Functional Genomics and Proteomics in Allergy Research

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Introduction

Asthma is a complex genetic disorder with a heterogeneous phenotype. With its rising incidence over the past three decades, asthma has emerged as an epidemic that currently affects nearly 155 million individuals worldwide [1–6]. The increase in asthma incidence encompasses all age and ethnic groups and, for reasons that remain unknown, the morbidity and mortality associated with asthma are disproportionately high among children, identifying asthma as the most common chronic disease in children in all developed countries. In the U.S., approximately 20 million people are diagnosed with asthma and, each year, the disease accounts for approximately 500,000 hospitalizations and nearly 5,000 deaths. Despite recent advances in medications used to treat the disease, the management of asthma is often complicated, and adequate control of symptoms is difficult to maintain [7,8]. Moreover, many of the drugs currently used to treat asthma (e.g., steroids, β-adrenergic agents, etc.) can have deleterious side effects and, in a number of individuals, medication overuse can actually lead to worsening of the disease. Thus, asthma represents a major health problem, particularly in children, and in order to ultimately develop more effective new strategies to treat the disease, a critical need exists to identify its underlying cause.

Although the cause of asthma is unknown, it is known to be attributed to the interactions between many genes and the environment and it has been suggested that genetics may contribute to as much as 60–80% of the inter-individual variability in therapeutic response to asthma medications. Numerous genetic studies have reported linkage or association with asthma and the asthma-associated phenotypes, atopy, elevated immunoglobulin E (IgE) levels, and bronchial hyper-responsiveness. In addition, specific alleles tagging cytokine/chemokine, remodeling or IgE regulating
genes have been shown to confer risk for these phenotypes. Although many studies reporting these observations are compelling, only a handful of genes have been uncovered that confer a meaningful risk of asthma based on the candidate gene or linkage approach. Moreover, the clinical implications of the genetic variations reported within the numerous candidate asthma genes with respect to asthma therapy remain largely undetermined.

The first major breakthrough in asthma discovery came through the cloning of the ADAM33 gene [9–11], a disintegrin that includes a metalloproteinase domain 33, now widely replicated within the asthma research community [9–12]. Discoveries of other asthma-related genes followed shortly, including that of DPP10 which encodes a homolog of dipeptidyl peptidases (DPPs) that cleave terminal dipeptides from cytokines and chemokines [13], PHF11, a PHD finger protein [9–11], and G protein-coupled receptor of asthma (GPRA) encoding the neuropeptide S (NPS)-NPS receptor 1 (NPSRI) [9–11]. These discoveries were fundamental to the notion that genetic/genomic factors may play key roles in the pathogenesis and pathobiology of complex disorders, such as asthma and atopy. These discoveries also stimulated interest in the study of gene–gene and gene–environment interactions as well as interest in re-sequencing these genes to pinpoint the actual functional/causative mutations involved. In addition, these variants may also influence treatment response.

It is widely accepted that common diseases, such as asthma, that have a strong but complex genetic component, together with variable drug response, are ideal targets for pharmacogenomic research [14–17]. Drugs that are in current use are not effective in all individuals, with relapse and severe adverse effects common in a high percentage of patients. The ability to analyze SNP patterns and expression levels of thousands of genes using oligonucleotide microarrays allows for a powerful screen of multiple molecular pathways simultaneously that may elucidate genes that determine drug response [18,19]. Generally, several genes are involved which, in conjunction with specific environmental factors, influence the efficacy of the drug response in some individuals and the potential for adverse events in others. In addition, the allelic interactions of the respective variants (i.e., SNP pattern) of the genes or gene pathways involved are highly complex and the resulting gene–gene and gene–environment interactions remain for the most part unexplained. Thus, it is no surprise that as many as two-third of patients with asthma may not attain full control of their symptoms despite modern therapies [20,21]. It also appears that about one-third of patients treated with inhaled corticosteroids (ICSs) may not achieve objective improvements in airway function or measures of airway reactivity [22]. A similar number of patients using oral corticosteroids develop osteoporosis [23–25]; cataracts and glaucoma are also reported side effects from ICS use [26–28]. In addition, approximately 5,000 asthma deaths occur in the US every year, which in large part is due to the use of long-acting β-agonists [29].

Drug responses vary widely between different populations and are also highly variable among individuals within the same population. A representative example is the observed response variability between asthma patients to β2-agonist therapy, where up to three quarters of the variability is genetically based, albeit different
among different ethnic groups [30]. Homozygosity for arginine at position 16 (the Arg/Arg genotype) of the β-adrenergic receptor predicts therapeutic response to β2-agonists in Puerto Ricans but not in Mexicans [31]. There is also evidence suggesting that variants in the β2-adrenergic receptor may explain differences in airway responsiveness in smokers versus nonsmokers [32]; this phenomenon is also evident in subjects using both ICSs and cigarettes [33]. Numerous candidate gene studies have been conducted in an attempt to unravel this mystery; however, the hunt for polymorphisms in candidate genes has not been productive thus far and the results from ongoing GWA studies in asthma are likely to fuel the interest of asthma investigators in the near future.

Polymorphisms can occur in coding and non-coding regions of genes, with their mechanism of action with respect to altered gene function generally remaining poorly understood. SNPs are by far the most commonly studied variants in pharmacogenetic/genomic studies [34]. Most disease associated variants are not expected to be directly functional themselves but instead are more likely to be in LD with the functional “smoking gun” mutations. Approximately 10 million SNPs are known to exist in the human genome, and they are stable over time [35, 36]. A different set of variants, known as “microsatellites,” constitute variable numbers of tandem repeats that may also produce functional changes or serve as markers for other changes in the genome. The potential effect of examining haplotypes, defined as varying combinations (similar to a barcode) of SNPs and/or variable numbers of tandem repeats over a linked region on a single chromosome, is also considered an informative way of studying disease susceptibility or drug response in pharmacogenomic association studies.

**Genetic/Genomic and Proteomic Studies in Asthma and Atopy**

Based on genetic, clinical, and epidemiological studies, asthma is well recognized as a common complex disorder with variable phenotype that is triggered by various environmental factors such as allergens, infectious agents, irritants, etc. [37–39]. As for other complex diseases, the genetics of asthma and atopy (its commonly related disorder) has been investigated using genome-wide linkage studies, with some followed by positional cloning, and candidate gene association studies. Many case-control studies have examined the association between one or more polymorphisms of a particular candidate gene and asthma/atopy phenotypes, with the candidate gene selected on the basis of its suspected role in the pathobiology of asthma or atopy. Such studies have included genes involved in innate immunity (e.g., toll-like receptors [TLRs], CD14, CARD15, etc.), inflammation (e.g., various cytokines, chemokines, etc.), lung function, growth and development (e.g., TGFB1, ADRB2, NOS1 and 3, SPINK5, etc.), and genes implicated as modifiers of responses to environmental exposures such as pollutants and tobacco smoke (e.g., GSTM1, GSTT1, and GSTP1) (see reviews [40–42]). Several studies have been consistent in demonstrating an association of asthma/atopy phenotypes with polymorphisms in
various candidate genes including the β2-adrenergic receptor gene (ADRB2) [43–45] and genes involved in the IL-4/IL-13 cytokine signaling pathway [46–49], which is importantly implicated in IgE switching and other immunoregulatory functions regulating atopic inflammation and asthma. Overall, however, the findings from the majority of these studies vary strikingly, and associations reported in some populations fail to be replicated in others. This discordance is not surprising given the phenotypic complexities of asthma and atopy, genetic background differences between the study populations, and the impact of the uncontrolled effects of environmental influences. Additionally, the findings in a number of studies may be complicated by type 1 errors and false positive results due to other differences between the study cases and controls (age, sex, race, etc.), genotyping errors, or insufficient control of multiple testing.

To date, approximately 20 genome-wide screens have been performed in different study populations to identify chromosomal regions that are linked to asthma/atopy and one or more of the related phenotypes of airway hyperresponsiveness (AHR), elevated IgE levels, and other allergy-associated phenotypic features. The findings from these studies (reviewed in refs [19,41,42,50,51]) also vary markedly, and are marred by inconsistencies due to such complicating factors as lack of sufficient statistical power, differences in study design, and inherent differences in the study populations. Overall, however, a number of these studies have been generally consistent in demonstrating linkage to certain chromosomal regions, identifying these loci as containing major genes influencing asthma and its associated phenotypes. These regions include genes whose transcripts are known to be biologically relevant in asthma, including the cytokine gene cluster on chromosome 5q, 11q (containing FcεRI-β), 12q (containing IFNγ and STAT6), and 16p (containing IL-4Rα). Moreover, in a study involving extended families with asthma in Iceland, significant linkage with an allele sharing lod score of 4.0 was demonstrated for an asthma susceptibility gene on chromosome 14q24 [52]. In other studies, suggestive linkage to asthma-related phenotypes also includes regions on 2q, 13q, and 6p (near the major histocompatibility complex [MHC]), and a genome screen of American families from different racial groups demonstrated weak linkage to broad regions on chromosomes 2q, 5q, 6p, 12q, 13q and 14q [19,40–42,50–53].

Linkage studies followed by positional cloning have recently identified some genes that had previously not been associated with asthma or atopy, including ADAM33, DPP10, PHF11, and GPRA. Two of these candidate genes, ADAM33 and GPRA, have generated considerable interest based on their potential roles in the pathobiology of asthma. ADAM33 encodes a disintegrin and metalloproteinase protein that mediates adhesion and proteolysis, and its detection in bronchial smooth muscle cells and in fibroblasts suggested its possible involvement in airway remodeling. In the original study, 19 SNPs in ADAM33 were found to have associations with asthma and AHR in affected sibling-pair families from the US and UK [9]. A series of replication studies that focused on a varying number of these SNPs were subsequently conducted in different study populations, including those from ethnically diverse backgrounds. The results for single SNPs and haplotypes have, in some
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studies, demonstrated impressive statistical significance. However, the SNPs used in many of these linkage studies have varied between the studies and, overall, the results have been inconsistent. Recently, a meta-analysis of all published studies demonstrated that, although the ST+7 variant of ADAM33 is significantly associated with asthma, the contribution of this locus to the risk of the population developing the disease is small [12]. The other candidate gene (for “GPRA”) was cloned following linkage association of asthma/atopy and elevated total serum IgE levels in Canadian and Finnish cohorts to seven common haplotypes spanning a 133-kb region on chromosome 7p15-p14 [11]. GPRA transcripts were subsequently detected in smooth muscle cells and in epithelial cells, with relatively enhanced expression in epithelial cells of asthmatic individuals [11]. Contrasting the original report, however, a subsequent study that genotyped a haplotype tagging SNP in a Korean population failed to find a significant association with the risk of asthma, atopy, total serum IgE, or AHR [54]. This failure of replication may be due to a variety of reasons that include differences in ethnic genetic background and environmental interaction, as well as the fact that only one haplotype tagging SNP was used in the latter study rather than a broader array of SNPs or haplotypes. As for ADAM33 and GPRA, further replication studies examining associations of the other candidate genes identified by positional cloning with asthma/atopy are ongoing, in addition to studies investigating the potential functional roles for these genes.

Recently, there has been a revolution occurring in SNP genotyping technology, with high-throughput genotyping methods allowing large volumes of SNPs ($10^3$–$10^6$) to be genotyped in large cohorts of patients and controls, therefore enabling large-scale GWA studies in complex diseases. Already with this technology, compelling evidence for genetic variants involved in type 1 diabetes [55–57], type 2 diabetes [57–61], age-related macular degeneration [62], inflammatory bowel disease [63], heart disease [64,65], and breast cancer [66] has been described. Even more recently, Moffat and colleagues reported the first GWA discovery in asthma [67]. In their study, they examined over 317,000 SNPs in 994 patients with childhood onset asthma and 1,243 non-asthmatics controls. They identified several markers on chromosome 17q21 that were reproducibly associated with childhood onset asthma in family and case-referent panels with a combined $P$ value $<10^{-12}$. In independent replication studies the 17q21 locus showed strong association with diagnosis of childhood asthma in 2,320 subjects from a cohort of German children ($P=0.0003$) and in 3,301 subjects from the British 1958 Birth Cohort ($P=0.0005$). They subsequently examined the relationships between markers at the 17q21 locus and transcript levels of genes in Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines from children who were being studied. The SNPs associated with childhood asthma were consistently and strongly associated ($P<10^{-22}$) in cis with transcript levels of ORMDL3, a member of a gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum. Accordingly, the study concluded that genetic variants regulating ORMDL3 expression are determinants of susceptibility to childhood asthma. Although these results need to be replicated by independent investigators, they present the first GWA results in asthma, with
significance level far exceeding any previous asthma gene study. It will be challenging but interesting to watch the translation work forthcoming on ORMDL3 in the near future [68]. Taken together, genes that have been associated with asthma and have been replicated in independent studies are shown in Table 1.

### Table 1: Asthma susceptibility genes established by replication and/or rigid-analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Variation</th>
<th>Phenotype</th>
<th>Odds ratios (95% CI)</th>
<th>Original study or meta-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAM33</td>
<td>20p</td>
<td>ST+7 allele</td>
<td>Asthma</td>
<td>1.46(1.21–1.76)</td>
<td>Van Eerdewegh et al. [9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F+1 allele</td>
<td>Asthma</td>
<td>1.20(1.00–1.45)</td>
<td>Van Eerdewegh et al. [9]</td>
</tr>
<tr>
<td>GPRA</td>
<td>7p15</td>
<td>GPRA-SNP</td>
<td>Asthma</td>
<td>2.25(2.00–3.10)</td>
<td>Laitinen et al. [11]</td>
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<tr>
<td></td>
<td></td>
<td>522363g&gt;c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD14</td>
<td>5q31</td>
<td>T-159 allele</td>
<td>Asthma</td>
<td>1.16(0.98–1.39)</td>
<td>Kedda et al. [112]</td>
</tr>
<tr>
<td>TNF</td>
<td>6p21</td>
<td>TNF-308A</td>
<td>Asthma</td>
<td>1.31(0.99–1.74)</td>
<td>Aoki T et al. [113]</td>
</tr>
<tr>
<td>B2AR</td>
<td>5q31</td>
<td>Gly16</td>
<td>Noct. Asthma</td>
<td>2.20(1.56–3.11)</td>
<td>Thakkinstian et al. [111]</td>
</tr>
<tr>
<td>ORMDL3</td>
<td>17q21</td>
<td>rs3894194</td>
<td>Asthma</td>
<td>1.68(1.25–2.26)</td>
<td>Moffatt et al. [67]</td>
</tr>
</tbody>
</table>

A Pharmacogenomic/Proteomic Perspective in Asthma and Atopy

The classes of anti-asthma medications that are available to patients include the bronchodilators, such as β₂-adrenergic agonists, and the anti-inflammatory agents, glucocorticoids (GCs) and leukotriene modifiers, with other drugs being rarely used. Pharmacogenomic studies on asthma are typically designed to determine whether the variations under study influence function with respect to these drugs. Most of these studies have been hypothesis driven and are based on a relatively small number of patients, thereby lacking power to assess factors that can confound genetic associations. A more broad-based non-hypothesis driven genome wide approach requires many more patients and is more costly; but is more likely to uncover novel variants in genes that influence or modify drug response. Thus, the GWA approach extends beyond the gene or pathway of interest and is used to screen for unknown disease or drug response variants. While these studies are in their infancy, it should be noted that a somewhat comparable approach was used to identify the association between the metalloproteinase gene, ADAM 33, and asthma [9]. To the extent that drug response is heritable, pharmacogenomics seeks to define the relationship between variability in the human genetic code and variability in response to pharmacologic interventions. Most studies to date have dealt with the signaling pathway from the receptor drug targets themselves, to the drug transporters and metabolizing enzyme cascades, focusing on the pharmacokinetic and pharmacodynamic characteristics of the drug in terms of clinical response measures (Fig. 1). The following section addresses the genetic diversity among individuals as it pertains to the receptor signaling pathways of the major drug classes used in asthma therapy.
β₂-Agonists

β₂-agonists are considered the first line therapy for bronchodilation and rapid relief from asthma symptoms [69]. β₂AR is also considered a putative candidate gene in the pathogenesis of asthma and related traits. Numerous studies have highlighted the important role of the airway smooth muscle in asthma [70, 71]. The sequence of β₂-AR has been known for many years and the effect of gene polymorphisms on the receptor functions has been thoroughly investigated [44, 72–77]. At least nine different point mutations have been found in the gene at nucleotide positions 46, 79, 100, 252, 491, 523, 1,053, 1,098 and 1,239 [73]. Four of these were found to cause changes in the encoded amino acids at residues at positions 16, 27, 34 and 164 with Arg16Gly and Gln27Glu being most frequent and showing most effect. Several studies have suggest a role for the β₂-AR in asthma pathogenesis [44, 74, 75]. Asthmatic children who are homozygous for Arg-16 have significantly greater (>fivefold) bronchodilator response to albuterol than homozygous Gly-16 individuals [44]. Similar results have been reported in multiple other populations suggesting they are real [74–77]. However, replication attempted in the Indian population reported exactly the opposite effect of these genotypes [78] and others have found either no difference between Gly-16 and Arg-16 receptor variants [70] or decrease in response in mild asthmatics carrying the homozygous Arg-16 genotype [79].

Regular use of β-agonist drugs has been reported to have detrimental effects on symptoms and lung function in double-blinded placebo-controlled studies [80]. Asthma patients carrying the Arg/Arg form may benefit by minimizing the use of both short-acting and long-acting β₂-agonists and Arg/Arg patients do not get benefit from the use of salmeterol, even when used concurrently with ICSs, and may develop worse airway function with chronic use of long-acting β₂-agonists [81]. Salmeterol may even provoke pro-inflammatory effect in Arg/Arg patients [81, 82]. Genotype–phenotype correlations may differ significantly across different ethnic groups as demonstrated by the association of the SNP at position 47 (Arg-19Cys)
with bronchodilator drug responsiveness in certain groups and not others [83]. Replication studies are needed to validate the differential role of this SNP on drug response in subjects of different ethnic background.

Reports suggest that 60% of asthma children who are homozygous for arginine at position 16 (Arg16/Arg16) may respond favorably to albuterol compared with only 13% in individuals homozygous for glycine at that position [84,85]. Others have not found such a striking difference, in studies including both pediatric [78] and adult patients [31]. In a study addressing haplotype diversity based on 13 SNPs in the β2-AR gene, different haplotypes were detected at the 5-prime end that differed significantly among different ethnic populations [72]. Interestingly, a relatively common haplotype that captured the Arg16 variant that was found to associate with decreased response to β2-agonists, showed the opposite effect in other cohorts [31,85], illustrating the important differences among subjects of different ethnic backgrounds. It is important to test for these variants in subjects who do not respond well to standard therapies, particularly if patients are using high doses of β2-agonists and controller medications and their asthma remains poorly controlled. The genetics of drug response traits is complex [86,87] and broader genomics approaches are needed to provide new insights into the molecular mechanisms of complex diseases and on how to optimize therapy for the individual patient.

**Leukotriene Modifiers**

The cysteinyl-leukotrienes, LTC4, LTD4 and LTE4, are lipoxygenase-derived eicosanoids and potent proinflammatory mediators that regulate contractile and inflammatory responses through G protein-coupled receptors. Cysteinyl-LTs have been causatively implicated in asthma and allergic rhinitis, and have also been shown to play a role in other inflammatory conditions, such as cardiovascular diseases, cancer and dermatitis. Variations of the promoter region of the 5-lipoxygenase (ALOX5) gene and the leukotriene C4 (LTC4) synthase gene have been well characterized and both have been associated with functional changes of these genes that affect drug response. Genetic variants have also been identified for the CysLT1 and CysLT2 receptors and are being examined in the context of asthma and atopy. Although several studies addressing the effects of variations in the LT pathway genes on responses to leukotriene modifier therapy have reported effects on drug response that may have clinical relevance, there are as many studies that have reported negative findings. Better powered studies are needed since meta-analysis on existing data is unlikely to sort this out.

**ALOX5**

The first committed enzyme in the leukotriene biosynthetic pathway is ALOX5. Several naturally occurring mutations are known to exist in the ALOX5 gene, including a variable number of tandem repeats in the promoter region of the gene.
that can modify transcription factor binding and reporter gene transcription. These microsatellites have been shown to code for the binding motif of the Sp1 and Egr1 transcription factors, thereby affecting the transcription rate of the gene [88]. Alterations in the number of tandem repeats have been shown to alter the efficiency of gene transcription such that any variation from the wild type decreased gene transcription at least in subjects with asthma [30]. Patients with mild-to-moderate asthma who were treated with a ALOX5 inhibitor and who carried at least one wild-type allele of the ALOX5 promoter locus were shown to have greater improvement in FEV1 than those without any wild-type alleles [89]. These data suggest that the absence of at least one copy of the wild-type allele creates a phenotype that is less responsive to leukotriene modifiers. While these results may sound intriguing with respect to pharmacogenetic applications, these variations account for only about 5% of the variability in response to leukotriene modifier therapy.

Leukotriene C4 (LTC4) synthase

The LTC4 synthase enzyme converts LTA4 to LTC4. The latter molecule is a critical mediator of the adverse reactions in aspirin-sensitive patients with asthma [90]. Substitution of A to C at the −444 site of the promoter of the gene is associated with three times the eosinophil-mediated LTC4 production in individuals with the wild-type genotype [91]. Asthma patients with variant LTC4 synthase genotypes receiving the leukotriene receptor antagonist zafirlukast for 2 weeks were found to have approximately 10% increase in FEV1, whereas patients with the wild-type genotype had a 12% reduction compared to the placebo group [91]. In contrast, no genotype effects were shown on AHR in patients on leukotriene modifier therapy [92]. As such, the observed differential response in FEV1 to leukotriene modifier therapy with respect to LTC4 synthase polymorphisms, suggests that this locus may help identify those who may benefit more from this therapy. Because variant LTC4 synthase genotypes are prevalent in patients with both aspirin-tolerant and -intolerant asthma [93], if the effects of this polymorphism are confirmed, its high prevalence may make it a useful predictor of response to this class of agents. Leukotriene-modifier drugs are widely used to treat asthma; however, there is growing evidence that the vast majority of asthma patients may not benefit from leukotriene antagonists when administered in combination with other therapies [94,95]. LTC4 receptor antagonist drugs have been found to be safe and well tolerated. In contrast, up to 5% of patients using ALOX5 inhibitors develop increases in liver function enzymes [96].

Corticosteroids

GCs are the most effective drugs available in asthma therapy [97]. In sensitive individuals, inhalation of GCs at doses <1,000 μg per day has been shown to have relatively little capacity to activate transcription within peripheral blood mononuclear
cells (PBMC) at concentrations found in plasma, and their action is thought to occur mainly within the lung [98]. This finding is in keeping with their relatively restricted systemic side effects at low or intermittent doses, whereas the repression of transcription factor activities, such as AP-1 and NF-B, in the airways concurs with their clinical efficacy in glucocorticoid sensitive (GC-S) patients [98]. In contrast, glucocorticoid resistant (GC-R) patients may suffer serious side effects because of escalation of drug doses caused by hypo-responsiveness. GC resistance has been defined as the lack of a response to a prolonged course of high-dose (0.5–1.0 mg/kg per day) oral GC [99,100]. Two forms of GC-R asthma have been reported, primary and acquired types [101–103]. The acquired form (type I) has been associated with abnormally reduced GC receptor ligand and DNA binding affinity, whereas type II GC-R asthma has been associated with primary GC receptor binding abnormality. In both forms, there is lack of GC-mediated inhibition of expression and release of molecules in PBMC, including the cytokines, interleukin (IL)-13, and IL-4 [101–103].

Modern asthma therapy is largely centered on ICSs with vast majority of patients demonstrating favorable response to therapy [104]. ICSs have been shown to mediate multiple beneficial effects in individuals with asthma but are also associated with multiple adverse effects. The mechanisms of action of ICSs are complex and remain incompletely characterized and only few pharmacogenomic studies have been reported. A candidate gene study in three study populations suggested a relationship between the response to ICSs and a polymorphism in the corticotropin-releasing hormone receptor 1 (CRHR1) gene [105]. Polymorphisms in CRHR1 were positively associated with significantly improved lung function after 8 weeks of ICS therapy. A haplotype in 27% frequency (GAT) showed modest increase in FEV₁ in response to ICSs in homozygous subjects in two out of the three populations whereas a single SNP correlated with similar improvement in the third population. The association of different SNPs in the same gene with changes in lung function suggests that the actual causal variant in CRHR1 remains to be discovered but that the three variants studied are imperfectly correlated markers in LD with a causal polymorphism. However, it is too early to tell whether the CRHR1 polymorphisms will be useful clinical predictors of response to ICSs.

A functional variant in the gene coding for transcription factor T-bet (T-box expressed in T cells) was recently reported by the same group [105], a finding that may be able to predict responsiveness to ICSs. A variant in the TBX21 gene associates with significant improvement in methacholine responsiveness in children with asthma who are being treated with ICSs. However, the minor allele frequency for this mutation (H33Q) is only 4.5%, suggesting that although the effect of the mutation may be large, it may only affect a small number of individuals.

In a study applying a high-density oligonucleotide microarray approach to search for differences in mRNA expression profiles in PBMC, from GC-S and GC-R asthma patients, gene expression was examined at baseline (resting PBMCs) and following treatment with a combination of IL-1β and TNFα [106]. In an attempt to further unveil genes that contribute to responsiveness of GC, in vitro effects of GC treatment on gene expression were compared in cells that were
activated with IL-1β and TNFα. The rationale for this strategy was based on the concepts, that the manifestations of asthma are, at least in part, channeled through the actions of IL-1β and TNFα [107,108], and that the efficacy of GCs in asthma is, at least in part, through its effect on the expression of genes that are modulated by proinflammatory cytokines [107]. The authors showed that GC responders could be separated from nonresponders with over 80% accuracy, by using the expression levels of only a few genes. The gene encoding the NFκB DNA binding subunit (NFKB1) was shown to confer the best predictive ability. A large number of genes are being translated after NFκB activation, including cytokines, chemokines, growth factors, cellular ligands, and adhesion molecules, many of which have been strongly associated with asthma and most of which react briskly to glucocorticoid therapy in sensitive individuals. Indeed, the efficacy of GC drugs in asthma is, at least in part, related to their efficacy in inhibiting transcription factors such as NFκB. Thus, NFκB is an exciting pharmacogenetic candidate and a growing body of evidence suggest it may be among the key culprit candidates in asthma [109,110].

Genomics/Proteomics Efforts in Pediatric Asthma and Atopy

Several Universities and Institutes have put together efforts to sample and store biological specimens for future genomic/proteomic research of complex medical disorders. At the Children’s Hospital of Philadelphia (CHOP), we recently launched a high-throughput pediatric genomic center, the Center for Applied Genomics (CAG), which is directed at high-throughput genetic/genomic analyses in children, with genotyping throughput of hundreds of DNA samples per day, on the high-resolution SNP genotyping platforms. The program was established with the aim of genotyping over 100,000 children in a couple of years, with major emphasis on asthma and other inflammatory disorders. The facility is coupled to electronic medical records with the health care network at CHOP for those patients who volunteer to participate. All personal information and data, including both phenotypes and genotypes, are thoroughly encrypted to ensure de-identification of the research. Over 40,000 subjects have been genotyped in the past 15 months at a SNP density of 550,000 per sample or higher. The diseases that are being examined include some of the most common complex pediatric disorders, including the inflammatory diseases, asthma, IBD, T1D, SLE, JIA and atopic dermatitis, in addition to obesity, attention deficit hyperactivity disorder and autism to name a few. In addition, extensive effort has been devoted towards high-resolution mapping of copy number variations (CNVs) in “healthy” individuals, wherein several thousand subjects and family trios have been examined, in order to better define “normal” CNVs of the genome, rendering it easier to assess both de novo alterations, as well as novel heritable CNVs, based on the family trios analysis, and addressing the role these variations play in disease. Finally, PBMCs from all patients are being harvested for future proteomic biomarker research that will be guided by the genomic results,
including through EBV cell lines that are genotyped at high-density and have a wealth of phenotypic information.

Since several of the diseases under study manifest themselves as inflammatory disorders (i.e., Asthma, IBD, JIA, T1D, SLE, AD) where the same underlying cell type may be involved in the pathogenesis, albeit in different organ systems, the notion that there may be a final common pathway involved that underlies the cellular perturbation in these disorders is highly compelling. Thus, an effort is underway directed at addressing the genetic/genomic/proteomic factors involved in these disorders “collectively.” This is likely to bear fruit, given the recent advances in the technology platforms that have made gene discovery highly robust. Thus, by applying a GWA approach to address the causes of some of these most common and complex diseases that we are challenged with every day, and we currently treat empirically, discovery can be made not only on those genetic factors that are specific for diseases such as asthma, but more importantly also on those factors that are common among many related genetic/inflammatory disorders. Moreover, apart from unveiling the mechanisms of these diseases themselves, a project of this size and scope is also in a position to dissect out the environmental factors that interact with the disease genes and constitute a gene-environment network that may underlie complex diseases and also address the pharmacogenomic and proteomic opportunities for those subjects who harbor these variants, through which we will establish biomarkers that will identify those who are most likely to benefit from a given therapy.

**Summary**

Asthma is a complex disorder with multiple phenotypes where multiple genes and environmental factors act together to cause the disease. Several genes have been associated with asthma, albeit, only a handful have been replicated in independent studies. The recent advances in genotyping technology, coupled with the fundamental information provided by the Human Genome and International HapMap Projects have revolutionized our abilities to search for disease genes. Although multiple laboratories are using the new genotyping approach, only one such study has already delivered significant findings [67].

Pharmacogenomics is a developing field with the principal objective of dissecting the effects of genetic variations on human drug responses. Until recently, pharmacogenetic studies were usually limited to investigations of a single polymorphism/gene (such as the B2AR gene) in small groups of individuals. With the development of GWA studies taking over the candidate gene and family-based linkage approaches, the results are expected to unveil the inter-individual variations that underlie differential drug response. It is anticipated that the new generation of drugs and diagnostics resulting from these efforts will lead to a major paradigm shift from conventional medicine to efficient predictive medicine.

The powerful combination of GWA coupled with ultra-high-throughput micro-array genotyping platforms, gene expression technologies, innovative bioinformatics,
and computational biology approaches is bringing such knowledge closer to reality as these integrative strategies enable scientists to pinpoint disease-causing gene pathways that may also influence differential responses to drugs. With optimal use of the HapMap dataset, future GWA studies conducted on large cohorts and replicated in different populations will uncover most major genes that confer disease susceptibility. The incorporation of pharmacogenetic data into clinical practice will guide risk assessment and treatment decision, and thereby revolutionize the practice of medicine for complex medical disorders such as asthma.

Future Perspective

With the successful completion of genome wide association (GWA) studies, numerous loci have been identified that associate with complex medical disorders. In order to pinpoint a disease mutation, resequencing of the genomic loci presents a natural extension. Ultra high throughput bi-directional resequencing of the corresponding linkage disequilibrium (LD) blocks (averaging 50 kb in Caucasians) for all candidate loci in genomic DNA derived from both cases and controls harboring the key SNP alleles and/or haplotypes that associate with the disease phenotype under study will enhance the chances that causal variants are identified and provide unprecedented information to fully understand and interpret the regions under study, unveiling the underlying causative mutations. Validation of SNP genotypes via direct sequencing will verify any newly discovered sequence variants directly by sequencing in both directions. New single nucleotide insertion or deletion alterations discovered during resequencing need to be analyzed in the context of the existing SNP data. The high-throughput sequencing systems available from Illumina, Roche and ABI allow for the sequencing of billion(s) of bases (1 Gb) per run in a matter of days. This represents a major advance in sequencing technologies as more established methodologies, such as capillary-based platforms, require many years to generate the same amount of data. No doubt, the whole genome sequencing approach will have a stunning impact on the practice of medicine within the next 5 years.

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