Review paper

Functional studies of genes involved in pathogenesis of aspirin-induced asthma

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Abstract

About one fifth of asthmatics have aspirin intolerance. There are several theories explaining pathogenesis of aspirin-induced asthma (AIA). According to the cyclooxygenase theory, aspirin inhibits cyclooxygenase enzymes leading to prostaglandins (PGs) biosynthesis inhibition. In turn, deficiency of PGE_2 has been considered as a triggering factor, resulting in overproduction of proinflammatory leukotrienes and therefore resulting in typical clinical symptoms. Equally important hypothesis seems to be 15-hydroxyeicosatetranoic acid release and diminished production of anti-inflammatory lipoxins after aspirin challenge. In the literature, there is also a lot of data about genetic mechanisms suggesting various gene involvement. This review presents a profile of genes whose involvement in the pathogenesis of bronchial asthma with aspirin hypersensitivity has been verified at the level of RNA and protein expression. Simultaneously, we have discussed several genes whose participation in the AIA is not completely understood due to the lack of functional studies.

Key words: asthma, aspirin, aspirin-induced asthma, arachidonic acid.

Introduction

Acetylsalicylic acid (ASA), also known as aspirin, was one of the first drugs obtained by chemical synthesis and is considered the foundation of the modern pharmaceutical industry [1]. Over 100 years of history, aspirin is widely used today, despite a number of other analgesic, antipyretic, antithrombotic and anti-inflammatory drugs which brought advances in pharmacology. Moreover, several recent studies showed another benefit of aspirin, namely it helps to prevent certain cancers and reduces the risk of death from cancer by 40% for colorectal cancer, 60% for esophageal cancer, 30% for lung cancer and 10% for prostate cancer [2].

Three years after introducing aspirin in the market, the first case of asthma exacerbation by aspirin was reported. Nowadays up to 20% of adult asthmatics are sensitive to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) [3]. A typical syndrome of AIA (aspirin-intolerant asthma), also known as an 'ASA triad' includes such symptoms as rhinorrhoea, nasal congestion and severe bronchospasm. These events are not immunological phenomena but are related to the pharmacological activity of ASA and other NSAIDs [4].

According to the European Academy of Allergy and Clinical Immunology (EAACI) and the Global Allergy and Asthma European Network (GA^2LEN), the diagnosis of AIA has to be confirmed by bronchial, nasal or oral provocation tests. Bronchial challenge is usually performed with lysine acetylsalicylate (L-ASA) that is more water-soluble than aspirin (40% vs. 0.3%) and better tolerated during inhalation [5]. In turn, oral challenge can elicit severe, sometimes life-threatening bronchospasm [6] and therefore, this test is done less frequently. There is also known an *in vitro* test (ASPItest) that measures ASA-induced 15-hydroxyeicosatetranoic acid (15-HETE) in peripheral blood. This method is based on numerous data indicating aspirin as a trigger of arachidonic acid metabolite 15-HETE in AS asthmatics, but not affecting 15-HETE release in AT asthmatics and healthy subjects [7]. The ASPItest does not require special expertise, equipment and seems to be highly sensitive and specific to confirm the history of aspirin sensitivity in asthmatic patients [8].

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Biochemical theories of aspirin-induced asthma

Inhibition of cyclooxygenase enzymes

So far, a widely accepted theory explaining the pathogenesis of AIA is a cyclooxygenase theory. According to it, aspirin inhibits intracellular COX enzymes which cause prostaglandin biosynthesis inhibition. It is now well recognized that there are at least two COX enzymes: COX-1 and COX-2, coded by 2 different genes [9] and *COX-1* gene is constitutively expressed, whereas *COX-2* is an inducible gene [10]. Cyclooxygenase-1 and cyclooxygenase-2 differ in their sensitivity to inhibition by aspirin because of the different structure of the active site [11]. The result of inhibition of COX is an imbalance between the synthesis of eicosanoids originating from cyclooxygenase pathway having a smooth muscle relaxant properties and the synthesis of lipoxygenase pathway eicosanoids shrinking bronchitis (15-HETE, LTB_4, cysteinyl leukotrienes – cysLTs), in favor of the latter.

Overproduction of cysteinyl leukotrienes

In the leukotriene pathway, arachidonic acid liberated from the phospholipid membrane is converted to leukotriene LTA_4 by ALOX5 and its cofactor ALOX5AP. Thus, further products, LTB_4 and cysLTs ($LTC_4/D_4/E_4$) seem to induce chemotaxis of inflammatory cells.

After aspirin challenge, a large increase in production of cysLTs is detectable in the bronchoalveolar lavage (BAL) fluid and urine of patients with AIA but not those with aspirin-tolerant asthma (ATA) [12, 13]. The LT synthesis inhibitors and selective cysLT receptor antagonists markedly attenuate aspirin-induced respiratory reactions [14, 15], whereas selective histamine H_1 antagonists have a little effect [16]. Therefore, cysLTs are considered as major mediators of AIA pathogenesis and can mediate bronchoconstriction, increase mucus secretion, vascular permeability and cellular infiltration [17, 18]. The AIA patients also show an increased basal production of cysLTs even under clinically stable conditions [19].

Overproduction of 15-hydroxyeicosatetraenoic acid

It is known that the production of 15-HETE in AIA patients is 3.6 fold higher than in ATA patients and that aspirin triggers 15-HETE release [7]. The substantial source of 15-HETE in this reaction seems to be 15-lipoxygenase (15-LOX) that is controlled by COX-1 [8]. Thus, inhibition of COX-1 and dysregulation of prostaglandin E_2 (PGE_2) production by aspirin results in activation of 15-LOX and 15-HETE production [7]. Overproduction of 15-HETE in AS asthmatics *inter alia* contributes to the induction of mucous glycoprotein secretion by human airway [20] and contraction of bronchial smooth muscles [21].

Diminished production of lipoxins

Lipoxins (LXs) are 15-lipoxygenase products that, in contrast to leukotrienes, have anti-inflammatory properties, i.e. inhibit chemotaxis, transmigration across endothelial and epithelial monolayers, diapedesis and superoxide anion generation by polymorphonuclear leukocytes (PMN) [22]. However, in the case of AIA and other chronic inflammatory diseases (such as chronic obstructive pulmonary disease), researchers suggest diminished capacity for LXs synthesis [23, 24]. Thus, impairment of a balance between lipoxins and leukotrienes production may be a key in the pathogenesis of aspirin hypersensitivity.

Other hypotheses associated with pathogenesis of aspirin-induced asthma

At high doses, there are properties of ASA that are independent of COX and prostaglandins inhibition. Few studies have shown that ASA is able to either activate the heat shock transcriptional factor [25] or inhibit either the mitogen-activated protein kinases p44Erk1 and p42Erk2 [26] and nuclear factor-κB [27].

There is also a theory suggesting that a chronic airway viral infection can alter expression of many cellular genes, including genes related to the arachidonic acid pathway [28] and virally infected cells become more prone to drug hypersensitivity [29].

Genetic theories of aspirin-induced asthma

Genes associated with the arachidonic acid pathway

In the leukotriene pathway, arachidonic acid is generated from phospholipids of cell membranes by cytosolic phospholipase A_2 ($cPLA_2$) and converted in two steps to leukotriene A_4 by 5-lipoxygenase. Next, LTA_4 is rapidly converted to LTB_4 by LTA_4 hydrolase and to cysLT: LTC_4 by LTC_4 synthase, which conjugates LTA_4 to reduced glutathione. After cellular export of LTC_4, the sequential cleavage of Glu and Gly provides LTD_4 and LTE_4 metabolites, respectively. So far, it has not been clarified which step in the leukotriene pathway is pivotal to the overproduction of cysLTs in patients with AIA. However, there is some noted correlation between the pathological increased level of cysLTs and altered expression of certain genes associated with the AA pathway.

The expression of *LTC4S* has been reported to be significantly higher in AIA than in ATA patients and normal controls [26]. Counts of cells expressing *LTC4S* were fivefold higher in AIA (11.5 ±2.2 cells/mm^2) than in ATA (2.2 ±0.7) and 18 fold higher than in normal control bronchial biopsies (0.6 ±0.4) [30]. From this, a fivefold higher LTC_4 synthase$^+$ cell count in AIA patients caused an ~200 fold enhanced sensitivity to inhaled aspirin [30]. Moreover, LTC4$^+$ cell counts in the bronchial submucosa

were related with basal levels of cysLTs in the BAL fluid, suggesting that higher cell counts can explain chronic cysLT overproduction and impaired baseline lung function in AIA patients [12]. In addition to the alteration of *LTC4S* expression in AIA, the literature also mentions data concerning the change in expression of the *COX2* gene.

COX2 mRNA expression turned out to be downregulated in nasal polyps from patients with AIA [31]. The mean level of *COX2* mRNA expression in nasal polyps from the AIA group (0.38 ±0.1) was significantly lower than in polyps (2.93 ±0.52) and nasal mucosa (2.1 ±0.54) from the ATA group [31]. The density of cells expressing COX2 was also significantly reduced in the subepithelial area of nasal polyps from AS patients as compared to the AT group (153 cells/mm^2 vs. 210 cells/mm^2) [32]. It is also known that impaired production of COX2 causes a chronic failure in the production of bronchoprotective PGE_2.

Genes encoding receptors for arachidonic acid metabolites

The biological actions of cysLTs occur by binding to their receptors, $cysLTR_1$ and $cysLTR_2$ on the surface of the target cells. Both receptors are G-protein coupled seven transmembrane receptors and are close to the locus for an increased risk of asthma in various populations [33, 34]. Most of proinflammatory actions of the cysteinyl leukotrienes are mediated by their binding to $cysLTR_1$ [35, 36]. *CysLTR1* is expressed in airway smooth muscle, eosinophils, macrophages, splenocytes [34] and an increased number of cells expressed in *CysLTR$_1$* was found in nasal mucosa in AIA patients with chronic rhinosinusitis (median, 542 cells/mm^2) compared to non-aspirin sensitive subjects (median, 116 cells/mm^2) [37]. There is also a study reporting genetic variants of the *cysLTR$_1$* promoter (–634 C>T, –475 A>C, –336 A>G), which are associated with AIA in Korean males [38]. This haplotype seems to contribute to increase the disease risk by elevating the expression level of *cysLTR1* in the asthmatic airway [38].

In turn, *CysLTR$_2$* is thought to be expressed in lung interstitial macrophages, eosinophils [39], mast cells [40], B and T lymphocytes and in lung smooth muscle [41]. It has recently been shown that the density of cells expressing $cysLTR_2$ was significantly higher in the subepithelial area of nasal polyps from AS patients as compared to nasal polyps from AT patients (394 cells/mm^2 vs. 125 cells/mm^2) [32]. An increased *cysLTR$_2$* expression in AIA may be explained by sequence variants on the promoter (–819 G>T) and on the 3'UTR (2078 C>T, 2534 A>G) which affect the efficiency of its transcription and stability of its mRNA [41].

Other equally important receptors of arachidonic acid pathway are E-prostanoid $(EP)_{1\text{-}4}$ receptors. Despite a global elevated expression of EP_1 and EP_2 receptors (but not EP_3 and EP_4) in the nasal epithelium in AIA and ATA, there was observed a significant reduction of the expression of EP_2 on a wide range of mucosal inflammatory leukocytes in the aspirin-sensitive as compared with tolerant patients [42].

Genes associated with eosinophilic inflammation

So far several genes have been found, which are associated with eosinophilic inflammation of the upper and lower airways. It is also known that eosinophilia is accompanied by aspirin hypersensitivity and the aspirin triad [43].

One of them is angiotensin I converting enzyme 1 (ACE) – a peptidase present in epithelial, endothelial cells [44] and it is related to the presence of kinins and substance P in lungs of asthmatics [45]. The decreased expression of ACE has been linked to the suppression of kininase II activity, resulting in the accumulation of kinins, substance P and prostaglandins [46]. This leads to bronchial hyperreactivity and airway eosinophilic inflammation in the asthmatic airway [47, 48]. It is possible that eosinophils that have toxic oxidizing effects may be associated with the degradation of the peptide in the epithelium [49]. Alternatively, a low level of *ACE* may limit degradation of a bioactive peptide with a chemotactic effect on eosinophils [49]. In addition to modified expression of ACE in asthmatic epithelium, one study demonstrated two SNPs –262 A>T and –115 T>C in the promoter region that are possibly involved in aspirin-induced asthma [50]. The association of –262 A>T is more pronounced and results in the decreased expression of *ACE* gene products in AIA [50].

Chemokine (C-C motif) receptor 3 (CCR3) seems to play a similar function in AIA, exactly in eosinophilic infiltration by [51]. In general, chemokines coordinate the recruitment and activation of leukocytes suitable for innate and adaptive immune responses [52]. However, recent evidence indicates that the effect of chemokines extends beyond their ability to modulate cell trafficking and involve such actions as angiogenesis, tissue remodeling and epithelial wound repair process [53]. In relation to AIA, there was a significant increase in the *CCR3* expression noted after aspirin challenge in comparison to the normal healthy subjects [54]. There is also evidence that *CCR3* gene polymorphism (–520 T/G) is associated with alteration of the gene expression and may exaggerate asthmatic symptoms after aspirin challenge [54].

NLRP3 gene (*NLR family*, *pyrin domain containing 3*) seems to be also included in the development of inflammation in the AIA. Such protein, *inter alia*, controls the activity of inflammatory caspase-1 by forming so-called inflammasomes [55] that are stimulated by pathogen-associated molecular patterns, microbial toxins, live bacteria, viruses and damage-associated molecular patterns [56]. In turn, activation of inflammasomes leads to autocatalytic processing and activation of caspase-1 that catalyzes cleavage of proinflammatory cytokines such as IL-1β and IL-18 [57]. It is also known that one *NLRP3* poly-

morphism (16974 C/T) has a significant association with AIA [58]. Functional analyses of 16974 C/T showed that this variant influenced a higher mRNA expression by altering expression enhancer activity or mRNA stability [58]. These observations suggest that *NLRP3* is involved in the hypersensitive immune reaction through gain-of-function variant [58].

Another gene that may be included in this group is *TBX21* (*T-box 21*). T-box 21 is expressed in IFN-γ producing Th1 cells [59] which have been suggested to protect against allergic responses by diminishing the activity of Th2 effector cells [60]. Therefore, decreased numbers of cells expressing T-box are in the airways of patients with allergic asthma [61]. In relation to AIA, it was found that –1993 T/C gene polymorphism affects the change of transcriptional regulation and consequently an increase in T-bet protein expression [57]. Moreover, the SNP mentioned above causes inappropriate Th1 responses in the airway and changed level of IFN-γ can contribute to the augmentation of allergic lung inflammation partly through the activation of eosinophils [62].

Gene involved in pathogenesis of nasal polyps

Rhinorrhoea and nasal congestion are usually the first symptoms of AIA. Many family studies indicate that a typical cold virus may trigger the onset of rhinitis. Thus, a chronic inflammatory process involving the upper airways may lead to development of nasal polyps.

It was previously reported that eosinophils are more activated in nasal polyp tissue of AIA compared to ATA patients and that the degree of eosinophilic inflammation of nasal polyp tissue is associated with the transforming growth factor β1 (TGF-β1) level [63]. The TGF-β is an anti-inflammatory cytokine and its decreased secretion could contribute to the development of a milieu in which allergic inflammation and asthma can develop [64]. In contrast to TGF-β anti-inflammatory effect, the appearance of this cytokine in ongoing allergic inflammation may lead to disease severity. Therefore, the mRNA and protein levels of TGF-β1 in eosinophils are elevated in patients with severe asthma compared to those with mild asthma, normal subjects [65] and elevated in response to allergen challenge [66].

Genes whose relationship with AIA is not yet clearly understood

In the literature, there is a lot of data concerning a gene polymorphism that might be associated with aspirin asthma, but there is no confirmation in functional studies. It is well known that point mutations do not have to mean changes in gene expression and function of protein.

The first protein whose function in the AIA has not yet been fully explained is fibrous sheath interacting protein 1 (FSIP1). The *FSIP1* gene is expressed in airway epithelium [67] and is regulated by amyloid β precursor protein (APP) [68]. It is also known that APP as an integral membrane protein is cleaved by α disintegrin and previously mentioned metalloproteinase 33 (ADAM33) as an asthma susceptibility gene. Further investigations have also reported one polymorphism of *FSIP1* (rs7179742) involved in increased susceptibility to AIA [69]. What is more, this variant is significantly associated with an increased fall rate of FEV_1 by aspirin provocation [69]. Some data also suggest that *FSIP1* might have an effect on AIA with a link to the *THBS1* gene. *THBS1* – thrombospondin 1 is located near *FSIP1* and generally is responsible for pulmonary response to oxidative stress in asthma [70].

Centrosomal protein 68 (CEP68), more precisely rs7572857 gene polymorphism, also seems to be a positive risk factor for the development of AIA [3]. The exact function of CEP68 in AIA has not yet been discovered, but it is known that this polymorphism more significantly affects the increase of FEV_1 decline in AIA than ATA patients [3].

Another gene whose connection with AIA is not yet explained is *SLC22A2*. Solute carrier family 22 (organic cation transporter) member 2 mediates the release of Ach (acetylcholine), which has been recognized as a novel regulator of airway remodeling [71] and one of the strongest bronchoconstrictors [72]. Moreover, airway epithelial cells possess various muscarine receptors and its dysregulation may lead to the development of asthma [71]. In the case of AIA, it has been shown that rs316021 gene polymorphism is significantly associated with the risk of AIA and rs3912161, SLC22A2-ht3 are significantly related to the maximum fall of FEV_1 [73]. Bronchospasm following the inhalation of aspirin in aspirin-intolerant patients with genetic polymorphisms of *SLC22A2* is probably bound to abnormal acetylcholine release [71].

ADAM33 belongs to a family of type 1 transmembrane metalloproteinases and is abundantly expressed in smooth muscle cells of airway tissue [74, 75]. About half of 34 ADAMs identified (including ADAM33) were predicted to be active proteinases based on the presence of the HEXXHXXGXXH zinc binding motif and the glutamic acid in the catalytic domain [76]. Multiple sequence variations within *ADAM33* were reported to be associated with asthma phenotype and BHR [77] but only ST+7, V-1 and V5 sites were significantly associated with AIA [74]. So far it has been unclear whether the functional activity of *ADAM33* is enhanced or decreased by these polymorphisms. Enhanced proteolytic activity could cause increased shedding of cytokine receptors but if the biological activity of ADAM33 was decreased, the number of certain receptors might be elevated (for example the number of cells with $cysLTR_1$ in the nasal mucosa in AIA patients is higher than in ATA patients [37]).

One of the stages of the process of airway remodeling in asthma is subepithelial fibrosis, caused by abnor-

mal deposition of ECM such as collagens and fibronectin in the basement membrane resulting in thickening of the subepithelial space [78]. In turn, thickening of membrane has been associated with asthma exacerbation, frequency and duration of symptoms and decline in FEV_1 [78]. In connection with AIA, a recent study has reported five gene polymorphisms (rs6949799, rs4727494, rs13233066, rs10279545, rs17470799) in *EMID2* (*EMI domain containing 2*) gene [79] encoding collagen γ1 chain. It has been also shown that its expression in undifferentiated mesenchymal cells [80] results in the epithelial-mesenchymal transitions, which are characteristic of remodeling response in asthma [81]. There is no clear explanation of the functional relationship between these SNPs and the AIA, but it is possible that these SNPs may contribute to abnormal, increased production of collagen affecting the airway limitation in AERD.

A correlation with chronic eosinophilic rhinosinusitis in AIA seems to show *IL-13* gene polymorphisms (–1510 A>C, 1055 C>T) [82]. Generally, IL-13 plays an important role in the development of allergic asthma [83] by inducing airway eosinophilia and hyperreactivity [84]. Functional analyses of the SNP –1055 C > T suggest that the minor allele is associated with enhanced promoter activity [82]. However, further studies are needed to explain a function played by these SNPs in development of rhinosinusitis in AIA.

Recently it has been shown that *TLR3* (toll like receptor 3) gene polymorphisms –299698 G>T and 293391 G>A are responsible for susceptibility to viral infections and therefore may induce asthma [85, 86]. In general, the function of the TLR family is to recognize conserved microbial structures and then to initiate the appropriate immune response. Eosinophils activated via TLR3 might be more able to recruit leukocytes to sites of inflammation and may cause exacerbation of allergic disease [87]. In healthy subjects, the activity of these lymphocytes is suppressed by PGE_2, but in AIA a shortage of PGE_2 causes an increase of cytotoxic reactions. Reactive oxygen species, toxic metabolites and released mediators precipitate asthma attacks [88].

Another protein associated with inflammation of airways in asthma is the adenosine A_1 receptor (*ADORA1*). It is also known that 1405 C>T polymorphism in the 3'-UTR confers susceptibility to AIA, while A102A has a protective effect [89]. Generally, adenosine levels are increased following challenge with allergens and in patients with asthma [90]. Moreover, inhalation of adenosine induces acute bronchoconstriction in asthmatics [91] which might be attenuated by lysine-aspirin inhalation [92]. At the biochemical level, adenosine is catabolized by adenosine deaminase (ADA) and blocking ADA induces accumulation of adenosine and thus severe inflammation [93]. In turn, the elimination of adenosine from inflammatory exudates using ADA reverses the anti-inflammatory effects of ASA [94]. Researchers speculate that ASA can change the levels of adenosine in the airways of asthmatics and that different effects observed between aspirin-tolerant subjects and patients with AIA are due to the altered expression of *ADORA1* [89].

It was previously known that LTC_4 is secreted from mast cells following Ca^{2+} influx through store-operated calcium release-activated calcium (CRAC) channels [95]. Thus, the airway smooth muscle cell contraction, caused by leukotriene overproduction, is regulated by changes in intracellular Ca^{2+} concentration. Moreover, leukotriene overproduction may contribute to induced recruitment of other immune cells, such as proinflammatory eosinophils. The L-type calcium channels are composed of 5 subunits and γ subunit is encoded by the *CACNG6* gene [96]. More recently, it has been demonstrated that rs192808 gene polymorphism of CACNG6 might be associated with the risk of AIA [97] and lead to abnormal Ca^{2+} concentration, providing a new connection between calcium channel and aspirin hypersensitivity in asthmatics.

HLA allele HLA DPB1*0301 associated with AIA

The HLA class II genes (HLA-DP, -DQ, -DR) of the human MHC are cardinal to the immune processing of exogenous antigens. Some genetic studies have demonstrated the strong positive association between the presence of HLA-DPB1*0301 and AIA. In the Polish population, frequency of HLA-DPB1*0301 was 19.5% in the AIA, compared with 5.2% in the normal controls (OR = 4.4, $p = 0.002$) and 4.4% in the asthmatic controls (OR = 5.3, $p = 0.0004$) [98]. In turn, in the Korean population, the frequency of this haplotype was 13.8% in patients with AIA compared to 2.2% in normal controls (OR = 8.3, $p < 0.0001$) and 4.1% in patients with ATA (OR = 5.2, $p = 0.004$) [99]. Moreover, carriers of DPB1*0301 tend to be female and exhibit typical clinical features of AIA, such as lower FEV_1 and higher prevalence of rhinosinusitis and/or nasal polyps [99]. Another study also suggests possible synergistic interactions between the TNF-α promoter polymorphisms (–1031 T>C, –863 C>T, –857 C>T) and HLA DPB1*0301, as the susceptible risk of AIA in patients compared with those carrying the HLA DRB1*0301 allele alone [100]. Similarly, *TBXA2R* gene polymorphism (795 T>C) showed a significant association with HLA DPB1*0301 in development of AIA [101]. However further studies are needed to investigate the mechanism how *TNF-α* and *TBXA2R* gene polymorphisms could interact with HLA DPB1*0301 allele in AIA.

Conclusions

In this review, we summarize a few genes whose relationship with AIA has been confirmed in functional studies and over a dozen gene polymorphisms that are suspected to be involved in the pathogenesis of AIA. A large number of genes whose relationship with AIA is not entirely explained suggests the need for conducting mul-

tiple studies to confirm this correlation. However, a milestone in solving the genetic mystery of AIA pathogenesis would be finding correlations among these genes.

References

1. Jones AW. Early drug discovery and the rise of pharmaceutical chemistry. Drug Test Anal 2011; 3: 337-44.
2. Rothwell PM, Fowkes FG, Belch JF, et al. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011; 377: 31-41.
3. Kim JH, Park BL, Cheong HS, et al. Genome-wide and follow-up studies identify CEP68 gene variants associated with risk of aspirin-intolerant asthma. PLoS One 2010; 5: e13818.
4. Jedrzejczak-Czechowicz M, Lewandowska-Polak A, Bienkiewicz B, et al. Involvement of 15-lipoxygenase and prostaglandin EP receptors in aspirin-triggered 15-hydroxyeicosatetraenoic acid generation in aspirin-sensitive asthmatics. Clin Exp Allergy 2008; 38: 1108-16.
5. Barranco P, Bobolea I, Larco JI, et al. Diagnosis of aspirin-induced asthma combining the bronchial and the oral challenge tests: a pilot study. J Investig Allergol Clin Immunol 2009; 19: 446-52.
6. Nizankowska E, Bestynska-Krypel A, Cmiel A, et al. Oral and bronchial provocation tests with aspirin for diagnosis of aspirin-induced asthma. Eur Respir J 2000; 15: 863-9.
7. Kowalski ML, Ptasinska A, Bienkiewicz B, et al. Differential effects of aspirin and misoprostol on 15-hydroxyeicosatetraenoic acid generation by leukocytes from aspirin-sensitive asthmatic patients. J Allergy Clin Immunol 2003; 112: 505-12.
8. Kowalski ML, Ptasinska A, Jedrzejczak M, et al. Aspirin-triggered 15-HETE generation in peripheral blood leukocytes is a specific and sensitive Aspirin-Sensitive Patients Identification Test (ASPITest). Allergy 2005; 60: 1139-45.
9. Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. J Allergy Clin Immunol 2003; 111: 913-21.
10. Smith WL, Dewitt DL. Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol 1996; 62: 167-215.
11. Gierse JK, McDonald JJ, Hauser SD, et al. A single amino acid difference between cyclooxygenase-1 (COX-1) and -2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. J Biol Chem 1996; 271: 15810-4.
12. Christie PE, Tagari P, Ford-Hutchinson AW, et al. Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Respir Dis 1991; 143: 1025-9.
13. Sladek K, Szczeklik A. Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. Eur Respir J 1993; 6; 391-9.
14. Christie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. Am Rev Respir Dis 1991; 144: 957-8.
15. Dahlen B, Kumlin M, Margolskee DJ, et al. The leukotriene-receptor antagonist MK-0679 blocks airway obstruction induced by inhaled lysine-aspirin in aspirin-sensitive asthmatics. Eur Respir J 1993; 6: 1018-26.
16. Phillips GD, Foord R, Holgate ST. Inhaled lysine-aspirin as a bronchoprovocation procedure in aspirin-sensitive asthma: its repeatability, absence of a late-phase reaction, and the role of histamine. J Allergy Clin Immunol 1989; 84: 232-41.
17. Henderson WR Jr. The role of leukotrienes in inflammation. Ann Intern Med 1994; 121: 684-97.
18. Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. N Engl J Med 1990; 323: 645-55.
19. Daffern PJ, Muilenburg D, Hugli TE, et al. Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses. J Allergy Clin Immunol 1999; 104: 559-64.
20. Marom Z, Shelhamer JH, Kaliner M. Effects of arachidonic acid, monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways in vitro. J Clin Invest 1981; 67: 1695-702.
21. Salari H, Schellenberg RR. Stimulation of human airway epithelial cells by platelet activating factor (PAF) and arachidonic acid produces 15-hydroxyeicosatetraenoic acid (15-HETE) capable of contracting bronchial smooth muscle. Pulm Pharmacol 1991; 4: 1-7.
22. Bonnans C, Levy BD. Lipid mediators as agonists for the resolution of acute lung inflammation and injury. Am J Respir Cell Mol Biol 2007; 36: 201-5.
23. Kupczyk M, Antczak A, Kuprys-Lipinska I, et al. Lipoxin A4 generation is decreased in aspirin-sensitive patients in lysine-aspirin nasal challenge in vivo model. Allergy 2009; 64: 1746-52.
24. Sanak M, Levy BD, Clish CB, et al. Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics. Eur Respir J 2000; 16: 44-9.
25. Jurivich DA, Sistonen L, Kroes RA, et al. Effect of sodium salicylate on the human heat shock response. Science 1992; 255: 1243-5.
26. Pillinger MH, Capodici C, Rosenthal P, et al. Modes of action of aspirin-like drugs: salicylates inhibit erk activation and integrin-dependent neutrophil adhesion. Proc Natl Acad Sci U S A 1998; 95: 14540-5.
27. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994; 265: 956-9.
28. Zhu H, Cong JP, Mamtora G, et al. Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. Proc Natl Acad Sci U S A 1998; 95: 14470-5.
29. Levy M. Role of viral infections in the induction of adverse drug reactions. Drug Saf 1997; 16: 1-8.
30. Cowburn AS, Sladek K, Soja J, et al. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest 1998; 101: 834-46.
31. Picado C, Fernandez-Morata JC, Juan M, et al. Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. Am J Respir Crit Care Med 1999; 160: 291-6.
32. Adamusiak A, Stasikowska-Kanicka O, Lewandowska-Polak A, et al. Expression of arachidonate metabolism enzymes and receptors in nasal polyps of aspirin hypersensitive asthmatics. Int Arch Allergy Immunol 2011; DOI: 10.1159/000329744.
33. Daniels SE, Bhattacharrya S, James A, et al. A genome-wide search for quantitative trait loci underlying asthma. Nature 1996; 383: 247-50.
34. Park JS, Jang AS, Park SW, et al. Protection of leukotriene receptor antagonist against aspirin-induced bronchospasm in asthmatics. Allergy Asthma Immunol Res 2010; 2: 48-54.

35. Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. N Engl J Med 1999; 340: 197-206.
36. Nicosia S, Capra V, Rovati GE. Leukotrienes as mediators of asthma. Pulm Pharmacol Ther 2001; 14: 3-19.
37. Sousa AR, Parikh A, Scadding G, et al. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. N Engl J Med 2002; 347: 1493-9.
38. Kim SH, Oh JM, Kim YS, et al. Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. Clin Exp Allergy 2006; 36: 433-9.
39. Mita H, Hasegawa M, Saito H, et al. Levels of cysteinyl leukotriene receptor mRNA in human peripheral leucocytes: significantly higher expression of cysteinyl leukotriene receptor 2 mRNA in eosinophils. Clin Exp Allergy 2001; 31: 1714-23.
40. Mellor EA, Frank N, Soler D, et al. Expression of the type 2 receptor for cysteinyl leukotrienes (CysLT2R) by human mast cells: Functional distinction from CysLT1R. Proc Natl Acad Sci U S A 2003; 100: 11589-93.
41. Early SB, Barekzi E, Negri J, et al. Concordant modulation of cysteinyl leukotriene receptor expression by IL-4 and IFN-gamma on peripheral immune cells. Am J Respir Cell Mol Biol 2007; 36: 715-20.
42. Ying S, Meng Q, Scadding G, et al. Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells. J Allergy Clin Immunol 2006; 117: 312-8.
43. Nasser SM, Pfister R, Christie PE, et al. Inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. Am J Respir Crit Care Med 1996; 153: 90-6.
44. Erdos EG. Angiotensin I converting enzyme and the changes in our concepts through the years. Lewis K. Dahl memorial lecture. Hypertension 1990; 16: 363-70.
45. Nieber K, Baumgarten CR, Rathsack R, et al. Substance P and beta-endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. J Allergy Clin Immunol 1992; 90: 646-52.
46. Overlack A. ACE inhibitor-induced cough and bronchospasm. Incidence, mechanisms and management. Drug Saf 1996; 15: 72-8.
47. Bucknall CE, Neilly JB, Carter R, et al. Bronchial hyperreactivity in patients who cough after receiving angiotensin converting enzyme inhibitors. Br Med J (Clin Res Ed) 1988; 296: 86-8.
48. Lindgren BR, Andersson RG. Angiotensin-converting enzyme inhibitors and their influence on inflammation, bronchial reactivity and cough. A research review. Med Toxicol Adverse Drug Exp 1989; 4: 369-80.
49. Roisman GL, Danel CJ, Lacronique JG, et al. Decreased expression of angiotensin-converting enzyme in the airway epithelium of asthmatic subjects is associated with eosinophil inflammation. J Allergy Clin Immunol 1999; 104: 402-10.
50. Kim TH, Chang HS, Park SM, et al. Association of angiotensin I-converting enzyme gene polymorphisms with aspirin intolerance in asthmatics. Clin Exp Allergy 2008; 38: 1727-37.
51. Stellato C, Brummet ME, Plitt JR, et al. Expression of the C-C chemokine receptor CCR3 in human airway epithelial cells. J Immunol 2001; 166: 1457-61.
52. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu Rev Immunol 2004; 22: 891-928.
53. Lukacs NW. Role of chemokines in the pathogenesis of asthma. Nat Rev Immunol 2001; 1: 108-16.
54. Kim SH, Yang EM, Lee HN, et al. Association of the CCR3 gene polymorphism with aspirin exacerbated respiratory disease. Respir Med 2010; 104: 626-32.
55. Ting JP, Willingham SB, Bergstralh DT. NLRs at the intersection of cell death and immunity. Nat Rev Immunol 2008; 8: 372-9.
56. Petrilli V, Dostert C, Muruve DA, et al. The inflammasome: a danger sensing complex triggering innate immunity. Curr Opin Immunol 2007; 19: 615-22.
57. Sutterwala FS, Ogura Y, Szczepanik M, et al. Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity 2006; 24: 317-27.
58. Hitomi Y, Ebisawa M, Tomikawa M, et al. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. J Allergy Clin Immunol 2009; 124: 779-85e6.
59. Akahoshi M, Obara K, Hirota T, et al. Functional promoter polymorphism in the TBX21 gene associated with aspirin-induced asthma. Hum Genet 2005; 117: 16-26.
60. Renauld JC. New insights into the role of cytokines in asthma. J Clin Pathol 2001; 54: 577-89.
61. Finotto S, Neurath MF, Glickman JN, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. Science 2002; 295: 336-8.
62. Ford JG, Rennick D, Donaldson DD, et al. IL-13 and IFN-gamma: interactions in lung inflammation. J Immunol 2001; 167: 1769-77.
63. Lee YM, Kim SS, Kim HA, et al. Eosinophil inflammation of nasal polyp tissue: relationships with matrix metalloproteinases, tissue inhibitor of metalloproteinase-1, and transforming growth factor-beta1. J Korean Med Sci 2003; 18: 97-102.
64. Hobbs K, Negri J, Klinnert M, et al. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. Am J Respir Crit Care Med 1998; 158: 1958-62.
65. Minshall EM, Leung DY, Martin RJ, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1997; 17: 326-33.
66. Redington AE, Madden J, Frew AJ, et al. Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. Am J Respir Crit Care Med 1997; 156: 642-7.
67. Harvey BG, Heguy A, Leopold PL, et al. Modification of gene expression of the small airway epithelium in response to cigarette smoking. J Mol Med (Berl) 2007; 85; 39-53.
68. Wen GY, Yang SY, Kaczmarski W, et al. Presence of hydroxysteroid dehydrogenase type 10 in amyloid plaques (APs) of Hsiao's APP-Sw transgenic mouse brains, but absence in APs of Alzheimer's disease brains. Brain Res 2002; 954: 115-22.
69. Kim JY, Kim JH, Park TJ, et al. Positive association between aspirin-intolerant asthma and genetic polymorphisms of FSIP1: a case-case study. BMC Pulm Med 2010; 10: 34.
70. Freishtat RJ, Benton AS, Watson AM, et al. Delineation of a gene network underlying the pulmonary response to oxidative stress in asthma. J Investig Med 2009; 57: 756-64.
71. Gosens R, Zaagsma J, Grootte Bromhaar M, et al. Acetylcholine: a novel regulator of airway smooth muscle remodelling? Eur J Pharmacol 2004; 500: 193-201.
72. Fujii T, Takada-Takatori Y, Kawashima K. Basic and clinical aspects of non-neuronal acetylcholine: expression of an independent, non-neuronal cholinergic system in lymphocytes and its clinical significance in immunotherapy. J Pharmacol Sci 2008; 106: 186-92.

73. Park TJ, Kim JH, Bae JS, et al. Possible association of SLC22A2 polymorphisms with aspirin-intolerant asthma. Int Arch Allergy Immunol 2011; 155: 395-402.
74. Sakagami T, Jinnai N, Nakajima T, et al. ADAM33 polymorphisms are associated with aspirin-intolerant asthma in the Japanese population. J Hum Genet 2007; 52: 66-72.
75. Zou J, Zhu F, Liu J, et al. Catalytic activity of human ADAM33. J Biol Chem 2004; 279: 9818-30.
76. Becherer JD, Blobel CP. Biochemical properties and functions of membrane-anchored metalloprotease-disintegrin proteins (ADAMs). Curr Top Dev Biol 2003; 54: 101-23.
77. Van Eerdewegh P, Little RD, Dupuis J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 2002; 418: 426-30.
78. Ward C, Pais M, Bish R, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax 2002; 57: 309-16.
79. Pasaje CF, Kim JH, Park BL, et al. A possible association of EMID2 polymorphisms with aspirin hypersensitivity in asthma. Immunogenetics 2011; 63: 13-21.
80. Yang Y, Haitchi HM, Cakebread J, et al. Epigenetic mechanisms silence a disintegrin and metalloprotease 33 expression in bronchial epithelial cells. J Allergy Clin Immunol 2008; 121; 1393-9.
81. Hackett TL, Warner SM, Stefanowicz D, et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. Am J Respir Crit Care Med 2009; 180: 122-33.
82. Palikhe NS, Kim SH, Cho BY, et al. IL-13 Gene polymorphisms are associated with rhinosinusitis and eosinophilic inflammation in aspirin intolerant asthma. Allergy Asthma Immunol Res 2010; 2: 134-40.
83. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. Science 1998; 282: 2258-61.
84. van der Pouw Kraan TC, van Veen A, Boeije LC, et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. Genes Immunol 1999; 1: 61-5.
85. Palikhe NS, Kim SH, Kim JH, et al. Role of toll-like receptor 3 variants in aspirin-exacerbated respiratory disease. Allergy Asthma Immunol Res 2011; 3: 123-7.
86. Ranjith-Kumar CT, Miller W, Sun J, et al. Effects of single nucleotide polymorphisms on Toll-like receptor 3 activity and expression in cultured cells. J Biol Chem 2007; 282: 17696-705.
87. Mansson A, Fransson M, Adner M, et al. TLR3 in human eosinophils: functional effects and decreased expression during allergic rhinitis. Int Arch Allergy Immunol 2010; 151: 118-28.
88. Szczeklik A. Aspirin-induced asthma as a viral disease. Clin Allergy 1988; 18: 15-20.
89. Kim SH, Kim YK, Park HW, et al. Adenosine deaminase and adenosine receptor polymorphisms in aspirin-intolerant asthma. Respir Med 2009; 103: 356-63.
90. Driver AG, Kukoly CA, Ali S, et al. Adenosine in bronchoalveolar lavage fluid in asthma. Am Rev Respir Dis 1993; 148: 91-7.
91. Cushley MJ, Tattersfield AE, Holgate ST. Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. Br J Clin Pharmacol 1983; 15: 161-5.
92. Crimi N, Polosa R, Magri S, et al. Inhaled lysine acetylsalicylate (L-ASA) attenuates the bronchoconstrictor response to adenosine 5'-monophosphate (AMP) in asthmatic subjects. Eur Respir J 1995; 8: 905-12.
93. Blackburn MR, Volmer JB, Thrasher JL, et al. Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation, and airway obstruction. J Exp Med 2000; 192: 159-70.
94. Cronstein BN, Montesinos MC, Weissmann G. Salicylates and sulfasalazine, but not glucocorticoids, inhibit leukocyte accumulation by an adenosine-dependent mechanism that is independent of inhibition of prostaglandin synthesis and p105 of NFkappaB. Proc Natl Acad Sci U S A 1999; 96: 6377-81.
95. Di Capite J, Shirley A, Nelson C, et al. Intercellular Ca2+ wave propagation involving positive feedback between CRAC channels and cysteinyl leukotrienes. FASEB J 2009; 23: 894-905.
96. Glossmann H, Striessnig J, Ferry DR, et al. Interaction between calcium channel ligands and calcium channels. Circ Res 1987; 61: I30-6.
97. Lee JS, Kim JH, Bae JS, et al. Association of CACNG6 polymorphisms with aspirin-intolerance asthmatics in a Korean population. BMC Med Genet 2010; 11: 138.
98. Dekker JW, Nizankowska E, Schmitz-Schumann M, et al. Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes. Clin Exp Allergy 1997; 27: 574-7.
99. Choi JH, Lee KW, Oh HB, et al. HLA association in aspirin-intolerant asthma: DPB1*0301 as a strong marker in a Korean population. J Allergy Clin Immunol 2004; 113: 562-4.
100. Kim SH, Ye YM, Lee SK, et al. Association of TNF-alpha genetic polymorphism with HLA DPB1*0301. Clin Exp Allergy 2006; 36: 1247-53.
101. Kim SH, Choi JH, Park HS, et al. Association of thromboxane A2 receptor gene polymorphism with the phenotype of acetyl salicylic acid-intolerant asthma. Clin Exp Allergy 2005; 35: 585-90.