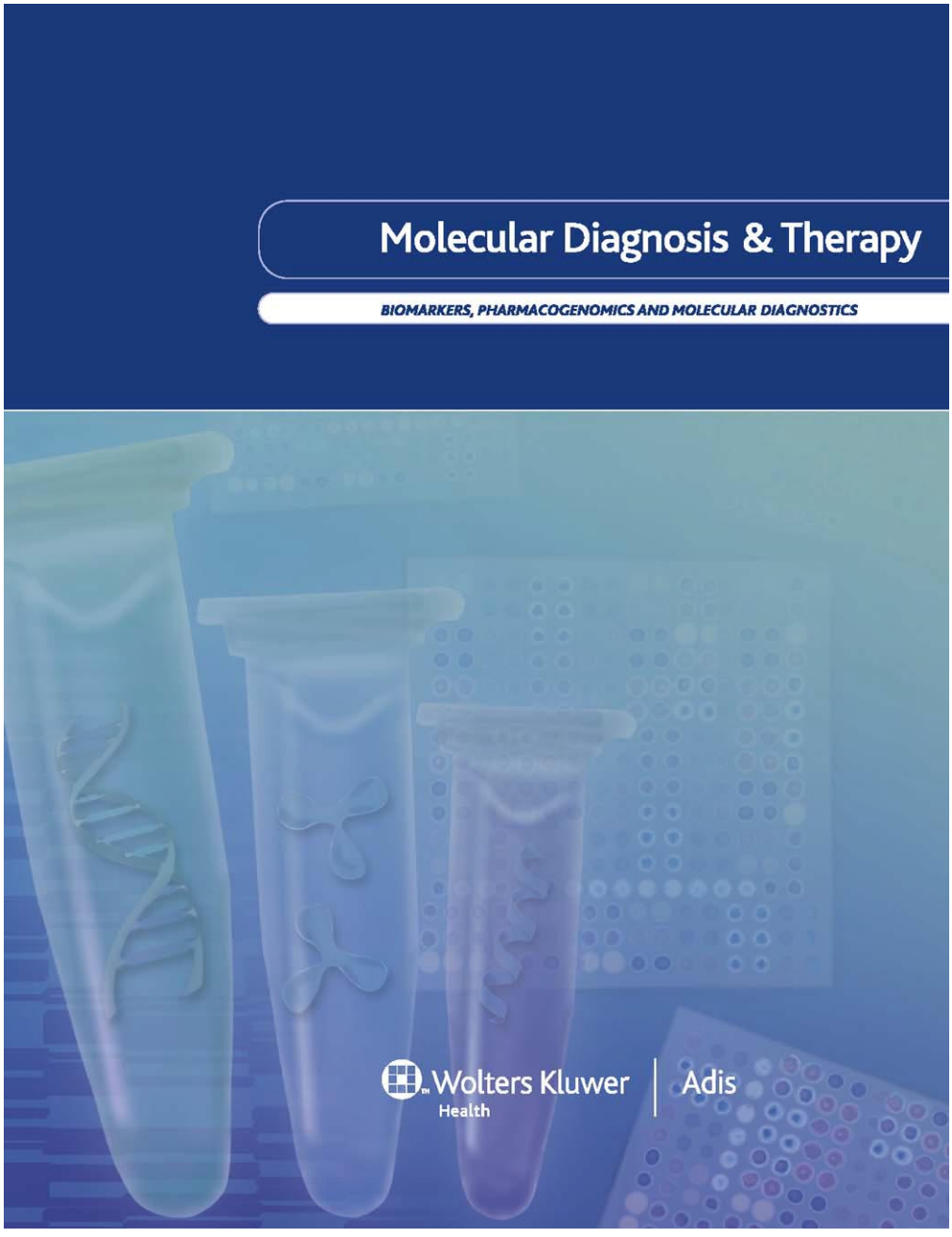

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Genetic Predictors of Response to Therapy in Childhood Asthma

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Contents

Abstract

Asthma is a common chronic condition in children, where the response to treatment can be heterogeneous within a population. Genetic variations may partly explain the inconsistent response to asthma treatment between individuals. There is a relatively small but consistent body of literature linking genetic variations to improved response to different classes of asthma treatment, including short- and long-acting β-agonists, corticosteroids, and leukotriene modifiers. In most cases, the advantage conferred by a single genetic mutation for treatment response is relatively small; the Arg16Gly single nucleotide polymorphism of the β2-adrenoceptor is the exception to this rule and is associated with a marked difference in response to short-acting β-agonists. Pharmacogenetic studies have only recently been undertaken in asthmatic individuals, and much more work is required before clinical applications arise. Future genome-wide association (GWA) studies and randomized controlled trials in genetically susceptible populations will determine whether asthma treatment can be tailored to an individual based on their DNA. The aim of the present paper is to review pharmacogenetic studies concerning asthma medications, with a primary focus on studies involving children.

1. Pharmacogenetics and Pharmacogenomics

Asthma is a common chronic condition that affects approximately 5 million people in the UK, including 1 million children.[1] There is a strong hereditary component to asthma causation, but environmental factors are also important.[2] There is no single genetic factor that causes asthma; rather, there are several genetic factors, each individually making a modest contribution to disease.[3] Childhood asthma is recognized as a complex condition where both genetic and environmental factors are involved.[2] There are many possible combinations of genetic and environmental factors implicated in asthma pathogenesis and the consequence of this is that the asthmatic phenotype is extremely heterogeneous; there is no

'typical' asthmatic. For example, two-thirds of asthmatic children are atopic and have coexisting allergic conditions such as hayfever, eczema, and food allergies, but approximately one-third are non-atopic. The natural history of childhood asthma is also heterogeneous; symptoms remit in some children as they grow older but symptoms develop in others as they approach adulthood.

In the same way that the asthma phenotype is heterogeneous within a given population, so the response to asthma treatment is also heterogeneous. Heterogeneity in response was demonstrated in the Pediatric Asthma Controller Trial (PACT), a double-blind, cross-over study where asthmatic children matched for all known confounders were treated with one of two asthma medications. Some children within the PACT population responded to both treatments but some only responded to one; response was evidenced as change in lung function[4] and level of symptoms.[5] It is probable that the variable response observed in the PACT study could be explained, at least in part, by genetic variants within the population, and this is the rationale for the study of pharmacogenetics in asthma. At the time of writing, no pharmacogenetics papers have arisen from the PACT study.

The concept that hereditary factors might influence response to treatment was proposed in the early 20th century by Archibald Garrod, and the term 'pharmacogenetics' was used by Friedrich Vogel in 1959.[6] Pharmacogenetics can be defined as the study of a *single* genetic variation that gives rise to differing therapeutic or adverse responses to drugs. Pharmacogenomics is an emerging science and can be defined as the study of *multiple* genetic variations that gives rise to differing therapeutic or adverse responses to drugs. The aim of pharmacogenetic and pharmacogenomic studies is to allow clinicians to predict which individuals will potentially benefit or come to harm from a given medication. Pharmacogenetic and pharmacogenomic studies use different methodologies but share a common underlying principle, and the two terms are often used interchangeably.

There are many instances of pharmacogenetics in practice. Warfarin is known to have considerable variability in dose-response and this is in part explained by variations in the genes coding for cytochrome P450 (*CYP2C9*) and vitamin K epoxide reductase complex subunit 1 (*VKORC1*).[7] In 1960, Evans et al.[8] described slow and fast metabolizers for isoniazid within a study population of 53 families. The difference was later attributed to differences in genetically-determined enzymatic acetylation rates in the liver. Variability in the response to β-adrenoceptor antagonists (β-blockers) is also in part due to genetic factors. A variation in the gene coding for the β1-adrenergic receptor may account for 20% of interpatient variability.[9] There are many more examples of genetic factors being important to drug response, and a comprehensive list of these can be found on the Pharmacogenomics Knowledge Base (PharmGKB) website (http://www.pharmgkb.org).

Genetic variations may influence the structure of a gene product (e.g. receptor or enzyme), resulting in altered drug pharmacokinetics (e.g. absorption, metabolism) and/or pharmacodynamics (e.g. binding to receptors). In general, most examples of pharmacogenetics involve altered pharmacodynamics with implications for toxicity in genetically susceptible individuals. With the exception of theophylline, asthma medications have a wide therapeutic index, and those genetic factors that might alter pharmacokinetics are currently not thought to be important in asthma treatment.

There is variable response to asthma treatment within a population, and genetic factors may account for as much as 80% of variability.[10] Better understanding of those genetic factors that affect treatment response is needed. Asthma pharmacogenetics has been studied for less than 20 years and remains a scientific field where the number of reviews exceeds the number of original papers. The study of asthma pharmacogenomics has an even shorter history, less than 10 years old, but already a number of challenges to the relevance of genetic factors to response to asthma treatment have been identified:

1. Asthma is a heterogeneous condition where there is no diagnostic test.
2. There is no 'gold standard' to measure response to asthma treatment.
3. Non-compliance may explain some apparent failure to respond to treatment.
4. The response to asthma treatment is often continuous, i.e. partial, and not dichotomous.
5. There is difficulty in establishing randomized clinical trials of sufficient sample size to detect the moderate effects of single polymorphisms on treatment response.

The aim of the present review is to describe what is currently known of pharmacogenetics and pharmacogenomics in childhood asthma. There are many more studies of asthma pharmacogenetics in adult populations and while many of these studies may be relevant to children, there are some important differences in asthma between adults and children. For example, asthma is much more common in women than in men, but in children it is more common in boys than in girls.[11] The present review will focus on pediatric studies but will mention studies from adult populations where no evidence is available from pediatric studies.

The present review will (i) provide an overview of how potential genetic factors are identified; (ii) describe genetic factors associated with response to treatment in asthmatic children; and (iii) briefly describe how future pharmacogenetic studies might be designed.

2. Identifying Genetic Factors – Finding the Needles in the Haystack

The human genome contains approximately 3 billion nucleic acid base pairs, where one million base pairs will include 12–15 genes.[12] The human genome is highly conserved, with less than 0.1% variation in DNA between two unrelated people. However, such is the size of the human genome, there are genetic variations occurring every 300 to 1000 base pairs,[13] or 10 million in total. The most common genetic variation (90%) occurs when a single nucleotide is substituted for another: a single nucleotide polymorphism (SNP). For example, a change from GAA to CAA will result in the amino acid glutamine appearing in the gene product instead of glutamic acid. A SNP can be described by one of two methods: the first describes the position and the substitution (e.g. Arg16Gly where the 'usual' or 'wild type' 16th amino acid in the β2-adrenoceptor, arginine, is replaced by glycine) and the second is a unique identifier called a 'RefSNP' or rs number. A variable number tandem repeat (VNTR), also known as a microsatellite, is a second, less common genetic variation, where a short sequence of nucleotides (between 10 and 100) is repeated 5–50 times. A VNTR where GAACAAGAACAA is repeated 10 times will result in a series of glutamic acid and glutamine amino acid molecules being inserted into the gene product. A third genetic variation is nucleotide deletion, which is relatively uncommon but more likely to have a profound effect on the protein coded, because each triplet downstream from the deletion will be wrongly read.

Over 90% of DNA is non-coding, i.e. does not have a gene product, though some of this noncoding DNA is important to regulation of RNA production. Therefore, approximately 90% of genetic variants are of little or no significance, and this has implications for genetic studies; the relevance of a genetic variation is uncertain until functionality is proven. An additional challenge in identifying important genetic variations is that many variations occur close to each other within a short length of DNA (i.e. are genetically linked and therefore inherited together), such that a genetic variation of apparent interest may indeed be functional, or may only be a marker for a truly important variation located close by.

There are essentially two strategies used to study the relationship between genetic factors and treatment response; gene-response (or candidate gene) studies and response-gene (or genome-wide) studies. Genome-wide studies can be subdivided into linkage-based and genome-wide association (GWA) studies. Some of the strengths and limitations of the two strategies are listed in table I.

Table I. Differences between candidate gene (gene-response) and genome-wide (response-gene) studies

Candidate gene (pharmacogenetic)	Genome-wide (pharmacogenomic)
Logical, hypothesis-driven	Not restricted to genes currently thought to be important
False-positive rate low	Many false positives
Does not consider influence of other genetic variations	Able to consider gene-gene interactions
Relatively low cost	Currently expensive
Small gene effects potentially missed	Better designed to identify modest gene effect

To date, candidate gene studies predominate and have investigated the potential role of variations in genes implicated in either asthma causation and/or response to asthma treatment. This is a straightforward, hypothesis-driven approach but cannot identify important genetic influences where none are suspected. The genome-wide approach makes no *a priori* assumptions; rather, it examines the whole genome looking for differences between affected and non-affected individuals. Genome-wide linkage studies look for areas of DNA variability within families that include affected and unaffected members. Since the family members are related and share common DNA, areas of DNA diversity are more likely to contain disease-associated genes. With this approach, the asthma gene *ADAM33* was identified on chromosome 20,[14] previously not a region of interest in genetic studies of asthma. GWA studies detect millions of SNPs in DNA from thousands of individuals who are participants in either family-based or case-control studies. The gene *ORMDL* was the first gene associated with asthma to be identified using GWA.[15] Inevitably, there is a large amount of data generated from GWA studies, including many false positive results and results that are truly positive but due to linkage; however, GWA studies are more able to detect genetic variations of moderate influence than genome-wide linkage studies. Additionally, the SNP of interest is identified in a GWA study whereas in a linkage study, further work is needed to identify the precise location of the important genetic variation(s).

In summary, detecting a genetic variation important to treatment response is challenging. The ideal genetic variation would have the following characteristics:

1. common, i.e. prevalence >10% in the general population;

2. located in coding DNA;
3. functionality proven;
4. influence on treatment response confirmed in several populations;
5. absence of linkage demonstrated;
6. a modest to large effect

In addition to these criteria, and arguably most important of all, the association must be confirmed in a clinical trial. For a comprehensive list of genes implicated in altered response to asthma treatment, the reader is directed to the Pharmacogenetics of Asthma Treatment website (www.pharmgat.org).

3. Pharmacogenetic Studies in Pediatric Asthma

There are at least three types of study designs with which to explore relationships between genetic variations and treatment response: (i) observational studies where groups categorized by genetic variation experience different outcomes after treatment with the same medication;[16,17] (ii) retrospective intervention studies where DNA is obtained after randomization to treatment/placebo;[18,19] (iii) prospective, randomized, control studies where treatment is assigned only to genetically susceptible/resistant individuals. The third study design is the gold standard but at the time of writing, only one such study has been published in an adult population with very mild asthma.[20]

The current literature will be reviewed by asthma treatment class. There are four classes of commonly used asthma medications: β-adrenergic receptor agonists (β-agonists), inhaled corticosteroids, leukotriene modifiers, and theophylline. Studies of relevance have been published for all classes except theophylline. Indications for prescribing different treatment classes are beyond the scope of the present review. The 2008 British Thoracic Society (BTS)/Scottish Intercollegiate Guidelines Network (SIGN) guideline[21] describes how these medications should be used in a step-wise manner in the context of increasing asthma severity.

3.1 β-Agonists

β-Agonists act at the β2-adrenoceptor (β2AR), which is located on the cell membrane. The two classes of β-agonists, short- and long-acting, differ by virtue of different affinities for the β2AR and also by being partial or complete agonists. Short-acting β-agonists (SABA) are the first-line asthma medication and the most common prescribed asthma medication. Approximately 5% of the entire UK pediatric population was prescribed a SABA in 2004.[22] Long-acting β-agonists (LABA) are used in more severe cases of asthma and were prescribed in 10% of children with asthma in 2004.[22] The *ADRB2* gene, coding for the β2AR, has at least nine variations (and possibly as many as 50), of which two are of greatest importance: SNPs at amino acid position 16 resulting in a substitution of arginine with glycine (Arg16Gly), and at position 27 where glutamine is substituted with glutamic acid (Gln27Glu). There appears to be at least two clinical consequences of genetic variations of the β2AR: first, altered SABA efficacy due to presumed altered binding with receptor, and second, altered down-regulation of β2ARs after prolonged treatment with β-agonists.

3.1.1 Short-Acting β-Agonists

In a study of 269 asthmatic and non-asthmatic children, individuals homozygous for *ADRB2* Arg16 were five times more likely to have a significant change in lung function after inhalation of a SABA (albuterol 180 μg) than those homozygous for Gly16.[16] The percentage of patients with a positive response to albuterol was intermediate in heterozygotes (figure 1). Approximately 15% of the population is homozygous for Arg16 and 40% for Gly16,[1] and thus this SNP is unusual by virtue of high frequency in the population and a large effect on phenotype. Arguably, no other SNP has subsequently been shown to have such a large effect on treatment response.

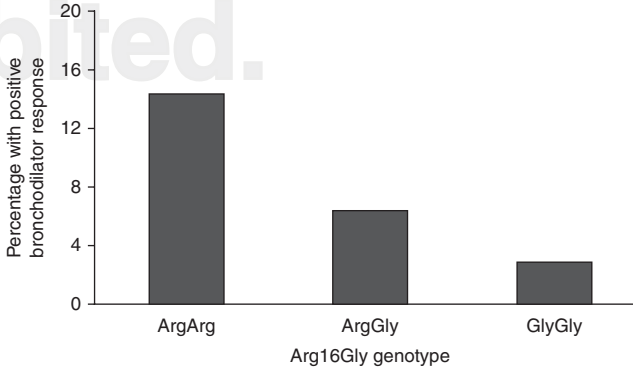

Fig. 1. Proportion of children with positive bronchodilator response categorized by *ADRB2* Arg16Gly genotype. Bronchodilator response was defined as ≥15.3% increase in forced expiratory volume in 1 second (FEV_1) after inhalation of albuterol 180 μg.[16]

1 By convention, the more frequent allele is termed the wild type, and is usually listed first in the SNP designation. However, with the *ADRB2* Arg16 Gly SNP, the Gly allele is more frequent than Arg. When the SNP was first described, the investigators thought the Arg was the wild type, and this categorization has remained despite the Gly allele being the 'true' wild type.

The Arg16Gly SNP is closely associated with response to SABA, but this relationship may be modified by other SNPs in the gene. The relevance of combinations (or haplotypes) of β2AR SNPs to SABA response was studied within a large cohort of asthmatic patients in the Childhood Asthma Management Program (CAMP).[23] CAMP was a study of 1024 mild/moderate asthmatic individuals aged 5–12 years.[24] Children were recruited from eight centers across the US in the late 1990s. Study participants were randomized to receive 4 years of treatment with either an inhaled corticosteroid (budesonide), inhaled cromone (nedocromil), or placebo. Phenotyping of the cohort included bronchodilator response (BDR, improvement in lung function after SABA treatment) and methacholine responsiveness (reduction in lung function after inhaling methacholine, an airway irritant). At a later assessment, study participants were genotyped and eight *ADRB2* SNPs were studied in relation to baseline lung function, BDR, and methacholine challenge.[23] When SNPs were studied in isolation, different SNPs were associated with response to bronchodilator and inhaled methacholine. In the haplotype analysis, three haplotypes were found to account for 90% of all haplotypes, confirming that the SNPs are in linkage.[23] Different haplotypes were associated with baseline lung function, BDR, and methacholine responsiveness. Haplotype analysis may be useful, but since the *ADRB2* SNPs are linked and the influence of the Arg16Gly SNP on BDR is so apparent, there is probably only marginal additional benefit to knowing the genotype in positions other than position 16 (i.e. Arg16Gly) in the context of BDR.

The potential of genotype to influence β2AR expression in the context of regular exposure to SABA has been demonstrated in a number of settings. First, an *in vitro* study of airway smooth muscle cells demonstrated that 24 hours of stimulation with a β-agonist resulted in 95% down-regulation of β2ARs in cells with the *ADRB2* Gly16 allele, 75% for the Arg16 allele, but only 30% for those expressing *ADRB2* Glu27.[25] Second, an Argentinian study of over 100 children measured BDR before and after 4 weeks of regular treatment with salbutamol.[26] Here, those children homozygous for Gln27 had attenuated BDR at the end of the study. A similar study in adults also found an association between the *ADRB2* genotype and respiratory outcome after 24 weeks of regular SABA treatment;[27] those adult asthmatic patients homozygous for Arg16 were at increased risk for serious exacerbations compared with other genotypes. The results of these two studies[26,27] are consistent since the Arg16 and Gln27 alleles are invariably coinherited. In a small study of 37 children admitted to an intensive care unit (ICU) for severe asthma and treated with nebulized and/or intravenous salbutamol, those homozygous for Gly16 were discharged from the ICU after a much shorter stay (a mean of 43 hours compared with 74 for those not homozygous for Gly16).[28]

Finally, in the only study of its type to date, Israel et al. completed a study where 16-weeks of treatment with SABA or placebo was randomized in individuals homozygous for Arg16 or Gly16.[20] The study population included 79 adults with mild asthma not requiring treatment with inhaled corticosteroids. While individuals homozygous for Gly16 had a favorable outcome when treated with regular SABA, those homozygous for Arg16 had more symptoms when randomized to SABA. In summary, the Arg16 allele seems to confer benefit in term of response to infrequent SABA treatment, but with regular/intensive SABA exposure, those carrying the Arg16 allele become less responsive; the underlying mechanism may involve receptor down-regulation.

The tone of the airway smooth muscle can be altered by stimulation of receptors other than the β2AR; for example, stimulation of the corticotropin-releasing hormone receptor (CRHR)-2 is associated with smooth muscle relaxation. The CAMP investigators used their dataset to test the hypothesis that genetic variations in the *CRHR2* gene are associated with altered BDR.[29] Of the 28 SNPs identified, three were associated with altered BDR within the CAMP study population. The authors sought to replicate their findings in two adult populations, and achieved this for one variant identified in the CAMP study (rs7793837) in one of the adult populations.

A third gene where SNPs may be associated with altered BDR is that coding for the enzyme arginase-1 (ARG-1).[30] ARG-1 activity is increased in asthma[31] and ARG-1 inhibitors may theoretically have a therapeutic role in asthma management.[32] In the CAMP population, one *ARG1* gene SNP (rs2781659) out of 64 tested was associated with BDR, and this was replicated in three confirmatory populations. There is the potential for variants of the *ADRB2*, *ARG1* and *CRHR2* genes to interact and influence SABA response; however, a study of such gene-gene interactions would require a very large number of participants.

3.1.2 Long-Acting β-Agonists

Down-regulation of the β2AR is thought to be relevant to tachyphylaxis with LABA use, i.e. enhanced down-regulation of receptors with continuous use. Individuals homozygous for Gly16 will be relatively resistant to tachyphylaxis since endogenous adrenalin will restrict the number of β2ARs. In contrast, individuals homozygous for Arg16 are likely to express more β2ARs and are theoretically more susceptible to harmful effects of receptor down-regulation after LABA use. This hypothesis is supported by a study of 546 asthmatic children in Scotland,

where those homozygous for Arg16 were twice as likely to have exacerbations as those homozygous for Gly16, and this risk rose to 3-fold if the child was treated with LABA.[17] There was some evidence that leukotriene receptor antagonist (LTRA) treatment protected Arg16 homozygotes who were receiving LABA from excessive exacerbations.[17]

Currently, LABAs are on the US FDA's top five list of most dangerous drugs. An adult study (the Salmeterol Multicenter Asthma Research Trial [SMART])[33] found excess deaths in those treated with LABA, and this prompted a 'black box' warning from the FDA. The number of unexpected asthma-related deaths was ten (13 in the group receiving LABA and three in the placebo group); a spoken presentation at the 2008 meeting of the American Thoracic Society reported no association between the Arg16Gly SNP and adverse outcomes within the SMART population. At the time of writing, there still remains concern about the wellbeing of children homozygous for Arg16 who are treated with LABA, although a recent study in adults found no increase in a number of adverse outcomes for Arg16 homozygoyes treated with LABA in combination with inhaled corticosteroids.[34]

3.2 Corticosteroids

Asthma is a steroid-sensitive condition, and treatment with inhaled corticosteroids is commonplace management in all but the mildest cases of asthma. Corticosteroids act by suppressing several pro-inflammatory immune pathways. Stimulation of the corticosteroid receptor, expressed at the cell nucleus, inhibits translation and transcription of pro-inflammatory mediators, including interleukin (IL)-4 and IL-5. Variations in gene coding for the corticosteroid receptor, and cytokines and their receptors, may plausibly influence response to corticosteroid treatment. There are at least four examples of genetic factors influencing response to inhaled corticosteroid treatment in children, all identified within the CAMP population.

Endogenous corticosteroids are produced in the adrenal gland under the regulation of the pituitary hormone adrenocorticotrophic hormone, the production of which is controlled by corticotrophin-releasing hormone (CRH). A study by Tantisira et al.[18] explored associations between three candidate genes and improvement in forced expiratory volume in 1 second (FEV_1) after starting treatment with inhaled corticosteroids. Importantly, the study also included patients from two adult populations in addition to CAMP; therefore, significant results are less likely to have arisen by chance or to be relevant to only one population. Of the three candidate genes, variants within the gene coding for the CRH receptor-1 (CRHR-1) were associated with improved lung function (FEV_1) in all three study populations. The investigators then looked for SNPs within the *CRHR1* gene and identified three in association with improved FEV_1.[18] One haplotype of three SNP variants, termed GAT, had a frequency of 30% and was associated with improved FEV_1. While lung function improved in all individuals after starting treatment with inhaled corticosteroids, children homozygous for the GAT haplotype had a 22% improvement in FEV_1 compared with 7% in those carrying no GAT haplotype. The GAT haplotype was only associated with improved FEV_1 in two study populations (including the CAMP population). Interestingly, in the study population where GAT was not associated with change in FEV_1, there was an association between one SNP of the *CRHR1* gene and improved FEV_1 that was not seen in the other two populations; one explanation for this apparent inconsistency is that genetic factors may not make a consistent contribution to treatment responses in every population. The mechanism for the *CRHR1* gene to influence response to corticosteroid treatment is not known. The authors[18] note that CRHR-1 may be important to CRH binding to mast cells, which are implicated in asthma pathogenesis. This study also points out the challenges in pharmacogenetics where response to treatment may be due to differences in SNPs between populations. Alternatively, these associations may be by chance and/or due to linkage with a third (functional) SNP.

T cells are important drivers of the inflammatory process seen in asthma, and the maturation of T cells is influenced by the transcription factor T-Box 21 (TBX21). A second study by Tantisira et al., again using the CAMP cohort, tested the hypothesis that a SNP (rs2240017; His33Gln) in the *TXB21* gene may affect response to corticosteroid treatment.[19] The hypothesis was based on studies undertaken in animals, where absence of the *TXB21* gene was associated with increased methacholine responsiveness. After treatment with inhaled corticosteroids, methacholine responsiveness in children homozygous for variants of the *TXB21* gene (comprising only 5% of the population) returned to normal non-asthmatic levels (figure 2). In contrast, methacholine responsiveness in the remainder of the population remained considerably abnormal compared with non-asthmatic patients.

A further pharmacogenetic study from the CAMP population looked at a potential interaction between the β2AR, treatment with inhaled corticosteroids, and BDR.[35] The rationale for the analysis was that β2AR stimulation initiates a pathway that requires activity of the enzyme adenylyl cyclase. Adenylyl cyclase activity can be upregulated with corticosteroid treatment. The authors hypothesized that a SNP in the

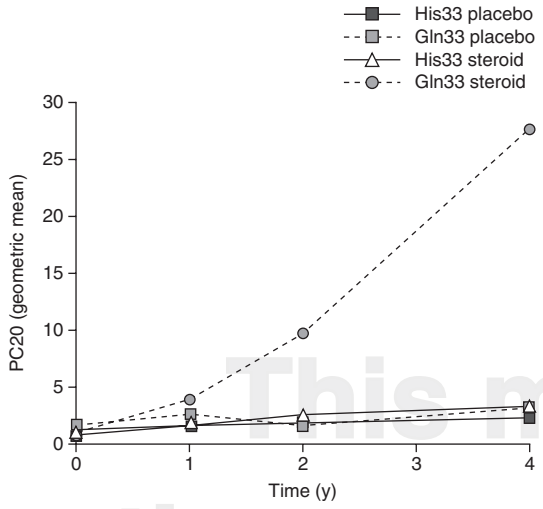

Fig. 2. Influence of the *TXB21* His33Gln SNP on response to treatment with inhaled corticosteroids, measured by airway responsiveness to inhaled methacholine (PC20, a hallmark for asthma). A higher PC20 corresponds with lower ('better') airway responsiveness. The mean PC20 of individuals treated with inhaled corticosteroids who also possessed a copy of the *TXB21* Gln33 variant (circles) improved significantly by the end of the first year of the study and continued to improve with time and ongoing corticosteroid usage. The mean PC20 value at 4 years for the Gln33 group treated with corticosteroids is within the range normally ascribed to individuals without the diagnosis of asthma. (Reproduced from Tantisira et al.[19] with permission. Copyright 2004 National Academy of Sciences, USA.)

gene encoding adenylyl cyclase 9 (*ADCY9*; also known as *AC9*) associated with increased enzymatic activity would only affect β2AR function (i.e. BDR) in association with corticosteroid treatment. Children with the Met722 variant allele in the *ADCY9* gene had increased BDR, but only if treated with corticosteroids. The Met722 allele is present in coding DNA and has 50% prevalence in the population; individuals who carry this mutation and have mild asthma symptoms but who do not appear to gain much relief from SABAs could benefit from treatment with corticosteroids.

The CAMP investigators explored the potential for inhaled corticosteroid treatment to confer increased risk of asthma exacerbations among genetically susceptible children.[36] The authors postulated that since increased serum IgE is associated with both inhaled corticosteroid treatment and asthma exacerbation, genetic variants in the IgE receptor gene (FCER2) may explain why some children have exacerbations despite corticosteroid treatment. One SNP (2206T/C), present in 25% of the population, was associated with a 3-fold increase in the likelihood of presenting to hospital with acute asthma compared with the wild type, but only in those randomized to inhaled corticosteroid treatment. Patients carrying the FCER2 2206C variant but randomized to other treatment were not at increased risk for hospitalization or attending the emergency department.

Corticosteroids have a wide spectrum of activity, and treatment response could plausibly be expected to be more complex than, for example, the response to SABAs. Studies that consider the influence of multiple genetic variations, rather than SNPs, are likely to yield insight into the complex mechanism of corticosteroid action in asthma. Corticosteroid resistance has been reported in adult asthmatic patients but is not a recognized problem in children, although pharmacogenetic studies may give insight into incomplete response to corticosteroids in children. On a practical note, the cost of detailed pharmacogenomic assays greatly exceeds the cost of a trial of an inhaled corticosteroid. For the foreseeable future, clinicians are likely to continue to using inhaled corticosteroids in children with asthma symptoms, blinded to knowledge of genetic factors that may predict treatment response.

3.3 Leukotriene Modifiers

Leukotrienes are phospholipids that are implicated in asthma causation. Leukotrienes stimulate bronchoconstriction and migration of eosinophils into tissues, and the pathway for their production is well described (figure 3). There are two classes of treatment directed at modifying the leukotriene pathway: LTRAs and 5-lipoxygenase inhibitors (5-LOIs). There are no pharmacogenetic studies relating to these drugs in pediatric populations, but these will be forthcoming given that some children have a better response to LTRAs than to inhaled corticosteroids, while other children do not respond to LTRAs at all.[4,5]

Studies in adults have looked at variants in the genes coding for 5-lipoxygenase (*ALOX5*) and leukotreine C_4 (LTC_4) synthase (*LTC4S*) and have tested the hypothesis that individuals with genetically-determined increased leukotriene production will benefit from treatment with leukotriene modifiers. There is a genetic variant (a VNTR) within the *ALOX5* gene that promotes transcription of the gene and is known to be

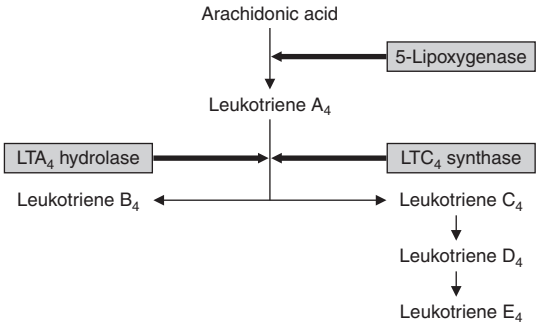

Fig. 3. The leukotriene pathway.

functional.[37] Individuals with the mutant allele associated with reduced gene product (5-lipoxygenase) failed to exhibit improved lung function after treatment with a 5-LOI compared with those who did not have the variant.[37] In contrast, in a second study[38] where individuals were treated with an LTRA, those carrying at least one mutant allele in the *ALOX5* gene promoter had less asthma symptoms after treatment with an LTRA than those with no mutant alleles. The treatment differed between the two studies (5-LOI[37,38] and LTRA[38]) and the results are therefore not directly comparable.

The study by Lima et al.[38] also looked at treatment response to LTRA in the context of polymorphisms in the *LTC4S* gene. Individuals with a SNP (rs730012) in the *LTC4S* gene promoter were at reduced risk for asthma exacerbations.[38] A third report has examined the relevance of candidate genes to response to leukotriene modifiers within two randomized, controlled studies.[39] The authors found that treatment with montelukast over 12 weeks was associated with a 2-fold increase in lung function among 10% of individuals with certain polymorphisms compared with the remaining 90% of the study population, who nonetheless also demonstrated some improvement relative to the placebo group.[39] These three studies[37-39] were conducted in relatively small populations where the frequency of genetic variations was low, but they nonetheless suggest that genetic factors are important to response to leukotriene modifiers.

The leukotriene pathway is a useful example of how gene-gene interactions may influence response to treatment. For example, mutations associated with increased 5-lipoxygenase and LTC_4S activity could act synergistically, and an individual with both variants might theoretically respond particularly well to leukotriene modifiers. Similarly, a 'favorable' mutation associated with increased LTC_4S activity could be irrelevant if genetic factors reduce 5-lipoxygenase activity further upstream. In this context, knowledge of a single SNP could be misleading when attempting to predict treatment response to leukotriene modifiers.

4. Future Directions

Response to treatment with asthma medications is an important but complicated outcome to measure in children. The present literature contains sufficient evidence to implicate genetic factors in response to asthma treatment. The literature also gives some insight into complexities in interpreting results from pharmacogenetic and pharmacogenomic studies. Gene-environment, gene-gene and treatment-treatment interactions and epigenetics (the study of factors that modify gene expression) may also be important to treatment response. Those studies that are able to consider the effects of more than one gene and polypharmacy are likely to yield novel insight into mechanisms for treatment response. These studies will need to be multicentered in order to enroll sufficient numbers of participants, who will have to be well characterized.

Once mechanisms are understood, prospective intervention studies in smaller populations will be designed to randomize treatment by each participant's genetic susceptibility to treatment response; these studies will determine the day-to-day practical benefit of pharmacogenetics. At present, there is no clinical application for pharmacogenetic and pharmacogenomic testing in children with asthma. In the future, as costs for DNA analysis fall, more treatment alternatives for asthma become available, and our understanding of how genetic factors influence response improves, then the clinician might be able to predict treatment response from a drop of blood.

5. Conclusions

This review of the current state of asthma pharmacogenetics in children can be summarized by the following take-home points:

- Pharmacogenomics and pharmacogenetics offer the potential to personalize asthma treatment, with implications for improved control and reduced adverse effects.
- Several genetic variations, each making a small contribution, influence an individual's response to most asthma treatments.
- To date, there is only one example of a single genetic variation with a relatively large influence on response to drug treatment, i.e. *ADRB2* Arg16Gly SNP and response to β-agonists.
- Prospective studies where participants are randomized to treatment/placebo by genotype are needed and will establish the role for pharmacogenetics/pharmacogenomics in asthma management.
- At present, there is not sufficient evidence to base clinical decisions on known genetic variations in asthmatic children.

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