Pharmacogenetics of asthma

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Abstract:

The paper reviews current knowledge on genetic background of action of drugs used in asthma treatment. In this aspect, glucocorticosteroids, leukotriene modifiers, β2 adrenoceptor agonists as well as methylxantines are discussed. The authors analyze different outcomes of treatment in subjects with non-wild-type genotype as compared to wild-type ones. For glucocorticosteroids, we focus on the polymorphism of corticotropin-releasing hormone receptor1 and the intracellularly located glucocorticoid receptor that exists in two variants (glucocorticoid receptor α – GRα and GRβ) created during alternative splicing of exon 9α to exon 9β. It is hypothesized that this polymorphism may be responsible for the reduced responsiveness to glucocorticosteroids in GRβ-predominant-subjects. For leukotriene, modifiers of two enzymes are reviewed: arachidonate 5-lipoxygenasae (ALOX5) and leukotriene C4 (LTC4) synthase. Especially LTC4Ss promoter has several described single nucleotide polymorphisms (SNPs), where the –444C is supposed to be associated with enhanced LTC4 production and, therefore, poorer response to leukotriene receptor antagonist treatment. β2 Adrenoceptor polymorphism has been widely studied recently. At least 55 SNPs have been identified, with Arg-Gly16 polymorphism and Gln-Glu27 polymorphism being the most frequent ones. It has been demonstrated that patients homozygous for Arg 16 produce significantly diminished response to β2 agonist treatment. Further, we discuss a possible role of CpG DNA motifs as adjuvants in immunotherapy of allergic diseases as well as modulators of children's immune system preventing development of allergic diseases. We conclude that, however there are medical disciplines where pharmacogenetics is in clinical use, in allergy and asthma we need further studies to evaluate potential risks and benefits.

Key words:
pharmacogenetics, asthma, β2-adrenoceptor, glucocorticosteroids, 5-lipoxygenase

Abbreviations: ALOX5 – arachidonate 5-lipoxygenase, CRHR1 – corticotropin-relesing hormone receptor1, CS – corticosteroids, ECP – eosinophil cationic protein, EPX – eosinophil peroxidase, GM-CSF – granulocyte macrophage colony-stimulating factor, GRα – glucocorticoid receptor α, GRβ – glucocorticoid receptor β, ICS – inhaled corticosteroids, IL – Interleukin, INF-γ – interferon γ, MBP – major basic protein, NF-AT –nuclear factor of activated T cells, NF-κB – nuclear factor κB, RANTES – regulated on activation, normal T – cell expressed and secreted, SNP – single nucleotide polymorphism, STAT6 – signal transductor and activator of transcription 6, TNF-α – tumor necrosis factor α, TLR – Toll-like receptor

Introduction

Bronchial asthma is one of the most common diseases worldwide. It is characterized by recurrent airway obstruction and bronchial hyperresponsiveness. In the pathophysiology of asthma, allergic inflammation plays central part. The allergic inflammation involves a number of cells among which eosinophils and activated T-cells play the most important role leading to

process amplification, bronchial hyperresponsiveness and eventually airway wall remodeling. Cells and mediators are activated after exposure to allergens. The allergens are presented by anitgen-presenting cells (APC) (i.e. dendritic cells, macrophages) to Th2 T cells stimulating them to release cytokines, such as interleukin (IL)-4, IL-13, IL-3, IL-5, granulocyte macrophage colony-stimulating factor (GM-CSF), regulated on activation, normal T-cell expressed and secreted (RANTES) and eotaxin. Cytokines produced by T-cells activate mast cells, eosinophils, basophils and B-cells producing immunoglobulin E (IgE). High-affinity IgE receptors FcεR1 on mast cells and basophils absorb Fc IgE, which binds circulating allergens causing mast cell degranulation.

Mediators released by mast cells such as LTC4, tryptase, histamine act on smooth muscle cells and small blood vessels leading to strong allergic reactions. Eosinophils, activated by Il-3, IL-5, GM-CSF, RANTES, eotaxin, release LTC4, major basic protein (MBP), eosinophil cationic protein (ECP) and peroxidase (EPX) which are toxic to epithelial cells. All these processes lead to airway wall remodeling and to chronic and acute airway narrowing.

Asthma genetics

Closer knowledge of genetics is crucial to its understanding. Population tests using genom-wide screen method show that DNA regions located on chromosomes 2p, 7q, 11q and especially 5q and 2q are important in asthma development [32]. Further, single-gene analyses indicate a significant correlation between the expression of IL-4 and IL-13 genes located on chromosome 5q13 and atopy prevalence [21, 40, 42, 63]. IL-4 gene promoter polymorphism (–590C/T) was first described in 1995. Its expression was assumed to correlate with higher IgE levels in asthmatics' serum but further investigations did not confirm that correlation [5, 20, 48, 49, 53, 59]. Quite recently a connection between -590T allele and higher risk of asthma development in the first year of life has been discussed [59, 69]. Polymorphism C/T -1111 of IL-13 genes is strictly related to atopic asthma, disturbed regulation of IL-13 production and increased nuclear proteins' binding (like Nf-AT) [64]. SNP (single nucleotide polymorphism) within coding region 4 (Arg → Glu 110) correlates with serum IL-13 level and is

important during receptor-ligand interaction [18]. A connection has been demonstrated between this polymorphism and elevated IgE levels in serum of 1399 children [12, 32], higher asthma prevalence in British and Japanese population [18] and with an elevated total and specific IgE level and atopic dermatitis [37] (but not asthma) only in a Chinese population [34]. Japanese asthmatics demonstrate a substitution Ile → Val 50 in the α chain of IL-4 and IL-13 receptors, which is caused by a SNP in the encoding gene on chromosome 16p12. This structural change results in the increased activation of STAT6 (signal transducer and activator of transcription 6), stimulation of transcriptional processes and CD 23 production and expression [26, 43].

In 2002, gene ADAM 33 was identified to be very important in asthma pathogenesis.[65] ADAM 33 belongs to a large family of ADAM proteins, which are mostly metalloproteinases. ADAM 33 is synthesized by fibroblasts and smooth muscle cells [65] and is related to fibroblasts, myofibroblasts and smooth muscle cell proliferation, which results in airway remodeling and hyperresponsiveness. The activity and level of expression of ADAM 33 gene might be responsible for structural and functional airway disturbances.

The knowledge of pathomechanisms and asthma genetics allows for further investigation of asthma treatment. A better understanding of asthma pathogenesis enables development of new or improved medications. Until now inhaled glucocorticosteroids (ICS), β2-mimetics and leukotriene modifiers have been the most important antiasthmatic. Since these drugs possess a wide spectrum of action, their use is almost always followed by adverse effects. Thanks to the pharmacogenetic research option, now we can try to identify patients who can take special advantage of the therapy and who will suffer less adverse effects.

Polymorphism of β2-adrenergic receptor

Currently, pharmacogenetics of β2-agonist based on β2-adrenoceptor polymorphism is the most widely explored. β2-Adrenoceptor is a member of the family of 7-transmembrane domain G-protein coupled receptors. It is built of 7 transmembrane spanning domains, 3 extracellular and 3 intracellular. The receptor gene is located on chromosome 5q31.32 [29]. In this loca-

tion, there are over 55 SNPs identified, with Arg-Gly16 polymorphism and Gln-Glu27 polymorphism being the most frequent ones [14, 16, 33]. Other polymorphisms, like Thr-Ile 164, Val-Met 34, BanI RFLP are relatively rare [13, 15, 45]. Both Gly16 and Glu27 polymorphisms are involved in higher agonist promoted down-regulation; moreover, Glu 27 is associated with stronger desensitization [14, 16]. It has been proven in several studies [25, 41], including a large prospective pharmacogenetic study [24], that patients homozygous for Arg 16, treated regularly or as needed with albuterol, get worse clinically and administration of the drug does not produce expected bronchodilatation. This suggested a diminished and still decreasing response in time of homozygous patients to β2 agonist treatment. Since the mechanisms of corticosteroid-β2-agonist co-operation has already been elucidated and also because during the above-mentioned experiments patients did not receive corticosteroids (CS), it came into question whether CS administration would change the response to β2 agonists [66].

Pharmacogenetics of leukotriene modifiers

There is a cascade of enzymes involved in leukotriene synthesis from arachidonic acid, but the most important enzymes for pharmacogenetics are by now ALOX5 and LTC4 synthase. 5-Lipoxygenase catalyzes the conversion of arachidonic acid to LTA4 [56]. The *ALOX-5* gene is located on chromosome 10q11.12 and its activity is associated with a number of repetitions of Sp1/Egr1 binding motifs in the promoter region. The promoter region contains five tandem motifs binding Sp1/Erg1 transcription factors (GGGCGG), that variation is called wild-type allele. Polimorphisms of this region are connected with addition or deletion of binding motifs and are known as non-wild-type alleles [23, 57]. Subjects with non-wild-type genotype are expected to have lower *ALOX-5* gene transcription, which leads to reduced enzyme production and finally to lower LTA4 levels [7, 27]. Patients treated with a 5-LO inhibitor (ABT-761) with non-wild-type allele at the *ALOX-5* promoter locus failed to respond to the therapy [7] because leukotriene pathway plays a minor role in their asthma patho-

genesis. Another important enzyme in the leukotriene pathway is LTC4 synthase, which is responsible for LTC4 formation from the arachidonic-acid backbone. Multiple *LTC4Ss* promoter SNPs have been identified [51, 52]. The A-444C variant (that means that adenine at -444 position is substituted by cytosine) is supposed to be associated with the enhanced LTC4 production. That is the reason for which asthmatic patients homozygous for A allele treated with zafirlukast (cysteinyl leukotriene receptor antagonists) had lesser FEV1 response than those with the C/C or C/A genotype [50]. There are some data suggesting an *ALOX-5* polymorphism contribution to cysteinyl leukotriene receptor antagonist responsiveness [10].

Theophylline pharmacogenetics

Theophylline is a second-line antiasthmatic drug with a narrow therapeutic window that needs close serum-level-monitoring. Overdose causes tachycardia, headache and nausea. When administered, it is metabolized by CYP1A2 (one of cytochrome P450 enzymes) to 1,3-dimethyl uric acid. Polymorphism of *CYP1A2* promoter is associated with changes in theophylline metabolism [44]. Transversion of G to A at -2964 position is connected with higher serum levels and decreased metabolism of theophylline. The increased serum levels of theophylline in Japanese asthmatics with SNP G-2964A have been shown, suggesting that this group of patients is more susceptible to cardiac side effects of theophylline and to them this drug should be administered with special caution if at all [44].

Pharmacogenetics of inhcorticosteroids

Also in the case of these potent and effective antiasthmatic drugs there is a group of individuals who are resistant to their effects. Tantisira et al. [61] suggested a correlation between ICS responsiveness and polymorphism of corticotropin-releasing hormone receptor1 (CRHR1). The Silverman group postulates that that gene polymorphism may be associated with an impairment of endogenous CS production and enhanced allergen-induced airway inflammation [55].

Some studies [61] indicate that *CRH1R* polymorphism is connected with lung function improvement (expressed as FEV1) after 8 weeks of ICS treatment. In two out of three analyzed populations with increased FEV1 after ICS therapy, one GAT haplotype (frequency 27%) was discovered. Individuals with the homozygous GAT/GAT haplotype had over twice FEV1 improvement compared to subjects with non-GAT haplotypes. These data can possibly explain individual response to ICS therapy but further investigations are needed to verify the clinical usefulness of these findings. The same group [60] demonstrated significant correlation between *TBX21* and ICS responsiveness. *TBX21* gene encodes transcription factor T-bet, which is responsible for induction of transformation of naive T lymphocytes into T helper cells (Th1) instead of Th2 cells. *TBX21* knockout mice demonstrate airway hyperresponsiveness, enhanced airway eosinophilia and remodeling [9]. Tantisira et al. [60] showed an interaction between the H33Q (replacement of histidine 33 with glutamine) mutation and ICS, which was associated with improved airway responsiveness. Cellular models suggest that H33Q could activate Th1 cytokine production (interferon γ – INF-γ) decreasing Th2 cytokine synthesis. It has been proved that GC inhibit the T-bet induction [47] but from the mouse model, we can suspect that H32Q mutation (mouse analog of H33Q) after GC stimulation correlates with increased INF-γ production.

GC can cause repression of gene transcription (transrepression). This phenomenon occurs in two situations. The first possibility is binding GC with negative GRE (nGRE), the second option is a direct interaction of the receptor with another nuclear transcription factor, like necrosis factor (NF)-κB, NF-AT or activated protein-1 (AP-1). Many anti-inflammatory activities of GC are based on this mechanism.

Also glucocorticoid receptor has been examined as a possible factor of GC resistance. The receptor is located intracellularly and exists in two variants (GRα and GRβ). These two variants are created during alternative splicing of exon 9α to exon 9β. GRα consists of 777 amino acids while in GRβ 50 carboxy terminal amino acids have been replaced by 15 amino acids encoded by exon 9β, which results in 742 amino-acid protein [8]. Both receptors are expressed in all human tissues and cells, but concentration of GRβ is lower than that of GRα. GRβ binds to DNA but not to CS and it may compete with GRα for binding to GRE. Although there are strong theoretical assumptions, there are no proofs that this polymorphism is responsible for reduced responsiveness to CS in clinical practice [3, 11, 39].

Many SNPs in the glucocorticoid receptor have been identified. Some may cause amino-acid substitutions, but only few were proven to cause familial glucocorticoid resistance [22, 38].

The role of CpG DNA in asthma therapy

Recently several research groups have shown an increasing interest in the DNA CpG motifs. They are supposed to be an additional pathway in asthma and allergy treatment. These structures include unmethylated cytosine and guanosine oligonucleotides repeated motifs (CpG) [17, 36, 46, 68] and are commonly found in bacteria. They are also localized in vertebrates and may be responsible for initiating antibacterial immunity, though. CpG stimulate antigen-presenting dendritic cells and natural killer cells producing IL-12, IFN-α/β, TNF-α and IL-18 [17, 30]. IL-12 determines differentiation of Th0 to Th1 cells and stimulates, due to an increased TNF-α and INF-α/β levels, NK cells and Th1 lymphocytes to produce of INF-γ [2, 6, 58]. INF-γ is a major microenvironmental element promoting Th1 cell activity and inhibiting Th2 cell proliferation [54]. CpG motifs, therefore, promote Th1-dependent processes in association with INF-γ synthesis and cytokines released by APC [2, 35]. The molecular mechanism of the interaction of CpG DNA with target cells has not been fully understood yet. It is supposed that CpG DNA interacts with a protein that belongs to the Toll-like receptor family (TLR9). The receptor is thought to be located intracellularly and to take part in immunological response to viruses, intracellular bacteria and parasites [19, 31, 67]. The polymorphism of *TLR9* gene has been recently investigated. The C-1237T polymorphism has been reported to have some asthma associations. On the other hand, Berghofer et al. did not find significant impact of *TLR9* polymorphism on TLR9 function. [1].

Many data prove that CpG DNA motifs are strong inducers of Th1-dependent reaction and inhibitors of Th2-dependent way. Some projects showed that asthmatic mice after administration of CpG DNA and after allergen exposure had lower airway eosinophilia, decreased specific B cell count and specific IgE pro-

duction [4, 28, 46, 54, 58]. A reduction of peribronchial and perivascular pulmonary inflammation and decreased airway hyperresponsivness after 6 weeks of exposure have been also described [47, 54, 58]. Further analyses showed local higher IL-12, INF-γ and lower IL-4 concentrations [58, 54].

Tighe et al. [62] administered purified Amba1 protein chemically linked to CpG DNA to mice. It led to Th1-based response to allergen, with production of Th1 IFN-γ-dependent IgG2a and IgG2b antibody isotypes, while administration of the same allergen without CpG pretreatment evoked a Th2 reaction. Also mice immunized with Amba1 that induced Th2-dependend reaction have inhibited IgE production after conjugate administration, what seems clinically relevant. These and many other findings suggest that CpG DNA motifs could be used as adjuvants in immunotherapy of allergic diseases but some further investigations in humans, testing especially adverse effects of a potential therapy are needed. In the future, modulation of children's immune system with CpG to prevent development of allergic diseases may be possible.

Conclusions

Pharmacogenetics is a field of research which helps us to understand variability of patients' responses to therapy from the point of view of genetic factors. It creates an opportunity to individualize pharmacotherapy, which is necessary to avoid adverse effects of treatment and to increase the number of medication responders. There are some disciplines of medicine where pharmacogenetics is already in clinical use but in the case of asthma, we still do not know much in this matter. Of all antiasthmatic drugs (like β2-agonists, ICS, leukotriene modifiers), β2-agonists are the best investigated, but we still need further studies to take advantage of the most up-to-date research achievements.

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