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

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Review paper

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 PGE₂ 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-hydroxyeicosatetraenoic 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 rhinorhoea, 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 (GA²LEN), 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-hydroxyeicosatetraenoic 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].
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 J 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 lipooxygenase pathway eicosanoids shrinking bronchitis (15-HETE, LTB4, cysteinyl leukotrienes – cysLTs), in favor of the latter.

**Overproduction of cysteinyk leukotrienes**

In the leukotriene pathway, arachidonic acid liberated from the phospholipid membrane is converted to leukotriene LTA4 by ALOX5 and its cofactor ALOX5AP. Thus, further products, LTB4 and cysLTs (LTC4/D4/E4) 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 H1 antagonists have a little effect [16]. Therefore, cysLTs are considered as major mediators of AIA pathogenesis and can mediate bronchoconstriction, increased 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 E2 (PGE2) 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 A2 (cPLA2) and converted in two steps to leukotriene A4 by 5-lipoxygenase. Next, LTA4 is rapidly converted to LTB4 by LTA4 hydrolase and to cysLT: LTC4 by LTC4 synthase, which conjugates LTA4 to reduced glutathione. After cellular export of LTC4, the sequential cleavage of Glu and Gly provides LTD4 and LTE4 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/mm²) 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 LTC4S synthase* cell count in AIA patients caused an ~200 fold enhanced sensitivity to inhaled aspirin [30]. Moreover, LTC4S$^+$ 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/mm² vs. 210 cells/mm²) [32]. It is also known that impaired production of COX2 causes a chronic failure in the production of bronchoprotective PGE2.

**Genes encoding receptors for arachidonic acid metabolites**

The biological actions of cysLTs occur by binding to their receptors, cysteinyI leukotrienes are mediated by their binding to cysLTR1 [35], 36]. CysLTR1 is expressed in airway smooth muscle, eosinophils, macrophages, splenocytes [34] and an increased number of cells expressed in CysLTR1 was found in nasal mucosa in AIA patients with chronic rhinosinusitis (median, 542 cells/mm²) compared to non-aspirin-sensitive subjects (median, 116 cells/mm²) [37]. There is also a study reporting genetic variants of the cysLTR1 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 of the receptor cell counts may explain increased risk of asthma in various populations [33, 34]. Most of proinflammatory actions of the cysteinyl leukotrienes extends beyond their ability to modulate cell trafficking and involve such actions as angiogenesis, tissue remodelling and epithelial wound repair process [53]. In relation to AIA, there was a significant increase in the expression level of cysLTR1 in the asthmatic airway [38].

In turn, CysLTR2 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 CysLTR2 was significantly higher in the subepithelial area of nasal polyps from AS patients as compared to nasal polyps from AT patients (394 cells/mm² vs. 125 cells/mm²) [32]. An increased cysLTR2 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 may be explained by sequence variants on the 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].

**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 aspirin-induced asthma 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 auto-catalytic 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**

Rhinorrhea 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 FEV1 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 FEV1 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-h13 are significantly related to the maximum fall of FEV1 [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 proteases based on the presence of the HEXXHXXXGXXH 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, V1 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 cysLTR1 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-
nal 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 FEV1 [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 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 293991 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 PGE2, but in AIA a shortage of PGE2 caus-es 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 A1 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 l-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 LTC4 is secreted from mast cells following Ca2+ 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 Ca2+ 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 Ca2+ 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 FEV1 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 DRBI*0301 allele alone [100]. Similarly, TBX42R 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 TBX42R 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 mile-
stone in solving the genetic mystery of AIA pathogenesis
would be finding correlations among these genes.

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