Pharmacogenetics of Asthma

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1. Introduction

Pharmacogenetics uses genetic information to help adjusting treatment for individual patients. It improves efficacy of therapy and enables avoiding side effects basing on genetic knowledge. Different asthmatic patients with similar disease severity, who are treated with the same medication, may respond to the therapy differently. After excluding non-genetic causes of such variability (like patient’s compliance, environmental and psychological factors), the most possible reason for the variability appears to be a different genetic structure. Changes in gene structure resulting in inter-individual dissimilarities, occur mostly as single nucleotide polymorphism (SNP). Different strategies play a role in searching and identifying SNPs, that influence pathogenesis of asthma and its response to treatment, (Kazani et al., 2010). One of the strategies involved is candidate gene studying, that focuses on finding genes responsible for therapy effectiveness as well as asthma development and its clinical severity (Moffatt & Cookson, 1997). Pharmacogenetics of asthma concentrates on genes coding: drug binding receptors, enzymes (important both in drug metabolism and metabolic cycles, eg. arachidonic acid cascade), chemokines, cytokines or growth factors relevant to asthma pathogenesis and pathophysiology. Genes need to be studied for known SNPs and new variants as well. When an SNP is found a thorough check for possible correlation between this polymorphism and disease phenotype or treatment response is needed. An expanded strategy for searching candidate genes involves screening of genes encoding proteins (enzymes) active in metabolic cycles important for drug response or key disease pathologies. In asthmatic patients this last method is often used to examine the leukotriene pathway in order to elucidate different patient reactions to leukotriene modifiers. Other options are genome-wide association studies that analyze genetic markers across the entire genome that may be connected with the phenotype. The identification of such a marker generated investigation of surrounding genes for SNPs related to the phenotype (Kazani et al., 2010). This procedure needs numerous and phenotypically well characterised populations and enables examination of the most frequent SNPs. There are some fields of medicine where pharmacogenetics is already in clinical use but in asthma treatment further investigation is still needed. This chapter reviews recent knowledge of pharmacogenetics of drugs commonly used in asthma treatment. We focus on bronchodilators, iCS (inhaled corticosteroids) and leukotriene modifiers.
2. Pharmacogenetics of antiasthmatic medications

2.1 Pharmacogenetics of β2-agonists

β2-adrenoreceptor (β2-ADR) agonists are fundamental relief medications and among the most important chronic treatments in asthma. These drugs exert their action by activation of β2-adrenoreceptors located among others on smooth muscle cells. This results in smooth muscle relaxation, airway dilatation and improved airflow. Depending on the duration of their action β2-agonistss are divided into two groups: short acting (SABA) and long acting β2-agonists (LABA). SABA are used exclusively as rescue medicines. They quickly reduce asthma symptoms: wheezing, shortness of breath and coughing. While LABA when used on a daily basis in combination with iCS help to improve asthma control. The side effects are common for both groups and these are: tachycardia, muscle tremble, mild hypokalaemia.

The β2-adrenoreceptor is a member of the 7-transmembrane domain G-protein coupled receptor family. It consists of seven transmembrane spanning domains, 3 extracellular and 3 intracellular (Fig. 1) (Dixon et al., 1986). Stimulation of β2-adrenoreceptor is G-protein dependent and results in activation of the second messenger, the adenylate cyclase. This in turn leads to an increase of cAMP level and smooth muscle relaxation. Another mechanism resulting from β2-adrenoreceptor stimulation is potassium channels opening by cAMP or directly by G-protein (Kowalski & Woszczek, 2002).

Fig. 1. Most common clinically relevant polymorphisms of the β2-adrenoreceptor (Ligget, 1997-changed). Black - nucleic acid deviations from wild type not resulting in nucleotide changes.
2.1.1 Polymorphisms of the $\beta_2$-adrenergic receptor

Examination of the intronless $\beta_2$-adrenoreceptor gene, which is located on chromosome 5q31.32 (Kobilka et al., 1987), revealed over 80 SPNs (Weiss et al., 2006). Two of these polymorphisms: Arg$\rightarrow$Gly16 (46A$\rightarrow$G) and Gln$\rightarrow$Glu27 (79C$\rightarrow$G) are the most frequent ones (see Figure 1.) (Green et al., 1994, 1995; Lee et al., 2004). Their occurrence results in receptor function change, different ligand binding and impaired signal transmission. The occurrence of the Gly16 gene variant is higher than that of the wild-type Arg16 and ranges between 67% in British asthmatics and 72% in British and American healthy subjects (Liggett, 1997; Tan et al., 1997; Lipworth et al., 1999). It has been estimated that the homozygous genotype Arg16 appears in 16% Caucasians and 25% Afro-Americans. Studies of Xie (Xie, et al., 2001) and co-workers revealed further differences between $\beta_2$-adrenoreceptor polymorphisms and ethnic groups. In a study, that examined 415 healthy subjects, Glu27 allele were the most frequent in Caucasian-Americans (34.8%). Other groups had much lower occurrence of this allele: Afro-Americans (20.7%) and Chinese (7.2%). Individuals with homozygous Glu27 genotype were mostly Caucasian-Americans (15.4%). This genotype occurred only in 4.9% African-Americans and was not observed in Chinese subjects (Xie, et al., 2001). Both Gly16 and Glu27 polymorphisms are involved in higher agonist promoted receptor down-regulation, moreover, Glu27 is related with a stronger desensitization of the receptor (Green et al., 1994, 1995). Another defined polymorphism: Thr$\rightarrow$Ile164 is associated with diminished affinity of $\beta_2$-agonist to the receptor, decreased adenylate cyclase binding and 50% shorter lasting salmeterol (one of the long acting beta2 agonists) effect (Green et al., 2001).

2.1.1.1 Correlation between $\beta_2$-adrenoreceptor gene polymorphism and short acting $\beta$-agonists action

Short acting $\beta$-agonists are drugs commonly used in asthma treatment, especially in asthma exacerbations or as regular rescue medications. However, they are not recommended as regular antiasthmatic drugs. Several studies demonstrated higher FEV1 increase (forced expiratory volume in the first second, a spirometric parameter used to determine the level of airways narrowing) increase after SABA (salbutamol) administration in homozygous Arg16 individuals as compared to heterozygous and homozygous Gly16 patients with polyinosis (Martinez et al., 1997; Woszczek et al., 2005). Different results were obtained during asthma exacerbation. Patients who were homozygous Arg16 had impaired SABA response compared to homozygous Gly16 individuals (Carroll et al., 2009). Systematic administration of SABA to Arg16 asthmatics caused deterioration of lung function (as evaluated with PEF - peak expiratory flow, another parameter used to monitor airway narrowing), that did not stop even with treatment discontinuation. In contrast patients homozygous for Gly16 demonstrated improved lung function (evaluated by PEF measurement as well) (Israel, 2000, 2004). Based on these studies it has been postulated that Arg16 homozygotes may be at higher risk during long-term SABA therapy. According to the 2010 updated GINA guidelines (Global Initiative for Asthma [GINA], 2010) regular long-term SABA treatment is not recommended for any individual. But due to relatively low differences in PEF-worsening between the two groups more research is needed to fully elucidate this problem.
2.1.1.2 Correlation between β2-adrenoreceptor gene polymorphisms and long acting β-agonists action

Long acting β2-agonists as opposed to SABA are drugs commonly used in long-term asthma therapy. There are several population studies suggesting increased risk of therapy with long acting β2-agonists in patients with the Arg16 homozygous genotype. However, no genotype is currently considered a direct contraindication for LABA treatment. Some patients treated with salmeterol, experienced rare but severe asthma exacerbations (Nelson et al., 2006). Further investigation suggested a dependence between Arg16 genotype and faster decline of lung parameters (FEV1) after LABA application (Nelson et al., 2006; Wechsler et al., 2006; Lee et al., 2004; Palmer et al., 2006). A good example is a study of Wechsler and co-workers comparing salmeterol response in individuals with asthma homozygous for arginine (Arg16) with glycine homozygous (Gly16) group of patients. Patients were divided in two groups. The first group was treated with salmeterol without iCS and the second continued iCS therapy while randomized for salmeterol. In both groups Arg16 patients didn’t draw benefit from salmeterol therapy comparing to Gly16 patients, which resulted in lower morning PEF, increased symptom scores and albuterol rescue use especially in trial without iCS. Present asthma treatment guidelines allow use of LABA only together with iCS since it has been demonstrated that iCS ameliorate the LABA effect. It is possible that in the future patients with Arg16Arg genotype will constitute a group requiring different treatment guidelines, but up to date therapeutic indications are uniform irrespective of the patient’s genotype.

2.1.1.3 Correlation between β2-adrenoreceptor gene polymorphisms and asthma exacerbations

It has been proven that exacerbations of asthma during short acting β-agonist therapy is related to β2-adrenoreceptor gene polymorphisms (Taylor et al., 2000). Recent studies reveal, that children and adolescent asthmatics with the Arg16 genotype suffer from asthma exacerbations more frequently than the Gly16 subpopulation (OR 2.05, 95% CI 1.19 to 3.53, p=0.010). This genotype-exacerbation correlation significantly increases after salmeterol treatment (OR 3.40, 95% CI 1.19 to 9.40, p=0.022) (Palmer et al., 2006). Other studies confirm the conclusion that risk of asthma exacerbation in the Arg16 group rises with higher doses and more frequent use of β2-agonists. Individuals with the Arg16 genotype receiving short- or long-acting β2-agonists on everyday basis had significantly higher risk of asthma exacerbation (OR 1.64, 95% CI 1.22 to 2.20, p=0.001) than patients with the Arg16 genotype taking β2-agonist less than once daily (Basu et al., 2009). According to the LARGE study patients with the Gly16 genotype have diminished bronchial hyperresponsiveness to metacholine after adding inhaled corticosteroids (at an average dose 480µg of beclomethasone daily) to salmeterol treatment (Wechsler et al., 2009). Arg16 genotype Afro-Americans have a lower chance for lung function improvement after co-administration of LABA and inhaled corticosteroids what may be related to more frequent prevalence of Arg16 polymorphism in this population (25%). This can also explain ethnic differences in asthma manifestation - more frequent severe asthma occurrence in Afro-Americans. According to Liggett (Liggett, SB., 2000) β2-adrenoreceptors in Gly16 subjects are down regulated at baseline by exposure to endogenous catecholamines what explains why reaction to exogenous β2-agonists is more evident in Arg16Arg individuals. At the same time however, Arg16 patients seem to have higher risk of asthma exacerbation especially
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during β2-agonist therapy. Despite these differences present guidelines [GINA, BTS (British Thoracic Society)] do not recommend checking the patients’ genotype before starting therapy. In our opinion LABA-treatment failure should be a recommendation for β2-adrenoreceptor genotype verification. This may increase both treatment effectiveness and safety. More research in this field is needed, however.

2.2 Pharmacogenetics of leukotriene modifiers

Leukotrienes are a family of polyunsaturated eicosatetraenoic acids that are derived from arachidonic acid in an enzymatic pathway called arachidonic acid cascade (see Figure 2.). In this pathway 5-lipoxygenase plays probably the most important role (Dixon et al., 1990). 5-lipoxygenase (5-LOX) catalyzes the conversion of arachidonic acid to leukotriene-A4 (LTA4) (Silverman & Drazen, 1999). All leukotrienes include cysteine and are called cysteinyl leukotrienes (with the exception of LTB4). Cysteiny1 leukotrienes bind to CysLT1 receptor causing among others airway smooth muscle contraction, eosinophilic influx and mucus hypersecretion. Another important enzyme in the leukotriene pathway is LTC4 synthase, which is responsible for LTA4 to LTC4 conversion. Leukotrienes have been shown to be potent pro-inflammatory mediators in asthma pathology (Chanarin & Johnston, 1994). They are produced and released by several types of inflammatory cells including eosinophils, neutrophils and mast cells.

![Fig. 2. The lipoxygenase pathway of leukotriene synthesis](https://www.intechopen.com)
Leukotriene modifiers are an important group of drugs in asthma treatment as well as in other diseases including allergic rhinitis. Based on their mode of action they can be divided into two groups: first - cysteine leukotrienes receptor antagonists (montelukast, zafirlukast, pranlukast and tomelukast), second - 5-lipoxygenase inhibitors (zileuton). These drugs show strong anti-inflammatory activity, ameliorate asthma clinical course and improve disease control with minimal or no side effects. Currently, they are listed in GINA 2010 (GINA, 2010) guidelines for asthma treatment as number two anti-inflammatory treatment (number one are still inhaled steroids), even though not all asthmatic patients benefit substantially from anti-leukotriene therapy. Based on the knowledge on leukotriene synthesis pathway, studies of genotype dependent therapeutic reactions have used strategies of candidate gene screening and examination of SNPs in genes encoding different proteins (enzymes) of the arachidonic acid cascade. To date, most investigations of the genetic factors which may affect therapy with anti-leukotriene drugs have focused on the 5-LOX enzyme and the LTC4 synthase. Possible genetic alterations of cysteine leukotriene receptors have also been investigated. The following paragraphs discuss the most important pharmacogenetic studies presenting major polymorphisms relevant in asthma and allergy as well as their impact on drug action.

2.2.1 Polymorphisms of the 5-lipoxygenase gene

The 5-LOX gene (ALOX5) is located on chromosome 10q11.12, contains 14 exons and its activity is associated with a number of repetitions of Sp1/Erg1 binding motifs in the promoter region (Hoshiko et al., 1990; Funk et al., 1989; Silverman et al., 1998). The promoter region containing five tandem motifs binding Sp1/Erg1 transcription factors (GGGCGG) is known as wild-type allele (Silverman et al., 1998). Polymorphisms of this region result from additions or deletions of binding motifs and are called non-wild-type alleles (In et al., 1997; Silverman et al., 1998). A polymorphism with one additional Sp1/Erg1 binding motif has been found in 35% of both asthmatic and non-asthmatic population (Fenech, A & Hall, IP., 2002). Further, 3% of the subjects without any copy of a wild-type allele are expected to have lower ALOX-5 gene transcription, which leads to reduced enzyme production and finally to lower LTA4 levels (Drazen et al., 1999; Kalayci et al., 2003). In consequence, the low level of cysteinyl leukotriene does not intensify allergic inflammation in asthma, but patients who do not have a wild-type allele, experience only 1% FEV1 improvement after 5-LOX inhibitor treatment comparing to wild-type patients (FEV1 improves up to 15-20%) and are considered non-responders for this type of therapy (Drazen et al., 1999). The same concerns to montelukast treatment (antagonist of cysteine leukotriene receptors): wild-type homozygous and heterozygous patients present benefit greatly from treatment (measured as FEV1 improvement (Telleria et al., 2008)), while non-wild-type are considered relative-non-responders. Other studies demonstrated however, that subjects with non-wild-type allele(s) treated with montelukast had reduced (73%) risk of asthma exacerbation (Lima et al., 2006). Because the role of leukotriene modifiers in asthma control increased significantly in the past five years, further studies are necessary to define responders and non-responders phenotypes. Defining standards of responding to leukotriene modifier therapy is extremely important at least in two subpopulations: in non-wild-type individuals, who in previous studies have not experienced treatment benefit and in patient with steroid resistance or at least with partially impaired response to iCS.
2.2.2 Polymorphisms of the leukotriene C4 synthase gene (LTC4S)

LTC4 synthase (LTC4S) belongs to S-glutathione synthases family and is responsible for leukotriene A4 and glutathione bonding. This reaction results in leukotriene C4 synthesis. LTC4 is a potent contracter of bronchial smooth muscles. The gene for LTC4 synthase is located on chromosome 5q35. In the promoter region of this gene several polymorphism have been described. One of the most important is substitution of nucleotide A by C in position 444 (−444A → C). The −444A → C SNP results in increased LTC4S gene transcription and therefore higher LTC4 level in eosinophils (Sampson et al., 2000; Sanak et al., 2000). This variant occurs more often in patients suffering from aspirin induced asthma (in patients with aspirin idiosyncrasy in general) (Sanak et al., 1997). Presence of the C nucleotide is also related to better response to cysteine leukotriene receptor 1 (LTRA1) blockers. During montelukast therapy 80% reduction of asthma exacerbation risk was observed in heterozygous individuals with C allele when compared to AA homozygous (Lima et al., 2006). Similar results were reported from a study in Japan, where patients with moderate, well controlled asthma, treated with inhaled corticosteroids, received pranlukast as an add-on treatment. Again, individuals with the C allele had more pronounced FEV1 improvement than AA homozygous patients (FEV1 improvement in C allele group 5.3% vs. in AA group 2.4%). Heterozygous population also showed higher values of bronchial dilatation after salbutamol usage (Asano et al., 2002).

2.2.3 Polymorphisms of the cysteine leukotriene receptors 1 and 2 genes (CYSTLTR1, CYSTLTR2)

The human CYSLT1 and CYSLT2 receptors have been characterized as G-protein coupled receptors (Lynch et al., 1999; Heise et al., 2000). The gene coding for the CYSLT1 receptor is located on chromosome X and the CYSTLT2 receptor gene maps to chromosome 13q14 (Lynch et al., 1999; Heise et al., 2000). Polymorphisms of these genes are studied in relation to the probability of asthma development. Previous data suggest however, that polymorphisms of CYSTLTR1 and CYSTLTR2 genes play a minor role in the determination of asthma severity and clinical symptoms’ expression (alike other genes encoding proteins related to leukotriene pathway) (Tantisira & Drazen, 2009). As for now there are no unequivocal results concerning polymorphisms of the CYSLT receptor genes in relation to anti-leukotriene treatment effects.

Although zileuton does not directly act through the CYSLT1 receptor, the possible correlation between this medication and CYSLT1R polymorphisms was also investigated. These studies, including genotype analysis of over five hundred patients treated with zileuton did not show any significant correlation between CYSLT1R gene polymorphisms and clinical response to therapy (Tantisira et al., 2009).

2.2.4 Polymorphisms of the ABCC1 gene

The ABCC1 gene (ATP-binding cassette, subfamily C, member 1) encodes MRP1 (Multiple Drug Resistance Protein 1) that takes a part in transmembrane LTC4 transport. This gene is located on chromosome 16p13.12 and demonstrates significant heterogeneity (Saito et al., 2002; van der Deen et al., 2005). One of the polymorphisms of this gene, that was thought to be correlated to drug response, namely rs119774, described by Lima et al. was related to a
significant FEV1 improvement in subjects receiving montelukast for 6 months (Lima et al., 2006). Heterozygous patients had a 24% FEV1 rise as compared to only a 2% improvement in homozygous individuals (Lima et al., 2006). Since there are no further studies of this correlation available data are insufficient to have any treatment implications. Again, further studies would help to elucidate whether the two phenotypes differ enough to justify different treatment regimens.

2.2.5 Polymorphism of LTA4 hydrolase gene

Hydrolase LTA4 is an enzyme that converts LTA4 to LTB4. The gene encoding this protein is located on chromosome 12q22. One of the known polymorphisms for this gene (rs2660845) involves a nucleotide change A->G at intron. Patients, whose genotypes contain at least one G allele (heterozygous), when treated with montelukast, have 4-5 higher risk of asthma exacerbation when compared to AA homozygous subjects (Lima et al., 2006). The pathogenetic mechanism of this phenomenon remains unclear. It has been hypothesized that this SNP causes a decreased enzyme activity that results in diminished LTB4 synthesis, therefore stimulating the LTC4-synthase pathway and leading to cysteine leukotriene synthesis (Lima et al., 2006) (Fig. 2).

There are big individual differences in response to leukotriene modifiers. All polymorphisms listed in paragraph 2.2 contribute to these differences. It remains extremely important to determine which patient subpopulation benefit most from the treatment.

2.2.6 Polymorphisms of the SLCO2B1 gene

The gene SLCO2B1 (solute carrier organic anion transporter family - 2B1) encodes the protein 2B1, that plays an important role in the active transport of organic anions through the intestinal wall. Protein 2B1 is thought to be a key transporter of montelukast through the intestinal wall. A recently described, common, SLCO2B1 polymorphism, namely rs12422149 935G→A (Arg312Gln), appears to relate to changes in montelukast pharmacokinetics. Specifically, individuals with this SNP have a significantly lower serum drug concentration (Mougey et al., 2009). So far, there are no data on other possible SLCO2B1 gene polymorphisms that could affect montelukast transport or serum level.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphisms with potential pharmacogenetic consequences during leukotriene modifier therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX5</td>
<td>10q11.12</td>
<td>Promoter Sp1/Egr1 binding motif (G+C rich sequence, i.e. -GGGCAG- ) different than 5 sequence repeats, -212 to -88 bp</td>
</tr>
<tr>
<td>LTC4S</td>
<td>5q35</td>
<td>Promoter -444A→C</td>
</tr>
<tr>
<td>CYSLTR1</td>
<td>Xq13.2-q21.1</td>
<td>927C→T</td>
</tr>
<tr>
<td>ABCC1</td>
<td>16p13.12</td>
<td>rs119774, G→A intron</td>
</tr>
<tr>
<td>LTA4H</td>
<td>12q22</td>
<td>rs2660845, A→G intron</td>
</tr>
<tr>
<td>SLCO2B1</td>
<td>11q13</td>
<td>rs12422149 935G→A</td>
</tr>
</tbody>
</table>

Table 1. Genes polymorphisms with potential pharmacogenetic consequences for leukotriene modifier therapy
Leukotriene modifiers are widely used in asthma treatment and they are orally administered which improves patients compliance and therefore efficacy. However, genes linked to their metabolism, drug-receptor interactions etc. have not intensively investigated. In our opinion, cytochrome P450, that metabolises both groups of leukotriene modifiers (especially CYP1A2 and CYP3A4), is a promising target. Studies investigating genetic variants of cytochrome P450 enzymes in relation to leukotriene modifiers response are necessary to establish possible dosing variations.

2.3 Pharmacogenetics of inhaled corticosteroids (iCS)

Corticosteroids are the most important and the most effective medication in asthma therapy. They are powerful anti-inflammatory agents in asthma management, mostly being “anti-eosinophilic”. Although many asthmatics derive therapeutic benefit from inhaled corticosteroids, many fail to respond or at least need to be treated with much higher doses. Despite iCS being considered a safe treatment, side effects of increased dosage may be clinically significant and include: adrenal suppression, osteoporosis, skin changes, cataract, and growth retardation in children. There at least two different mechanisms of CS resistance, but both are still under investigation.

Candidate gene studies were used to determine the pharmacogenetics of response to inhaled corticosteroids.

2.3.1 Glucocorticoid receptor

Corticosteroids exert their action by binding to the glucocorticoid intracellular receptor (GR), a nuclear receptor. The GR gene is located on the long arm of chromosome 5 (5q31-32). Members of the superfamily of nuclear receptors share a structural pattern containing a short central DNA-binding domain, a variable N-terminal domain as well as a C-terminal, which is the steroid hormone binding part, and a transcription regulator (Beato et al., 1996; Gronemeyer, 1992). There are two different GR isoforms known: one consisting of 777 (called GRα) and the other of 742 amino acids called GRβ. These isoforms are created during alternative splicing of the GR pre-mRNA (Bamberger et al., 1996). GRβ varies from the other isoform only in the length of C-terminal domain, which is shorter by five amino acids. This results in reduced glucocorticoid binding affinity of the GRβ receptor. Both receptors are expressed in all human cells, but GRβ plays a regulatory role and its concentration is much lower than that of GRα. Although there is no evidence to support that this polymorphism is responsible for reduced responsiveness to GC in clinical practice, this concept has been widely discussed and related studies are currently carried out (Brogan et al., 1999; Gagliardo et al., 2000; Malmstrom et al., 1999). In the cytoplasm, the glucocorticoid receptor is linked with several regulatory proteins, with the heat shock protein (hsp90), p59 immunophilin and p23 phosphoprotein being the most important (Smith & Toft, 1993). GR and hsp90 coupling enables ligand (CS) binding to the receptor and facilitates correct receptor “maturation” after synthesis (Smith & Toft, 1993). After CS binding the complex GR/hsp90 is disunited and activated GR/CS translocates to the nucleus and binds to DNA via the central domain consisting of two “zinc fingers” (Mitchell & Tjian, 1989). On the DNA side the fragment interacting with GR is called GRE (glucocorticoid response elements). This is one of the two mechanisms for CS to stimulate or inhibit transcription and therefore mRNA
synthesis. The other mechanism involves intra-cytoplasmic interaction of GR/CS with transcription factors, resulting in the blockage of their activity and consequently hampering transcription of several inflammatory agents as cytokines and chemokines, simplifying synthesis of anti-inflammatory agents (Barnes, 1996).

The importance of iCS in asthma treatment made the glucocorticoid receptors gene polymorphisms the obvious target of pharmacogenetic studies. However, despite the large number of researchers involved and the considerable funds devoted by both academic and industrial teams, only few polymorphisms have been discovered until now: Val→Asp641, that results in a three-fold lower binding affinity for dexamethasone, Val→Ile729 - with four-fold decrease in dexamethasone activity and Asn→Ser363 that results in higher activity to exogenous corticosteroids (Hurley et al., 1991; Malchof et al., 1993; Huizenga et al., 1998). From published studies we know, that patients with GR gene polymorphisms Val→Asp641 and Val→Ile729 may be predisposed to a relatively decreased response to CS therapy (Koper et al., 1997; Lane et al., 1994). A three marker long haplotype G-A-T (frequency 23% in general population; G allele - BclI SNP, A allele - intron B 33389, T allele - intron B 33388) was described in 2004 by Stevens and coworkers. It is associated with enhanced GC sensitivity measured as low postdexamethasone cortisol (frequency 41%). Subjects homozygous for G-A-T had over twofold FEV1 improvement after CS treatment compared to heterozygous or non-G-A-T haplotypes (Tantisira et al., 2004).

However, all these studies have not demonstrated a correlation between GR polymorphisms and corticosteroid resistance in asthma. Corticosteroid resistance does not seem to be dependent on a single GR gene polymorphism.

2.3.2 Polymorphisms of the CRHR1 gene (corticotropin releasing hormone receptor type 1)

In contrast to the GR gene polymorphism studies, CRHR1 investigations seem to yield more promising results. The CRHR1 gene is the major receptor for corticotropin that in turn is the key regulator of corticosteroids synthesis and catecholamine production. The gene for CRHR1 is located on chromosome 17q12-22, in the genomic region linked to asthma in a genome-wide-screen (Zandi et al., 2001). Most important data came from studies by Tantisira et al (Tantisira et al., 2004) that analyzed 14 genes connected with biological pathways of corticosteroids in three large groups of patients. Study participants were recruited from several other clinical trials studying the use of inhaled corticosteroids in asthma. The first group consisted of 470 adult individuals and was encoded AD (Adult Study), the second included 336 adult patients - ACRN (Asthma Clinical Research Network), and the third one included 311 children from CAMP (Childhood Asthma Management Program). This project revealed a significant correlation between lung function improvement after inhaled corticosteroid therapy and SNPs (rs1876828, rs242939 and rs242941) and haplotype occurrence within the CRHR1 gene, especially rs242941 (G→T, intron located) polymorphism, in all populations. In the AD population homozygous individuals with this polymorphism had average FEV1 improvement, higher than homozygous patients lacking this SNP. Similar results were obtained for the paediatric population that was studied. These data can contribute to our
understanding of the diverse patient reactions to iCS treatment but further investigation is still necessary.

2.3.3 Polymorphisms of the TBX21 gene (T-box expressed in T cells)

Another gene modulating inhaled corticosteroids action is TBX21 that encodes T-bet transcription factor (Tantisira et al., 2004). It plays an important role in balancing lymphocyte subpopulations, enhancing Th1 and inhibiting Th2 clone formation. TBX21 knockout mice develop bronchial hyperresponsiveness, enhanced airway eosinophilia and faster airway remodelling (Finotto et al., 2002) proving that TBX21 is crucial for asthma protection.

So far one clinically important SNP, His→Glu33 (H33Q), was found within this gene’s area in mice. Cellular models suggest that the H33Q allele can activate Th1 cytokine production (including interferon γ - INFγ) that in turn decreases Th2 cytokine synthesis providing a stable protection against asthma and allergy development. Surprisingly enough, it has been demonstrated that corticosteroids are able to inhibit T-bet induction (Refojo et al., 2003) resulting in Th2 domination. These findings still require a direct in vivo confirmation.

Studies in children (CAMP population) showed 4.5% occurrence in general population of homozygous Glu33 individuals. The presence of even one copy of this allele in subjects treated with iCS was associated with a significant decrease in airway hyperresponsiveness (measured as PC20) as compared to His33His homozygous subjects and individuals not iCS-treated (Tantisira et al., 2004).

2.3.4 Polymorphisms of the FCER2 gene (Fc fragment of IgE, low affinity II, receptor for (CD23))

The FCER2 gene encodes a protein, which is the low-affinity receptor for IgE and a key molecule for B-cell activation and growth. FCER2 gene polymorphism was predicted to bronchial hyperresponsiveness and IgE-mediated allergic diseases. Within this gene three SNPs have been described, all connected to higher risk of severe asthma and asthma exacerbations in spite of inhaled corticosteroids therapy. The polymorphism 2206T→C occurs relatively often (allelic frequency 0.26 in Caucasians and 0.44 in black population) and was carefully analyzed for potential association to inhaled corticosteroids therapy response (Tantisira et al., 2007). The presence of the C allele increases three- to four-fold the risk of severe asthma exacerbations. This effect was confirmed both in Afro-American and Caucasian individuals being under iCS therapy.

2.3.5 Polymorphism of AC9 gene (cyclase adenylate 9)

Although adenylate cyclase is activated via the β2-adrenoreceptor it may also influence inhaled corticosteroids reaction. Individuals carrying the polymorphism Ile→Met 772 demonstrate increased bronchial dilatation after SABA when treated with corticosteroids compared to wild-type individuals (isoleucine in 772 position) (Tantisira et al., 2005). This substitution results in a loss of function. Met772 has lower basic as well as beta2-mediated adenylyl cyclase activities compared to Ile772.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphism with potential pharmacogenetic consequences during iCS therapy</th>
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<tr>
<td>CRHR1</td>
<td>17q12-22</td>
<td>rs242941 (G→T, intron)</td>
</tr>
<tr>
<td>TBX21</td>
<td>17q21.32</td>
<td>His→Glu33 (H33Q)</td>
</tr>
<tr>
<td>FCER2</td>
<td>19p13.3</td>
<td>2206T→C</td>
</tr>
<tr>
<td>AC9</td>
<td>16p13.3-13.2</td>
<td>Ile→Met 772</td>
</tr>
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Table 2. List of gene polymorphism examples that could have pharmacogenetic consequences during corticosteroids therapy

### 2.4 Pharmacogenetics of anticholinergic treatment

#### 2.4.1 Polymorphisms of the muscarinic receptor

Anticholinergics are used mainly in chronic obstructive pulmonary disease (COPD) but sometimes also in asthma as second line bronchodilators. Anticholinergics are antagonists of muscarinic receptors: M1, M2 and M3. SNPs have been found in coding regions of M2 and M3 receptors (Fenech, 2001). The expression of M2 and M3 receptors is dependent on transcription regulation in the gene promoter region and polymorphisms have been demonstrated in both promoter regions (Fenech, 2004; Donfack, 2003). Furthermore different expression of M2 receptor may be related to various number of dinucleotide CA repetitions in gene promoter region. None of these changes has been investigated in relation to bronchodilation in asthma or COPD yet.

### 3. Conclusions

Genome analysis, candidate gene studies and SNP investigation represent a new approach to pharmacological treatment in all chronic diseases. Genetically defined differences combined with clinical phenotyping lead to treatment personalization. At this point “personalization” means selecting different treatment regimens for different groups of patients. The more knowledge on pharmacogenetics and general genetics we have, the smaller these groups are likely to be.

iCS remain the mainstream therapy in asthma. Despite intensive research in this field there is only one biological treatment available for asthma and allergy (anti-IgE monoclonal antibody). Several other have been suggested and underwent pre-clinical or clinical tests, but to prove either their effectiveness and safety.

All the studies presented in this chapter have aimed at the identification and characterization of subgroups of asthmatic patients that will derive optimal therapy benefit while minimizing or eliminating drug side effects. The ultimate goal in the pharmacogenetics of antiasthmatic medication is to enable the optimization of individual therapies from the very start, maximizing efficacy without exposing patients to side effects. That would also significantly improve patient’s treatment compliance. The amount of clear data from pharmacogenetics of antiasthmatic drugs is still limited due, among others, to the vast number of genotype variants in different populations.
Combined research of the past decade seems to suggest that either asthma has several phenotypes distinct in terms of inflammatory mechanisms (eosinophilic versus neutrophilic, IL-17 dependent vs non-dependent etc.) or “asthma” is rather a group of respiratory diseases with similar symptomatology than a uniform disease. Computerized multivariate analysis has to be employed in the process of defining clinically relevant disease phenotypes of asthma before effective pharmacogenetic research can be undertaken.

4. References


GINA (Global Initiative for Asthma, updated 2010)


Pharmacogenetics of Asthma

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The rapidly evolving field of Pharmacogenetics aims at identifying the genetic factors implicated in the inter-individual variation of drug response. These factors could enable patient sub-classification based on their treatment needs thus expediting drug development and promoting personalized, safer and more effective treatments. This book presents Pharmacogenetic examples from a broad spectrum of different drugs, for different diseases, which are representative of different stages of evaluation or application. It has been designed so as to serve both the unfamiliar reader through explanations of basic Pharmacogenetic concepts, the clinician with presentation of the latest developments and international guidelines, and the research scientist with examples of Pharmacogenetic applications, discussions on the limitations and an outlook on the new scientific trends in this field.

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