

Review article

Leukotriene pathway genetics and pharmacogenetics in allergy

Leukotrienes (LT) are biologically active lipid mediators known to be involved in allergic inflammation. Leukotrienes have been shown to mediate diverse features of allergic conditions including inflammatory cell chemotaxis/activation and smooth muscle contraction. Cysteinyl leukotrienes (LTC₄, LTD₄ and, LTE₄) and the dihydroxy leukotriene LTB₄ are generated by a series of enzymes/proteins constituting the LT synthetic pathway or 5-lipoxygenase (5-LO) pathway. Their function is mediated by interacting with multiple receptors. Leukotriene receptor antagonists (LTRA) and LT synthesis inhibitors (LTSI) have shown clinical efficacy in asthma and more recently in allergic rhinitis. Despite growing knowledge of leukotriene biology, the molecular regulation of these inflammatory mediators remains to be fully understood. Genes encoding enzymes of the 5-LO pathway (i.e. *ALOX5*, *LTC4S* and *LTA4H*) and encoding for LT receptors (*CYSLTR1/2* and *LTBR1/2*) provide excellent candidates for disease susceptibility and severity; however, their role remains unclear. Preliminary data also suggest that 5-LO pathway/receptor gene polymorphism can predict patient responses to LTSI and LTRA; however, the exact mechanisms require elucidation. The aim of this review was to summarize the recent advances in the knowledge of these important mediators, focusing on genetic and pharmacogenetic aspects in the context of allergic phenotypes.

N. P. Duroudier, A. S. Tulah, I. SayersDivision of Therapeutics and Molecular Medicine,
University of Nottingham, Nottingham, UKKey words: allergy; association; genetics; G-protein
coupled receptors; leukotriene.Dr Nathalie P Duroudier
Division of Therapeutics and Molecular Medicine
D-Floor, South Block
Queen's Medical Centre
Nottingham NG7 2UH
UK

Accepted for publication 30 July 2008

Leukotrienes (LT) are a family of potent eicosanoid lipid mediators with central importance in disease processes such as inflammation and proliferation most notably observed in allergic conditions like asthma (1). There are two general classes of LT according to the presence of a cysteine residue in their amino acid chain: the cysteinyl leukotrienes (CysLTs) including LTC₄, LTD₄ and LTE₄ and the dihydroxy leukotriene LTB₄ (2).

The 5-lipoxygenase pathway

Leukotrienes are produced in a multi-step enzyme pathway called the 5-lipoxygenase (5-LO) pathway, which is active in leucocytes such as neutrophils, eosinophils, mast cells and monocytes (Fig. 1). Arachidonic acid is the precursor for LT synthesis and is hydrolysed from the plasma membrane by cytosolic phospholipase A₂ (and other isoforms) (3, 4) in a calcium-dependent process (5). Upon activation, the rate-limiting enzyme 5-LO oxygenates first free arachidonic acid into the unstable intermediate 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is then either hydrolysed to 5-hydroxyeicosatetraenoic acid (5-HETE) or transformed into the unstable epoxide leukotriene A₄ (LTA₄) by forming a conjugated triene

system through dehydration (6). 5-LO translocates from either the nucleus (in macrophages) or cytosol (in neutrophils) to the nuclear envelope in response to cell activation (7, 8). This movement occurs as 5-LO is dependent on a 5-LO activating protein (FLAP) for its function. FLAP remains associated with the nuclear membrane (9) and acts as a 'transfer protein' presenting arachidonic acid to 5-LO, a system which allows the favourable conversion of 5-HPETE to LTA₄ compared to 5-HETE (10). Both 5-LO and FLAP are expressed in cells of myeloid lineage (11) restricting the pathway to these cell types. LTA₄ can be further metabolised to the cysteinyl leukotrienes (CysLTs) or LTB₄. The specific glutathione *S*-transferase leukotriene C₄ synthase (LTC₄S) conjugates LTA₄ to form LTC₄ which can then be rapidly converted to LTD₄ by a gamma-glutamyl transpeptidase and to LTE₄ by a dipeptidase once exported out the cell by membrane transport proteins such as multi-drug related protein 1 (MRP1) (12–15). A specific zinc metallohydrolase, LTA₄ hydrolase (LTA₄H) is responsible for the conversion of LTA₄ to LTB₄ (16). CysLTs and LTB₄ act on different G-protein coupled receptors (GPCRs). Each bind to at least two different receptors: CysLTs to CYSLTR1 and CYSLTR2 and LTB₄ to LTB₄R1 and LTB₄R2 (or BLT1 and BLT2) (17).

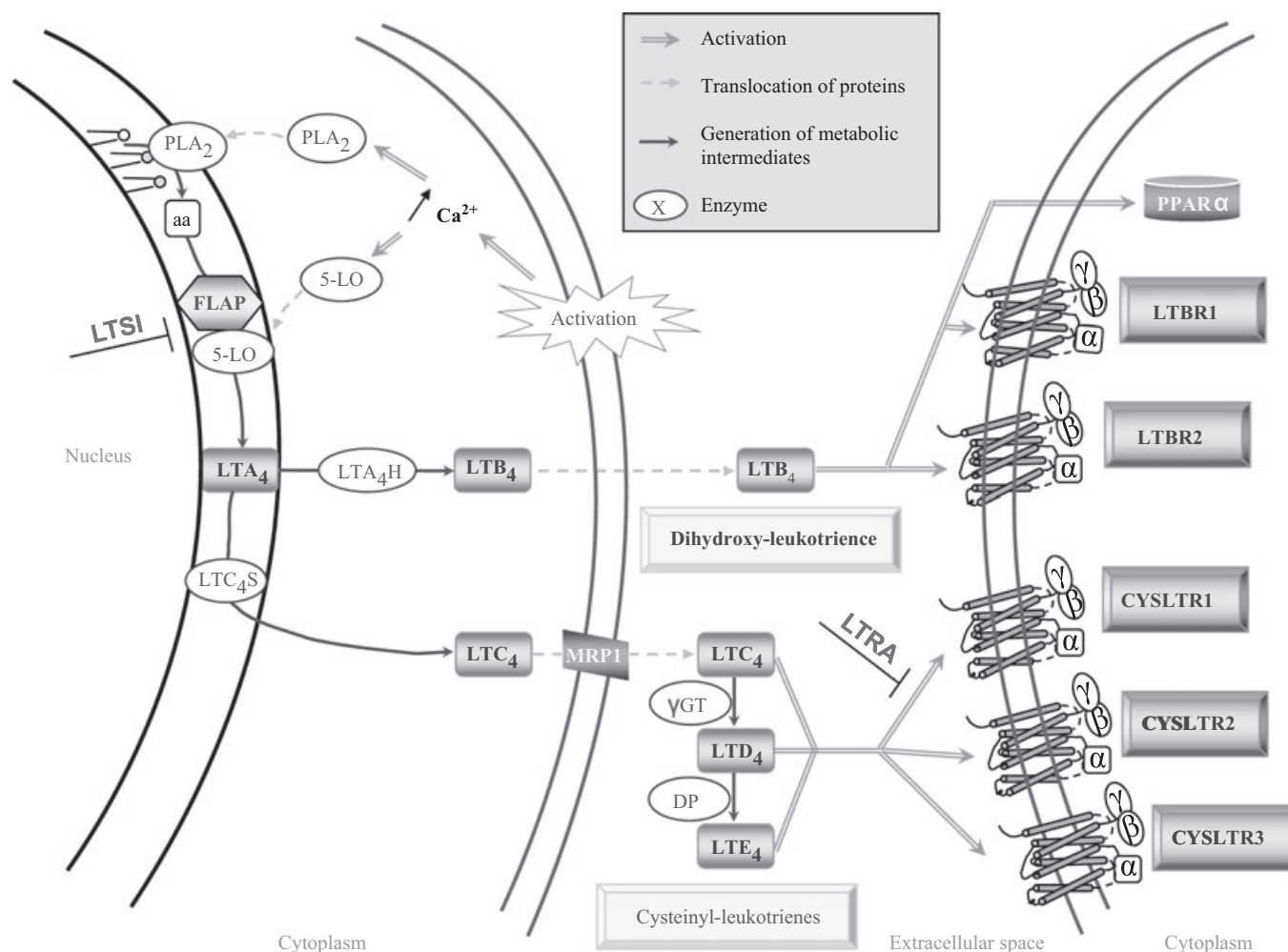


Figure 1. The 5-lipoxygenase pathway. See text for description. 5-LO, 5-lipoxygenase; aa, arachidonic acid; CYSLTR, cysteinyl leukotriene receptor; DP, dipeptidase; FLAP, 5-lipoxygenase activating protein; γ-GT, γ-glutamyl transpeptidase; LTBR, leukotriene B₄ receptor; LTA₄, LTB₄, LTC₄, LTD₄, LTE₄, leukotriene A₄, B₄, C₄, D₄, E₄; LTA₄H, LTA₄ hydrolase; LTC₄S, LTC₄ synthase; LTRA, leukotriene receptor antagonists; LTSI, leukotriene synthesis inhibitor; MRP1, multi-drug resistant protein 1; PLA₂, phospholipase A₂; PPARα, peroxisome proliferator activated receptor alpha.

Regulation of LT synthesis

Leukotriene synthesis is restricted to cells of myeloid lineage as this is where 5-LO and FLAP are expressed. Different cells also express LTC₄S or LTA₄H affecting which LT is synthesised. LTC₄S is expressed in eosinophils and mast cells whereas neutrophils produce LTB₄ as their major LT. There are also cells that produce both (18). LTA₄H is expressed ubiquitously in most tissues. In addition, the interaction between different cell types with cooperation of their enzymes can allow platelets (19) and vascular endothelial cells (20) or erythrocytes (21) to produce LTC₄ or LTB₄, respectively, from LTA₄ transferred from neutrophils (transcellular metabolism). Subcellular localisation of the enzymes/proteins is important. 5-LO, FLAP and LTC₄S all co-localise to the nuclear envelope in a calcium-dependent process upon cell activation, bringing 5-LO into the proximity of its

substrate and LTC₄S to allow its preferential action (9, 22). 5-LO is concentrated in the cytosol of resting neutrophils, but is located in the nucleus of alveolar macrophages and mast cells (23). This localisation is possible because of three nuclear localisation signals (24, 25). The mechanisms involved here are still not fully understood, but there is evidence for cells with nuclear 5-LO to produce more LTB₄ than the same cells with cytoplasmic 5-LO (26). Phosphorylation by protein kinase (mitogen-activated protein) A on Ser523 can decrease 5-LO activity, whereas phosphorylation on Ser271 by MAP kinase (mitogen-activated protein) activated protein 2 and on Ser663 by extracellular signal-regulated kinase can increase it (27–29).

Leukotrienes are lipid mediators derived from arachidonic acid by a sequence of reactions catalysed by the enzymes of the 5-LO pathway. The pathway is activated by many different stimuli including biological messengers

such as cytokines and immune complexes and environmental stimuli like antigens and microbes. There are multiple levels of regulation including at the genetic and biological level.

LTs and allergic inflammation

Cysteinyl leukotrienes have been shown to be involved in most of the key features of asthma including airway smooth muscle constriction (30, 31), vasodilation and increased microvascular permeability of vascular smooth muscle (32), leading to plasma exudation hence tissue oedema, mucus secretion (33) and inhibition of human respiratory cilia activity (34), airway remodelling (35), bronchial hyperresponsiveness (BHR) (17) and increased survival of inflammatory cells (36, 37). CysLT synthesis has been directly linked to asthma pathogenesis as there is extensive evidence that CysLT production is increased in asthma subjects, particularly during an exacerbation or upon challenge (1).

CysLTs are also key mediators in allergic rhinitis (AR) (38). In addition to their inflammatory effects similar to those seen in asthma, they modulate nasal allergic inflammation and clinical symptoms (especially sneezing and rhinorrhea) via activation of CYSLTR1 in nasal mucosa, in particular in vascular endothelial cells and interstitial cells (eosinophils, mast cells, macrophages and neutrophils) (39). In addition, CysLTs have been shown to participate in the process of allergic sensitisation by enhancing dendritic cell-stimulated antigen presentation (40). There is also evidence of enhanced CysLT production in the pathogenesis of atopic dermatitis (AD). Enhanced spontaneous and stimulated releasability of LTC₄ from basophils and eosinophils isolated from AD patients is increased compared with healthy controls and increased production of LTs has been reported in the skin of atopic patients after allergen-specific challenge (41).

LTB₄ has been proposed to play a role in a variety of acute and chronic inflammatory diseases including AD (42), asthma (1) and chronic obstructive pulmonary disease (COPD) (43). LTB₄ is one of the most powerful

chemotactic agents known to date (44) and its role in not only the recruitment but also activation and survival of neutrophils to the site of tissue injury (45) suggests that the mediator may amplify proinflammatory circuits *in vivo*. Indeed, LTB₄ is overproduced in the airways of both asthma and COPD patients with levels correlating to the severity of asthma observed (46) and this LTB₄ overproduction and subsequent interaction with its receptors causes the migration of inflammatory cells (mast cells, lymphocytes and eosinophils) into the airways (44).

It is important to note that in addition to a role in allergic inflammation, prominent expression of LT in the brain, adrenals, heart and vascular endothelium suggests additional unrecognised functions of those mediators, as shown by their involvement in brain injury (47), chronic cardiovascular diseases (48), cardiac anaphylaxis and ischemic myocardial infarction (MI) (48). Interestingly, a contribution to host defence against infection by augmenting the ability of macrophages and neutrophils to phagocytose microorganisms has also been suggested.

There is extensive evidence supporting a key role for LTs in allergen-induced inflammatory diseases such as asthma and AR which has been further confirmed by the clinical efficacy of strategies targeting LT production and activity (Table 1).

LT modifier therapy

Asthma

Three selective CYSLTR1 antagonists (LT receptor antagonists, LTRAs), have been used in clinic practice in asthma therapy for nearly 10 years (Table 1): Pranlukast (Onon[®]; Ono Pharmaceutical Co, Ltd, Osaka City, Tokyo, Japan), Montelukast (Singulair[®]; Merck & Co, Inc, Whitehouse Station, New Jersey, USA) and Zafirlukast (Accolate[®]; AstraZeneca Pharmaceuticals LP, Wilmington, Delaware, USA). Although there are differences in potency and pharmacokinetics (49), LTRAs have similar effects. As they are administered orally, LTRAs are of particular value in improving patient compliance. They are also well tolerated as there have

Table 1. Leukotriene modifier therapy in human disease

Modifier(s)	Target	Used in clinical practice for	Potential therapeutic effects on (in clinical trials)
FLAP inhibitors: DG-031, BAYx1005	FLAP		MI, COPD, chronic bronchitis
5-LO inhibitor: Zileuton	5-LO	Asthma	COPD
LTB ₄ R antagonists: LY293111	LTB ₄ receptors	Asthma, COPD	Psoriasis, COPD, atherosclerosis, cancer
LTTRAs: Montelukast, Pranlukast, Zafirlukast	CysLT receptors	Asthma, AR	COPD, interstitial lung disease, chronic urticaria, AD, allergic fungal disease, nasal polyposis, paranasal sinus disease, migraine, respiratory syncytial virus postbronchiolitis, systemic mastocytosis, cystic fibrosis, pancreatitis, vulvovaginal candidiasis, cancer, atherosclerosis, eosinophils cystitis, otitis media, capsular contracture, eosinophilic gastrointestinal disorders

5-LO, 5-lipoxygenase; AD, atopic dermatitis; AR, allergic rhinitis; COPD, chronic obstructive pulmonary disease; CysLT, cysteinyl leukotriene; FLAP, 5-lipoxygenase activating protein; LTB₄, leukotriene B₄; LTB₄R, leukotriene B₄ receptor; LTTRA, leukotriene receptor antagonists; MI, myocardial infarction.

been only few class-specific side effects identified (headaches and gastrointestinal problems), rarely severe enough to cause discontinuation of the therapy (1). LTRAs are efficient in inhibiting bronchoconstrictor challenge, in particular to allergen, exercise, irritants and aspirin as well as inhaled LTD₄ (50). They reduce the early and late response to allergens by approximately 50% (51). Studies comparing the effects of LTRAs with placebo in patients with mild-to-moderate symptomatic asthma have consistently shown the active drug to be more effective than placebo in improving several outcome measures including lung function tests, quality of life, night-time awakenings and asthma symptoms (52). Despite significant benefit compared with placebo, LTRAs are less effective than low doses of inhaled corticosteroids in chronic asthma (53). In the USA, the LT synthesis inhibitor (LTSI) Zileuton which targets 5-LO has been introduced to clinical practice (54). Although the effects are short lived, Zileuton blocks 70% of CysLT production. However, this drug has been associated with severe liver toxicity, limiting its clinical usefulness (55).

Allergic rhinitis

Montelukast has also been approved in the USA for treatment of seasonal and perennial AR (56). The anti-leukotriene significantly reduces day time nasal symptoms (congestion, rhinorrhea, pruritus and sneezing), day time eye symptoms, night time symptoms (difficulty sleeping, night-time awakenings and congestion on awakening) and peripheral eosinophilia (56). In addition, Zafirlukast has generated interest as it significantly improved nasal airway resistance by 68% in patients with AR (57) and reduced nasal congestion in patients allergic to cat allergens (58).

Because of the postulated role of LTs in the pathogenesis of other diseases, anti-leukotrienes are now being evaluated for other disorders (59) such as AD (41) and atherosclerosis (60) (Table 1). Although LTRAs and Zileuton are the only anti-leukotrienes used in clinical practice to date, others are in development (61, 62). Japanese studies have experimented with blocking the LTB₄–LTB₄R interaction with the LTB₄ receptor antagonist LY293111 to prevent the chemotaxis of neutrophils into the asthmatic lung (63). This provides the potential for therapy in the future. DeCode Genetics has licensed the FLAP inhibitor DG-031 (veliflapon) created by Bayer and observed that the inhibitor reduced the incidence of MI by reducing the production of LTB₄ (64).

In a randomised, double-blind, placebo controlled, 12-week trial on 895 patients with mild-to-moderate asthma, Malmstrom et al. observed that, like other anti-asthmatic drugs, the patient response to montelukast was heterogeneous (53). The reasons for this selective response pattern are not known, but may be due to many factors such as patient compliance, disease severity, co-morbidity, misdiagnosis, drug interactions and patient

environment (especially for allergic phenotypes). Another factor may affect not only patient response but also the disease: the patient's genetic profile. For this reason, the genes encoding LT synthesising enzymes, adaptor proteins and receptors are being studied for pharmacogenetic influences on LTRA and LTSI responses.

Targeting of LTs has shown clinically significant improvements in asthma and more recently allergic rhinitis particularly as add-on therapies. Interestingly, the patient response is very variable and may in part be due to the patient's genetic profile. A better understanding of the molecular genetics of the key components of the 5-LO pathway is thus needed.

Molecular genetics of the 5-LO pathway

LT synthesis

There are many proteins involved in the formation of LTs (see Fig. 1 and Table 2). The gene encoding 5-LO (*ALOX5*) spans 82 kb on 10q11.2 (65). The 85 kDa protein encoded is composed of a 673 amino acid polypeptide (66). The transcription initiation site is located 65 bp upstream of the ATG and there are no TATA or CAAT sequences in the promoter (67). The gene is regulated at the transcriptional level (68) with basal mRNA levels remaining constant, but which rise rapidly during cell activation (69, 70). Another level of regulation is the calcium-dependent translocation of 5-LO to the membrane from the cytosol to arrange the protein closer to its substrate (which is derived from the membrane) (8).

FLAP is critical for the function of 5-LO. The 162 amino acid and 18 kDa protein is encoded by the 31 kb gene *ALOX5AP*. *ALOX5AP* is composed of five exons and four much larger introns (71) and is located on chromosome 13q12 (72). Analysis of cDNA from

Table 2. Molecular characteristics of the genes encoding for the 5-LO pathway proteins and receptors

Genes	Chromosomal location	Gene length (kb)	Exon (no.)	Protein (no. of residues)
<i>ALOX5</i>	10q11.2	82	14	673
<i>ALOX5AP</i>	13q12	31	5	162
<i>LTC4S</i>	5q35	2.5	5	150
<i>LTA4H</i>	12q22	35	19	610
<i>CYSLTR1</i>	Xq13-q21	54.9	3	337
<i>CYSLTR2</i>	13q14	2.8	1	346
<i>LTB4R1</i>	14q11.2-q12	5	3	352
<i>LTB4R2</i>	14q11.2-q12	2.8	1	389
<i>PPARA</i>	22q12-q13.1	83.7	9	468
<i>MRP1</i>	16p13.1	6.5	30	1531

ALOX5, 5-lipoxygenase; *ALOX5AP*, 5-lipoxygenase activating protein; *CYSLTR 1–2*, cysteinyl leukotriene receptor 1–2; *LTA4H*, LTA₄ hydrolase; *LTB4R 1–2*, leukotriene B₄ receptor 1–2; *LTC4S*, LTC₄ synthase; *MRP1*, multi-drug resistant protein 1; *PPARA*, peroxisome proliferator activated receptor alpha.

mammalian species has shown the protein to be highly conserved (73). Protein analysis has indicated regions more conserved between mammals (such as residues 74–123 which form the loop between transmembrane domains 2 and 3). Characterisation of the 5' regulatory regions has identified a TATA box and glucocorticoid response elements as well as AP-1 (activator protein 1) and AP-2 transcription factor recognition sequences (71).

Both 5-LO and FLAP act upon arachidonic acid, which is released from membrane phospholipids by cytosolic phospholipase A₂ (PLA₂). This 85 kDa protein composed of 749 amino acids is encoded by a gene on 1q25 (PLA2G4) (74). While the genomic structure is not fully understood, there are transcription factor binding sites for NF-kappa B, AP-1, *polyomavirus enhancer A*-binding protein-3 (*PEA-3*) and NF-IL6. Crystal structure analysis identified Ser228 as involved in catalytic activity (75).

The two enzymes responsible for either CysLT or LTB₄ formation are LTC₄S and LTA₄H respectively. LTC₄S is an 18 kDa (150 amino acid) protein (76) encoded by *LTC4S*, a 2.5 kb gene with five exons which resides on 5q35 (77). The protein contains two consensus protein kinase C phosphorylation sites along with an *N*-glycosylation site and three putative transmembrane spanning regions (78). LTC₄S belongs to the same family as FLAP as it shows 31% identity and a similar hydropathy profile. Promoter analysis has identified three predicted transcription start sites at -96, -69 and -66 relative to the ATG. Multiple transcription factor recognition sequences have also been identified (Sp1, AP-1, AP-2 and GATA-1) (77). *LTA4H* is a 35 kb gene residing on chromosome 12q22 and contains 19 exons (79). It encodes a 69 kDa enzyme from a 1833 bp open reading frame (ORF) of 610 amino acids. Promoter analysis identified a transcription start site at -151 relative to the ATG (79) and multiple transcription factor binding sites. The gene can be alternatively spliced by skipping an 83 bp exon in the 3' coding region which produces a smaller 59 kDa protein with a different C-terminal (80).

LTB₄ receptors

There are two known receptors for LTB₄ named LTB₄R1 and LTB₄R2 (or BLT1 and BLT2). LTB₄R1 is a high-affinity LTB₄-specific receptor (K_d of 0.15 nM) expressed mainly on leucocytes. Despite being the high-affinity receptor, 12(R)-HETE and 20-hydroxyl LTB₄ can (with much less potency than LTB₄) agonize the receptor (81). LTB₄R2 is less-specific, able to bind other eicosanoids and is expressed more ubiquitously (82). The structure of these *LTB4R* genes is yet to be fully resolved. *LTB4R1* is a small gene on chromosome 14q11.2-q12. Currently, three exons have been reported, but analysis in epithelial cells has identified four different 5' untranslated exons producing transcripts 5–7.5 kb in length (81, 83). Cloning of the cDNA expressed in leucocytes identified a 1056 bp ORF

coding for a 43 kDa polypeptide of 352 amino acids. More recently, alternatively spliced variants have been identified involving a deletion of the 100 N-terminal amino acids or deletion of 39 internal amino acids (residues 43–81) (84). Analysis of the promoter region of *LTB4R1* highlighted the presence of another ORF 3 kb upstream coding for a 358 amino acid polypeptide which has 45% homology to *LTB4R1* (85). This gene, *LTB4R2* is expressed as a major transcript of 2.5 kb (86). *LTB4R2* is closely related to its mouse homolog with 93% identity. The close location of these genes indicates that they are likely to be the result of gene duplication (87). LTB₄R1 and LTB₄R2 show high homology in transmembrane domains 3 and 7 suggesting that this is the site for LTB₄ binding (85). Agonist binding leads to the activation of a heterotrimeric guanine-nucleotide binding protein (or G-protein) made of an α -, β - and γ -subunit, which can transduce the signal from the plasma membrane to the cytoplasm. Binding to the LTB₄Rs lead to the activation of phospholipase C leading to inositol-1,4,5-trisphosphate and calcium production and inhibition of adenylyl cyclase (81).

Transcription of *LTB4R1* is regulated in a tissue-specific manner in macrophages and eosinophils (cells important for its chemotactic and proliferative function). The promoter contains recognition sequences for the ubiquitous transcription factor Sp-1 at -50 important for basal transcription but not tissue-specific regulation (88).

LTB₄ has also been found to be a ligand for the nuclear hormone receptor peroxisome proliferator-activated receptor alpha (PPAR α). The *PPARA* gene spans 83.7 kb and resides on chromosome 22q12-q13.1. It is expressed in tissues with a high fatty acid concentration such as the muscle, heart, kidney and liver. Activation of this receptor leads to the expression of genes containing PPAR responsive elements in their promoters (the consensus sequence AGGTCAXAGGTCA) that controls the catabolism of eicosanoids (LTs and prostanooids) (89). This produces a system of negative feedback (90, 91).

CysLT receptors

Two different CysLT-specific GPCRs have been identified based on their pharmacological profiles, CYSLTR1 and CYSLTR2. Affinity studies for CYSLTR1 agonists in transfected cells or in isolated human bronchial preparations have identified the rank order of potency: LTD₄ > LTC₄ > LTE₄ (EC₅₀ value: approximately 10⁻⁹, 10⁻⁸ and 10⁻⁷ M, respectively), but these results are not consistent between studies and receptor properties and function defined by functional studies differ between species and cell type (92). CYSLTR1 is antagonised by Montelukast, Pranlukast, Zafirlukast, BAY u9773 and MK-571. LTE₄ has a 10-fold lower affinity for CYSLTR2 than LTC₄ and LTD₄ and is antagonised by the CYSLTR1/2 antagonist BAY u9773 (93, 94).

The *CYSLTR1* and *CYSLTR2* genes have been cloned (93, 95). *CYSLTR1* is 337 amino acids, 38.5 kDa and encoded by a gene on Xq13-21. There is 32% amino acid identity to the purinoreceptor P2Y1 and 28% identity to the LTB₄R1 receptor (95, 96). Cloning and sequencing of the cDNA indicated an intronless coding sequence. The genomic organisation of the human *CYSLTR1* gene is only partially characterised and remains unclear. Transcripts are approximately 1.5 kb long comprising three exons with the ORF in exon 3 (92). Receptor mRNA is highly expressed in the spleen and peripheral blood leucocytes, less strongly in the lung, bronchus, small intestine, colon, skeletal muscle, pancreas and placenta (95, 96). Immunohistochemistry, Northern and reverse transcriptase-polymerase chain reaction analyses located the receptor in lung smooth muscle cells [including pulmonary artery smooth muscle (97)] and interstitial macrophages, with little or no expression in epithelial cells (95, 98).

CYSLTR2 resides on chromosome 13q14 and has been characterised by three different groups (93, 99, 100). The gene is 2548 bp long encoding a 346 amino acids polypeptide. Expression occurs in monocytes, B-lymphocytes, airway smooth muscle and eosinophils through the identification of mRNA (95, 98). The human *CYSLTR2* protein is weakly expressed in lung smooth muscle cells and the strongest expression of the receptor in the lung was seen in interstitial macrophages. Moderate expression could be detected in the immune system, in particular the spleen, lymph nodes and peripheral blood leucocytes (especially monocytes and eosinophils).

The *CYSLTR1* and *CYSLTR2* genes show little nucleotide sequence homology and there is only 37% amino acid identity at the protein level. A third *CYSLTR*, *CYSLTR3*, not antagonised by any current antagonists has been suggested (101). Based on relatedness to the *CYSLTRs* and P2Y families, this could be the dual-receptor GPR17 (102).

As the genes encoding the key components of the 5-LO pathway are being elucidated, an increasing number of studies have investigated the genetic and pharmacogenetic role of polymorphisms in these genes in allergy.

LT genetics, asthma and allergy susceptibility

Asthma and allergy genetic association studies have been performed on the 5-LO pathway genes and the first two CysLT receptors, but interest is now growing towards other enzymes and LTB₄ receptors. These data are summarised in Tables 3 and 4.

ALOX5 and *ALOX5AP*

Work has focused on investigating *ALOX5* and *ALOX5-AP* as 5-LO is the key enzyme in LT synthesis which needs to interact with FLAP prior to LT synthesis. The *ALOX5* 5'UTR has been well characterised and by resequencing

asthma patient DNA samples mutations in the region 176–146 bp upstream of the ATG (in a GC-rich region) with deletion of one, or two or addition of one zinc finger (Sp1/Egr-1) binding sites have been identified which results in reduced Sp1/Egr-1 transcription factor binding and gene transcription (103, 104). Mutations of the Sp1 repeat in the promoter are associated with airway hyper-responsiveness, but not asthma susceptibility (105, 106). Using 341 asthma enriched families, no association between the *ALOX5* Sp1 polymorphism and asthma or asthma-related phenotypes was identified (107). However, the Sp1 polymorphism has been suggested as a predictor of disease severity (108). Analysis of a Korean population ($n = 397$) with an *ALOX5* haplotype, ht1-(G-C-G-A), covering single nucleotide polymorphisms (SNPs) –1708 G>A and coding region exon 1 21 C>T, exon 2 270 G>A and exon 13 1728 A>G has shown association with aspirin intolerant asthma (AIA) [$p = 0.01$, odds ratio (OR) = 5.0 and 95% confidence interval (CI) of 1.54–17.9] (109). Recently, a 760 G>A (E254K) polymorphism has been described in exon 6 which results in a residue change from negative to positive in the C-terminal catalytic domain. This SNP was only seen in bronchial asthma patients (6% frequency) and not in a cohort of nonallergic subjects indicating that it may be associated with bronchial asthma (110).

Two polymorphisms have been identified in the *ALOX5AP* promoter, a –336 (G>A) and a poly(A) repeat at position –169 to –146 yielding 19A and 23A alleles (107). No associations were observed with asthma in a Caucasian cohort of 341 asthma families or basal transcription levels in a promoter reporter analysis (107). However, association was observed with asthma in a Japanese study with a greater frequency of the 21A allele in asthmatics compared to controls, which was statistically significant ($p = 0.035$) (111). The significance of this finding is questionable for the small size of the study (71 subjects and 71 controls). The *ALOX5AP* gene has now been completely sequenced and multiple SNPs have been identified (112). In asthma, intronic SNPs (SG13S114, SG13S89 and SG13S41) have been associated with asthma and atopy phenotypes indicating that markers in the gene are predictors for disease susceptibility (113). There has been no extensive investigation into 3'UTR SNPs; however, analysis of a 218 A>G SNP in the Korean population failed to find associations with asthma (109).

LTA4H and *LTC4S*

Mutation screening of the *LTA4H* gene identified several SNPs spanning *LTA4H* (114). Association with asthma and atopy phenotypes was identified for SNP rs1978331 (intron 11) and a haplotype encompassing rs1978331-rs17677715-rs2540482-rs2660845-rs240475 (113). *LTC4S* encodes for the main enzyme specific to CysLT synthesis and has been extensively studied, especially a

Table 3. Allergic disease genetic association studies for 5-LO pathway genes

Gene	Polymorphism	Location	Population	Association	Reference
<i>ALOX5AP</i>	18A–21A repeats	P	Japanese (71A, 71C)	Yes, asthma	Koshino et al. (111)
	–336 G>A, 23A–19A repeats	P P	UK Caucasian (341 families, <i>n</i> = 1503)	No, asthma and atopy	Sayers et al. (107)
	23A–19A repeats	P	Korean (107AIA, 109 ATA, 114 C)	No, AIA	Kim et al. (106)
	SG13S25 G>A SG13S114 T>A SG13S89 G>A SG13S32 C>A rs380327 C>A SG13S41 A>G SG13S35 G>A	5'UTR I I I I I 3'UTR	UK Caucasian (341 families, <i>n</i> = 1503)	Yes, SG13S114, SG13S89, SG13S41 and haplotypes with asthma and atopy related phenotypes	Holloway et al. (113)
	218 A>G	3'UTR	Korean (93 AIA, 181 ATA, 123 C)	No, AIA	Choi et al. (109)
			Japanese (55 AIA, 63 ATA, 53 C)	No, AIA	Kawagishi et al. (105)
			UK Caucasian (341 families, <i>n</i> = 1503)	No, asthma and atopy	Sayers et al. (107)
	Sp1 repeat	P	Korean (107 AIA, 109 ATA, 114 C)	No, asthma Yes, severity of AHR in AIA	Kim et al. (106)
<i>ALOX5</i>			Turkey (624 A)	Yes, severity and exercise induced asthma	Kalayci et al. (108)
	–1708 G>A 21 C>T	P C	Korean (93 AIA, 181 ATA, 123 C)	Yes, AIA for both SNPs	Choi et al. (109)
	760 G>A	C	Japanese (180 A, 150 C)	Yes, asthma	Bai et al. (110)
			Polish (47 AIA, 64 ATA, 42 C)	Yes, AIA	Sanak et al. (118)
<i>LTC4S</i>			Polish (76 AIA, 110 ATA, 75 C)	Yes, AIA	Sanak et al. (117)
			Japanese (60 AIA, 100 ATA, 110 C)	Yes, AIA vs ATA	Kawagishi et al. (105)
			USA Caucasian (61 AIA, 33 ATA, 137 C)	No, AIA	Van Sambeek et al. (125)
			Polish (26 AIA, 33 ATA)	No, AIA	Mastalerz et al. (122)
			Korean (159 AIA, 116 AICU)	No, AIA	Kim et al. (149)
			Korean (110 AIA, 125 ATA, 125 C)	No, AIA	Choi et al. (120)
	–444 A>C (rs730012)	P	Spanish (123 A, 43 AIA, 76 ATA, 103 C)	No, asthma, AIA	Isidoro-Garcia et al. (121)
			Korean (93 AIA, 181 ATA, 123 C)	No, AIA	Choi et al. (109)
			Australian Caucasian (604 A, 67 AIA, 496 ATA, 458 C)	Yes, asthma	Kedda et al. (115)
			Japanese (349 A, 171 C)	No, asthma	Asano et al. (119)
			Spanish Caucasian (130 A, 78 C)	No, asthma	Sanz et al. (123)
			UK Caucasian (341 families, <i>n</i> = 1503)	No, asthma and atopy	Sayers et al. (124)
			UK Caucasian (23 SA, 31 C)	Yes, severe asthma	Sampson et al. (116)
			Turkish (85 AR, 95 C)	Yes, AR	Eskandari et al. (126)
	–1072 G>A	P	UK Caucasian (341 families, <i>n</i> = 1503)	No, asthma and atopy	Sayers et al. (124)
			Korean (110 AIA, 125 ATA, 125 C)	No, AIA	Choi et al. (120)
	IVS1-10	I	Australian Caucasian (604 A, 67 AIA, 496 ATA, 458 C)	No, asthma	Kedda et al. (115)
	10 G>A	C	Japanese (141 AL, 110 NAL)	Yes, allergic disease (asthma, AD, AR)	Yoshikawa et al. (127)

Table 3. (Continued)

Gene	Polymorphism	Location	Population	Association	Reference
<i>LTA4H</i>	rs2540475 C>T	5'UTR	UK Caucasian (341 families, <i>n</i> = 1503)	Yes, rs1978331 and haplotypes with asthma and atopy	Holloway et al. (113)
	rs2660845 A>G	5'UTR			
	rs2540482 A>G	5'UTR			
	rs17677715 T>C	I			
	rs1978331 T>C	I			

3'UTR, 3' untranslated region; 5'UTR, 5' untranslated region; A, asthma; AD, atopic dermatitis; AIA, aspirin intolerant asthma; AICU, aspirin intolerant chronic urticaria; AL, allergic; *ALOX5*, 5-lipoxygenase; *ALOX5AP*, 5-lipoxygenase activating protein; AR, allergic rhinitis; ATA, aspirin tolerant asthma; C, controls (population); C, coding region (location); I, intronic; *LTA4H*, leukotriene A4 hydrolase; *LTC4S*, leukotriene C4 synthase; NAL, nonallergic; P, promoter; SA, severe asthma; SNP, single nucleotide polymorphism.

Table 4. Allergic disease genetic association studies for CysLT receptor genes

Gene	Polymorphism	Location	Population	Association	Reference
<i>CYSLTR1</i> †	–945 C>T (rs321029)	P	Korean (105 AIA, 110 ATA, 125 C)	Yes, TCG haplotype with AIA (males), total IgE (females) and atopy (females) No, BHR and FEV ₁	Kim et al. (131)
	–786 A>C (rs2637204)	P	Japanese (137 families with A, <i>n</i> = 466, 48 families with AR, <i>n</i> = 188)	No, asthma and AR	Zhang et al. (130)
	–647A>G (rs2806489)	P			
	–945 C>T (rs321029)	P	Korean (159 AIA, 116 AICU)	Yes, AIA vs AICU	Kim et al. (149)
	927 T>C (rs320995)	C	Korean (93 AIA, 181 ATA, 123 C)	No, AIA, ATA	Choi et al. (109)
			Spanish Caucasian (130 A, 78 C)	Yes, alone and in combination with LTC4S SNP –444 C allele, asthma (males)	Sanz et al. (123)
			Spanish Caucasian [87 A (41 with AD), 79 C]	Yes, asthma and asthma + AD vs A + no AD (males)	Arriba-Mendez et al. (128)
			UK Caucasian (341 families)	No, asthma, atopy and related phenotypes Yes, atopy severity (females)*	Hao et al. (129)
	899 G>A (G300S)	C	Tristan de Cunha (52 AL vs 60 C, 54 A vs 58 C)	Yes, atopy Yes, asthma (females)	Thompson et al. (132)
<i>CYSLTR2</i>	–1220 A>C	P	Japanese (137 families, <i>n</i> = 466)	Yes, atopic asthma	Fukai et al. (134)
	M202V	C	Tristan de Cunha (52 AL vs 60 C)	Yes, atopy (M202V)	Thompson et al. (137)
	S237L	C			
	601A>G	C	Caucasian (359 and 384 families)	Yes, asthma (both cohorts)*	Pillai et al. (136)
	–819 T>G 2078 C>T 2534 A>G 2545 + 297 A>G	5'UTR 3'UTR 3'UTR 3'FR	Korean (490A, 152C)	Yes, –819 T>G, 2078 C>T, 2534 A>G and haplotypes, AIA vs ATA No, asthma	Park et al. (135)

3'FR, 3' flanking region; 3'UTR, 3' untranslated region; 5'UTR, 5' untranslated region; A, Asthma; AD, atopic dermatitis; AIA, aspirin intolerant asthma; AICU, aspirin intolerant chronic urticaria; AL, allergic; AR, allergic rhinitis; ATA, aspirin tolerant asthma; C, controls (population); C, coding region (location); *CYSLTR 1–2*, cysteinyl leukotriene receptor 1–2; I, intronic; P, promoter; SNP, single nucleotide polymorphism.

Unless stated otherwise (*), phenotypes were associated with the minor allele.

†*CYSLTR1* promoter SNPs are located from the exon 4 3'end as described by Duroudier et al. (133).

polymorphism located –444 upstream of the ATG translation start site (–444 A>C, rs730012). Approximately half of the published genetic studies found the C allele associated with asthma, asthma severity or aspirin

intolerance (105, 115–118). The other studies, including two also investigating another promoter polymorphism (–1072 G>A) did not detect an association with those same phenotypes (109, 119–125) (see Table 3). Interest-

ingly, in Caucasian populations, Kedda et al. observed an association of the C allele (−444) with asthma ($p = 0.03$, 1062 individuals), but not asthma severity or aspirin intolerance (115). Sampson et al. and Isidoro-García et al. observed an increased frequency of this allele in severe asthma (116, 121), although this did not reach significance for the latter. Sanak et al. also detected an association of the same allele with aspirin sensitivity in two separate studies (153 and 261 individuals) (117, 118), whereas Mastalerz et al. and Van Sambeek et al. did not (59 and 201 individuals respectively) (122, 125). In Asian populations, aspirin sensitive asthma patients showed a higher frequency of the C allele in one study (270 individuals, $p = 0.042$), but this could not be reproduced elsewhere (109, 120). Discordances in the results are seen among the same ethnic populations (Asian and Caucasian). This raises issues regarding study design, particularly related to the relatively small size of the populations studied.

In a Turkish population of 180 individuals, heterozygotes for the −444 T>C SNP were at higher risk of developing AR (OR = 2.18; 95% CI:1.173–4.053; $p = 0.014$) (126). In addition, the minor allele of the recently identified coding polymorphism 10 G>A (Glu4Lys) was associated with allergic diseases ($p = 0.046$) among 251 Japanese individuals (127).

CysLT receptors

There are many discrepancies among studies investigating *CYSLTR1* genetics. This is confounded by the fact that the gene is located on the X chromosome. In a Caucasian Spanish population, Sanz et al. reported the minor allele of the synonymous coding SNP 927 T>C (rs320995) to be associated with asthma (130 asthma vs 78 controls) (123), but Arriba-Mendez et al. observed this association only in male subjects (87 asthma vs 79 controls, $p < 0.008$) (128), whereas two other groups could not detect any association in a Caucasian British and a Korean population (109, 129). Although this was not reproduced in a Japanese study (137 families) (130), Kim et al. found an association of the three SNPs in the *CYSLTR1* promoter with aspirin intolerant asthma among male Koreans (340 individuals, $p < 0.03$) (131). The authors also observed an association of these same alleles with atopy among female aspirin intolerant asthmatic individuals ($p = 0.03$) (131). Interestingly, we have preliminary evidence that a promoter SNP is associated with atopy incidence in a large Caucasian population (unpublished data, Duroudier et al.) In addition, in a family-based association study (341 Caucasian families), the coding SNP 927 T>C which is in high linkage disequilibrium with the promoter SNP rs2806489 was associated with atopy severity ($p = 0.015$), which was more significant in female asthma subjects ($p = 0.0087$) (129). In a high prevalence asthmatic population with a founder effect, Thompson et al. observed the minor allele

of the coding polymorphism 899 G>A (Gly300Ser) to be associated with both asthma ($p = 0.005$) and atopy (OR = 4; 95% CI:1.2–13.3) in female subjects (132). Other studies have detected an association of the promoter or coding SNPs with allergic phenotypes such as high total IgE ($p = 0.003$) (131) and AD (OR = 4.97; 95% CI:1.29–19.13) (128), which could not be reproduced elsewhere (129, 130). Overall, despite these discrepancies, most studies agree that there is an association between *CYSLTR1* genetic variation and allergic phenotypes and that this association is gender specific. Furthermore, the functionality of the associated promoter SNPs (tagged in the same linkage disequilibrium block) found that the minor allele (−647, rs2806489) caused a two-fold decrease in reporter expression compared to the A allele when examined using promoter-reporter analyses in human monocytes (133).

Multiple SNPs within *CYSLTR2* have been found to be in association with atopic asthma or aspirin sensitivity within asthma subjects (134–137). Interestingly, a coding region variant (601 A>G) results in a decreased potency for LTD₄ and was shown to be under transmitted to asthma subjects using 384 asthma families (136). The Met201Val variant was also associated with atopy (137).

LTD₄ receptors and PPARA

Polymorphisms have been reported in the coding regions of *LTB4R1* (138) including G>T (Ala79Ser) and T>C (Phe346Leu) and in *LTB4R2* G>A (Gly196Asp), but we have not observed these in the Caucasian population (unpublished data, A.S. Tulah and I. Sayers). The role of *LTB4R1* and *LTB4R2* SNPs in asthma and allergy susceptibility remains to be resolved at this time. The PPAR α is a nuclear hormone receptor with a cysteine-rich zinc finger DNA-binding region. Cloning and sequencing of the gene identified two non-synonymous SNPs in the DNA-binding domain (139). Leu162Val which is normally conserved between species, makes the protein unresponsive to low ligand concentrations compared to the wild-type, whilst the mutant allele has also been associated with higher plasma total and low-density lipoprotein (LDL)-apolipoprotein B and LDL-cholesterol in a French-Canadian population (140). The Arg131Gln polymorphism which is present at a lower frequency slightly raises constitutive activity of the receptor in response to the ligand. A G>A synonymous SNP in exon 7 was also identified (140). To date, association with allergy phenotypes has not been explored.

There is accumulating evidence that polymorphism spanning genes involved in leukotriene production and activity influence allergic disease phenotypes. As inflammatory diseases are complex, genetic and pharmacogenetic effects are likely to be caused by several, perhaps numerous genes, each with a small overall contribution and relative risk. Each of these genes is likely to be necessary but by themselves not sufficient to have a

Table 5. Leukotriene pharmacogenetic studies

Gene	Polymorphism	Location	Population	Association	Reference
<i>ALOX5AP</i>	rs3803278 C>T rs12721458 C>G rs3803277 A>C	I I I	USA (79% Caucasian, 174 A)	No, FEV ₁ , morning PEF (montelukast)	Klotsman et al. (145)
	23A–19A repeats 218 A>G	P 3'UTR	Korean (89 AIA)	No, FEV ₁ , PEF (montelukast)	Kim et al. (144)
<i>ALOX5</i>			USA Caucasian (221 A)	Yes, FEV ₁ (ABT-761)	Drazen et al. (68)
			USA Caucasian (61 A)	Yes, asthma exacerbations (montelukast)	Lima et al. (142)
	Sp1 repeat	P	USA (79% Caucasian, 174 A)	Yes, PEF (montelukast)	Klotsman et al. (145)
			UK Caucasian (52 AA)	No BHR (montelukast, zafirlukast)	Fowler et al. (143)
			Korean (89 AIA)	No, FEV ₁ , PEF (Montelukast)	Kim et al. (144)
	Intron 2 A>G (rs2115819)	I	USA Caucasian (61 A)	Yes, FEV ₁ (montelukast)	Lima et al. (142)
	rs4987105 T>C rs4986832 G>A	- -	USA (79% Caucasian, 174 A)	Yes, PEF (montelukast) for both SNPs	Klotsman et al. (145)
	rs2229136 A>G rs228064 A>G	- -		No, FEV ₁ , PEF (montelukast)	
	-1708 G>A 21 C>T 270 G>A (rs2228064) 1728 A>G (rs2229136)	P C C C	Korean (89 AIA)	No, FEV ₁ , PEF (montelukast)	Kim et al. (144)
<i>LTC4S</i>			UK Caucasian (23 SA, 31 C)	Yes, FEV ₁ , FVC, PEF (zafirlukast, $p = ns$)	Sampson et al. (116)
			Japanese (50 A)	Yes, FEV ₁ (pranlukast)	Asano et al. (119)
			Polish (26 AIA, 33 ATA)	Yes, daytime symptoms, morning PEF (montelukast, $p = ns$)	Mastalerz et al. (122)
			USA (12 A)	Yes, % change in FE(NO) (montelukast, $p = ns$)	Whelan et al. (146)
	-444 A>C (rs730012)	P	USA Caucasian (61 A)	Yes, asthma exacerbations (montelukast)	Lima et al. (142)
			USA Caucasian (150 A)	No, FEV ₁ , BHR (AMP or methacholine) (zafirlukast)	Currie et al. (147)
			USA (79% Caucasian, 174 A)	No, FEV ₁ , PEF (montelukast)	Klotsman et al. (145)
			Korean (100 EIA)	No, FEV ₁ (montelukast)	Lee et al. (148)
			Korean (89 AIA)	No, FEV ₁ , PEF (montelukast)	Kim et al. (144)
	-1072 G>A	P	Korean (89 AIA)	No, FEV ₁ , PEF (montelukast)	Kim et al. (144)
<i>LTA4H</i>	rs2660845 A>G	5'UTR	USA Caucasian (61 A)	Yes, asthma exacerbations (montelukast)*	Lima et al. (142)
<i>CYSLTR1</i> [†]	-945 C>T (rs321029) 927 T>C (rs320995)	P	Korean (89 AIA)	Yes, FEV ₁ , PEF (montelukast)	Kim et al. (144)
	927 T>C (rs320995)	C	Korean (100 EIA)	No, FEV ₁ (montelukast)	Lee et al. (148)
<i>CYSLTR2</i>	rs912278 T>C rs912277 T>C	C C	USA (79% Caucasian, 174 A)	Yes, PEF (montelukast) for both SNPs	Klotsman et al. (145)
<i>MRP1</i>	Intron 1 C/T rs119774	I	USA Caucasian (61 A)	Yes, FEV ₁ (montelukast)	Lima et al. (142)

3'UTR, 3' untranslated region; A, Asthma; AA, allergic asthma; AIA, aspirin intolerant asthma; *ALOX5*, 5-lipoxygenase; *ALOX5AP*, 5-lipoxygenase activating protein; AMP, adenosine monophosphate; ATA, aspirin tolerant asthma; BHR, bronchial hyperresponsiveness; C, controls (population); C, coding region (location); *CYSLTR 1–2*, cysteinyl leukotriene receptor 1–2; EIA, exercise induced asthma; FE(NO), fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; I, intronic; *LTA4H*, leukotriene A4 hydrolase; *LTC4S*, leukotriene C4 synthase; *MRP1*, multi-drug resistant protein 1; ns, not significant; P, promoter; PEF, peak expiratory force; SA, severe asthma; SNP, single nucleotide polymorphism; (-), not provided. Unless stated otherwise (*), phenotypes were associated with the minor allele. [†]*CYSLTR1* promoter SNPs are located from the exon 4 3' end as described by Duroudier et al. (133).

significant effect (141). Therefore, in addition to single SNP analyses, there is accumulating evidence to suggest that gene haplotypes over the 5-LO pathway may be functionally more relevant for activity.

Haplotype and multi-SNP analyses

Helgadottir and colleagues conducted a genome-wide linkage scan to search for genes associated with MI in an Icelandic cohort (112). *ALOX5AP* was shown to have the most significant association localised to a four SNP haplotype covering 33 kb designated HapA (SG13S25, SG13S114, SG13S89 and SG13S32). In a similar manner, *LTA4H* was investigated and HapK (defined by SNPs rs1978331, rs1767715, rs2540482, rs2660845 and rs2540475) showed association with MI (114). Interestingly, these haplotypes predicted LTB₄ production in stimulated PBMCs (peripheral blood mononuclear cells) (112) suggesting that polymorphisms within *ALOX5AP* and *LTA4H* are genetic determinants of LTB₄ production and may have relevance to airway disease such as asthma. Indeed, haplotype analysis with SNPs spanning *ALOX5-AP* and *LTA4H* identified association or suggestive association with asthma-related phenotypes such as BHR, forced expiratory volume in 1s (FEV₁), to total IgE and atopy (113).

Three groups have investigated combinations of SNPs from several genes of the 5-LO pathway. Associations of the *CYSLTR2* -819 G, 2078 T, 2534 G alleles with aspirin sensitivity were greater when combined with the *LTC4S* -444 C allele (135). Similarly, the combination of TA (927 *CYSLTR1*/-444 *LTC4S*) was significantly less common in male patients with asthma than in controls (OR = 0.37; p = 0.039) (123). Finally, GC carriers (*ALOX5* SG13S41G and *LTA4H* rs1978331C) had an increased risk of developing asthma (OR = 2.17, 95% CI:1.41–3.32) (113).

LT pharmacogenetics

To date, there have been twelve studies specifically addressing the contribution of 5-LO pathway and receptor gene polymorphisms to clinical outcomes following LT modifier therapy (Table 5). Using a retrospective study of 221 asthmatics, the pharmacogenetic significance of the *ALOX5* Sp1/Egr1 polymorphism was assessed; those possessing only mutant *ALOX5* alleles were relatively resistant to treatment with the LTSI ABT-761 (68). Mean FEV₁ improved by approximately $18.8 \pm 3.6\%$ (n = 64) for wild-type homozygotes and $23.3 \pm 6\%$ (n = 40) for heterozygotes compared with $-1.2 \pm 2.9\%$ (n = 10) in homozygote mutant individuals (68). Similarly, Lima *et al.* showed a significantly higher FEV₁ response to the LTRA montelukast for the *ALOX5* rs2115819 SNP GG homozygotes

compared to the AA and AG genotypes (p = 0.017) (142). Another study found no polymorphism specific bronchodilator responses between homozygous wild-types and heterozygotes for the *ALOX5* Sp1 polymorphism in 52 asthma subjects (143). Kim *et al.* found no significant association between LTRA requirement and polymorphisms in the *ALOX5* gene and Klotzman *et al.* identified an 18–25% improvement in peak expiratory flow for mutant alleles of *ALOX5* SNP rs4987105 and rs4986832 (p = 0.01 for both), whereas wild-type patients only showed a 10% improvement (144, 145). These data illustrated that genes regulating the expression of LTs could influence the efficacy of therapeutics that target their synthesis and/or activity and further studies are required to determine the role of *ALOX5* polymorphisms on these responses.

For a *LTA4H* rs2660845 G > A SNP, possession of the minor G allele gave a greater likelihood of experiencing an asthma exacerbation, which was further increased in the homozygote state (OR for GA and GG > 4, p = <0.01) while taking LTRA (142).

Five of the nine published pharmacogenetic studies on the *LTC4S* -444 A > C polymorphism showed an improved response to LTRAs from patients carrying the C allele (116, 119, 122, 142, 146), although results were not always statistically significant (116, 122, 146) (perhaps partly due to the small number of individuals in the populations studied). The other four published studies could not reproduce these findings (144, 145, 147, 148). As these results were obtained in different populations and using different end points, this suggests that, although not sufficient to explain the variation in patient response to LTRAs, this polymorphism is a strong candidate and is potentially exerting its influence with other gene polymorphisms.

Interestingly, Lima *et al.* published for the first time a pharmacogenetic study of *MRP1*, one of the LTC₄ transmembrane transporters to the extracellular space. The presence of the T allele (intronic SNP, rs119774) significantly improved the response of 61 asthmatics to Montelukast (FEV₁) (142). This SNP has also shown an association with BHR (Methacholine) in asthma subjects (p = 0.003) using 341 asthma families (113).

To our knowledge, only three pharmacogenetic studies investigating *CYSLTRs* have been published. In a Korean population of 100 children with exercise-induced asthma, the synonymous coding *CYSLTR1* polymorphism T > C (rs320995) did not affect the efficacy of montelukast to improve FEV₁ (148), but the minor alleles of both the promoter and coding SNPs [C > T (rs321029) and T > C (rs320995)] were associated with requirement for montelukast in another Korean population of 89 aspirin intolerant asthmatics (p < 0.02) (144). Carriers of the variants of two polymorphisms in the *CYSLTR1* gene (rs91227 and rs912278) responded better to montelukast (145).

Overall, these pharmacogenetic studies provide preliminary evidence that polymorphisms spanning genes

from the 5-LO pathway may influence clinical outcomes of anti-leukotriene therapies in allergic disease.

Future implications

In the past decade, it has become clear that LTs are key inflammatory mediators and new roles in the pathogenesis of a number of diseases are being discovered. Leukotriene modifiers are already used in clinical practice for asthma and AR and are in trial for other diseases including AD. Despite the efficacy of these drugs, the patient response remains highly variable and the mechanisms of action of LTs remain to be elucidated. As the genes of the key components of the 5-LO pathway are being characterised, one could hypothesise that genetic variation in those genes could affect disease risk or severity and/or patient response to anti-leukotrienes, i.e. not only predispose to allergy but also predispose to a more LT-dependent allergy.

Discrepancies between studies are likely to be due to the experimental design of the studies, which were performed in populations from different ethnic origins, using different criteria to characterise the same phenotypes (e.g. atopy defined as elevated specific IgE or positive skin prick test) and looked at SNPs in isolation rather than in haplotypes. This reflects the importance of ethnicity in such studies as population stratification often

significantly affects the effect of a polymorphism, whether genetic or pharmacogenetic, constituting a crucial element of more personalised health care. The population sizes are also of significance as most of the studies described used small numbers of individuals, creating the potential for type I and type II errors. Despite these discrepancies, overall, polymorphisms in the *ALOX5*, *LTC4S* and *CYSLTR1* genes seem likely to have a real genetic and/or pharmacogenetic effect with relevance to allergy. However, individual polymorphisms may be predicted to have a small influence in complex multifunctional diseases. Combinations of polymorphisms across the entire pathway are therefore more likely to be responsible for disease susceptibility/severity and variability of the patient response to anti-leukotrienes.

Another interesting element arising out of these studies is the gender-specificity seen with *CYSLTR1* polymorphisms located on chromosome X. This is of major importance regarding not only the administration of anti-leukotriene therapies but also the signalling of CysLTs. The origin of this gender segregation could have several causes such as X-inactivation or a sex-specific regulator (oestrogen).

In conclusion, polymorphisms in the genes encoding enzymes and receptors of the 5-LO pathway are likely to have a genetic and/or pharmacogenetic impact in allergic disease. However, the relevance of these associations needs to be asserted with larger more robustly designed studies.

References

- Wenzel SE. The role of leukotrienes in asthma. *Prostaglandins Leukot Essent Fatty Acids* 2003;**69**:145–155.
- Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 1983;**220**:568–575.
- Dennis EA. Diversity of group types, regulation, and function of phospholipase A2. *J Biol Chem* 1994;**269**:13057–13060.
- Peters-Golden M, Song K, Marshall T, Brock T. Translocation of cytosolic phospholipase A2 to the nuclear envelope elicits topographically localized phospholipid hydrolysis. *Biochem J* 1996;**318**(Pt 3):797–803.
- Channon JY, Leslie CC. A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A2 with membrane in the macrophage cell line RAW 264.7. *J Biol Chem* 1990;**265**:5409–5413.
- Rouzer CA, Matsumoto T, Samuelsson B. Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A4 synthase activities. *Proc Natl Acad Sci USA* 1986;**83**:857–861.
- Brock TG, McNish RW, Peters-Golden M. Translocation and leukotriene synthetic capacity of nuclear 5-lipoxygenase in rat basophilic leukemia cells and alveolar macrophages. *J Biol Chem* 1995;**270**:21652–21658.
- Rouzer CA, Kargman S. Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A23187. *J Biol Chem* 1988;**263**:10980–10988.
- Woods JW, Evans JF, Ethier D, Scott S, Vickers PJ, Hearn L et al. 5-lipoxygenase and 5-lipoxygenase-activating protein are localized in the nuclear envelope of activated human leukocytes. *J Exp Med* 1993;**178**:1935–1946.
- Abramovitz M, Wong E, Cox ME, Richardson CD, Li C, Vickers PJ. 5-lipoxygenase-activating protein stimulates the utilization of arachidonic acid by 5-lipoxygenase. *Eur J Biochem* 1993;**215**:105–111.
- Reid GK, Kargman S, Vickers PJ, Mancini JA, Leveille C, Ethier D et al. Correlation between expression of 5-lipoxygenase-activating protein, 5-lipoxygenase, and cellular leukotriene synthesis. *J Biol Chem* 1990;**265**:19818–19823.
- Deville P, Baccard N, Advenier C. Leukotrienes, leukotriene receptor antagonists and leukotriene synthesis inhibitors in asthma: an update. Part I: synthesis, receptors and role of leukotrienes in asthma. *Pharmacol Res* 1999;**40**:3–13.
- Kuwano M, Toh S, Uchiumi T, Takano H, Kohno K, Wada M. Multidrug resistance-associated protein subfamily transporters and drug resistance. *Anticancer Drug Des* 1999;**14**:123–131.

14. Lam BK, Owen WF Jr, Austen KF, Soberman RJ. The identification of a distinct export step following the biosynthesis of leukotriene C4 by human eosinophils. *J Biol Chem* 1989;**264**:12885–12889.
15. Li L, Lee TK, Meier PJ, Ballatori N. Identification of glutathione as a driving force and leukotriene C4 as a substrate for oatp1, the hepatic sinusoidal organic solute transporter. *J Biol Chem* 1998;**273**:16184–16191.
16. Haeggstrom JZ. Structure, function, and regulation of leukotriene A4 hydrolase. *Am J Respir Crit Care Med* 2000;**161**(2 Pt 2):S25–S31.
17. Nicosia S, Capra V, Rovati GE. Leukotrienes as mediators of asthma. *Pulm Pharmacol Ther* 2001;**14**:3–19.
18. Henderson WR Jr. The role of leukotrienes in inflammation. *Ann Intern Med* 1994;**121**:684–697.
19. Maclouf JA, Murphy RC. Transcellular metabolism of neutrophil-derived leukotriene A4 by human platelets. A potential cellular source of leukotriene C4. *J Biol Chem* 1988;**263**:174–181.
20. Feinmark SJ, Cannon PJ. Endothelial cell leukotriene C4 synthesis results from intercellular transfer of leukotriene A4 synthesized by polymorphonuclear leukocytes. *J Biol Chem* 1986;**261**:16466–16472.
21. McGee JE, Fitzpatrick FA. Erythrocyte-neutrophil interactions: formation of leukotriene B4 by transcellular biosynthesis. *Proc Natl Acad Sci USA* 1986;**83**:1349–1353.
22. Peters-Golden M, McNish RW. Redistribution of 5-lipoxygenase and cytosolic phospholipase A2 to the nuclear fraction upon macrophage activation. *Biochem Biophys Res Commun* 1993;**196**:147–153.
23. Brock TG. Regulating leukotriene synthesis: the role of nuclear 5-lipoxygenase. *J Cell Biochem* 2005;**96**:1203–1211.
24. Jones SM, Luo M, Healy AM, Peters-Golden M, Brock TG. Structural and functional criteria reveal a new nuclear import sequence on the 5-lipoxygenase protein. *J Biol Chem* 2002;**277**:38550–38556.
25. Jones SM, Luo M, Peters-Golden M, Brock TG. Identification of two novel nuclear import sequences on the 5-lipoxygenase protein. *J Biol Chem* 2003;**278**:10257–10263.
26. Brock TG, McNish RW, Bailie MB, Peters-Golden M. Rapid import of cytosolic 5-lipoxygenase into the nucleus of neutrophils after in vivo recruitment and in vitro adherence. *J Biol Chem* 1997;**272**:8276–8280.
27. Luo M, Jones SM, Phare SM, Coffey MJ, Peters-Golden M, Brock TG. Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523. *J Biol Chem* 2004;**279**:41512–41520.
28. Werz O, Burkert E, Fischer L, Szellas D, Dishart D, Samuelsson B et al. Extracellular signal-regulated kinases phosphorylate 5-lipoxygenase and stimulate 5-lipoxygenase product formation in leukocytes. *FASEB J* 2002;**16**:1441–1443.
29. Werz O, Klemm J, Samuelsson B, Radmark O. 5-lipoxygenase is phosphorylated by p38 kinase-dependent MAPKAP kinases. *Proc Natl Acad Sci USA* 2000;**97**:5261–5266.
30. Bisgaard H, Groth S, Madsen F. Bronchial hyperreactivity to leukotriene D4 and histamine in exogenous asthma. *Br Med J (Clin Res Ed)* 1985;**290**:1468–1471.
31. Drazen JM. Comparative contractile responses to sulfidopeptide leukotrienes in normal and asthmatic human subjects. *Ann NY Acad Sci* 1988;**524**:289–297.
32. Walch L, Norel X, Gascard JP, Brink C. Functional studies of leukotriene receptors in vascular tissues. *Am J Respir Crit Care Med* 2000;**161**(2 Pt 2):S107–S111.
33. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro. *Am Rev Respir Dis* 1982;**126**:449–451.
34. Bisgaard H, Pedersen M. SRS-A leukotrienes decrease the activity of human respiratory cilia. *Clin Allergy* 1987;**17**:95–103.
35. Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003;**111**(Suppl. 1):S18–S34 (discussion S34–36).
36. Lee E, Robertson T, Smith J, Kilfeather S. Leukotriene receptor antagonists and synthesis inhibitors reverse survival in eosinophils of asthmatic individuals. *Am J Respir Crit Care Med* 2000;**161**:1881–1886.
37. Spinozzi F, Russano AM, Piattoni S, Ageo E, Bistoni O, de Benedictis D et al. Biological effects of montelukast, a cysteinyl-leukotriene receptor-antagonist, on T lymphocytes. *Clin Exp Allergy* 2004;**34**:1876–1882.
38. Mygind N, Dahl R, Bisgaard H. Leukotrienes, leukotriene receptor antagonists, and rhinitis. *Allergy* 2000;**55**:421–424.
39. Shirasaki H, Kanaizumi E, Watanabe K, Matsui T, Sato J, Narita S et al. Expression and localization of the cysteinyl leukotriene 1 receptor in human nasal mucosa. *Clin Exp Allergy* 2002;**32**:1007–1012.
40. Okunishi K, Dohi M, Nakagome K, Tanaka R, Yamamoto K. A novel role of cysteinyl leukotrienes to promote dendritic cell activation in the antigen-induced immune responses in the lung. *J Immunol* 2004;**173**:6393–6402.
41. Friedmann PS, Palmer R, Tan E, Ogboli M, Barclay G, Hotchkiss K et al. A double-blind, placebo-controlled trial of montelukast in adult atopic eczema. *Clin Exp Allergy* 2007;**37**:1536–1540.
42. Thorsen S, Fogh K, Broby-Johansen U, Sosndergaard J. Leukotriene B4 in atopic dermatitis: increased skin levels and altered sensitivity of peripheral blood T-cells. *Allergy* 1990;**45**:457–463.
43. Chung KF. Inflammatory mediators in chronic obstructive pulmonary disease. *Curr Drug Targets Inflamm Allergy* 2005;**4**:619–625.
44. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ. Leukotriene B4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 1980;**286**:264–265.
45. Lee E, Lindo T, Jackson N, Meng-Choong L, Reynolds P, Hill A et al. Reversal of human neutrophil survival by leukotriene B(4) receptor blockade and 5-lipoxygenase and 5-lipoxygenase activating protein inhibitors. *Am J Respir Crit Care Med* 1999;**160**:2079–2085.
46. Profita M, Giorgi RD, Sala A, Bonanno A, Riccobono L, Mirabella F et al. Muscarinic receptors, leukotriene B4 production and neutrophilic inflammation in COPD patients. *Allergy* 2005;**60**:1361–1369.
47. Hu H, Chen G, Zhang JM, Zhang WP, Zhang L, Ge QF et al. Distribution of cysteinyl leukotriene receptor 2 in human traumatic brain injury and brain tumors. *Acta Pharmacol Sin* 2005;**26**:685–690.
48. Folco G, Rossoni G, Buccellati C, Berti F, Maclouf J, Sala A. Leukotrienes in cardiovascular diseases. *Am J Respir Crit Care Med* 2000;**161**(2 Pt 2):S112–S116.
49. Barnes PJ. Anti-leukotrienes: here to stay? *Curr Opin Pharmacol* 2003;**3**:257–263.

50. Dahlen SE, Malmstrom K, Nizankowska E, Dahlen B, Kuna P, Kowalski M et al. Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: a randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* 2002;**165**:9–14.
51. Currie GP, Lipworth BJ. Bronchoprotective effects of leukotriene receptor antagonists in asthma: a meta-analysis. *Chest* 2002;**122**:146–150.
52. Jarvis B, Markham A. Montelukast: a review of its therapeutic potential in persistent asthma. *Drugs* 2000;**59**:891–928.
53. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Pineiro A, Wei LX et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. *Ann Intern Med* 1999;**130**:487–495.
54. Horwitz Randy J, McGill Kelly A, Busse William W. The Role of Leukotriene Modifiers in the Treatment of Asthma. *Am J Respir Crit Care Med* 1998;**157**:1363–1371.
55. Joshi EM, Heasley BH, Chordia MD, Macdonald TL. In vitro metabolism of 2-acetylbenzothiothiophene: relevance to zileuton hepatotoxicity. *Chem Res Toxicol* 2004;**17**:137–143.
56. Busse W, Kraft M. Cysteinyl leukotrienes in allergic inflammation: strategic target for therapy. *Chest* 2005;**127**:1312–1326.
57. Piatti G, Ceriotti L, Cavallaro G, Ambrosetti U, Mantovani M, Pistone A et al. Effects of zafirlukast on bronchial asthma and allergic rhinitis. *Pharmacol Res* 2003;**47**:541–547.
58. Phipatanakul W, Eggleston PA, Conover-Walker MK, Kesavanathan J, Sweitzer D, Wood RA. A randomized, double-blind, placebo-controlled trial of the effect of zafirlukast on upper and lower respiratory responses to cat challenge. *J Allergy Clin Immunol* 2000;**105**:704–710.
59. Riccioni G, Bucciarelli T, Mancini B, Ilio CD, D'Orazio N. Antileukotriene drugs: clinical application, effectiveness and safety. *Curr Med Chem* 2007;**14**:1966–1977.
60. Whatling C, McPheat W, Herslof M. The potential link between atherosclerosis and the 5-lipoxygenase pathway: investigational agents with new implications for the cardiovascular field. *Expert Opin Investig Drugs* 2007;**16**:1879–1893.
61. Evans JF, Ferguson AD, Mosley RT, Hutchinson JH. What's all the FLAP about: 5-lipoxygenase-activating protein inhibitors for inflammatory diseases. *Trends Pharmacol Sci* 2008;**29**:72–78.
62. Rubin P, Mollison KW. Pharmacotherapy of diseases mediated by 5-lipoxygenase pathway eicosanoids. *Prostaglandins Other Lipid Mediat* 2007;**83**:188–197.
63. Evans DJ, Barnes PJ, Spaethe SM, van Alstyne EL, Mitchell MI, O'Connor BJ. Effect of a leukotriene B4 receptor antagonist, LY293111, on allergen induced responses in asthma. *Thorax* 1996;**51**:1178–1184.
64. Funk CD. Leukotriene modifiers as potential therapeutics for cardiovascular disease. *Nat Rev Drug Discov* 2005;**4**:664–672.
65. Funk CD, Funk LB, FitzGerald GA, Samuelsson B. Characterization of human 12-lipoxygenase genes. *Proc Natl Acad Sci USA* 1992;**89**:3962–3966.
66. Matsumoto T, Funk CD, Radmark O, Hoog JO, Jornvall H, Samuelsson B. Molecular cloning and amino acid sequence of human 5-lipoxygenase. *Proc Natl Acad Sci USA* 1988;**85**:26–30.
67. Funk CD, Hoshiko S, Matsumoto T, Radmark O, Samuelsson B. Characterization of the human 5-lipoxygenase gene. *Proc Natl Acad Sci USA* 1989;**86**:2587–2591.
68. Drazen JM, Yandava CN, Dube 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**:168–170.
69. Ring WL, Riddick CA, Baker JR, Munafo DA, Bigby TD. Lymphocytes stimulate expression of 5-lipoxygenase and its activating protein in monocytes in vitro via granulocyte macrophage colony-stimulating factor and interleukin 3. *J Clin Invest* 1996;**97**:1293–1301.
70. Steinhilber D, Brungs M, Radmark O, Samuelsson B. Transforming growth factor-beta and 1,25-dihydroxyvitamin D3 induce 5-lipoxygenase activity during myeloid cell maturation. *Adv Prostaglandin Thromboxane Leukot Res* 1995;**23**:449–451.
71. Kennedy BP, Diehl RE, Boie Y, Adam M, Dixon RA. Gene characterization and promoter analysis of the human 5-lipoxygenase-activating protein (FLAP). *J Biol Chem* 1991;**266**:8511–8516.
72. Yandava CN, Kennedy BP, Pillari A, Duncan AM, Drazen JM. Cytogenetic and radiation hybrid mapping of human arachidonate 5-lipoxygenase-activating protein (ALOX5AP) to chromosome 13q12. *Genomics* 1999;**56**:131–133.
73. Vickers PJ, O'Neill GP, Mancini JA, Charleson S, Abramovitz M. Cross-species comparison of 5-lipoxygenase-activating protein. *Mol Pharmacol* 1992;**42**:1014–1019.
74. Tay A, Simon JS, Squire J, Hamel K, Jacob HJ, Skorecki K. Cytosolic phospholipase A2 gene in human and rat: chromosomal localization and polymorphic markers. *Genomics* 1995;**26**:138–141.
75. Sharp JD, Pickard RT, Chiou XG, Manetta JV, Kovacevic S, Miller JR et al. Serine 228 is essential for catalytic activities of 85-kDa cytosolic phospholipase A2. *J Biol Chem* 1994;**269**:23250–23254.
76. Penrose JF, Gagnon L, Goppelt-Strube M, Myers P, Lam BK, Jack RM et al. Purification of human leukotriene C4 synthase. *Proc Natl Acad Sci USA* 1992;**89**:11603–11606.
77. Penrose JF, Spector J, Baldasaro M, Xu K, Boyce J, Arm JP et al. Molecular cloning of the gene for human leukotriene C4 synthase. Organization, nucleotide sequence, and chromosomal localization to 5q35. *J Biol Chem* 1996;**271**:11356–11361.
78. Lam BK, Penrose JF, Freeman GJ, Austen KF. Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. *Proc Natl Acad Sci USA* 1994;**91**:7663–7667.
79. Mancini JA, Evans JF. Cloning and characterization of the human leukotriene A4 hydrolase gene. *Eur J Biochem* 1995;**231**:65–71.
80. Jendraschak E, Kaminski WE, Kiehl R, von Schacky C. The human leukotriene A4 hydrolase gene is expressed in two alternatively spliced mRNA forms. *Biochem J* 1996;**314**(Pt 3):733–737.
81. Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T. A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. *Nature* 1997;**387**:620–624.
82. Tager AM, Luster AD. BLT1 and BLT2: the leukotriene B(4) receptors. *Prostaglandins Leukot Essent Fatty Acids* 2003;**69**:123–134.
83. Owman C, Nilsson C, Lolait SJ. Cloning of cDNA encoding a putative chemoattractant receptor. *Genomics* 1996;**37**:187–194.

84. Einstein R, Jordan H, Zhou W, Brenner M, Moses EG, Liggett SB. Alternative splicing of the G protein-coupled receptor superfamily in human airway smooth muscle diversifies the complement of receptors. *Proc Natl Acad Sci USA* 2008;**105**:5230–5235.
85. Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. A second leukotriene B(4) receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. *J Exp Med* 2000;**192**:421–432.
86. Kamohara M, Takasaki J, Matsumoto M, Saito T, Ohishi T, Ishii H et al. Molecular cloning and characterization of another leukotriene B4 receptor. *J Biol Chem* 2000;**275**:27000–27004.
87. Yokomizo T, Izumi T, Shimizu T. Leukotriene B4: metabolism and signal transduction. *Arch Biochem Biophys* 2001;**385**:231–241.
88. Kato K, Yokomizo T, Izumi T, Shimizu T. Cell-specific transcriptional regulation of human leukotriene B(4) receptor gene. *J Exp Med* 2000;**192**:413–420.
89. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARalpha-leukotriene B4 pathway to inflammation control. *Nature* 1996;**384**:39–43.
90. Krey G, Braissant O, L'Horsset F, Kalkhoven E, Perroud M, Parker MG et al. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* 1997;**11**:779–791.
91. Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr Opin Lipidol* 1997;**8**:159–166.
92. Brink C, Dahlen SE, Drazen J, Evans JF, Hay DW, Nicosia S et al. International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. *Pharmacol Rev* 2003;**55**:195–227.
93. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS et al. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 2000;**275**:30531–30536.
94. Labat C, Ortiz JL, Norel X, Gorenne I, Verley J, Abram TS et al. A second cysteinyl leukotriene receptor in human lung. *J Pharmacol Exp Ther* 1992;**263**:800–805.
95. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999;**399**:789–793.
96. Sarau HM, Ames RS, Chambers J, Ellis C, Elshourbagy N, Foley JJ et al. Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. *Mol Pharmacol* 1999;**56**:657–663.
97. Walch L, Norel X, Back M, Gascard JP, Dahlen SE, Brink C. Pharmacological evidence for a novel cysteinyl-leukotriene receptor subtype in human pulmonary artery smooth muscle. *Br J Pharmacol* 2002;**137**:1339–1345.
98. Figueroa DJ, Breyer RM, Defoe SK, Kargman S, Daugherty BL, Waldburger K et al. Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* 2001;**163**:226–233.
99. Nothacker HP, Wang Z, Zhu Y, Reinscheid RK, Lin SH, Civelli O. Molecular cloning and characterization of a second human cysteinyl leukotriene receptor: discovery of a subtype selective agonist. *Mol Pharmacol* 2000;**58**:1601–1608.
100. Takasaki J, Kamohara M, Matsumoto M, Saito T, Sugimoto T, Ohishi T et al. The molecular characterization and tissue distribution of the human cysteinyl leukotriene CysLT(2) receptor. *Biochem Biophys Res Commun* 2000;**274**:316–322.
101. Tudhope SR, Cuthbert NJ, Abram TS, Jennings MA, Maxey RJ, Thompson AM et al. BAY u9773, a novel antagonist of cysteinyl-leukotrienes with activity against two receptor subtypes. *Eur J Pharmacol* 1994;**264**:317–323.
102. Ciana P, Fumagalli M, Trincavelli ML, Verderio C, Rosa P, Lecca D et al. The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *EMBO J* 2006;**25**:4615–4627.
103. 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;**99**:1130–1137.
104. Silverman ES, Du J, De Sanctis GT, Radmark O, Samuelsson B, Drazen JM et al. Egr-1 and Sp1 interact functionally with the 5-lipoxygenase promoter and its naturally occurring mutants. *Am J Respir Cell Mol Biol* 1998;**19**:316–323.
105. Kawagishi Y, Mita H, Taniguchi M, Maruyama M, Oosaki R, Higashi N et al. Leukotriene C4 synthase promoter polymorphism in Japanese patients with aspirin-induced asthma. *J Allergy Clin Immunol* 2002;**109**:936–942.
106. Kim SH, Bae JS, Suh CH, Nahm DH, Holloway JW, Park HS. Polymorphism of tandem repeat in promoter of 5-lipoxygenase in ASA-intolerant asthma: a positive association with airway hyper-responsiveness. *Allergy* 2005;**60**:760–765.
107. 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;**33**:1103–1110.
108. Kalayci O, Birben E, Sackesen C, Keskin O, Tahan F, Wechsler ME et al. ALOX5 promoter genotype, asthma severity and LTC production by eosinophils. *Allergy* 2006;**61**:97–103.
109. Choi JH, Park HS, Oh HB, Lee JH, Suh YJ, Park CS et al. Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase. *Hum Genet* 2004;**114**:337–344.
110. Bai C, Matsui E, Ohnishi H, Kimata K, Kasahara K, Kaneko H et al. A novel polymorphism, E254K, in the 5-lipoxygenase gene associated with bronchial asthma. *Int J Mol Med* 2008;**21**:139–144.
111. Koshino T, Takano S, Kitani S, Ohshima N, Sano Y, Takaishi T et al. Novel polymorphism of the 5-lipoxygenase activating protein (FLAP) promoter gene associated with asthma. *Mol Cell Biol Res Commun* 1999;**2**:32–35.
112. 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**:233–239.
113. Holloway JW, Barton SJ, Holgate ST, Rose-Zerilli MJ, Sayers I. The role of LTA4H and ALOX5AP polymorphism in asthma and allergy susceptibility. *Allergy* 2008;**63**:1046–1053.
114. Helgadottir A, Manolescu A, Helgason A, Thorleifsson G, Thorsteinsdottir U, Gudbjartsson DF et al. A variant of the gene encoding leukotriene A4 hydrolase confers ethnicity-specific risk of myocardial infarction. *Nat Genet* 2006;**38**:68–74.

115. Kedda MA, Shi J, Duffy D, Phelps S, Yang I, O'Hara K et al. Characterization of two polymorphisms in the leukotriene C4 synthase gene in an Australian population of subjects with mild, moderate, and severe asthma. *J Allergy Clin Immunol* 2004;**113**: 889–895.
116. Sampson AP, Siddiqui S, Buchanan D, Howarth PH, Holgate ST, Holloway JW et al. Variant LTC(4) synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predicts clinical response to zafirlukast. *Thorax* 2000;**55**(Suppl. 2):S28–S31.
117. Sanak M, Pierzchalska M, Bazan-Socha S, Szczeklik A. Enhanced expression of the leukotriene C(4) synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000;**23**:290–296.
118. Sanak M, Simon HU, Szczeklik A. Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997;**350**: 1599–1600.
119. Asano K, Shiomi T, Hasegawa N, Nakamura H, Kudo H, Matsuzaki T et al. Leukotriene C4 synthase gene A(-444)C polymorphism and clinical response to a CYS-LT(1) antagonist, pranlukast, in Japanese patients with moderate asthma. *Pharmacogenetics* 2002;**12**:565–570.
120. Choi JH, Kim SH, Bae JS, Yu HL, Suh CH, Nahm DH et al. Lack of an association between a newly identified promoter polymorphism (-1702G > A) of the leukotriene C4 synthase gene and aspirin-intolerant asthma in a Korean population. *Tohoku J Exp Med* 2006;**208**:49–56.
121. Isidoro-Garcia M, Davila I, Moreno E, Lorente F, Gonzalez-Sarmiento R. Analysis of the leukotriene C4 synthase A-444C promoter polymorphism in a Spanish population. *J Allergy Clin Immunol* 2005;**115**:206–207.
122. Mastalerz L, Nizankowska E, Sanak M, Mejza F, Pierzchalska M, Bazan-Socha S et al. Clinical and genetic features underlying the response of patients with bronchial asthma to treatment with a leukotriene receptor antagonist. *Eur J Clin Invest* 2002;**32**:949–955.
123. Sanz C, Isidro-Garcia M, Davila I, Moreno E, Laffond E, Lorente F. Analysis of 927T > C CYSLTR1 and -444A > C LTC4S polymorphisms in patients with asthma. *J Investig Allergol Clin Immunol* 2006;**16**:331–337.
124. Sayers I, Barton S, Rorke S, Beghe B, Hayward B, Van Eerdeghe P et al. Allelic association and functional studies of promoter polymorphism in the leukotriene C4 synthase gene (LTC4S) in asthma. *Thorax* 2003;**58**:417–424.
125. Van Sambeek R, Stevenson DD, Baldasaro M, Lam BK, Zhao J, Yoshida S et al. 5' flanking region polymorphism of the gene encoding leukotriene C4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J Allergy Clin Immunol* 2000;**106**(1 Pt 1):72–76.
126. Eskandari HG, Unal M, Ozturk OG, Vayisoglu Y, Muslu N. Leukotriene C4 synthase A-444C gene polymorphism in patients with allergic rhinitis. *Otolaryngol Head Neck Surg* 2006;**134**:997–1000.
127. Yoshikawa K, Matsui E, Kaneko H, Fukao T, Inoue R, Teramoto T et al. A novel single-nucleotide substitution, Glu 4 Lys, in the leukotriene C4 synthase gene associated with allergic diseases. *Int J Mol Med* 2005;**16**:827–831.
128. Arriba-Mendez S, Sanz C, Isidoro-Garcia M, Davila I, Laffond E, Horeno E et al. 927T/C polymorphism of the cysteinyl-leukotriene type-1 receptor (CYSLTR1) gene in children with asthma and atopic dermatitis. *Pediatr Allergy Immunol* 2006;**17**:323–328.
129. Hao L, Sayers I, Cakebread JA, Barton SJ, Beghe B, Holgate ST et al. The cysteinyl-leukotriene type 1 receptor polymorphism 927T/C is associated with atopy severity but not with asthma. *Clin Exp Allergy* 2006;**36**:735–741.
130. Zhang J, Migita O, Koga M, Shibasaki M, Arinami T, Noguchi E. Determination of structure and transcriptional regulation of CYSLTR1 and an association study with asthma and rhinitis. *Pediatr Allergy Immunol* 2006;**17**:242–249.
131. Kim SH, Oh JM, Kim YS, Palmer LJ, Suh CH, Nahm DH et al. Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. *Clin Exp Allergy* 2006;**36**:433–439.
132. 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;**17**:539–549.
133. Duroudier NP, Sayers I, Castagna CC, Fenech AG, Halapi E, Swan C et al. Functional polymorphism and differential regulation of CYSLTR1 transcription in human airway smooth muscle and monocytes. *Cell Biochem Biophys* 2007;**47**:119–130.
134. Fukai H, Ogasawara Y, Migita O, Koga M, Ichikawa K, Shibasaki M et al. Association between a polymorphism in cysteinyl-leukotriene receptor 2 on chromosome 13q14 and atopic asthma. *Pharmacogenetics* 2004;**14**:683–690.
135. Park JS, Chang HS, Park CS, Lee JH, Lee YM, Choi JH et al. Association analysis of cysteinyl-leukotriene receptor 2 (CYSLTR2) polymorphisms with aspirin intolerance in asthmatics. *Pharmacogenet Genomics* 2005;**15**: 483–492.
136. Pillai SG, Cousens DJ, Barnes AA, Buckley PT, Chiano MN, Hosking LK et al. A coding polymorphism in the CYSLT2 receptor with reduced affinity to LTD4 is associated with asthma. *Pharmacogenetics* 2004;**14**:627–633.
137. Thompson MD, Storm van's Gravesande K, Galczynski H, Burnham WM, Siminovich KA, Zamel N et al. A cysteinyl leukotriene 2 receptor variant is associated with atopy in the population of Tristan da Cunha. *Pharmacogenetics* 2003;**13**:641–649.
138. Iida A, Saito S, Sekine A, Takahashi A, Kamatani N, Nakamura Y. Japanese single nucleotide polymorphism database for 267 possible drug-related genes. *Cancer Sci* 2006;**97**:16–24.
139. Sapone A, Peters JM, Sakai S, Tomita S, Papiha SS, Dai R et al. The human peroxisome proliferator-activated receptor alpha gene: identification and functional characterization of two natural allelic variants. *Pharmacogenetics* 2000;**10**:321–333.
140. Vohl MC, Lepage P, Gaudet D, Brewer CG, Betard C, Perron P et al. Molecular scanning of the human PPARα gene: association of the L162V mutation with hyperapobetalipoproteinemia. *J Lipid Res* 2000;**41**:945–952.
141. Whittaker PA. Genes for asthma: much ado about nothing? *Curr Opin Pharmacol* 2003;**3**:212–219.
142. 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* 2006;**173**:379–385.
143. Fowler SJ, Hall IP, Wilson AM, Wheatley AP, Lipworth BJ. 5-Lipoxygenase polymorphism and in-vivo response to leukotriene receptor antagonists. *Eur J Clin Pharmacol* 2002;**58**:187–190.

144. Kim SH, Ye YM, Hur GY, Lee SK, Sampson AP, Lee HY et al. CysLTR1 promoter polymorphism and requirement for leukotriene receptor antagonist in aspirin-intolerant asthma patients. *Pharmacogenomics* 2007;**8**:1143–1150.
145. Klotzman M, York TP, Pillai SG, Vargas-Irwin C, Sharma SS, van den Oord EJ et al. Pharmacogenetics of the 5-lipoxygenase biosynthetic pathway and variable clinical response to montelukast. *Pharmacogenet Genomics* 2007;**17**:189–196.
146. Whelan GJ, Blake K, Kissoon N, Duckworth LJ, Wang J, Sylvester JE et al. Effect of montelukast on time-course of exhaled nitric oxide in asthma: influence of LTC₄ synthase A(-444)C polymorphism. *Pediatr Pulmonol* 2003;**36**:413–420.
147. Currie GP, Lima JJ, Sylvester JE, Lee DKC, Cockburn WJR, Lipworth BJ. Leukotriene C₄ synthase polymorphisms and responsiveness to leukotriene antagonists in asthma. *Br J Clin Pharmacol* 2003;**56**:422–426.
148. Lee SY, Kim HB, Kim JH, Kim BS, Kang MJ, Jang SO et al. Responsiveness to montelukast is associated with bronchial hyperresponsiveness and total immunoglobulin E but not polymorphisms in the leukotriene C₄ synthase and cysteinyl leukotriene receptor 1 genes in Korean children with exercise-induced asthma (EIA). *Clin Exp Allergy* 2007;**37**:1487–1493.
149. Kim SH, Yang EM, Park HJ, Ye YM, Lee HY, Park HS. Differential contribution of the CysLTR1 gene in patients with aspirin hypersensitivity. *J Clin Immunol* 2007;**27**:613–619.