Genetics of aspirin induced asthma

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Aspirin induced asthma (AIA) is more common in women than in men. The first symptoms of the disease are often a viral respiratory infection which is followed by a prolonged and perennial rhinitis. Nasal congestion, anosmia, and rhinorrhea are accompanied by nasal polyps which are found on physical examination in about half the patients. Within a few years bronchial asthma develops, characterised by aspirin intolerance. Aspirin precipitates life threatening attacks of asthma accompanied by rhinorrhea, conjunctive congestion, and facial and neck flushing. The trait of aspirin sensitivity, despite avoidance of non-steroidal anti-inflammatory drugs to which patients usually have cross sensitivity, frequently remains for the patient’s lifetime.

Familial history of aspirin intolerance

Although asthma, nasal polyps, and aspirin intolerance are considered as an inherited disease in the catalogue of Mendelian Inheritance in Man, reports on familial occurrence of AIA are rather scarce. Miller described a pair of sisters with the trait. In one of the families described by Lockey et al consanguinity of the parents suggested a recessive inheritance pattern. In the other family a late onset of AIA and discordance between identical twins raised the possibility of an interaction between environmental and genetic factors. Another multiple family with mild asthma sensitive aspirin and dominant inheritance of the trait was described by von Maur et al. An early onset of asthma in most affected members of the family and a lack of symptoms of sinusitis or nasal polyposis distinguished this familial variant of AIA.

In almost 400 patients studied in the European Network on Aspirin Induced Asthma’s familial occurrence of aspirin hypersensitivity was reported in 5.1%. In families with multiple cases of AIA, affected individuals were usually siblings. The course of their disease was characterised by an intense rhinitis and was more severe, as scored by the number of admissions to hospital, than in singleton families. We recently consulted two sisters aged 27 and 20 years with aspirin intolerance who had reported rhinitis since the age of 12 years. In the older sister aspirin challenge precipitated an asthma attack. The younger sister was suffering from perennial rhinitis and had blood eosinophilia. Following aspirin challenge she developed marked nasal symptoms leading to a profound decrease in nasal airflow and ocular irritation, but without bronchial obstruction. Both sisters shared the variant allele of leukotriene C4 (LTC4) synthase associated with AIA. Although their parents and some other family members were atopic or had moderate eosinophilia, the aspirin challenge was negative in all.

Eosinophilic inflammation of airways in AIA

Signs of persistent inflammation of the bronchi in patients with AIA are characterised by eosinophilic infiltration, desquamation of epithelia, increased cytokine production, and expression of adhesion molecules. Bronchial biopsy specimens reveal a fourfold increase in eosinophils in patients with AIA compared with aspirin tolerant asthmatic subjects. Mast cell counts are variable, probably reflecting activation of this cell population. Increased eosinophilia in the bronchi is accompanied by increased expression of interleukin (IL)-5, a cytokine directing maturation, activation, recruitment, and prevention of eosinophil apoptosis. Since the frequency of atopy is similar in patients with AIA and in aspirin tolerant asthmatics, persistent eosinophilic inflammation has to result from another mechanism. A chronic viral infection and/or autoimmunisation are likely events preceding the onset of AIA. In fact, many patients with AIA have a low titre of anti-DNA antibodies, enhanced IgG synthesis, and associated HLA antigens.

Infiltrating eosinophils are a site of cysteinyl leukotriene (cys-LT) synthesis, as demonstrated by immunostaining of LTC4 synthase. The overexpression of LTC4 synthase in bronchial biopsy specimens of patients with AIA correlates with aspirin sensitivity and is several times higher than in aspirin tolerant asthmatic subjects.

Lipid mediators of asthma and aspirin sensitivity

Despite numerous attempts, no immune system mediated hypersensitivity to aspirin has been found in patients with AIA. In addition, cross sensitivity to other non-steroidal anti-inflammatory drugs (NSAIDs), represented by a variety of chemical compounds, would suggest that any immune response dependent mechanism is improbable. Cyclo-oxygenase (COX) is a common target for the action of NSAIDs. Careful comparison of inhibitory spectra of several NSAIDs suggests that inhibition of COX-1 rather than COX-2 triggers bronchoconstriction in sensitive patients. Measurements of COX metabolites in the body fluids, especially in bronchoalveolar lavage fluid, demonstrate diminished biosynthesis of prostaglandin E (PGE), in patients with AIA. Administration of aspirin further suppresses PGE production, but this is not reflected by inhibition of the synthesis of other prostanooids as can be seen on COX inhibition in aspirin.
LTC4 synthase (LTC4S) promoter sequence between nucleotides –454 and –434

**Figure 1** LTC4 synthase (LTC4S) copies per 100ng total RNA calculated on the basis of semiquantitative competitive RT-PCR (sensitivity threshold 50 copies). Patients grouped according to their LTC4S genotypes. • = homozygotes CC or heterozygotes AC; ○ = homozygotes AA. ATA = aspirin tolerant asthma; AIA = aspirin induced asthma.

**Figure 2** LTC4 synthase (LTC4S) promoter sequence upstream to the translation start. Allele \( \text{A} \) has an AP2 transcription factor core sequence (–441 to –446). Allele \( \text{C} \) has the same AP2 binding motif which overlaps with a H4TF-2 binding (–448 to –444) site created by –444 A → G transversion.

tolerant asthmatics. PGE2 is a probable trigger for asthmatic attacks in AIA; inhibition of its synthesis removes PGE2, mediated restraints on inflammatory cells. This prostaglandin, in addition to its anti-inflammatory properties, can relax smooth muscles. Genetic screening of COX genes has not revealed any alterations to explain this phenomenon. PGE2 synthase, which is an inducible and glutathione dependent enzyme, has been newly cloned but no studies in patients with AIA are yet available.

LTC4 synthase seems to be the most important bronchoconstrictor mediator of asthma in aspirin sensitive disease. Urinary excretion of LTE4 is increased by 2–10 times in aspirin sensitive asthmatics compared with aspirin tolerant patients. During inhalatory aspirin challenge, a further transient increase in LTE4, excretion in the urine is observed in patients with AIA. Cys-LTs can be released into the nasal cavity and bronchi after local deposition of aspirin. The first step in the biosynthesis of cys-LTs is accomplished by 5-lipoxygenase (5-LO) and 5-LO associated protein (FLAP). Expression of 5-LO is modulated by a genetic variation in the SP1 transcription factor binding motif of the 5-LO gene promoter and AIA have been found. Immunostaining of bronchial biopsy specimens for 5-LO and FLAP have not revealed any difference with regard to aspirin sensitivity. Thus, only LTC4 synthase, a terminal enzyme for cys-LT production, is the pathway constituent distinguishing patients with AIA. Overexpression of LTC4 synthase could result from an intense enzyme induction in cells infiltrating bronchi of patients with AIA, but the possibility of a genetic mechanism facilitating the LTC4 synthase gene transcription has been tested.

**Genetic polymorphism of LTC4 synthase controls biosynthesis of leukotrienes**

LTC4 synthase is expressed in eosinophils, basophils, and macrophages. In some other cells such as the epithelia, endothelia, and platelets the enzyme is an orphan because of lack of 5-LO expression. In these cells production of LTC4 synthase depends on external sources of LTA4. The LTC4 synthase gene has been cloned and its sequence, molecular organisation, and functional analysis have been described. A common allelic variant of the LTC4 synthase gene distinguished by the transversion of adenine (A) to cytosine (C), 444 bases upstream from the translation start, was found during screening of the 5' region of the gene. In a population of asthmatic patients with more than 60 individuals in each group, the allele C frequency was 39% in patients with AIA but 26% in aspirin tolerant asthmatics and 25% in healthy controls. Semiquantitative studies of LTC4 synthase transcripts in peripheral blood eosinophils (fig 1) revealed increased numbers of mRNA copies in patients with AIA. This increase correlated with the presence of the allelic C variant of LTC4 synthase. By functional studies, vectors carrying either the C or A allele of the 5' end of the LTC4 synthase gene and expressed in eukaryotic cell lines showed that allele C produced a moderate (25%) enhancement of the reporter gene transcription. A difference in binding of transcription factors created by the transversion has been analysed by oligonucleotide shift assay. Incubations of double strand oligonucleotides encompassing the transcription with nuclear extracts had a three times higher affinity of transcription factor AP2 for allele C. In addition, because of the overlap of the DNA motifs, allele C can interact with another transcription factor (fig 2), namely H4TF-2. An interaction of cytokine induced AP2 with a cell cycle dependent H4TF-2 is probably the cause of the higher expression of LTC4 synthase transcripts in circulating eosinophils of patients with AIA. The nature of the interaction which orchestrates LTC4 synthase expression in inflammatory cells of patients with AIA has not been fully elucidated. A transient increase in urinary LTE4 after aspirin challenge in sensitive patients (fig 3) is significant only in carriers of the C allele. Homozygotes for LTC4 synthase AA genotype are characterised by a lower dose of lysyl-aspirin (6.65 (SD 7.45) versus 30.0 (47.0) mg) to provoke a fall in FEV1 of 20% (PD20). This negative correlation of aspirin PD20 with LTC4 synthase genotype (p≤0.001) strongly suggests a complex mechanism for the disease, with a subpopulation of patients highly sensitive to aspirin as a result of a more efficient triggering mechanism.
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were grouped according to their LTC4 synthase genotypes: 

Figure 3 Urinary excretion of LTE4 in ng/mg creatinine 
in patients with AIAA. Error bars = standard error of mean.

Conclusions

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