126,129).

TABLE 3-4 - BASELINE CHARACTERISTICS OF STUDY RECRUITS

	Montelukast group (n=669)			Placebo group (n=677)		
	5/5	5/x+x/y	Total	5/5	5/x+x/y	Total
n (%)	416 (62%)	253 (38%)	669 (100%)	426 (63%)	251 (37%)	677 (100%)
Height (cm)	90·0 (10·3)	89·8 (10·5)	89·9 (10·4)	89·9 (10·5)	91·8 (11·7)	90·6 (11·0)
Weight (kg)	14·0 (3·0)	13·9 (3·7)	14·0 (3·3)	14·0 (3·3)	14·6 (3·8)	14·2 (3·5)
Age (years)	2·6 (1·1)	2·5 (1·1)	2·6 (1·1)	2·6 (1·1)	2·8 (1·2)	2·7 (1·1)
Male sex	262 (63%)	164 (65%)	426 (64%)	276 (65%)	161 (64%)	437 (65%)
Ethnic origin						
White	335 (81%)	179 (71%)	514 (77%)	338 (79%)	174 (69%)	512 (76%)
Black	5 (1%)	14 (6%)	19 (3%)	4 (1%)	14 (6%)	18 (3%)
Asian	55 (13%)	37 (15%)	92 (14%)	58 (14%)	46 (18%)	104 (15%)
Other	21 (5%)	23 (9%)	44 (7%)	26 (6%)	17 (7%)	43 (6%)
Preterm birth (<37 weeks)	58 (14%)	40 (16%)	98 (14%)	56 (13%)	42 (17%)	98 (15%)
Birth weight (<2500g)	51 (12%)	28 (11%)	79 (12%)	42 (10%)	28 (11%)	70 (10%)
Food allergy	64 (15%)	44 (18%)	108 (16%)	64 (15%)	47 (19%)	111 (17%)
Drug allergy	26 (6%)	12 (5%)	38 (6%)	23 (6%)	19 (8%)	42 (6%)
Itchy rash (>6 months, ever)*	98 (23%)	64 (25%)	162 (24%)	104 (25%)	60 (24%)	164 (25%)
Eczema (ever)†	207 (49%)	121 (48%)	328 (48%)	215 (52%)	134 (53%)	349 (52%)
History of asthma in mother	156 (37%)	95 (38%)	251 (37%)	141 (34%)	89 (35%)	230 (34%)
History of asthma in father	126 (30%)	73 (29%)	199 (29%)	126 (30%)	81 (32%)	207 (31%)
Age at first wheeze (months)	12·4 (9·8)	13·5 (10·5)	12·8 (10·1)	12·4 (10·4)	13·6 (11·5)	12·9 (10·8)
Children with episodic viral wheeze	296 (71%)	181 (72%)	477 (71%)	295 (69%)	191 (76%)	486 (72%)
Children with multitrigger wheeze	120 (29%)	72 (28%)	192 (29%)	131 (31%)	60 (24%)	191 (28%)
Interval between onset of URTI and wheezing (h)‡	31·6 (27·4)	28·8 (25·2)	30·5 (26·6)	27·3 (23·4)	28·2 (26·0)	27·7 (24·4)
Children with more than one hospital admission for wheeze in the past year	363 (87%)	216 (85%)	579 (87%)	351 (82%)	203 (81%)	554 (82%)

Oral corticosteroid courses in past yr	2·0 (1·9)	1·8 (1·8)	1·9 (1·8)	1·9 (1·9)	1·8 (2·0)	1·9 (2·0)
USMA in previous year	5·5 (4·3)	5·4 (4·1)	5·4 (4·2)	5·7 (5·3)	5·6 (4·6)	5·6 (5·1)
Continuous inhaled corticosteroids	118 (28%)	66 (26%)	184 (28%)	144 (34%)	69 (27%)	213 (31%)

Data are mean (SD) or n (%), unless otherwise indicated. USMA=unscheduled medial attendance for wheeze. URTI=upper-respiratory-tract infection.

*A question to parents from the International Study of Asthma and Allergies in Childhood questionnaire was used to identify symptoms suggestive of eczema.

†Eczema from birth was based on parental report to recruiting investigator at enrolment.

‡Based on parental report of the usual interval between URTI and onset of wheezing.

3.6 Assessment of treatment compliance

Patient carers were asked to return empty/unused/expired sachets to the sponsor in self-addressed prepaid envelopes to assess compliance, however returns were too low to yield meaningful data. Compliance was encouraged and informally monitored via phone calls.

3.7 Efficacy results and tabulations of patient data

3.7.1 Primary outcome

There was no difference between montelukast and placebo for the primary outcome, need for unscheduled medical attendance (Table 3-5).

TABLE 3-5 - EFFECT SIZE AND CONFIDENCE INTERVAL - PRIMARY OUTCOME

	Montelukast Group	Placebo Group	Adjusted incidence rate ratio (95% CI)	P-value
Analysis population (N (%))	652 (50%)	656 (50%)		
Unscheduled medical attendance for wheeze episodes (mean, (SD))	2·0 (2·6)	2·3 (2·7)	0·88 (0·77 to 1·01)	0·06

Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group, a random effect for individual to account for overdispersion with follow up time fitted as the exposure. Follow up time is based on time from randomisation until either 12 month end of trial date or date of last phone call. Primary outcome data is taken from the phone call which occurred every two months, and confirmed from diary cards and primary and secondary care records. Children were included in the analysis if they had at least one phone call recorded and follow up time is then fitted as an exposure in the model.

3.7.2 Secondary outcomes

TABLE 3-6 - SUBGROUP ANALYSIS OF TREATMENT RESPONSE IN THE 5/5 AND [5/X + X/Y] STRATA

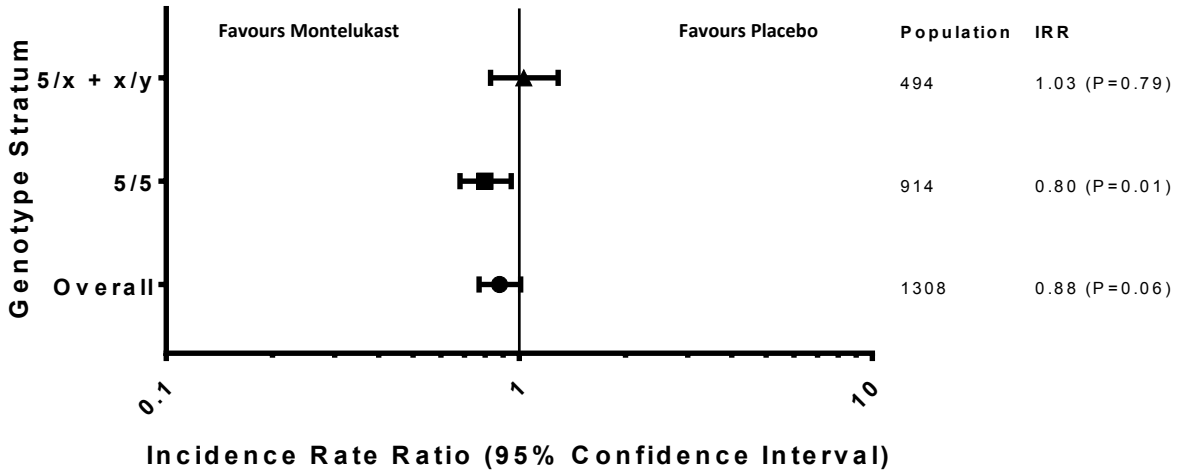
	Montelukast Group		Placebo Group		Adjusted incidence rate ratio (IRR), (95% CI)	p-value	p-value (interaction)
USMA in 5/5 stratum, Mean (SD)	2·0	(2.7)	2·4	(3.0)	0·80 (0·68 to 0·95)	0·01	0·08
USMA in [5/x+x/y] stratum, Mean (SD)	2·0	(2.5)	2.0	(2.3)	1·03 (0·83 to 1.29)	0·79	

USMA=unscheduled medical attendance for wheeze episodes.

Genotype stratified subgroup analysis suggested an interaction between ALOX5 promoter polymorphism and the primary outcome, in that subjects homozygous for the 5-repeat, wild type allele appeared to have increased unscheduled medical attendances on placebo when

compared to those with variant genotypes, and this was reduced to the baseline for variant genotype subjects on treatment with montelukast (Figure 3-2). However the study was not powered to identify so small an effect (2.10.2) and the test for interaction was therefore non-significant (Table 3-6).

FIGURE 3-2 - FOREST PLOT OF UNSCHEDULED MEDICAL ATTENDANCES BY GENOTYPE STRATUM



There was no effect on USMA when data were analysed by alternative genotype strata, wheeze phenotype, or use of reported use of inhaled steroids at study entry (Table 3-7).

TABLE 3-7 - OTHER PRE-SPECIFIED SUBGROUP ANALYSES OF TREATMENT EXPOSURE

	Montelukast; Mean (SD)		Placebo; Mean (SD)		P-value (interaction)
USMA in [5/5+5/x] stratum	2·0	(2·6)	2·3	(2.8)	0·93
USMA in [x/y] stratum	1.7	(1.8)	1.9	(2.0)	
Inhaled Corticosteroid Use at baseline	2·0	(3.0)	2·0	(2.3)	0·09
No Inhaled Corticosteroid Use at baseline	2.0	(2.2)	2.5	(3.0)	
Multitrigger Wheeze	2.1	(3.0)	2.0	(2.5)	0.19
Episodic Viral Wheeze	2.0	(2.4)	2.3	(2.9)	

USMA=unscheduled medical attendance for wheeze episodes. Multitrigger wheeze=phenotype where wheeze can occur in absence of a viral cold. Episodic viral wheeze=phenotype characterised by wheeze occurring only in the context of a viral cold.

TABLE 3-8 - SECONDARY OUTCOMES

		Montelukast		Placebo		IRR, OR, or HR (95% CI)	p-value
Time to first USMA for wheeze - days (Median;IQR)	Any (N=1294)	147	50- 365	130	38-N/A	HR; 0·89 (0·78-1·02)	0·09
	Hosp Admission (N=1305)	N/A	202-N/A	N/A	144-N/A	HR; 0.82 (0.68-0.99)	0.04
	Emergency Dept (N=1308)	N/A	N/A	N/A	N/A	HR; 0.89 (0.53-1.52)	0.68
	Unscheduled GP (N=1297)	257	64-365	240	68-365	HR; 0.94 (0.81-1.09)	0.41
Children with one or more USMA (N (%))		426	(65%)	456	(70%)	OR; 0·83 (0·66-1·04)	0·10
Need for rescue oral steroids; courses per child ** (Mean (SD))		0·26	(0·7)	0.33	(0·9)	IRR; 0·75 (0·58-0·98)	0·03
Wheeze episodes Mean (SD)**		2·7	(2·9)	2·6	(3·0)	IRR; 1·02 (0.91-1·16)	0·68
Duration of wheeze episodes; days (Mean (SD))		5·2	(4·0)	5.4	(3·9)	IRR; 0·97 (0.89 -1·06)	0·53
Duration of hospital admission; days per		1·8	(1·3)	1·7	(1·1)	IRR; 1·05 (0·94-1·18)	0·40

child.*** (Mean (SD))						
Symptom days/wheeze episode (Mean (SD))	4·9	(3·5)	4·8	(3·8)	IRR; 0.96 (0·88-1·05)	0·36

Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group, a random effect for individual to account for overdispersion, with follow up time fitted as the exposure. Follow up time is based on time from randomisation until either 12 month end of trial date or date of last phone call. An interaction term was included to assess whether there is a differential treatment effect dependent on genetic stratum.

*7 participants were missing dates for USMA and 7 participants had their first medical attendance on the day of randomisation and are hence excluded. Time to first USMA data was analysed using a Cox regression model with fixed effects for stratification factor and treatment group (See Figure 3-3).

**Analysis included all children. 446 children had no diary data and these were considered to have no wheeze and cold episodes. The analysis was repeated treating these patients as missing and there was no difference in the incidence rate ratio between treatment and placebo.

***Duration of each hospital admission is analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual with follow up time fitted as the exposure.

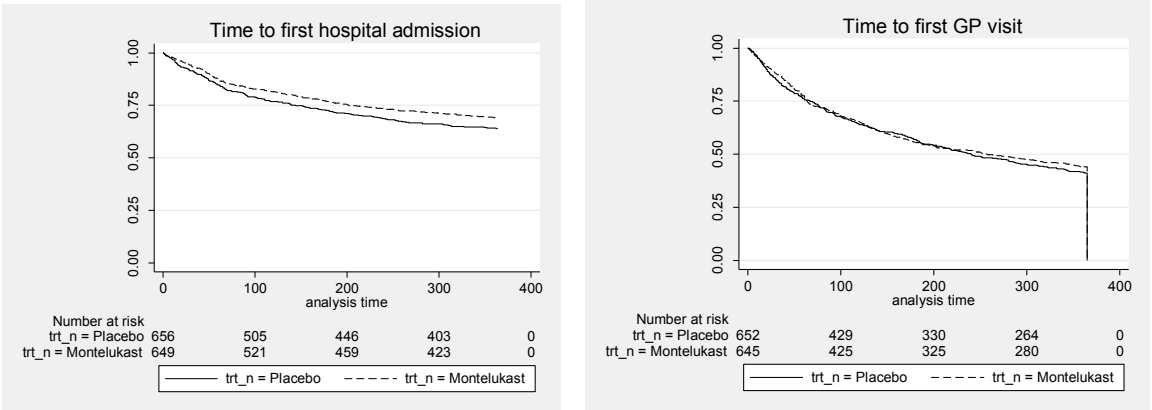
IRR; incidence rate ratio, OR; odds ratio, HR; hazard ratio, IQR; interquartile range, SD; standard deviation, USMA; unscheduled medical attendance for wheeze episodes. N/A = no value observed as insufficient proportion of children experienced an event within follow-up period.

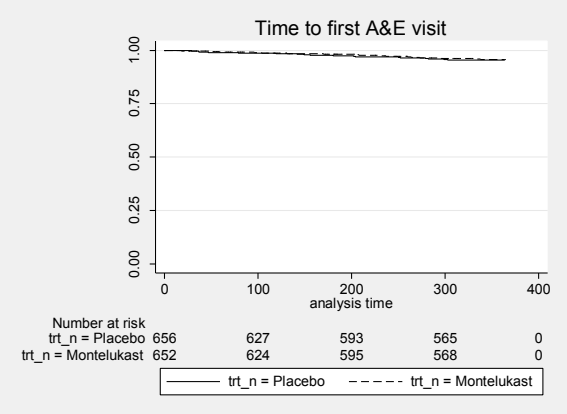
3.7.3 Concomitant medication use

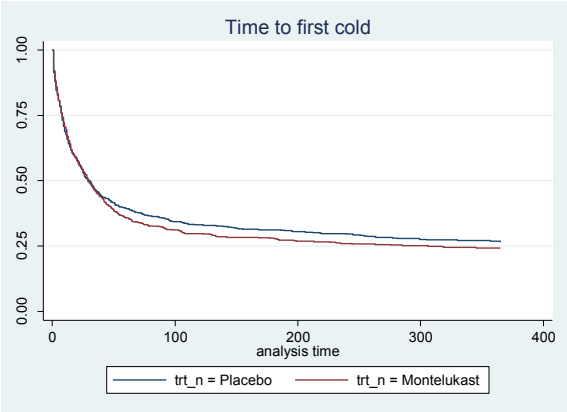
Subjects were permitted to use any concomitant medications excluding leukotriene receptor antagonists. A record was kept of concomitant medication usage. There was no difference in reported salbutamol usage between treatment groups. A statistically significant reduction in oral corticosteroid usage was observed in montelukast treated subjects (P=0.03, Table 3-8).

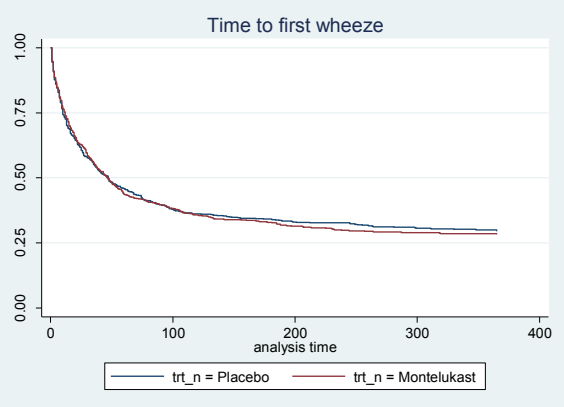
3.7.4 Survival analyses by treatment arm

FIGURE 3-3 - KAPLAN-MEIER CURVES OF TIME TO FIRST TREATMENT OR DISEASE EVENT









Kaplan-Meier curves were plotted with available data for a number of pre-specified secondary outcomes. There was no significant difference between placebo and active treatment for any of the outcomes depicted. A&E visits culminating in hospital admission are coded as an admission not as an A&E visit. Frequency of event was as expected: Cold > Wheeze > GP visit > Hosp admission. There was no difference in USMA (GP/A&E/admission duration) between treatment arms.

3.7.5 Safety evaluation

3.7.5.1 Adverse events (AEs)

Table 3-9 below shows adverse events reported during the conduct of the trial. Section A shows a breakdown by intensity, followed by category (section B) for all adverse events. Subsequent sections (C-G) reflect the likelihood, as assessed by the (blinded) local Principal Investigator, that the AE was attributable to the trial drug. Of the 940 adverse events reported in the study, 657 (70%) were classified as definitely not related to study drug, 179 (19%) as probably not related, 93 (10%) as possibly related, 11 (1%) as probably related, and no adverse event was definitely related. We recorded one serious adverse event, which was a skin reaction in a child allocated to placebo. The distribution of adverse events was similar between groups. There were no recorded deaths.

TABLE 3-9 - ADVERSE EVENTS

	Montelukast		**Placebo (N=677)**		**Total (N=1346)**	
	N	(%)	N	(%)	N	(%)
Total number of events	397	(100%)	543	(100%)	940	(100%)
Total number of participants	197	(29%)	235	(35%)	432	(32%)
A) Intensity	**397**		**543**		**940**	

Mild	314	(79%)	426	(78%)	740	(79%)
Moderate	77	(19%)	108	(20%)	185	(20%)
Severe	6	(2%)	9	(2%)	15	(2%)
B) Category	**397**		**543**		**940**	
Minor injury	27	(7%)	22	(4%)	49	(5%)
Gastrointestinal	86	(22%)	122	(22%)	208	(22%)
Upper respiratory tract infection	73	(18%)	103	(19%)	176	(19%)
Central nervous system	25	(6%)	46	(8%)	71	(8%)
Minor infection	87	(22%)	107	(20%)	194	(21%)
Allergy	16	(4%)	20	(4%)	36	(4%)
Cutaneous	32	(8%)	54	(10%)	86	(9%)
Respiratory	34	(9%)	54	(10%)	88	(9%)
Haematological	5	(1%)	7	(1%)	12	(1%)
Genitourinary	10	(3%)	6	(1%)	16	(2%)
Major injury	2	(1%)	1	(<1%)	3	(<1%)
Musculoskeletal	0		1	(<1%)	1	(<1%)
C) Total number of events: definitely not related	**281**		**376**		**657**	
Minor injury	27	(10%)	22	(6%)	49	(7%)
Gastrointestinal	40	(14%)	62	(16%)	102	(16%)
Upper respiratory tract infection	63	(22%)	88	(23%)	151	(23%)
Central nervous system	8	(3%)	10	(3%)	18	(3%)
Minor infection	76	(27%)	91	(24%)	167	(25%)
Allergy	13	(5%)	16	(4%)	29	(4%)
Cutaneous	18	(6%)	32	(9%)	50	(8%)
Respiratory	25	(9%)	47	(13%)	72	(11%)
Haematological	2	(1%)	2	(1%)	4	(1%)
Genitourinary	7	(2%)	4	(1%)	11	(2%)
Major injury	2	(1%)	1	(<1%)	3	(<1%)
Musculoskeletal	0		1	(<1%)	1	(<1%)
D) Total number of events: probably not related	**80**		**99**		**179**	
Minor injury	0		0			0
Gastrointestinal	26	(33%)	33	(33%)	59	(33%)
Upper respiratory tract infection	10	(13%)	15	(15%)	25	(14%)
Central nervous system	5	(6%)	8	(8%)	13	(7%)
Minor infection	11	(14%)	16	(16%)	27	(15%)
Allergy	3	(4%)	4	(4%)	7	(4%)
Cutaneous	10	(13%)	13	(13%)	23	(13%)
Respiratory	9	(11%)	7	(7%)	16	(9%)
Haematological	3	(4%)	1	(1%)	4	(2%)
Genitourinary	3	(4%)	2	(2%)	5	(3%)
Major injury	0		0			0

Musculoskeletal	0		0			0
E) Total number of events: possibly related	**33**		**60**		**93**	
Minor injury	0		0			0
Gastrointestinal	19	(58%)	23	(38%)	42	(45%)
Upper respiratory tract infection	0		0			0
Central nervous system	10	(30%)	25	(42%)	35	(38%)
Minor infection	0		0			0
Allergy	0		0			0
Cutaneous	4	(12%)	8	(13%)	12	(13%)
Respiratory	0		0			0
Haematological	0		4	(7%)	4	(4%)
Genitourinary	0		0			0
Major injury	0		0			0
Musculoskeletal	0		0			0
F) Total number of events: probably related	**3**		**8**		**11**	
Minor injury	0		0		0	
Gastrointestinal	1	(33%)	4	(50%)	5	(45%)
Upper respiratory tract infection	0		0		0	
Central nervous system	2	(67%)	3	(38%)	5	(45%)
Minor infection	0		0		0	
Allergy	0		0		0	
Cutaneous	0		1	(13%)	1	(9%)
Respiratory	0		0		0	
Haematological	0		0		0	
Genitourinary	0		0		0	
Major injury	0		0		0	
Musculoskeletal	0		0		0	
G) Total number of events: definitely related	**0**		**0**		**0**	

3.7.5.2 Safety conclusions

This study supports the position that Montelukast is safe in this age group. No excess of adverse events was observed in the treatment group, nor were any novel adverse events identified over and above those known prior to study commencement.

3.7.6 Health economic outcomes

The health economic analysis was dependent upon a demonstrable treatment effect. In the absence of a treatment effect of montelukast further analysis was deemed unwarranted, however, the implication is that montelukast is not overall cost-effective in this population.

4 RESULTS - MECHANISMS DATA

4.1 Overview

In this section I describe the exploratory mechanistic data. I compare urinary excretion of LTE_4 and other eicosanoids at baseline and during wheezing exacerbation, and between preschool wheezing children and non-wheezing controls. Previous studies have shown an increase in uLTE4 during preschool wheezing exacerbation(38,41), I attempted to replicate this finding, and hypothesized that baseline or exacerbation uLTE4 might associate with montelukast response and/or ALOX5 promoter genotype. I also describe patterns observed in other eicosanoid mediators measured in this population. I go on to describe and explore the associations observed between eicosanoid pathway SNPs and montelukast response, wheeze phenotype, and other selected measures. This was an exploratory rather than a hypothesis-driven investigation.

4.2 Urine eicosanoids

4.2.1 Urinary LTE_4 by ALOX5 status

Urinary eicosanoids were evaluated at baseline and, in a subset of recruits, during exacerbation. Baseline urine was analysed by genotype stratum (Figure 4-1). There was a statistically significant increase in baseline leukotriene activation in subjects with no wild type (5 repeat) ALOX5 promoter allele. This is contrary to the direction that might be predicted from the (non-significant) genotype:efficacy interaction suggested in Table 3-6. The numbers in the x/y group are very small, thus this observation must treated with caution.

4.2.2 Effect of age and atopic status on urinary eicosanoids - healthy controls

In order to give context to the values obtained within our preschool wheezing cohort we assessed $uLTE_4$ and other eicosanoids in non-wheezing atopic and non-atopic children of all ages.

71 recruited children provided an adequate urine sample for analysis. Baseline characteristics are summarised in Table 4-1. There was a significant inverse correlation between age and urinary levels for all nine eicosanoids (Pearson's rank correlation (ρ), $P<0.05$, Table 4-3). Figure 4-2 depicts this effect for 13,14-dihydro-15-keto-tetranor-PgE_2, a Prostaglandin E_2 metabolite. In this small sample there was no difference in any urinary eicosanoid between atopic and non-atopic non-wheezing children (Table 4-2).

FIGURE 4-1 - URINARY LTE_4 BY ALOX5 PROMOTER GENOTYPE

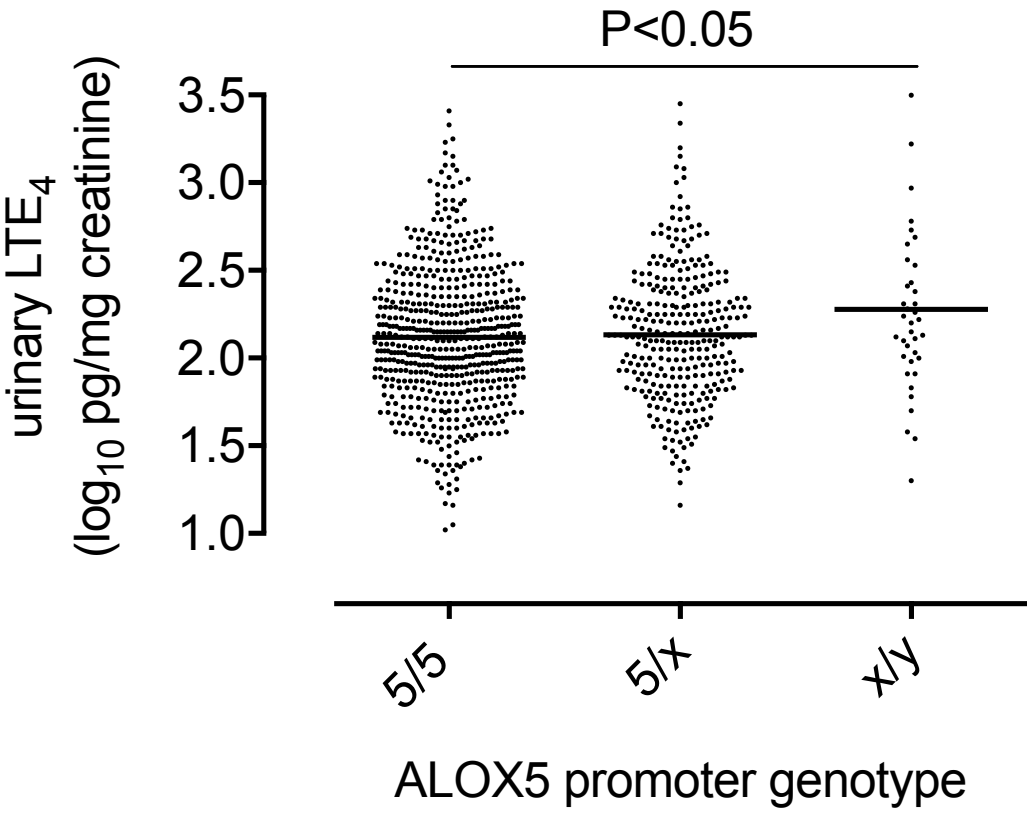


TABLE 4-1 - BASELINE CHARACTERISTICS OF NON-WHEEZING CONTROLS

	Female		Male	
Group	n	Age/years (mean ± SEM)	n	Age/years (mean ± SEM)
Total	31	6.09 ± 0.58	40	5.66 ± 0.50
Atopic	13	5.91 ± 0.93	15	5.78 ± 0.85
Non-Atopic	18	6.41 ± 0.78	25	5.58 ± 0.64

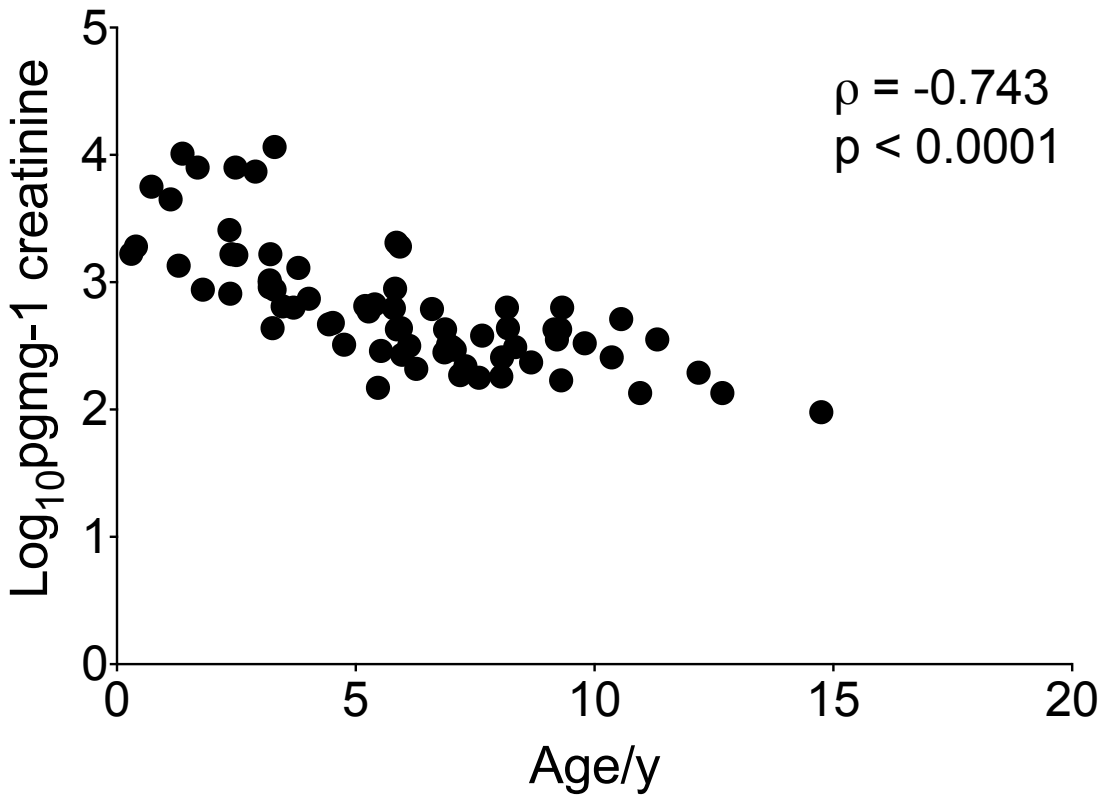
TABLE 4-2 - EICOSANOID MEDIATORS IN ATOPIC AND NON-ATOPIC NON-WHEEZING CONTROLS

	$Log_{10}pgmg^{-1}$ Cr (mean ± SEM)		P (two-tailed)
Eicosanoid Mediator	Atopic (n=28)	Non-Atopic (n=43)	Students t-test
LTE_4	2.15 ± 0.09	1.98 ± 0.06	0.1087
13,14-dihydro-15-keto-PgE_2	2.81 ± 0.06	2.76 ± 0.04	0.4918
13,14-dihydro-15-keto-PgD_2	2.41 ± 0.07	2.35 ± 0.05	0.4775
13,14-dihydro-15-keto-tetranor-PgE_2	2.82 ± 0.09	2.79 ± 0.07	0.7721
13,14-dihydro-15-keto-tetranor-PgD_2	2.79 ± 0.10	2.78 ± 0.08	0.9167
Tetranor-PgE-M	4.42 ± 0.06	4.41 ± 0.04	0.8552
Tetranor-PgD-M	3.87 ± 0.05	3.77 ± 0.06	0.2528
15-deoxy-delta12,14-PgJ_2	1.86 ± 0.09	1.80 ± 0.06	0.6072
9a,11b-PgF2	2.83 ± 0.03	2.75 ± 0.03	0.0793

TABLE 4-3 - CORRELATION BETWEEN AGE AND URINARY EICOSANOIDS (NON-WHEEZING CONTROLS)

Eicosanoid Mediator ($Log_{10}pgmg^{-1}$ Cr)	Pearson's ρ	95% confidence interval	P (two-tailed)	Number of XY Pairs
LTE_4	-0.2929	-0.4925 to -0.0639	0.0132	71
13,14-dihydro-15-keto-PgE_2	-0.5219	-0.6733 to -0.3286	<0.0001	71
13,14-dihydro-15-keto-PgD_2	-0.4466	-0.6157 to -0.2381	<0.0001	71
13,14-dihydro-15-keto-tetranor-PgE_2	-0.7432	-0.8322 to -0.6169	<0.0001	71
13,14-dihydro-15-keto-tetranor-PgD_2	-0.6559	-0.7712 to -0.4989	<0.0001	71
Tetranor-PgE-M	-0.2583	-0.4637 to -0.0266	0.0296	71
Tetranor-PgD-M	-0.2873	-0.4879 to -0.0579	0.0151	71
15-deoxy-delta12,14-PgJ_2	-0.6324	-0.7687 to -0.4411	<0.0001	55[8]
9a,11b-PgF2	-0.3163	-0.5118 to -0.0896	0.0072	71

FIGURE 4-2 - DECLINE IN 13,14-DIHYDRO-15-KETO-TETRANOR-PGE_2 WITH AGE



4.2.3 Urinary eicosanoids in preschool wheezing children

The 9 eicosanoid mediators indicated were measured in 949 subjects at baseline. They were analysed in relation to one another, and against a range of putative predictors including age, sex, atopic status, urinary cotinine, genetics and other eicosanoids. According to the T-2 screening questionnaire there were 19 demographic variables against which to analyse these data.

[8] Analysis of 15-deoxy-delta12,14-PgJ_2 failed on this run.

TABLE 4-4 - SUMMARY STATISTICS AND AGE CORRELATION FOR URINARY EICOSANOIDS (PRESCHOOL WHEEZE)

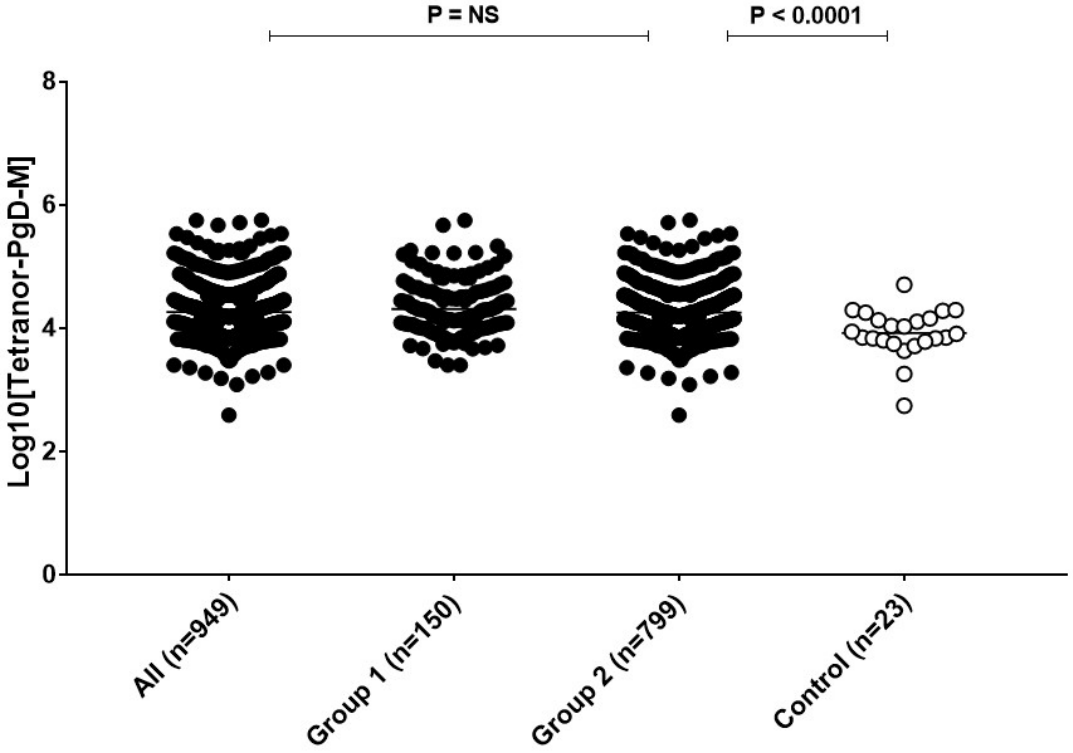
Eicosanoid Mediator ($Log_{10}pgmg^{-1}$ Cr)	N	Mean ± SEM	95% CI	Pearson's ρ with age	95% CI of ρ	P (2-tailed)
LTE_4	949	2.13 ± 0.01	2.10-2.16	-0.1298	-0.1918 to -0.0667	<0.0001
13,14-dihydro-15-keto-PgE_2	949	2.86 ± 0.01	2.84-2.89	-0.3237	-0.3795 to -0.2655	<0.0001
13,14-dihydro-15-keto-PgD_2	949	2.50 ± 0.01	2.48-2.53	-0.3212	-0.3771 to -0.2629	<0.0001
13,14-dihydro-15-keto-tetranor-PgE_2	949	3.00 ± 0.01	2.98-3.03	-0.3202	-0.3762 to -0.2619	<0.0001
13,14-dihydro-15-keto-tetranor-PgD_2	949	3.09 ± 0.01	3.07-3.12	-0.3225	-0.3784 to -0.2643	<0.0001
Tetranor-PgE-M	949	4.43 ± 0.01	4.41-4.45	-0.3995	-0.4517 to -0.3446	<0.0001
Tetranor-PgD-M	949	4.26 ± 0.01	4.24-4.29	-0.293	-0.3501 to -0.2337	<0.0001
15-deoxy-delta12,14-PgJ_2	949	2.07 ± 0.02	2.04-2.10	-0.2101	-0.2701 to -0.1484	<0.0001
9a,11b-PgF_2	949	2.88 ± 0.01	2.86-2.90	-0.2337	-0.293 to -0.1727	<0.0001

Baseline urinary eicosanoids did not vary with cotinine (not shown), while all markers were inversely correlated with age (Table 4-4), although to a lesser extent than in non-wheezing controls. Urinary tetranor PgD-M was elevated in preschool wheezing children compared with non-wheezing controls (Table 4-5), and this relationship was consistent regardless of atopic status (Table 4-6) or recruiting centre (Figure 4-3). The elevation was not modulated by acute exacerbation (Table 4-8), but was reduced in subjects receiving maintenance inhaled steroids (ICS) (Table 4-6, Figure 4-4).

TABLE 4-5 - EICOSANOID MEDIATORS IN PRESCHOOL WHEEZERS AND NON-WHEEZING CONTROLS

	$Log_{10}pgmg^{-1}$ creatinine (mean ± SEM)		P (two-tailed)
Eicosanoid Mediator	Preschool Wheezing (n=949)	Non-wheezing control (n=23)	Unpaired t-test
LTE_4	2.13 ± 0.01	2.17 ± 0.13	0.6769
13,14-dihydro-15-keto-PgE_2	2.86 ± 0.01	2.92 ± 0.06	0.4503
13,14-dihydro-15-keto-PgD_2	2.50 ± 0.01	2.55 ± 0.09	0.5922
13,14-dihydro-15-keto-tetranor-PgE_2	3.00 ± 0.01	3.16 ± 0.10	0.0912
13,14-dihydro-15-keto-tetranor-PgD_2	3.09 ± 0.01	3.16 ± 0.13	0.3924
Tetranor-PgE-M	4.43 ± 0.01	4.49 ± 0.07	0.4501
Tetranor-PgD-M	4.26 ± 0.01	3.92 ± 0.08	<0.0001*
15-deoxy-delta12,14-PgJ_2	2.07 ± 0.02	2.08 ± 0.13	0.9293
9a,11b-PgF_2	2.88 ± 0.01	2.84 ± 0.05	0.4934

FIGURE 4-3 - URINARY TETRANOR PGD-M BY SUBGROUP

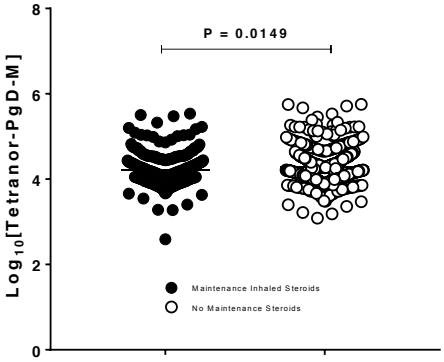


Tetranor PgD-M elevation was consistent in preschool wheezing children, regardless of recruiting centre (P<0.0001, 1-way ANOVA). Group 1 were recruited in the London host study site, Group 2 were recruited in other sites. Children on maintenance inhaled corticosteroids had reduced tetranor-PgD-M compared with steroid-naïve subjects (P = 0.01)

TABLE 4-6 - URINARY TETRANOR-PGD-M BY CLINICAL SUBGROUP

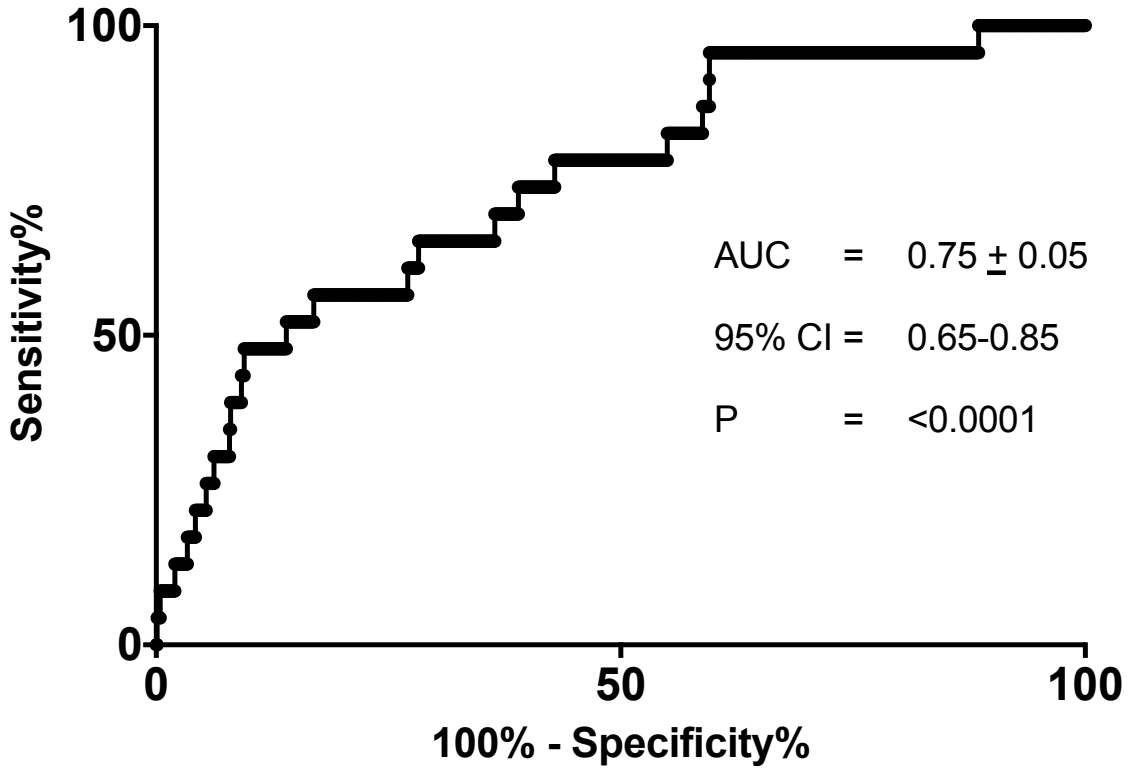
Clinical Subgroup	N	Mean ± SEM	95% CI
All preschool wheeze (PSW)	949	4.26 ± 0.01	4.24-4.29
Group 1	150	4.31 ± 0.04	4.24-4.38
Group 2	799	4.25 ± 0.01	4.23-4.28
All Age-matched controls	23	3.92 ± 0.08	3.75-4.09
Atopic controls	7	3.95 ± 0.13	3.63-4.28
Non-atopic controls	16	3.91 ± 0.10	3.69-4.13
Preschool wheeze + ICS	342	4.22 ± 0.02	4.18-4.26
Preschool wheeze - ICS	607	4.28 ± 0.01	4.25-4.31

FIGURE 4-4 - TETRANOR PGD-M BY USE OF MAINTENANCE ICS



Given the associations suggested in Table 4-6 and Figure 4-4, a receiver-operator characteristic (ROC) curve was generated for Log_{10}[Tetranor PgD-M] against presence of wheeze and wheeze phenotype. Tetranor PgD-M had some potential utility in predicting wheeze (Figure 4-5).

FIGURE 4-5 - ROC CURVE OF TETRANOR PGD-M AND PRESENCE OF WHEEZE



The optimum likelihood ratio (1.83) for prediction of wheeze was at Log_{10}[Tetranor PgD-M] <4.159. The modest area under the curve may reflect the small number of non-wheezing control (n=23) compared with wheezing (n=949) subjects. Tetranor PgD-M had no utility in predicting wheeze phenotype (not shown). Because linear regression showed that 13,14-dihydro-15-keto-PgD_2 and 13,14-dihydro-15-keto-PgE_2 were related to atopic disease (Table 4-7), I repeated the ROC analysis for these metabolites and neither showed utility in predicting wheeze (not shown).

4.2.4 Association of urinary eicosanoids with select demographic/phenotypic traits

All 9 urinary eicosanoids were standardized and log transformed and regressed on candidate predictor variables including age, sex, ethnicity, baseline unscheduled medical attendance, hospital admission, oral steroids courses in the preceding 12 months, maintenance inhaled corticosteroids, urinary cotinine, BMI z-score, preterm birth, low birth weight, allergic status, eczema, in utero and household tobacco exposure, parental asthma and wheeze phenotype in 949 preschool wheezing children. Regression coefficients and associated p-values of significant terms are indicated in Table 4-7. The correlation plot in Figure 4-6 shows the relationships between the respective eicosanoids.

FIGURE 4-6 - CORRPLOT™ OF CORRELATION BETWEEN URINARY EICOSANOIDS

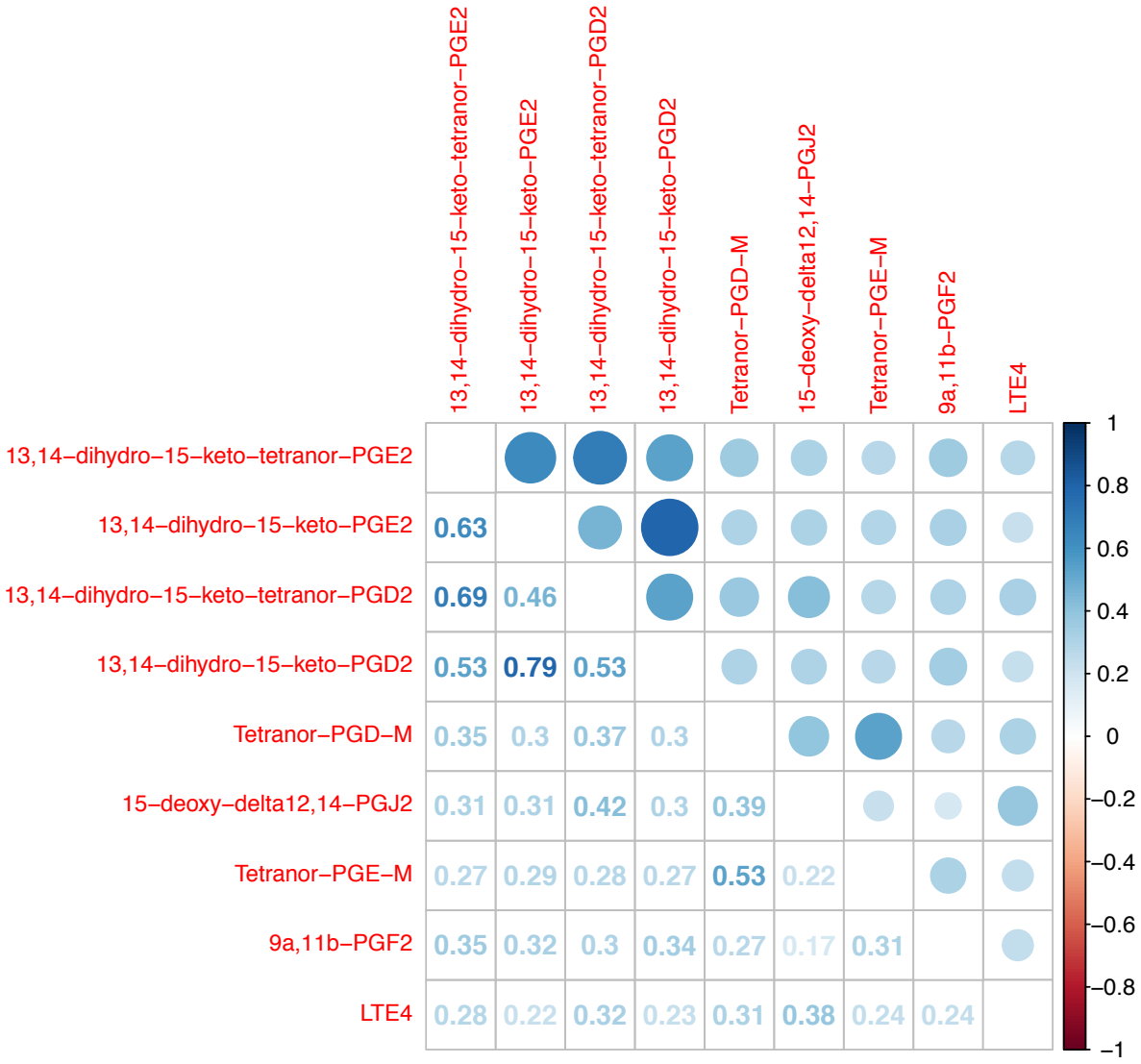


TABLE 4-7 - LINEAR REGRESSION OF URINARY EICOSANOIDS ON SELECT PREDICTOR TRAITS

	Age		Itchy Rash in last 6 months		Multitrigger Wheeze	
Eicosanoid Mediator	β-coefficient	p-value	β-coefficient	p-value	β-coefficient	p-value
LTE_4	-0.1439	<0.0001	-0.0828	0.3494	0.1293	0.0899
13,14-dihydro-15-keto-PgE_2	-0.2621	<0.0001	-0.4042	<0.0001	0.2857	0.0002
13,14-dihydro-15-keto-PgD_2	-0.2454	<0.0001	-0.3756	<0.0001	0.2297	0.0017
13,14-dihydro-15-keto-tetranor-PgE_2	-0.2877	<0.0001	-0.2625	0.0034	0.0916	0.2341
13,14-dihydro-15-keto-tetranor-PgD_2	-0.2826	<0.0001	-0.1313	0.1449	-0.0155	0.8414
Tetranor-PgE-M	-0.3394	<0.0001	-0.0998	0.2573	-0.0399	0.5989
Tetranor-PgD-M	-0.2554	<0.0001	-0.1641	0.0758	0.0293	0.7123
15-deoxy-delta12,14-PgJ_2	-0.1787	<0.0001	-0.1459	0.1005	0.0743	0.3308
9a,11b-PgF_2	-0.2339	<0.0001	-0.2087	0.0263	0.1001	0.2152

As per section 4.2.3 all eicosanoid mediators decreased with increasing age. In addition 13,14-dihydro-15-keto-PgD_2 and E_2 associated with multitrigger wheeze and recent itchy rash (a proxy for atopic dermatitis). Together these observations are suggestive of a role in atopy, although the exact mechanism is unclear (Table 4-7). There was no association between any eicosanoid mediator and urinary cotinine (not shown).

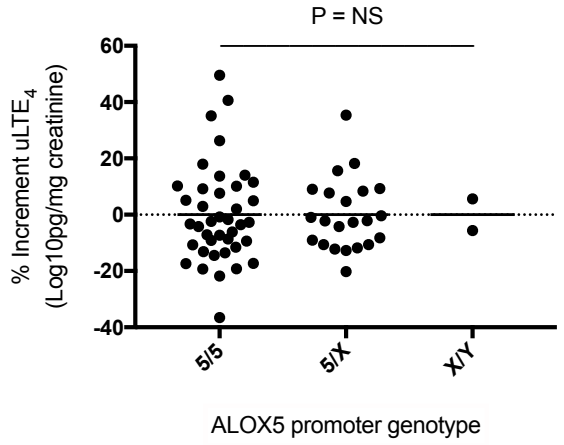
4.2.5 Effect of acute wheeze exacerbation on urinary eicosanoids

An unselected subset of study participants provided a urine sample during an acute wheezing exacerbation as well as at baseline. Exacerbation values were compared with baseline by paired t-test in 64 subjects. The results are as indicated in Table 4-8. In this small sample there was a significant elevation in LTE_4 during exacerbations, consistent with previous work by our group and others(38,41), while no effect was seen when increment was analysed according to ALOX5 promoter stratum (Figure 4-7). Tetranor-PgD-M was not altered in exacerbation despite the observed constitutive elevation in wheezing subjects (Figure 4-3).

TABLE 4-8 - BASELINE VS EXACERBATION URINARY EICOSANOIDS

Eicosanoid Mediator ($Log_{10}pgmg^{-1}$ Cr)	N	Baseline Mean ± SEM	95% CI	Exacerbation Mean ± SEM	95% CI	Increment (%)	P-value Paired t-test
LTE_4	64	2.11 ± 0.05	2.01 - 2.20	2.22 ± 0.05	2.13 - 2.32	7.71	0.0253
13,14-dihydro-15-keto-PgE_2	64	2.92 ± 0.04	2.84 - 3.00	2.91 ± 0.04	2.83 - 3.00	0.53	0.8147
13,14-dihydro-15-keto-PgD_2	64	2.53 ± 0.05	2.44 - 2.63	2.54 ± 0.05	2.44 - 2.64	1.34	0.8796
13,14-dihydro-15-keto-tetranor-PgE_2	64	3.09 ± 0.05	3.00 - 3.18	2.99 ± 0.06	2.87 - 3.10	-2.47	0.0952
13,14-dihydro-15-keto-tetranor-PgD_2	64	3.09 ± 0.05	3.00 - 3.18	3.03 ± 0.04	2.95 - 3.11	-0.94	0.2351
Tetranor-PgE-M	64	4.43 ± 0.04	4.36 - 4.51	4.44 ± 0.04	4.36 - 4.52	0.55	0.8699
Tetranor-PgD-M	64	4.25 ± 0.04	4.16 - 4.33	4.17 ± 0.04	4.09 - 4.25	-1.25	0.1750
15-deoxy-delta1214-PgJ_2	64	2.04 ± 0.07	1.90 - 2.17	2.02 ± 0.06	1.89 - 2.14	2.33	0.7386
9a11b-PgF_2	64	2.91 ± 0.04	2.84 - 2.99	2.88 ± 0.04	2.79 - 2.96	-0.59	0.4143

FIGURE 4-7 - % INCREMENT IN ULTE$_4$ BY ALOX5 PROMOTER STRATUM



4.2.6 ROC curves of LTE_4 increment vs USMA in montelukast treated subjects

Placebo-treated children with paired LTE4 sample data were classified according to numbers of USMA during follow-up and ROC curves generated from the percentage LTE_4 increment:

Analysis 1: subjects with ≥5 or <5 USMA/year at baseline
Analysis 2: subjects with ≥5 or <5 USMA/year during follow-up
Analysis 3: subjects with or without USMA during follow-up
Analysis 4: Montelukast treated subjects with ≥5 or <5 USMA/year during follow-up
Analysis 5: Montelukast treated subjects with ≥2 or <2 USMA/year during follow-up
Analysis 6: Montelukast treated subjects with or without USMA/year during follow-up

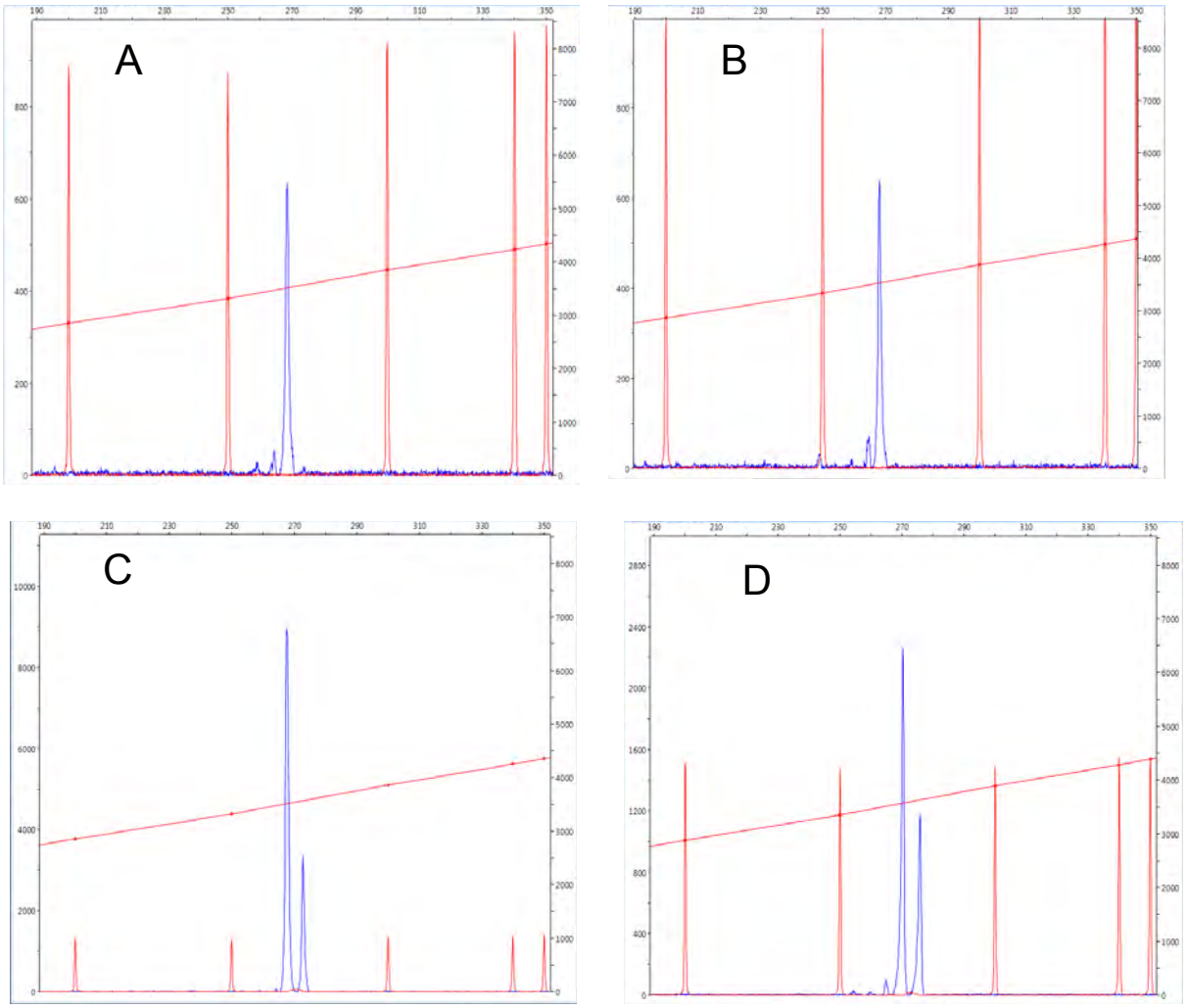
None of the above analyses yielded a statistically significant or clinically useful predictive model for either baseline wheezing frequency or response to montelukast (Appendix 8.10, Figure 8-8).

4.3 Genetic analysis

4.3.1 ALOX5 genotyping

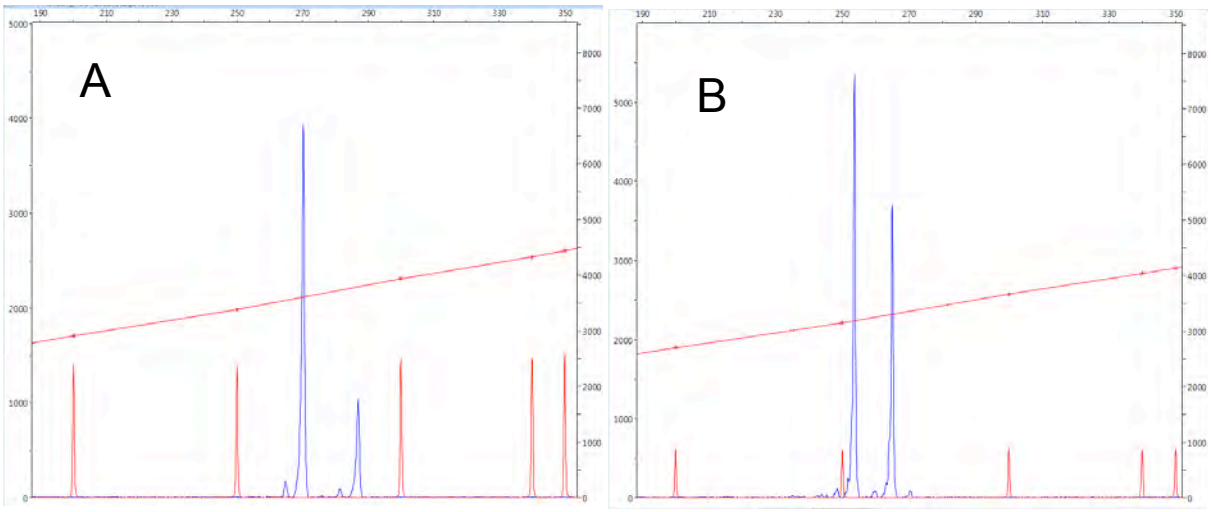
All 1366 saliva samples referred to the laboratory were genotyped successfully. However, two samples were placed in the inappropriate stratum. In one case, a genotype was called as 5/5 and the individual placed in stratum A. On review it was noted that this sample had not amplified well, and on repeat was shown to have a genotype of 5/6 (Figure 4-8). In a second case the genotype was called as 4/5 but through clerical error the stratum was entered as A. In both cases, medication had been dispensed before the correction could be made.

FIGURE 4-8 - POOR AMPLIFICATION GENOTYPE ERROR



A and B: poor amplification - allele 5 only, C and D: good amplification - alleles 5 and 6 revealed

FIGURE 4-9 - RARE GENOTYPE ELECTROPHEROGRAMS



A = 5/8 B = 2/4

4.3.1.1 Rare and novel genotypes

Table 4-9 shows the frequency of reported ALOX5 genotypes. As expected the 5/5 wild type genotype predominated. We reported three rare genotypes (the 2/4, 2/8 and 5/8 alleles[9]) and to our knowledge the current work is the first to report the presence of an 8-repeat allele (Figure 4-9).

4.3.1.2 ALOX5 genotype by reported ethnicity

ALOX5 genotype was compared to self-reported ethnicity (Table 4-9, Figure 4-10, Figure 4-11, Figure 4-12). There was marked genotypic variation between ethnicities, with black subjects having a lower frequency of 5/5 alleles than white and Asians, and also having the highest frequency of x/y alleles (particularly 3-repeat alleles). This observation is only partially consistent with Mougey *et al*. who found that overall 14.8% (40/270) of children (28% of 135 African Americans) carried two non-5-repeat variant alleles (143) i.e. an x/y genotype. The discrepancy in % x/y genotype in the total cohort (x/y % = 4.5, Figure 4-10) may reflect a lower ethnic heterogeneity in the current study, with white subjects (% x/y = 0.2) forming 75% of the population compared with $\leq$ 50% in the Mougey cohort. Owing in part to the relatively small minority ethnic populations in the current study a clinical correlate has not been established however it is recognised that black subjects have relatively poor asthma outcomes(172,173) which may result from a more severe phenotype(174) and the observed increase in $uLTE_4$ in the (albeit rather small) x/y group warrants investigation as a putative explanatory mechanism.

[9] The subject with the 3/8 allele (Table 4-9) did not provide permission for data use and is recorded as 'other'.

TABLE 4-9 - ALOX5 PROMOTER POLYMORPHISM GENOTYPE BY PARENT-REPORTED ETHNICITY

Genotype	White	Black	Asian	Bangladeshi	Mixed	Other	All
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
3/3	0 (0.00)	4 (10.81)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.29)
3/4	1 (0.10)	2 (5.41)	0 (0.00)	1 (0.75)	2 (2.78)	1 (2.70)	7 (0.51)
3/5	4 (0.39)	6 (16.22)	0 (0.00)	0 (0.00)	10 (13.89)	4 (10.81)	24 (1.76)
3/6	0 (0.00)	2 (5.41)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.15)
3/7	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.39)	1 (2.70)	2 (0.15)
4/4	18 (1.75)	0 (0.00)	8 (13.34)	5 (3.73)	2 (2.78)	1 (2.70)	34 (2.49)
4/5	285(27.78)	10 (27.03)	18 (30)	33 (24.63)	11 (15.28)	7 (18.92)	364 (26.65)
4/6	6 (0.58)	0 (0.00)	1 (1.67)	2 (1.49)	0 (0.00)	1 (2.70)	10 (0.73)
5/5	677 (65.98)	9 (24.32)	27 (45)	83 (61.94)	43 (59.72)	19 (51.35)	858 (62.81)
5/6	30 (2.92)	4 (10.81)	5 (8.33)	10 (7.46)	3 (4.17)	2 (5.41)	54 (3.95)
6/6	0 (0.00)	0 (0.00)	1 (1.67)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
2/4	1 (0.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
5/8	1 (0.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
5/7	2 (0.19)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.15)
2/5	1 (0.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
3/8	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.70)	1 (0.07)
Total	1026 (100)	37 (100)	60 (100)	134 (100)	72 (100)	37 (100)	1366* (100)
5/5	677 (65.98)	9 (24.32)	27 (45.00)	83 (61.94)	43 (59.72)	19 (51.35)	858 (62.81)
5/X	323 (31.48)	20 (54.05)	23 (38.33)	43 (32.09)	24 (33.33)	13 (35.14)	446 (32.65)
X/Y	26 (0.19)	8 (21.62)	10 (16.67)	8 (5.97)	5 (6.94)	5 (13.51)	62 (4.54)
Total	1026 (100)	37 (100)	60 (100)	134 (100)	72 (100)	37 (100)	1366* (100)

FIGURE 4-10 - ALOX5 GENOTYPE STRATUM BREAKDOWN

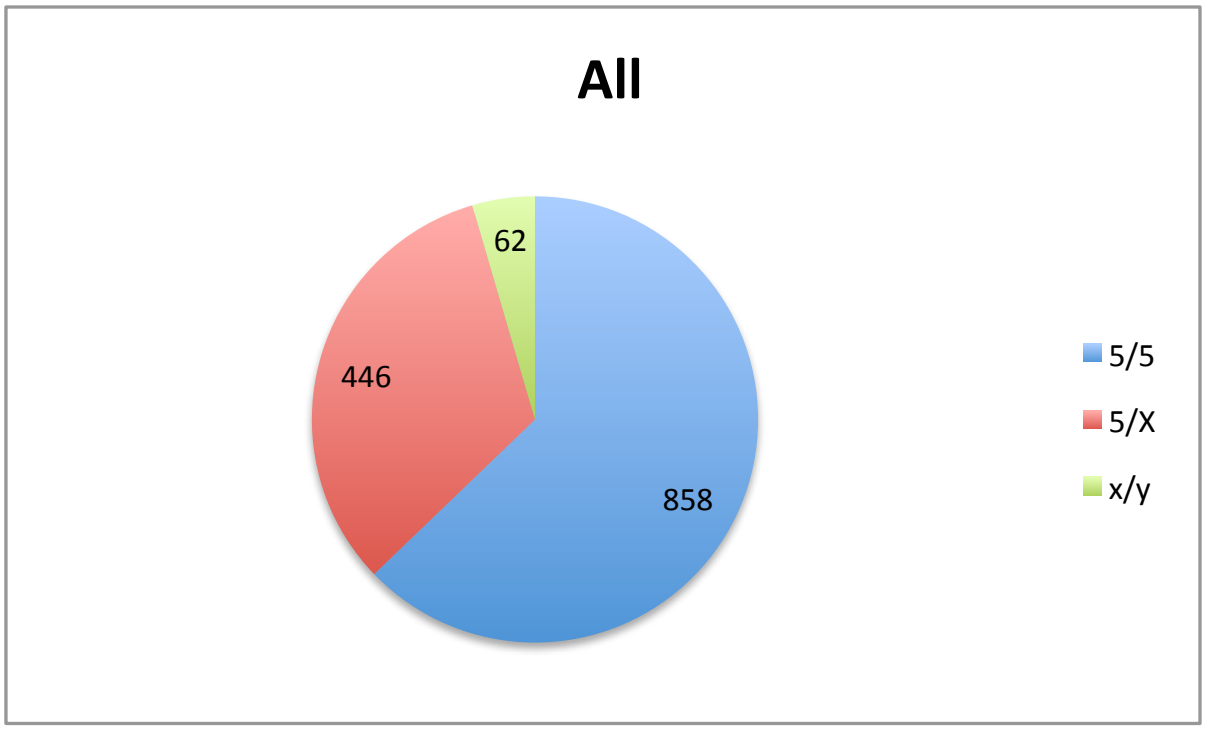


FIGURE 4-11 - CHARTS OF ETHNICITY AGAINST ALOX5 STATUS

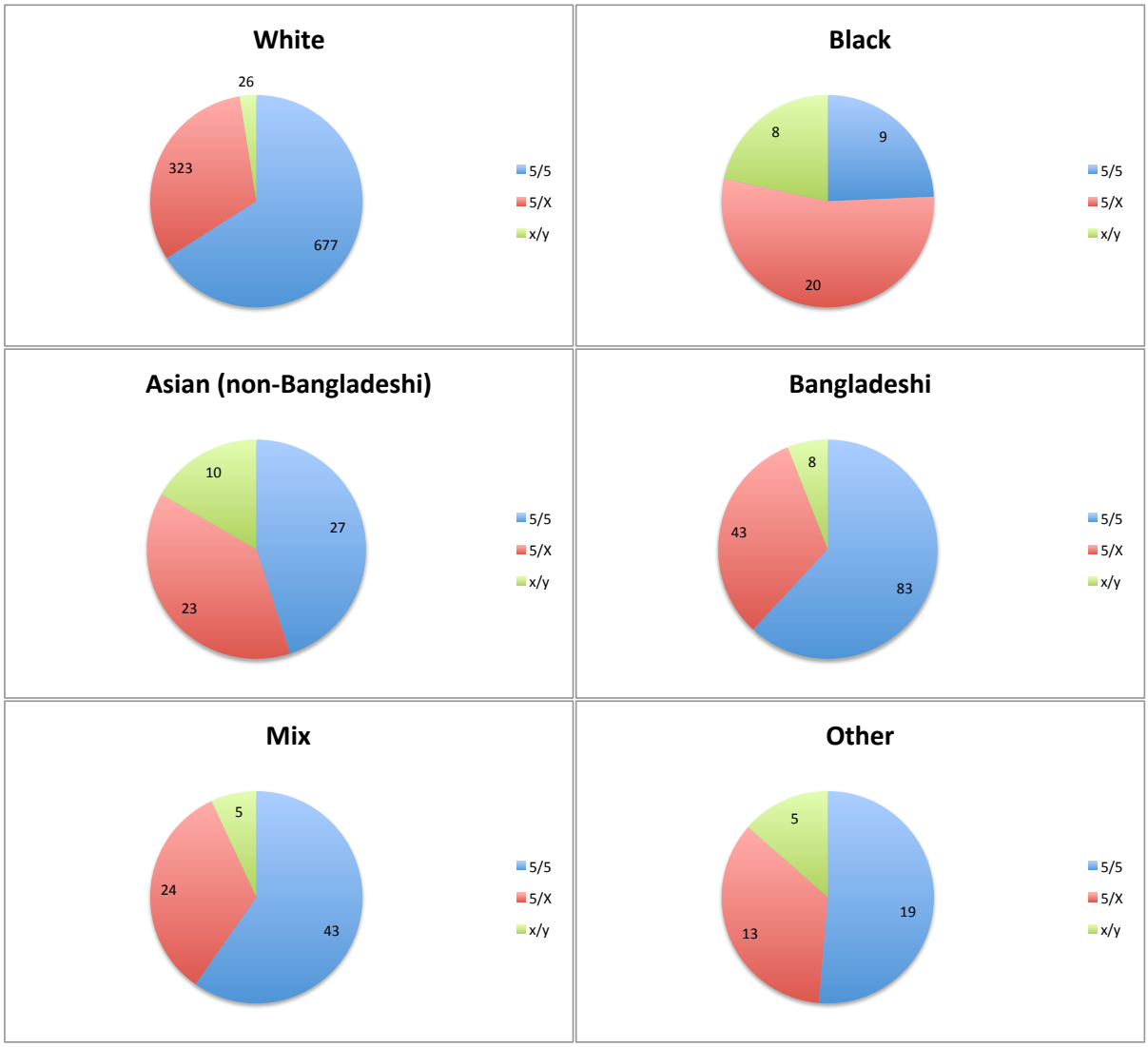
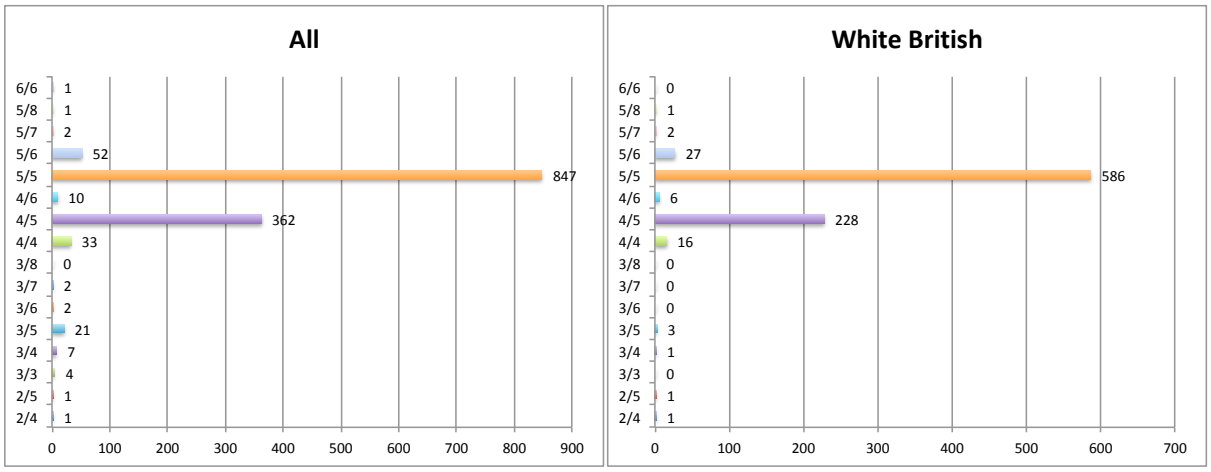
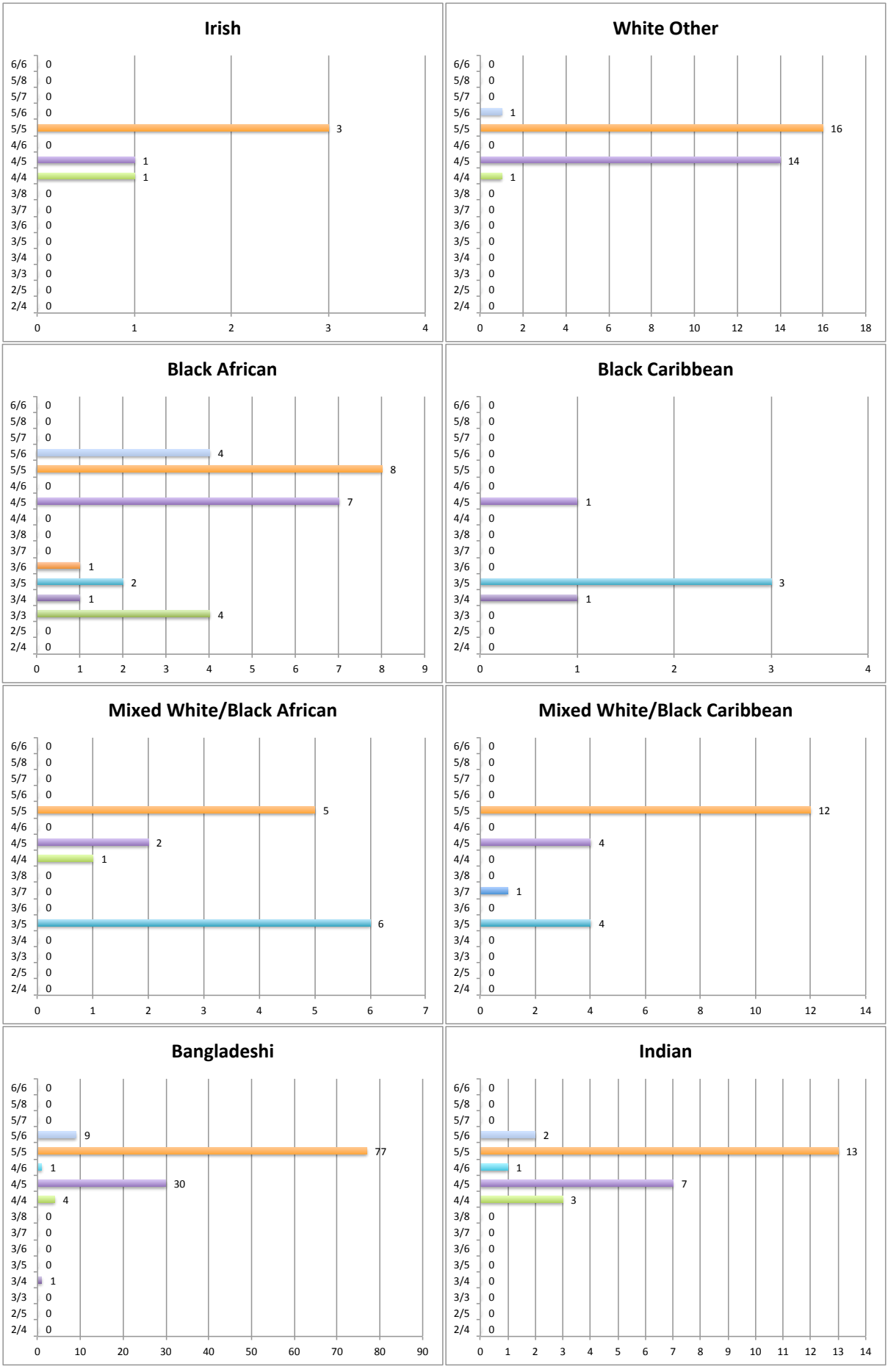


FIGURE 4-12 - DETAILED ETHNIC BREAKDOWN OF ALOX5 PROMOTER POLYMORPHISM COPY NUMBER

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5/7 0
5/6 0
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4/6 0
4/5 1
4/4 1
3/8 0
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3/5 0
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2/4 0
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5/7 0
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4/6 0
4/5 14
4/4 1
3/8 0
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Black African
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5/8 0
5/7 0
5/6 4
5/5 8
4/6 0
4/5 7
4/4 0
3/8 0
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3/4 1
3/3 4
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2/4 0
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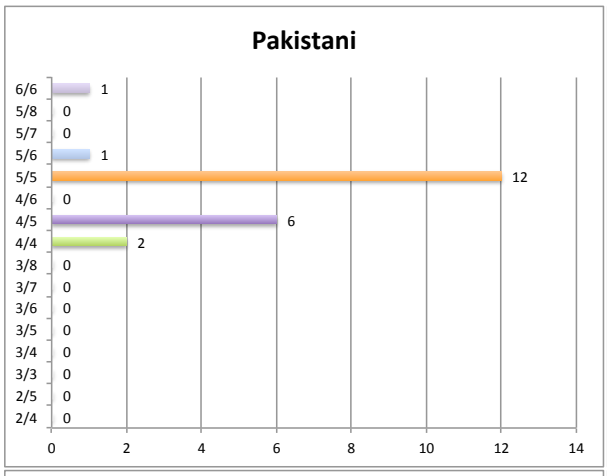
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2/4 0
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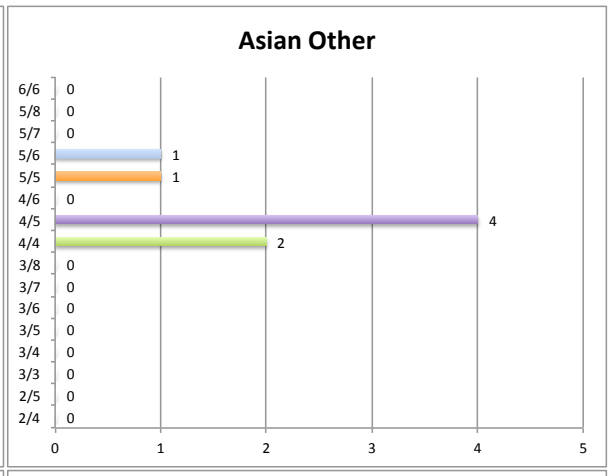
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2/4 0
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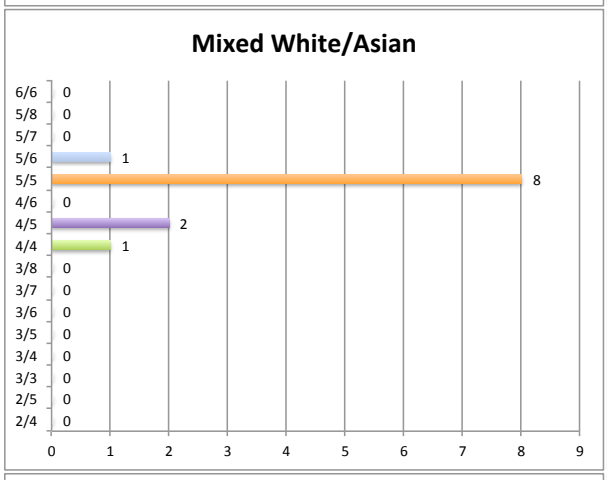
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5/5 8
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4/5 2
4/4 1
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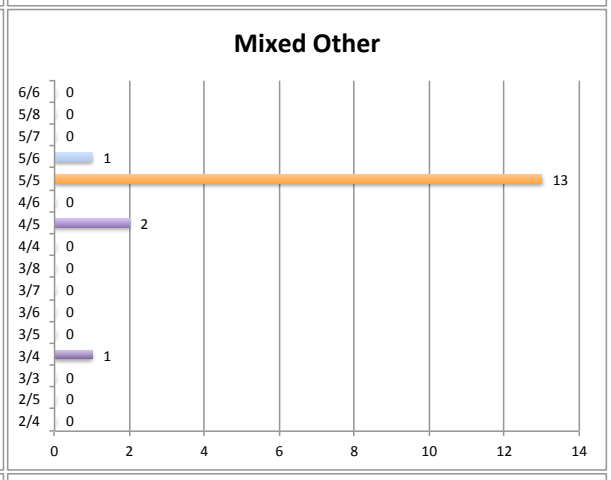
3/6 0
3/5 0
3/4 0
3/3 0
2/5 0
2/4 0
0 1 2 3 4 5 6 7 8 9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3/3 0
2/5 0
2/4 0
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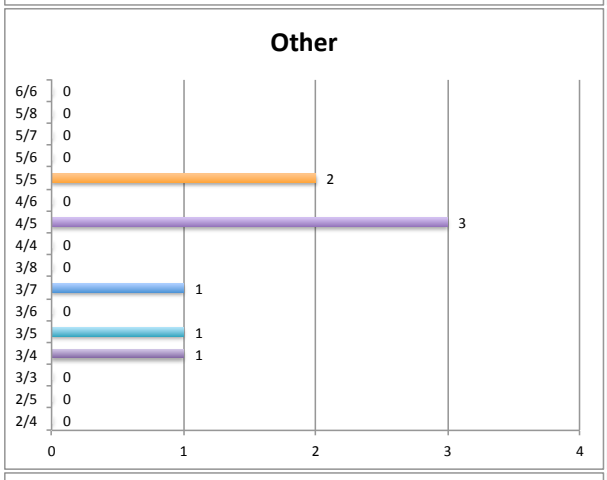
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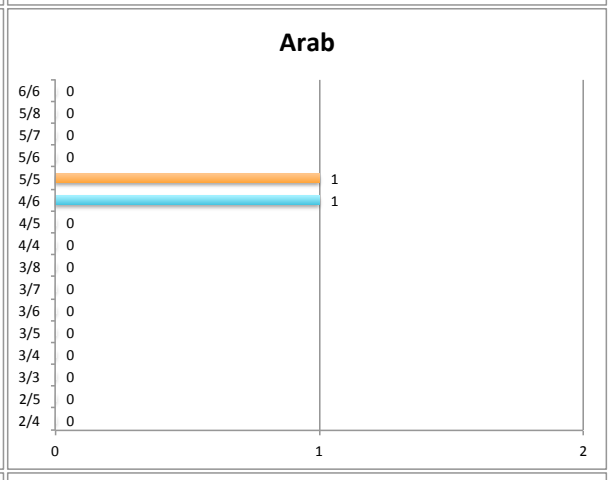
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5/8 0
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5/6 0
5/5 1
4/6 1
4/5 0
4/4 0
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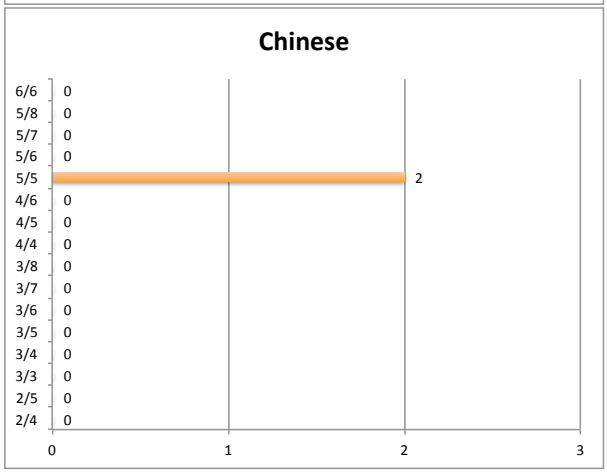
3/7 0
3/6 0
3/5 0
3/4 0
3/3 0
2/5 0
2/4 0
0 1 2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3/3 0
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2/4 0
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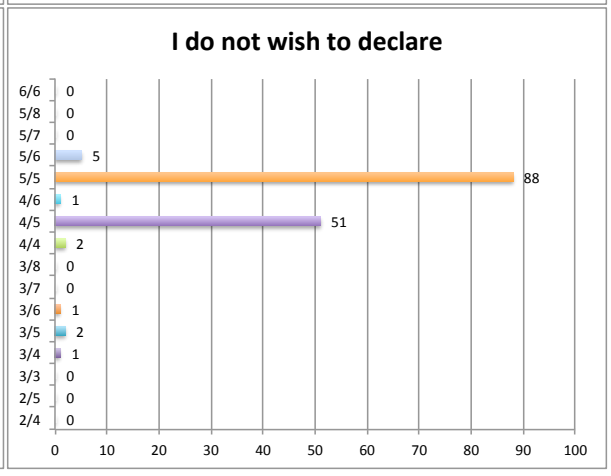
I do not wish to declare
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3/7 0
3/6 1
3/5 2
3/4 1
3/3 0
2/5 0
2/4 0
0 10 20 30 40 50 60 70 80 90 100

4.3.2 SNP analysis

Subject salivary DNA was subject to exploratory analysis for 143 eicosanoid pathway single nucleotide polymorphisms (SNPs) (Appendix 8.9) - based on previous work related to eicosanoid biology(130,138,157,160–164,175–178). In order to minimise the effects of multiple testing and yield less conservative FDR cut off thresholds, SNPs with linkage disequilibrium >0.8 to SNPs in the final analysis were excluded. Additionally, SNPs from CYSLTR1 (which is on the X-chromosome) were removed from the analysis, as several males had heterozygous genotypes, and a preliminary run with the additive genotypic model did not reveal any significance. The remaining SNPs were tested for association with the primary outcome, selected secondary outcomes and also with the various urinary eicosanoid markers.

4.3.2.1 SNP analysis and primary outcome

The exploratory SNPs (including the ALOX5 promoter polymorphism) were tested for association with the primary outcome, unscheduled medical attendances over the 12-month follow-up period. The analysis screened for significance of the effect of the interaction between genotype and intervention (montelukast or placebo). This analysis yielded signal for two SNPs: rs12422149 (SLCO2B1) and rs2526564 (LTB4R2) with significant p-values at an FDR cut off of 0.1 (Table 4-10). Results for both of these SNPs indicated gene:treatment interactions suggestive of a differential effect of montelukast on children with the minor allele; i.e. children with the minor allele had higher unscheduled medical attendances if receiving placebo. Table 4-11 shows the linear regression for the primary outcome stratified by treatment allocation, with significant p-values at FDR cut off of 0.15 observed in the same two SNPs.

TABLE 4-10 - EICOSANOID PATHWAY SNPS AND FOLLOW-UP UNSCHEDULED MEDICAL ATTENDANCES

SNP	CHR	Gene	A1	TEST	N	BETA ± SE	95% CI	STAT	P
rs12422149	11	SLCO2B1	A	RECxintervention	892	-7.364 ± 2.183	-11.64 to -3.085	-3.373	0.0008
rs2516564	14	LTB4R2	T	RECxintervention	888	-2.88 ± 0.9342	-4.711 to -1.049	-3.083	0.0021
Genomic Inflation factor:	<1								
Model:	USMAfu = β0 + β1(REC) + β2(SEX) + β3(age) + β4(intervention) + β5(REC*intervention)								
USMAfu:	the number of unscheduled medical attendances in the 12m following recruitment								
REC term:	1 if recessive, 0 if not								
Intervention:	1 if placebo, 2 if montelukast								
Null hypothesis:	USMAfu is not associated with the interaction between the homozygous minor allele and intervention, controlled for age and sex								
FDR cut-off at q:	0.05	0.1	0.15	0.2					
	0.001724138	0.003448276	0.005172414	0.006896552					

USMAfu = Unscheduled medical attendances during WAIT study follow-up; CHR = chromosome; BETA = regression coefficient of interaction(β5); SE = standard error, A1 = recessive allele, 95% CI = 95% confidence interval, FDR = False Discovery Rate

TABLE 4-11 - LINEAR REGRESSION OF EICOSANOID PATHWAY SNPS AND PRIMARY OUTCOME

			Placebo			Montelukast			Placebo: Montelukast	
SNP	Chr	Gene	Beta ± SE	T-stat	P	Beta ± SE	T-stat	P	Z-stat	P
rs12422149	11	SLCO2B1	6.2126 ± 1.3797	4.5029	<0.0001	-0.1033 ± 1.0357	-0.0998	NS	3.661	0.0003
rs2516564	14	LTB4R2	2.3440 ± 0.6199	3.7812	0.0002	-0.7446 ± 0.5914	-1.2590	NS	3.605	0.0003
Genomic Inflation factor:			<1							

Model:	USMAfu = b0 + b1(REC) + b2(SEX) + b3(age), stratified by placebo and montelukast arms
REC term:	1 if recessive, 0 if not
Null hypothesis 1:	USMAfu is not associated with the homozygous minor allele, controlled for age/sex, in the placebo arm
Null hypothesis 2:	USMAfu is not associated with the homozygous minor allele, controlled for age/sex, in the montelukast arm
Null hypothesis 3:	There is no difference in the association with USMAfu between treatment arms for the homozygous minor allele, controlled for age/sex

What columns represent:				
BETA (Placebo)	regression coefficient of recessive term in placebo arm			
P (Placebo)	P-value for recessive term in placebo arm			
BETA (Montelukast)	regression coefficient of recessive term in montelukast arm			
P (Montelukast)	P-value for recessive term in montelukast arm			
P (Plac:Montelukast)	P-value for difference in USMAfu between placebo and montelukast arms, under the recessive model			
FDR cut-off at q:	0.05	0.1	0.15	0.2
1 SNP	0.000862069	0.001724138	0.002586207	0.003448276
2 SNPs	0.001724138	0.003448276	0.005172414	0.006896552

TABLE 4-12 - DATA SUMMARY OF USMA BY SELECTED SNPS

Gene:SNP	Genotype	Placebo		Montelukast	
		N	USMA (Mean ± SEM)	N	USMA (Mean ± SEM)
SLCO2B1: rs12422149	GG	382	2.36 ± 0.15	365	2.10 ± 0.14
	GA	68	2.34 ± 0.27	80	1.66 ± 0.21
	AA	4	9.00 ± 3.24	6	1.83 ± 0.70
LTB4R2: rs2516564	CC	293	2.17 ± 0.16	278	2.10 ± 0.16
	CT	142	2.47 ± 0.22	148	1.91 ± 0.20
	TT	21	5.00 ± 1.04	19	1.26 ± 0.44

Each SNP was analysed for the primary outcome stratified by the following four groups: homozygous for the major allele, homozygous for the minor allele - to test for recessive effects, and heterozygous and homozygous major and heterozygous and homozygous minor (e.g. for rs12422149 the four stratification groups would be GG, AA, [GG + GA] and [AA + GA]) to look for dominant effects. By this analysis several SNPs had P<0.05, but none below the FDR cut off level at q=0.2 of 0.0034, thus allowing for multiple testing no signal was observed.

Looking at summary data for each SNP we see:

- The increased USMA in SNP rs12422149 (SLCO2B1) is suspect because only 10 subjects (Table 4-12) have the minor allele, and the observed difference in unscheduled medical attendances is driven by the 4 patients with this allele who also received placebo, which *may* be due to outliers.
- For SNP rs2516564 (LTB4R2) there are 40 study subjects (Table 4-12) with the minor allele, making the positive result for this SNP more convincing.

Scrutiny of the observed increase in USMA in subjects with the minor allele in the highlighted SNPs indicates that the effects are in the placebo-treated group. Surmising that the recessive genotypes are more severely affected without montelukast, but respond to montelukast, we hypothesized that there would be an observable effect in USMA at baseline for these two SNPs (4.3.2.2).

4.3.2.2 SNP analysis and pre-trial unscheduled medical attendances

To validate this effect the reported frequency of unscheduled medical attendances in the 12 months preceding study enrolment was reviewed (Table 4-13). There was no significant difference in reported unscheduled medical attendances prior to treatment in children associated with the presence of the minor allele at either locus, contrary to the effect observed during the study.

TABLE 4-13 - EICOSANOID PATHWAY SNPS AND BASELINE UNSCHEDULED MEDICAL ATTENDANCES

SNP	Chromosome	Gene	A1	TEST	N	BETA	STAT	P
rs2660880	12	LTA4H	A	REC	931	7.498	2.476	0.01346
rs9315045	13		C	REC	936	-1.257	-2.023	0.04333
rs4503649	13	ALOX5AP	A	REC	937	1.208	2.045	0.04111
rs3935644	13		T	REC	937	1.185	2.185	0.02915
rs4254165	13		G	REC	931	1.009	2.149	0.0319
rs912277	13	CYSLTR2	C	REC	931	3.93	2.048	0.04086

Genomic Inflation factor:	<1			
Model:	USMAbase = β0 + β1(REC) + β2(SEX) + β3(age)			
REC term:	1 if recessive, 0 if not			
Null hypothesis:	The number of USMA 12m prior to recruitment is not associated with homozygous minor allele, controlled for age and sex			
What columns represent:				
BETA:	Regression coefficient of recessive term			
Unadjusted P-value	Unadjusted P-value for recessive term			
Adjusted P-value	P-value for recessive term adjusted for genomic control			
FDR cut-off at q:	0.05	0.1	0.15	0.2
	0.000862069	0.001724138	0.002586207	0.003448276

Table 4-13 shows a selection of SNPs all of which had P<0.05 for association with unscheduled medical attendance at baseline, however none fell below the FDR cut-off

adjusted P-value of 0.0034 at q-value 0.2, suggesting but not confirming an association; these are different to those highlighted as significant for follow-up USMA (4.3.2.1). Possible reasons for this inconsistency include recall bias (the possibility that questionnaire-reported unscheduled attendances are inaccurate compared to prospectively recorded attendances during trial follow-up), or perhaps differential treatment (perhaps with montelukast) for some or all of the year prior to enrolment, however the possibility that the effect is spurious, driven by outliers (particularly in the small SLCO2B1 minor allele population) cannot be excluded.

4.3.2.3 SNP analysis and urinary leukotriene E_4

Asymptomatic (baseline) values for urinary leukotriene E_4 were reviewed for association with the SNP panel. SNPs with linkage disequilibrium >0.8 were excluded to optimise false discovery rate (FDR) cut-offs in the intervention analysis. Symptomatic (exacerbation) values for urinary leukotriene E_4 showed no association with any SNP tested, however this may reflect the small numbers of symptomatic samples.

TABLE 4-14 - SNP ASSOCIATION WITH ASYMPTOMATIC ULTE$_4$

SNP	Chr/ Gene	A1	TEST	N	BETA	St Err	95% CI	STAT	Unadj P-value	Adjusted T stat	Adj P-value
VNTR	Chr: 10 Gene: ALOX5	X	REC	678	0.241	0.096	0.053 - 0.429	2.518	0.0121	2.449	0.0146
rs3824613		T	REC	672	0.300	0.116	0.073 - 0.526	2.594	0.0097	2.523	0.0119
rs2115819		C	REC	673	0.081	0.036	0.011 - 0.151	2.275	0.0232	2.213	0.0273
Genomic Inflation factor:			1.057132654								
Model:	$Log_{10}(uLTE4A) = \beta0 + \beta1(REC) + \beta2(SEX) + \beta3(age)$										
REC term:	1 if recessive, 0 if not										
Null hypothesis:	Log_{10} of asymptomatic [$uLTE_4$] is not associated with homozygous minor allele, controlled for age & sex										
What columns represent:											
BETA:	Regression coefficient of recessive term										
Unadj P-value	Unadjusted P-value for recessive term										
Adj P-value	P-value for recessive term adjusted for genomic control										
False Discovery Rate cut-off at q:	0.05	0.001190476	0.1	0.002380952	0.15	0.003571429	0.2	0.004761905			

As indicated, P-values for 3 SNPs, including the ALOX5 VNTR promoter polymorphism are below 0.05, but above the FDR cut off of 0.0034 at q-value = 0.2. There *may* therefore exist an association between these SNPs and uLTE4, but the current sample is not powered to confirm this, accounting for multiple comparisons.

4.3.2.4 SNP analysis and other urinary eicosanoids

Given this finding, and the impossibly low FDR cut-offs required to correct for screening multiple SNPs against multiple urinary metabolites, no further analysis of urinary eicosanoid vs arachidonic acid pathway SNPs was performed.

5 RESULTS - QUALITATIVE DATA

5.1 Overview

This section describes the outputs from the semi-structured interview. These data are somewhat non-traditional in a trial of this kind, but offer an insight into the experience of patients and families, particularly those from ethnic minorities, as regards clinical research and medical illness; it is to be hoped that they may perhaps inform the design and conduct of future research and clinical interventions.

5.2 Patterns of participation

At the time of commencement of the Qualitative Study (QS), 139 children had been enrolled in the Parent Study (PS). Bangladeshi participants were relatively overrepresented in the parent study. Bangladeshi parents taking part in the parent study were less likely than parents of non-Bangladeshi ethnicity to be interviewed for the QS. Table 5-1 shows ethnically delineated differences in study participation.

TABLE 5-1 - PATTERNS OF PARTICIPATION BY ETHNIC GROUP(179)

	Bangladeshi	White British	Other*	Total
Percentage of Local <5 Population by ethnic group	9,280 (50%)	3,094 (17%)	6,376 (33%)	18,750 (100%)
Children enrolled in Parent Study at time of QS	94 (68%)	24 (17%)	21 (15%)	139 (100%)
Parents consenting to QS at time of enrolment to Parent Study (% of parents enrolled, 95% CI)	48 (51%, 41-61)	17 (71%, 51-85)	20 (95%, 76-100)	85
Qualitative interview completed (% of parents enrolled, 95% CI)	20 (21%, 14-31)	10 (42%, 24-61)	12 (57%, 37-76)	42

*Other interviewed parents: African (n=6), Caribbean (n=2), South American (n=1), Middle Eastern (n=1), Chinese (n=1)

5.3 Qualitative study participants

Of the 85 parents who gave written consent to structured interview at parent study enrolment only half subsequently participated in a face-to-face interview. The reasons for non-participation are as stated in Table 5-2. The remaining 42 parents agreed to a face-to-face interview, which took place over a seven month period. There is no ideal sample size for qualitative studies (180) and there was a sufficient number of interviews within this opportunistic sample to achieve data saturation.

TABLE 5-2 - REASONS FOR DECLINE OR NON-RESPONSE TO REQUEST FOR INTERVIEW

Reasons given for decline or no response to request for qualitative interview	Number of parents
No response	14
Declined - no reason given	11
Declined - no time (employment-related)	4
Declined - unable to speak English	5
Declined - no time (heavily pregnant or caring for newborn)	4
Declined - annual or religious or imminent extended holiday	5
Total number of parents	43

TABLE 5-3 - CHARACTERISTICS OF QUALITATIVE INTERVIEW PARTICIPANTS

		Bangladeshi	White UK	Other
Demographics				
Male		6	2	2
Female		14	8	10
Age in years (mean (SD))		35 (7.8)	34 (6.2)	36 (4.3)
Language				
1st Language	Bengali/Sylheti	19		
	English	1	10	4
	French			2
	Arabic			2
	Mandarin			1
	Creole			1
	Portuguese			2
Fluency in spoken English[10]	Excellent	5	10	4
	Good	1	0	0
	Fair	5	0	5
	Poor	9	0	3
Socioeconomic				
Educational attainment	Left before 16 years	1		
	GCSE or equivalent	8	6	3
	A level or equivalent	1	0	3
	Graduate degree	1	4	3
	Postgraduate degree	1		2
	Not answered	8		1
Occupation of highest earner in family	Higher managerial, administrative & professional	1	2	2
	Intermediate	3	1	3
	Routine and manual	8	2	3
	Student			1
	Not answered	8	5	3
	Total	20	10	12

The majority of respondents were female. Bangladeshi participants had poorer spoken English than other groups and were less inclined to disclose their level of schooling, perhaps indicating sensitivity regarding poor educational attainment. Most households reported at least one working parent, but the numbers engaged in full time, part time, or shift work were unclear due to guarded responses.

5.4 Qualitative interview themes

Themes emerging from the interviews included:

1. Reasons parents gave for enrolling their child in the trial;
2. Participating parents' experience of the consent process and understanding of written and verbal information provided at the outset of the trial, in particular their understanding of the randomisation process;
3. Participating parents' understanding and response to the collection of genetic information;

[10] Interviewer's judgment

4. The nature of participating parents' consultations with other people before deciding to take part;

Given the sizable local Bangladeshi population, distinctions between Bangladeshi respondents and other groups were noted.

Parents of children in all groups reported anxiety related to their helplessness during wheezing attacks, often driven by their experience at the first hospital presentation. Major fears were of the potential for death or major longterm disability. These concerns drove a preoccupation with monitoring their child's health, together with anxiety about the potential progression of a cold to a wheeze with the attendant hospital visit, investigations and treatment. These fears were important drivers for participation in the parent study, with hopes that participation might contribute to improved treatment for wheeze. Box 5-1 shows interview extracts relevant to this theme.

BOX 5-1 - ANXIETIES ABOUT WHEEZE IN CHILDREN

When I am putting him in the shower he was difficult to breath, he goes (makes gasping sound) with his hand in his mouth and I was scared and then I had to call an ambulance…he stayed in hospital for two days. (F-Brazilian)

He wasn't too good they said we have to keep him in and he had oxygen up his nose. It was horrible. So he was in hospital for three days. That was the worst three days of my life. (F-UK)

I'm so worried. My God. I know asthma may kill so I'm very worried (F-Bangladeshi)

'My worst fear was that if I'm not with him or something like that….not breathe or…I don't know, I don't know much about asthma. I don't want him to get that. (F-Egyptian)

'I can remember saying to (husband) very clearly if he dies don't come and collect me because I don't want to leave without him' (F-Caribbean)

'I really didn't know how bad it was and how it can affect a child. And I really didn't know it was going to be the start of this long process of hospital after hospital after hospital.' (F-UK)

'I am looking for a final treatment for her because this disease is not good for her health you know so I am looking for much better treatment for her and to find a treatment which is better for her whole life.' (FM-Bangladeshi).

This was the emotional context within which parents were invited to enrol their child into the parent study. Parents reported being approached while inpatients or during follow-up appointments in primary or secondary care. Most children within the QS were already on prescribed medication for wheeze.

Half of the parents (11/20 Bangladeshi, 6/10 UK, 4/12 other) said that their primary reason for enrolling their child was that they hoped it would benefit their child in curtailing or curing the wheeze. A subset of these believed that the trial medicine represented an individual treatment regime for their child, perhaps conflating research with personal treatment. Others

also viewed it as a route to additional information, treatments and medical attention by skilled physicians (Box 5-2).

A third (15/42, of which 5/20 Bangladeshi, 4/10 UK, 6/12 other) said that their aim was to help other children by contributing to the advancement of medical knowledge (although benefit to others was secondary to a consideration of potential benefit to their own child). Only four parents (2/10 UK, 2/12 other) voiced a wholly altruistic outlook by explicitly recognising that the results of the trial would be unlikely to directly benefit their own child.

A few parents (2/20 Bangladeshi, 1/10 UK, 0/12 other) based their decision to participate in the trial primarily on their trust in the research team, these families appeared not to differentiate between trial researcher and healthcare provider roles. Parents felt reassured that they could opt out of the trial at any time, and particularly if their child experienced side effects.

BOX 5-2 - OTHER REASONS FOR TAKING PART IN PARENT STUDY

Benefit to child

I wanted to see if it helps my daughter, to see if it got rid of her wheeze. (F-Bangladeshi)

They said if you do this study your daughter is going to get better. (F-Bangladeshi)

It's an extra medicine for my daughter that will help her, and it helps her stay at home rather than going to the GP or hospital all the time. (F-Bangladeshi)

A very good way of you know, getting him seen by good doctors … and hopefully getting answers you're looking for' (F-UK)

Benefit to others

[My child] won't really benefit but from it …this is obviously a trial so that they can try and prescribe this medicine in the future for children. (F-UK)

It's good for the future. All children. Not for her [child] because she has already got it now, but yes, all children of the world. (F-African)

Hopefully it's good for other children and good for her. (M-Chinese)

Trust in clinicians

I thought like, you know, it's from hospital, obvious it's good for him. So the doctor knows better than us. (F-Bangladeshi)

Being in control

It's reassuring that they kept saying that at any time we can pull out. (F-Bangladeshi)

They explained to me that the main side effect was sleep like sleep disturbance erm... and obviously if it was too much then just stop. (F-UK)

Beliefs about the acceptability to their child and the effects of the substance they were given - whether montelukast or placebo - were clearly important motives in maintaining or discouraging continuing participation (Box 5-3). 4/20 Bangladeshi parents (but no others) believed there would be no side effects, reporting that this was what the trial researcher had told them. Even parents with a well-informed understanding of the trial process said they would consider withdrawing their child if they believed the medication was not having a beneficial effect. Three (one from each ethnic group) had already decided to discontinue, because their child did not like the medication or because it did not appear to be effective, or because of perceived adverse effects. Three more (2/10 UK, 1/12 other) said they would consider dropping out for similar reasons if they believed that their child had been allocated the placebo drug, or if the medication seemed ineffective or harmful, indicating that subject recruitment and retention is driven strongly by the perceived likelihood of personal benefit.

BOX 5-3 - EFFECTS AND ACCEPTABILITY OF MEDICATION

They just told me there's not going to be there, there is no side effects at all. (M-Bangladeshi)

Yes medicine he doesn't like. (F-Bangladeshi)

I don't want there to be any side effects. Yes everything has got side effects but, it's the sleeping part and the behaviour that was another thing. I didn't want that to change. (F-UK)

I think our first step would be if I thought he wasn't on the medicine, getting the medicine prescribed somewhere else. (F-UK)

I mean the only reason I would come out of the trial was if I thought there was any erm... negative side effects. And we're now on our second dose of medicine and he's been totally fine. (F-UK)

I'd go back to the hospital...and tell them the medicine you give to me maybe don't do anything. (F-African)

The wheezing is still there and it was not going away, so I just said, I just stopped giving to him, I said I didn't think it was helping him at all. (F-Caribbean)

5.4.1 Information and consent

TABLE 5-4 - INFORMATION AND CONSENT

	Bangladeshi	White UK	Other*	Total
Satisfied with initial information	20 (100%)	10 (100%)	12 (100%)	42/42 (100%)
Personally Read PIS	11/20 (55%)	6/10 (60%)	4/12 (33%)	21/42 (50%)
Had PIS read to them	5/20 (25%)	4/10 (40%)	6/12 (50%)	15/42 (36%)
Understanding of randomisation	5/20 (25%)	7/10 (70%)	3/12 (25%)	14/42 (33%)
Awareness that DNA sample taken	6/20 (30%)	9/10 (90%)	9/12 (75%)	24/42 (57%)

All parents reported satisfaction with the initial trial information they had received and that all queries were answered adequately. Information retention was poor however, and by the

time of the interview few could recall significant detail about the parent study. The decision to give consent was strongly influenced by the meeting with the clinical team.

The patient information sheet (PIS) was translated from English into Bengali but not into other languages as Bangladeshis were by far the largest local non-English speaking minority, with a disproportionately reduced likelihood of English in comparison to other, rarer language groups (Table 5-3). Many respondents had low literacy in Bengali, thus interviewees were often evasive regarding their reading of the PIS, suggesting that this was an area of sensitivity. Just 7 (41%) of 17 Bangladeshi respondents who gave direct answers claimed to have read the leaflet (compared with 15/16 (93%) of non-Bangladeshis); a further 7 said they had not and 3 said that other family members had read it for them. Box 5-4 shows comments made about the PIS. The length and detail of the PIS appeared to discourage reading in some (mainly Bangladeshi) respondents, placing the emphasis more firmly on personal interaction with researchers for communication of trial information.

BOX 5-4 - COMMENTS ABOUT THE PATIENT INFORMATION SHEET

Lots of pages. Yeah, little bit I read…He explained me nicely that time. I understand what he's saying but I can't tell you now. I can't remember all of it. (F-Bangladeshi)

Some of the first page and second page we did and then we was happy with this. We read we are so happy some of the paragraphs is very nice but it carried on and on. (M- Bangladeshi)

That time I was very busy and I don't have time to read it, and when I had time I forgot. (F-Bangladeshi)

I understood what she explained so I didn't really bother to read that much. (F-Bangladeshi)

Yes of course, I read everything. Erm, I did read it, I could have read more, erm but I am one of the people who reads everything. I am used to reading complicated stuff in my work anyway. (M-UK)

They were good explanatory, there was a lot of them but it's not the same as talking to somebody saying well look I'm worried about and then they'll they put me right. I had a better understanding and you can't ask a question on a bit of paper. (F-UK)

It told me everything I need to know to be able to start the trial. (F-Black UK)

Er....can't remember. Something. I have to think... because it was a long time ago. (F-Egyptian)

I read it, well both myself and my partner read it and we did find it like yeah it was absolutely fine for us. (F-Caribbean)

I read it….Just first the introduction, the introduction this research. (M-Chinese)

5.4.2 Understanding the research process

Just over a third of parents understood the principle of randomisation to some degree (5/20 Bangladeshi, 7/10 White UK, 3/12 Others)(Box 5-5). Bangladeshi families were least aware

that a DNA sample had been taken from their child (6/20 Bangladeshi were aware, 9/10 UK, 9/12 Others). Despite poor comprehension of personalised medicine and genetic testing concepts most respondents viewed the genetic component of the study positively.

BOX 5-5 - UNDERSTANDING AND ACCEPTANCE OF RANDOMISATION AND GENETIC STRATIFICATION

Randomisation

I totally don't know if the powder is the ..er..blank one (M-Chinese)

Well they said they were going to test so many people with this and so many people with that and then get the results and see what. (F-Bangladeshi)

We are in a trial and we could be given a placebo or cure and that's done on a group of kids. (M-Middle Eastern)

Yes, so I could have a treatment that is sherbet in other words. (F-Black UK)

DNA component

They did tell me [its purpose] at the time but I really can't remember. (F-Bangladeshi)

They told me, eh, I can't remember, sorry. (F-African)

It's only if it didn't hurt him, it was only a swab from his mouth so no, that was fine. (F-UK)

As long as it's not invasive (F-Egyptian)

I'm sure it's only used for the medical and not generally. I think the only time it would be concerning is like I said if they were going to share the information. (F-Bangladeshi)

I haven't really thought about it. It's just part of the one part of the study that needs to be that they're looking at. I don't think there's anything sinister being done. Everyone's going to end up on a DNA database somewhere. (F-UK)

5.4.3 Consulting others

There were some differences between ethnic groups in how decisions were made to enrol their child in the trial. Some decided to consent as soon as they were approached but others sought advice from other people. Some (4/20) Bangladeshi respondents reported that they relied entirely on the medical profession to guide them but they were the only group to express this. Some non-Bangladeshi respondents were able to call upon medically qualified family members for advice, or made use of the Internet and other sources of pharmaceutical information. Very few respondents (3/42), all of White UK ethnicity, reported receiving negative views about the trial from family or friends. Box 5-6 shows relevant extracts.

BOX 5-6 - OTHER SOURCES OF INFORMATION AND REASSURANCE

It's from hospital obvious it's good for him. He…the doctor knows better than us. (F-Bangladeshi)

I told him [my husband] and he said OK if you want to go you can go. (F-Bangladeshi)

I looked at the internet I think……where I work we've got an old copy of the BNF so I looked at that. (M-UK)

I was pretty certain I think.. but his dad was a bit more reluctant because he's sort of thought it was a trial medicine.... and then when I explained that montelukast was already a drug... and if he has asthma and it gets progressively worse, there's a good chance it will be prescribed anyway, so.., (F-UK)

5.5 Qualitative study summary

From this study, Bangladeshi families appear particularly motivated to participate in clinical trials despite understanding of study concepts being limited by educational attainment or language. The decision to participate was driven primarily by rapport with the researcher, with quality of study literature being of less importance. Where a study population has a Bangladeshi (or perhaps South Asian) bias particular emphasis should be placed on face-to-face verbal explanation of trial concepts and procedures. Further detail regarding qualitative study outcomes is available via open access online(181).

6 DISCUSSION AND OVERALL CONCLUSIONS

6.1 Discussion of study design

Study design reflects previous work in this area. Short of meta-analysis, an adequately powered double blind placebo controlled randomised controlled trial is the gold standard for assessing therapeutic efficacy. The unique aspect of this study was the attempt not only to assess whether intermittent montelukast was effective in preschool wheeze, but also to investigate whether genetic mutations affecting the synthesis of the cysteinyl leukotrienes (the endogenous ligand for its target receptor) influenced its efficacy. Previous retrospective studies have suggested a role for ALOX5 polymorphisms in leukotriene production, wheeze severity(143) and montelukast efficacy(130,139). However, this is the first study to prospectively test this association. Prospective genetic stratification was necessary to address this pharmacogenetic question in that randomisation within strata ensures a 50:50 montelukast:placebo split in each genotype group. Additionally, this approach effectively negates the impact of any confounding variables, even where these may segregate along genetic lines, such as biological or environmental traits associated with a particular ethnic group. Therefore, as a method to address the role of ALOX5 promoter polymorphism this approach is sound, however this narrow focus has limitations as a means to identify any alternative response predictor. The exploratory mechanistic aspects of the study to some extent mitigate these limitations; Firstly, LTE_4 is the final excretion product of cys-LT metabolism and would be expected to mirror *any* valid genetically-determined augmented montelukast response (ALOX5-related or otherwise) with a rise in excretion (assuming an effect mediated by increased cys-LT activation and not by altered montelukast metabolism), i.e. if ALOX5 genotype influences montelukast response it must do so by altered cys-LT activation at rest or during wheeze exacerbation. Secondly, the exploration of plausible SNPs in previously implicated genes permits hypothesis generation as regards alternative responsive phenotypes, while measurement of other eicosanoid mediators against the primary outcome may identify alternative markers of montelukast response, or perhaps even novel therapeutic targets.

The lack of montelukast effect in this study may be a true depiction of drug efficacy, or it may reflect flaws in trial design and conduct. There follows a critique of the study with consideration of its limitations.

6.1.1 Selection of study population

There exists a fundamental conflict between pragmatism in population selection, which lends itself to broader applicability of study results, and more focused inclusion criteria, which may increase the likelihood that an effect is detected, assuming an accurate a priori hypothesis on treatment responsive phenotype. Bacharier and Robertson both purged their studies of

potential bronchiolitics by excluding children under 2; *if* montelukast is ineffective in bronchiolitis(182) then the current study may be confounded by inclusion of infants and younger pre-schoolers. The study population was relatively healthy in comparison to previous cohorts; the Preempt study stipulated a higher frequency of wheeze exacerbations prior to enrolment, requiring a minimum of three unscheduled medical attendances for study inclusion(39). Whilst more relaxed severity criteria facilitate recruitment and broaden study applicability, a higher baseline USMA rate reduces the potential for a type 2 error in determining montelukast efficacy. The current study required two previous wheezing episodes, with only one episode medically confirmed; with hindsight, stricter severity criteria for study inclusion may have been a wiser study design, although recruitment might have been compromised. Earlier work shows augmented cys-LT activity in atopic preschool wheezers during exacerbation(38,41), hence limiting eligibility to those with elevated mAPI and/or elevated $uLTE_4$ while wheezing *might* increase probability or magnitude of a montelukast effect. The hypothesis that ALOX5 promoter polymorphism would determine montelukast response derived from Lima *et al*., who saw a 73% reduction in exacerbations in adults with variant promoter polymorphism copy numbers(130). Despite this, it is possible that other cys-LT pathway variants (such as FLAP(163,183) or the coactosin-like protein (132) or perhaps ALOX5 promoter methylation(136,184)) might have greater influence on montelukast effect and perhaps be more valid stratification criteria than ALOX5 genotype.

6.1.2 Intervention

A valid montelukast effect may have been missed for a number of reasons regarding IMP administration.

Firstly, while dosing regimen reflects the SPC(124), based on data indicating plasma montelukast concentrations higher than those seen in adults in recommended doses, it remains possible that the accepted paediatric dosing strategy is suboptimal, or perhaps that genetic variants affecting pharmacodynamics (185) might influence validity of dosing advice.

Secondly, patients should be advised to avoid ingestion of citrus in the temporal vicinity of montelukast administration, as this blanket advice could usefully apply regardless of SLCO2B1 genotype(186).

Subjects were advised to commence IMP at the start of a viral cold or wheezing episode. The signs and symptoms that indicate an impending viral wheeze episodes vary between subjects(187), as does the threshold at which parents will initiate treatment. These variations may act at random, or they may segregate with genes influencing montelukast efficacy or wheeze severity. They may be universal to all subjects, but if so, the effect may to cause an

undue delay in initiation of treatment, negating any effect achievable by prompt cys-LT blockade.

Compliance was indirectly and incompletely estimated, reliant as it was on parental report rather than dose counting devices, or accurate packaging returns. It is to be hoped that active and placebo IMP were sufficiently similar to preclude differential compliance, but it must also be recognised that although there was no evidence of discrepant adverse events(Table 3-9), the documented adverse effect profile of montelukast(124) has potential to skew compliance.

Subjects were not limited in their use of concomitant medications. Placebo-treated patients appear to have had greater recourse to oral corticosteroids (an accepted primary outcome in other similar studies), a potential confounding effect that may have diluted any increase in USMA. This should have been accounted for in the definition of the outcome measures and expected effect sizes.

6.1.3 Mechanistic investigations

A role of ALOX5 promoter sequence polymorphism on cys-LT activity (and LTRA response) has been suspected since In *et al*. found reduced ALOX5 mRNA transcription with non-wild 5/5 copy numbers in vitro(134) however, the direction of effect has differed with study design and population and may be ethnically divergent with confounding interactions with other loci.

6.1.3.1 Stratification genotypes

The current study was stratified by ALOX5 promoter polymorphism in keeping with Lima *et al*. who found a 73% reduction in exacerbation risk in montelukast-treated adult subjects not homozygous for the wild type 5 copies of the ALOX5 promoter sequence(130). Mougey *et al*.(143) found a similar direction of effect in school-aged children, who had greater asthma-related morbidity but poorer lung function with non-5/5 copy number alleles. While it was hoped that such a large effect might be replicable in preschool children, alternative stratification might usefully have been considered since Telleria *et al*. found a contrary effect, with 5/5 and 5/x copy numbers conferring superior montelukast response(139), as did Drazen *et al*.(188). These contradictory findings might reflect a complex interaction with other ethnogenetic or environmental factors, and a pilot study within this population, perhaps incorporating uLTE4 estimation as a proxy for probability of efficacy (spirometry is unreliable at this age, while USMA frequency is too low for a pilot outcome) could have given specific evidence to support or refute the (contradicted) study hypothesis (that efficacy would be greater in the variant copy number stratum [5/x + x/y]).

6.1.3.2 Leukotriene hypothesis

It was hypothesized that (regardless of relationship with ALOX5 promoter copy number) ALOX5 activity, cys-LT production (assessed by baseline [$uLTE_4$]), disease severity, and montelukast response would co-segregate, but the current study found highest cys-LT levels in the genotype group that had poorest montelukast response (Figure 4-1). This apparent inconsistency mirrors that seen by Kalayci *et al*., where increased ALOX5 activity and cys-LT synthesis was found in wild type subjects, but with paradoxically milder disease(189). These observations challenge assumptions around the relationship between baseline cys-LT activity and disease severity; the observed discordance between cys-LT production and symptom severity may indicate that baseline cys-LT levels are actually protective, that they do not predict exacerbation-related cys-LT rise, that cys-LT activity affects wheezing severity differently in different ages or ethnic groups, or that population pathophysiologic heterogeneity allows for severe disease unrelated to cys-LT activity, and thus to montelukast response.

It has been suggested that ALOX5 Sp1 binding motif copy number might influence cys-LT activity (and perhaps therefore montelukast response) in a dose dependent fashion. To our knowledge there is no evidence that this is the case, and in any case stratification at this resolution would require a much larger and more complex trial design. Previous studies assessing effects of ALOX5 promoter Sp1-binding sequence repeat number have employed broad categories, such as 5/5 homozygotes (wild type) vs other, or those possessing one or more wild type allele [5/5 + 5/x] vs those with none. Analysis or stratification of therapeutic response, [$uLTE_4$], or wheeze severity by narrower allele categories is compromised by very low minor allele frequencies (Table 4-9).

6.1.4 Statistical/analytical issues

The study was limited in that, although adequately powered to address the efficacy of intermittent montelukast in preschool wheeze, it had the power to detect only a rather substantial interaction between genotype and efficacy. As such, the suggestion (P=0.01) of differential efficacy in the 5/5 stratum is not mathematically robust when exposed to a test for interaction (P=0.08, Table 3-6) as per the pre-specified analysis; that is, while the apparently significant P-value in the 5/5 stratum would be valid in a standalone study, the more appropriate means to test for differential effectiveness is via a specific test for interaction, which was negative. This issue is discussed in more detail by Wang *et al*. in a 2007 report(190). The interquartile range for time to first unscheduled medical attendance (USMA) was not calculable as less than 75% of children had an USMA.

6.2 Primary outcome

This study is overall negative for the primary outcome, indicating no benefit from intermittent montelukast in preschool children with wheeze. This supports the recent findings of Valovirta *et al*.(129), who compared intermittent and regular montelukast with placebo in a large, well executed study and found no benefit. In the most recent published study Nagao *et al*. found a benefit of regular montelukast in preschool wheeze, however this study had marked limitations, including small size, open label design, and restrictive exclusion criteria; it cannot be construed to influence the debate on the role of montelukast and is mentioned for purposes of completeness only(191). There was an increased time to first USMA requiring hospital admission for wheeze in the montelukast group (but not for other types of USMA), and an increased use of rescue oral corticosteroids (Table 3-8), however the study was not powered to demonstrate these effects, and the patchiness of the effect makes its validity questionable. The decreased recourse to oral steroids in the montelukast-treated population *may* indicate a genuine montelukast effect (OCS requirement is a recognised primary outcome in its own right (77)) however the discrepant use of OCS between groups may also have had a confounding effect by reducing the USMA rate in the placebo group, thus attenuating the differential in the primary outcome. There was no apparent influence of wheeze phenotype, use of inhaled steroids at baseline, or alternative genotype stratum on USMA (Table 3-7). Important caveats to these observations are firstly that wheeze phenotype was based on parental reporting which is retrospective, subjective(192) and subject to recall bias (though this was not prominent in a review of data from the COPSAC birth cohort(193)); secondly that preschool children are known to move between phenotypes (and back again) with time(15), and that inhaled steroid usage was assessed in a strictly binary fashion, mean historic and concurrent daily dose of inhaled steroids was not assessed in this study. The incidence rate ratio seen in the montelukast group compared with placebo was 0.88 (P=0.06) in favour of montelukast, not meeting statistical significance. A larger trial might have power to identify a difference of this magnitude but the marginal clinical benefit may not justify the exercise, this should be considered in the design of future studies.

6.2.1 Systematic review of primary outcome

In addition to the basic review of literature presented in the opening chapter, I wished to formally compare the primary outcome of this study to existing reports, and thus undertook a systematic review of previously published data based on that recently described by Ducharme *et al.*(194). I searched MEDLINE®, Current Contents, PubMed and the Cochrane Library. The search terms used were ‘wheez* or asthm*’, ‘preschool or pre-school’, ‘randomised’ or ‘randomized’ or ‘randomly’ or ‘trial’, ‘leukotriene*’ or ‘anti-leukotriene’ or ‘antileukotriene’ or ‘montelukast’. In addition, ‘viral wheeze’ or ‘viral-wheeze’, ‘young children’ and ‘infant’, ‘intermittent’, ‘pre-emptive’, ‘preemptive’ were included. Trials selected used a

similar methodology to that reported in this study; a placebo-controlled design to assess intermittent montelukast with respect to unscheduled medical attendances for wheezing in preschool children over a 12-month follow-up. Three studies were selected for the combined analysis; Robertson *et al.*(2007)(39), Valovirta *et al.*(2011)(129) and Bacharier *et al.*(2008)(195). In addition, I searched EMBASE, SCOPUS, MEDLINE and the Cochrane Airways Group trials register for details of trials that may have been published following the review by Ducharme *et al.*(194). No further studies were identified.

The published reports were reviewed for details on pre-specified outcomes. The study by Robertson *et al.* (129) reported unscheduled healthcare resource utilizations in the group of children who received at least one episode of treatment as a primary outcome, in children between 2-14 years of age. Raw data from this study were provided by personal communication, from which we calculated the mean and standard deviation for the number of episodes requiring an unscheduled medical attendance for each child, within the subgroup of children aged 2-5 years. The study by Bacharier *et al.*(195) enrolled children aged 1-6 years, with number of unscheduled visits to primary care offices, urgent care or emergency departments and hospitalisations for acute wheezing episodes as a secondary outcome. The mean and standard deviation for these episodes was published in their report, additionally the number of children with one or more events for this outcome was confirmed by personal correspondence. Original data was not received from the study of Valovirta *et al.*(129). They included children aged 6 months - 5 years, and reported a secondary outcome for "adjusted rate of asthma attacks", with an attack defined within the statement *"The start of an asthma attack was the first day the patient's symptoms required HRU"*. Overall, the three studies were comparable in terms of study design (placebo-controlled trial), randomisation, concealment of allocation, inclusion criteria and duration of follow-up (12 months) to those in the current trial (Table 6-1).

A meta-analysis was performed using the inverse variance fixed effect method to calculate the summary weighted risk ratio with 95% confidence intervals. The analysis was performed using RevMan™ version 5.3 (196). We compared the mean and standard deviation for number of episodes requiring an unscheduled medical attention per child in each study, comparing experimental (intermittent montelukast) and control (placebo) groups, using a fixed effects model for mean difference as per the review by Ducharme *et al.*(194). The overall analysis of 2783 preschool children shows no overall benefit for intermittent montelukast therapy in reducing the need for unscheduled medical attention for a wheezing episode (weighted mean difference (WMD) -0.10, 95% CI -0.26 to -0.06, p=0.21, Figure 6-1). In the absence of original data from Valovirta *et al.*(129) we conducted a sensitivity analysis excluding the results from this study. This resulted in a change to the overall conclusion of

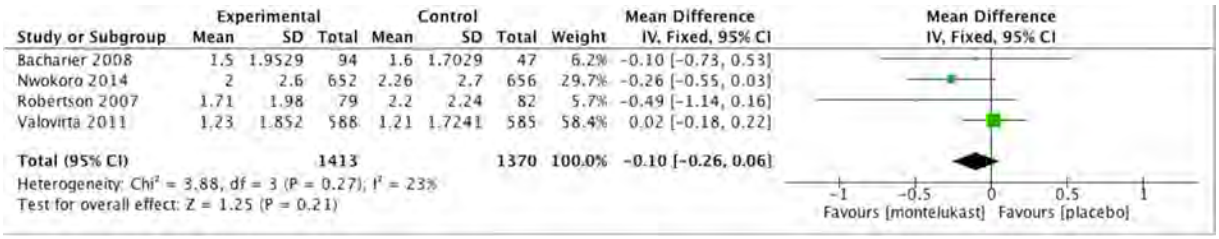
the analysis, showing a small favourable effect for montelukast (WMD -0.27, 95%CI -0.51 to -0.03, p=0.03, data not shown). This discrepancy reflects the inadequate number of studies addressing this issue, Valovirta is the largest relevant trial to date and was robustly negative (although post hoc analysis suggested a montelukast effect in the subgroup aged 2-5y), and in the context of such a relatively small combined population has potential to alter overall conclusions. It is also important to consider this influence in the light of the discussion regarding the effects of population ethnic heterogeneity raised later in section 6.3.

TABLE 6-1 - ADDITIONAL STUDIES INCLUDED IN SYSTEMATIC REVIEW

Study	Inclusion criteria	Design	Follow-up	Data provided	Outcome	Outcome definition
Bacharier *et al.*2008	Age 12-59 months 2 or more episodes of wheeze with RTI in past year	3 arm double-blind RCT. 7d intermittent montelukast 4mg od po vs. budesonide vs. placebo. Parent-initiated therapy.	12 months	Yes - for montelukast and placebo subgroups	Secondary outcome: number of unscheduled visits for acute wheezing episodes	Visit to primary care office, urgent care, ED or hospitalisation
Robertson *et al.*2007	Age 2-14 years Doctor diagnosed intermittent asthma Between 3-6 exacerbations in past 12 months	2 arm double blind RCT. 7-20d (as needed) montelukast 4mg od po (2-5 yr subgroup) vs. placebo. Parent-initiated therapy.	12 months	Yes - full data set provided. Analysis of 2-5 years subgroup performed.	Primary outcome: unscheduled health care resource utilisation (HRU)	Unscheduled visit to GP, specialist Paediatrician, ED or admission to hospital specific for asthma
Valovirta *et al.*2011	Age 0.5-5 years episodes of asthma symptoms in past 12 months: 2-4 if under 2 years, 3-6 if over 2 years, at least 1 episode in previous 6 months	3 arm double-blind RCT. 12 days montelukast vs. daily montelukast vs. placebo. Parent-initiated based on symptom calendar.	12 months	No	Secondary outcome: adjusted rate for number of asthma attacks	Start of an asthma attack defined as the first day the patient's symptoms required an HRU - only one attack was counted per "episode"

RTI; respiratory tract infection, RCT; randomised controlled trial, ED; emergency department, GP; General Practitioner, HRU; healthcare resource utilisation

FIGURE 6-1 - MEAN GROUP DIFFERENCE IN NEED FOR USMA FOR PRESCHOOL WHEEZE



Mean group difference of children requiring unscheduled medical attention for pre-school wheezing. Mean group difference (fixed effects model) for the number of exacerbations of pre-school wheeze, defined by need for unscheduled medical attention, experienced by children comparing intermittent montelukast therapy with placebo. The width of the horizontal line indicates the 95% CI around the point estimate, and the area of the point estimate (square) is a representation of the relative weight of the study in the overall analysis. The pooled summary (diamond) represents the overall result, with the vertical line representing no overall effect (RR=1.0).

6.2.2 Subsequent reviews

Kaiser *et al*. conducted a wide-ranging review (77) of treatment options for recurrent preschool wheeze, but did not include studies comparing montelukast with placebo, preferring to focus on intermittent or continuous inhaled corticosteroid as the main comparator. In addition, the review was limited to studies in which the need for rescue oral corticosteroids was an outcome. From this study the overwhelming body of existing evidence continues to favour intermittent and regular inhaled corticosteroids over montelukast or placebo in this age group, however the findings are limited by the narrowness of the evidence considered, which excluded the current study and indeed cited only one (Bacharier, 2008) of the four studies quoted in our review.

The 2015 Cochrane review by Brodlie *et al*. looked specifically at maintenance and intermittent LTRA in episodic viral preschool wheeze, rather than the broader population featured in the current study. The focus on LTRA rather than ICS makes it a necessary complement to Kaiser *et al*. This phenotype stratified approach included data from Bisgaard *et al*. (published and unpublished) as well as the studies from our review, but the overall outcome was unchanged, with no evidence to support the routine use of intermittent or indeed maintenance montelukast in preschool (episodic viral) wheezing children(127).

A recent review by Hussein *et al*. pertinent to the role of montelukast in preschool wheeze reprised our study, also including the 2005 work by Bisgaard *et al*. (126)(excluded from our review due to use of regular rather than intermittent montelukast). Their analysis of intermittent montelukast against USMA did not identify any additional studies beyond those in our review, and thus supported our conclusion that overall montelukast is not an effective treatment for preschool wheeze. They call for future studies to be powered to identify responsive subgroups should such exist(128). Closer analysis hints at differential response in those children aged 24-59 months(39,125,126), with studies (including Nwokoro *et al*.)

including younger children more likely to be negative(129,195,197), perhaps indicating a reduced efficacy in bronchiolitis compared with classical preschool wheeze.

6.2.3 Interpretation of primary outcome results

Despite the repeated unfavourable outcomes of reviews into the role of montelukast in preschool wheeze, mechanistic, anecdotal and clinical trial evidence supporting a treatment effect persists. One randomised trial reports that intermittent montelukast in preschool wheeze is efficacious(195), while another reports no benefit(129). An additional trial (albeit in older subjects) had too few wheeze episodes to inform clinical practice(139). The current study sought to determine the efficacy of intermittent montelukast in preschool wheeze using need for unscheduled medical attendances for wheezing as the primary outcome. We speculate that inconsistent trial data to date are due, in part, to marked heterogeneity in response to montelukast. The implied montelukast-responsive subgroup has yet to be identified, but trial stratification according to candidate response markers (be they genetic, biochemical, physiologic or clinical) is a potential method by which to do so. The recent work by Fitzpatrick *et al*. informs both treatment choice and the design of future trials; employing a blinded three way crossover design they compared response to LTRA, daily ICS and intermittent ICS in preschool wheezing, identifying a significantly better response to daily ICS than to the other options. Post hoc interrogation of the data according to prespecified potential determinants of differential response indicated that aeroallergen sensitization and peripheral serum eosinophilia (in keeping with the mAPI(14)) increased the likelihood of preferential response to ICS(198). These readily measurable phenotypic traits, if validated in appropriately stratified prospective trials, might support parents and clinicians in PSW treatment decisions.

6.3 ALOX5 promoter polymorphism effect

Since studies in adults report that copy numbers of the GGGCGG Sp1-binding motif in the arachidonate 5-lipoxygenase (ALOX5) gene promoter (either 5/5, 5/x, or x/y, where x and y ≠ 5) are associated with heterogeneity in montelukast response(130,139), we stratified the trial by 5/5 and [5/x + x/y] genotype. In doing so we found that montelukast is not superior to placebo in an unselected preschool wheezing population, but that the data *hint* at improved response to intermittent (as required) therapy in children with the 5/5 genotype. The direction of this possible response was contrary to Lima's finding(130) but consistent with Telleria(130,139), however the test for genotype:efficacy interaction was not confirmatory (Table 3-6, Figure 3-2) and a larger or targeted study limited to 5-repeat homozygotes may have greater power to clarify this issue. Of note, in our study and others there was a higher proportion of x/y heterozygotes (and fewer 5/5 homozygotes) in black subjects than in other groups, and this, in conjunction with the white European ethnic preponderance may have influenced the primary outcome result, as a relative bias towards white children (and thus

wild type ALOX5 promoter) might be expected to favour an overall negative result (if the study hypothesis was correct). Comparison of population allele frequencies, however, shows highly variant x/y%; e.g. 14.8% Mougey(143), 19.7% Telleria(139), 9.6% Lima(130) compared to 4.5% in the current study(197), thus if ALOX5 promoter is of genuine influence then study comparisons (there have been a number of systematic reviews) should take this variation into account. Variant alleles are more common in those of non-white European subjects (Table 4-9), thus consideration should be given to other (unrecognised?) ethnically divergent confounders, which may be both genetic and environmental.

6.4 Exploratory SNP analysis

In addition to exploration of the previously noted $uLTE_4$ preschool wheeze association, we performed exploratory assessment of a panel of eicosanoid markers present in urine and of putative genetic markers implicated in the eicosanoid mediator pathway. The aim was to identify potential therapeutic targets or predictors of response phenotype. The analysis of multiple SNPs against multiple biomarkers left the study open to multiple testing errors, and we were obliged to account for this by using adjusted p-value thresholds to reduce the false discovery rate. With this in mind, the only SNPs to yield results of interest ($P<0.0034$) were rs12422149 (SLCO2B1) and rs2516564 (LTB4R2).

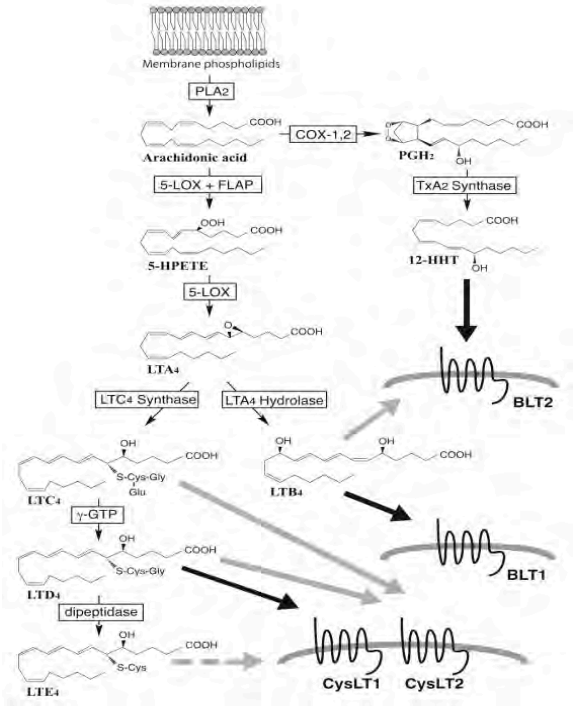
6.4.1 Significance of rs12422149 (SLCO2B1)

rs12422149 (935G>A) is a non-synonymous variant in gene SLCO2B1 encoding a change from arginine to glutamine at position 312 in OATP2B1. OATP2B1 is an organic anion transporter implicated in the pharmacokinetics of montelukast. Mougey *et al*. (175) reported that the presence of the minor allele at this locus was associated with reduced plasma montelukast concentrations and asthma symptom utility index scores after 1 month and 6 months of montelukast treatment, and that co-administration of montelukast with citrus juice reduced concentrations (via flavonoid glycoside-induced suppression of OATP2B1-mediated absorption, not increased clearance/metabolism) in GG homozygotes (major allele) but not in AG heterozygotes(heterozygous minor allele)(186). Conversely, neither Tapaninen *et al*. nor Kim *et al*. found any influence of this polymorphism on montelukast pharmacokinetics (185,199). We found that children with the minor allele had higher USMA if receiving placebo, indicating a baseline (treatment-independent) effect of rs12422149 that is not predicted from previous reports of OATP2B1 function. To date there is no mechanistic connection between SLCO2B1 variants and baseline wheeze susceptibility, thus this effect may be spurious (plausible given the small numbers with the minor allele in this study), it may suggest a role for OATP2B1 in transport of a putative endogenous mediator, or perhaps SLCO2B1 is in linkage disequilibrium with and thus is a marker for a gene with a role in preschool wheezing susceptibility.

6.4.2 Significance of rs2516564 (LTB4R2)

rs2516564 is a 5' UTR variant in LTB4R2 (BLT2) on chromosome 14. LTB4R2 encodes Leukotriene B_4 receptor 2, initially recognised as a relatively low affinity G-protein coupled receptor for the eicosanoid ligand leukotriene B_4, but subsequently found to have stronger affinity and selectivity for other arachidonic acid metabolites, specifically 12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid (12-HHT), which itself does not bind to LTB4R1 (BLT1). This suggests that the primary role of LTB4R2 lies outwith the LTB_4 pathway. LTB_4 binds with high affinity to LTB4R1, exerting pro-inflammatory effects including promotion of neutrophil chemotaxis and survival. LTB_4 is elevated in sputum, BAL fluid, blood and exhaled breath condensate derived from asthmatic subjects. LTB4R1 -/- mice exhibit diminished airway hyperresponsiveness, pulmonary inflammation and mucus secretion after allergen sensitization and challenge compared with wild type mice, and increased density of LTB4R1 positive CD8+ T cells are seen in the BAL fluid of asthmatic human subjects(200). The primary ligand for Leukotriene B_4 receptor 2 is 12-HHT, derived from arachidonic acid via the Cyclooxygenase:Thromboxane A2 synthase route (Figure 6-2). While LTB_4 appears pro-inflammatory in the lung, stimulation of LTB4R2 by either LTB_4 or more potently 12-HHT has mixed effects; LTB4R2 on mast cells mediates their recruitment to sites of tissue inflammation, whereas work in a mouse model suggests anti-inflammatory effects(200).

FIGURE 6-2 - SYNTHESIS AND RECEPTOR TARGETS OF LEUKOTRIENE B_4 AND 12-HHT



Reproduced with permission from Yokomizo and Liu(232)

A common model for allergic airways disease is the ovalbumin-sensitized mouse. BAL fluid 12-HHT levels increase in response to ovalbumin challenge, associated with increased ovalbumin-specific IgE, airway hyperresponsiveness, airway cytokine release (IL-4, 5 and 13) and eosinophilia. In LTB4R2 -/- mice the ovalbumin-induced IL-13 response is enhanced, with no impact on IL-4, IL-5, IFN-γ or serum specific IgE; this suggests that LTBR42 activation in some way constitutively suppresses IL-13 mediated allergic inflammation(201). In the current study, placebo-treated children homozygous for the recessive allele at rs12422149 on LTB4R2 recorded increased USMA during follow-up as compared to dominant allele homozygotes, and the work of Watanabe *et al*.(200) and Matsunaga *et al*.(201) provides a mechanism for this observation. Given the close proximity of LTB4R1 and LTB4R2 on chromosome 14 it is also possible that the latter

exerts an inhibitory effect on the former, that rs2516564 tags for a functional SNP within LTB4R1, or perhaps that variant LTB4R2 indirectly influences substrate flux down the ALOX5 or COX routes, with consequent effects on cys-LT activity and montelukast response.

6.5 Urinary eicosanoid observations

6.5.1 Eicosanoids in normal children

In the 71 non-wheezing control subjects atopic status did not appear to influence urinary eicosanoid levels, but there was evidence of a negative age correlation with all metabolites (Table 4-3, Figure 4-2)(202). The clinical significance of this is unclear, but in this small sample it is a consistent effect and may possibly reflect declining levels of exposure or inflammatory response to minor (often viral) stimuli, or perhaps the gradual acquisition of immunological tolerance observed as childhood progresses. This highlights the importance of acquiring robust, population-specific, age-sensitive normative data on any putative childhood biomarker.

6.5.2 Urinary LTE_4 in preschool wheezing children

Montelukast functions through competitive inhibition of cys-LT action at cys-LTR1, and leukotriene activation has been associated with preschool wheezing illness (38,41). As such, elevated cys-LT levels (indicated by urinary LTE_4) either at baseline or during exacerbation might associate with montelukast response. With the exception of possible effects on receptor number or function or montelukast pharmacokinetics, any genetic or clinical responsiveness factor is likely to be mediated via increased leukotriene activation, either through enhanced production or reduced degradation. We therefore measured $uLTE_4$ in trial subjects at baseline and during exacerbation (where possible). Baseline $uLTE_4$ was elevated in subjects without a 5-repeat allele in the ALOX5 promoter polymorphism (Figure 4-1), in keeping with the both the findings of Mougey *et al*. in older children(143) and the *a priori* hypothesis inferred from Lima *et al*.(130), but contrary to the direction of effect predicted by the observed non-significant gene-treatment interaction (Table 3-6, a tendency to increased response in 5/5 subjects). Such a small and mechanistically inconsistent effect could not reliably influence the targeting of treatment without robust replication in further trials with corroborating clinical outcome measures. Although (in keeping with previous studies(38,41)) uLTE4 was elevated during wheeze exacerbation, there was no association observed between $uLTE_4$ measured during exacerbation and ALOX5 genotype (Figure 4-7), and no interaction with urinary cotinine measured on the same sample (not shown). At the time of writing ALOX5 promoter genotype has no role in predicting montelukast response in preschool wheeze either alone or in combination with baseline or exacerbation $uLTE_4$.

6.5.3 Urinary tetranor PgD-M

The negative age correlation observed in non-wheezing controls was replicated in the larger preschool wheezing population. There was no association with urinary cotinine, despite 17% (162/949) of subjects' parents reporting in utero smoke exposure and 27% (258/949) admitting household passive tobacco smoke exposure. There is no data on household and in utero exposure in the control population. Urinary Tetranor PgD-M was elevated in preschool wheezing children and reduced in those receiving regular inhaled corticosteroids.
9α-hydrox-11,15-dioxo-2,3,4,5-tetranorprostan-1,20-dioic acid (Tetranor PgD-M) is a major metabolite of prostaglandin D_2 (PgD_2), with evidence that urinary excretion accurately reflects in vivo biosynthesis(91), and that urinary levels associate specifically with airway biosynthesis(203). PgD_2 is recognised as a pro-inflammatory mediator in adult asthma, as indicated in section 1.3.2.2, and urinary tetranor-PgD-M is elevated in stable adult asthmatics with intermittent symptoms(203). The reduction in tetranor PgD-M noted in children receiving maintenance ICS combined with the advent of orally available antagonists of PgÖD2 action with clinical bronchodilator efficacy in adults(92), lend both credence and clinical significance to this observation.

Unlike $uLTE_4$, in this sample tetranor PgD-M did not increase during wheeze exacerbations. If, then, PgD_2 and cys-LTs are implicated (as seems plausible from the available evidence) in preschool wheeze pathophysiology, then one hypothesis involves constitutive elevation of airway PgD_2 (from presumed mucosal mast cell abundance) providing inflammatory priming in pathologic synergy with (virus-)triggered intermittent increased cys-LT activation. This mechanism is supported by rodent work (204,205) showing eosinophilic airway inflammation in response to intratracheal PgD_2 or dsDNA (mimicking viral replication) which leads to increased PgD_2 and consequent airway inflammation, by Malmström *et al*. who found that mucosal mast cell density (mast cells are the primary source of PgD_2) in infancy correlated with preschool wheeze at age 3(21), and by Brannan *et al*., who abrogated mannitol induced bronchoconstriction in older asthmatics through formoterol or cromoglicate-induced mast cell stabilisation ((evidenced by reduced PgD_2 metabolite excretion(206)). Xue *et al*.(207) provide a detailed discussion of the mechanisms of the postulated PgD_2:cys-LT synergy. This could go some way to explain the imperfect and complementary roles of ICS and LTRA in preschool wheeze, with the relative importance of each varying with individual pathophysiologic bias.

6.6 The role of arachidonic acid 5-lipoxygenase promoter genotype in montelukast responsiveness in wheezing preschool children - a summary

The search for an effective therapy for preschool wheezing illness is hampered by the lack of a clearly defined phenotype with robust biomarkers. This study espoused a pragmatic

approach, recruiting a heterogeneous population encompassing several aetiologies, in the hope that inhibition of leukotriene activity might address a mechanistic pathway common to these probably distinct but overlapping clinical entities. There is evidence to implicate the cysteinyl leukotrienes in a proportion of preschool wheezing disease(38,41) and a greater success in assessing $uLTE_4$ during exacerbation (as opposed to at baseline) might have shed light on the validity of this hypothesis and thus the viability of montelukast as a therapeutic target. The lack of a clear ALOX5:$uLTE_4$ correlation may reflect a lesser than anticipated importance of ALOX5 promoter polymorphism genotype, or perhaps that differences in $uLTE_4$ excretion become more significant during exacerbation compared to convalescence. The leukotriene pathway is complex, and it is possible that several mutations in combination(116,130,131,158), perhaps with an environmental(141) or epigenetic influence(208) play a more important role in determining leukotriene activity and montelukast response in this population than ALOX5 alone.

6.6.1 The role of montelukast in treatment of preschool wheeze

This study does not progress the debate on the role of montelukast in preschool wheeze. It cannot be recommended routinely for intermittent or even regular use, but given its accepted safety, tolerability, low cost and convenience, an "n of one" trial remains justifiable if one accepts the premise that one or more montelukast-responsive subgroups exists, could we but identify them. An important caveat to this approach is that a trial of withdrawal of montelukast therapy is essential after a suitable interval (3 months seems reasonable), to exclude type 1 error due to coincident spontaneous improvement in wheezing frequency.

6.6.2 Preschool wheeze treatment recommendations

Recent reviews by Brodlie(127), Kaiser(77) and Hussein(128) (discussed in Section 6.2.2) do not materially alter the recommendations from the 2008 ERS consensus(11). Castro-Rodriguez *et al*. recently conducted a more comprehensive review of the main therapeutic options in preschool wheeze(209). In summary, they reiterate the lack of evidence for either rescue oral corticosteroids or maintenance or intermittent montelukast in preschool wheeze, while allowing for a cautious therapeutic trial of LTRA given the acknowledged phenotypic heterogeneity; they support a role for daily ICS or intermittent high dose ICS (with no longterm impact on linear growth), and call for head-to-head trials of ICS vs LTRA(195,198), and also of LTRA vs LABA(67) as add-on therapy to maintenance ICS for preschool wheeze. Taken in the round the available evidence suggests that the anecdotal ubiquity of montelukast prescribing is not supported by proof of efficacy in most cases. Therefore, as per the 2014 ERS consensus statement update(10), the majority of preschool wheezing children with intermittent symptoms should be managed with as required SABA, with low-medium dose daily ICS added if maintenance therapy is required due to either frequency or severity of wheezing symptoms. This approach is broadly endorsed (with regional

caveats/variations) by the major recognised international guideline bodies, and seems unlikely to change without significant new evidence(65,210). In light of the uncertainty regarding ADRB2 polymorphisms SABA usage should be strictly "as required" and kept at a minimum and consideration should be given to a "LABA holiday" in children with poor control (perhaps with enhanced corticosteroid cover) while on maintenance LABA therapy.

6.7 Suggestions for future research

6.7.1 Montelukast

6.7.1.1 Cys-LT activity

Future work should aim to replicate the effect seen in the 5/5 stratum, i.e. to conduct a similar trial with ALOX5 promoter region 5 Sp1-binding repeat homozygosity a prerequisite for study entry. In the aftermath of the current study, and eager to keep the successful clinical trial team together if a 'sequel' were to prove imminent, our group submitted a funding proposal to the NIHR; this application was ultimately unsuccessful, and a copy is included in this work (Appendix 8.11). Given the observed increase in LTE_4 during wheezing attacks, consideration should be given to stratification of montelukast response trials by urinary LTE_4 levels measured during exacerbation (or perhaps measured following standardised airways challenge). A similar approach employing exhaled breath condensate(211) or sputum measurements(212) may give a more accurate reflection of airway-specific cys-LT activity, and may thus provide more effective trial stratification. The impact of potential confounders such as air pollution or tobacco smoke exposure on cys-LT activity (including epigenetic influence(208)) may also prove of interest.

6.7.1.2 Montelukast pharmacology

Alongside determinants of cys-LT activity, consideration should also be given to montelukast pharmacokinetics. OATP2B1 is an organic anion transporter encoded by gene SLCO2B1 and linked to montelukast concentration and symptomatic response(175). SLCO2B1 polymorphisms have been shown to segregate along ethnic lines(213), raising the distant possibility of more facile (but controversial) guides to treatment choice. We found that polymorphism rs12422149 in SLCO2B1 may influence baseline unscheduled medical attendances for wheeze. While a supporting mechanistic link between SLCO2B1 genotype and baseline wheeze frequency is not apparent, scrutiny of adjacent loci for mechanistically plausible SNPs in linkage disequilibrium with rs12422149 may yield therapeutic or phenotyping targets.

6.7.2 Novel cys-LT receptors

GPR99 and PY21Y should be fully characterised, with a view to trials stratified by receptor genotype, or the development of orally available antagonists.

6.7.3 Other leukotriene pathway genes

Other genes in the cys-LT pathway, such as ALOX5AP and LTA4 Hydrolase are potential therapeutic targets or response markers.

6.7.4 Genome wide association studies

Our speculative candidate gene approach to montelukast responsiveness in preschool wheeze has yielded results that are at best tenuous. GWA techniques are recognised as an increasingly affordable method to identify genotype:phenotype associations. Application to existing birth cohorts and associated biobank data can identify SNPs with greater reliability and replicability than other approaches. Examples pertinent to preschool wheeze include Bonnelykke *et al*.(214), which used Danish Biobank data to identify confirm previously recognised asthma susceptibility loci including GSDMB, IL33, RAD50 and IL1RL1 as well as the novel CDHR3 (encoding cadherin-related family member 3), a protein highly expressed in airway epithelium. The large numbers of genes under investigation necessitate P-values several orders of magnitude smaller than acceptable in conventional studies, a challenge that can be mitigated by studying larger sample sizes or by increasing phenotypic specificity, as in studies targeting preschool wheeze or steroid responsiveness(214,215) rather than asthma as an undifferentiated whole. A GWAS of a composite cohort with known montelukast response phenotype (perhaps comprising participants of the studies included in the various meta-analyses(127,128) previously cited) might therefore suggest candidate responsive subgroups, or lend epidemiological support to putative novel cys-LT receptors. In the long term this approach seems more likely to yield useful results than candidate gene alternatives.

6.7.5 Prostaglandin D_2 blockade

PgD_2 is implicated in wheezing disease, both mechanistically and through clinical trials data. Barnes *et al*. (92) established that blockade of CRTH2 resulted in increased pre-bronchodilator FEV-1 after a small 28-day crossover trial, with some suggestion of reduced eosinophilic inflammation, while Maher *et al.* showed cough fibre stimulation was mediated by PgD_2 in both *in vivo* and *in vitro* animal models(90). Tetranor PgD-M (11,15-dioxo-2,3,4,5-tetranorprostan-1,20-dioic acid), the main urinary metabolite of PgD_2(91), was elevated in preschool wheezing children in comparison with controls (Table 3-14, Figure 3-6). There was no influence of acute wheezing exacerbation, atopic status or urinary cotinine, but wheezers receiving inhaled steroids had lower tetranor PgD-M. Given that urinary tetranor PgD-M reflects PgD_2 activity, CRTH2 blockade or perhaps mast cell stabilization with sodium

cromoglicate may prove to be an effective treatment in preschool wheezing children; additionally, elevated baseline urinary PgD-M in preschool wheeze may indicate a highly responsive subpopulation of children analogous to that predicted with symptomatic urinary LTE_4 levels and montelukast response (6.7.1.1); a study stratified by baseline urinary tetranor PgD-M (or perhaps by CRTH2 receptor polymorphisms(216,217)) should be considered, using oral sodium cromoglicate until anti-CRTH2 agents suitable for childhood use become available(218).

6.7.6 Beyond montelukast

This work has demonstrated logically inconsistent relationships between ALOX5 promoter polymorphism status, montelukast efficacy and leukotriene production. The assumptions upon which the study hypotheses are predicated (that homozygous wild type ALOX5 promoter genotype associates with low cys-LT activity and poor montelukast response) are not consistently supported by this work or by the other published literature. While considerations of study population choice (atopic children and those over 2 may be better targets) and stratification strategy (the most appropriate genetic/biomarker/phenotypic stratification is unclear) may explain some of the discrepancies in study outcomes, taking the available evidence in its entirety the unavoidable conclusion is that montelukast does not have a role in the majority of children with preschool wheeze. As such, the utility of significant future investment in identification of the presumed (and by no means excluded) responsive subgroup must now be called into question, as the size of said group must be small, and the cost and therapeutic index of montelukast are such that the time-limited “n of one” trial (6.6.1) could be considered to be a more cost-effective and appropriate use of finite health science resources.

Moving on from montelukast, as it seems we must, treatment options in preschool wheeze remain limited. Since this study was completed the role of oral corticosteroid has been revisited, but the observed 3-hour reduction in length of hospital stay(219) must be weighed against a course of prednisolone in therapeutic efficacy, adverse effect, social and health economic terms. There remains no evidence that steroids can modify risk of recurrence, persistence or progression of symptoms except in specific viral aetiologies(220). Elsewhere macrolide antibiotics, with their acknowledged anti-inflammatory properties, have also shown early promise but have yet to cement a place in the wheezing armamentarium(221–223).

Perhaps the most exciting avenue of exploration for preschool wheeze therapy includes the emerging PgD_2 antagonists(92,218,224), particularly in the context of the observed elevation in PgD_2 metabolites in preschool wheezing children(225). Replication of the safety and efficacy observed in pilot studies may justify investment in large-scale paediatric trials.

7 REFERENCES

1. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. Clinical Practice Guideline: The Diagnosis, Management, and Prevention of Bronchiolitis. Pediatrics. 2014;134(5):e1474–502.
2. Kuehni CE, Davis A, Brooke AM, Silverman M. Are all wheezing disorders in very young (preschool) children increasing in prevalence? Lancet. 2001/06/19. 2001;357:1821–5.
3. Grigg J, Silverman M. Wheezing disorders in young children: one disease or several phenotypes? In: Frey U, Gerritsen J, editors. European Respiratory Monograph 37: Respiratory Diseases in Infants and Children. United Kingdom: European Respiratory Society Journals; p. 153–69.
4. Davies G, Paton JY, Beaton SJ, Young D, Lenney W. Children admitted with acute wheeze/asthma during November 1998-2005: a national UK audit. Arch Dis Child. 2008/05/24. 2008;93:952–8.
5. Stevens CA, Wesseldine LJ, Couriel JM, Dyer AJ, Osman LM, Silverman M. Parental education and guided self-management of asthma and wheezing in the pre-school child: a randomised controlled trial. Thorax. 2002/01/26. 2002;57:39–44.
6. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson children's respiratory study: 1980 to present. J Allergy Clin Immunol. 2003 Mar 11;111(4):661–75.
7. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and Wheezing in the First Six Years of Life. Vol. 332, New England Journal of Medicine. 1995. p. 133–8.
8. Spycher BD, Silverman M, Pescatore AM, Beardsmore CS, Kuehni CE. Comparison of phenotypes of childhood wheeze and cough in 2 independent cohorts. J Allergy Clin Immunol. 2013;132(5):1058–67.
9. Collins SA, Pike KC, Inskip HM, Godfrey KM, Roberts G, Holloway JW, et al. Validation of novel wheeze phenotypes using longitudinal airway function and atopic sensitization data in the first 6 years of life: Evidence from the Southampton Women's survey. Pediatr Pulmonol. 2013;48(7):683–92.
10. Brand PLP, Caudri D, Eber E, Gaillard EA, Garcia-Marcos L, Hedlin G, et al. Classification and pharmacological treatment of preschool wheezing: Changes since 2008. Eur Respir J. 2014 Apr 1;43(4):1172–7.
11. Brand PLP, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: An evidence-based approach. Eur Respir J. 2008;32(4):1096–110.
12. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, et al.

Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med. 2006 May;354(19):1985–97.

13. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med. 2000;162:1403–6.
14. Guilbert TW, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, et al. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. J Allergy Clin Immunol. 2004;114(6):1282–7.
15. Schultz A, Devadason SG, Savenije OEM, Sly PD, Le Souëf PN, Brand PLP. The transient value of classifying preschool wheeze into episodic viral wheeze and multiple trigger wheeze. Acta Paediatr Int J Paediatr. 2010;99(1):56–60.
16. Simpson A, Tan VYF, Winn J, Svensén M, Bishop CM, Heckerman DE, et al. Beyond atopy: Multiple patterns of sensitization in relation to asthma in a birth cohort study. Am J Respir Crit Care Med. 2010;181(11):1200–6.
17. Lazic N, Roberts G, Custovic A, Belgrave D, Bishop CM, Winn J, et al. Multiple atopy phenotypes and their associations with asthma: Similar findings from two birth cohorts. Allergy Eur J Allergy Clin Immunol. 2013;68(6):764–70.
18. Saglani S, Payne DNR, Nicholson AG, Scallan M, Haxby E, Bush A. The safety and quality of endobronchial biopsy in children under five years old. Thorax. 2003;58(12):1053–7.
19. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without TH2 cytokines. J Allergy Clin Immunol. 2012;129(4).
20. O'Reilly R, Ullmann N, Irving S, Bossley CJ, Sonnappa S, Zhu J, et al. Increased airway smooth muscle in preschool wheezers who have asthma at school age. J Allergy Clin Immunol. 2013;131(4).
21. Malmström K, Pelkonen AS, Malmberg LP, Sarna S, Lindahl H, Kajosaari M, et al. Lung function, airway remodelling and inflammation in symptomatic infants: outcome at 3 years. Thorax. 2011;66(2):157–62.
22. Malmström K, Malmberg LP, O'Reilly R, Lindahl H, Kajosaari M, Saarinen KM, et al. Lung function, airway remodeling, and inflammation in infants: Outcome at 8 years. Ann Allergy, Asthma Immunol. 2015;114(2):90–6.
23. Jochmann A, Artusio L, Robson K, Nagakumar P, Collins N, Fleming L, et al. Infection and inflammation in induced sputum from preschool children with chronic airways diseases. Pediatr Pulmonol. 2016 Dec 1;51(8):778–86.
24. Krawiec ME, Westcott JY, Chu HW, Balzar S, Trudeau JB, Schwartz LB, et al. Persistent wheezing in very young children is associated with lower respiratory inflammation. Am J Respir Crit Care Med. 2001;163(6):1338–43.

25. Pavord ID, Ward R, Woltmann G, Wardlaw AJ, Sheller JR, Dworski R. Induced sputum eicosanoid concentrations in asthma. Am J Respir Crit Care Med. 1999;160(6):1905–9.
26. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. J Allergy Clin Immunol. 2011;127(1):153–60, 160-9.
27. Gaillard EA, McNamara PS, Murray CS, Pavord ID, Shields MD. Blood eosinophils as a marker of likely corticosteroid response in children with preschool wheeze: Time for an eosinophil guided clinical trial? Vol. 45, Clinical and Experimental Allergy. 2015. p. 1384–95.
28. Fleming L, Wilson N, Regamey N, Bush a. Use of sputum eosinophil counts to guide management in children with severe asthma. Thorax. 2012;67(3):193–8.
29. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: A randomised controlled trial. Lancet. 2002;360(9347):1715–21.
30. Fleming L, Tsartsali L, Wilson N, Regamey N, Bush a. Sputum inflammatory phenotypes are not stable in children with asthma. Thorax. 2012;67(8):675–81.
31. Lu M, Wu B, Che D, Qiao R, Gu H. FeNO and asthma treatment in children: a systematic review and meta-analysis. Medicine (Baltimore). 2015;94(4):e347.
32. Gomersal T, Harnan S, Essat M, Tappenden P, Wong R, Lawson R, et al. A systematic review of fractional exhaled nitric oxide in the routine management of childhood asthma. Pediatr Pulmonol. 2016 Mar;51(3):316–28.
33. Sonnappa S, Bastardo CM, Saglani S, Bush A, Aurora P. Relationship between past airway pathology and current lung function in preschool wheezers. Eur Respir J. 2011 Dec;38(6):1431–6.
34. NICE. Measuring fractional exhaled nitric oxide concentration in asthma : NIOX MINO, NIOX VERO and NObreath. National Institute of Health and Care Excellence; 2014.
35. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Piippo-Savolainen E, Korppi M. Eosinophil activity in infants hospitalized for wheezing and risk of persistent childhood asthma. Pediatr Allergy Immunol. 2010 Feb 1;21:96–103.
36. Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: Randomised controlled trial. Lancet. 2003 Nov 1;362(9394):1433–8.
37. Smit HA, Pinart M, Antó JM, Keil T, Bousquet J, Carlsen KH, et al. Childhood asthma prediction models: a systematic review. Lancet Respir Med. 2015 Dec;3(12):973–84.
38. Oommen A, Grigg J. Urinary leukotriene E4 in preschool children with acute clinical viral wheeze. Eur Respir J. 2003/02/07. 2003;21:149–54.
39. Robertson CF, Price D, Henry R, Mellis C, Glasgow N, Fitzgerald D, et al. Short-

course montelukast for intermittent asthma in children: a randomized controlled trial. Am J Respir Crit Care Med. 2006/11/18. 2007 Feb 15;175(4):323–9.

40. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294(5548):1871–5.
41. Marmarinos A, Saxoni-Papageorgiou P, Cassimos D, Manoussakis E, Tsentidis C, Doxara A, et al. Urinary leukotriene E4 levels in atopic and non-atopic preschool children with recurrent episodic (viral) wheezing: a potential marker? J Asthma. 2014 Dec 24;1–6.
42. Sanak M, Bochenek G, Faber J, Plutecka H, Szczeklik A. Elevated urinary leukotriene E4 excretion in asthma: a comparison of HPLC-mass spectrometry and ELISA. Allergy. 2010;65(5):663–4.
43. Glowacka E, Jedynak-Wasowicz U, Sanak M, Lis G. Exhaled eicosanoid profiles in children with atopic asthma and healthy controls. Pediatr Pulmonol. 2013;48:324–35.
44. Ducharme FM, Lemire C, Noya FJD, Davis GM, Alos N, Leblond H, et al. Preemptive use of high-dose fluticasone for virus-induced wheezing in young children. N Engl J Med. 2009;360(4):339–53.
45. Todd G, Dunlop K, McNaboe J, Ryan MF, Carson D, Shields MD. Growth and adrenal suppression in asthmatic children treated with high-dose fluticasone propionate. Lancet. 1996;348(9019):27–9.
46. Holden K, Roland D, Staley K, Gaillard E. Are raised blood eosinophils during acute wheeze associated with exacerbation frequency in preschool children? Eur Respir J. 2015 Oct 30;46(suppl 59).
47. Ortega VE, Meyers DA, Bleecker ER. Asthma pharmacogenetics and the development of genetic profiles for personalized medicine. Pharmgenomics Pers Med. 2015 Jan 16;8:9–22.
48. Chavasse JPGR, Seddon P, Bara A, McKean MC. Short acting beta2-agonists for recurrent wheeze in children under two years of age. Cochrane Database Syst Rev. 2009;4(1).
49. Nicklas RA. Paradoxical bronchospasm associated with the use of inhaled beta agonists. J Allergy Clin Immunol. 1990;85(5):959–64.
50. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J Clin Invest. 1997 Dec 15;100(12):3184–8.
51. Silverman EK, Kwiatkowski DJ, Sylvia JS, Lazarus R, Drazen JM, Lange C, et al. Family-based association analysis of β2-adrenergic receptor polymorphisms in the Childhood Asthma Management Program. J Allergy Clin Immunol. 2003;112(5):870–6.
52. Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, et al.

Complex promoter and coding region β2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci U S A. 2000;97(19):10483–8.

53. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, et al. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. Am J Respir Crit Care Med. 2005 Mar 15;171(6):563–70.
54. Taylor DR, Drazen JM, Herbison GP, Yandava CN, Hancox RJ, Town GI. Asthma exacerbations during long term β agonist use: influence of β2 adrenoceptor polymorphism. Thorax. 2000;55:762–7.
55. Israel E, Chinchilli VM, Ford JG, Boushey HA, Cherniack R, Craig TJ, et al. Use of regularly scheduled albuterol treatment in asthma: Genotype-stratified, randomised, placebo-controlled cross-over trial. Lancet. 2004 Oct 23;364(9444):1505–12.
56. Royal College of Physicians. Why Asthma Still Kills: the National Review of Asthma Deaths (NRAD) Confidential Enquiry Report. 2014;
57. Basu K, Palmer CNA, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic β2-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. J Allergy Clin Immunol. 2009;124(6):1188–94.
58. Palmer CNA, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 β2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax. 2006 Nov;61(11):940–4.
59. Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human β2-adrenergic receptor impart distinct agonist-promoted regulatory properties. Biochemistry. 1994;33(32):9414–9.
60. Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of β2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. AmJRespirCell MolBiol. 1995;13(1):25–33.
61. de Paiva AC, Marson FA de L, Ribeiro J, Bertuzzo C. Asthma: Gln27Glu and Arg16Gly polymorphisms of the β2-adrenergic receptor gene as risk factors. Allergy, Asthma Clin Immunol. 2014 Feb 5;10(1):8.
62. Ortega VE, Hawkins GA, Peters SP, Bleecker ER. Pharmacogenetics of the beta 2-adrenergic receptor gene. Immunol Allergy Clin North Am. 2007;27(4):665–84; vii.
63. Walker JKL, DeFea KA. Role for β-arrestin in mediating paradoxical β2AR and PAR2 signaling in asthma. Curr Opin Pharmacol. 2014;16(1):142–7.
64. Chen M, Hegde A, Choi YH, Theriot BS, Premont RT, Chen W, et al. Genetic deletion of β-arrestin-2 and the mitigation of established airway hyperresponsiveness in a murine asthma model. Am J Respir Cell Mol Biol. 2015 Sep 1;53(3):346–54.
65. British Thoracic Society. British Guideline on the Management of Asthma. Scottish Intercoll Guidel Netw. 2016;

66. Turner S, Francis B, Vijverberg S, Pino-Yanes M, Maitland-van der Zee AH, Basu K, et al. Childhood asthma exacerbations and the Arg16 β2-receptor polymorphism: A meta-analysis stratified by treatment. J Allergy Clin Immunol. 2016 Jul;138(1):107–113.e5.
67. Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA, et al. Tailored second-line therapy in asthmatic children with the Arg16 genotype. Clin Sci. 2013 Apr 1;124(8):521–8.
68. Salpeter SR, Buckley NS, Ormiston TM, Salpeter EE, FE S, GK C, et al. Meta-analysis: effect of long-acting beta-agonists on severe asthma exacerbations and asthma-related deaths. Ann Intern Med. 2006 Jun 20;144(12):904–12.
69. Salpeter SR, Wall AJ, Buckley NS. Long-acting Beta-Agonists with and without Inhaled Corticosteroids and Catastrophic Asthma Events. Am J Med. 2010;123(4).
70. Stempel DA, Szefler SJ, Pedersen S, Zeiger RS, Yeakey AM, Lee LA, et al. Safety of Adding Salmeterol to Fluticasone Propionate in Children with Asthma. N Engl J Med. 2016 Sep 1;375(9):840–9.
71. Griffiths B, Ducharme FM. Combined inhaled anticholinergics and short-acting beta2-agonists for initial treatment of acute asthma in children (Review). Griffiths B, editor. Vol. 2013, Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2013. p. i-98.
72. Teoh L, Cates CJ, Hurwitz M, Acworth JP, van Asperen P, Chang AB. Anticholinergic therapy for acute asthma in children. In: Teoh L, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2012. p. i-44.
73. Szczepankiewicz A, Breborowicz A, Sobkowiak P, Kramer L, Popiel A. Association of A/T polymorphism of the CHRM2 gene with bronchodilator response to ipratropium bromide in asthmatic children. Pneumonol Alergol Pol. 2009;77(1):5–10.
74. De Benedictis FM, Bush A. Corticosteroids in respiratory diseases in children. Vol. 185, American Journal of Respiratory and Critical Care Medicine. American Thoracic Society; 2012. p. 12–23.
75. Aksoy MO, Mardini IA, Yang Y, Bin W, Zhou S, Kelsen SG. Glucocorticoid effects on the β-adrenergic receptor-adenylyl cyclase system of human airway epithelium. J Allergy Clin Immunol. 2002 Mar;109(3):491–7.
76. Panickar J, Lakhanpaul M, Lambert PC, Kenia P, Stephenson T, Smyth A, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. N Engl J Med. 2009;360(4):329–38.
77. Kaiser S V, Huynh T, Bacharier LB, Rosenthal JL, Bakel LA, Parkin PC, et al. Preventing Exacerbations in Preschoolers With Recurrent Wheeze: A Meta-analysis. Pediatrics. 2016;137(6):1–15.
78. van Wonderen KE, Geskus RB, van Aalderen WMC, Mohrs J, Bindels PJE, van der

Mark LB, et al. Stability and predictiveness of multiple trigger and episodic viral wheeze in preschoolers. Clin Exp Allergy. 2016 Jun 1;46(6):837–47.

79. Carroll WD, Wildhaber J, Brand PL. Parental concerns about inhaled corticosteroid use and the impact on asthma control: Comparison of Canadian families' attitudes with global reports in the Room to Breathe survey. Chest. 2010 Oct;138(4):312A.
80. Farzan N, Vijverberg SJH, Arets HG, Raaijmakers JAM, Maitland-van der Zee AH. Pharmacogenomics of inhaled corticosteroids and leukotriene modifiers: a systematic review. Vol. 47, Clinical and Experimental Allergy. 2017. p. 271–93.
81. Haczku A, Takeda K, Hamelmann E, Loader J, Joetham A, Redai I, et al. CD23 exhibits negative regulatory effects on allergic sensitization and airway hyperresponsiveness. Am J Respir Crit Care Med. 2000 Mar;161(3 I):952–60.
82. Riffo-Vasquez Y, Spina D, Thomas[2] M, Gilbey T, Kemeny[2] DM, Page CP. The role of CD23 on allergen-induced IgE levels, pulmonary eosinophilia and bronchial hyperresponsiveness in mice. Clin Exp Allergy. 2000;30(30):728–38.
83. Tantisira KG, Silverman ES, Mariani TJ, Xu J, Richter BG, Klanderman BJ, et al. FCER2: A pharmacogenetic basis for severe exacerbations in children with asthma. J Allergy Clin Immunol. 2007 Dec 1;120(6):1285–91.
84. Rogers AJ, Tantisira KG, Fuhlbrigge AL, Litonjua AA, Lasky-Su JA, Szefler SJ, et al. Predictors of poor response during asthma therapy differ with definition of outcome. Pharmacogenomics. 2009 Aug;10(8):1231–42.
85. Koster ES, Maitland-Van Der Zee AH, Tavendale R, Mukhopadhyay S, Vijverberg SJH, Raaijmakers JAM, et al. FCER2 T2206C variant associated with chronic symptoms and exacerbations in steroid-treated asthmatic children. Allergy Eur J Allergy Clin Immunol. 2011 Dec;66(12):1546–52.
86. Euler US. {Ü}ber die Spezifische Blutdrucksenkende Substanz des Menschlichen Prostata- und Samenblasensekretes. Klin Wochenschr. 14(33):1182–3.
87. Cao H, Xiao L, Park GY, Wang X, Azim AC, Christman JW, et al. An improved LC-MS/MS method for the quantification of prostaglandins E2 and D2 production in biological fluids. Anal Biochem. 2008;372(1):41–51.
88. Patrignani P, Patrono C. Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. Biochim Biophys Acta. 2015 Apr;1851(4):422–32.
89. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 Selectively Induces Chemotaxis in T Helper Type 2 Cells, Eosinophils, and Basophils via Seven-Transmembrane Receptor CRTH2. J Exp Med. 2001;193(2):255–62.
90. Maher SA, Birrell MA, Adcock JJ, Wortley MA, Dubuis ED, Bonvini SJ, et al. Prostaglandin D2 and the role of the DP1 , DP2 and TP receptors in the control of airway reflex events. Eur Respir J. 2015;45:1108–18.

91. Song W-L, Wang M, Ricciotti E, Fries S, Yu Y, Grosser T, et al. Tetranor PGDM, an abundant urinary metabolite reflects biosynthesis of prostaglandin D2 in mice and humans. J Biol Chem. 2008 Jan 11;283(2):1179–88.
92. Barnes N, Pavord I, Chuchalin A, Bell J, Hunter M, Lewis T, et al. A randomized, double-blind, placebo-controlled study of the CRTH2 antagonist OC000459 in moderate persistent asthma. Clin Exp Allergy. 2011/07/19. 2012;42:38–48.
93. Rumzhum NN, Ammit AJ. Cyclooxygenase 2: its regulation, role and impact in airway inflammation. Clin Exp Allergy. 2016;46(46):397–410.
94. Maher SA, Birrell MA, Belvisi MG. Prostaglandin E2 mediates cough via the EP3 receptor: implications for future disease therapy. Am J Respir Crit Care Med. 2009 Nov 15;180(10):923–8.
95. Sweatman WJF, Collier HOJ. Effects of Prostaglandins on Human Bronchial Muscle. Nature. 1968 Jan 6;217(5123):69–69.
96. Torres R, Picado C, de Mora F. The PGE2–EP2–mast cell axis: An antiasthma mechanism. Mol Immunol. 2015;63(1):61–8.
97. Zaslona Z, Peters-Golden M. Prostanoids in Asthma and COPD: Actions, Dysregulation, and Therapeutic Opportunities. Chest. 2015 Nov;148(5):1300–6.
98. Picado C. Aspirin-intolerant asthma: role of cyclo-oxygenase enzymes. Allergy. 2002 Aug;57 Suppl 7(4):58–60.
99. Hernandez JM, Janssen LJ. Revisiting the usefulness of thromboxane A2 modulation in the treatment of bronchoconstriction in asthma. Can J Physiol Pharmacol. 2015 Feb;93(2):111–7.
100. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature. 1976 Oct 21;263(5579):663–5.
101. Kellaway CH, Trethewie ER. The liberation of a slow-reacting smooth muscle stimulating substance in anaphylaxis. Q J Exp Physiol. 1940;30:121–45.
102. Brocklehurst WE. The release of histamine and formation of a slow reacting substance (SRS-A) during anaphylactic shock. J. Physiol.151:416–435. J Physiol. 1960;151:416–35.
103. Samuelsson B, Hammarström S, Murphy RC, Borgeat P. Leukotrienes and Slow Reacting Substance of Anaphylaxis (SRS-A). Allergy. 1980 Jul;35(5):375–81.
104. Dahlén SE, Hedqvist P, Hammarström S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. Nature. 1980 Dec 4;288(5790):484–6.
105. Murphy RC, Gijon MA. Biosynthesis and metabolism of leukotrienes. Biochem J. 2007;405:379–95.
106. Drazen JM, O'Brien J, Sparrow D, Weiss ST, Martins MA, Israel E, et al. Recovery of leukotriene E4 from the urine of patients with airway obstruction. Am Rev Respir Dis.

1992;146(1):104–8.

107. Arm JP, O'Hickey SP, Spur BW, Lee TH. Airway Responsiveness to Histamine and Leukotriene E4 in Subjects with Aspirin-induced Asthma. Am Rev Respir Dis. 1989 Jul;140(1):148–53.
108. Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, et al. Urinary Leukotriene E 4 Concentrations Increase after Aspirin Challenge in Aspirin-sensitive Asthmatic Subjects. Am Rev Respir Dis. 1991 May;143:1025–9.
109. Berges-Gimeno MP, Simon RA, Stevenson DD. The effect of leukotriene-modifier drugs on aspirin-induced asthma and rhinitis reactions. Clin Exp Allergy. 2002 Oct;32(10):1491–6.
110. Park JS, Jang AS, Park SW, Lee YM, Uh ST, Kim YH, et al. Protection of leukotriene receptor antagonist against aspirin-induced bronchospasm in asthmatics. Allergy Asthma Immunol Res. 2010 Jan;2(1):48–54.
111. Israel E, Cohn J, Dubé L, Drazen JM, Ratner P, Pleskow W, et al. Effect of Treatment With Zileuton, a 5-Lipoxygenase Inhibitor, in Patients With Asthma. JAMA. 1996 Mar 27;275(12):931.
112. Miligkos M, Bannuru RR, Alkofide H, Kher SR, Schmid CH, Balk EM. Leukotriene receptor antagonists versus placebo in the treatment of asthma in adults and adolescents: a systematic review and meta-analysis. Ann Intern Med. 2015;163(10):756–67.
113. Laidlaw TM, Boyce JA. Cysteinyl leukotriene receptors, old and new; implications for asthma. Clin Exp Allergy. 2012 Sep;42(9):1313–20.
114. Thompson MD, Capra V, Takasaki J, Maresca G, Rovati GE, Slutsky AS, et al. A functional G300S variant of the cysteinyl leukotriene 1 receptor is associated with atopy in a Tristan da Cunha isolate. Pharmacogenet Genomics. 2007 Jul;17(7):539–49.
115. Thompson MD, Storm van's Gravesande K, Galczenski H, Burnham WM, Siminovitch KA, Zamel N, et al. A cysteinyl leukotriene 2 receptor variant is associated with atopy in the population of Tristan da Cunha. Pharmacogenetics. 2003 Oct;13(10):641–9.
116. Thompson MD, Capra V, Clunes MT, Rovati GE, Stankova J, Maj MC, et al. Cysteinyl Leukotrienes Pathway Genes, Atopic Asthma and Drug Response: From Population Isolates to Large Genome-Wide Association Studies. Front Pharmacol. 2016;7(December):299.
117. Bäck M, Powell WS, Dahlén SE, Drazen JM, Evans JF, Serhan CN, et al. Update on leukotriene, lipoxin and oxoeicosanoid receptors: IUPHAR Review 7. Vol. 171, British Journal of Pharmacology. 2014. p. 3551–74.
118. Maekawa A, Balestrieri B, Austen KF, Kanaoka Y. GPR17 is a negative regulator of the cysteinyl leukotriene 1 receptor response to leukotriene D4. Proc Natl Acad Sci U

S A. 2009;106(28):11685–90.

119. Maekawa A, Kanaoka Y, Xing W, Austen KF. Functional recognition of a distinct receptor preferential for leukotriene E4 in mice lacking the cysteinyl leukotriene 1 and 2 receptors. Proc Natl Acad Sci U S A. 2008;105(43):16695–700.
120. Kanaoka Y, Maekawa A, Austen KF. Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. J Biol Chem. 2013;288(16):10967–72.
121. Bankova LG, Lai J, Yoshimoto E, Boyce JA, Austen KF, Kanaoka Y, et al. Leukotriene E4 elicits respiratory epithelial cell mucin release through the G-protein-coupled receptor, GPR99. Proc Natl Acad Sci U S A. 2016;113(22):6242–7.
122. Foster HR, Fuerst E, Lee TH, Cousins DJ, Woszczek G. Characterisation of P2Y12 Receptor Responsiveness to Cysteinyl Leukotrienes. Bozza PT, editor. PLoS One. 2013 Mar 5;8(3):e58305.
123. Paruchuri S, Tashimo H, Feng C, Maekawa A, Xing W, Jiang Y, et al. Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. J Exp Med. 2009;206(11):2543–55.
124. Sandoz Limited. Montelukast Sodium 4 mg Oral Granules - Summary of Product Characteristics (SPC) - (eMC) [Internet]. Electronic Medicines Compendium. 2016 [cited 2017 Mar 4]. Available from: https://www.medicines.org.uk/emc/medicine/27591
125. Knorr B, Franchi LM, Bisgaard H, Vermeulen JH, LeSouef P, Santanello N, et al. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5 years. Pediatrics. 2001;108(3):E48.
126. Bisgaard H, Zielen S, Garcia-Garcia ML, Johnston SL, Gilles L, Menten J, et al. Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. Am J Respir Crit Care Med. 2005 Feb 15;171(4):315–22.
127. Brodlie M, Gupta A, Rodriguez-Martinez CE, Castro-Rodriguez JA, Ducharme FM, Mckean MC. Leukotriene receptor antagonists as maintenance and intermittent therapy for episodic viral wheeze in children. Brodlie M, editor. Cochrane Database Syst Rev. 2015 Oct 19;2015(10):CD008202.
128. Hussein HR, Gupta A, Broughton S, Ruiz G, Brathwaite N, Bossley CJ. A meta-analysis of montelukast for recurrent wheeze in preschool children. Eur J Pediatr. 2017 Jun 1;176(7):963–9.
129. Valovirta E, Boza ML, Robertson CF, Verbruggen N, Smugar SS, Nelsen LM, et al. Intermittent or daily montelukast versus placebo for episodic asthma in children. Ann Allergy, Asthma Immunol. 2011;106(6):518–26.
130. Lima JJ, Zhang S, Grant A, Shao L, Tantisira KG, Allayee H, et al. Influence of Leukotriene Pathway Polymorphisms on Response to Montelukast in Asthma. Am J Respir Crit Care Med. 2005/11/19. 2006;173:379–85.

131. Lima JJ. Treatment heterogeneity in asthma: genetics of response to leukotriene modifiers. Mol Diagn Ther. 2007/04/03. 2007;11:97–104.
132. Basavarajappa D, Wan M, Lukic A, Steinhilber D, Samuelsson B, Radmark O. Roles of coactosin-like protein (CLP) and 5-lipoxygenase-activating protein (FLAP) in cellular leukotriene biosynthesis. Proc Natl Acad Sci. 2014;111(31):11371–6.
133. Rådmark O, Werz O, Steinhilber D, Samuelsson B. 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. Vol. 1851, Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids. Elsevier; 2015. p. 331–9.
134. In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. J Clin Invest. 1997 Mar 1;99(5):1130–7.
135. Sayers I, Barton S, Rorke S, Sawyer J, Peng Q, Beghe B, et al. Promoter polymorphism in the 5-lipoxygenase (ALOX5) and 5-lipoxygenase-activating protein (ALOX5AP) genes and asthma susceptibility in a Caucasian population. Clin Exp Allergy. 2003 Aug 1;33(8):1103–10.
136. Uhl J, Klan N, Rose M, Entian KD, Werz O, Steinhilber D. The 5-lipoxygenase promoter is regulated by DNA methylation. J Biol Chem. 2002;277(6):4374–9.
137. Tse SM, Tantisira K, Weiss ST. The pharmacogenetics and pharmacogenomics of asthma therapy. Pharmacogenomics J. 2011 Dec;11(6):383–92.
138. Klotsman M, York TP, Pillai SG, Vargas-Irwin C, Sharma SS, van den Oord EJCGCG, et al. Pharmacogenetics of the 5-lipoxygenase biosynthetic pathway and variable clinical response to montelukast. Pharmacogenet Genomics. 2007 Mar;17(3):189–96.
139. Telleria JJ, Blanco-Quiros A, Varillas D, Armentia A, Fernandez-Carvajal I, Jesus Alonso M, et al. ALOX5 promoter genotype and response to montelukast in moderate persistent asthma. Respir Med. 2008 Jun 1;102(6):857–61.
140. Cai C, Yang J, Hu S, Zhou M, Guo W. Relationship between urinary cysteinyl leukotriene E4 levels and clinical response to antileukotriene treatment in patients with asthma. Lung. 2007 Apr 28;185(2):105–12.
141. Rabinovitch N, Reisdorph N, Silveira L, Gelfand EW. Urinary leukotriene E_4 levels identify children with tobacco smoke exposure at risk for asthma exacerbation. J Allergy Clin Immunol. 2011 Aug 8;128(2):323–7.
142. Rabinovitch N, Graber NJ, Chinchilli VM, Sorkness CA, Zeiger RS, Strunk RC, et al. Urinary Leukotriene E4 /Exhaled Nitric Oxide Ratio and Montelukast Response in Childhood Asthma. J Allergy Clin Immunol. 2010;126:544–5.
143. Mougey E, Lang JE, Allayee H, Teague WG, Dozor AJ, Wise RA, et al. ALOX5 polymorphism associates with increased leukotriene production and reduced lung function and asthma control in children with poorly controlled asthma. Clin Exp Allergy.

2013;43:512–20.

144. Dal Negro RW, Visconti M, Micheletto C, Tognella S, Guerriero M, Dal Negro RW. Reference urinary LTE 4 levels in normal individuals: a pilot study. Eur Ann Allergy Clin Immunol. 2011 Feb;43(1):22–8.
145. Adamek-Guzik T, Guzik TJ, Czerniawska-Mysik G, Korpanty G, Mastalerz L, Radwan J, et al. Urinary leukotriene levels are increased during exacerbation of atopic eczema/dermatitis syndrome. Relation to clinical status. Allergy. 2002;57(8):732–6.
146. Mason S, Hussain-Gambles M, Leese B, Atkin K, Brown J. Representation of South Asian people in randomised clinical trials: analysis of trials' data. Br Med J. 2003;326(7401):1244–5.
147. Hussain-Gambles M, Atkin K, Leese B. Why ethnic minority groups are under-represented in clinical trials: a review of the literature. Health Soc Care Community. 2004;12(5):382–8.
148. Netuveli G, Hurwitz B, Levy M, Fletcher M, Barnes G, Durham SR, et al. Ethnic variations in UK asthma frequency, morbidity, and health-service use: A systematic review and meta-analysis. Vol. 365, Lancet. 2005. p. 312–7.
149. Patton MQ. Qualitative Evaluation and Research Methods. Vol. 14, Qualitative Evaluation and Research Methods. Wiley Subscription Services, Inc., A Wiley Company; 1990. 169-186 p.
150. Sheikh A, Halani L, Bhopal R, Netuveli G, Partridge MR, Car J, et al. Facilitating the recruitment of minority ethnic people into research: qualitative case study of South Asians and asthma. PLoS Med. 2009;6(10):e1000148.
151. Shilling V, Young B, Chappuy H, Doz F, Blanche S, Gentet J, et al. How do parents experience being asked to enter a child in a randomised controlled trial? BMC Med Ethics. 2009;10(1).
152. Ferguson PR. Patients' perceptions of information provided in clinical trials. J Med Ethics. 2002;28(1):45–8.
153. Woodgate RL, Yanofsky RA. Parents' experiences in decision making with childhood cancer clinical trials. Cancer Nurs. 2010 Jan;33(1):11–8.
154. Glaser BG. Basics of Grounded Theory Analysis. Sociology Press; 1992. 129 p.
155. Pope C, Ziebland S, Mays N, Bryman A, Burgess R, Miles M, et al. Qualitative research in health care. Analysing qualitative data. BMJ. 2000;320(7227):114–6.
156. Richards TJ, Richards L. The transformation of qualitative method: computational paradigms and research processes. In: Fielding NG, Lee RM, editors. Using Computers in Qualitative Research. 2nd ed. London: Sage Publications UK; 1994. p. 38–53.
157. Duroudier NP, Tulah AS, Sayers I. Leukotriene pathway genetics and pharmacogenetics in allergy. Allergy Eur J Allergy Clin Immunol. 2009 Jun;64(6):823–

39.
158. Tantisira KG, Lima J, Sylvia J, Klanderman B, Weiss ST. 5-lipoxygenase pharmacogenetics in asthma: overlap with Cys-leukotriene receptor antagonist loci. Pharmacogenet Genomics. 2009;19(3):244–7.
159. Tantisira KG, Drazen JM. Genetics and pharmacogenetics of the leukotriene pathway. J Allergy Clin Immunol. 2009 Sep;124(3):422–7.
160. Helgadottir A, Manolescu A, Thorleifsson G, Gretarsdottir S, Jonsdottir H, Thorsteinsdottir U, et al. The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet. 2004;36(3):233–9.
161. Crosslin DR, Shah SH, Nelson SC, Haynes CS, Connelly JJ, Gadson S, et al. Genetic effects in the leukotriene biosynthesis pathway and association with atherosclerosis. Hum Genet. 2009;125(2):217–29.
162. Geiger E V., Doehring A, Kirchhof A, Lötsch J, Rn J, Tsch L. Functional variants of the human 5-lipoxygenase gene and their genetic diagnosis. Prostaglandins Leukot Essent Fat Acids. 2009 May 1;80(5–6):255–62.
163. Tcheurekdjian H, Via M, De Giacomo A, Corvol H, Eng C, Thyne S, et al. ALOX5AP and LTA4H polymorphisms modify augmentation of bronchodilator responsiveness by leukotriene modifiers in Latinos. J Allergy Clin Immunol. 2010 Oct;126(4):853–8.
164. Holloway JW, Barton SJ, Holgate ST, Rose-Zerilli MJ, Sayers I. The role of LTA4H and ALOX5AP polymorphism in asthma and allergy susceptibility. Allergy Eur J Allergy Clin Immunol. 2008;63(8):1046–53.
165. SeattleSNPs Database [Internet]. [cited 2017 Mar 17]. Available from: http://pga.gs.washington.edu
166. NIEHS SNPs [Internet]. [cited 2017 Mar 17]. Available from: http://egp.gs.washington.edu
167. Smith SM, Maughan PJ. SNP genotyping using KASPar assays. Methods Mol Biol. 2015;1245:243–56.
168. Ehrlich R, Kattan M, Godbold J, Saltzberg DS, Grimm KT, Landrigan PJ, et al. Childhood asthma and passive smoking. Urinary cotinine as a biomarker of exposure. Am Rev Respir Dis. 1992;145(3):594–9.
169. Henderson FW, Reid HF, Morris R, Wang O-L, Hu PC, Helms RW, et al. Home Air Nicotine Levels and Urinary Cotinine Excretion in Preschool Children. Am Rev Respir Dis. 1989 Jul;140(1):197–201.
170. NIHR, MHRA. General Practice Research Database [Internet]. [cited 2018 Mar 3]. Available from: http://www.gprd.com/home/default.html
171. MRC Biostatistics Unit. WINBUGS 1.4.x [Internet]. 2003 [cited 2015 May 10]. Available from: http://www.mrc-bsu.cam.ac.uk/software/bugs/the-bugs-project-winbugs/

172. Wechsler ME, Castro M, Lehman E, Chinchilli VM, Sutherland ER, Denlinger L, et al. Impact of race on asthma treatment failures in the Asthma Clinical Research Network. Am J Respir Crit Care Med. 2011 Dec 1;184(11):1247–53.
173. El-Ekiaby A, Brianas L, Skowronski ME, Coreno AJ, Galan G, Kaeberlein FJ, et al. Impact of race on the severity of acute episodes of asthma and adrenergic responsiveness. Am J Respir Crit Care Med. 2006 Sep 1;174(5):508–13.
174. Koo S, Gupta A, Fainardi V, Bossley C, Bush A, Saglani S, et al. Ethnic Variation in Response to IM Triamcinolone in Children with Severe Therapy-Resistant Asthma. Chest. 2016;149(1):98–105.
175. Mougey EB, Feng H, Castro M, Irvin CG, Lima JJ. Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. Pharmacogenet Genomics. 2009 Feb;19(2):129–38.
176. Kang M-J, Kwon J-W, Kim B-J, Yu J, Choi W-A, Shin Y-J, et al. Polymorphisms of the PTGDR and LTC4S influence responsiveness to leukotriene receptor antagonists in Korean children with asthma. J Hum Genet. 2011 Apr 10;56(4):284–9.
177. Kim JH, Lee SY, Kim HB, Jin HS, Yu JH, Kim BJ, et al. TBXA2R gene polymorphism and responsiveness to leukotriene receptor antagonist in children with asthma. Clin Exp Allergy. 2008;38(1):51–9.
178. Jinnai N, Sakagami T, Sekigawa T, Kakihara M, Nakajima T, Yoshida K, et al. Polymorphisms in the prostaglandin E2 receptor subtype 2 gene confer susceptibility to aspirin-intolerant asthma: A candidate gene approach. Hum Mol Genet. 2004 Oct 27;13(24):3203–17.
179. UK Office for National Statistics. 2011 Census: Custom Age, Gender & Ethnicity Tool [Internet]. NOMIS. 2011 [cited 2018 Apr 5]. Available from: https://www.towerhamlets.gov.uk/Documents/Borough_statistics/Research-tools-and-guidance/Tool-2011-Census-TH-Ethnicity-by-Age.xlsx
180. Huberman A, Miles M. The Qualitative Researcher's Companion. Sage Publications; 2002. 410 p.
181. Macneill V, Nwokoro C, Griffiths C, Grigg J, Seale C. Recruiting ethnic minority participants to a clinical trial: A qualitative study. BMJ Open. 2013;3(4):1–8.
182. Liu F, Ouyang J, An S, Liu S, Yang B, Xiong W, et al. Leukotriene inhibitors for bronchiolitis in infants and young children. Cochrane Database Syst Rev. 2015 Mar 16;(3).
183. Via M, Tcheurekdjian H, González Burchard E. Role of interactions in pharmacogenetic studies: leukotrienes in asthma. Pharmacogenomics. 2013;14(8):923–9.
184. Rådmark O, Werz O, Steinhilber D, Samuelsson B. 5-Lipoxygenase: regulation of

expression and enzyme activity. Trends Biochem Sci. 2007 Jul 1;32(7):332–41.

185. Kim KA, Lee HM, Joo HJ, Park IB, Park JY. Effects of polymorphisms of the SLCO2B1 transporter gene on the pharmacokinetics of montelukast in humans. J Clin Pharmacol. 2013;53(11):1186–93.
186. Mougey EB, Lang JE, Wen X, Lima JJ. Effect of Citrus Juice and SLCO2B1 Genotype on the Pharmacokinetics of Montelukast. J Clin Pharmacol. 2011 May 1;51(5):751–60.
187. Rivera-Spoljaric K, Chinchilli VM, Camera LJ, Zeiger RS, Paul IM, Phillips BR, et al. Signs and Symptoms that Precede Wheezing in Children with a Pattern of Moderate-to-Severe Intermittent Wheezing. J Pediatr. 2009;154(6).
188. Drazen JM, Yandava CN, Dubé L, Szczerback N, Hippensteel R, Pillari A, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. Nat Genet. 1999;22(2):168–70.
189. Kalayci O, Birben E, Sackesen C, Keskin O, Tahan F, Wechsler ME, et al. ALOX5 promoter genotype, asthma severity and LTC4 production by eosinophils. Allergy Eur J Allergy Clin Immunol. 2006 Nov 30;61(1):97–103.
190. Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in Medicine — Reporting of Subgroup Analyses in Clinical Trials. N Engl J Med. 2007 Nov 22;357(21):2189–94.
191. Nagao M, Ikeda M, Fukuda N, Habukawa C, Kitamura T, Katsunuma T, et al. Early control treatment with montelukast in preschool children with asthma: A randomized controlled trial. Allergol Int. 2018 May 16;67(1):72–8.
192. Cane RS, Ranganathan SC, McKenzie SA. What do parents of wheezy children understand by 'wheeze'? Arch Dis Child. 2000 Apr;82(4):327–32.
193. Vissing NH, Jensen SM, Bisgaard H. Validity of information on atopic disease and other illness in young children reported by parents in a prospective birth cohort study. BMC Med Res Methodol. 2012 Oct 22;12(1):160.
194. Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. Vol. 383, The Lancet. 2014. p. 1593–604.
195. Bacharier LB, Phillips BR, Zeiger RS, Szefler SJ, Martinez FD, Lemanske RF, et al. Episodic use of an inhaled corticosteroid or leukotriene receptor antagonist in preschool children with moderate-to-severe intermittent wheezing. J Allergy Clin Immunol. 2008;122(6):1127–35.
196. Review Manager (RevMan)[Computer Program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration; 2014.
197. Nwokoro C, Pandya H, Turner S, Eldridge S, Griffiths CJ, Vulliamy T, et al. Intermittent montelukast in children aged 10 months to 5 years with wheeze (WAIT trial): A multicentre, randomised, placebo-controlled trial. Lancet Respir Med. 2014;2(10):796–803.

198. Fitzpatrick AM, Jackson DJ, Mauger DT, Boehmer SJ, Phipatanakul W, Sheehan WJ, et al. Individualized therapy for persistent asthma in young children. J Allergy Clin Immunol. 2016;138(6):1608–1618.e12.
199. Tapaninen T, Karonen T, Backman JT, Neuvonen PJ, Niemi M. SLCO2B1 c.935G>A single nucleotide polymorphism has no effect on the pharmacokinetics of montelukast and aliskiren. Pharmacogenet Genomics. 2013 Jan;23(1):19–24.
200. Watanabe M, Machida K, Inoue H. A turn on and a turn off: BLT1 and BLT2 mechanisms in the lung. Expert Rev Respir Med. 2014;8(4):381–3.
201. Matsunaga Y, Fukuyama S, Okuno T, Sasaki F, Matsunobu T, Asai Y, et al. Leukotriene B4 receptor BLT2 negatively regulates allergic airway eosinophilia. FASEB J. 2013 Aug;27(8):3306–14.
202. Kantsedikas I, Hussain S, Nwokoro C, Brugha R, Sanak M, Grigg J. Urinary eicosanoid levels in healthy children. In: Clinical and Experimental Allergy. 2013. p. 1435–6.
203. Daham K, Song W-LL, Lawson JA, Kupczyk M, Gülich A, Dahlén S-EE, et al. Effects of celecoxib on major prostaglandins in asthma. Clin Exp Allergy. 2011;41(1):36–45.
204. Shiraishi Y, Asano K, Nakajima T, Oguma T, Suzuki Y, Shiomi T, et al. Prostaglandin D2-induced eosinophilic airway inflammation is mediated by CRTH2 receptor. J Pharmacol Exp Ther. 2005 Mar 1;312(3):954–60.
205. Shiraishi Y, Asano K, Niimi K, Fukunaga K, Wakaki M, Kagyo J, et al. Cyclooxygenase-2/Prostaglandin D2/CRTH2 Pathway Mediates Double-Stranded RNA-Induced Enhancement of Allergic Airway Inflammation. J Immunol. 2008 Jan 1;180(1):541–9.
206. Brannan JD, Gulliksson M, Anderson SD, Chew N, Seale JP, Kumlin M. Inhibition of mast cell PGD2 release protects against mannitol-induced airway narrowing. Eur Respir J. 2006 May 1;27(5):944–50.
207. Xue L, Fergusson J, Salimi M, Panse I, Ussher JE, Hegazy AN, et al. Prostaglandin D2 and leukotriene E4 synergize to stimulate diverse Th2 functions and Th2 cell/neutrophil crosstalk. J Allergy Clin Immunol. 2015;135(5):1358–1366e11.
208. Rabinovitch N, Singh A, Jiang RW, Wong SW, Tebbutt S, Carlsten C. Diesel exhaust in asthmatics modulates leukotriene E4 and airflow through epigenetic changes. Eur Respir J. 2014 Sep 1;44(Suppl_58):P1499-.
209. Castro-Rodriguez JA, Custovic A, Ducharme FM. Treatment of asthma in young children: evidence-based recommendations. Asthma Res Pract. 2016;2(1):1–11.
210. Reddel HK, Bateman ED, Becker A, Boulet LP, Cruz AA, Drazen JM, et al. A summary of the new GINA strategy: A roadmap to asthma control. European Respiratory Journal. 2015;46(3):622–39.
211. Bucchioni E, Csoma Z, Allegra L, Chung KF, Barnes PJ, Kharitonov SA. Adenosine 5′-

monophosphate increases levels of leukotrienes in breath condensate in asthma. Respir Med. 2004;98(7):651–5.

212. Fernandez-Nieto M, Sastre B, Sastre J, Lahoz C, Quirce S, Madero M, et al. Changes in sputum eicosanoids and inflammatory markers after inhalation challenges with occupational agents. Chest. 2009;136(5):1308–15.
213. Man M, Farmen M, Dumaual C, Teng CH, Moser B, Irie S, et al. Genetic Variation in Metabolizing Enzyme and Transporter Genes: Comprehensive Assessment in 3 Major East Asian Subpopulations With Comparison to Caucasians and Africans. J Clin Pharmacol. 2010 Aug 1;50(8):929–40.
214. Bønnelykke K, Sleiman P, Nielsen K, Kreiner-Møller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet. 2014;46(1):51–5.
215. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, et al. Genomewide Association between *GLCCI1* and Response to Glucocorticoid Therapy in Asthma. N Engl J Med. 2011;365(13):1173–83.
216. Huang JL, Gao PS, Mathias RA, Yao TC, Chen LC, Kuo ML, et al. Sequence variants of the gene encoding chemoattractant receptor expressed on Th2 cells (CRTH2) are associated with asthma and differentially influence mRNA stability. Hum Mol Genet. 2004 Sep 14;13(21):2691–7.
217. Sanz C, Isidoro-García M, Dávila I, De Pedro MP, De Arriba Méndez S, Padrón J, et al. A new PTGDR promoter polymorphism in a population of children with asthma. Pediatr Allergy Immunol. 2009;20(2):151–6.
218. Santini G, Mores N, Malerba M, Mondino C, Macis G, Montuschi P. Investigational prostaglandin D2 receptor antagonists for airway inflammation. Expert Opin Investig Drugs. 2016 Jun 2;25(6):639–52.
219. Foster SJ, Cooper MN, Oosterhof S, Borland ML. Oral prednisolone in preschool children with virus-associated wheeze: a prospective, randomised, double-blind, placebo-controlled trial. Lancet Respir Med. 2018;6(2):97–106.
220. Lukkarinen M, Lukkarinen H, Lehtinen P, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after first rhinovirus wheeze: A 7-year follow-up. Pediatr Allergy Immunol. 2013 May 1;24(3):237–43.
221. Mandhane PJ, Paredes P, De Silbernagel Z, Aung YN, Williamson J, Lee BE, et al. Treatment of preschool children presenting to the emergency department with wheeze with azithromycin: A placebo-controlled randomized trial. PLoS One. 2017;12(8):1–15.
222. Stokholm J, Chawes BL, Vissing NH, Bjarnadóttir E, Pedersen TM, Vinding RK, et al. Azithromycin for episodes with asthma-like symptoms in young children aged 1-3 years: A randomised, double-blind, placebo-controlled trial. Lancet Respir Med. 2016;4(1):19–26.

223. Grigg J. Antibiotics for preschool wheeze. Lancet Respir Med. 2016 Jan 1;4(1):2–3.
224. Santus P, Radovanovic D. Prostaglandin D2 receptor antagonists in early development as potential therapeutic options for asthma. Vol. 25, Expert Opinion on Investigational Drugs. 2016. p. 1083–92.
225. Grigg J, Whitehouse A, Pandya H, Turner S, Griffiths CJ, Vulliamy T, et al. Urinary prostanoids in preschool wheeze. Eur Respir J. 2017 Feb;49(2):1601390.
226. Nwokoro C, Grigg J. Preschool wheeze, genes and treatment. Paediatr Respir Rev. 2018 Dec;
227. Nwokoro C, Pandya H, Turner S, Eldridge S, Griffiths CJ, Vulliamy T, et al. Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate 5-lipoxygenase (ALOX5) promoter genotype: a multicentre, randomised, placebo-controlled trial. Effic Mech Eval. 2015;2(6):51.
228. Szefler SJ, Ando R, Cicutto LC, Surs W, Hill MR, Martin RJ. Plasma histamine, epinephrine, cortisol, and leukocyte beta-adrenergic receptors in nocturnal asthma. Clin Pharmacol Ther. 1991 Jan;49(1):59–68.
229. Wikimedia Commons contributors. File:Eicosanoid synthesis.svg. Wikimedia Commons, the free media repository; 2016.
230. Murphey LJ, Williams MK, Sanchez SC, Byrne LM, Csiki I, Oates JA, et al. Quantification of the major urinary metabolite of PGE2 by a liquid chromatographic/mass spectrometric assay: Determination of cyclooxygenase-specific PGE2 synthesis in healthy humans and those with lung cancer. Anal Biochem. 2004;334(2):266–75.
231. Christie WW. Prostanoids – prostaglandins, prostacyclins and thromboxanes: chemistry and biology. [Internet]. Marangoni A (University of G, editor. A Lipid Primer. The American Oil Chemists Society; 2014 [cited 2017 Feb 21]. p. 1–11. Available from: http://lipidlibrary.aocs.org/Primer/content.cfm?ItemNumber=39316
232. Liu M, Yokomizo T. The role of leukotrienes in allergic diseases. Allergol Int. 2015 Jan;64(1):17–26.

8 APPENDICES

8.1 Appendix 1 - Study locations

The study was primarily conducted in the locations indicated in Table 8-1 - Primary study locations. However additional recruiting sites (8.1.1) were subsequently added, with a number of GP practices acting as patient identification centres (PICs) (8.1.2). The role of PICs was restricted to identification of potential candidates under local regulations, no recruitment or other study specific activity occurred at these venues.

TABLE 8-1 - PRIMARY STUDY LOCATIONS

Location	Study Activity
The Royal London Hospital Queen Mary, University of London	Study design, sponsorship, management, monitoring, data storage and archiving IMP storage and dispatch DNA extraction ALOX5 genotyping Qualitative and quantitative manuscript production
University Hospitals Leicester	Subject recruitment IMP storage and dispatch
Royal Aberdeen Children's Hospital	Subject recruitment IMP storage and dispatch
Novalabs, Leicester	IMP and placebo production
Kbiosciences, Hitchin	Eicosanoid pathway SNP genotyping
Jagiellonian University, Krakow	Urinary eicosanoid estimation
King's College London	Urinary cotinine estimation

8.1.1 Local Investigators in secondary care centres

Dr Christopher Upton (Norfolk and Norwich University Hospitals NHS Trust), Dr Maria O'Callaghan (Barts Health NHS Trust, Whipps Cross Hospital), Dr S. Murthy Saladi (Countess of Chester NHS Foundation Trust), Dr Catherine Tuffrey (Portsmouth Hospitals NHS Trust), Dr Sheng-Ang Ho (East Cheshire NHS Trust), Dr Robert Ross Russell (Cambridge University Hospitals NHS Trust), Dr Anil Tuladhar (North Tees and Hartlepool NHS Trust), Dr Edwin Osakwe (Oxford Radcliffe Hospitals NHS Trust), Dr Paul McNamara (Alder Hey Children's NHS Trust), Dr James Y Paton (NHS Lothian University Hospitals, Royal Hospital for Sick Children), Dr Mansoor Ahmed (Burton Hospitals NHS Foundation Trust), Dr John Alexander (University Hospital of North Staffordshire NHS Trust), Dr Deepthi Jyothish (Birmingham Children's Hospital NHS Trust), Dr John Scanlon (Worcestershire Acute NHS Trust), Dr Edward Simmonds (University Hospitals of Coventry NHS Trust), Dr James Crossley (Chesterfield Royal NHS Foundation Trust), Dr Shakeel Rahman (Harrogate and District NHS Foundation Trust), Professor Harish Vyas (Nottingham University Hospitals NHS Trust), Dr Will Carroll (Royal Derby Hospitals NHS Trust), Dr Diarmuid P Kerrin (Barnsley NHS Foundation Trust), Dr Hazel Evans (Southampton University Hospitals NHS

Trust), Dr Anna Mathew (Western Sussex NHS Hospitals Trust), Dr Anne Prendiville (Royal Cornwall Hospital Trust), Professor Mark Everard (Sheffield Children's NHS Foundation Trust), Dr Lakshmi Chilukuri (St Helens and Knowsley Teaching Hospitals NHS Trust), Dr Sharryn Gardner (Southport and Ormskirk NHS Trust), Dr Gary Ruiz (King's College Hospital Foundation NHS Trust), Dr Simon Langton Hewer (University Hospitals Bristol NHS Trust), Dr Peter DeHalpert (Royal Berkshire NHS Foundation Trust), Dr Paul Seddon (Brighton and Sussex University Hospitals NHS Trust), Dr Tim Adams (NHS Ayrshire & Arran), Dr David Cremonesini (Hinchingbrooke Health Care NHS Trust), Dr Jonathan Garside (Calderdale and Huddersfield NHS Trust), Dr Anil Shenoy (Bradford Teaching Hospitals NHS Trust), Dr Matthew Babirecki (Airedale NHS Foundation Trust), Dr Anne Ingram (Luton & Dunstable Hospital NHS Trust), Dr John Furness (County Durham and Darlington NHS Trust), Dr David Lacy (Wirral University Teaching Hospital NHS Trust), Dr Mike Linney (Western Sussex Hospitals NHS Trust).

8.1.2 Patient identification centres

Springfield GP-led Health Centre, Lower Clapton Practice, The Lawson Practice, Neaman Practice, Elm Practice, Sandringham Practice, Queensbridge Group Practice, Latimer Health Centre, Statham Grove Surgery. In the Tower Hamlets Primary Care Trust; Strouts Place Medical Centre, Jubilee Street Practice, Wapping Health Centre, East One Health, Barkantine Health Centre, Blithehale Health Centre, Albion Health Centre, Chrisp Street Practice, Bromley-By-Bow Health Centre, XX Place Surgery, St Andrews Health Centre, Mission Practice.

8.2 Appendix 2 - Publications

8.2.1 Conference abstracts

1) Royal College of Paediatrics and Child Health Annual Meeting. Glasgow. Research in Action - Improving Care by Improving Research Session. 30th April 2015. Delivering WAIT across multiple settings. Oral Presentation. Brady, C
2) Royal College of Paediatrics and Child Health Annual Meeting. Glasgow. Research in Action - Improving Care by Improving Research Session. 30th April 2015. The WAIT Study of parent determined oral montelukast therapy for pre-school wheeze - Introduction to the study, Key findings, and plans for further research. Oral Presentation. Grigg, J.
3) John Price Respiratory Conference. London. 24th March 2015. The Wheeze and Intermittent Treatment (WAIT) trial: Results and what next? Oral Presentation. Nwokoro, C.
4) Royal College of Paediatrics and Child Health Annual Meeting. Glasgow. Medicines for Children Research Network Session. 5th June 2013. WAIT - working together to deliver a large paediatric trial. Oral Presentation. Nwokoro, C.
5) Royal College of Paediatrics and Child Health Annual Meeting. Glasgow. British Paediatric Respiratory Society Session. 6th June 2013. Recruiting ethnic minority participants to a clinical trial: qualitative study. Oral Presentation. Nwokoro, C.
6) Barts and the London School of Medicine and Dentistry. Paediatric Research Seminar. 14th Feb '13. Lipid Mediators in Preschool Wheezing Disorders. Oral Presentation. Nwokoro, C.
7) European Respiratory Society Annual Congress - Vienna - September 2012. Urinary Eicosanoids and Preschool Wheeze Phenotype. Oral Presentation. Nwokoro, C.

8.2.2 Peer-reviewed papers

1) Preschool wheeze, genes and treatment. Nwokoro C, Grigg J. Paediatric Respiratory Reviews; 2017 [In Press](226)
2) Urinary prostanoids in preschool wheeze. Grigg J, Whitehouse A, Pandya H.... and Nwokoro C. Eur Respir J 2017 Feb 2;49(2). (225)
3) Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate 5-lipoxygenase (*ALOX5*) promoter genotype: a multicentre, randomised, placebo-controlled trial. Nwokoro C *et al*. NIHR Journals Library; 2015 Nov(227).

4) Intermittent montelukast in children aged 10 months to 5 years with wheeze (WAIT trial): a multicentre, randomised, placebo-controlled trial. Nwokoro C *et al*. Lancet Respir Med. Oct 2014; 2(10): 796-803.(197)

5) Recruiting ethnic minority participants to a clinical trial: a qualitative study. MacNeill V, Nwokoro C *et al*. BMJ Open. 2013; 3(4): e002750.(181)

8.3 Appendix 3 - Funding, ethics committee and regulatory approvals

8.3.1 Integrated research application form (abbreviated, 1-12 of 48 pages)

Welcome to the Integrated Research Application System

IRAS Project Filter

The integrated dataset required for your project will be created from the answers you give to the following questions. The system will generate only those questions and sections which (a) apply to your study type and (b) are required by the bodies reviewing your study. Please ensure you answer all the questions before proceeding with your applications.

Please enter a short title for this project (maximum 70 characters)
Wheeze And Intermittent Treatment; WAIT

1. Is your project research?

(•) Yes ( ) No

2. Select one category from the list below:

(•) Clinical trial of an investigational medicinal product
( ) Clinical investigation or other study of a medical device
( ) Combined trial of an investigational medicinal product and an investigational medical device
( ) Other clinical trial or clinical investigation
( ) Study administering questionnaires/interviews for quantitative analysis, or using mixed quantitative/qualitative methodology
( ) Study involving qualitative methods only
( ) Study limited to working with human tissue samples, other human biological samples and/or data *(specific project only)*
( ) Research tissue bank
( ) Research database

If your work does not fit any of these categories, select the option below:

( ) Other study

2a. Please answer the following question:

Is this trial subject to advice from the Expert Advisory Group on Clinical Trials and the Commission on Human Medicine prior to authorisation from MHRA? ( ) Yes (•) No

2b. Please answer the following question:

Is this a trial of a gene therapy medicinal product? ( ) Yes (•) No

2c. Please answer the following question(s):

a) Does the study involve the use of any ionising radiation? ( ) Yes (•) No
b) Will you be taking new human tissue samples (or other human biological samples)? (•) Yes ( ) No
c) Will you be using existing human tissue samples (or other human biological samples)? ( ) Yes (•) No

3. In which countries of the UK will the research sites be located? *(Tick all that apply)*

[x] England
[ ] Scotland
[ ] Wales
[ ] Northern Ireland

3a. In which country of the UK will the lead NHS R&D office be located:

(•) England
( ) Scotland
( ) Wales
( ) Northern Ireland
( ) This study does not involve the NHS

4. Which review bodies are you applying to?

[x] NHS/HSC Research and Development offices
[ ] Social Care Research Ethics Committee
[x] Research Ethics Committee
[x] Medicines and Healthcare products Regulatory Agency (MHRA) – Medicines
[ ] Gene Therapy Advisory Committee (GTAC)
[ ] National Information Governance Board for Health and Social Care (NIGB)
[ ] Ministry of Justice (MoJ)

5. Will any research sites in this study be NHS organisations?

(•) Yes ( ) No

5a. Do you want your application to be processed through the NIHR Coordinated System for gaining NHS Permission?

(•) Yes ( ) No

If yes, you must complete and submit the NIHR CSP Application Form immediately after completing this project filter, before proceeding with completing and submitting other applications.

6. Do you plan to include any participants who are children?

(•) Yes ( ) No

7. Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? *The guidance notes explain how an adult is defined for this purpose.*

( ) Yes (•) No

8. Do you plan to include any participants who are prisoners or young offenders in the custody of HM Prison Service in England or Wales?

( ) Yes (•) No

9. Is the study, or any part of the study, being undertaken as an educational project?

( ) Yes (•) No

10. Is this project financially supported by the United States Department for Health and Human Services?

( ) Yes (•) No

11. Will identifiable patient data be accessed outside the clinical care team without prior consent at any stage of the project (including identification of potential participants)?

( ) Yes (•) No

Integrated Research Application System
Application Form for Clinical trial of an investigational medicinal product

NHS National Patient Safety Agency
National Research Ethics Service

Application to NHS/HSC Research Ethics Committee

The Chief Investigator should complete this form. Guidance on the questions is available wherever you see this symbol displayed. We recommend reading the guidance first. The complete guidance and a glossary are available by selecting Help.

Short title and version number: (maximum 70 characters - this will be inserted as header on all forms)
Wheeze And Intermittent Treatment; WAIT

Please complete these details after you have booked the REC application for review.

REC Name:
South East REC

REC Reference Number: 09/H1102/110
Submission date: 20/10/2009

PART A: Core study information

1. ADMINISTRATIVE DETAILS

A1. Full title of the research:

Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.

A3. Chief Investigator:

	Title	Forename/Initials	Surname
	Professor	Jonathan	Grigg
Post	Professor of Paediatric Respiratory Medicine		
Qualifications	BSc, MBBS, MRCP(UK), MD, FRCPCH		
Employer	Queen Mary University London/Barts and the London School of Medicine		
Work Address	4 Newark Street London 4 Newark Street		
Post Code	E12AT		
Work E-mail	j.grigg@qmul.ac.uk		
* Personal E-mail	kleinert@blueyonder.co.uk		
Work Telephone	02078822206		
* Personal Telephone/Mobile	07787550774		

Fax	02078825555

** This information is optional. It will not be placed in the public domain or disclosed to any other third party without prior consent.*

A copy of a current CV (maximum 2 pages of A4) for the Chief Investigator must be submitted with the application.

A4. Who is the contact on behalf of the sponsor for all correspondence relating to applications for this project?
This contact will receive copies of all correspondence from REC and R&D reviewers that is sent to the CI.

Title	Forename/Initials	Surname
Mr	Gerry	Leonard

Address	Head of Resources, Joint R&D Barts and the London/QMUL 5 Walden Street
Post Code	E1 2EF
E-mail	gerry.leonard@bartsandthelondon.nhs.uk
Telephone	0207 882 7260
Fax	

A5-1. Research reference numbers. *Please give any relevant references for your study:*

Applicant's/organisation's own reference number, e.g. R & D (if available):	08/43/03
Sponsor's/protocol number:	006983 QM
Protocol Version:	1
Protocol Date:	09/10/2009
Funder's reference number:	08/43/03
International Standard Randomised Controlled Trial Number (ISRCTN):	
ClinicalTrials.gov Identifier (NCT number):	
European Clinical Trials Database (EudraCT) number: 2009-015626-11	
Project website:	http://www.ihse.qmul.ac.uk/chspctu/Current%20projects/External%20Studies

Ref.Number Description	Reference Number

A5-2. Is this application linked to a previous study or another current application?

○ Yes ◉ No

Please give brief details and reference numbers.

2. OVERVIEW OF THE RESEARCH

To provide all the information required by review bodies and research information systems, we ask a number of specific questions. This section invites you to give an overview using language comprehensible to lay reviewers and members of the public. Please read the guidance notes for advice on this section.

A6-1. Summary of the study. *Please provide a brief summary of the research (maximum 300 words) using language easily understood by lay reviewers and members of the public. This summary will be published on the website of the National Research Ethics Service following the ethical review.*

A quarter of all UK children will have at least one attack of wheeze during the preschool period (10 months to 5 years 11 months of age). Severe attacks of wheeze in these young children are usually triggered by viral-colds. The aim of this trial is to assess whether the intermittent use of montelukast, a blocker of a substance that narrows the airways called leukotriene, is an effective treatment strategy in preschool wheeze. A further aim of the trial is to determine whether there is a subgroup of children that is highly responsive to montelukast due to differences in their genetic ability to produce leukotriene. The gene that we will focus on is "ALOX5", since there is evidence that variations in ALOX5 alter montelukast responsiveness in adult asthmatics. Parents will start the trial medication (either active or inactive granules given once a day) whenever their child develops a cold, and stop the medication when their child's wheeze resolves. Parents will also give the trial medication for wheeze between colds. For each family the trial will last 12 months, and during this period children will continue to receive standard inhaled therapy. During this period we will assess the number of unscheduled attendances to a medical practitioner for wheeze. At the end of the trial, we will determine whether montelukast is effective and whether there is a difference in response to montelukast between the children with a particular variant of the ALOX5 gene. We will also assess whether responsiveness to montelukast is affected by other genes and their relationship between genes and the amount of leukotrienes excreted in the urine.

A6-2. Summary of main issues. *Please summarise the main ethical and design issues arising from the study and say how you have addressed them.*

Informed consent in a paediatric population: The parent or legal representative of the child will have an interview with the investigator, or a designated member of the investigating team, during which opportunity will be given to understand the objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted. They will be provided with written information and contact details of the study nurse, who will be able to provide further information about the trial, and will be made aware of their right to withdraw the child from the trial at any time without the child or family being subject to any detriment in the child's treatment. Children will receive information, according to their capacity of understanding. In this trial's case limited, child friendly information will be available in the subgroup of children over 4 years of age, if considered appropriate.

Montelukast is currently licensed for use in preschool children (6 months to 5 years) as a continuous (i.e. daily) "add on" therapy to inhaled steriods, or as a monotherapy where inhaled steriods cannot be given. No child will be denied the standard effective therapy for wheeze in this age group i.e. "as required" inhaled short acting beta 2 agonist (salbutamol). The reported side effects of montelukast are mild, with a slight excess of headache, ear infection, sore throat, and upper respiratory infection reported in paediatric studies. These side effects have been reported with continuous use and are probably much less likely to occur with intermittent therapy. Montelukast can be safely given with all other anti-asthma medications. All children will receive "as required" inhaled salbutamol, and if clinically indicated, may receive regular inhaled corticosteroids. A child may be withdrawn from the trial they experience a serious adverse event which necessitates withdrawal, or if continuous oral montelukast is prescribed.

Benefits; A recent audit of "asthma" admission in children covering 67 hospitals during the period 1998-2005, found that 75% of 9,429 admissions were for preschool wheeze. Since this audit was based in secondary care-based audit, it underestimates the total number unscheduled attendances for preschool wheeze. A therapy that reduces the number of severe attacks will therefore have a major benefit to the NHS and children.

3. PURPOSE AND DESIGN OF THE RESEARCH

A8. Type of medicinal trial:

- ☐ Clinical trial of an unlicensed investigational medicinal product
- ☑ Clinical trial of a licensed medicinal product in new conditions of use (different from those in the SmPC, i.e. new target population, new dosage schemes, new administration route, etc.)
- ☐ Clinical trial of a licensed medicinal product used according to the SmPC
- ☐ Other (please specify)

A9. Phase of medicinal trial: *(Tick one category only)*

Human pharmacology (Phase I)	○ Yes	○ No
Therapeutic exploratory trial (Phase II)	○ Yes	○ No
Therapeutic confirmatory trial (Phase III)	◉ Yes	○ No
Therapeutic use trial (Phase IV)	○ Yes	○ No

A10. What is the principal research question/objective? *Please put this in language comprehensible to a lay person.*

The principal objective of this research is to determine whether intermittent parent-initiated treatment with oral montelukast in preschool children with a history of wheeze, reduces the need for unscheduled medical attention for wheeze. To assess this treatment will be started by parents or guardians i) at the onset of every cold and continued for a minimum of 7 days or until wheeze has resolved for 48 hours, and ii) for every episode of wheeze not associated with a viral cold, and stopped when symptoms have resolved for 48 hours. For each child, the trial will last 12 months.

A11. What are the secondary research questions/objectives if applicable? *Please put this in language comprehensible to a lay person.*

Respiratory morbidity

- Number of days with parent-reported wheeze over the 12 month trial period
- Number of admissions to hospital over the 12 month trial period
- Duration of admissions to hospital over the 12 month trial period
- Time to first attack of wheeze
- Number of unscheduled GP consultations for wheeze
- Duration of episodes by diary card
- Severity of episodes by diary card
- Parent's overall impression of efficacy of IMP (trial medication)

Health service use

- Unscheduled GP consultation with exacerbation of wheeze, expressed as time from randomisation to first attendance and annual attendance rate
- A&E attendance with wheeze exacerbation, expressed as time from randomisation to first attendance and annual attendance rate
- Unscheduled hospital admission with wheeze exacerbation, expressed as time from randomisation to first admission and annual rate of admissions
- Total duration of hospital admissions for exacerbation of wheeze

Adverse events

- Severe adverse events
- Withdrawal from the trial
- Mortality due to exacerbation of asthma
- Mortality due to respiratory infection
- All-cause mortality

Medication use

- Use of oral corticosteroids, expressed as number of courses taken per year, and proportion of children receiving at least one course of oral corticosteroids during the trial
- Use of inhaled relief medication (salbutamol), expressed as total number of occasions used over 12 month period, and mean number per wheeze episode
- Use of inhaled corticosteroids (ICS), expressed as mean daily dose of beclometasone equivalent over the 12 month trial period
- Regular prescription of inhaled ICS over the 12 month trial period

Inflammatory outcomes

- Association between baseline urinary cysteinyl leukotriene level and:ALOX5 status, Other polymorphisms of leukotriene genes, Previous phenotype of wheeze (viral-triggered episodic vs. multi-trigger, Responsiveness to montelukast

Genetic parameters

- Differential responsiveness to montelukast for the primary outcome in the stratum with ALOX5 promoter polymorphism [5/5], compared with the stratum with the ALOX5 [5/x + x/x]" genotype.
- Differential responsiveness to montelukast for the primary outcome resulting from other polymorphisms in genes

influencing leukotriene synthesis, leukotriene metabolism and leukotriene activity.

Economic outcomes

- Costs incurred by parents due to wheeze episode (including costs of travel to health care facility, childcare, and days absence from work)
- Costs of medical care provided for exacerbation of wheeze

Qualitative outcomes (parental)

- Attitudes towards genetic testing in order to personalise therapy
- Acceptability of parent-initiated therapy for preschool wheeze
- Experience of using the trial medication
- Difficulties/advantages of the parent-initiated approach
- Views on parent information sheet

A12. What is the scientific justification for the research? *Please put this in language comprehensible to a lay person.*

A quarter of preschool children between 1 and 5 yrs of age will develop at least one attack of wheeze. The majority of affected children have several attacks of wheeze trigged by viral-colds, with minimal or no symptoms between attacks. A minority of preschool children will also wheeze between colds. Preschool wheeze is a major clinical problem, with significant costs to primary and secondary care. There are at least 2 clinical patterns of preschool wheeze; episodic viral-triggered wheeze (i.e. wheeze that starts around the time of a cold and resolved rapidly)which affects the majority of affected children, and multiple trigger wheeze (i.e. wheeze that not only is triggered by colds - but by other things such as exercise, change in weather, and excitement) which affects the minority. A promising therapy for both clinical patterns of wheeze is montelukast (trade name; Singulair), the only blocker of the potent airway narrowing substance called cysteinyl leukotrienes (cLT) that is licensed for young children. An important role of cLT in preschool wheeze was suggested by our study which showed increased levels of urinary cLT's during attacks of preschool wheeze. A study relevant to "multi-trigger" preschool wheeze is a recent trial of montelukast in 689 young children where regular oral montelukast given over a 12 month period reduced the rate of attacks of wheeze. There may also be a role for montelukast in episodic (viral) preschool wheeze since a trial found that regular daily use of oral montelukast over 12 months reduced the rate of preschool wheezing episodes by 32% compared with placebo. Since most preschool wheeze is intermittent, intermittent therapy may be a more effective alternative to continuous therapy. Indeed in trial that including both preschool and school-age children (n=220), we found that montelukast, started only at the onset of colds, and stopped when the wheeze resolved, reduced the need for unscheduled health-care resource utilizations. The aim of this trial is therefore to assess whether parent-initiated montelukast therapy is efficacious in preschool wheeze.

The beneficial effect of montelukast found in the trials to date has been to date clinically "modest". The overall modest benefit of montelukast may because some children respond very well to montelukast, while others do not respond at all. Recent evidence from adults with asthma suggests that this marked variation in responsiveness is due to differences in genes controlling the synthesis, action, and metabolism of LTs . The first step in LT production is the conversion of LTA4 by membrane bound 5-lipoxygenase (ALOX5). The gene ALOX5 controls leukotriene synthesis, and is under the control of another area of DNA called the "ALOX5 promoter". Within this promoter are "SP1 transcription factor-binding motifs" and the number of these motifs affects the activity of ALOX5 and thereby LT production. Children with 5 "SP1 repeats" in the ALOX5 promoter are classified as having the "wild" type, with children carrying other numbers of repeats having the "mutant" genotype. Although we do not know whether this variation is important in children with wheeze, studies in adults have found that subjects carrying the variant number of repeats have a 73% reduction in the risk of having an asthma attack on montelukast compared with those with the wild-type. We hypothesise that the overall effect of parent-initiated montelukast therapy will be relatively modest, but that there will be a highly-responsive subgroup of children that can be defined by their ALOX5 promotor status. In this trial, we therefore include a stratification step for ALOX5 promoter polymorphism status, to ensure that an equal number of children with the variant and wild type number of SP1 repeats receive placebo and active medication.

A13. Please give a full summary of your design and methodology. *It should be clear exactly what will happen to the research participant, how many times and in what order. Please complete this section in language comprehensible to the lay person. Do not simply reproduce or refer to the protocol. Further guidance is available in the guidance notes.*

At the start of the trial, a member of the GP's child's usual care team, or a hospital paediatric clinician, will identify potentially eligible children from reviewing surgery and accident and emergency records. By letter, phone call or direct conversation a member the child's direct care team will then approach the child's parents or guardian, to ask if they would like to be contacted about the study by a member of the research team. Individuals who agree to be contacted about the study will then be contacted by a research nurse or research assistant, who will briefly describe the study to them, and ask them if they would like to read a parent information sheet (PIS). The research nurse or research

assistant will then post or give a PIS to patients expressing an interest in the study; those who subsequently confirm their interest in participation will be re-contacted and offered a screening appointment (visit #1) at a study site. A second invitation letter will be posted to individuals who do not respond to the first invitation letter. Potential participating parents will be offered a screening appointment. They will also be asked to bring their child's usual asthma medication with them when they attend the screening visit.

At the screening visit, an investigator, research nurse or research assistant will obtain written informed consent to participate in the trial from parents who are willing to take part in the study. The eligibility of children to participate in the study will then be assessed. The parents of all eligible will be asked to complete baseline assessments of their child's wheeze status including recording of baseline demographic and clinical data and details of concomitant medications, measurement of weight and height, taking a salivary sample using the Oragene paediatric collection system for extraction of DNA and assessment of leukotriene-associated genes, obtaining a urine sample for leukotriene analysis. A follow-up appointment will be arranged for the issue of the trial medication.

DNA will then be extracted from the salivary sample, and children assigned to either ALOX5 promoter polymorphism "5/5", or "[5/x and x/x]" genotype. Extracted DNA will be stored for batch analysis of 50 polymorphisms in 10 genes encoding components of the LT biosynthetic pathway and the LT receptors. The research nurse or research assistant will then assign a randomisation number to that child, and withdraw the corresponding box from pharmacy on behalf of that subject. The research nurse or research assistant will then arrange to meet with parents, medications will be reviewed, and if all baseline data has been collected satisfactorily, issue parents the box containing 50 sachets of the trial medication. Children whose parents are willing to participate but who do not meet eligibility criteria (i.e. no wheeze attack within 3 months) at their initial screening visit may be reassessed if they subsequently meet the eligibility criteria at some time in the future. Parents will be taught how to use the granules, reminded how to use the inhaled "as required" salbutamol metered dose inhaler and spacer.

At this point, in a subgroup of 30 families, we will also do a more extensive interview to establish their attitudes towards genetic testing to individualise therapy, acceptability of parent-initiated therapy for preschool wheeze, and the expected advantages and disadvantages of using the trial medication. In these families a further qualitative study will be done at the end of the study (12 months) to establish changes in their attitudes towards genetic testing to individualise therapy, acceptability of parent-initiated therapy for preschool wheeze, their experience of using the trial medication, and the difficulties and advantages with a parent-initiated approach. Either one parent will be interviewed or, if they prefer, a joint interview with both parents will be done. Where possible, interviews will be done at the parental home. Interviewing, transcribing, and analysis of interviews will be done by a research assistant.

Parent will then be contacted by telephone monthly (i.e. a total of 12 times) by the research nurse or research assistant to check whether the parent has started the , the numbers of days the trial mediation was used, use of healthcare resources, concomitant medications, adverse events, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Parents will be asked to phone the research nurse when they start and stop each course of the trial medication via a freephone number. Data recorded are the number of days of wheeze, GP attendance, admission to hospital, need for additional asthma therapy, adverse events, procedures, days lost from childcare, and parent days lost from work.

A14-1. In which aspects of the research process have you actively involved, or will you involve, patients, service users, and/or their carers, or members of the public?

- [x] Design of the research
- [x] Management of the research
- [x] Undertaking the research
- [ ] Analysis of results
- [x] Dissemination of findings
- [ ] None of the above

Give details of involvement, or if none please justify the absence of involvement.
Parents have been involved at all stages of this trial's design. First, we surveyed 98 parents to determine whether they considered our primary outcome to be the most appropriate. The majority considered "severe attacks of wheeze" to be the most appropriate outcome. Second, a mother of a child with preschool wheeze has advised us in the protocol design. Third, we have embedded a qualitative study of parental attitudes towards genetic testing, acceptability of

parent-initiated therapy for preschool wheeze, their experience of using the trial medication, any difficulties they experienced with this approach, and their views on pharmacogenetic studies in this age group. Fourth, at each of the recruitment centres we will establish parental advisory groups (PAG) responsible for advising researchers on the best way of approaching and involving parents in the trial, and on disseminating its results.

4. RISKS AND ETHICAL ISSUES

RESEARCH PARTICIPANTS

A17-1. Please list the principal inclusion criteria (list the most important, max 5000 characters).

• age ≥ 10 months and ≤ 5 years on the day of the first dose of IMP.
• two or more attacks of parent-reported wheeze,
• at least one attack with wheeze validated by a clinician
• the most recent attack within the last 3 months.
• contactable by telephone and able to attend one face-to-face review for issue of IMP
• parent or guardian able to give written informed consent for their child participate in the study.

A17-2. Please list the principal exclusion criteria (list the most important, max 5000 characters).

•• any other chronic respiratory condition diagnosed by a clinician including structural airway abnormality (e.g. floppy larynx) and cystic fibrosis
• any chronic condition that increases vulnerability to respiratory tract infection such as severe developmental delay with feeding difficulty
• history of neonatal chronic lung disease
• current continuous oral montelukast therapy
• in a trial using an IMP within the previous 3 months prior to recruitment.

RESEARCH PROCEDURES, RISKS AND BENEFITS

A18. Give details of all non-clinical intervention(s) or procedure(s) that will be received by participants as part of the research protocol. *These include seeking consent, interviews, non-clinical observations and use of questionnaires.*

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.
2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?
3. Average time taken per intervention/procedure (minutes, hours or days)
4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Consent process for trial at screening visit (#1)	1	0	30	Parents will be given ample time to review the parent information sheet and will have the opportunity, if they wish, to ask questions to a study clinican either directly or via telephone prior to consent.
Medical History at screening visit (#1)	1	0	10	Details will include the child's previous pattern of wheeze, family history of asthma, and treatment
Issuing trial medication at visit #2	1	0	10	Parents will be taught on the use of the trial medication
Monthly telephone call from research nurse	12	0	5	Parents will be asked whether they have used the trial medication, number of days used, need for unscheduled medical attention, and any questions answered.
Phone conversation initiated by parent if medication is started and when it is stopped.	4	0	5	Number of attacks of wheeze is estimated here. Parents will leave a message on a freephone number

A19. Give details of any clinical intervention(s) or procedure(s) to be received by participants as part of the research protocol. *These include uses of medicinal products or devices, other medical treatments or assessments, mental health interventions, imaging investigations and taking samples of human biological material. Include procedures which might be received as routine clinical care outside of the research.*

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.
2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?
3. Average time taken per intervention/procedure (minutes, hours or days).
4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Salivary sample for DNA at screening Visit #1.	1	0	2	This will use the Oragene infant collection system
Urine sample for urinary leukotriene at Visit #2	1	0	5	Parents will be given a cardboard potty or a urine bag at Visit #1 and asked to collect and store urine in the freezer prior to visit #2

A20. Will you withhold an intervention or procedure, which would normally be considered a part of routine care?

( ) Yes (•) No

A21. How long do you expect each participant to be in the study in total?

The total study duration for each participant (child) is 12 months.

A22. What are the potential risks and burdens for research participants and how will you minimise them?

For all studies, describe any potential adverse effects, pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Only describe risks or burdens that could occur as a result of participation in the research. Say what steps would be taken to minimise risks and burdens as far as possible.
Ethical research must seek to minimise potential inconvenience, discomfort and risk that children and their parents may experience during the course of a study. The principle inconveniences of the study arise from the time spent by parents to attend for the Screening Visit 1 and Visit 2 where the trial medication will be issued. We have sought to minimise this by providing reimbursement of reasonable travel expenses incurred as a result of participation in the study. The principle discomfort involved arises from collecting samples for DNA analysis. We have sought to minimise this by using a specially designed saliva collection system for infants (Oragene-child/infant collection system) which does not need scraping of the buccal mucosa. The risks associated with montelukast therapy are very low, with no significant risks reported. We have sought to minimise risks further by review by the data monitoring committee of accumulating data relating to adverse events. Montelukast is licensed for use in preschool children (6 months to 5 years) as a continuous "add on" therapy. Side effects of montelukast are mild, with a slight excess of headache, ear infection, sore throat, and upper respiratory infection reported in paediatric studies. These side effects have been reported with continuous use and are probably less likely to occur with intermittent therapy. Montelukast can be safely given with all other anti-asthma medications. No child will be denied the main effective therapy for wheeze in this age group i.e. "as required" inhaled short-acting beta 2 agonist (salbutamol). All children will receive "as required" inhaled salbutamol, and if clinically indicated, may receive regular inhaled corticosteroids. A child may be withdrawn from the trial they experience a serious adverse event which necessitates withdrawal, or if continuous oral montelukast is prescribed.

A23. Will interviews/ questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

( ) Yes (•) No

A24. What is the potential for benefit to research participants?

If intermittent montelukast is effective in reducing severe wheeze attacks, then half of children in this study, who would not have normally received intermittent montelukast, would have additional benfit over and above standard care. Children receiving placebo in the trial will not have been disadvantaged over normal standard care since both "as required" inhaled bronchodilator (salbutamol) and regular inhaled steriods may be continued during the trial.

A25. What arrangements are being made for continued provision of the intervention for participants, if appropriate, once the research has finished? *May apply to any clinical intervention, including a drug, medical device, mental health intervention, complementary therapy, physiotherapy, dietary manipulation, lifestyle change, etc.*

At the end of the study, there will be an option for a clinican to continue to prescribe montelukast as licenced, if clinically indicated. As described above, montelukast is a licenced medication for preschool and school age children.

A26. What are the potential risks for the researchers themselves? *(if any)*

None

RECRUITMENT AND INFORMED CONSENT

In this section we ask you to describe the recruitment procedures for the study. Please give separate details for different study groups where appropriate.

A27-1. How will potential participants, records or samples be identified? Who will carry this out and what resources will be used?*For example, identification may involve a disease register, computerised search of GP records, or review of medical records. Indicate whether this will be done by the direct healthcare team or by researchers acting under arrangements with the responsible care organisation(s).*

Recruitment in primary care will use the East London Academic- (under Professor Griffiths) and Norwich Academic-primary care networks (under Professor Price). To identify children in primary care, pratices (Participant Identification Centres) will search their medical records on GP computer systems for children aged 10 to 60 months with Read coded records suggestive of wheeze and wheezing disorders including asthma or prescription for asthma therapy. A letter of invitation for assessment of eligibility, signed by the child's GP, will be sent to parents of children with a record of doctor-diagnosed wheezing illness.

In secondary care at the Royal London Hospital (under Professor Grigg), University Hospitals of Leicester Children's Hospital Accident and Emergency and General Wards (under Dr Pandya), the Norfolk and Norwich University Hospitals NHS Trust Accident and Emergency Department (under Professor Price), and the Royal Aberdeen Children's Hospital Accident and Emergency Department and General Wards (under Dr Turner), paediatricians will prospectively screen children presenting to their outpatient clinics, accident and emergency departments, and admitted to the wards. Once children have been identified, an invitation letter will be sent or given to families from their GP, or hospital consultant as appropriate. Parents expressing an interest will be sent by post or given in person the information sheet, followed by a telephone call inviting them to attend their local trial centre, at a convenient time. Parental visits will be at; 1) the Barts and the London Children's Hospital, 2) The University Hospital of Leicester Children's Hospital, 3) the Royal Aberdeen Children's Hospital, and 4) the Norfolk and Norwich University Hospital.

A27-2. Will the identification of potential participants involve reviewing or screening the identifiable personal information of patients, service users or any other person?

(•) Yes ( ) No

Please give details below:
Potential children who may be candidates for this trial will be discussed by members of the clinical team looking after them as part of their routine clinical care.

A27-4. Will researchers or individuals other than the direct care team have access to identifiable personal information of any potential participants?

8.3.2 Ethics committee approval

NHS
National Research Ethics Service
South East Research Ethics Committee
South East Coast Strategic Health Authority
Preston Hall
Aylesford
Kent
ME20 7NJ

Telephone: 01622 713048
Facsimile: 01622 885966

23 November 2009

Professor Jonathan Grigg
Professor of Paediatric Respiratory Medicine
Queen Mary University London/Barts and the London School of Medicine
4 Newark Street
London
E12AT

Dear Professor Grigg

Study Title:	**Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.**
REC reference number:	**09/H1102/110**
Protocol number:	**1**
EudraCT number:	**2009-015626-11**

The Research Ethics Committee reviewed the above application at the meeting held on 11 November 2009. Thank you for attending to discuss the study.

Ethical opinion

The committee started by commending you on your application.

The committee stated that they had not been provided with the topics that were to be covered in the interview process.

You stated that the interview was covered in the protocol. You went on to say that the questions had been created using feedback from parents.

The committee drew your attention to the PIS and stated that it would need to be amended to contain details regarding dosage, side effects, the length of treatment and the risk of overdose.

You agreed with this and went on to state that it would also cover what would happen if a participant vomited out the drug.

The committee asked about the length of time between the start of treatment and the point at which the researcher would be contacted.

You stated that the parents / guardians of the participant would contact the researcher at the start of treatment and then contact would be made again, 7-10 days after that. Contact would subsequently be made on a monthly basis.

The committee suggested that a 'What will happen when I initiate Therapy?' section should be added to the PIS.

You agreed to this.

The committee then asked how it would be judged if the child participant really had a cold.

You stated that this would be down to the parents and suggested that they were experienced and knowledgeable enough to make the judgement. In addition to this, parents would be given training on specific triggers to watch out for.

The committee asked what would happen if the treatment was started when the child participant didn't actually have a cold.

You agreed that this could happen - but assured the committee that it was a safe medication.

The members of the Committee present gave a **favourable ethical opinion** of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

a) The PIS should indicate that travel expenses will be covered.
b) In pt. 1 of the PIS it states that steroid tablets do not work. This statement should be removed.
c) The PIS should be proofread throughout. There a few errors that need correcting (for example, the word 'sue' appears instead of the word 'use').
d) The consent form needs to have boxes inserted so that participants have definite areas to tick.
e) The PIS needs to advise parents of participants who hold private medical insurance covering the child that they should inform their insurance companies that they are taking part in the trial.
f) At A53 on the application it states that a lay summary of findings will be offered. In the PIS it states that this summary may be requested. The PIS should be amended to read that the summary will be offered.
g) The PIS is missing information relating to the possible side effects of the treatment. It also needs to provide information on the length of treatment and the possible risks of overdose.
h) A 'What will happen when I initiate Therapy?' section should be added to the PIS.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering Letter		19 October 2009
REC application		19 October 2009
Protocol	1	01 October 2009
Investigator CV	Prof Jonathan Grigg	
Participant Information Sheet: Parent (PartISWAIT)	1	01 October 2009
Participant Information Sheet: Child	1	01 October 2009
Participant Consent Form: Parent / Guardian (PCFWAIT)	1	01 October 2009
Letter of invitation to participant	1	01 October 2009
GP/Consultant Information Sheets	1	08 October 2009
Evidence of insurance or indemnity		19 October 2009
Sample Diary/Patient Card	1	08 October 2009
Summary of Product Characteristics	SPCmontelukast	12 March 2009
Sponsorship Approval Letter		19 October 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Prof C Katona declared a non-specific interest in this study.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out

the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1102/110	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr L. Alan Ruben
Chair

Email: dean.beattie@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers"

Copy to: *Mr Gerry Leonard*

8.3.3 Medicines and healthcare products regulatory agency (MHRA) approval

Safeguarding public health

MHRA

Dr J Grigg
BARTS AND THE LONDON NHS TRUST
2 NEWARK STREET
LONDON
E1 2AT
UNITED KINGDOM

22/02/2010

Dear Dr J Grigg

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	21313/0024/001-0001
Eudract Number:	2009-015626-11
Product:	Singulair Paediatric 4mg Granules
Protocol number:	006983 QM

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 12/02/2010.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
Market Towers 1 Nine Elms Lane London SW8 5NQ
T 020 7084 2000 F 020 7084 2353 www.mhra.gov.uk

An executive agency of the Department of Health

8.3.4 Sponsor approval

Sponsorship Approval Letter

Joint Research and Development Office
5 Walden Street
Whitechapel
London E1 2AN

Tel: 0207 882 7250
Fax: 0207 882 7276

Prof. Jonathan Grigg
Prof. of Paediatric Respiratory Medicine
Queen Mary University of London
ICMS
The Blizard building
4 Newark St
E1 2AT

19th October 2009

Dear Professor Grigg,

This letter is to confirm that **Queen Mary University of London** will act as a sponsor for the project stated below.

Project Title: Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype: WAIT

Chief Investigator: Professor J Grigg

ReDA No: 006539 QM

Sponsorship will remain in effect until the completion of the project and the ongoing responsibilities of the Chief Investigator as stated in the sponsorship agreement have been met.

Should the Chief Investigator fail to notify the Joint R&D Office of a substantial amendment to the project, this may result in incorrect indemnity or sponsorship cover and therefore the project may not be fully covered.

The sponsor may terminate this arrangement with immediate effect if:

- It is reasonably of the opinion that the project should cease in the interests of the safety of participants or staff involved in the project.
- The Chief Investigator is no longer (for whatever reason) able to act as Chief Investigator and no mutually acceptable replacement can be found.
- The Chief Investigator does not adhere to the responsibilities stated in the conditions of sponsorship letter.

Please see page 2 for more details of the conditions of sponsorship of the Chief Investigator.

For Multicentre Projects

It is the responsibility of all **Principal Investigators at each site** to ensure

- That they and all members of the research team comply with all current regulations applicable to the performance of the project including but not limited to, the NHS Research Governance Framework for Health and Social Care (April 2005), the most recent version of the World Medical Association Declaration of Helsinki, the UK Medicines for Human Use (Clinical Trials) Regulations (2004) and subsequent amendments, Good Clinical Practice Guidelines, the Human Tissue Act (2004) and the Data Protection Act (1998).
- Indemnity for negligent harm is obtained from their employing organisation.
- **Serious Adverse Events (SAE's) and Suspected Unexpected Serious Adverse Reactions (SUSAR's) must be reported within a working day of learning** of the event to the Chief Investigator at the Lead site and to your own local Research and Development Office. If applicable, the incident should also be reported using the Trust specific incident reporting procedure.
- If the project is a clinical trial of a medicinal product, a sponsorship agreement with the Sponsor is signed.
- **Please use page 2 – the conditions of sponsorship of the Chief Investigator as a guideline for good research practice and ensure you adhere to your own Trust policies and R&D arrangements.**

Yours sincerely,

8.3.5 Protocol amendments

The study underwent a number of protocol and other amendments. Where an amendment has not been subject to ethics committee or MHRA scrutiny it has been deemed non-substantial by the sponsor. These amendments are summarised in the list below and detailed in Table A. Approval letters are available but for brevity are not included here.

1. Change to meet initial Ethics committee conditions (before study commenced)
    a. PIS update to include dosage, duration, side effect, rx duration, action if vomited, overdosage outcomes.
    b. Update regarding parent-researcher contact time
    c. "What will happen when I initiate therapy section" added
    d. PIS states travel expenses will be covered.
    e. Statement re: inefficacy of steroids removed
    f. Typographical errors corrected
    g. Informed Consent form has checkboxes added.
    h. Advice to parents re: contacting their insurers added.
    i. Update to say that lay summary of findings will be offered
2. Change to allow specific tests in Aberdeen (EBC, LF, SPT, FeNO - never performed).
3. Diary card changes designated as minor amendment.
4. Permission to repackage meds into smaller boxes due to reduced supply
5. Invitation sheet amended with "or has been prescribed meds for wheeze" to explain why child has been identified as a possible participant.
6. Multiple new site additions.
7. Removal of DSMC charter from protocol.
8. Removal of Aberdeen extra tests from protocol (these were never performed).
9. Addition of cover letters for primary care and hospital identified patients.
10. PIS amended to state that montelukast is not a new/experimental drug.
11. Addition of a recruitment poster.
12. Addition of a GP invitation letter.
13. Update to GP recruited/not-recruited letters (tidier format, reference to website).
14. Update to allow medications sourced from outside the EU.
15. Update to introduce parent reminder sheet (an aide-memoire)
16. Update to allow Novalabs to have a primary packaging role.
17. Final protocol update
    a. To reflect multicentre nature of trial (esp pharmacy)
    b. To allow 24 hrs OR an overnight stay for parental consideration of PIS.
    c. Removal of reference to weighing salbutamol canisters.

d. To allow 2nd urine specimens to be collected in other sites as well as London
e. To clarify status of viral wheezing episodes as not being adverse events.
f. Remove ambiguity re: need for parents to contact trial team when starting medicine.
g. Remove reference to PCTU in PIS

18. Addition of failed contact letters

TABLE 8-2 - TABLE OF PROTOCOL AMENDMENTS

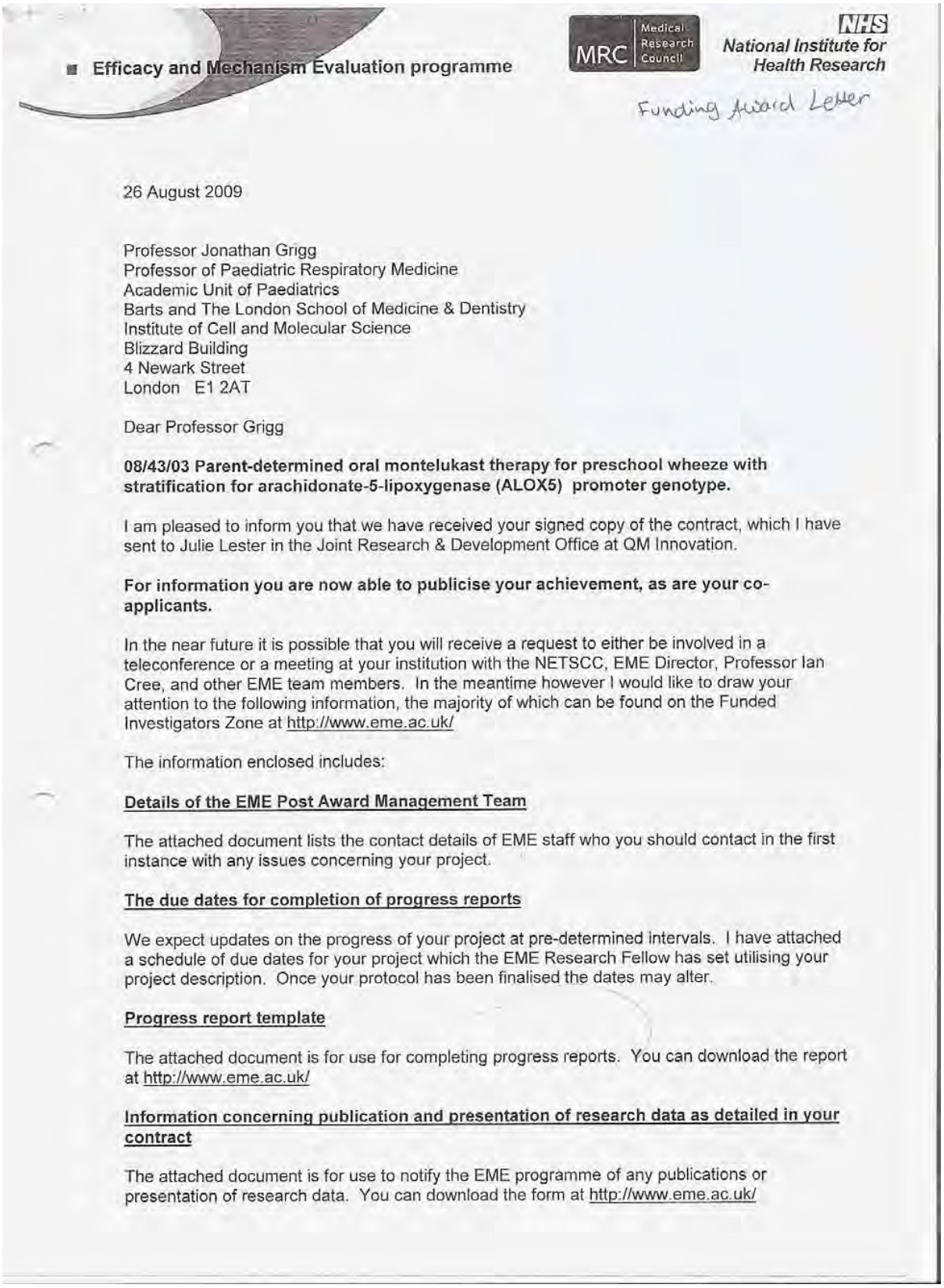
Amendment Identifiers:			Description of Protocol Version Change or Amendment	Date Submitted to REC	Date of Approval	New Detailed Protocol Version Date
Ame	REC/MHRA #	Type				
1	Protocol No 1	Major	(Initial Protocol Submission to REC)	1/10/09	23/11/09	V1 - 1/10/09
N/A		N/A	Internal minor revisions - not submitted	N/A	N/A	V2-3 - 23/04/10-11/9/10
2	Change to PIS	N/A	Update to meet initial REC-imposed approval conditions	12/9/10 (from cover letter)	25,27/10/10 (received dates, no approval date given)	V4 - 7/6/10
3	(1)	Major	Amendment to allow additional tests in Aberdeen	13/10/10 (from cover letter)	27/10/10 (not 27/7/10 as per REC letter)	V5 - 26/7/10
4	N/A	Minor	Diary Card Minor Amendment	N/A	Internal	
5	Nil - email approval	Minor MHRA	MHRA NSA to split boxes	11/3/11	11/3/11	
6	Parent Invitation Sheet - Change in text	Minor	NSA Parent Invitation Sheet v3	30/03/11	5/4/11	
7	Amendment No 2, Protocol v6	Major	1. New Sites, Principal Investigators, Local Collaborators (PCT) added to protocol. 2. Research nurses, Statistician details added to protocol. 3. DSMC charter removed from protocol for clarity - stored separately in TMF. 4. GP Cover letter to parent (with reminder letter) added to improve primary care recruitment and protect patient information. 5. Aberdeen amendment (Protocol 5) removed from protocol. 5. Hospital attendee cover letter added to clarify information sheet. 6. Text: "Montelukast is not a new medicine and has been licensed as safe for use in young children for several years." Added to PIS V2	11/3/11	14/4/11	V6 - 10/3/11
8	Amendment No 3, protocol v6	Major	WAIT GP Letter v1 081009* → WAIT GP Letter v2 13 April 2011 GP Invitation Letter v1 13 April 2011 WAIT Poster v1 13 April 2011 (REC letter says 081011 - which is in the future and hence erroneous)	13/4/11	28/04/11	
9	21313/0024/001-0003	Major MHRA	Amendment to allow use non-EU sourced IMP	14/4/11	6/5/11	

Amendment Identifiers:			Description of Protocol Version Change or Amendment	Date Submitted to REC	Date of Approval	New Detailed Protocol Version Date
Ame	REC/MHRA #	Type				
10	Amendment No 4, protocol v6	Major	New sites and Parent reminder sheet v1	1/6/11	22/6/11	
11	Amendment 5, Protocol No 6	Major	New sites	24/6/11	12/7/11	
12	21313/0024/001/004	Major MHRA	To allow Novalabs to package unsacheted raw material from manufacturer	30/6/11	13/7/11	

Amendment Identifiers:			Description of Protocol Version Change or Amendment	Date Submitted to REC	Date of Approval	New Detailed Protocol Version Date
Ame	REC/MHRA #	Type				
13	Amendment No 5, protocol v7	Major	Replacement of protocol v6 with v7. This updates the protocol to reflect: Additional trial sites Updated medicines distribution and replacement procedure - to allow dispensing from local sites and account for varying date and need for replacement. Update timing of interview to reflect convenience of subject being paramount. Update to allow 24 hr **or** an overnight stay as minimum duration for consideration of trial information before consent to enter trial - to account for occasional lack of documentation of timing of initial approach, and also to allow recruitment following an overnight admission rather than enforcing a home visit or parental clinic visit. This suggestion has met with approval from parents. Update to remove reference to weighing of salbutamol canisters. Usage is estimated from parental reporting in diary cards. This change was recommended by the trial steering committee as weighing canisters was deemed impractical. Update to suggest 2nd urine specimens (during illness) be collected at all sites rather than solely the Royal London Hospital. Update to specify that viral and wheezing episodes that trigger IMP use not be deemed as adverse events, as this temporal association is dictated by the protocol instructions to parents (they are told to use the IMP when their child has a cold or wheeze). Replacement of "Parent Reminder Sheet v1 250511.doc" with "Parent reminder sheet v2 240611.doc". This removes the erroneous reference to parents contacting trial team when starting medicine. Replacement of "Parent Information Sheet v2 290311.doc" with "Parentinformationsheet-version3 220611.doc". This corrects some misinformation regarding trial procedure (details in 1a-g and in tracked changes version). In particular, references to the clinical trials unit are removed as they are not actually involved in the study. Addition of new sites with associated PIs to the study protocol as an appendix (this forms a separate amendment as discussed with Sophie Vella). Addition of documents: "Wait failed contact letter - general v1 130611.doc" "Wait failed contact letter - medicines v1 130611.doc" For use when telephone contact proves impossible.	24/6/11	17/8/11	
14	(1)	Major	We would like to request approval for the addition of a consent form to allow the use of patient data on children who are not recruited to the study. This will allow us to ascertain the characteristics of those who are not recruited as well as those who are.	22/7/11	Rejected 17/8/11	

Amendment Identifiers:			Description of Protocol Version Change or Amendment	Date Submitted to REC	Date of Approval	New Detailed Protocol Version Date
Ame	REC/MHRA #	Type				
15	Amendment No 6, protocol v6	V7	New Sites	29/7/11	4/8/11	V7 - 24/6/11
16	Nil stated	Major	PIS V3→V4 to explicitly state that specimens will be sent to London To state that a copy of the information will be stored in London To modify consent form for unrecruited patient data retention (see 14)	24/8/11	26/10/11	
17	AM14	Minor	Modification to make WAIT Clinic Letter v2 → 2.1 (generalizable)	3/10/11	1/11/11	
18	Nil stated	Major	Close out letter and certificate of thanks	23/9/11	26/10/11	
19	Minor - Diary Card v4	Minor	Minor modifications diary card v 3→4	24/1/12	22/6/12	
20	AM15	Major	New Sites (Hinchingbrooke, Kilmarnock, etc.)	6/3/12 (*n.b. REC letter stated erroneous receipt date due to revised submission)	7/3/12	
21	16	Major	Additional PI (Chester)	9/3/12 (REC letter says 20/3/12)	4/4/12	
22	Addition of sites (Bradford Teaching hospitals trust and Calderdale and Huddersfield Trust)	Major	New Sites (Huddersfield, Bradford)	22/6/12	6/7/12	
23	Extension of study until 30.4.14.	Minor	Study Extension	20/6/12	21/6/12	
24	Amendment - Protocol v8	Major	Protocol V8 - to allow repeat urines/testing for urinary cotinine	27/6/12	17/7/12	V8 - 20/6/12
25	23	Major	New Sites(Luton, Airedale, Warrington, Durham)	16/7/12 (REC letter says 24/6/12)	15/8/12	
26	17	Major	Change of PI - Royal Berkshire	2/4/12	4/4/12	

8.3.6 Funding award letter



Efficacy and Mechanism Evaluation programme

MRC Medical Research Council

NHS National Institute for Health Research

Funding Award Letter

26 August 2009

Professor Jonathan Grigg
Professor of Paediatric Respiratory Medicine
Academic Unit of Paediatrics
Barts and The London School of Medicine & Dentistry
Institute of Cell and Molecular Science
Blizzard Building
4 Newark Street
London E1 2AT

Dear Professor Grigg

08/43/03 Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.

I am pleased to inform you that we have received your signed copy of the contract, which I have sent to Julie Lester in the Joint Research & Development Office at QM Innovation.

For information you are now able to publicise your achievement, as are your co-applicants.

In the near future it is possible that you will receive a request to either be involved in a teleconference or a meeting at your institution with the NETSCC, EME Director, Professor Ian Cree, and other EME team members. In the meantime however I would like to draw your attention to the following information, the majority of which can be found on the Funded Investigators Zone at http://www.eme.ac.uk/

The information enclosed includes:

Details of the EME Post Award Management Team

The attached document lists the contact details of EME staff who you should contact in the first instance with any issues concerning your project.

The due dates for completion of progress reports

We expect updates on the progress of your project at pre-determined intervals. I have attached a schedule of due dates for your project which the EME Research Fellow has set utilising your project description. Once your protocol has been finalised the dates may alter.

Progress report template

The attached document is for use for completing progress reports. You can download the report at http://www.eme.ac.uk/

Information concerning publication and presentation of research data as detailed in your contract

The attached document is for use to notify the EME programme of any publications or presentation of research data. You can download the form at http://www.eme.ac.uk/

Submission of a final report

Although due some time in the future, I would like to draw your attention to details on the EME website (http://www.eme.ac.uk/) concerning the submission of your final report. Please read these carefully as financial penalties may be incurred if you do not follow the guidelines.

I also enclose a Post Award Management pamphlet for your information.

EME Experts Group

The EME programme expects all applicants and co-applicants to become members of the "EME Experts Group". This group may be called upon to act as peer reviewers for other EME projects in the future. If you haven't done so already please follow the link to complete the experts form. http://www.eme.ac.uk/getinvolved/referee.htm and also ensure that your co-applicants complete the form.

In my previous letter I requested nominations for Chairs and membership of the TSC and DMEC and details of your ISRCTN.

Once they become available please also remember to send the ethics approval documentation, CTA approvals, project management plan and final protocol approved by the Ethics Committee.

I know you are dealing with these requests and I look forward to receiving them in due course.

Please do keep me informed of developments and progress with obtaining the above and I shall be in contact regarding these too as your project nears its start date. If you have any queries in the meantime please don't hesitate to contact me.

Yours sincerely

Jane Sinclair
Programme Manager
NETSCC, EME

8.4 Appendix 4 - Informed consent process

All subjects participated with the understanding and written consent of their parent or guardian. Where necessary a translator was employed. The information sheet (6.5.1) and consent form (6.5.2) are below.

8.4.1 Patient information sheet

Centre Name: Royal London Hospital ☐

Centre Number: _____ (as appropriate)

The "WAIT" Study; Wheeze And Intermittent Treatment

We are inviting parents[11] and their children to take part in a research study. Before you decide if you would like to take part it is important that you understand why the research is being done and what it will mean for you and your child. This information sheet gives all of the important information about our trial. We have divided this information sheet into two parts:

Part One

Tells you the purpose of the research and what will happen if you decide to take part.

Part Two

Gives you more detailed information about how the study will be organised.

Part 1

What is the purpose of the study?

Attacks of wheeze (the noise we make when our airways become narrowed) are very common in children under 6 years of age (we call this preschool wheeze). Most of these attacks happen during colds, but in some children wheeze can happen between colds as well. We know that most young children grow out of their wheeze after the age of 6 years. At the moment we don't know the best way to stop these attacks of wheeze but we think that a medicine called "Montelukast" will make the attacks of wheeze less severe. Montelukast stops a substance in the body called "leukotriene" from narrowing the airways and causing wheeze. It is already licensed as safe for young children - but at the moment is used only as an "add on" to regular inhaled steroids and it has to be given every day.

[11] Where we use the word 'Parent' we mean people who have parental responsibility, which may include a legal representative (guardian).

We think that Montelukast may be helpful in preschool wheeze on its own, and that regular daily use may not be necessary. We have designed this study to see if Montelukast, if started by parents at the first sign of a cold and stopped when children are better, may prevent wheeze becoming so bad that your child needs to see your GP or emergency doctor. To see if montelukast really works in the way we think we have to give some children the active medicine - montelukast coated onto granules of sugar taken by mouth once a day - and some children the sugar granules only (with no montelukast). No one knows in advance which one your child will get. This sort of study is called a "randomised controlled trial".

Studies of montelukast in adults with asthma have shown that genetic make-up affects whether someone responds very well, or not so well to montelukast. Another aim of our trial is to measure the amount of leukotriene produced by the body and the genes that control it to see if we can identify children who may respond very well to montelukast.

What is the drug, device or procedure that is being tested?

The medicine that we are testing is called montelukast. Its "trade name" is "Singulair". Montelukast is not a new medicine and has been licensed as safe for use in young children for several years. It comes as granules in individually packaged sachets and can be given either directly into the mouth, or mixed with a spoonful of cold or room temperature soft food (e.g. apple sauce, ice cream, carrots or rice). The granules consist of a sugar core with a fine coating of the drug. Each dose of montelukast granules stops the airway narrowing effect of leukotriene for 24 hours - so you only need to give it once a day. Some children will be given the core sugar granules, but without the coating of montelukast, this is called a placebo medicine and has no effect. These are packed so they look and taste exactly the same as montelukast granules.

Why has my child been chosen?

Your child has been asked to take part in this study because he/she has had at least 2 episodes of wheeze. Your General Practitioner (GP), specialist asthma nurse or hospital doctor thinks your child might be suitable to take part in this study and wants to refer them to the research team to assess this. We will be recruiting 1300 children for this study from a number of children's hospitals across the UK as well as from GP practices.

Does my child have to take part?

No, taking part is completely voluntary. It is up to you to decide whether or not to take part. Even if you do agree to join, you can drop out at any time without giving a reason. A decision to leave the study, or a decision not to take part, will not in any way affect the standard of

care you and your child receive now or in the future. If you change your mind about staying in the study we would appreciate it if you would let us know. The study doctor may also stop your child from taking the study treatments at any time if they feel it is best for them to do so. However, if this happens, they will still want to carry on collecting information from your child if you both agree.

What will happen to my child if we agree to take part and how long will it take?
If you do take part, you will be given this information sheet to keep and be asked to sign a consent form. We would like your child to remain in the study for a year. If you agree to take part, you will have another visit to receive the medication. After that we will be contacting you by phone or email only. We may ask some of you to allow us a more extended interview about parents' views on the study and if so we will visit your home at the end of the study. Each visit will last under an hour. We will now explain what will happen at each of the visits.

Visit 1[12].
If you are interested in taking part, and are satisfied with the explanations from your research team, you will be asked to sign a consent form at your first clinic visit. You will be given a copy of this information sheet and your signed consent forms to keep. Once consent has been given, you and your child will be asked some questions to make sure that they are suitable to join. The research doctor or nurse will want to know about your child's wheezing symptoms. They will ask some questions about your child's medical history and what other medicines they are taking. We will check that you can use the salbutamol (blue) inhaler properly so that they are getting the right amount of medication each time they use it. The doctor or nurse will also collect a saliva sample from your child using a specially designed mini-sponge which is entirely painless. The saliva will be analysed for genes (DNA) for leukotrienes. We will give you a container to collect some urine on the day of visit number 2. We will measure the amount of leukotrienes in your child's urine.

The amount of leukotriene in our urine can be affected by exposure to tobacco smoke (this can come from the air breathed out by smokers nearby - it does not mean that you or your child is a smoker) and so we will also measure levels of cotinine (produced if we are exposed to tobacco smoke) in the urine samples. This will make it easier for us to understand the results of the urinary leukotriene measurement. If you like we will tell you the cotinine result at the end of the study. The amount of leukotriene in the urine may also vary with time or illness, so we will collect a repeat sample if you come into hospital with wheeze, and also at

[12] The study group will pay a £10 contribution to clinic visit travel expenses for you and your child.

the end of your year in the study at a time when your child is well. We will only do this if it is convenient to you.

Visit 2.

If you are happy to, and the doctor or nurse says you are suitable to take part, we will invite you to come for a second visit at a convenient time for you - normally within 2 weeks of the first visit. We may be able to visit your home, if you agree. We will then give you a box of sachets and instruction on how to use them. We will also give you a simple diary card to fill out when/if you have to use the medicine. The doctor or nurse will talk to you about it and answer any questions you have. There is space to write down anything you think is important for the nurse to know next time you see them. If you agree, we will let your GP know that your child has been enrolled into a study.

Your study doctor and/or nurse may ask your permission to make a sound recording of the interview when we give you the trial medicine and at the end of the study. This is because in a small number of parents we would like to find out their views on parent-guided medicines and whether we can improve the experience of parent and children in future studies. This is an “add on” study; you can take part in the main study without agreeing to this.

Phone Calls

You will be contacted once every two months by the research nurse. She will check whether you have used the trial medicine, and whether you have visited your GP or the Hospital. If you child has had an attack of wheeze she will ask you about the effect on the family, including things such as additional child care and days off work. If you have to use the trial mediation we will ask you to post to us the completed diary card (and empty sachets) using a provided freepost envelope.

Replacement Medicines

If your child uses all their medicines, or the medicines reach their use-by date, we will contact you to provide you with a replacement box. If we do not contact you (or the medicines are lost or damaged) please contact us on the number provided. Do not attempt to get replacement medications from your GP or hospital doctor.

Extended Interview

If you have agreed to the optional extended interview a researcher will contact you and arrange a time that is convenient to you.

At 12 months

The study finishes for your child. We will ask you to send back all the used and unused medicine sachets.

What does my child have to do if we agree to take part?
Your child will need to provide a saliva sample and urine sample(s).
You should give your child the study medicine if they get a cold or wheeze attack
There is nothing unpleasant or painful involved in the study.
You will need to complete a symptom diary during attacks of wheeze.
You should tell the research doctor or nurse about any other medicines your child is taking. It is important to make sure that any other doctor your child visits knows that they are taking part in this study. Names and contact telephone numbers of the people running this study will be in the diary which is issued to you at your first visit. The study doctor will write to your GP and let them know that you are taking part in the research study.

What will happen when I start treatment?
You will give your child 1 sachet of medication either directly into the mouth or mixed with cold or room temperature food from the start of every cold or wheezing attack.
You will continue to give this every day for 10 days, even if your child gets better.
You will complete a simple 10-day diary card for every course of medicine.
If your child vomits after taking the medicine no additional dose should be given, and the vomit should be recorded on the diary chart.
You should give all other medicines to your child as normal.
You will inform the research team (see contact details) that you have commenced the study medicine by sending back the completed diary card at the end of the 10 days.

What are the alternatives for treatment?
Your child will receive the standard (normal) treatment for preschool wheeze of “as needed” inhaled salbutamol (the blue inhaler). If a doctor thinks that your child needs to have regular inhaled steroids, these may be given without affecting the trial. If a doctor thinks that you child also need daily montelukast, this can also be given, but in this case we will stop the trial medicine and, with your permission continue to collect information about the number of wheeze episodes.

What are the side effects of any treatment received when taking part?
There are very few side effects reported with continuous montelukast. A possible side effect is a mild tummy upset and increased thirst. Some older children have had mild headaches.
If your child accidentally takes too much montelukast the symptoms are similar to those already described. There may also be some increased sleepiness or agitation in some

children. If your child takes an overdose of any medication you should seek medical advice. There is no evidence of longterm harm from montelukast overdose.

What are the other possible disadvantages and risks of taking part?
Some parents might worry that if their child is given the placebo (inactive) medicine they won't be getting enough medicine to manage their wheeze. However, every child in the study will get the normal standard care of inhaled salbutamol as well as other medicines that their doctor prescribes. Only children enrolled in the study are eligible to have "as required" montelukast.

What are the possible benefits of taking part?
During the study we will check that all of the children are well at every visit/phone call. At any time during the study your GP or hospital doctor may decide to change your child's medicine or stop the study medicine. We are conducting this research so that we can know how best to treat children with preschool wheeze. We cannot promise that taking part will help your child personally, but your child will not be disadvantaged in any way. The information we get might help to improve the treatment of other children with preschool wheeze in the future.

What happens when the research study stops?
It may be some time after your child has completed the study before the results from all of the children taking part are known. We have to wait until the end of the whole study before we can analyse the results. Once the results are known we will write to you personally and tell your GP.

What if there is a problem?
Any complaint about the way you or your child have been dealt with during the study or any possible harm you might suffer will be addressed appropriately. Information relating to this is detailed in Part Two. If you have any complaints about this research study, please contact the appropriate Patient Advice and Liaison Service (PALS) office.

Will my child's taking part be kept confidential?
Yes. All of the information about your child's participation in this study will be kept confidential. The details are included in Part Two.

Contact details:
You will be able to contact a member of the research team to discuss any questions or concerns you may have and/or to get help. Please contact:

Research Nurse:	Research Nurse Name
Tel:	Researcher Number
Email:	Researcher@email.address.uk
Research Doctor:	Doctor Name
Tel:	Researcher Number
Email:	Researcher@email.address.uk
Patient Advice and Liaison Service:	PALS
	PALS Postal Address
Tel:	PALS contact 1
Fax:	PALS contact 2
Minicom:	PALS contact 3
E-mail:	PALS@email.address.uk

This completes Part One of the Information sheet. If the information in part One has interested you and you are considering participation, please continue to read the additional information in Part Two before making any decisions

Part Two

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/s being studied. If this happens, your study doctor will tell you and your child about it and discuss whether you both want to, or should, continue in the study. If you or your doctor decides that you should not carry on, your research doctor will discuss the reasons with you and make arrangements for your child's medical care to continue outside the study. If you decide to continue in the study you (and your child if appropriate) will be asked to sign updated consent forms. If the study is stopped for any other reason, you will be told why and your child's continuing care will be arranged.

What will happen if my child or I don't want to carry on with the research?

If at any point you decide to withdraw from the study, we will ask that you return all of the unused study medications to us. You can withdraw from treatment but continue to be followed up and have information collected.

Following withdrawal from the study, the research doctor will talk to you about whether they need to find out what medications your child was taking during the study to enable

appropriate follow-on treatment. Your child will then be treated as per standard local clinical procedures. All data collected up until the time of withdrawal will be anonymised[13] and included in the study analysis, unless you specifically state otherwise.

What if there is a problem?

If you have a concern about any aspect of this study you should contact the researchers who will do their best to answer any questions (contact numbers are in Part One).

If you are still unhappy after you have spoken to them and wish to complain formally, you can do this through the NHS Complaints Procedure.

If you have a complaint about a study doctor or nurse you have seen at the hospital, you can contact the Patient Advice and Liaison Service (PALS) department at the hospital for help.

If you wish to complain about a General Practitioner you have seen as part of this study, then you should contact the Primary Care Trust they belong to. Your study nurse will be able to help you with this if you want.

In the event that something goes wrong and your child is harmed during the research study the normal NHS complaints mechanism will be available to you. Additionally, if harm arises as a result of the design or management of this study, even if no one is at fault, you may have grounds for legal action against, or compensation from, the study sponsor: Queen Mary University of London. Please ask your doctor or research nurse for more information on this if you have any questions.

If your child is harmed due to hospital staff negligence then you may have grounds for a legal action against the hospital where those staff are employed. However, you may have to pay your own legal costs.

Will my child's participation in this study be kept confidential?

All information that is collected about you and your child during this study is considered to be confidential and giving this information to someone else ('a third party') is not allowed with the exceptions noted below.

The paper files used to record information in this study will be labelled with a unique study number.

Medical information may be given to your child's doctor or appropriate medical personnel responsible for their welfare.

The paper files used to record information in this study will also be stored securely in a locked cabinet and the information will then be entered into a secure computer database file. This file will be labelled with your child's number but NOT their name. A copy of the information in the paper files will be stored securely by the research team at the coordinating

[13] Anonymised means that a number will be used instead of your child's name so that no one will know that the information is about them.

study centre at Queen Mary University London. This is to ensure that all the information regarding the study remains accessible and secure for later analysis of the study results, and to check accuracy of information.

When your child finishes taking part in the study we will need to find out what treatment they were taking so that they can inform your GP. To do this, we will have to link your child's trial number to their name but this link will still be kept separate to all of the other information collected about them in the study. The trial team will ensure that confidentiality is preserved.

If you join the study, some parts of your child's medical records and the data collected for the study will be looked at by representatives of regulatory authorities and by authorised people from other NHS bodies to check that the study is being carried out correctly. Your child's medical records will be checked at the hospital and will not be removed. All authorised individuals have a duty of confidentiality to you and your child as research participants and nothing that could reveal your child's identity will be disclosed outside the research site. By signing the consent form you are giving permission for this to happen.

In the event of the results of the study being sent to Health Authorities or published, all of your child's records will be kept confidential and your child's name will not be disclosed to anyone outside of the hospital.

All documents and files relating to the study will be stored confidentially either at your local study site or the main study site or both for a maximum period of 20 years.

Involvement of the General Practitioner/family doctor (GP)

With your consent, the study doctor will write to your child's GP to let them know that they are taking part in the study. In some circumstances your GP will already know since he/she will have sent out your invitation letter. The study doctor may ask your child's GP for further medical information about them if necessary.

All patients (children) who are registered in the study will have follow up data collected about them. The information requested will all be related to your child's wheezing control and the research team will ask your GP to give them access to this data. By signing the attached consent form, you are agreeing for your GP to share this information with the research team.

I have private health insurance - does this make a difference?

You should inform your health insurance provider that your child has been enrolled into the study. They may wish to speak with the study group, in which case they can be provided with our contact details. Study involvement should not affect your insurance cover.

What will happen to any samples my child gives?

Your child's DNA and urine sample will be transferred to Queen Mary University London for testing and will be identified only by a special number to maintain your child's anonymity.

Will any genetic tests be done?

We will measure only the genes that affect how leukotrienes work in the body. Your child's sample will be collected by a researcher and sent directly to the laboratory at Queen Mary University London where it will be stored. Within 2 weeks we will measure the ALOX5 gene (a leukotriene gene). A DNA sample will be securely stored with a label that gives a subject number only (so that it cannot be directly linked to your child) and within 2 years sent to an external laboratory (KB Bio Science) for analysis of all the other genes that are associated with leukotrienes. Your child's sample will always be labelled with a special number, instead of their name, so no-one will know that it belongs to them. Once we have analysed it for leukotriene genes, the DNA sample will be disposed of and not kept.

What will happen to the results of the research study?

The results are likely to be published in the year following the end of the study. Your child's confidentiality will be ensured at all times and they will not be identified in any publication. At the end of the study the group results will be made available to you and/or your GP (should you wish). They will also be published on the National Institute of Health Research (NIHR) website.

Who is organising and funding the research?

The study is sponsored by Queen Mary University of London. This study is funded by the Efficacy and Mechanism Evaluation Programme of the UK Department of Health. Each participating research site has been allocated funds to pay for a researcher for this study, for the provision of general office supplies and to support pharmacy costs.

Who has reviewed the study?

The trial protocol has received the favourable opinion of the South East Research Ethics Committee

THANK YOU FOR READING THIS INFORMATION SHEET. WE HOPE YOU HAVE FOUND THE INFORMATION HELPFUL.

8.4.2 Informed consent form

Serial Number: I__I__I-I__I__I__I__I

	Parent/Guardian Consent Form (v5, 31.07.2012)	**Please initial box**
1	I confirm that I have read and understand the information sheet dated 31.07.12 (v5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2	I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without my care/my child's care or legal rights being affected.	☐
3	I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by responsible individuals from the Barts and the London Clinical Trials Unit, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my child's records.	☐
4	I agree to my child's GP being informed of my child's participation in the study.	☐
5	I agree to participate in a recorded interview about my views.[14]	☐
6	I agree for my child to take part in this study.	☐
7	I do not wish my child to/my child is ineligible to take part in this study but I am happy for their information as recorded today to be used by	☐

[14] Delete if not applicable to this centre

the study team under the terms stated in the Information sheet.

_____		
Name of Patient		
_____	_____	_____
Name of Parent	Signature	Date
_____	_____	_____
Name of Researcher	Signature	Date
_____	_____	_____
Name of Principal Investigator	P.I. Countersignature	Date

1 copy for parent, 1 for researcher site file, 1 to be kept in patient (child's) notes

8.5 Appendix 5 - Case report forms (CRFs)

All CRF copyright is owned by the WAIT team. All may be reproduced with appropriate accreditation and citation of their origin.

FIGURE 8-1 - T-2 ASSESSMENT AND RANDOMISATION CRF AND AIDE-MEMOIRE

NHS National Institute for Health Research **WAIT** mcrn PCRN

T -2 ASSESSMENT & RANDOMISATION CRF (Copy 1 – Trial Manager, Copy 2 – Local Site File)

Serial number: I I I I I I Site: I I I (e.g LO, AB, LE)

Researcher Initials:I__I__I Date of THIS Visit: I__I__/__I__/__I__I

CONSENT TO USE DATA: Yes ☐ No ☐ **If NO – DO NOT COMPLETE**

INCLUSION CRITERIA

Age between 10 months and 5 years:	Yes ☐	No ☐
Doctor-diagnosed wheeze, EVER:	Yes ☐	No ☐
Wheeze in the preceding three months:	Yes ☐	No ☐
At least two episodes of wheeze, EVER:	Yes ☐	No ☐
Parent contactable by phone:	Yes ☐	No ☐

EXCLUSION CRITERIA

Regular Montelukast	Yes ☐	No ☐
History of neonatal chronic lung disease	Yes ☐	No ☐
In a drug trial in the preceding three months	Yes ☐	No ☐
Clinician-diagnosed chronic respiratory illness Including structural airway anomaly and CF:	Yes ☐	No ☐
Any other chronic illness predisposing to respiratory infection (including developmental delay with feeding difficulty):	Yes ☐	No ☐

If you have ticked any GREYED-OUT boxes do not register this child for the WAIT study

INFORMED CONSENT TO ENTER STUDY:

Parent and child information sheets reviewed:	Yes ☐	No ☐
Informed consent form signed:	Yes ☐	No* ☐

*If no, please state the reason:
Did not want to take part in a genetic study: ☐
Concerned about confidentiality: ☐
Other (please specify): ☐ _____

If informed consent is NOT given do not collect samples, but please collect demographic data on page 2. If informed consent IS given collect samples as per guidance and also complete administration section on page 3

STUDY VISIT CONDUCTED BY:

Researcher Signature:_____ Print Name:_____ I__I__/__I__/__I__I

I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.

PI Signature_____ Print Name:_____ I__I__/__I__/__I__I

NHS National Institute for Health Research **WAIT** mcrn PCRN

T -2 ASSESSMENT & RANDOMISATION CRF (Copy 1 – Trial Manager, Copy 2 – Local Site File)

Serial number: I__I__I__I__I Site: I__I__I (e.g LO, AB, LE)

Researcher Initials:I__I__I Date of THIS Visit: I__I__/__I__/__I__I

Weight:	I__I__I.I__Ikg	Height:	I I I I.I Icm	DOB:	I I / I / I I I	Sex	M ☐ F ☐

Risk factors

Birth, Atopy and Family History	Yes	No
Preterm Birth < 37wk gestation	☐	☐
Birth weight < 2500g	☐	☐
Allergy:		
Food	☐	☐
Drug	☐	☐
Itchy rash for > 6 months, ever	☐	☐
Eczema, ever	☐	☐
Tobacco Exposure:		
In utero	☐	☐
In household* (*any household smoking contact)	☐	☐
Daycare attendance	☐	☐
Immunisation Status:		
Pneumococcus	☐	☐
Influenza	☐	☐
History of Asthma		
Mother:	☐	☐
Father:	☐	☐

Pre-study Illness and Therapy	Yes	No
Age at 1st wheeze episode	I__Iy I__I__Im	
Wheezes only with viral URTI (episodic)	☐	☐
Wheezes at other times (multitrigger)	☐	☐
Interval between onset of URTI and wheezing:	I__I__I hr	
Admitted to hospital for wheeze:		
In last year?	☐	☐
Ever?	☐	☐
No of courses of systemic steroids in last year	I__I__I	
No of unscheduled medical attendances for wheeze in last year?	I__I__I	
Preventer therapy:		
None	☐	☐
Antileukotriene agents	☐	☐
Maintenance Inhaled Steroids	☐	☐
Episodic inhaled Steroids	☐	☐

Ethnicity

Asian or Asian British	Mixed	Black or Black British	White	Other Ethnic Group
☐ Bangladeshi	☐ White & Asian	☐ African	☐ British	☐ Chinese
☐ Indian	☐ White & Black African	☐ Caribbean	☐ Irish	☐ Any other ethnic group
☐ Pakistani	☐ White & Black Caribbean	☐ Any other Black background	☐ White other	☐ I do not wish to disclose my ethnic origin
☐ Any other Asian background	☐ Mixed other			

Saliva sample collected:	Yes ☐	No ☐	Date collected:	I I / I I / I I
Saliva sample posted to laboratory:	Yes ☐	No ☐	Date sent:	I__I__/__I__/__I__I
Urine sample collected:	Yes ☐	No ☐	Date collected:	I__I__/__I__/__I__I

STUDY VISIT CONDUCTED BY:

Researcher Signature:_____ Print Name:_____ I__I__/__I__/__I__I

I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.

PI Signature Print Name: I I / I I / I I

NHS National Institute for Health Research **WAIT** mcrn PCRN

T -2 ASSESSMENT & RANDOMISATION CRF (Copy 1 – Trial Manager, Copy 2 – Local Site File)

Serial number: I__I__I__I__I Site: I__I__I (e.g LO, AB, LE)

Patient Initials: I__I__I__I Researcher Initials:I__I__I Date of THIS Visit: I__I__/__I__/__I__I

ADMINISTRATION (ONLY COMPLETE IF RECRUITED TO STUDY) – Do not send this page to trial coordinator

Full Name:	I_____I		
House/flat number:	I_____I		
Address 1:	I I		
Address 2:	I_____I		
Address 3:	I_____I		
Postcode:	I I I I I I I I I		
Mobile:	I__I__I__I__I__I__I__I__I__I__I__I		
Landline:	I__I__I__I__I__I__I__I__I__I__I__I		
Email:	I_____I		
T0 visit booked?:	Yes ☐	No ☐	Date: I__I__/__I__/__I__I
Inhaler technique assessed:	Yes ☐	No ☐	
Further advice/training provided as necessary:	Yes ☐	No ☐	

STUDY VISIT CONDUCTED BY:

Researcher Signature: Print Name: I I / I I / I I

I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.

PI Signature_____ Print Name:_____ I__I__/__I__/__I__I

Please scan and forward pages 1-2 only to Trial coordinator via secure email on xxxxxxxx@nhs.net as soon as possible after T-2 visit. Keep this page in local site file with consent forms.

NHS National Institute for Health Research **WAIT** mcrn PCRN

T-2 VISIT RESEARCHER AIDE-MEMOIRE

BEFORE THE VISIT

- Ensure that you have access to:
  - a stadiometer and scales
  - universal containers and urine collection apparatus
  - Ice box and ice
  - a genotek kit
  - specimen label sheets
  - T-2 proforma
  - Consent form
  - Information sheets
- Ensure that appropriate **language arrangements** are in place if English is not the parents' first language.

DURING THE VISIT

CRF and consent

- Check that parents understand what you are saying, review information sheet and seek informed consent
- If informed consent is not granted then seek consent to use data short of administrative section
- Complete CRF up to the administrative section FOR ALL children (including weight and height), even if they do not agree to take part if consent is provided.
- Leave one copy of consent form with parents.
- Sign and gain PI countersignature on each page that is completed

Specimens – if informed consent gained

- Review sample collection guide
- Collect urine, decant into 2 x 1ml aliquots label with serial number and put on ice immediately.
- Finally collect and label DNA sample with serial number

Administration – if informed consent gained

- Complete the administration section on page 3 of the CRF including:
  - Administrative data
  - Checking and correcting Inhaler technique as necessary
  - Arranging T0 medicines dispensing visit
  - Signing off on CRF
  - Copy consent form and give a copy to parents

AFTER THE VISIT

CRF and consent

- Researcher completing to ensure their sign off is complete (*N.B.* researcher signing form must be delegated on the site delegation log to take consent/complete CRFs).
- CRF pages 1-2 to be **countersigned by local PI**, scanned and secure emailed via nhs.net to xxxxxxxx@nhs.net in London as soon as possible **(any delay will delay stratum allocation).**
- Remember to keep one copy of consent form for local site file (consent and CRF) and give one copy to parents (consent form only).
- London Lab will allocate stratum to complete CRF T-2.

Specimens

- DNA sample to be posted **urgently** with request form in the pre-addressed envelope provided. An electronic copy of the request form must be sent to the trial coordinator on Cnwokoro@nhs.net
- Urine sample to be taken urgently on ice to be taken to local freezer and frozen at -70 or below for batch courier to London lab.

Stratification and Randomisation

- Trial laboratory technician should analyse DNA samples and complete stratification and inform researcher.
- PI should complete prescription with stratum based on above.
- Research nurse should deliver prescription to local pharmacy
- Local Pharmacist to complete prescription form, allocate IMP number and dispense trial drug for collection by local researcher
- Local researcher should convey IMP to parent.

FIGURE 8-2 - T0 TRIAL ENTRY CRF AND AIDE-MEMOIRE

NHS National Institute for Health Research **WAIT** mcrn PCRN

T0 TRIAL ENTRY CRF – PART A (Copy 1 – Trial Manager, Copy 2 – Local Site File)

Serial number: I__I__I__I__I Site: I__I__I (e.g. AB, LE, LO etc.) Subject Number (IMP): I__I__I__I__I

Patient Initials: I__I__I__I Researcher Initials:I__I__I Date of THIS Visit: I__I__/__I__/__I__I

1) CRF Documentation

Tick if you have seen the signed and countersigned:

☐ i) Consent Form
☐ ii) CRF T-2
☐ iii) Prescription Form

2) Samples

Tick if you have:

☐ i) Collected DNA sample
☐ ii) Collected urine sample
☐ iii) Explained the need for additional urine samples on attendance at ED

3) Diary Card

Tick if you have:

☐ i) Provided and labelled diary cards (x5)
☐ ii) Explained their usage
☐ iii) Explained procedure for return

4) Medication

Tick if you or soemone else have (on this or a prior visit)

☐ i) Checked salbutamol MDI and spacer availability
☐ ii) Checked MDI/spacer technique
☐ iii) Checked understanding of appropriate salbutamol usage
☐ iv) Checked IMP number matches number written by pharmacy on prescription
☐ v) Provided and explained use of IMP
☐ vi) Explained procedure for return of IMP
☐ vii) Explained procedure for replacement of IMP

5) Communication

Tick if you have:

☐ i) Provided local contact number and email
☐ ii) Explained indications for contact (solely trial-related and **including suspected drug reactions**, contact local NHS for acute health advice).
☐ iii) Provided pre-addressed jiffy bag for return of IMP/empty salbutamol canisters/diary cards

Researcher Signature:_____ Print Name:_____ Date: I__I__/__I__/__I__I

I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.

PI Signature:_____ Print Name:_____ Date: I__I__/__I__/__I__I

NHS National Institute for Health Research **WAIT** mcrn PCRN

T0 VISIT RESEARCHER AIDE-MEMOIRE

BEFORE THE VISIT

- Ensure that you have seen the signed and countersigned consent form, CRF T-2, and prescription form
- Ensure that you have collected the trial drug in good time from your local pharmacy and that the subject number on the box matches that on the prescription and CRF T0.
- Ensure that you have a copy of the CRF T0 and have **completed sections 1 and 2 in advance**.
- Ensure that you have copies of the diary card and understand its use.
- Ensure that appropriate **language arrangements** are in place if English is not the parents' first language.

DURING THE VISIT

Diary Card

- Explain the use of the diary card
- Provide one diary card per course of medication (usually five per box)
- Explain diary card return procedure

Medication

- Check the parents' possession of and knowledge of the use of spacer and MDI (may be brief if already performed e.g. on ward/at T-2 visit)
- Give IMP to parents and explain when and how to use and return it

Communication

- Give advice and information regarding researcher contact (including email and phone contact numbers – in PIS).
- Explain what to do if there are concerns regarding drug reactions or trial participation (contact local healthcare provider if child acutely unwell, contact researcher otherwise).

T0 Trial Entry CRF

- Work systematically through CRF

AFTER THE VISIT

- Researcher completing to ensure their sign off is complete (*N.B.* researcher signing form must be delegated on the site delegation log to take consent/complete CRFs)
- Keep one copy of the CRF in the local site file, send one copy to the London Trial coordinator.
- Send a recruited/not recruited letter to the GP and put a copy of this and the consent form in the clinical notes if appropriate.

FIGURE 8-3 - T2-T12 BIMONTHLY PHONE CALL CRF AND MEDICAL ATTENDANCE VERIFICATION CRF

NHS National Institute for Health Research **WAIT** mcrn PCRN

BIMONTHLY PHONECALL CRF (Copy 1 – Local Site file – **send to trial manager on completion)**

Serial number: I__I__I__I__I Site: I__I__I__I__I

Patient Initials: I__I__I__I Subject Number(IMP): I__I__I__I__I

Phonecall T+2m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

Number of IMP initiations? I__I__I
Total days used? I__I__I
Indications reminder☐ Diary card reminder☐
Number of days school/childcare missed I__I__I
Days taken off work (any carer) I__I__I
Other medications used _____

Number I__I__I and dates (below) of GP/Hosp attendances
Hosp name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I,
GP name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I
Adverse Events:☐ (if ticked complete follow AE guidance as per protocol)

Phonecall T+4m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

Number of IMP initiations? I__I__I
Total days used? I__I__I
Indications reminder☐ Diary card reminder☐
Number of days school/childcare missed I__I__I
Days taken off work (any carer) I__I__I
Other medications used _____

Number I__I__I and dates (below) of GP/Hosp attendances
Hosp name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I,
GP name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I
Adverse Events:☐ (if ticked complete follow AE guidance as per protocol)

Phonecall T+6m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

Number of IMP initiations? I__I__I
Total days used? I__I__I
Indications reminder☐ Diary card reminder☐
Number of days school/childcare missed I__I__I
Days taken off work (any carer) I__I__I
Other medications used _____

Number I__I__I and dates (below) of GP/Hosp attendances
Hosp name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I,
GP name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I
Adverse Events:☐ (if ticked complete follow AE guidance as per protocol)
(returns reminder)☐

Phonecall T+8m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

Number of IMP initiations? I__I__I
Total days used? I__I__I
Indications reminder☐ Diary card reminder☐
Number of days school/childcare missed I__I__I
Days taken off work (any carer) I__I__I
Other medications used _____

Number I__I__I and dates (below) of GP/Hosp attendances
Hosp name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I,
GP name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I
Adverse Events:☐ (if ticked complete follow AE guidance as per protocol)

Phonecall T+10m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

Number of IMP initiations? I__I__I
Total days used? I__I__I
Indications reminder☐ Diary card reminder☐
Number of days school/childcare missed I__I__I
Days taken off work (any carer) I__I__I
Other medications used _____

Number I__I__I and dates (below) of GP/Hosp attendances
Hosp name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I,
GP name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I
Adverse Events:☐ (if ticked complete follow AE guidance as per protocol)

Phonecall T+12m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

Number of IMP initiations? I__I__I
Total days used? I__I__I
Indications reminder☐ Diary card reminder☐
Number of days school/childcare missed I__I__I
Days taken off work (any carer) I__I__I
Other medications used _____

Number I__I__I and dates (below) of GP/Hosp attendances
Hosp name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I,
GP name_____ I__I__/__I__/__I__I, I__I__/__I__/__I__I,
I__I__/__I__/__I__I, I__I__/__I__/__I__I, I__I__/__I__/__I__I
Adverse Events:☐ (if ticked complete follow AE guidance as per protocol)
(returns reminder)☐

Sign-off letter posted ☐ **IMP was helpful** Y ☐ N ☐

Researcher Signature:_____ Print Name:_____ Date: I__I__/__I__/__I__I

I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.

PI Signature:_____ Print Name:_____ Date: I__I__/__I__/__I__I

NHS National Institute for Health Research **WAIT** mcrn PCRN

MEDICAL ATTENDANCE VERIFICATION (Copy 1 – Local File – **send to trial manager on completion)**

Serial number: I__I__I__I__I Site: I__I__I__I__I

Patient Initials: I__I__I__I Subject Number(IMP): I__I__I__I__I

Phonecall T+2m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1	H/G				
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					

Phonecall T+4m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					

Phonecall T+6m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					

Phonecall T+8m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					

Phonecall T+10m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					

Phonecall T+12m Date I__I__/__I__/__I__I Time I__I__:__I__I (24hr) Caller Initials I__I__I

	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					

Researcher Signature:_____ Print Name:_____ Date: I__I__/__I__/__I__I

I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.

PI Signature:_____ Print Name:_____ Date: I__I__/__I__/__I__I

FIGURE 8-4 - 10-DAY PARENT DIARY CARD

NHS National Institute for Health Research **WAIT** NHS National Institute for Health Research

PARENT DIARY CARD

Patient Serial Number__-____

INSTRUCTIONS

START the trial medicine when your child has a **COLD** or you think they will have a **WHEEZE** attack

CONTINUE it for **10 days** even if your child is well

- **Complete this diary card every time you start the trial medicine**
- **Complete the card at the END of each day for 10 days**
- **Stop the card when you stop the trial medicine**
- **Post the card back to us in the freepost envelope**
- **Remember to send back your empty sachets with this card.**

Subject No. (IMP) I__I__I__I__I	Study Site I__I__I	Card Number I__I__I
Patient Initials: I__I__I__I	Researcher Initials: I__I__I	Date Given/Posted I__I__/__I__/__I__I

NHS National Institute for Health Research **WAIT** NHS National Institute for Health Research

DAY 1 OF MEDICINE

SUN MON TUES WED THUR FRI SAT

DATE: I__I__/__I__/20__I__I TIME: __I__:__I__

The questions below refer to the past 24 hours.

Please answer as well as you can remember

Please answer the questions by ticking (√) Yes or No →	Y	N
Did your child wheeze in the last 24 hours?		
Did your child have a cold in the last 24 hours?		
Did you give your child the TRIAL medicine TODAY?		
Did your child vomit the medicine TODAY?		
Did your child miss school or nursery TODAY?		
Did **ANYONE** stay home to look after your child TODAY?		
Did your child see a doctor or nurse TODAY?		
Did you give your child the blue inhaler in the last 24 hrs		
If yes? How many times did you give it to them in the last 24 hours?		
On average, how many puffs did you give them each time?		

NHS National Institute for Health Research **WAIT** NHS National Institute for Health Research

DAY 10 OF MEDICINE

SUN MON TUES WED THUR FRI SAT

DATE: I__I__/__I__/20__I__I TIME: __I__:__I__

The questions below refer to the past 24 hours.

Please answer as well as you can remember

Please answer the questions by ticking (√) Yes or No →	Y	N
Did your child wheeze in the last 24 hours?		
Did your child have a cold in the last 24 hours?		
Did you give your child the TRIAL medicine TODAY?		
Did your child vomit the medicine TODAY?		
Did your child miss school or nursery TODAY?		
Did **ANYONE** stay home to look after your child TODAY?		
Did your child see a doctor or nurse TODAY?		
Did you give your child the blue inhaler in the last 24 hrs		
If yes? How many times did you give it to them in the last 24 hours?		
On average, how many puffs did you give them each time?		

NHS National Institute for Health Research **WAIT** NHS National Institute for Health Research

TRIAL MEDICINE COMMENTS

(write anything you would like to tell us about the medicine)

OTHER MEDICINES TAKEN THIS WEEK

Medicine	Dose	Days	Doses per day

THANK YOU FOR COMPLETING THIS DIARY.

NOW PLEASE RETURN IT IN THE FREEPOST ENVELOPE PROVIDED. THIS WILL INFORM YOUR RESEARCHER THAT YOU HAVE USED THE MEDICINE.

RESEARCHER PHONE NUMBER_____

Diary card was printed and bound in 12-page card-covered A6 booklet by LEA printers, Orpington, Kent, UK (www.leaprinters.co.uk).

FIGURE 8-5 - NON-SERIOUS ADVERSE EVENT CRF

NSAE - SINGULAIR

Site number: I__I__I__I Subject number: I__I__I__I__I Researcher Initials:I__I__I

Patient Initials: I__I__I__I Date of THIS Visit/Call: I__I__/__I__/__I__I

NONSERIOUS ADVERSE EXPERIENCE

If AE resulted in Death, if AE is immediately Life-Threatening, results in Persistent or Significant Disability/Incapacity, results in Hospitalization or Prolongs an Existing Hospitalization, is a Congenital Anomaly/Birth Defect, a Cancer, the result of an Overdose, or Other Important Medical Event, enter event on the SAE form.

Were there any nonserious AEs since last visit/phonecall?
None ☐ *or complete the form below* Date information obtained: _____ *DD-Mon-YYYY*

Clinical AE Term	Check if Worsening Condition	Onset Date DD Mon-YYYY	Stop Date *(or check box if continuing)* DD Mon-YYYY	Duration *(If less than 24 hours)* Specify number of hours minutes or seconds	Intensity 1 = Mild 2 = Moderate 3 = Severe	Action Taken on Primary Test Drug Due to AE: 1 = None 2 = Interrupted 3 = Discontinued 4 = Reduced	Did primary test drug cause AE? *(Refer to Guidelines for Causality)* 1 = Definitely not 2 = Probably not 3 = Possibly 4 = Probably 5 = Definitely
	☐		☐ Continuing	Hrs ☐ Min ☐ Sec ☐			
	☐		☐ Continuing	Hrs ☐ Min ☐ Sec ☐			
	☐		☐ Continuing	Hrs ☐ Min ☐ Sec ☐			
	☐		☐ Continuing	Hrs ☐ Min ☐ Sec ☐			
	☐		☐ Continuing	Hrs ☐ Min ☐ Sec ☐			

NONSERIOUS LABORATORY or OTHER DIAGNOSTIC PROCEDURES

TYPE OF AE	LABORATORY OR OTHER DIAGNOSTIC PROCEDURES *(To describe **Lab AE** use the term "Increased" or "Decreased")*	Check if Worsening Condition	Date lab specimen obtained or special exam performed DD Mon-YYYY	Action Taken on Primary Test Drug Due to AE: 1 = None 2 = Interrupted 3 = Discontinued 4 = Reduced	Did primary test drug cause AE? *(Refer to Guidelines for Causality)* 1 = Definitely not 2 = Probably not 3 = Possibly 4 = Probably 5 = Definitely
Lab ☐ Other ☐		☐			
Lab ☐ Other ☐		☐			
Lab ☐ Other ☐		☐			

Comments:

FIGURE 8-6 - SERIOUS ADVERSE EVENT CRF

SERIOUS ADVERSE EVENT REPORTING FORM
(BLT/QM sponsored trial)

Once you have become aware of a SAE or SUSAR, please scan & email/fax this signed form to the Research Governance & GCP Manager: **020 7882 7276** (or to the trial co-ordinator's fax number if multi site project) WITHIN a working day of learning of the event for SUSARs and within the time line outlined in the protocol approved by the MHRA and REC if expected SAEs. It is the CI's responsibility to inform the MREC of the SUSARs. If this event is a SUSAR, request an acknowledgment email of receipt of this form, from the JRO, print it and file it in your TMF.

Report type:	Initial ☐ Follow up ☐
If the project is multi-site, the section below should be completed by the Main site Trial coordinator prior to sending the template to the sites	
Full title of the study:	Parent-determined oral montelukast therapy for preschool wheeze with stratificatiobn for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.
Name of sponsor:	BLT ☐ QMUL ☐
Sponsor R&D Number:	EudraCT Number:2009-015626-11
MREC Number:	09/H1102/110
Chief Investigator:	Name: Prof J Grigg Phone No:02078822206/07787550774 Email address: j.grigg@qmul.ac.uk
Is this a double blind study?	Yes If Yes are the code break procedures in place with pharmacy? Yes
Name of ALL IMPs and/or medical devices	IMP 1: Montelukast IMP 3: IMP 2: IMP 4:
This section should be completed by the SITE:	
Subject identification code:	Patient/initials (first, last): _____
DOB: (Day/Month/Year) (___/___/____)	Sex: M ☐ F ☐
Patient's Age:	
Principal Investigator:	Name: Phone No: Email address:
Trial Co-ordinator local site:	Name: Phone No: Email address:
Name of reporting host institution:	Trust/ Institution name: Site number:
Date of site becoming aware of the event	__/__/____ Onset date of SAE: Resolution date of SAE:
Event Description (e.g. body site, symptoms) (*please use separate form for each event)	Event*: Severity: Mild ☐ Moderate ☐ Severe ☐
Type of SAE	Results in Death ☐ Life threatening ☐ Hospitalisation or prolongation of hospitalisation ☐ Persistent or significant disability or incapacity ☐ Congenital anomaly or birth defect ☐ "Other" important medical event ☐ If "Other", please describe: ..
	The co-ordinator needs to replace IMP 1,2,3,4 by the actual name of the IMP prior to sending the template to the sites.
Is the SAE **likely** to be a reaction to one of the	IMP 1 likely or possibly **Related** ☐ Unrelated ☐ IMP 2 likely or possibly **Related** ☐ Unrelated ☐

IMPs or medical device in the trial?	IMP 3 likely or possibly **Related** ☐ Unrelated ☐ IMP 4 likely or possibly **Related** ☐ Unrelated ☐
Is the SAE expected? *Expected reactions will be found in the Investigator Brochure, SmPC(http://emc.medicines.org.uk/) and/or protocol.*	IMP 1 **Expected** ☐ Unexpected ☐ IMP 2 **Expected** ☐ Unexpected ☐ IMP 3 **Expected** ☐ Unexpected ☐ IMP 4 **Expected** ☐ Unexpected ☐
Is the SAE due to the progression of an underlying illness?	Yes ☐ No ☐ Is the SAE related to the trial CONDUCT? Yes ☐ No ☐
Names of non IMPs concomitant medicines:	
Names of concomitant diseases:	
Is the event classified as a SUSAR? (ie, RELATED to one of the IMPs and UNEXPECTED)	Yes ☐ No ☐ **If Yes, please also complete CIOMS form** http://www.jazmp.si/files/farmakovigilanca/ObrazecPoro%C4%8DanjeNUZ_CIOMS_angl.doc . **also on page 4. If Yes, please give the batch number of each of the IMPs related to the SAE:** IMP 1: Batch Number: IMP 2: Batch Number: IMP 3: Batch Number: IMP 4: Batch Number:
Action taken with study treatment:	IMP 1 Continued ☐ Reduced ☐ Increased ☐ Temporary stop ☐ Permanent stop* ☐
	IMP 2 Continued ☐ Reduced ☐ Increased ☐ Temporary stop ☐ Permanent stop* ☐
	IMP 3 Continued ☐ Reduced ☐ Increased ☐ Temporary stop ☐ Permanent stop* ☐
	IMP4 Continued ☐ Reduced ☐ Increased ☐ Temporary stop ☐ Permanent stop* ☐
Did the PI withdraw the patient from the study?	Yes ☐ No ☐
Outcome of SAE:	Resolved ☐ Resolved with sequelae* ☐ *specify sequelae _____ Improved ☐ Persisting ☐ Worsened ☐ Fatal ☐ (date of death___ /____/___) Unknown ☐
	If fatal, copy of post-mortem available? Yes ☐ No ☐
Person completing the form if not the PI	Name: Phone No: Email address: Signature: Date:
Investigator's Name:	Print :
Investigator's Signature	Date:

Additional information requested by the CI's team for this project:

	CI's team, please customise this table prior to sending the form to the sites.
	Please add as many rows as required.

For Multi-site trials only

Date form RECEIVED by CI's team from external site: (___/___/____) (*This date will be DAY 1 for SUSARs*)	Reviewed by: Date:

For R&D Office use only

Date form RECEIVED by R&D team: (___/___/____)	Reviewed by: Date reviewed:
For SUSAR only:	Date reported to the MHRA:

Adverse Event (AE) Recording & Reporting
An AE occurs during a RESEARCH project, what do I do next?

Is the research project a Clinical Trial of an Investigational Medicinal Product (CTIMP)?
For guidance please see: www.ct-toolkit.ac.uk/route_maps/stations.cfm?current_station_id=287&view_type=map
If ANY doubts please email your protocol to ctdhelpline@mhra.gsi.gov.uk and cc the JRO on that email.

Yes / No →
1. Record AE in the study file and source documentation.
2. Follow up AE until resolved (if applicable).
3. SAEs in non CTIMPs that are related to the project and unexpected should be reported to the main ethics committee. "NRES report of serious adverse event form". www.corec.org.uk/applicants/apply/docs/Safety_Report_Form_(nonCTIMPs)v2.0.doc

Is it a serious adverse event (SAE)?
A SAE is defined as any untoward medical occurrence or effect that results in either death, is life threatening, requires hospitalisation or prolongation of hospitalisation, results in persistent or significant disability or incapacity or is a congenital anomaly or birth defect. Please note that all 'near misses' should also be reported via the Trust Incident form.

Yes / No →
1. Record the AE in the study file (Case Report Form) and source documentation (patient's notes)
2. Follow up AE until it is resolved (if applicable)

Is the SAE likely to be a REACTION to the investigational medicinal product (IMP)?
All AE judged by either the reporting investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as ADVERSE REACTION (AR).

Yes / No →
1. RECORD SAE in study file (Case report form) and source documentation (patient's notes).
2. Inform the trial sponsor within the time line stated in the protocol (Unless agreed in the protocol that EXPECTED events do not need REPORTING). If BLT/ QMUL is the sponsor, scan and email the signed SAE form or fax it to the R&D Office on 020 7882 7276.
3. A template BLT/QMUL SAE form is provided for BLT/QM sponsored trials.
4. Follow up SAE until resolved (if applicable).
5. The SAE may need reporting to the ethics committee,www.nres.npsa.nhs.uk/applicants/guidance

Is the SAR expected?
Reactions are considered EXPECTED if they are listed in the Investigators Brochure (IB), summary of product characteristics (SmPC) or in the protocol.

No / Yes →
1. RECORD SAE in study file (Case report form) and source documentation (patient's notes).
2. Inform the trial sponsor within the time line stated in the protocol (Unless agreed in the protocol that EXPECTED events do not need REPORTING). If BLT/ QMUL are the sponsor, scan and email the signed SAE form or fax it to the R&D Office on 020 7882 7276.
3. A template SAE form is provided for BLT/QM sponsored trials.
4. Follow up SAE until resolved (if applicable).
5. The SAE may need reporting to the ethics committee, see link for guidance www.nres.npsa.nhs.uk/applications/guidance

This event is a SUSAR (Suspected Unexpected Serious Adverse Reaction) Actions to be taken

1 The PI to record the event in the study file (Case report form) and source documentation (patient's notes).
2 The PI to complete sponsor SAE reporting form and CIOMS: http://www.cioms.ch/cioms.pdf
3 The PI to scan & email/Fax (020 7882 7276) the **signed** SAE form to the sponsor, **as soon as possible and within a working day**. The PI to make contact with the sponsor and ensure that the SAE reporting form has been received if the event is a SUSAR.
4 The PI to inform the REC using cover sheet safety report to main REC.
5 If the trial is multi-site, the CI has to inform the PIs on all sites.
6 The sponsor reports the SUSAR to the MHRA, within 7 days for death and life-threatening SUSARs and within 15 days for all other SUSARs
7 The sponsor to email to the PI an acknowledgment of receipt of this form (if the event is a SUSAR).
8 Follow up the SUSAR and record information in source documentation & compile annual safety report for sponsor. (Due date of the annual safety report is the anniversary date on the "notice of acceptance letter" from the MHRA.)

CIOMS
http://www.jazmp.si/files/farmakovigilanca/ObrazecPoro%C4%8DanjeNUZ_CIOMS_angl.doc

SUSPECT ADVERSE REACTION REPORT

I. REACTION INFORMATION

1. PATIENT INITIALS (first, last)	1a. COUNTRY	2. DATE OF BIRTH Day	Month	Year	2a. AGE Years	3. SEX	4-6 REACTION ONSET Day	Month	Year	8-12 CHECK ALL APPROPRIATE TO ADVERSE REACTION

7 + 13 DESCRIBE REACTION(S) (including relevant tests/lab data)

☐ PATIENT DIED
☐ INVOLVED OR PROLONGED INPATIENT HOSPITALISATION
☐ INVOLVED PERSISTENT OR SIGNIFICANT DISABILITY OR INCAPACITY
☐ LIFE THREATENING
☐ CONGENITAL ANOMALY
☐ OTHER MEDICALLY IMPORTANT CONDITION

II. SUSPECT DRUG(S) INFORMATION

14. SUSPECT DRUG(S) (include generic name)		20. DID REACTION ABATE AFTER STOPPING DRUG? ☐ YES ☐ NO ☐ NA
15. DAILY DOSE(S)	16. ROUTE(S) OF ADMINISTRATION	21. DID REACTION REAPPEAR AFTER REINTRO-DUCTION? ☐ YES ☐ NO ☐ NA
17. INDICATION(S) FOR USE		
18. THERAPY DATES (from/to)	19. THERAPY DURATION	

III. CONCOMITANT DRUG(S) AND HISTORY

22. CONCOMITANT DRUG(S) AND DATES OF ADMINISTRATION (exclude those used to treat reaction)

23. OTHER RELEVANT HISTORY (e.g. diagnoses, allergies, pregnancy with last menstrual period, etc.)

IV. MANUFACTURER INFORMATION

24a. NAME AND ADDRESS OF MANUFACTURER		26-26a. NAME AND ADRESS OF REPORTER (INCLUDE ZIP CODE)
ORIGINAL REPORT NO.	24b. MFR CONTROL NO.	
24c. DATE RECEIVED BY MANUFACTURER	24d. REPORT SOURCE ☐ STUDY ☐ LITERATURE ☐ HEALTH PROFESSIONAL ☐ REGULATORY AUTHORITY ☐ OTHER	
DATE OF THIS REPORT	25a. REPORT TYPE ☐ INITIAL ☐ FOLLOW-UP	

FIGURE 8-7 - WITHDRAWAL CRF

NHS National Institute for Health Research **WAIT** mcrn PCRN Primary Care Research Network

TRIAL WITHDRAWAL CRF

Serial number: I__I__I__I Site: I__I__I (AB, LE, LO, GP) Subject number (IMP):I__I__I__I__I

Patient Initials: I__I__I__I Researcher Initials:I__I__I Date of THIS Visit: I__I__/__I__/__I__I

(Circle as appropriate)

1.	Has the participant withdrawn from:	Treatment Only	*(i.e. Placebo/Montelukast)*	0
		Trial	*(i.e. Treatment and Follow-Up)*	1

2.	Date of withdrawal	Day ☐☐ Month ☐☐ Year ☐☐☐☐

3.	Reason for withdrawal *(Circle all that apply)*	Eligibility criterion no longer met (Specify:_____)	1
		Death of participant *(SAE no.____)*	2
		Other adverse event (AE/SAE no.____)	3
		Deterioration of pre-existing medical condition	4
		Poor adherence to treatment	5
		Perceived lack of efficacy of medication	6
		Unable to locate participant/carer	7
		Other (Specify:_____)	8

4.	Withdrawal decision initiated by: *(Circle all that apply)*	Chief Investigator (CI)	1
		Principal Investigator (PI)	2
		Referring Investigator	3
		Carer	4
		Participant	5
		Other (Specify:_____)	6

5.	Would the PI have independently recommended treatment withdrawal ?	No	0
		Yes	1

6.	Permission given to use data collected:	No, use of all data collected to date denied	1
		Yes, partial permission to use data up to withdrawal (Specify:_____)	2
		Yes, permission to use all data up to withdrawal	3
		Yes, permission to collect and use all follow-up data	4

7.	Treatment code broken: *(Not unless absolutely necessary)*	No	0
		Yes (Emergency Unblinding Request no._____)	1

8.	Signature of Researcher	
	Signature of Principal Investigator	

8.6 Appendix 6 - Study drugs

8.6.1 Wait trial investigator brochure - montelukast sodium oral granules 4mg

WAIT Trial Investigator's Brochure – Montelukast Sodium Oral Granules 4mg

Table of Contents

1. NAME OF THE MEDICINAL PRODUCT

SINGULAIR® Paediatric 4 mg Granules

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One sachet of granules contains montelukast sodium, which is equivalent to 4 mg montelukast. For a full

4.4 Special warnings and precautions for use

The diagnosis of persistent asthma in very young children (6 months - 2 years) should be established by a paediatrician or pulmonologist.

Patients should be advised never to use oral montelukast to treat acute asthma attacks and to keep their usual appropriate rescue medication for this purpose readily available. If an acute attack occurs, a short-acting inhaled β - agonist should be used. Patients should seek their doctors' advice as soon as possible if they need more inhalations of short-acting β-agonists than usual.

Montelukast should not be abruptly substituted for inhaled or oral corticosteroids.

There are no data demonstrating that oral corticosteroids can be reduced when montelukast is given concomitantly.

In rare cases, patients on therapy with anti-asthma agents including montelukast may present with systemic eosinophilia, sometimes presenting with clinical features of vasculitis consistent with Churg-Strauss syndrome, a condition which is often treated with systemic corticosteroid therapy. These cases usually, but not always, have been associated with the reduction or withdrawal of oral corticosteroid therapy. The possibility that leukotriene receptor antagonists may be associated with emergence of Churg-Strauss syndrome can neither be excluded nor established. Physicians should be alert to eosinophilia, vasculitic rash, worsening pulmonary symptoms, cardiac complications, and/or neuropathy presenting in their patients. Patients who develop these symptoms should be reassessed and their treatment regimens evaluated.

4.5 Interaction with other medicinal products and other forms of interaction

Montelukast may be administered with other therapies routinely used in the prophylaxis and chronic treatment of asthma. In drug-interactions studies, the recommended clinical dose of montelukast did not have clinically important effects on the pharmacokinetics of the following medicinal products: theophylline, prednisone, prednisolone, oral contraceptives (ethinyl estradiol/norethindrone 35/1), terfenadine, digoxin and warfarin.

The area under the plasma concentration curve (AUC) for montelukast was decreased approximately 40% in subjects with co-administration of phenobarbital. Since montelukast is metabolised by CYP 3A4, caution should be exercised, particularly in children, when montelukast is co-administered with inducers of CYP 3A4, such as phenytoin, phenobarbital and rifampicin.

In vitro studies have shown that montelukast is a potent inhibitor of CYP 2C8. However, data from a clinical drug - drug interaction study involving montelukast and rosiglitazone (a probe substrate representative of medicinal products primarily metabolised by CYP 2C8) demonstrated that montelukast does not inhibit CYP 2C8 *in vivo*. Therefore, montelukast is not anticipated to markedly alter the metabolism of medicinal products metabolised by this enzyme (e.g., paclitaxel, rosiglitazone, and repaglinide.)

General recommendations. The therapeutic effect of SINGULAIR on parameters of asthma control occurs within one day. Patients should be advised to continue taking SINGULAIR even if their asthma is under control, as well as during periods of worsening asthma.

No dosage adjustment is necessary for patients with renal insufficiency, or mild to moderate hepatic impairment. There are no data on patients with severe hepatic impairment. The dosage is the same for both male and female patients.

SINGULAIR as an alternative treatment option to low-dose inhaled corticosteroids for mild, persistent asthma:

Montelukast is not recommended as monotherapy in patients with moderate persistent asthma. The use of montelukast as an alternative treatment option to low-dose inhaled corticosteroids for children 2 to 5 years old with mild persistent asthma should only be considered for patients who do not have a recent history of serious asthma attacks that required oral corticosteroid use and who have demonstrated that they are not capable of using inhaled corticosteroids (see section 4.1). Mild persistent asthma is defined as asthma symptoms more than once a week but less than once a day, nocturnal symptoms more than twice a month but less than once a week, normal lung function between episodes. If satisfactory control of asthma is not achieved at follow-up (usually within one month), the need for an additional or different anti-inflammatory therapy based on the step system for asthma therapy should be evaluated. Patients should be periodically evaluated for their asthma control.

SINGULAIR as prophylaxis of asthma for 2 to 5 year old patients in whom the predominant component is exercise-induced bronchoconstriction.

In 2 to 5 year old patients, exercise-induced bronchoconstriction may be the predominant manifestation of persistent asthma that requires treatment with inhaled corticosteroids. Patients should be evaluated after 2 to 4 weeks of treatment with montelukast. If satisfactory response is not achieved, an additional or different therapy should be considered.

Therapy with SINGULAIR in relation to other treatments for asthma.

When treatment with SINGULAIR is used as add-on therapy to inhaled corticosteroids, SINGULAIR should not be abruptly substituted for inhaled corticosteroids (see section 4.4).

10-mg film-coated tablets are available for adults 15 years of age and older.

5-mg chewable tablets are available for paediatric patients 6 to 14 years of age.

4-mg chewable tablets are available as an alternative formulation for paediatric patients 2 to 5 years of age.

4.3 Contraindications

Hypersensitivity to the active substance or to any of the excipients.

4.4 Special warnings and precautions for use

The diagnosis of persistent asthma in very young children (6 months - 2 years) should be established by a paediatrician or pulmonologist.

Patients should be advised never to use oral montelukast to treat acute asthma attacks and to keep their usual appropriate rescue medication for this purpose readily available. If an acute attack occurs, a short-acting inhaled β - agonist should be used. Patients should seek their doctors' advice as soon as possible if they need more inhalations of short-acting β-agonists than usual.

Montelukast should not be abruptly substituted for inhaled or oral corticosteroids.

There are no data demonstrating that oral corticosteroids can be reduced when montelukast is given concomitantly.

In rare cases, patients on therapy with anti-asthma agents including montelukast may present with systemic eosinophilia, sometimes presenting with clinical features of vasculitis consistent with Churg-Strauss syndrome, a condition which is often treated with systemic corticosteroid therapy. These cases usually, but not always, have been associated with the reduction or withdrawal of oral corticosteroid therapy. The possibility that leukotriene receptor antagonists may be associated with emergence of Churg-Strauss syndrome can neither be excluded nor established. Physicians should be alert to eosinophilia, vasculitic rash, worsening pulmonary symptoms, cardiac complications, and/or neuropathy presenting in their patients. Patients who develop these symptoms should be reassessed and their treatment regimens evaluated.

4.5 Interaction with other medicinal products and other forms of interaction

Montelukast may be administered with other therapies routinely used in the prophylaxis and chronic treatment of asthma. In drug-interactions studies, the recommended clinical dose of montelukast did not have clinically important effects on the pharmacokinetics of the following medicinal products: theophylline, prednisone, prednisolone, oral contraceptives (ethinyl estradiol/norethindrone 35/1), terfenadine, digoxin and warfarin.

The area under the plasma concentration curve (AUC) for montelukast was decreased approximately 40% in subjects with co-administration of phenobarbital. Since montelukast is metabolised by CYP 3A4, caution should be exercised, particularly in children, when montelukast is co-administered with inducers of CYP 3A4, such as phenytoin, phenobarbital and rifampicin.

In vitro studies have shown that montelukast is a potent inhibitor of CYP 2C8. However, data from a clinical drug - drug interaction study involving montelukast and rosiglitazone (a probe substrate representative of medicinal products primarily metabolised by CYP 2C8) demonstrated that montelukast does not inhibit CYP 2C8 *in vivo*. Therefore, montelukast is not anticipated to markedly alter the metabolism of medicinal products metabolised by this enzyme (e.g., paclitaxel, rosiglitazone, and repaglinide.)

4.6 Pregnancy and lactation

Use during pregnancy

Animal studies do not indicate harmful effects with respect to effects on pregnancy or embryonal/foetal development.

Limited data from available pregnancy databases do not suggest a causal relationship between SINGULAIR and malformations (i.e. limb defects) that have been rarely reported in worldwide post marketing experience.

SINGULAIR may be used during pregnancy only if it is considered to be clearly essential.

Use during lactation

Studies in rats have shown that montelukast is excreted in milk (see section 5.3). It is not known if montelukast is excreted in human milk.

SINGULAIR may be used in breast-feeding mothers only if it is considered to be clearly essential.

4.7 Effects on ability to drive and use machines

Montelukast is not expected to affect a patient's ability to drive a car or operate machinery. However, in very rare cases, individuals have reported drowsiness or dizziness.

4.8 Undesirable effects

Montelukast has been evaluated in clinical studies in patients with persistent asthma as follows:

- 10-mg film-coated tablets in approximately 4000 adult patients 15 years of age and older
- 5-mg chewable tablets in approximately 1750 paediatric patients 6 to 14 years of age
- 4-mg chewable tablets in 851 paediatric patients 2 to 5 years of age, and
- 4-mg granules in 175 paediatric patients 6 months to 2 years of age.

Montelukast has been evaluated in a clinical study in patients with intermittent asthma as follows:

- 4 mg granules and chewable tablets in 1038 paediatric patients 6 months to 5 years of age

The following drug-related adverse reactions in clinical studies were reported commonly (≥1/100 to <1/10) in patients treated with montelukast and at a greater incidence than in patients treated with placebo:

Body System Class	Adult Patients 15 years and older (two 12-week studies; n=795)	Paediatric Patients 6 to 14 years old (one 8-week study; n=201) (two 56-week studies; n=615)	Paediatric Patients 2 to 5 years old (one 12-week study; n=461) (one 48-week study; n=278)	Paediatric Patients 6 months up to 2 years old (one 6-week study; n=175)
Nervous system disorders	headache	headache		hyperkinesia
Respiratory, thoracic, and mediastinal disorders				asthma
Gastro-intestinal disorders	abdominal pain		abdominal pain	diarrhoea
Skin and subcutaneous tissue disorders				eczematous dermatitis, rash
General disorders and administration site conditions			thirst	

With prolonged treatment in clinical trials with a limited number of patients for up to 2 years for adults, and up to 12 months for paediatric patients 6 to 14 years of age, the safety profile did not change.

Cumulatively, 502 paediatric patients 2 to 5 years of age were treated with montelukast for at least 3 months, 338 for 6 months or longer, and 534 patients for 12 months or longer. With prolonged treatment, the safety profile did not change in these patients either.

The safety profile in paediatric patients 6 months to 2 years of age did not change with treatment up to 3 months.

The following adverse reactions have been reported in post-marketing use:

Infections and infestations: upper respiratory infection.

Blood and lymphatic system disorders: increased bleeding tendency.

Immune system disorders: hypersensitivity reactions including anaphylaxis, hepatic eosinophilic infiltration.

Psychiatric disorders: dream abnormalities including nightmares, hallucinations, insomnia, somnambulism, irritability, anxiety, restlessness, agitation including aggressive behaviour or hostility, tremor, depression, suicidal thinking and behaviour (suicidality) in very rare cases.

Nervous system disorders: dizziness drowsiness, paraesthesia/hypoesthesia, seizure.

Cardiac disorders: palpitations.

Respiratory, thoracic and mediastinal disorders: epistaxis.

Gastro-intestinal disorders: diarrhoea, dry mouth, dyspepsia, nausea, vomiting.

Hepatobiliary disorders: elevated levels of serum transaminases (ALT, AST), hepatitis (including cholestatic, hepatocellular and mixed pattern liver injury).

Skin and subcutaneous tissue disorders: angiooedema, bruising, urticaria, pruritus, rash, erythema nodosum.

Musculoskeletal and connective tissue disorders: arthralgia, myalgia including muscle cramps.

General disorders and administration site conditions: asthenia/fatigue, malaise, oedema, pyrexia.

Very rare cases of Churg-Strauss Syndrome (CSS) have been reported during montelukast treatment in asthmatic patients (see section 4.4).

4.9 Overdose

No specific information is available on the treatment of overdose with montelukast. In chronic asthma studies, montelukast has been administered at doses up to 200 mg/day to adult patients for 22 weeks and in short term studies, up to 900 mg/day in patients for approximately one week without clinically important adverse experiences.

There have been reports of acute overdose in post-marketing experience and clinical studies with montelukast. These include reports in adults and children with a dose as high as 1000 mg (approximately 61 mg/kg in a 42 month old child). The clinical and laboratory findings observed were consistent with the safety profile in adults and paediatric patients. There were no adverse experiences in the majority of overdose reports. The most frequently occurring adverse experiences were consistent with the safety profile of montelukast and included abdominal pain, somnolence, thirst, headache, vomiting, and psychomotor hyperactivity.

It is not known whether montelukast is dialysable by peritoneal- or haemo-dialysis.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Leukotriene receptor antagonist

ATC-code: R03D C03

The cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4) are potent inflammatory eicosanoids released from various cells including mast cells and eosinophils. These important pro-asthmatic mediators bind to cysteinyl leukotriene receptors (CysLT) found in the human airway and cause airway actions, including bronchoconstriction, mucous secretion, vascular permeability, and eosinophil recruitment.

Montelukast is an orally active compound which binds with high affinity and selectivity to the $CysLT_1$ receptor. In clinical studies, montelukast inhibits bronchoconstriction due to inhaled LTD_4 at doses as low as 5 mg. Bronchodilation was observed within 2 hours of oral administration. The bronchodilation effect caused by a β-agonist was additive to that caused by montelukast. Treatment with montelukast inhibited both early- and late-phase bronchoconstriction due to antigen challenge. Montelukast, compared with placebo, decreased peripheral blood eosinophils in adult and paediatric patients. In a separate study, treatment with montelukast significantly decreased eosinophils in the airways (as measured in sputum). In adult and paediatric patients 2 to 14 years of age, montelukast, compared with placebo, decreased peripheral blood eosinophils while improving clinical asthma control.

In studies in adults, montelukast, 10 mg once daily, compared with placebo, demonstrated significant improvements in morning FEV_1 (10.4% vs 2.7% change from baseline), AM peak expiratory flow rate (PEFR) (24.5 L/min vs 3.3 L/min change from baseline), and significant decrease in total β-agonist use (-26.1% vs -4.6% change from baseline). Improvement in patient-reported daytime and night-time asthma symptoms scores was significantly better than placebo.

Studies in adults demonstrated the ability of montelukast to add to the clinical effect of inhaled corticosteroid (% change from baseline for inhaled beclometasone plus montelukast vs beclometasone, respectively for FEV_1: 5.43% vs 1.04%; β-agonist use: -8.70% vs 2.64%). Compared with inhaled beclometasone (200 µg twice daily with a spacer device), montelukast demonstrated a more rapid initial response, although over the 12-week study, beclometasone provided a greater average treatment effect (% change from baseline for montelukast vs beclometasone, respectively for FEV_1: 7.49% vs 13.3%; β-agonist use: -28.28% vs -43.89%). However, compared with beclometasone, a high percentage of patients treated with montelukast achieved similar clinical responses (e.g., 50% of patients treated with beclometasone achieved an improvement in FEV_1 of approximately 11% or more over baseline while approximately 42% of patients treated with montelukast achieved the same response).

In an 8-week study in paediatric patients 6 to 14 years of age, montelukast 5 mg once daily, compared with placebo, significantly improved respiratory function (FEV_1 8.71% vs 4.16% change from baseline; AM PEFR 27.9 L/min vs 17.8 L/min change from baseline) and decreased "as-needed" β-agonist use (-11.7% vs +8.2% change from baseline).

In a 12-month study comparing the efficacy of montelukast to inhaled fluticasone on asthma control in paediatric patients 6 to 14 years of age with mild persistent asthma, montelukast was non-inferior to fluticasone in increasing the percentage of asthma rescue-free days (RFDs), the primary endpoint. Averaged over the 12-month treatment period, the percentage of asthma RFDs increased from 61.6 to 84.0 in the montelukast group and from 60.9 to 86.7 in the fluticasone group. The between group difference in LS mean increase in the percentage of asthma RFDs was statistically significant (-2.8 with a 95% CI of -4.7, -0.9), but within the limit pre-defined to be clinically not inferior. Both montelukast and fluticasone also improved asthma control on secondary variables assessed over the 12 month treatment period:

- FEV_1 increased from 1.83 L to 2.09 L in the montelukast group and from 1.85 L to 2.14 L in the fluticasone group. The between-group difference in LS mean increase in FEV_1 was -0.02 L with a 95% CI of -0.06, 0.02. The mean increase from baseline in % predicted FEV_1 was 0.6% in the montelukast treatment group, and 2.7% in the fluticasone treatment group. The difference in LS means for the change from baseline in the % predicted FEV_1 was significant: -2.2% with a 95% CI of -3.6, -0.7.
- The percentage of days with β-agonist use decreased from 38.0 to 15.4 in the montelukast group, and from 38.5 to 12.8 in the fluticasone group. The between group difference in LS means for the percentage of days with β-agonist use was significant: 2.7 with a 95% CI of 0.9, 4.5.
- The percentage of patients with an asthma attack (an asthma attack being defined as a period of worsening asthma that required treatment with oral steroids, an unscheduled visit to the doctor's office, an emergency room visit, or hospitalisation) was 32.2 in the montelukast group and 25.6 in the fluticasone group; the odds ratio (95% CI) being significant: equal to 1.38 (1.04, 1.84).
- The percentage of patients with systemic (mainly oral) corticosteroid use during the study period was 17.8% in the montelukast group and 10.5% in the fluticasone group. The between group difference in LS means was significant: 7.3% with a 95%CI of 2.9; 11.7.

In a 12-week, placebo-controlled study in paediatric patients 2 to 5 years of age, montelukast 4 mg once daily improved parameters of asthma control compared with placebo irrespective of concomitant controller therapy (inhaled/nebulised corticosteroids or inhaled/nebulised sodium cromoglycate). Sixty percent of patients were not on any other controller therapy. Montelukast improved daytime symptoms (including coughing, wheezing, trouble breathing and activity limitation) and night-time symptoms compared with placebo. Montelukast also decreased "as-needed" β-agonist use and corticosteroid rescue for worsening asthma compared with placebo. Patients receiving montelukast had more days without asthma than those receiving placebo. A treatment effect was achieved after the first dose.

In a 12-month, placebo-controlled study in paediatric patients 2 to 5 years of age with mild asthma and episodic exacerbations, montelukast 4 mg once daily significantly ($p \leq 0.001$) reduced the yearly rate of asthma exacerbation episodes (EE) compared with placebo (1.60 EE vs. 2.34 EE, respectively). [EE defined as ≥ 3 consecutive days with daytime symptoms requiring β-agonist use, or corticosteroids (oral or inhaled), or hospitalisation for asthma]. The percentage reduction in yearly EE rate was 31.9%, with a 95%

CI of 16.9, 44.1.

In a placebo-controlled study in paediatric patients 6 months to 5 years of age who had intermittent asthma but did not have persistent asthma, treatment with montelukast was administered over a 12-month period, either as a once-daily 4 mg regimen or as a series of 12-day courses that each were started when an episode of intermittent symptoms began. No significant difference was observed between patients treated with montelukast 4 mg or placebo in the number of asthma episodes culminating in an asthma attack, defined as an asthma episode requiring utilization of health-care resources such as an unscheduled visit to a doctor's office, emergency room, or hospital; or treatment with oral, intravenous, or intramuscular corticosteroid.

Efficacy of montelukast is supported in paediatric patients 6 months to 2 years of age by extrapolation from the demonstrated efficacy in patients 2 years of age and older with asthma, and is based on similar pharmacokinetic data, as well as the assumption that the disease course, pathophysiology and the medicinal product's effect are substantially similar among these populations.

Significant reduction of exercise-induced bronchoconstriction (EIB) was demonstrated in a 12-week study in adults (maximal fall in FEV_1 22.33% for montelukast vs 32.40% for placebo; time to recovery to within 5% of baseline FEV 44.22 min vs 60.64 min). This effect was consistent throughout the 12-week study period. Reduction in EIB was also demonstrated in a short term study in paediatric patients 6 to 14 years of age (maximal fall in FEV_1 18.27% vs 26.11%; time to recovery to within 5% of baseline FEV_1 17.76 min vs 27.98 min). The effect in both studies was demonstrated at the end of the once-daily dosing interval.

In aspirin-sensitive asthmatic patients receiving concomitant inhaled and/or oral corticosteroids, treatment with montelukast, compared with placebo, resulted in significant improvement in asthma control (FEV_1 8.55% vs -1.74% change from baseline and decrease in total β agonist use -27.78% vs 2.09% change from baseline).

5.2 Pharmacokinetic properties

Absorption. Montelukast is rapidly absorbed following oral administration. For the 10-mg film-coated tablet, the mean peak plasma concentration (C_{max}) is achieved 3 hours (T_{max}) after administration in adults in the fasted state. The mean oral bioavailability is 64%. The oral bioavailability and C_{max} are not influenced by a standard meal. Safety and efficacy were demonstrated in clinical trials where the 10-mg film-coated tablet was administered without regard to the timing of food ingestion.

For the 5-mg chewable tablet, the C_{max} is achieved in 2 hours after administration in adults in the fasted state. The mean oral bioavailability is 73% and is decreased to 63% by a standard meal.

After administration of the 4-mg chewable tablet to paediatric patients 2 to 5 years of age in the fasted state, C_{max} is achieved 2 hours after administration. The mean C_{max} is 66% higher while mean C_{min} is lower than in adults receiving a 10-mg tablet.

The 4-mg granule formulation is bioequivalent to the 4-mg chewable tablet when administered to adults in the fasted state. In paediatric patients 6 months to 2 years of age, C_{max} is achieved 2 hours after administration of the 4-mg granules formulation. C_{max} is nearly 2-fold greater than in adults receiving a 10-

mg tablet. The co-administration of applesauce or a high-fat standard meal with the granule formulation did not have a clinically meaningful effect on the pharmacokinetics of montelukast as determined by AUC (1225.7 vs 1223.1 ng•hr/mL with and without applesauce, respectively, and 1191.8 vs 1148.5 ng•hr/mL with and without a high-fat standard meal, respectively).

Distribution. Montelukast is more than 99% bound to plasma proteins. The steady-state volume of distribution of montelukast averages 8-11 litres. Studies in rats with radiolabelled montelukast indicate minimal distribution across the blood-brain barrier. In addition, concentrations of radiolabelled material at 24 hours post-dose were minimal in all other tissues.

Biotransformation. Montelukast is extensively metabolised. In studies with therapeutic doses, plasma concentrations of metabolites of montelukast are undetectable at steady state in adults and children.

In vitro studies using human liver microsomes indicate that cytochrome P450 3A4, 2A6 and 2C9 are involved in the metabolism of montelukast. Based on further *in vitro* results in human liver microsomes, therapeutic plasma concentrations of montelukast do not inhibit cytochromes P450 3A4, 2C9, 1A2, 2A6, 2C19, or 2D6. The contribution of metabolites to the therapeutic effect of montelukast is minimal.

Elimination. The plasma clearance of montelukast averages 45 ml/min in healthy adults. Following an oral dose of radiolabelled montelukast, 86% of the radioactivity was recovered in 5-day faecal collections and <0.2% was recovered in urine. Coupled with estimates of montelukast oral bioavailability, this indicates that montelukast and its metabolites are excreted almost exclusively via the bile.

Characteristics in patients. No dosage adjustment is necessary for the elderly or mild to moderate hepatic insufficiency. Studies in patients with renal impairment have not been undertaken. Because montelukast and its metabolites are eliminated by the biliary route, no dose adjustment is anticipated to be necessary in patients with renal impairment. There are no data on the pharmacokinetics of montelukast in patients with severe hepatic insufficiency (Child-Pugh score>9).

With high doses of montelukast (20- and 60-fold the recommended adult dose), a decrease in plasma theophylline concentration was observed. This effect was not seen at the recommended dose of 10 mg once daily.

5.3 Preclinical safety data

In animal toxicity studies, minor serum biochemical alterations in ALT, glucose, phosphorus and triglycerides were observed which were transient in nature. The signs of toxicity in animals were increased excretion of saliva, gastro-intestinal symptoms, loose stools and ion imbalance. These occurred at dosages which provided>17-fold the systemic exposure seen at the clinical dosage. In monkeys, the adverse effects appeared at doses from 150 mg/kg/day (>232-fold the systemic exposure seen at the clinical dose). In animal studies, montelukast did not affect fertility or reproductive performance at systemic exposure exceeding the clinical systemic exposure by greater than 24-fold. A slight decrease in pup body weight was noted in the female fertility study in rats at 200 mg/kg/day (>69 - fold the clinical systemic exposure). In studies in rabbits, a higher incidence of incomplete ossification, compared with concurrent control animals, was seen at systemic exposure>24-fold the clinical systemic exposure seen at the clinical dose. No

abnormalities were seen in rats. Montelukast has been shown to cross the placental barrier and is excreted in breast milk of animals.

No deaths occurred following a single oral administration of montelukast sodium at doses up to 5000 mg/kg in mice and rats (15,000 mg/m^2 and 30,000 mg/m^2 in mice and rats, respectively), the maximum dose tested. This dose is equivalent to 25,000 times the recommended daily adult human dose (based on an adult patient weight of 50 kg).

Montelukast was determined not to be phototoxic in mice for UVA, UVB or visible light spectra at doses up to 500 mg/kg/day (approximately>200-fold based on systemic exposure).

Montelukast was neither mutagenic in *in vitro* and *in vivo* tests nor tumorigenic in rodent species.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Mannitol

Hyprolose (E 463)

Magnesium stearate

6.2 Incompatibilities

Not applicable.

6.3 Shelf life

2 years.

6.4 Special precautions for storage

Store in the original package in order to protect from light and moisture.

6.5 Nature and contents of container

Packaged in polyethylene/aluminium/polyester sachet in:

Cartons of 7, 20, 28 and 30 sachets.

Not all pack sizes may be marketed.

6.6 Special precautions for disposal and other handling

Any unused product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

Merck Sharp & Dohme Limited

Hertford Road, Hoddesdon, Hertfordshire EN11 9BU, UK

8. MARKETING AUTHORISATION NUMBER(S)

PL 0025/0440

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

14 February 2003/ 25 August 2007

10. DATE OF REVISION OF THE TEXT

November 2010

LEGAL CATEGORY

POM

SPC.SGA-OG.10.UK.3247.ii-052

8.6.2 Montelukast certificate of analysis

Nova Laboratories Limited

Certificate Of Analysis

Product:	Montelukast 4mg Paediatric Granules
Batch Number:	0880x001

RELEASE TESTS

Test	Method Reference	Acceptance criteria	Result	Pass / Fail
Appearance	CM0880	White Granules	White Granules	Pass
Montelukast Identificaion (HPLC)	CM0880	Positive response at the retention time for Montelukast	Positive response at the retention time for Montelukast	Pass
Montelukast content	CM0880	3.6 to 4.4mg per sachet	4.3mg per sachet	Pass
Uniformity of Mass (Ph Eur 2.9.5)	CM0880	Complies with Ph Eur	Conforms	Pass
Total Viable Count	SOP1034	TAMC: NMT 2000 cfu/g TYMC: NMT 200 cfu/g	< 4 cfu / g < 4 cfu / g	Pass

Julie Walker
Head of Quality

Date: 08SEP10

Audrey Holt
Quality Systems Manager

Date: 08SEP10

Nova Laboratories Limited, Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YL, UK
Tel: +44 (0) 116 223 0100 Fax: +44 (0) 116 223 0101

8.6.3 Placebo certificate of analysis

Nova Laboratories Limited

Certificate Of Analysis

Product:	Placebo to match Montelukast 4mg Paediatric Granules
Batch Number:	0891x001

RELEASE TESTS

Test	Method Reference	Acceptance criteria	Result	Pass / Fail
Appearance	CM0891	White Granules	White Granules	Pass
Absence of Montelukast by HPLC	CM0891	No response at the retention time for Montelukast	No response at the retention time for Montelukast	Pass
Uniformity of Mass (Ph Eur 2.9.5)	CM0891	Complies with Ph Eur	Conforms	Pass
Total Viable Count	SOP1034	TAMC: NMT 2000 cfu/g TYMC: NMT 200 cfu/g	< 4 cfu / g < 4 cfu / g	Pass

Julie Walker
Head of Quality

Date: 08 SEP 10

Audrey Holt
Quality Systems Manager

Date: 08 SEP 10

Safeguarding public health

MHRA

INSPECTION REPORT

Nova Laboratories Ltd
Martin House
Gloucester Crescent
Wigston
Leicester
LE18 4YL

Medicines and Healthcare Products Regulatory Agency
2A Hunter House
57 Goodramgate
YORK YO1 7FX
Telephone: 01904 610556 Fax: 01904 625430

Head Office:
Inspection & Standards Division,
Medicines and Healthcare products Regulatory Agency
151 Buckingham Palace Road, Victoria, London SW1W 9SZ
Tel: 020 3080 6000

GMP INSPECTION OF Nova Laboratories Ltd

SECTION A INSPECTION REPORT SUMMARY

Inspection requested by: Routine fee based re-inspection

Scope of inspection: EU Guide to GMP

Licence or Reference Number: MIA, MIA(IMP), MS and ManSA 13581

Licence Holder/Applicant: Nova Laboratories Ltd

Details of Product(s)/ Clinical trials/Studies:
Variety of aseptically prepared sterile products, for Clinical Trials or hospital specials.
Extemporaneously prepared non-sterile products as specials.

Activities carried out by company:

	Y/N
Manufacture of Active Ingredients	N
Manufacture of Finished Medicinal Products	Y
Manufacture of Intermediate or Bulk	Y
Packaging	Y
Importing	N
Laboratory Testing	Y
Batch Certification and Batch Release	Y
Other: *Specials and IMP activities*	Y

Name and Address of site(s) inspected: Nova Laboratories Ltd
Martin House, Gloucester Crescent,
Wigston Leicester LE18 4YL

Site Contact: Dr Peter White peter.white@novalabs.co.uk

Date(s) of Inspection: 11-15th July 2011

Lead Inspector: Vicki Pike

Accompanying Inspector(s): N/A

References: Insp GMP/IMP 13581/4097-0015

Final Conclusion/Recommendation:

The site operates to a satisfactory level of GMP. A GMP certificate shall be issued. Please refer to Annex 1 for re-inspection frequency.

Name and Dated Signature of Lead Inspector:

Signed: Vicki Pike **Dated:** 16/08/11

8.7 Appendix 7 - Statistical analysis plan

The statistical analysis plan was finalised prior to locking of the trial database in January 2014, however minor formatting changes occurred up to February 2014. Page 30 is in fact blank and is therefore excluded for the purposes of brevity.

Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype (WAIT)

Statistical Analysis Plan

Version: 2.0
Date: 18th February 2014

Person(s) contributing to the analysis plan	
Name(s) and position(s)	Clare Rutterford Trial Statistician Sandra Eldridge Senior Statistician Chinedu Nwokoro
Authorisation	
Position	**Chief or principal investigator**
Name	**Jonathan Grigg**
Signature	
Date	
Position	**Senior trial statistician**
Name	**Sandra Eldridge**
Signature	
Date	
Position	**Independent statistician***
Name	**NA**
Tick once reviewed	**NA**
Date	DD/MMM/YYYY

*This will normally be the Trial Steering Committee (TSC) statistician, but if there is no TSC the DMC statistician may sign off the analysis plan, provided there has been no interim unblinded analysis.

1. INTRODUCTION

1.1. Purpose of statistical analysis plan

The purpose of this document is to provide details of the statistical analyses and presentation of results to be reported within the principal paper(s) of the WAIT trial. Subsequent papers of a more exploratory nature (including those involving baseline data only) will not be bound by this strategy but will be expected to follow the broad principles laid down in it. Any exploratory, post-hoc or unplanned analyses will be clearly identified in the respective study analysis report.

The structure and content of this document provides sufficient detail to meet the requirements identified by the International Conference on Harmonisation (ICH) and the PCTU SOP (PCTU/07).

The following were reviewed in preparation for writing this document:

Trial protocol version 7 24/06/2011

ICH E9 Guidance on statistical principals for clinical trials

ICH E3 Structure and content of clinical study reports

CONSORT guidelines for the reporting of randomised trials

PCTU_DM_04 Standard Operating Procedures (SOP) for: Data Entry, Quality Control, Data Extraction and Database lock

1.2. Members of the writing committee

Clare Rutterford (CR) was primarily responsible for (i) writing the Statistical Analysis Strategy and (ii) writing the computer code implementing the analysis strategy and (iii) implementing the strategy at the point of analysis all under the guidance of Professor Sandra Eldridge (SE).

This document has been developed prior to examination of trial data and will not be implemented prior to final approval and after the database has been locked to changes.

1.3. Summary

1.4. Changes from planned analysis in the protocol

- During November 2011 eleven WAIT participants were randomised not in accordance with the predefined schedule. The DMC recommended the inclusion of these 11 incorrectly randomized participants in the analysis and a sensitivity analysis without them included.
- Five participants were randomised with the incorrect genotype recorded at stratification and will be analysed as randomised.

- One participant AB161 was randomised and allocated a box of IMP; however they did not receive the medication and were then found to be ineligible. They shall be excluded from the analysis
- A couple of children received the wrong box of medication during the trial (approximately three doses). They shall be analysed as randomised
- A handful of participants were withdrawn prior to receiving study medication. Their study medication was reallocated to future participants. CR expressed concern whether this affected the allocation schedule that may distort the balance of the Active/Placebo blocks. Consensus was that the numbers were small so any effect will be negligible and the participants should be analysed as randomised.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study objectives

2.1.1. Primary objectives

1.To determine whether intermittent treatment with oral montelukast in preschool children reduces the need for unscheduled medical attention (GP visit, hospital attendance, hospital admission) for wheeze.

2.1.2. Secondary objectives

2.To determine whether the effect of treatment on the primary analysis is different depending upon ALOX5 status (5/5 vs. 5/x and x/x).

3.To determine whether intermittent treatment with oral montelukast in preschool children reduces the time to first medical attendance.

4.To determine whether intermittent treatment with oral montelukast in preschool children reduces the need for each type of medical attention for wheeze: hospital admissions; hospital attendance; and GP visits.

5.To determine whether intermittent treatment with oral montelukast in preschool children reduces the time to first occurrence of each type of medical attention for wheeze: hospital admissions; hospital attendance; and GP visits.

6.To determine whether intermittent treatment with oral montelukast in preschool children reduces the duration of hospital admissions.

7.To determine whether intermittent treatment with oral montelukast in preschool children reduces the number of episodes, duration and time to first event of wheeze and cold.

8.To determine whether intermittent treatment with oral montelukast in preschool children reduces the need for alternative medications (Steroids, Salbutamol).

9.To describe the safety profile of montelukast.

10.To describe parents opinion of treatment efficacy

11.To describe compliance to medication

12.To determine whether baseline urinary eicosanoid level is different across baseline groups: ALOX5 status (A or B), leukotriene genes and, type of wheeze (episodic, multitrigger).
NOTE ANALYSIS DETAIL NOT CONTAINED IN THIS PLAN

13.To determine whether montelukast is cost effective. **NOTE ANALYSIS DETAIL NOT CONTAINED IN THIS PLAN**

2.1.3. Exploratory objectives

14.To determine whether the effect of treatment on the primary analysis is different depending upon ALOX5 status (categorised as (5/5 vs. 5/x) vs. x/x).

2.1. Outcome measures

2.1.1. Primary outcomes

The number of times a child attends for an unscheduled medical opinion (a summation of hospital admissions, attendances, GP visits,) with respiratory problems over a 12 month period as confirmed from clinical records

2.1.2. Secondary outcomes

Breakdown of unscheduled medical opinion

Hospital admissions:

- Number of hospital admissions over the 12 month period as recorded at each phone call
- Duration of hospital admissions as recorded at each phone call
- Time from randomisation date to date of first hospital admission as recorded at each phone call

Hospital admission for wheeze:

- Number of hospital admissions over the 12 month period as recorded at each phone call
- Time from randomisation date to date of first hospital admission as recorded at each phone call

Hospital attendance for wheeze:

- Number of hospital attendances (A&E) over the 12 month period as recorded at each phone call

- Time from randomisation date to date of first hospital attendance (A&E) as recorded at each phone call

Unscheduled GP visit for wheeze:

- Number of unscheduled GP visits over the 12 month period as recorded at each phone call
- Time from randomisation date to date of first unscheduled GP visit as recorded at each phone call

Description of wheezing episodes

Wheeze:

- Number of wheeze episodes* as recorded on the diary card
- Time to first episode* of wheeze as recorded on the diary card
- Duration of wheeze episodes* as recorded on the diary card

Cold

- Number of cold episodes* as recorded on the diary card
- Time to first episode* of cold as recorded on the diary card
- Duration of cold episodes* as recorded on the diary card

***Definition of episode of wheeze and cold: The duration of an episode is defined as the days from the start of symptoms until the last days of symptoms (includes both start and stop day) followed by a period of 5 symptom free days.**

Medication use

Steroids (OCS):

- The number of courses per year (and total number of days) as recorded on the diary card. Each mention of use on a separate diary card indicates a course.
- The proportion receiving none vs. any during the trial as recorded on the diary card or in the phonecall data.

Steroids (ICS):

- Proportion starting ICS during the trial as recorded on the diary card or phonecall data (baseline data (T2) indicates whether child was on ICS at the start of the trial)

Salbutamol:

- Total number of puffs overall per episode of wheeze as recorded on the diary card
- Total number of puffs (Salbutamol use per year)

Investigational Medicinal Product (IMP) usage:

- The number of IMP initiations (whether for wheeze or cold).
- Mean sachets (IMP use) per episode (wheeze or cold) as recorded on the diary card
- Compliance calculated from diary card, number dispensed and number returned

2.1.3. Inflammatory outcomes

- Baseline and exit urinary eicosanoid level
- Leukotriene genes (approximately 150 genes)

Note: this data is not stored on the main trial database and the analysis is not included within this plan

2.1.4. Safety outcomes

- The number of withdrawals from the trial per group
- Serious adverse events per group
- Adverse events per group
- All cause mortality per group
- Mortality due to exacerbation of asthma per group
- Mortality due to respiratory infection per group

2.1.5. Economic outcomes

Costs due to wheeze:

Unit costs will be assigned for the cost of medical attendances, medicines and time off work. The analysis of economic and qualitative outcomes is not contained within this analysis plan.

3. STUDY METHODS

3.1. Overall study design and plan

Target for randomisation: 650 intervention and 650 control participants

Date of first randomisation: 25/10/2010

Date of last randomisation: 27/12/2012

Trial design: Individually randomized, parallel group

Blinding: Participants and their treating clinician are blind to treatment allocation

Randomised Interventions: Montelukast vs. placebo

Allocation ratio: 1:1

3.2. Selection of study population

Inclusion Criteria

- age ≥ 10 months and ≤ 5 years on the day of consent.
- two or more attacks of parent-reported wheeze.
- at least one attack with wheeze validated by a clinician
- the most recent attack within the last 3 months.
- contactable by telephone and able to attend one face-to-face review
- parent or guardian able to give written informed consent for their child to participate in the study.

Exclusion Criteria

- any other chronic respiratory condition diagnosed by a clinician including structural airway abnormality (e.g. floppy larynx) and cystic fibrosis
- any chronic condition that increases vulnerability to respiratory tract infection such as severe developmental delay with feeding difficulty or sickle cell disease
- history of neonatal chronic lung disease
- current continuous oral montelukast therapy
- in a trial using an IMP in the previous 3 months prior to recruitment.

3.3. Method of treatment assignment and randomisation

Randomisation was stratified according to ALOX5 promoter polymorphism status. This yielded two groups:

Group I Children with the [5/5] ALOX5 promoter polymorphism genotype.
Group II Children with [5/x and x/x]" ALOX5 promoter polymorphism genotype; where x is > or < than 5 SP1 repeats.

Children (participants) in each of these two genotype groups were assigned consecutive randomisation numbers from randomised permuted blocks of 10. Within each block equal numbers of children were randomly allocated to placebo and active treatment. When all numbers from the first block had been assigned a new block of randomisation numbers was allocated to that genotype group, until a total of 1300 children in groups 1 and 2 combined had been assigned a randomisation number. If a randomisation number was assigned to a child who did not subsequently take any dose

of IMP, the IMP bearing that randomisation number was returned to pharmacy, and the randomisation number may have been assigned to another child (participant).

3.4. Treatment masking (Blinding)

This was a double-blind trial: neither subject nor investigator was aware of a subject's allocation. Active and placebo batches of IMP had identical packaging, labelling and appearance.

3.5. Sample size determination

This trial is powered to detect a clinically significant difference in the number of attacks of wheeze between intervention and control arms. We also have some power to detect differential responsiveness (in terms of the primary outcome) to montelukast in the stratum with ALOX5 promoter polymorphism [5/5], compared with the stratum with the ALOX5 [5/x and x/x]" genotype.

Data on mean (0.76) and standard deviation (1.22) of number of attacks come from data from the UK General Practitioner Research Database on courses of oral steroids (a proxy for number of episodes). These data follow an overdispersed Poisson distribution. To take account of this we used markov chain Monte Carlo simulation in WinBUGs to estimate sample sizes required: (WinBUGS Version 1.4. 2003 Available from: http://www.mrc-bsu.cam.ac.uk/bugs/welcome.shtml). To detect a 33% drop in attack rate requiring medical attention, with a power of 90% and at a significance level of 5%, and a 6% loss to follow up, we require 1050 children in total. A 33% drop in attack rates equates to an attack rate of 0.51 for the treatment group. The clinical significance of these changes is that approximately four children will need to be treated to prevent one clinically severe attack. A sample size of 1200 gives just over 80% power at the 5% significance level to detect an interaction between treatment and genotype if the effect is a 60% reduction in the [5/x plus x/x] and a 20% reduction in the [5/5] stratum. Assuming a 6% dropout, 1300 children will need to be recruited.

4. DATA COLLECTION

4.1. Baseline

Demographics

Height in cm

Weight in Kg

Age in years

Sex (Male; Female)

Stratum (A or B)

Ethnicity (Asian or Asian British; Mixed; Black or Black British; White; Other)

Risk factors: Birth, Atopy and Family History (Yes, No)

Preterm birth <37 wk gestation; Birth weight<2500g; Food allergy; Drug allergy; itchy rash for >6 months; Eczema; Tobacco exposure in utero; Tobacco exposure in household; daycare attendance; immunisation status for Pneumococcus; imunisation status for influenza; history of asthma mother; history of asthma father.

Pre-study illness and therapy (Yes/No)

Episodic wheeze; multitrigger wheeze; admitted to hospital in last year; ever admitted to hospital; Preventer therapy none; Preventer therapy antileukotriene; Preventer therapy Maintenance inhaled steroids; Preventer therapy episodic inhaled steroids

Age at first wheeze in months

Interval between onset of URTI and wheezing (hours)

Number of courses of systemic steroids in the last year

Number of unscheduled medical attendances for wheeze in last year

Pre-existing conditions

Medical condition

Date of diagnosis

Resolved/ongoing

Current treatment

4.2. Follow up

Unscheduled medical attendance

Phone call data: Type of attendance (A&E; Hospital; GP; Pharmacist; Other)

Phone call data: Duration of visit (calculated from date of admission and date of discharge)

Description of wheezing episodes

Diary card: Wheeze in the last 24 hours (Yes/No)

Diary card: Date of diary card entry

Diary card: Duration of wheeze episodes will be calculated where wheeze in the last 24 hours has been ticked over consecutive days

Diary card: Total duration of wheeze days over follow-up period

Medication use Steroids (OCS)

Diary card: Date

Diary card: Medication (where medication includes Prednisolone and its variations)

Diary card: Dose

Diary card: Units

Diary card: Days

Diary card: Doses per day

Phone call data: Other medications used (where medication includes Prednisolone and its variations)

Medication use Steroids (ICS)

Diary card: Date

Diary card: Medication

Diary card: Dose

Diary card: Units

Diary card: Days

Diary card: Doses per day

Phone call data: Other medications used

Medication use Salbutamol

Diary card: Date

Diary card: blue inhaler used today?

Diary card: How many times blue inhaler used?

Diary card: How many puffs when blue inhaler used?

Phone call data: Other medications used (where medication includes salbutamol and its variations)

Medication use IMP

Diary card: Date

Diary card: Wheeze in last 24 hours (Yes/No)

Diary card: Cold in last 24 hours (Yes/No)

Diary card: Trial medicine used today (Yes/No)

Phone call data: Number of IMP initiations

Phone call data: Total days used

Adverse events and serious adverse events

Clinical AE term (categorised as; minor injury, GI, URTI, CNS, minor infection, allergy, cutaneous, respiratory, haem)

SAE term

SAE expected (Yes/No)

Start date

End date

Date of death

Duration in hours

Intensity (Mild, Moderate, Severe)

Action taken (none, interrupted, discontinued, reduced)

Related to study drug (Definitely not, probably not, possibly, probably, definitely)

SAE resolved (resolved, resolved with sequelae)

Sequelae details

Outcome (improved, persisting, worsened, fatal, unknown)

Withdrawals

Withdrawal (from treatment or trial)

Date of withdrawal

Reason for withdrawal (eligibility no longer met, death of participant, other adverse event, deterioration of pre-existing condition, Poor adherence to treatment, Perceived lack of efficacy, unable to locate participant, other)

Withdrawal decision by (CI, PI, Referring investigator, Carer, Participant, other)

Permission to use data (do not use any data, use partial data up to withdrawal, use all data up to withdrawal, collect and use all follow up data)

Code broken (Yes/No)

4.3. Timing of data collection

Each child (participant) was followed up for 12 months post randomisation with data collection taking place at 2, 4, 6, 8, 10 and 12 months.

5. GENERAL ISSUES FOR STATISTICAL ANALYSIS

All analyses will be conducted two sided and significance interpreted at the 5% significance level.

5.1. Blinding of the statistical analysis

The statistical analysis will be conducted unblinded so that the appropriate treatment code can be used in the models fitted.

5.2. Analysis populations

5.2.2. Intent-to-treat population

The intention-to-treat (ITT) sample is defined for this trial as all participants randomized into the trial included in the intervention group to which they were randomised.

5.2.3. Available-case population

The available Case (AC) sample is defined for this trial as all participants randomized into the trial included in the intervention group to which they were randomised where outcome data are available.

5.2.4. Per protocol population

The Per Protocol (PP) sample is defined as the available case sample with those participants who discontinue IMP or were randomised incorrectly being excluded.

5.2.5. Safety population

The safety population includes all participants.

5.2.6. Other populations

Two populations are described for the sensitivity analyses described in section 8.5. The first is based on the ITT population replacing any stratification factors that were incorrectly defined at randomisation with the corrected values.

The second is based on the ITT population with the exclusion of 11 incorrectly randomised participants.

5.3. Database

5.3.1. Description

The data were entered into and stored in a Microsoft Access database. Data were entered by trial staff who were blind to treatment group.

5.3.2. Data quality

Source data verification is performed for 10% of CRFs by the trial team.

5.3.3. Database freeze and lock

Once the trial team have completed all data entry and checking. The statistician responsible for the analysis will conduct or oversee additional data checks. These include things such as range checks, logical and consistency checks which may not be picked up by checks performed at the individual level. Procedures implemented to database lock will be followed in accordance with the relevant SOP (PCTU_DM_04 Standard Operating Procedures (SOP) for: Data Entry, Quality Control, Data Extraction and Database lock)

Analysis will take place when the database is considered final.

5.4. Analysis software

The analysis will be carried out using Stata version 12.0.

5.5. Methods for withdrawals, loss to follow-up and missing data

Those participants who withdraw and provide permission to use their data will be included in the analysis up to the point of withdrawal.

For the primary outcome phonecall data, at the time of writing (prior to unblinding) we have:

Full 12 months data on 1134/1347(84%)

29/1347 (2%) participants withdrew before the first 2 month phonecall and have no data collected as expected

12/1347 (0.9%) do not have any follow up data and this is being queried with the sites

Partial follow up data is available for 172 (13%). 44 of these participants did not formally withdraw from follow up. This is being queried with the sites

After data cleaning we expect the levels of missing data to improve. Due to these relatively low levels of missing data, and that the follow up time for each participant is to be included in the analysis no imputation of the missing data will be performed

5.6. Method for handling centre effects

We do not anticipate there to be any affect of centre and this will not be adjusted for in the analysis

5.7. Method for handling randomisation stratification or minimisation factors

The randomisation was stratified by genotype and this will be included as a covariate in all analyses.

5.8. Method for handling clustering effects

Some outcomes are collected at the level of episode, (duration of wheeze episode, duration of cold episode, duration of hospital admission) therefore we have episode data within children. In these cases a random effect is included for child.

5.9. Method for selecting other variables that will be adjusted for

All analysis will only be adjusted for genotype (see section 2.7).

5.10. Multiple comparisons and multiplicity

No formal method will be used to account for multiple comparisons. All comparisons will be defined within this document *a–priori* and all will be reported.

5.11. Method for handling non-adherence

Analysis of all primary and secondary outcomes will be performed on an intention-to-treat basis. A Complier Average Causal Effect (CACE) analysis and per protocol analysis will also be conducted for the primary analysis.

5.12. Method for handling time-varying interventions

Not applicable

5.13. Method for handling outliers and influential points

Where any outliers are identified they will be investigated to determine whether they are true recorded values or a data entry error. Where outliers are identified as a true recorded value, an assessment will be made as to whether there are clear quality indications to remove them. If such indications exist, the outliers will be removed. If such indications do not exist, the analysis will be performed both including and excluding the outlier to assess the robustness of the conclusions.

5.14. Data from external sources

Not applicable

5.15. Derived and computed variables

All derived and computed variables will be documented in the analysis programmes. The primary outcome is a summation of all types of medical attendances across the entire trial, for each participant.

The primary outcome, and the breakdown of unscheduled medical opinion, will be taken solely from the phone call data as this data has been confirmed against clinical records.

Medication use data may be recorded on either the phone call CRF and/or the diary card. A medication will be defined as being used if it appears in either of these two records.

Medical attendance data was collected strictly within 12 months, as calculated from the date of randomisation. Participants who do not experience an event are censored at exactly 12 months of follow up or the point of withdrawal from follow up.

Any diary data collected outside of the 12 month follow up will be excluded from the analysis. Participants who do not experience episodes of cold or wheeze will be censored at the point of 12 months from randomisation or withdrawal from medication, as diary cards are not completed for those not taking IMP.

6. DESCRIPTIVE ANALYSES

The proposed tables to be populated during the analysis can be found in the appendix

6.1. Participant flow

Participant throughput will be summarized in a CONSORT diagram.

6.2. Representativeness of sample

Information unavailable to make this comparison

6.3. Baseline comparability of randomised groups

See table 1 in the appendix for the variables to be used in these comparisons.

6.3.1. Demographics

6.3.2. Prior and concurrent medications

6.3.3. Baseline and screening conditions

6.3.4. Baseline medical history

6.3.5. Baseline physical exam

6.3.6. Cluster characteristics if cluster randomised

6.3.7. Characteristics of care providers where applicable

6.4. Comparison of losses to follow-up

See table 2 in the appendix

6.5. Comparison of compliance to treatment and protocol

Compliance to treatment will be summarised as the number of returned used sachets of medication.

6.6. Emergency or accidental unblinding of randomised treatment

All unblindings will be summarised by treatment group

7. INTERIM ANALYSES AND SAFETY MONITORING ANALYSES

7.1. Purpose of interim analyses

No interim analyses of the data were planned or conducted.

7.2. Monitoring plan

A Data Monitoring Committee was initiated at the beginning of the study. This committee met three times during the course of the study and saw accumulating data by treatment group on recruitment, safety and efficacy. All data was presented descriptively with no hypothesis testing.

7.3. Stopping rules

Not applicable

7.4. Measures taken to minimize bias

Not applicable

7.5. Adjustment for p-values

Not applicable

7.6. Interim analysis for sample size adjustment

Not applicable

8. ANALYSIS OF PRIMARY OUTCOME

8.1. Definition of outcome measure

The primary outcome for each participant is the total number of unscheduled medical attendances over the course of the trial.

8.2. Descriptive statistics for outcome measure

The primary outcome will be summarised for each treatment group as the total number of events and corresponding median length of follow up time per treatment group.

Data will be presented as mean (sd) or median (interquartile range) depending upon the distribution of the data.

8.3. Primary analysis

The primary analysis will be a Poisson regression model with the follow up time of each individual fitted as an exposure variable and with a random effect for individual to account for overdispersion.

The incident rate ratio (IRR) for the treatment effect and corresponding 95% confidence interval will be presented. An IRR of less than 1 indicates a benefit of Montelukast in reducing the rate of unscheduled medical attendance needed.

8.4. Assumption checks and actions to be taken if assumptions do not hold

The fit of the model will be compared to a model without a random effect using the likelihood ratio test, and the fit will be assessed using diagnostic plots (residuals versus fitted values), alternative distributions to the Poisson such as the Negative binomial or removal of the random effect shall be considered where necessary for improved fit.

8.5. Other analysis supporting the primary (inc. sensitivity analyses)

The primary analysis will be performed on the per-protocol population and using a CACE analysis.

8.5 It will be repeated replacing any stratification factors that were incorrectly defined at randomisation with the corrected values (see section 1.4).

It will be repeated with exclusion of 11 incorrectly randomised participants (see section 1.4).

9. ANALYSIS OF SECONDARY OUTCOMES

9.1. Definition of outcome measure

- individual type of medical attendance: (hospital admission, hospital attendance (a&e), and GP visit)
- Duration (in days) of hospital admission
- Number of wheeze episodes
- Total duration of wheeze episode

- The number of steroid (OCS) courses per year
- The number of IMP courses per year
- first hospital admission
- first hospital attendance (A&E)
- first GP visit
- first episode of wheeze
- proportion receiving no steroids (OCS) vs. any during the trial
- Proportion starting steroids (ICS) during the trial
- Salbutamol use per year
- Salbutamol use per episode of wheeze per year

9.2. Descriptive statistics for outcome measure

Each outcome will be summarised for each treatment group as the total number of events or average duration of episode.

Data will be presented as mean (sd) or median (interquartile range) depending upon the distribution of the data.

9.3. Secondary analysis

The primary analysis will be repeated for each of the following secondary outcomes:

1. individual type of medical attendance: (hospital admission, hospital attendance (a&e), and GP visit)
2. Duration (in days) of hospital admission
3. Number of wheeze episodes
4. Duration of wheeze episode
5. The number of steroid (OCS) courses per year
6. The number of IMP courses per year

Time to event data will be summarised using Kaplan Meier plots. The treatment effect will be evaluated using a Cox regression model. The Hazard Ratio (HR) for the treatment effect and corresponding 95% confidence interval will be presented. A HR of less than 1 indicates a benefit of Montelukast in reducing the time to first event.

1. first hospital admission
2. first hospital attendance (A&E)
3. first GP visit
4. first episode of wheeze

Binary outcomes will be analysed with logistic regression

1. proportion receiving no steroids (OCS) vs. any during the trial
2. Proportion starting steroids (ICS) during the trial

9.4. Assumption checks and actions to be taken is assumptions do not hold

The assumption of proportional hazards for the cox regression model will be checked using the methods proposed by Grambsch and Therneau (14). If this assumption is violated, alternative methods will be used.

See section 8.4 for Poisson regression assumption checks.

9.5. Other analysis supporting the secondary (inc. sensitivity analyses)

None

10. SAFETY AND TOLERABILITY ANALYSES

Adverse event data will be summarised with descriptive statistics.

10.1. Intervention exposure

The number of participants receiving medication will be summarised per treatment group.

10.2. All Adverse events

See table 7 in the appendix

10.3. Adverse events leading to withdrawal

See table 2 in the appendix

10.4. Serious adverse events

See table 8 in the appendix

10.5. Clinical laboratory evaluations

There are no AEs defined by laboratory evaluations

11. SUBGROUP ANALYSES

11.1. Definition of outcome measure

For each participant, the total number of unscheduled medical attendances over the course of the trial.

11.2. Definition of subgroups

The primary analysis will be repeated to assess whether there is a differential effect of treatment by:

- Genotype, categorised as 5/5 vs (5/x and x/x) and alternatively as (5/5 and 5/x) vs x/x
- Whether ICS taken at baseline (yes,No)
- Episodic vs multitrigger wheeze at baseline

11.3. Sample size justification for the subgroup analysis

The study has been powered to detect a specific interaction effect.

11.4. Descriptive analysis for subgroups

The mean and standard deviation of the number of unscheduled medical attendances will be summarised for each ALOX5 genotype and each treatment group

11.5. Method of analysis

The primary analysis will be repeated including an interaction term between treatment and stratum. The significance of the interaction term assessed.

12. AMENDMENTS TO VERSION X

13. REFERENCES

14. APPENDICIES

This document was created based on the Mental Health and Neuroscience Clinical Trials Unit (MH&N CTU) analysis strategy template (version 1.5;13/02/2008)

Appendix: Statistical Analysis Report Template

CONSORT Flow Diagram

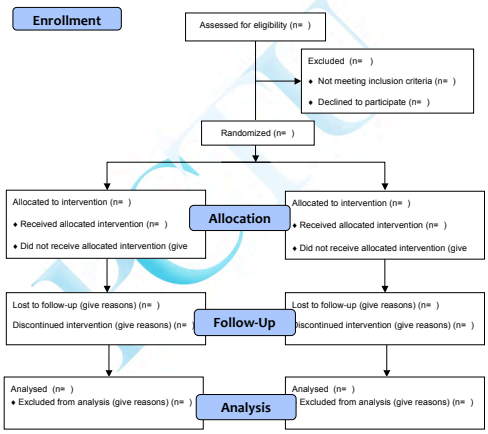


Table 1: Baseline comparability of treatment groups

ITT population	Montelukast N=			Placebo N=		
	5/5	5/x and x/x	Total	5/5	5/x and x/x	Total
Height (cm)						
Weight (Kg)						
Age (years)						
Gender						
Male						
Female						
Stratum						
A						
B						
Ethnicity						
Asian or Asian British						
Mixed						
Black or Black British						
White						
Other						
Pre existing conditions						
X						
Y						
Z						
Age at first wheeze (months)						

Interval between onset of URTI and wheezing (hours)	
Episodic wheeze	
Multi-trigger wheeze	
Admitted to hospital in last year	
Admitted to hospital ever	
Preventer therapy	
None	
Antileukotriene	
Maintenance inhaled steroids	
Episodic inhaled steroids	
Risk factors	
Preterm birth <37 wk gestation	
Birth weight<2500g	
Food allergy	
Drug allergy	
Itchy rash for > 6 months	
Eczema	
Tobacco exposure in utero	
Tobacco exposure in household	
Daycare attendance	
Immunised for Pneumococcus	
Immunised for influenza	
History of asthma in mother	

History of asthma in father	
Numbers are N(%) or mean (SD)	

This table is to be repeated for the alternative stratification (5/5 and 5/x) versus (x/x).

Table 2: losses to follow up

ITT population	Montelukast N=			Placebo N=		
	Trial Treatment		Total	Trial Treatment		Total
Reason for withdrawal						
Eligibility no longer met						
Death						
Other adverse event						
Deterioration of pre-existing condition						
Poor adherence to treatment						
Perceived lack of efficacy						
Unable to locate participant						
Other						
Decision made by						
CI						
PI						
Referring investigator						
Carer						
Participant						
Other						
Permission to use data						
Do not use any data						

Use partial data up to withdrawal	
Use all data up to withdrawal	
Collect and use all follow up data	
Numbers are N (%)	

Table 3 Primary analysis: unscheduled medical attendances for wheeze over 12 months

ITT population	Montelukast N=	Placebo N=	Adjusted IRR (95% CI)[1]	p-value
Follow up time (days)				
Number of :				
Any medical attendance				
All				
Straum A				
Stratum B				
Stratum 5/5 and 5/x				
Stratum D x/x				
ICS at baseline				
Multitrigger vs episodic wheeze				
Hospital admissions				
Hospital attendances (A&E or admission)				
Unscheduled GP visits				
Parents considered medication to be				

efficacious, N(%)

Data are mean (SD)

[1] Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual to account for overdispersion with follow up time fitted as the exposure. An interaction term has been included to assess whether there is a differential treatment effect dependent on stratum.

Table 4: Episodes of cold and wheeze

ITT population	Montelukast N=	Placebo N=	IRR (95% CI)[1]	p-value
Number of:				
Wheeze episodes				
Cold episodes				
Days wheezing				
Returned used medication sachets				
Duration of:				
Wheeze episodes (days)				
Hospital admission (days)				

Data are mean (SD)

[1] Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual to account for overdispersion with follow up time fitted as the exposure. Duration of each hospital admission is analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual with follow up time fitted as the exposure.

Table 5: Time to first event of unscheduled medical attendance, wheeze or cold

ITT population	Montelukast N=	Placebo N=	HR (95% CI)	p-value
Time (in days) to first:				
Hospital admission				
Hospital attendance (A&E or admission)				
Unscheduled GP visit				
Episode of wheeze				
Episode of a cold				

Data are median (IQR)

Data are analysed using a Cox regression model with fixed effects for stratification factor and treatment group

Table 6: Medication usage

ITT population	Montelukast N=	Placebo N=	IRR or OR (95% CI)	p-value
Steroids (OCS)				
[1]Number of courses, mean (SD)				
[2]Proportion receiving OCS, N (%)				
Steroids (ICS)				
[2]Proportion starting, N (%)				

Salbutamol

[1]Number of puffs used per episode, mean(SD)

Total puffs used per year

Investigational Medicinal Product

[1]Number of initiations, mean (SD)

[1]Number of sachets per episode, mean (SD)

Number of sachets used per year

[1]Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group and a random effect for individual to account for overdispersion with follow up time fitted as the exposure.

[2] Data are analysed using logistic regression with fixed effects for stratification factor and treatment group

Table 7 Total adverse events per group

Safety population	Montelukast N=	Placebo N=
All events		
Minor injury		
GI		
URTI		
CNS		
Minor infection		
Allergy		
Cutaneous		
Respiratory		
Haem		

Possibly, probably or definitely related		
Minor injury		
GI		
URTI		
CNS		
Minor infection		
Allergy		
Cutaneous		
Respiratory		
Haem		

Data are n (%)

Table 8: Serious Adverse events per group

Safety population	Montelukast N=	Placebo N=
Death		
XXX		
XXX		
…		

Data are n (%)

8.8 Appendix 8 - Standard operating procedures

8.8.1 Sample collection SOP

Barts and The London
School of Medicine and Dentistry

Standard Operating Procedures (SOP) for: Clinical Procedures for the WAIT Study (Wheeze and Intermittent Treatment)			
SOP Number:	**1**	Version Number:	**1**
Effective Date:	**15/09/2011**	Review Date:	**01/04/2012**

Author:	**Cassie Brady**

Authorisation:	
Name / Position	**Professor J. Grigg**
Signature	
Date	**15/08/2011**

Purpose and Objective:
To ensure the correct procedure for genetic swabs and urine sampling/ for the WAIT Study.

SOP Text

	Responsibility	Activity
1.	CI/PI/Research Nurse	At T-2 visit obtain Informed Consent and ensure this has been signed (by parent/guardian) and researcher and/or PI prior to commencing sample (DNA/urine) collection. Sampling will occur immediately following consent process.
2.	PI/Research Nurse	Prepare child and family appropriately prior to sampling. Obtain saliva samples (5 oral sponges soaked in saliva) then cut the sponge tip off the stick and put them in the container provided. Fix the lid securely to release the preserving medium. (Please see appendix 1 for technique). Ensure that cap is secured parallel to the base to prevent leakage. Once lid is secure apply an adhesive label with the allocated serial number on it (e.g. LO-0000, LE-0000, AB-0000) to the top or side of the container. Date and sign the label and document on the T-2 Assessment and CRF (Case record form) page 2 that samples have been taken and sent to the Lab. The genetic swab is only required to be taken **once** at the time of consent (Visit1). Only if there is a problem extracting the DNA for genotyping from the original sample, then another sample will be required. All equipment required is stored in WAIT Study Offices. **Equipment needed for DNA sampling are**: **Oragene.DNA Kit** (Part 1, Oragene is a **container** for collection of human saliva samples) and DNAgenotek (Part 2 of the kit, are 5 x swabs for Saliva collection from Young Children)

		Arrange for sample delivery to laboratory for processing. • Complete electronic PDF request form and email to c.nwokoro@nhs.net • Print a copy – place one half with the sample for posting, keep the other in your investigator site file with the patient's CRFs. • Place each labelled sample in the prepaid/pre-addressed sample envelope provided (with PDF request form and a a small amount of absorbent material (such as gauze swabs) and post/hand deliver (London team) promptly. Laboratory is located at the Blizard Institute Institute of Cell and Molecular Science Barts and The London School of Medicine and Dentistry The Blizard Building 4 Newark St, Whitechapel, London, E1 2AT, UK. Laboratory contacts are, Dr. Thomas Vulliamy (0207 882 2623) and Iain Dickson.(0207 882 2616) Urine sample is collected at visit 1 providing the child is free from viral illness (they don't have a cold or viral wheeze). All recruits will provide a second urine if they present to A&E or are admitted to a ward at the Royal London Hospital with a viral wheeze. Equipment needed is a Mid Stream Urine Collection Set/U-Bag Urine collector to collect the sample. From the sample the PI/Research Nurse transfers 1-1.8ml of urine into each of 2 CryoTubes with a syringe/pipette. Label both tubes with adhesives labels or hand write (in permanent marker) the trial ID and initials and date and sign them. The sample is immediately stored on ice and transferred to the -70 degrees freezer located at each site immediately after clinic. (Aim to have it transferred from ice to the freezer within an hour of being collected) Document collection in the urine collection log. Urine samples will be shipped at the end of the study on dry ice to the Jagiellionian University Laboratory in Poland. For external sites urines collected will be stored on site in a -70-80deg freezer. (checked during site monitoring visit)
3.	PI/Research Nurse	Document collection of samples in Subjects CRF Document height and weight on T-2 CRF (this is routinely recorded by clinic staff)

Version	Reason for Change	Date Authorised

T -2 Sampling Instructions and Proforma

PREPARING FOR SALIVA COLLECTION

DO

- Check consent form before proceeding.
- Be careful - do not leave child unattended with saliva sponges.
- Be nice – explain that sampling may tickle the gums "like a toothbrush" but will be fun and will not take long.
- Allow 10 minutes after drinking water or 15 minutes after breastfeeding before collecting sample.
- Allow children able to spit to spit directly into the *Oragene* base-unit (grey) to supplement DNA collection.
- Seal the Oragene container after 15 minutes have passed.

DO NOT

- Rub directly on the child's teeth – this increases the proportion of bacterial DNA collected.
- Use any sponges other than those provided.
- Exceed 15 minutes before placing the saliva-soaked sponges in the *Oragene* vial

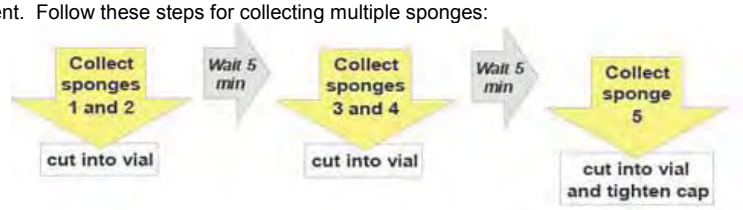
SALIVA COLLECTION

1. Place the saliva sponge into the child's mouth in the cheek pouch (the space between the gums and the inner cheek). Gently move the saliva sponge around the upper and lower cheek pouches on both sides of the mouth to soak up as much saliva as possible. There is no need to 'scrape' the inner cheek with saliva sponges – simply collect as much saliva as possible from the cheek pouches. The sponge will absorb more saliva if it is left in the child's mouth for a longer time (up to 60 seconds).

2. Once collected, cut the sponge into the blue base of the *Oragene•DNA* kit as follows. Place the sponge firmly against the bottom of the kit between the tooth and the kit wall (see pictures to left). This action will ensure that the sponge tip remains in the container during the cutting action. Using the scissors provided, cut the narrow part of the handle just above the sponge.



3. For the collection of up to 5 saliva sponge samples from the same child, repeat steps 1 and 2. Follow the sequence shown in the diagram below. A rest period of about 5 min between each collection of 2 sponges is helpful. To prevent the saliva samples from drying out, cap the vial (see step 4) within 15 min of the first collection. If you have not had a chance to collect all 5 sponges within 15 minutes, you may carefully re-open the kit. If you remove the cap be sure that the inside is facing upwards when putting it on any surface. Do not spill the content. Follow these steps for collecting multiple sponges:



FLEXIBILITY IS KEY WITH YOUNG CHILDREN – IT IS MORE IMPORTANT TO COLLECT THE MAXIMUM VOLUME OF SALIVA – IDEALLY USING FIVE SPONGES, THAN TO FOLLOW THESE TIMINGS TO THE LETTER. THE CLOSER TO THIS PROTOCOL WE CAN REMAIN WITHOUT CAUSING UNDUE STRESS TO THE CHILD THE BETTER.

4. Carefully cap the kit and tighten it firmly. Once the Oragene•DNA liquid is released from the cap, it will preserve the DNA collected by the sponge(s). Samples may need to repeated if the kit is not correctly capped.

Ensure that the cap is secured parallel to the base to prevent leakage in transit.

5. Invert gently 5 times to mix the sample. Place in envelope provided with some absorbent material (gauze swabs are ideal) and post with request form. Request form should also be emailed as instructed on form.

T -2 Sampling Instructions and Proforma

URINE SAMPLE COLLECTION

DO

- Check consent form before proceeding.
- Be careful - do not upset child or parent.
- Be nice – always.
- Allow breastfeeding children to feed, as this often triggers urination.
- Be opportunist, be ready to catch urine at a moment's notice, especially with infants and young children.
- Have icebox, syringe and universal container prepared, labelled with date and registration number and ready to hand.
- Place specimen on ice **immediately** after collection. Warm samples cause spurious leukotriene results.

DO NOT

- Leave urine at room temperature once collected.

URINE COLLECTION

Under 2s:

1. With parental assistance if appropriate, change nappy and apply urine bag (under 2s) on arrival.

Over 2s:

2. Open cardboard potty and give to parent (they may know best how to secure a urine sample from their child).
3. Some over 2s may be better with a urine bag or other method, take guidance from the parents and apply your experience.

All:

4. Use syringe/pipette to decant urine from bag/potty to 2 x 1ml cryotubes labelled with full patient serial number. Take care not to fill container above indicator line as this allows no space for volume expansion on freezing.
5. **PLACE CONTAINER ON ICE IMMEDIATELY AND TRANSFER TO -70 FREEZER AS SOON AS POSSIBLE**
6. If urine specimen is not provided before end of visit, leave the potty/urine bag (plus a spare), syringe/pipette and cryotubes and request that parents decant and **freeze specimens immediately on production**, these can then be collected at a later date (T0 visit). **Urine must be collected and frozen in cryotubes.**
7. Complete specimen collection proforma before ending the visit.

BASELINE URINE SAMPLE – TRANSPORT

Royal London Hospital:

1. Transport specimens on ice to London -70 freezer on day of collection.
2. Specimens taken out of hours should be maintained frozen (in domestic freezer if necessary) until transfer.
3. Complete urine specimen collection log.

Other Sites:

1. Transport specimens on ice to designated hospital freezer. Specimens must remain frozen at -70.
2. Specimens will be couriered in batches on ice to London Laboratory.
3. Complete urine specimen collection log.

SYMPTOMATIC URINE SAMPLE – TRANSPORT (On attendance at secondary care only)

1. Specimens should be labelled with addressograph/serial number and date and frozen immediately followed by transfer to -70 freezer as soon as possible.

DNA genotek

Shipping Recommendations for Exempt Specimen Samples

Summary of recommendations

For samples that are not expected to be pathogenic, the following packaging is recommended for shipping the container with the collection specimen:

- Capped DNA Genotek collection tube, disc, or vial (with or without secondary rigid plastic container)
- A liquid-tight bag with biohazard logo to hold the capped container
- Absorbent material in the liquid-tight bag sufficient to soak up at least 4 mL of liquid
- An outer mailing envelope labeled as either "Exempt Human Specimen" or "Exempt Animal Specimen"

This complete kit is available for purchase from DNA Genotek, including all relevant labels and instructions. *Please contact sales@DNAgenotek.com or reference our website at www.dnagenotek.com for different mailer product options.*

Background

Air transportation of diagnostic specimens is governed under authority of the International Civil Aviation Organization (ICAO) and its regulations are published by the International Air Transport Association (IATA). Since courier services designated as "ground" may involve an air transport segment, the IATA publications are broadly applicable to both air and ground shipment.

The IATA Dangerous Goods Manual was revised on January 1, 2005, and most recently amended according to Addendum III, issued on July 5, 2005. This Addendum introduces the following guidance:

3.6.2.2.3.6 Patient specimens for which there is minimal likelihood that pathogens are present are not subject to these Regulations if the specimen is transported in a packaging which will prevent any leakage and which is marked with the words "Exempt human specimen" or "Exempt animal specimen", as appropriate. The packaging must meet the following conditions:

(a) The packaging must consist of three components:
  (1) a leak-proof primary receptacle(s);
  (2) a leak-proof secondary packaging; and
  (3) an outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm × 100 mm;

(b) For liquids, absorbent material in sufficient quantity to absorb the entire contents must be placed between the primary receptacle(s) and the secondary packaging so that, during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the cushioning material;

(c) When multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them.

NOTE:

In determining whether a patient specimen has a minimal likelihood that pathogens are present, an element of professional judgment is required to determine if a substance is exempt under this paragraph. That judgment should be based on the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions.

Examples of specimens which may be transported under this paragraph include the blood or urine tests to monitor cholesterol levels, blood glucose levels, hormone levels, or prostate specific antigens (PSA); tests required to monitor organ function such as heart, liver or kidney function for humans or animals with non-infectious diseases, or therapeutic drug monitoring; tests conducted for insurance or employment purposes and are intended to determine the presence of drugs or alcohol; pregnancy tests; biopsies to detect cancer; and antibody detection in humans or animals.

For those collecting samples which may not fit the definition above, more stringent requirements for transportation apply. These include the use of a rigid outer container, application of UN2814 (Category A pathogens) or UN3373 (Category B pathogens) labels, and demonstration of compliance with pressure tests. More information is available upon request from DNA Genotek, directly from IATA, or possibly from your local carrier.

DNA genotek
www.dnagenotek.com
info@dnagenotek.com
Tel.: (613)723-5757

8.8.2 Urinary eicosanoid measurement SOP (Krakow)

Marek Sanak, Anna Gielicz 2009-08-02

Standard Operating Procedure: Urinary eicosanoids measurements:

Platform: High performance liquid chromatography - mass spectrometry
Gas chromatography - mass spectrometry

Sample requirements: frozen urine, 2 aliquots of 1 mL (Eppendorf tubes)
Sample preparation: urine (from the first morning micturition, or sampled using a schedule of the clinical protocol) immediately transferred to the lab in 50 mL disposable jar.
Preprocessing: if clear aliquot into 2 Eppendorf tubes 1 mL each, label, storage: frozen at -70°C in the freezer, otherwise - centrifugation 5 000g, 10 min swinging bucket rotor, then aliquot
Stability: tested for 2 years storage, no decay of eicosanoids

Urinary creatinine measurement: use one aliquot, thaw on ice or in the fridge (4°C, 3-5 hrs), required 200 uL, measure using the standard protocol and Vitros 350 Chemistry System (Ortho Diagnostics).

Organic phase extraction:
Thaw on ice or in the fridge (4°C, 3-5 hrs; batch up to 20 samples). Adjust pH to 3.5 with 1 N HCl (30 - 80 uL), check pH using the narrow range pH stick. Add internal deuterated standards mix containing: LTE_4-d_3 (2 ng), tetranor-PGE-M-d_6 (10 ng), tetranor-PGD-M-d_6 (10 ng), 13,14-dehydro,15-keto PGE_2-d_4 (1 ng), 13,14-dehydro,15-keto PGD_2-d_4 (1 ng), 13,14-dehydro,15-keto-tetranor-PGE_2-d_4 (1 ng), 13,14-dehydro,15-keto-tetranor-PGD_2-d_4 (1 ng), 9α11β-PGF_2-d_4 (1 ng), 15-deoxy,delta-12,14-PGJ_2-d_4 (1 ng) in methanol - 10 uL of the mix. If uric acid precipitate present - spin 10 min 10 000 g at 4°C (microcentrifuge) and transfer supernatant to fresh tube. Mix in a conical 10 mL tube with 1 mL tertiary-butylmethyl-ether (TBM), vortex 2 min, spin as before. Collect upper organic phase to fresh tube, repeat extraction with another 1 mL TBM, combine organic phases. Dry at room temperature under nitrogen flow (1 L/min) for 30 min. Dissolve in 60 ml methanol and immediately proceed with analysis.
High-performance liquid chromatography - tandem mass spectrometry
Equipment: autosampler (Shimadzu Sil-2-AC), reverse phase column (Zorbax Eclipse XDB C-18, Agilent Technologies) stabilized thermally at 37 °C, multiple reaction monitoring mode

(MRM) tandem mass spectrometry (Qtrap 4000, Applied Biosystems) equipped with electrospray ion source negative ionization mode, use batch protocol for urinary eicosanoids. Test: inject 10 uL of internal standard mix. Check for area under the peak > 20 000.

Injection: 10 uL of methanol extract

Elution: gradient consisting of two mobile phases: A) acetonitrile/water/acetic acid (20/80/0.01) and B) acetonitrile /iso-propanol/acetic acid (55/45/0.01) using the flow rate 0.11ml/min

Gas chromatography - mass spectrometry

Equipment: single quadrupole mass spectrometer (Engine 5989B series II Hewlett Packard, Palo Alto, CA, USA), 15 m capillary column, gas-chromatography negative-ion chemical ionization mode (GC-NICI-MS). Use protocol for urinary prostanoids.

Three step derivatisation: to pentafluorobenzyl ester, trimethylsilyl esters, and methoxyoxime, and subsequent purification by a thin-layer chromatography (TLC).

Following methanol elution from the silica of TLC

Injection 2 uL of the eluate

Data analysis:

HPLC-MS:

ion pairs:

- LTE_4-d_3 441-336 and LTE_4 438-333
- tetranor-PGE-M-d_6 tetranor-PGD-M-d_6 333-315 and tetranor-PGE-M tetranor-PGD-M 327-309 (different retention time)
- 13,14-dehydro,15-keto PGE_2-d_4 and 13,14-dehydro,15-keto PGD_2-d_4 355-337 and 13,14-dehydro,15-keto PGE_2 and 13,14-dehydro,15-keto PGD_2 351-333 (different retention time)
- 13,14-dehydro,15-keto-tetranor-PGE_2-d_4 and 13,14-dehydro,15-keto-tetranor-PGD_2 301-283 and 13,14-dehydro,15-keto-tetranor-PGE_2 and 13,14-dehydro,15-keto-tetranor-PGD_2 297-279 (different retention time)
- 15-deoxy,delta-12,14-PGJ_2-d_4 319-275 and 15-deoxy,delta-12,14-PGJ_2 315-271

GC-MS

- 9α11β-PGF_2-d_4 573 and 9α11β-PGF_2 569

Integrate area under the peak (AUP) for the analyte and corresponding internal standard (IS). Calculate from the formula: IS_{amount}*($AUP_{analyte}$/AUP_{IS}). Report as divided by urinary creatinine concentration in pg/mg creatinine.

8.8.3 Case report form (CRF) completion SOP

Case Report Form (CRF) Completion Guidelines

- CRFs should ONLY be completed by someone authorised to do so on the site delegation log
- CRFs should always be completed during or at the study visit

When completing CRFs DO:

- Write clearly and legibly using **black** ballpoint pen
- Fill in the header information on each page, never submit a CRF page without adding the patient identifiers (trial reference number and initials) and visit details to that sheet
- Always record dates in the requested format – for WAIT it's dd/mm/yyyy (i.e. 04/06/2008 for 4th June 2008)
- Use the correct unit of measure (i.e. kg for weight, cm for height)
- Completely fill in each box provided using a leading '0' for numerical data or a '–' for alphabetical (i.e. enter a dash between the initials of a patient with no middle name 'A-B')
- Avoid abbreviations/acronyms (unless standard medical abbreviations or pre-agreed) and use only recognised terminology
- Make sure you use the same terminology or description if recording information on different CRFs about the same event (e.g. you record an AE on the Adverse Events CRF and then record the medication prescribed to treat that AE on the Concomitant Medications CRF)
- When you are describing an event, for example an adverse event, make sure you and your reader are completely clear in what you mean. Avoid any ambiguity.
- Ensure all data entries are consistent with any source documents, i.e. the first place the information is recorded, if the CRF isn't the source document for that data (e.g. patient's medical notes are usually the source documents for medical examination information).
- Always ask for & record concomitant medications and adverse events at each visit/phone call – get as much information as you can from the patient and their carer and enter that onto the appropriate CRF.
- Ensure that CRF pages are numbered where appropriate (i.e. numbering is left blank for completion at site on the Medical History, Concomitant Medications and Adverse Event CRFs as you may use more than one for an individual patient over the course of the trial)
- Make sure all CRFs are signed & dated by a researcher/s authorised to do so on the site delegation log.
- Scan and email all completed CRF pages via secure nhs.net mail to cnwokoro@nhs.net as soon as is possible and then file the original.

When completing CRFs DO NOT:

- Write outside of the designated boxes* – any comments should be made in the appropriate comments section on the CRF. *Except to initial and date any corrections (see below)
- Record incomplete dates – if you don't know enter 'UK' (i.e. UK/06/2008 if you know the month and year but not the day)
- Leave any fields blank. Use the following as appropriate and explain the reason in the appropriate comments section on the CRF:
  - → **N/A** (*not applicable*): use when field does not apply
  - → **N/D** (*not done*): use when a process or procedure was not done
  - → **N/R** (*not recorded*): use when the procedure was known to be done, but the data is unavailable or not written down
  - → **UK** (*unknown*): use when there is no other explained reason for the missing data
- Cover up or obscure incorrect data with a new entry or correction! To make a correction:
  - → Cross out the incorrect entry with a single straight line so that the original text can still be read
  - → Enter the correct data above or next to it
  - → Initial & date the correction, for example....

    ~~50.8 kg~~ 30.8 kg *04/06/08 SP*
  - → Give an explanation for the correction if it is not immediately obvious why it was made.

8.8.4 Laboratory quality assurance SOP (London)

Standard Operating Procedures (SOP) for: **WAIT Trial QA/QC**			
SOP Number:	**12**	Version Number:	**1**
Effective Date:		Review Date:	

Author:	**Iain Dickson**

Authorisation:	
Name / Position	**Dr Tom Vulliamy**
Signature	
Date	

Purpose and Objective:
To document quality assurance/quality control (QA/QC) procedures taking place within the WAIT trial.

SOP Text

	Responsibility	Activity
1.	Lab Technician	**Sample Receipt –** When a sample arrives, it is checked over for packaging, labelling and for any leaks. This is documented for each sample in the 'WAIT Sample Receipt' log, kept in filing cabinet GWHD-6, in the paediatric write-up area.
2.	Lab Technician	**Sample Processing –** All samples are amplified in duplicate. All samples are also run alongside positive standards. Three of these standards were used to validate the method (see 'Method Validation' in the WAIT trial lab site file) and were sequenced to confirm their genotype. They are as follows: S1-W001– 5/5 genotype S2-2535 – 4/5 genotype S3-2551 – 3/5 genotype A fourth standard with the 5/6 genotype is also run with all samples. This standard originated from a trial sample which was found to have the 5/6 genotype. DNA from this saliva sample was re-extracted and is labelled with the same trial number followed by a (2), e.g. LO-140(2). As a standard, it will therefore appear on the genotyping worksheet as, for example, S4-LO140(2).
3.	Lab Technician	**Repeat Testing –** When there is a low number of samples to be analysed and space on the genotyping plate, randomly picked old trial samples are re-amplified and re-genotyped. This is demonstrated on the genotyping worksheet by 'QA/QC' in the margins next to the samples being re-run. Periodically, a whole 'QA/QC' run may take place where all the samples on the plate are re-genotyped. This again will denoted by 'QA/QC' on the worksheet.
4.	Lab Technician	**Results Reporting –** All results in the WAIT trial are double checked by another member of the lab staff, Dr. Tom Vulliamy. Before a report or sample result is sent out, Dr. Vulliamy will look over the raw data and double check the genotype result, as well as the stratification. Please see the 'Results Reporting SOP',

8.9 Appendix 9 - List of SNPs

8.9.1 Test SNPs

TEST SNPS					
Gene	**Gene Function/Protein**	**SNP ID**	**SNP**	**References**	**Associations**
ALOX5	5-LO	**ALOX5_1**	rs2029253 (A>G)	Lima et al. 2006; Crosslin et al. 2009	Atherosclerosis
		ALOX5_2	rs2115819 (A>G)	Lima et al. 2006; Crosslin et al. 2009; Geiger et al. 2009	Atherosclerosis
		ALOX5_3	rs745986 (A>G)	Lima et al. 2006	
		ALOX5_4	rs892691 (G>A)	Lima et al. 2006; crosslin et al. 2009	Atherosclerosis
		ALOX5_5	rs4987105	Klotsman et al. 2006; Geiger et al 2009	Peak Expiratory Flow Rate
		ALOX5_6	rs4986832	Klotsman et al. 2006; Giger et al 2009	Peak Expiratory Flow Rate
		ALOX5_7	rs2228064	Klotsman et al. 2006	
		ALOX5_8	rs1864414	Crosslin et al. 2009	
		ALOX5_9	rs3824613	Crosslin et al. 2009	
		ALOX5_10	rs1369214	Crosslin et al. 2009	Atherosclerosis
		ALOX5_11	rs10900215	Crosslin et al. 2009	Coronary Artery Disease
		ALOX5_12	rs3780906	Crosslin et al. 2009	
		ALOX5_13	rs3740107	Crosslin et al. 2009	Coronary Artery Disease
		ALOX5_14	rs1487562	Crosslin et al. 2009	Coronary Artery Disease
		ALOX5_15	rs1565096 (A>G)	Geiger et al. 2009	
		ALOX5_16	rs10751382 (A>G)	Geiger et al. 2009	
		ALOX5_17	rs7099684	Tag SNP	
		ALOX5_18	rs934187	Tag SNP	
		ALOX5_19	rs4948672	Tag SNP	
		ALOX5_20	rs12264801	Tag SNP	
		ALOX5_21	rs3824612	Tag SNP	
		ALOX5_22	rs10900213	Tag SNP	
		ALOX5_23	rs7896431	Tag SNP	
		ALOX5_24	rs2228065	Duroudier et al. 2009	Asthma
cysLTR1	cys-LT receptor 1	**CYSLTR1_1**	rs321081 (G>A)	Lima et al. 2006	
		CYSLTR1_2	rs320995	Lima et al. 2006; Klotman et al. 2006; Kim et al. 2007	
		CYSLTR1_3	rs321029 (G>A)	Lima et al. 2006; Kim et al. 2007	
		CYSLTR1_4	rs321092 (A>G)	Lima et al. 2006	
		CYSLTR1_5	rs320988	Tag SNP	
		CYSLTR1_6	rs2637204	Duroudier et al. 2009	
		CYSLTR1_7	rs2806489	Duroudier et al. 2009	
		CYSTLR1_8	rs321007	Tag SNP	
cysLTR2	cys-LT receptor 2	**CYSLTR2_1**	rs912278	Klotsman et al. 2006	Peak Expiratory Flow Rate
		CYSLTR2_2	rs912277	Klotsman et al. 2006	Peak Expiratory Flow Rate
LTA4H	LTA4 Hydrolase	**LTA4H_1**	rs2247570 (T>C)	Lima et al. 2006	
		LTA4H_2	rs2660845 (A>G)	Lima et al. 2006; Crosslin et al. 2009	
		LTA4H_3	rs2660880	Crosslin et al. 2009	
		LTA4H_4	rs6538697	Crosslin et al. 2009	Coronary Artery Disease
		LTA4H_5	rs1978331	Holloway et al. 2008; Crosslin et al. 2009	Asthma phenotypes
		LTA4H_6	rs17677715	Crosslin et al. 2009	
		LTA4H_7	rs2660898	Crosslin et al. 2009	
		LTA4H_8	rs2540482	Crosslin et al. 2009	
		LTA4H_9	rs2540475	Crosslin et al. 2009	
		LTA4H_10	rs2660895	Tag SNP	
		LTA4H_11	rs2540497	Tag SNP	
		LTA4H_12	rs2540491	Tcheurekdjian et al. 2010	Spirometry - FEV1
		LTA4H_13	rs2540487	Tcheurekdjian et al. 2010	Spirometry - FEV1
		LTA4H_14	rs2540500	Tag SNP	
LTC4S	LTC4 Synthase	**LTC4S_1**	rs272431 (G>T)	Lima et al. 2006	
		LTC4S_2	rs272440	Lima et al. 2006	
		LTC4S_3	rs730012 (A>C)	Lima et al. 2006; Klotman et al. 2006	
MRP1	Multiple drug resistant protein 1	**MRP1_1**	rs152033 (C>T)	Lima et al. 2006	
	(leukotriene C4 transporter)	**MRP1_2**	rs1967120 (A>G)	Lima et al. 2006	
		MRP1_3	rs212081 (G>A)	Lima et al. 2006	
		MRP1_4	rs215066 (G>A)	Lima et al. 2006	
		MRP1_5	rs2239996(A>G)	Lima et al. 2006	
		MRP1_6	rs246221V_V (T>C)	Lima et al. 2006	
		MRP1_7	rs35587N_N (T>C)	Lima et al. 2006	
		MRP1_8	rs3887893 (T>C)	Lima et al. 2006	
		MRP1_9	rs4148356R_Q (G>A)	Lima et al. 2006	
ALOX5AP	5-LO Activating Protein	**ALOX5AP_1**	rs3803277	Klotsman et al. 2006	
		ALOX5AP_2	rs17216473	Crosslin et al. 2009	Coronary Artery Disease
		ALOX5AP_3	rs10507391	Holloway et al. 2008; Crosslin et al. 2009; Tcheurekdjian et al. 2010	Asthma phenotypes
		ALOX5AP_4	rs4769874	Holloway et al. 2008; Crosslin et al. 2009	Asthma phenotypes/Myocardial Infarction
		ALOX5AP_5	rs9551963	Crosslin et al. 2009; Tcheurekdjian et al. 2010	Spirometry - FEV1
		ALOX5AP_6	rs9315050	Holloway et al. 2008; Crosslin et al. 2009	Asthma phenotypes
		ALOX5AP_7	rs4073259	Helgadottir et al. 2004	Myocardial Infarction
		ALOX5AP_8	rs9506352	Tag SNP	
		ALOX5AP_9	rs9315049	Tag SNP	
		ALOX5AP_10	rs9579646	Tag SNP	
		ALOX5AP_11	rs10162089	Tag SNP	
		ALOX5AP_12	rs12429692	Tag SNP	
		ALOX5AP_13	rs4075131	Tag SNP	
		ALOX5AP_14	rs3935644	Tag SNP	
		ALOX5AP_15	rs9315045	Tag SNP	
		ALOX5AP_16	rs9579648	Tag SNP	
		ALOX5AP_17	rs9315048	Tag SNP	
		ALOX5AP_18	rs4503649	Tag SNP	
		ALOX5AP_19	rs4254165	Tag SNP	
PLA2G4A	Phospholipase A2	**PLA2_1**	rs3736741	Klotsman et al. 2006	
		PLA2_2	rs2307200	Klotsman et al. 2006	
CYP3A4	Cytochrome P450 isoform	**CYP3_1**	rs1057910	Klotsman et al. 2006	
	(drug metabolism)	**CYP3_2**	rs2740574	Klotsman et al. 2006	
CYP2C9	Cytochrome P450 isoform	**CYP2**	rs1799853	Klotsman et al. 2006	
ADRB2	B2-adrenergic receptor	**ADRB2_1**	rs1042713	Klotsman et al. 2006	
		ADRB2_2	rs1042714	Klotsman et al. 2006	Spirometry - FEV1
		ADRB2_3	rs1042711	Klotsman et al. 2006	Spirometry - FEV1
NR3C1	Glucocorticoid receptor	**NR3C_1**	rs6188	Klotsman et al. 2006	Spirometry - FEV1
		NR3C_2	rs6196	Klotsman et al. 2006	Spirometry - FEV1
		NR3C_3	rs6190	Klotsman et al. 2006	
SLCO2B1	organic anion transporter 2B1	**SLCO2B**	rs12422149	Mougey et al. 2009	Plasma montelukast levels
PTGDR	prostaglandin D2 receptor	**PTGDR**	rs803010	Kang et al. 2011	Montelukast response
TBXA2R	Thromboxane A2 receptor	**TBX_1**	rs4523	Kim et al. 2007	Asthma
		TBX_2	rs1131882	Kim et al. 2007	
		TBX_3	rs4807491	Kim et al. 2007	
PTGS1	COX 1	**PTGS1_1**	rs4240474	Tag SNP	
		PTGS1_2	rs4273915	Tag SNP	
		PTGS1_3	rs8046	Tag SNP	
		PTGS1_4	rs1213266	Tag SNP	
		PTGS1_5	rs7866582	Tag SNP	
		PTGS1_6	rs3842798	Tag SNP	
		PTGS1_7	rs3842787	Tag SNP	
		PTGS1_8	rs10306194	Tag SNP	
PTGS2	COX 2	**PTGS2_1**	rs2066826	Tag SNP	
		PTGS2_2	rs5275	Tag SNP	
		PTGS2_3	rs2206593	Tag SNP	
		PTGS2_4	rs5277	Tag SNP	
		PTGS2_5	rs2745557	Tag SNP	
LTB4R	Leukotriene B receptor 1	**LTB4R_1**	rs2224122	Tag SNP	
		LTB4R_2	rs1046587	Tag SNP	
LTB4R2	Leukotriene B receptor 2	**LTB4R2**	rs2516564	Tag SNP	
PTGER2	EP2	**PTGER2_1**	uS5 (rs708494)	Jinai et al. 2004; Szczeklik et al. 2008	
		PTGER2_2	uS7 (rs708495)		
		PTGER2_3	uS10 (rs17125318)		
		PTGER2_4	rs2075797		
		PTGER2_5	rs1353411		
PTGER3	prostaglandin E3 receptor	**PTGER3**	rs7551789	Kim et al. 2007	
PTGIR	Prostacyclin receptor (IP)	**PTGIR**	rs1126510	Kim et al. 2007	

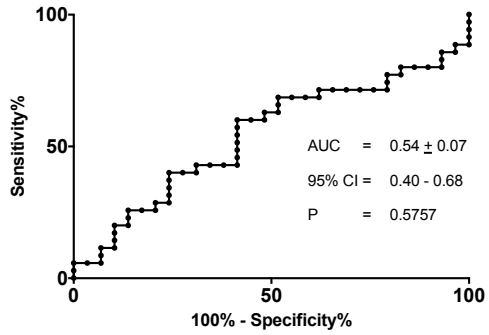
8.9.2 Control SNPs

CONTROL SNPs					
N/A	Random control SNP	**RAND_1**	rs7782389		
N/A	Random control SNP	**RAND_2**	rs997279		
N/A	Random control SNP	**RAND_3**	rs2826003		
N/A	Random control SNP	**RAND_4**	rs7562047		
N/A	Random control SNP	**RAND_5**	rs7232792		
N/A	Random control SNP	**RAND_6**	rs2733262		
N/A	Random control SNP	**RAND_7**	rs11746926		
N/A	Random control SNP	**RAND_8**	rs7132743		
N/A	Random control SNP	**RAND_9**	rs6770096		
N/A	Random control SNP	**RAND_10**	rs7591449		
N/A	Random control SNP	**RAND_11**	rs1442293		
N/A	Random control SNP	**RAND_12**	rs3796644		
N/A	Random control SNP	**RAND_13**	rs2128238		
N/A	Random control SNP	**RAND_14**	rs10454231		
N/A	Random control SNP	**RAND_15**	rs7776785		
N/A	Random control SNP	**RAND_16**	rs11735827		
N/A	Random control SNP	**RAND_17**	rs9900426		
N/A	Random control SNP	**RAND_18**	rs527705		
N/A	Random control SNP	**RAND_19**	rs7875663		
N/A	Random control SNP	**RAND_20**	rs221454		
N/A	Random control SNP	**RAND_21**	rs778233		
N/A	Random control SNP	**RAND_22**	rs2074175		
N/A	Random control SNP	**RAND_23**	rs3824781		
N/A	Random control SNP	**RAND_24**	rs2236687		
N/A	Random control SNP	**RAND_25**	rs2153747		
N/A	Random control SNP	**RAND_26**	rs470411		
N/A	Random control SNP	**RAND_27**	rs12280701		
N/A	Random control SNP	**RAND_28**	rs11809289		
N/A	Random control SNP	**RAND_29**	rs6599689		
N/A	Random control SNP	**RAND_30**	rs6017870		

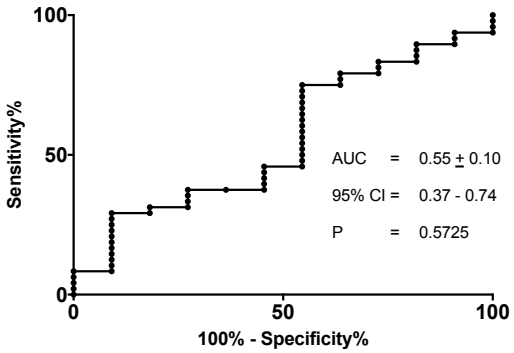
8.10 Appendix 10 - Additional data

FIGURE 8-8 - ROC CURVES (1-6) OF ULTE$_4$ PERCENTAGE INCREMENT AGAINST USMA

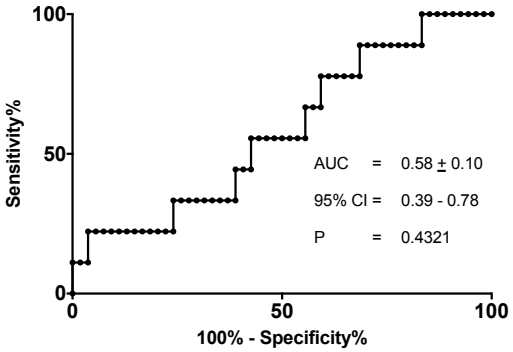
1) >5/≤5 USMA at baseline



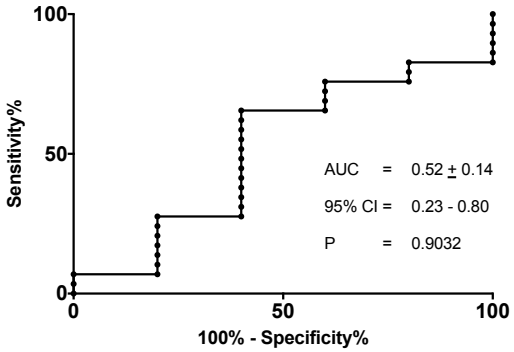
2) >5/≤5 USMA in follow-up period



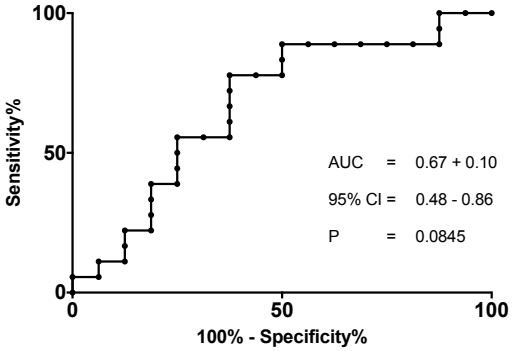
3) With or without USMA in follow-up period



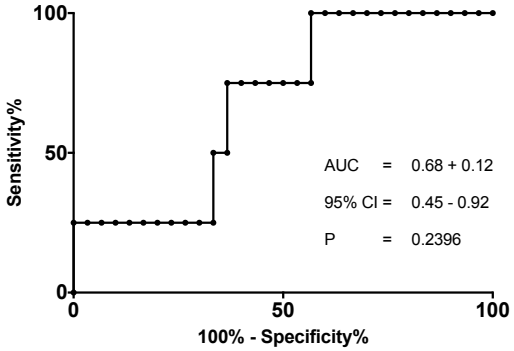
4) >5/≤5 USMA in follow-up period - on montelukast



5) >2/≤2 USMA in follow-up period - on montelukast



6) With or without USMA in follow-up period - on montelukast



Receiver-Operator Characteristic curves of uLTE4 percentage increment against:

1) Subjects with ≥5 or <5 USMA/year at baseline
2) Subjects with ≥5 or <5 USMA/year during follow-up
3) Subjects with or without USMA during follow-up
4) Montelukast treated subjects with ≥5 or <5 USMA/year during follow-up
5) Montelukast treated subjects with ≥2 or <2 USMA/year during follow-up
6) Montelukast treated subjects with or without USMA/year during follow-up

8.11 Appendix 11 - Study proposal - montelukast for preschool wheeze in ALOX5 5/5 homozygotes

NIHR Health Technology Assessment Programme

NHS National Institute for Health Research

HTA EXPRESSION OF INTEREST - RESEARCH DETAILS

Programme Name	HTA
Funding Opportunity	HTA CET EOI to Full
Call	15/08 HTA CET Open Call, HTA EOI to Full Form, closing 6 May 2015
Host Organisation	Barts & The London Queen Mary's School of Medicine & Dentistry

Research Title
Intermittent montelukast for preschool wheeze in children with the 5/5 ALOX-5 promoter genotype; a randomised placebo controlled trial.

Research Type	Primary Research
Proposed start date, end date (duration)	From: 01/02/2016 to: 31/01/2019 (36 months)
How did you hear about this call?	Other (specify below) Recommended to submit by Chair of EME programme
Estimated research costs requested (not including NHS support & treatment costs)	£
Estimated NHS support & treatment costs	£

Contact Information	
Details of Chief Investigator	Professor Jonathan Grigg
Job Position	Professor of Paediatric Respiratory Medicine
Email / Phone	j.grigg@qmul.ac.uk 020 7882 2206
Organisation	Barts & The London Queen Mary's School of Medicine & Dentistry

CO-APPLICANTS

Will you be using co-applicants in your proposal? All co-applicants cited in this section **must have agreed** to be part of this proposal	Yes

Name	Position Held	Role in this project	Department	Organisation
Professor David Price	Professor of Primary Care Respiratory Medicine	Trial Steering Committee	Academic Primary Care	University Court of the University of Aberdeen
Dr Stephen Turner	Senior Lecturer in Paediatrics (Clinical)	Local PI, Trial Steering Committee	The Institute of Applied Health Sciences	University Court of the University of Aberdeen
Dr Hitesh Pandya	Senior Lecturer in Paediatrics (Clinical)	Local PI, Trial Steering Committee	Infection, Immunity and Inflammation	University of Leicester
Dr Tom Vulliamy	Senior Lecturer in Molecular Biology	Overseeing genotyping	Blizard Institute	Barts & The London Queen Mary's School of Medicine & Dentistry
Dr Nick Croft	Senior Lecturer in Paediatrics (Clinical)	Lead for BartsHealth Clinical Research Centre and local CRN	Blizard Institute	Barts & The London Queen Mary's School of Medicine & Dentistry
Professor Chris Griffiths	Professor of Primary Care,	Trial Steering Committee, Lead for Asthma UK Centre	Blizard Institute	Barts & The London Queen Mary's School of Medicine & Dentistry
Dr Anita Patel	Chair in Health Economics	Health Economic Assessment, Trial Steering Committee	Blizard Institute	Barts & The London Queen Mary's School of Medicine & Dentistry

Please declare any conflicts or potential conflicts of interest that you or your co-applicants may have in undertaking this research, including any relevant, non-personal & commercial interests that could be perceived as a conflict of interest.

Professor Grigg has received honoraria from Novartis as a member of an advisory board for an asthma medication (not a leukotriene blocker) and received honoraria from GSK for advice on an asthma medication study design.

Professor Price has board membership with Aerocrine, Almirall, Amgen, AstraZeneca, Boehringer Ingelheim, Chiesi, Meda, Mundipharma, Napp, Novartis, and Teva; consultancy with Almirall, Amgen, AstraZeneca, Boehringer Ingelheim, Chiesi, GSK, Meda, Mundipharma, Napp, Novartis, Pfizer, and

Please declare any conflicts or potential conflicts of interest that you or your co-applicants may have in undertaking this research, including any relevant, non-personal & commercial interests that could be perceived as a conflict of interest.
Teva; grants/grants pending with UK National Health Service, British Lung Foundation, Aerocrine, AstraZeneca, Boehringer Ingelheim, Chiesi, Eli Lilly, GSK, Meda, Merck, Mundipharma, Novartis, Orion, Pfizer, Respiratory Effectiveness Group, Takeda, Teva, and Zentiva; payments for lectures/speaking: Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, Cipla, GSK, Kyorin, Meda, Merck, Mundipharma, Novartis, Pfizer, SkyePharma, Takeda, and Teva; payment for manuscript preparation: Mundipharma and Teva; patents (planned, pending or issued): AKL Ltd; payment for the development of educational materials: GSK, Novartis; stock/stock options: shares in AKL Ltd which produces phytopharmaceuticals and owns 80% of Research in Real Life Ltd and its subsidiary social enterprise Optimum Patient Care; payment for travel/accommodations/meeting expenses from Aerocrine, Boehringer Ingelheim, Mundipharma, Napp, Novartis, and Teva; funding for patient enrollment or completion of research: Almirall, Chiesi, Teva, and Zentiva; peer reviewer for grant committees: Medical Research Council (2014), Efficacy and Mechanism Evaluation programme (2012), HTA (2014); unrestricted funding for investigator-initiated studies: Aerocrine, AKL Ltd, Almirall, Boehringer Ingelheim, Chiesi, Meda, Mundipharma, Napp, Novartis, Orion, Takeda, Teva, Zentiva.

PATIENT AND PUBLIC INVOLVEMENT

Were patients and the public actively involved in identifying the research topic or prioritising the research questions?	Yes
Were patients and the public actively involved in preparing this application?	Yes

Describe how Patient and Public Involvement has informed and/or influenced the development of the application

A need for more therapeutic trials in preschool wheeze was identified by the NIHR CRN's Respiratory and Cystic Fibrosis Clinical Study Group.

In January 2015 a PPI/E event was organised by the BartsHealth Children's Clinical Research Facility (http://www.bartshealth.nhs.uk/research/facilities/childrens-clinical-research-facility/). Seven parents of children with preschool wheeze attended. A facilitated discussion covered parents' views on current treatment of preschool wheeze, their views on our WAIT trial's results, and options for further studies. There was an overwhelming preference for intermittent therapy ("it is impossible to give regular treatment when you know that your child only wheezes with colds"). Parents unanimously agreed that a study of intermittent montelukast in the 5/5 ALOX5 genotype should be done. This parent group will help write the trial protocol and will directly advise the trial steering committee.

The lay summary in this outline application was written by members of the Asthma UK Centre for Applied Research Patient Advisory Group (AUKCAR PAG). This group will develop the parent information sheets and final protocol.

PREVIOUS APPLICATION HISTORY

Relevant NETS Programmes previous application information (since 1st April 2012)

Other funders previous application information

SUMMARY OF THE RESEARCH PROPOSAL

Rationale for the Research

(Please refer to the guidance notes for further information on this section)

If applying for researcher-led funding you must not include any information that enables any individual or team associated with your application to be identified in this section.

1. **What is the problem being addressed?**
2. **Why is the research important in terms of improving the health of the public and/or to patients and the NHS?**
   For commissioned calls where you are responding to an advertised topic, please describe how your proposal meets the specification of the brief.
3. **How does the existing literature support this proposal?**
4. **What is the research question?**

1. What is the problem being addressed?
Health Need. A third of all children will have at least one episode of wheeze before their fourth birthday (1) . Wheeze in preschool children (10 mo to 5 yr) is not the same disease as atopic asthma, for example it is not associated with atopy. The typical pattern is that wheeze is only triggered by viral-colds, and for the majority it resolves by school age (1). Thus therapies of proven efficacy in atopic asthma cannot be assumed to be efficacious in preschool wheeze. The high prevalence of preschool wheeze is reflected by NHS hospital admissions data showing that preschoolers account for the greatest number of admissions across this age range. Thus it is estimated that preschool wheeze utilises at least 0.15% of the total healthcare budget in the UK (2).

Sustained interest. The prevalence of preschool wheeze in the UK is not decreasing. Indeed between 1990 and 2000, there was a significant increase in the prevalence of reported preschool wheeze in the UK from 16% to 29% (3). Furthermore, there is no evidence that licensing of regular low dose inhaled corticosteroids for preschool children has impacted on either the prevalence or severity of wheeze episodes – since they remain the dominant case of hospitalisations for wheeze in the NHS for any age group (http://www.hscic.gov.uk/).

New knowledge. The proposed trial will definitively assess the cost effectiveness of intermittent montelukast, a licensed generic medication, in a genotype that covers over half of all children with preschool wheeze. It has therefore the potential for immediate national and international impact. Proof of effectiveness would represent the first ever use of genotyping to target a medication for wheeze.

Scientific knowledge. The proposed trial is directly suggested by a recent EME-funded study of intermittent montelukast in preschool wheeze (4). The study addresses the new hypothesis that the 5/5 genotype is montelukast responsive. The trial's results will have direct applicability to targeting intermittent montelukast to preschool children, and opens the possibility of future studies in genetic targeting of montelukast (both intermittent and continuous) in older children and adults with atopic asthma.

2. Why is the research important in terms of improving the health of the public and/ or to patients and the NHS?
Because wheeze in young children is characterised by asymptomatic periods interspersed with short episodes of wheeze, anti-inflammatory therapies started at the onset of each viral cold and discontinued when symptoms resolve (i.e. intermittent therapy) is of major interest. In 2 major trials, we found that intermittent use of oral corticosteroids is ineffective (5) (6). By contrast, intermittent high-dose inhaled fluticasone (a potent corticosteroid) has been reported to reduce the risk of clinically severe preschool wheeze episodes by up to 30%, but this strategy is associated with clinically

Rationale for the Research

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1. **What is the problem being addressed?**
2. **Why is the research important in terms of improving the health of the public and/or to patients and the NHS?**
   For commissioned calls where you are responding to an advertised topic, please describe how your proposal meets the specification of the brief.
3. **How does the existing literature support this proposal?**
4. **What is the research question?**

significant suppression of growth (7) – and therefore has not been adopted. By contrast, the oral cysteinyl leukotriene blocker montelukast does not suppress children's growth and is effective in reducing wheeze severity in atopic asthma. Thus establishing the effectivness of intermittent montelukast in preschool wheeze would have a major impact on the NHS by reducing number of unscheduled medial attendances.

3. How does the existing literature support this proposal?
Evidence; Data on the efficacy of intermittent montelukast in preschool wheeze are conflicting. Robertson et al (8)in children aged 2 to 14 years showed that intermittent montelukast, when given over a 12-month period, reduces unscheduled use of acute health-care resources by 38%. Beneficial effects of intermittent montelukast were also seen in a subgroup analysis limited to the preschool age range. By contrast, Bacharier et al (9) reported that intermittent montelukast therapy over 12 months does not decrease wheeze severity in young children or need for oral corticosteroid therapy; and Valovirta et al (10) reported no beneficial effect of a 12 month course of intermittent montelukast on wheeze attacks in preschool children. To address this, the EME funded the Wheeze And Intermittent Therapy (WAIT) trial. Researchers randomly assigned children to receive intermittent montelukast (n=669) or placebo (n=677) (4). The primary outcome was need for unscheduled medical attendances for wheezing episodes (USMA). Primary outcome data were available for 1308 (96%) children. Overall, there was no difference in USMA for wheezing episodes between children in the montelukast and placebo groups (mean 2.0 [SD 2.6] vs 2.3 [2.7]; incidence rate ratio [IRR] 0.88, 95% CI: 0.77–1.01; p=0.06). In a subsequent meta-analysis, no benefit was found of a 12 month period of intermittent montelukast therapy on USMA (4). However, the summary statistic favors intermittent montelukast in all but one of the included studies (the exception is the Valovirta study, where the observed USMA rate was lower than that expected (10)).

Responsive subgroups; Heterogeneity of response is one explanation for a lack of consistency in trials of intermittent montelukast. Indeed, a trial of continuous montelukast for asthmatic children found that although there was an overall benefit, a third of children allocated to montelukast had no improvement (11). The WAIT trial therefore sought to assess both overall efficacy of intermittent montelukast and to identify potentially responsive children. This was achieved this by stratifying by the addition/deletion repeat polymorphism in the ALOX5 promoter. This polymorphism results in variation in the number of SP1 transcription factor-binding motifs, which in turn alters transcription factor binding, and influences production of cysteinyl leukotrienes. Five SP1 repeats in the ALOX5 promoter represent the "wild" type, with other numbers (x) of repeats reflecting the "mutant" genotype. In the WAIT trial we chose strata suggested by the study of Lima et al (12) who reported that adults with either x/x, or 5/x, had a 73% reduction in the risk of having an asthma attack whilst receiving on montelukast compared with those with the 5/5 "wild-type" allele. However the optimal grouping ALOX-5 promoter genotype for responsiveness remains unclear since Telleria et al (13) reported that montelukast decreases asthma exacerbations in the 5/5 but not the x/x genotype. The WAIT trial found that USMA episodes were reduced in children allocated to intermittent montelukast in the 5/5 stratum (2·0 [2·7] vs. 2·4 [3·0]; IRR 0·80, 95% CI 0·68–0·95; p=0·01), but not the 5/x+x/x stratum (2·0 [2·5] vs. 2·0 [2·3]; 1·03, 0·83–1·29;

Rationale for the Research

(Please refer to the guidance notes for further information on this section)

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2. **Why is the research important in terms of improving the health of the public and/or to patients and the NHS?**
   For commissioned calls where you are responding to an advertised topic, please describe how your proposal meets the specification of the brief.
3. **How does the existing literature support this proposal?**
4. **What is the research question?**

p=0·79, p interaction=0·08). Thus although there was no overall benefit of intermittent montelukast but, there was, in contradiction to the researchers' original hypothesis, there was evidence of montelukast responsiveness in the 5/5 genotype.

What is the research question?
Whether intermittent montelukast is superior to placebo in reducing unscheduled attendances for wheeze in preschool children with the 5/5 ALOX-5 promoter genotype and to assess whether this strategy is cost effective.

HTA remit. The study maps to the HTA since we are assessing effectiveness in a representative population of children with preschool wheeze. If superiority over placebo is established by the proposed study, then use within the NHS will be driven by its cost-effectiveness. The study's outcome is parent/guardian centered, addressing the overwhelming preference of parents for intermittent therapy, and the high prevalence of preschool wheeze episodes.

Scientific Abstract

If applying for researcher led funding you must not include any information that enables any individual or team associated with your application to be identified in this section.

Design; randomised, placebo controlled, multi-centre trial.
Setting; secondary care with treatment initiated by parents at home. The trial will use 3 major recruitment hubs supplemented by 40 secondary centres spokes linked to CRN's that participated in the sucessful WAIT trial (described in the online supplement in reference (4)).
Target population; inclusion criteria; children 10 months to 5 years, 2 or more previous episodes of wheeze, 5/5 polymorphism of the ALOX5 promoter. Exclusion criteria; pre-existing respiratory, currently receiving continuous oral montelukast.
Health technology. Intermittent montelukast in children with preschool wheeze with the 5/5 ALOX5 genotype. Children will be randomly assigned to receive montelukast or placebo over the 12-month study period. Parents will start trial medication at the onset of each viral cold or wheezing episode and stop after 10 days. Investigators will phone parents bi-monthly to ask about medical attendances.
Primary outcome; number of unscheduled medical attendances for wheezing episodes (USMA). The European Medicines Agency states that "the primary endpoint in the pre-school age group can only be a clinical one: such as number of exacerbations, and number of hospitalisations for wheeze exacerbations".
http://www.ema.europa.eu/docs/en_GB/document_library/Minutes/2012/11/WC500135121.pdf. The Comet database referenced publication limits recommendations for wheeze outcomes to older asthmatic children.
Measurements:

Sample size: 1712 children will be randomised to intervention or placebo. This will be sufficient to detect a 20% fall in USMA from 2.0 to 1.6 per year, with 90% power and 5% significance and

Scientific Abstract

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assuming a drop out rate of 6% taking into account extra Poisson variation assuming the coefficient of variation of 1.15. This will require 2800 children to be screened assuming (from our previous study) that 63% will have the 5/5 ALOX5 genotype.
Pathways: the 2014 BTS SIGN Guideline for Asthma states "there is some limited evidence that leukotriene antagonists may be used intermittently in children with episodic asthma". Genotyping preschool children prior to prescribing intermittent montelukast is entirely practicable. First, delaying issuing a prescription until genotype is known is clinically acceptable. Second, a single centre can genotype samples from across the UK within 1 week. Third, a buccal swab requires no training and is acceptable to parents.
Project timetables: 0 to 4 months; trial set up including approvals. 5 to 23 months; recruitment and follow up. 155 children to be genotyped and 95 children with the 5/5/ genotype enrolled per month (total 1712 enrolled). 23 to 35 months; follow up, 35 to 36 months; close down and reporting.
Expertise: the trial will be delivered by a multi disciplinary team that have delivered major independent trials in preschool wheeze. The team also includes a NIHR Clinical Research Network recruitment lead, a Senior Lecturer in Molecular Biology, and an embedded parent/guardian advisory group who will advise on all aspects of trial design and delivery.
Dissemination. This study is supported by the Asthma UK Centre for Applied Research who will support the dissemination of results.

Summary (in Plain English)

If applying for researcher led funding you must not include any information that enables any individual or team associated with your application to be identified in this section.

Young children less than 6 years of age (preschool) often get very wheezy when they get a cold. The reason is the cold virus narrows the small breathing tubes in the chest and it's quite upsetting for children and frightening to their parents or guardians. We want to find a more effective way of dealing with this type of wheeze. The wheeze that affects preschool children is different to 'allergic asthma' seen in older children since it goes away by the time children reach school age, and is not caused by allergy. Nevertheless, for some preschool children wheezing can be so bad that their parents need to ask their GP for help, and in some cases children even need to go to hospital.

We're interested in 'intermittent treatments" that can lessen the wheeze. The first signs of a cold warns parents to start the treatment which they then stop when the wheeze ends (usually around 10 days). At the moment, there are two intermittent treatments that could be used- inhaled steroids, and an anti-inflammatory medicine taken by mouth called montelukast. Intermittent inhaled steroids seem to work, but unfortunately stop children growing normally because of the high doses needed. That leaves oral montelukast. Used intermittently, montelukast doesn't affect growth - but clinical trials have not consistently shown that it really can reduce the severity of wheeze.
Interestingly, montelukast seems to work very well in some preschool children, but not at all in others. Also, studies of adults with allergic asthma suggest that those with particular variations in a gene called ALOX5 do better on montelukast.

Researchers in previous study that was published last year thought that variations in the ALOX5 gene might account for some preschool children with wheeze doing better. In a large trial in a group of preschool children that looked whether those who took montelukast granules needed less medical attention for wheeze than a control group who got identical looking inactive granules. Before doing the trial, they also found out about each child's ALOX 5 gene. The researchers found that giving montelukast didn't reduce wheeze in these children. However, children with a variation called '5/5' in their ALOX 5 gene did wheeze less with montelukast. This was surprising, because it isn't the same variation that responds to montelukast in adults with asthma.

What's next?
This is why we now want to do a new trial – looking again at intermittent montelukast, but this time

Summary (in Plain English) If applying for researcher led funding you must not include any information that enables any individual or team associated with your application to be identified in this section.
only in children with the 5/5 ALOX5 gene variation (about 60% of all preschool children have this variation). The children will get a 12-month course of intermittent montelukast. Parents will be asked to start the 10-day course of trial medicine every time their child gets a cold. We'll be testing whether children on the montelukast have to see a GP or go to hospital less often than those in the control group receiving identical inactive granules. If we find that intermittent montelukast does make preschool children with this gene variation wheeze less, we'll have to see if it's cost-effective for the NHS. We'll need to work out whether the benefit seen outweighs the combined cost of testing for the 5/5 ALOX5 variation and montelukast. To run the trial, we'll use a wide network of hospitals across the country. If we show that analysing genes to target wheeze therapy does work, this will be the first evidence for 'personalised wheeze treatment' in any age group

CLINICAL TRIALS UNIT PARTICIPATION

Is a Clinical Trials Unit involved with this research proposal?	Yes

If applicable, please describe how you have worked with a Clinical Trials Unit in developing your application and what support they will provide if funding is approved.
The Queen Mary University of London's Pragmatic Clinical Trials Unit helped develop this application and will fully support all aspects of the running of the proposed trial.

Clinical Trials Unit (CTU)	Does the CTU hold a UKCRC registration?	UKCRC Reg. No	Is the CTU receiving CTU support funding from NIHR?

UPLOADS

The following pages contain the following uploads:

Upload Name
CV of Lead Applicant (One side of A4 only)
References
Flowchart
Cover Letter

CURRICULUM VITAE

Jonathan Grigg BSc, MBBS, MRCP (UK), MD, FRPCH, Professor of Paediatric Respiratory and Environmental Medicine, Queen Mary University of London, Deputy Director, Blizard Institute, Honorary Consultant Paediatrician, BartsHealth NHS Trust, Deputy Director North Thames CLAHRC

SELECTED ONGOING GRANTS (CI; Chief Investigator)

2014-2017	ActionResearch *Asthma controller medication* **Co-I £197,000**
2014- 2017	Medical Research Council *Breath Africa*. **Co-I £540,000**
2014-2019	NIHR *CLAHRC* **CI for QMUL £9,000,000**
2013-2018	Asthma UK. *National Asthma Research Centre* **Co-I £2,000,000**
2013-2016	Medical Research Council. *Air pollution and dendritic cells*. **CI £800,000**
2013-2017	Medical Research Council. *Cookstove intervention to prevent pneumonia in children*. **Co-I £2,300,000**
2015-2017	Colt foundation. *Welding fumes and pneumococcal infection* **CI £95,000**

SELECTED PUBLICATIONS

1) Oommen A, **Grigg J**. Urinary leukotriene E_4 in preschool children with acute clinical viral-wheeze. *European Respiratory Journal*. 2003;21:149-154.

2) Oommen A, McNally T, **Grigg J**. Eosinophil activation in preschool viral-wheeze. *Thorax*. 2003;58:876-879.

3) Oommen A, Lambert PC, **Grigg J**. Efficacy of a short course of oral prednisolone for viral-wheeze in children aged 1-5 years: randomised controlled trial. *Lancet* 2003;362:1433-1438.

4) Pierse N, Rushton L, Harris R, Kuehni CE, Silverman M, **Grigg J**. Locally-generated particulate pollution and respiratory symptoms in young children. *Thorax*. 2006. 61:216-20.

5) Kulkarni N, Pierse N, Rushton L, **Grigg J**. Carbon in airway macrophages and lung function in children. *New England Journal of Medicine*. 2006 Jul 6;355:21-30

6) Panickar J, Lakhanpaul M, Lambert PC, Kenia P, Stephenson T, Smyth A, **Grigg J**. Oral Prednisolone for Preschool Children with Acute Virus-Induced Wheezing *New England Journal of Medicine* 2009;360;329-338

7) MacNeill V, Nwokoro C, Griffiths C, **Grigg J**, Seale C. Recruiting ethnic minority participants to a clinical trial: qualitative study. *British Medical Journal Open* 2013 Apr 8;3(4). pii: e002750.

8) Nwokoro C, Pandya P, Turner S, Eldridge S, Griffiths CJ, Vulliamy T, Price P, Sanak M, Holloway JW, Brugha R, Koh L, Dickson I, Rutterford C, **Grigg J**. Intermittent montelukast in preschool children with wheeze: a randomised placebo controlled trial stratified for arachidonate 5-lipoxygenase promoter genotype (WAIT trial) *The Lancet Respiratory Medicine* 2014 Oct;2(10):796-803

References

1 Bush A, Grigg J, Saglani S. Managing wheeze in preschool children. BMJ 2014; 348:g15

2 Stevens CA, Turner D, Kuehni CE, et al. The economic impact of preschool asthma and wheeze. Eur Respir J 2003; 21:1000-1006

3 Kuehni CE, Davis A, Brooke AM, et al. Are all wheezing disorders in very young (preschool) children increasing in prevalence? Lancet 2001; 357:1821-1825

4 Nwokoro C, Pandya H, Turner S, et al. Intermittent montelukast in children aged 10 months to 5 years with wheeze (WAIT trial): a multicentre, randomised, placebo-controlled trial. Lancet Respir Med 2014

5 Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: randomised controlled trial. Lancet 2003; 362:1433-1438

6 Panickar J, Lakhanpaul M, Lambert PC, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. N Engl J Med 2009; 360:329-338

7 Ducharme FM, Lemire C, Noya FJ, et al. Preemptive use of high-dose fluticasone for virus-induced wheezing in young children. N Engl J Med 2009; 360:339-353

8 Robertson CF, Price D, Henry R, et al. Short-course montelukast for intermittent asthma in children: a randomized controlled trial. Am J Respir Crit Care Med 2007; 175:323-329

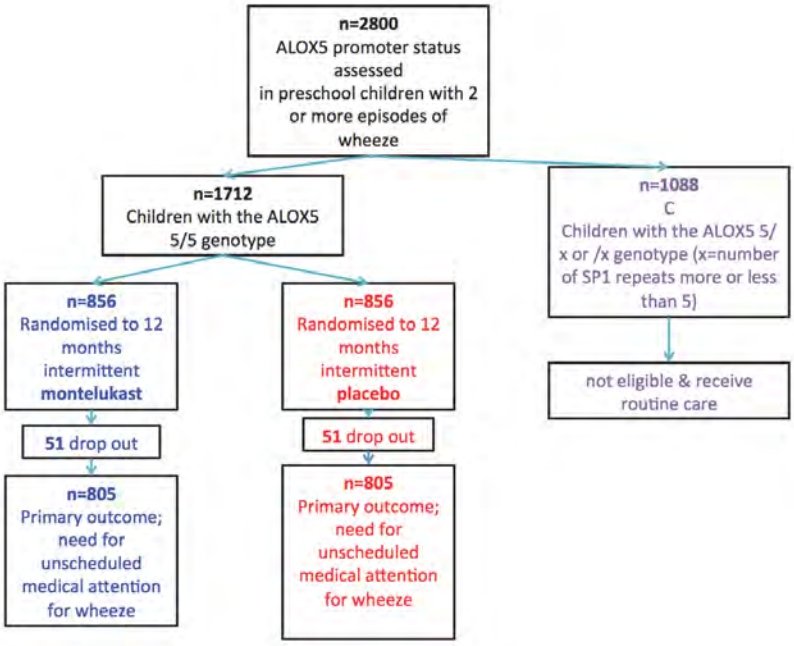
9 Bacharier LB, Phillips BR, Zeiger RS, et al. Episodic use of an inhaled corticosteroid or leukotriene receptor antagonist in preschool children with moderate-to-severe intermittent wheezing. J Allergy Clin Immunol 2008; 122:1127-1135 e1128

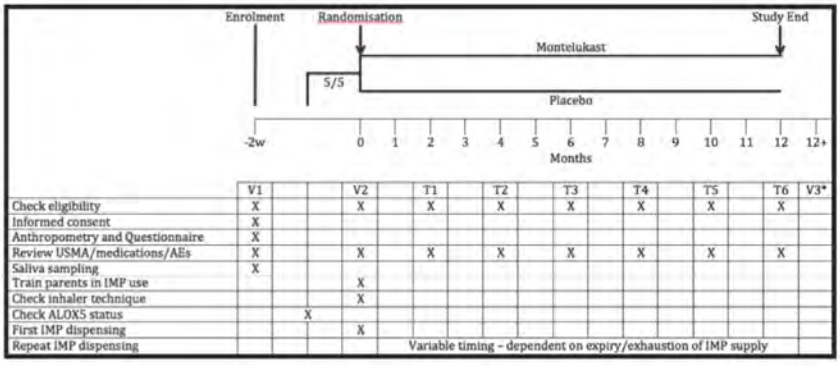
10 Valovirta E, Boza ML, Robertson CF, et al. Intermittent or daily montelukast versus placebo for episodic asthma in children. Ann Allergy Asthma Immunol 2011; 106:518-526

11 Zeiger RS, Szefler SJ, Phillips BR, et al. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. J Allergy Clin Immunol 2006; 117:45-52

12 Lima JJ. Treatment heterogeneity in asthma: genetics of response to leukotriene modifiers. Mol Diagn Ther 2007; 11:97-104

13 Telleria JJ, Blanco-Quiros A, Varillas D, et al. ALOX5 promoter genotype and response to montelukast in moderate persistent asthma. Respir Med 2008; 102:857-861





	V1			V2		T1		T2		T3		T4		T5		T6	V3*
Check eligibility	X			X		X		X		X		X		X		X	
Informed consent	X																
Anthropometry and Questionnaire	X																
Review USMA/medications/AEs	X			X		X		X		X		X		X		X	
Saliva sampling	X																
Train parents in IMP use				X													
Check inhaler technique				X													
Check ALOX5 status			X														
First IMP dispensing				X													
Repeat IMP dispensing	Variable timing - dependent on expiry/exhaustion of IMP supply																

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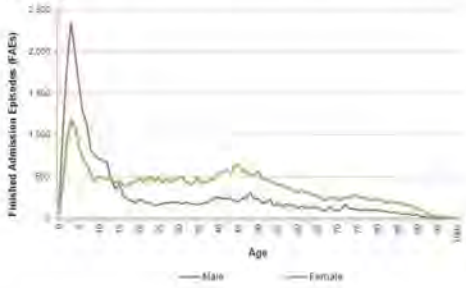
29th April 2015

Professor Sallie Lamb
Chair
HTA Clinical Evaluation and Trials Board

Re; Outline Application (online) Intermittent montelukast for preschool wheeze in children with the 5/5 ALOX-5 promoter genotype; a randomised placebo controlled trial.

Dear Sallie,
I am delighted to enclose this outline application, which is informed by our recent EME-funded WAIT trial. The main outcome of the WAIT trial is that, although intermittent montelukast is not effective in preschool wheeze, we found evidence for responsiveness in the subgroup of children with the ALOX 5/5 genotype (a genotype that covers 60 % of the target population). We now wish to determine the cost effectiveness of intermittent montelukast in children with this genotype.

We think this application may be of interest to your panel since;
i) preschool wheeze is a major cause of UK hospital admissions for wheeze (see graph below)

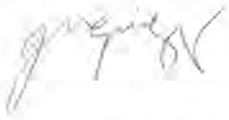


ii) because of the intermittent nature of preschool wheeze, delay in initiating therapy

while ALOX5 genotype status is being assessed, is clinically acceptable.
iii) there is equipoise on the effectiveness of intermittent montelukast in the ALOX5 5/5 genotype – since the responsive genotype in the WAIT trial is different to that originally hypothesised (albeit there are data from atopic asthmatics to suggest montelukast responsiveness of the 5/5 genotype).
iv) we have an outstanding track record for delivering major independent trials in preschool wheeze.

I look forward to hearing your panel's views.

Best wishes,
Yours sincerely,



Jonathan (Jonathan Grigg) BSc, MB BS, MRCP (UK), MD, FRCPCH.