Genetic polymorphisms and associated susceptibility to asthma

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Abstract: As complex common diseases, asthma and allergic diseases are caused by the interaction of multiple genetic variants with a variety of environmental factors. Candidate-gene studies have examined the involvement of a very large list of genes in asthma and allergy, demonstrating a role for more than 100 loci. These studies have elucidated several themes in the biology and pathogenesis of these diseases. A small number of genes have been associated with asthma or allergy through traditional linkage analyses. The publication of the first asthma-focused genome-wide association (GWA) study in 2007 has been followed by nearly 30 reports of GWA studies targeting asthma, allergy, or associated phenotypes and quantitative traits. GWA studies have confirmed several candidate genes and have identified new, unsuspected, and occasionally uncharacterized genes as asthma susceptibility loci. Issues of results replication persist, complicating interpretation and making conclusions difficult to draw, and much of the heritability of these diseases remains undiscovered. In the coming years studies of complex diseases like asthma and allergy will probably involve the use of high-throughput next-generation sequencing, which will bring a tremendous influx of new information as well as new problems in dealing with vast datasets.

Keywords: genome-wide association study, high-throughput next-generation sequencing, allergy, environmental irritant, allergen

Introduction

Asthma is a chronic inflammatory condition of the lungs characterized by excessive responsiveness of the lungs to stimuli in the forms of infections, allergens, and environmental irritants.

Due to the variability of the disease and lack of generally agreed-on standards for diagnosis, it can be difficult to estimate the prevalence of asthma. Further, variations in practice from country to country complicate worldwide estimates. In the USA, it is estimated that at least 22.9 million Americans suffer from the condition. Asthma is the leading chronic illness in US children, with 6.8 million affected in 2006.¹ It is estimated that 300 million individuals suffer from asthma worldwide, with increased prevalence in both adults and children in recent decades.² Prevalence is rising in locations where rates were previously low and variation in rates from country to country appears to be diminishing.³ Twin studies have shown that there is a genetic element to asthma susceptibility, with heritability of the condition estimated at between 36% and 77%.³,⁴ Since the publication of the first study linking a genetic locus to asthma in 1989, more than 100 candidate genes have been reported in connection to asthma or asthma-related phenotypes such as bronchial hyperresponsiveness and elevated levels...
of serum immunoglobulin (Ig) E. Initial studies were usually candidate-gene analyses, examining the role of specific loci in asthma in a hypothesis-based manner. A few loci were identified in a hypothesis-independent manner through traditional linkage analysis. Recently, the application of genome-wide association (GWA) studies has led to the hypothesis-independent identification of a much larger list of loci associated with asthma.

Functional categories revealed through genetic analyses
Before describing the loci identified through various study designs, it would be useful to summarize the findings of the last 25 years of genetics research in asthma. The numerous genome-wide linkage, candidate gene, and GWA studies performed on asthma and asthma-related phenotypes have resulted in an increasingly large list of genes implicated in asthma susceptibility and pathogenesis. This list can be categorized into broad functional groups, from which several themes have emerged (reviewed previously).

T_\text{H}_2-mediated cell response
Given the appreciation of asthma as a disease of dysregulated immunity and its connection to atopy and allergic disease, it is perhaps unsurprising that genes controlling the development and regulation of the immune response have been implicated in asthma. T helper (T\text{H}_2) cell-mediated adaptive immune responses have been widely recognized as a crucial component of allergic disease. Pathways involved in T\text{H}_2 cell differentiation and function have been extensively studied in asthma candidate-gene association studies. Additionally, single nucleotide polymorphisms (SNPs) in many of these genes have been associated with asthma and other allergic phenotypes. Genes important for T\text{H}_1 versus T\text{H}_2 T cell polarization, such as GATA3, TBX21, IL4, IL4RA, STAT6, and IL12B, have been implicated in asthma and allergy.\textsuperscript{9,20} The genes encoding interleukin (IL)-13 and the beta-chain of the IgE receptor FceRI are well-recognized contributors to asthma susceptibility.\textsuperscript{10,12,21–24}

Inflammation
Unsurprisingly, several genes involved in inflammation have been associated with asthma. Genes for the cytokine IL-18\textsuperscript{25} and its receptor IL18R\textsuperscript{13} have been implicated, as has the general mediator of inflammation tumor necrosis factor alpha.\textsuperscript{27} Molecular mediators of inflammation have also been implicated, with the identification of leukotriene C4 synthase and other enzymes involved in the generation of leukotrienes, such as ALOX-5.\textsuperscript{28–30}

Environmental sensing and immune detection
A second class of associated genes is involved in the detection of pathogens and allergens. These genes include pattern-recognition receptors and extracellular receptors, such as CD14, toll-like receptor (TLR) 2, TLR4, TLR6, TLR10, and intracellular receptors, such as nucleotide-binding oligomerization domain-containing 1 (NOD1/CARD4).\textsuperscript{31–36} Additional studies have strongly associated variations in the human leukocyte antigen (HLA) class II genes with asthma and allergen-specific IgE responses.\textsuperscript{21}

Airway remodeling
A variety of genes involved in mediating the response to allergic inflammation and oxidant stress on the tissue level appears to be an important contributor to asthma susceptibility. Examples include a disintegrin and metalloproteinase domain-containing protein 33 (ADAM33), which is expressed in lung fibroblasts and smooth muscle cells; the alpha-1 chain of a specific collagen (COL1A1); DPP10, a potentially inactive serine protease; and G protein-coupled receptor for asthma (GPR41), activation of which upregulates metalloproteinase expression in the lung.\textsuperscript{37–40}

Bronchoconstriction
Acute asthma episodes involve constriction of the airways. Genes encoding proteins involved in this process have been identified as susceptibility loci for asthma. These loci include CHRNA3/5, which encodes a receptor for acetylcholine; PDE4D, which encodes a phosphodiesterase with enzymatic activity that generates molecular mediators of smooth muscle cell constriction; and NOS1, which encodes a nitric oxide synthase.\textsuperscript{41–43}

Epithelial barrier function
Studies of asthma genetics have raised new interest in the body’s first-line of immune defense, the epithelial barrier, in the pathogenesis of asthma. Mutations in the filaggrin (FLG) gene were initially identified in the rare single-gene disorder ichthyosis vulgaris;\textsuperscript{44} however, loss-of-function variants were reported subsequently to be strongly associated with atopic dermatitis, eczema, and asthma, both dependent on and independent of atopic dermatitis.\textsuperscript{45–48} Filaggrin, a protein involved in keratin aggregation, is not expressed in the bronchial mucosa,\textsuperscript{49} which has led others to suggest that asthma susceptibility in patients with loss-of-function FLG variants may be due to allergic sensitization that occurs after breakdown of the epithelial barrier.\textsuperscript{50} Several epithelial
Genes with important roles in innate and adaptive immune function have also been implicated in asthma. These genes include defensin-beta1 (DEFB1; an antimicrobial peptide), uteroglobin/Clara cell 16-kD protein (CC16) (an inhibitor of dendritic cell-mediated T_{h}2-cell differentiation), and several chemokines (CCL-5, -11, -24, and -26) involved in the recruitment of T cells and eosinophils.31–37

Overview of genetic analyses of asthma

Most of the published reports examining genetic contributions to asthma have been candidate-gene studies. Over 100 loci have been associated with asthma through candidate-gene studies, in which specific genes are investigated for their involvement in the phenotype based on their suspected roles or plausible hypothetical contributions to disease. The loci identified in candidate-gene studies of asthma and associated phenotypes have been extensively reviewed elsewhere.38–40 Among the genes identified in candidate studies are various cytokines and cytokine-signaling proteins involved in T cell survival, proliferation, and differentiation; genes involved in lung function, development, and response to stimuli; receptors for detection of microbial products; genes involved in epithelial barrier function and innate immunity;41,43 and molecules involved in responses to the environment.44–46 Genes that have been extensively replicated include the beta2 adrenergic receptor (ADRB2) gene;47–49 the cytokines, receptors, signaling proteins, and transcription factors involved in T_{h}1 and T_{h}2 differentiation of T cells, such as IL4, IL4RA, IFNG, IFNGR1, STAT6, GATA3, and TBX21;13,14,15,17–20 and genes involved in the cellular responses that characterize atopic disease, such as IL13 and FCER1B.12,13,22–24 Many genes identified through candidate-gene studies have failed to be replicated, either because replication has never been attempted or due to failure of replication in subsequent experiments. Failure of replication is a considerable complication in the genetic analysis of asthma.8 Genes that have been well replicated in candidate-gene studies examining asthma are summarized in Table 1.

Genome-wide linkage studies rely on families of affected and unaffected individuals and use the differentially shared regions of inherited chromosomes to track genetic markers that segregate with the disease status. Genes within disease-associated regions become candidates for further study or for positional cloning of the disease-causing variant. Linkage studies are hypothesis-independent experiments, allowing for the identification of truly novel and previously unsuspected disease-associated variants. Due to the requirement for large family cohorts, genome-wide linkage studies can be difficult and expensive to perform, and are often sufficiently powered to detect only variants with large effects. Linkage studies have identified multiple well-replicated chromosomal regions that contain genes of biological relevance to asthma and allergic disease, including the cytokine cluster on chromosome 5q (containing IL3, IL5, and granulocyte/macrophage colony-stimulating factor [GMCSF]), FCER1B on 11q, interferon g (IFNG) and STAT6 on 12q, and IL4R (the IL-4Ra chain, also part of the IL-13R) on 16p. Linkage studies followed by positional cloning have identified a comparatively small set of novel asthma susceptibility loci, including CYFIP2,67 DPP10,68 HLAG,69 PHF11,70 GPRA,71 and ADAM33.72 As molecules with plausible (and potentially drug-targetable) roles in the lung pathology of asthma, GPRA and ADAM33 have generated considerable interest.73 Genes identified through genome-wide linkage analyses are summarized in Table 2.

The availability of high-density genotyping arrays and comparatively low costs of applying such technology to increasingly large patient and control cohorts have led to the development of a third kind of genetics experiment: the GWA study. Large numbers of SNPs can be screened in large numbers of individuals and assessed for association with a disease state. As with linkage analyses, GWA studies are hypothesis-independent study designs, allowing the discovery of the contributions of novel loci. Currently, more than 30 GWA studies have been published using asthma, allergy, or related phenotypes such as serum IgE levels or blood eosinophil counts as endpoints. Many of these reports do not report any loci that reach the required level of statistical significance to be considered true GWA results. However, the reports of suggestive associations are valuable, as are reports of failures to replicate previously published results. The loci identified through GWA studies that have reached high statistical significance are summarized in Table 3. This list has grown rapidly in the last few years, as the arrays available for genotyping provide more SNPs for analysis and as researchers collaborate to assemble larger and more completely controlled cohorts to add more statistical power to their analyses.

The first GWA study that focused on bronchial asthma as an endpoint was reported in 2007.74 Markers on chromosome 17q21 were reproducibly associated with childhood-onset asthma. The findings were replicated in German and British cohorts. Independent replication of the 17q21 association has been reported in multiple populations of
diverse ethnic backgrounds. This locus contains the genes ORMDL3 and GSDMB and variable expression of both was linked to asthma susceptibility.

A case-control GW A study of North American asthmatics of European ancestry from the Childhood Asthma Management Program (CAMP) cohort has also been reported. The strongest association found was to variants of the PDE4D gene on chromosome 5q12, which encodes a bronchially expressed phosphodiesterase. The association of PDE4D to asthma was not observed in individuals of African descent. In a separate study, GW A data from the CAMP cohort was investigated for replication of previously reported candidate-gene associations. Thirty-nine genes were investigated with five SNP-based associations replicating to a nominal significance in the IRAK-3, PHF11, IL10, ITGB3, ORMDL3, and IL4R genes. Another GWA study on allergic asthma in children 6 years of age has recently been reported. No single SNP achieved genome-wide significance, but one SNP in an intron of PDE11A was cited as potentially interesting. PDE11A encodes a phosphodiesterase related to PDE4D, suggesting that this family of proteins may play a broader role in asthma pathogenesis.

### Table 1 Well-replicated loci identified through candidate-gene studies

| Gene   | Chromosomal locus | Function                                                                 |
|--------|-------------------|--------------------------------------------------------------------------|
| IL10   | 1q31-q32          | Cytokine – immune regulation                                              |
| CTLA4  | 2q33              | Control/inhibition of T cell responses/immune regulation                 |
| IL13   | 5q31              | induces T<sub>H</sub>2 effector functions                                 |
| IL4    | 5q31.1            | T<sub>H</sub>2 differentiation                                             |
| CD14   | 5q31.1            | Microbe detection – recognizes pathogen associated molecular patterns     |
| HAVCR1 | 5q33.2            | T cell responses – hepatitis A virus receptor                             |
| LTC4S  | 5q35              | Leukotriene synthase – inflammatory mediator                              |
| LTA    | 6p21.3            | Inflammatory mediator                                                    |
| TNF    | 6p21.3            | Inflammatory mediator                                                    |
| HLA-DRB1| 6p21             | Major histocompatibility complex class II – antigen presentation           |
| HLA-DQB1| 6p21             |                                                                           |
| HLA-DPB1| 6p21             |                                                                           |
| FCER1B | 11q13             | Receptor for IgE – atopy                                                  |
| IL18   | 11q22.2-q22.3     | Inflammation                                                             |
| STAT6  | 12q13             | IL-4 and IL-13 signaling                                                 |
| CMA1   | 14q11.2           | Chymase – mast cell expressed serine protease                            |
| IL4R   | 16p12.1-p12.2     | Alpha chain of receptors for IL-4 and IL-13                               |
| FLG    | 1q21.3            | Epithelial integrity and barrier function                                |
| SPINK5 | 5q32              | Epithelial serine protease inhibitor                                      |
| CC16   | 11q12.3-q13.1     | Potential immunoregulatory function – epithelial expression              |
| NOS1   | 12q24.2-q24.31    | Nitric oxide synthase – cellular communication                           |
| CCL11  | 17q21.1-q21.2     | Eotaxin-1 – eosinophil chemoattractant                                   |
| CCL5   | 17q11.2-q12       | RANTES – chemoattractant for T cells, eosinophils, basophils             |
| GSTM1  | 1p13.3            | Detoxification, removal of products of oxidative stress                   |
| ADRB2  | 5q31-q32          | Smooth muscle relaxation                                                 |
| GPRA   | 7p14.3            | Regulation of metalloprotease expression, neuronal effects               |
| NAT2   | 8p22              | Detoxification                                                            |
| GSTP1  | 11q13             | Detoxification, removal of products of oxidative stress                  |
| ACE    | 17q23.3           | Regulation of inflammation                                               |
| TBX2R  | 19p13.3           | Platelet aggregation                                                     |
| TGFBI  | 19q13.1           | Influences cell growth, differentiation, proliferation, apoptosis        |
| ADAM33 | 20p13             | Cell–cell and cell–matrix interactions                                   |
| GSTT1  | 22q11.23          | Detoxification, removal of products of oxidative stress                  |

Abbreviations: IgE, immunoglobulin E; IL, interleukin; RANTES, regulated and normal T cell expressed and secreted; T<sub>H</sub>2, Thelper.
An association was reported between several SNPs in the *transducin-like enhancer of split 4* (TLE4) gene on chromosome 9q and asthma in a population of 492 Mexican children with asthma, but, again, these associations did not reach genome-wide significance. However, the investigators replicated these findings in an independent cohort of 177 Mexican case-parent trios. TLE4 had not previously been linked to the pathogenesis of asthma, but does play a role in early B cell development.

Association of asthma with SNPs in multiple genes was reported in a GW A study containing more than 10,000 asthmatics and 16,000 controls. SNPs in several loci achieved genome-wide significance, including *IL1RL1* and *IL18R*, *HLA-DQ*, *IL33*, *SMAD3*, and *IL2RB*. The authors observed association with the previously reported *ORMDL3/GSDMB* locus on chromosome 17 only in childhood-onset asthma. Many of these genes have direct or indirect roles in T cell responses (*IL2RB*, *HLA-DQ*) and the development of T_{h}1 (*IL18R1*) or T_{h}2 (*IL33*) responses.

A GW A study from our group was recently reported on a series of pediatric asthma patients consisting of North American cases of European ancestry with persistent asthma requiring daily inhaled glucocorticoids for symptom control, and matched controls without asthma. In this study, in addition to the previously reported 17q21 locus, we uncovered association to a novel asthma locus on chromosome 1q31. The locus contains *DENND1B*, a gene that is expressed by natural killer cells and dendritic cells. The association of *DENND1B* with asthma replicated in a cohort of African Americans, although the associated allele at each SNP was the alternative allele to that associated with asthma in the discovery set. Allele reversal at shared-risk loci can be attributed to differences in the underlying genomic architecture at the loci between populations of different ancestry. The *DENND1B* gene has since been replicated in Crohn’s disease and in primary biliary cirrhosis.

A GW A study examining pediatric asthma in a Japanese discovery cohort and Japanese and Korean replication cohorts recently confirmed the role of the *HLA* locus in these populations. Additionally, this study identified *TSLP* on chromosome 5, along with a gene-rich region on chromosome 12 and the *USP38-GAB1* region on chromosome 4.

Four loci were identified in a GW A study that examined Australian cases and controls in combination with large numbers of genotyped samples from the GABRIEL (A Multi-disciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community) Consortium and multiple in silico cohorts. Reported statistically significant loci were *IL6R* on chromosome 1, *C11orf30/LRRC32* on chromosome 11, *PRKG1* on chromosome 10, and *RPL32P28/OR7E156P* on chromosome 13. The locus on chromosome 11 was also associated with atopy among asthmatics.

A recent meta-analysis examined three ethnically diverse North American populations (European American, African American or African Caribbean, and Latino), searching for asthma susceptibility loci that replicated across ethnic cohorts. Four previously identified loci were identified in this study (17q21, *IL1RL1*, *TSLP*, *IL33*), although this is the first report that has shown they are shared across three ethnic groups. Additionally, the *PYHIN1* locus was identified as a new susceptibility locus in African Americans.

Several GW A studies have been reported using intermediate phenotypes and quantitative traits, rather than asthma itself, as study endpoints. The first report used GWAs to identify variants that modulate serum protein levels. A promoter SNP in the *CHISL1* gene that encodes the chitinase-like protein YKL-40 was shown to influence serum YKL-40 levels and to be weakly associated with asthma, bronchial responsiveness, and pulmonary function in the Hutterite population. A GW A study showed significant association of the *FCER1A* and *RAD50* genes with expression of CHISL1, and evidence for association of the *STAT6* gene with IgE levels. IgE levels are closely correlated with the clinical expression and severity of both asthma and allergy. The *RAD50* variants were further shown to be associated with increased risk of asthma and atopic eczema. Several loci (*IL4R*, *FCER1A*, *IL13*, *STAT6*, and *HLA*) with known functions in T_{h}2 and allergic responses were associated with IgE levels in another recent GW A study.

Eosinophils are leukocytes that play an important role in the initiation and propagation of inflammatory signals. This makes them probable mediators of inflammatory disease and a GW A study was performed examining blood eosinophil counts.
Table 3 Genome-wide association study loci referenced in this review

| Reported gene | Locus   | Top single nucleotide polymorphism | Endpoint analyzed                          | Reference          |
|---------------|---------|------------------------------------|--------------------------------------------|--------------------|
| ORMDL3        | 1q12    | rs7216389                          | Asthma                                     | Moffatt et al[21]  |
| CHI3L1        | 1q32.1  | rs4950928                          | Asthma/YKL-40 serum levels                 | Ober et al[20]     |
| IL1RL1        | 2q12.1  | rs1420101                          | Asthma/blood                               | Gudbjartsson et al[21] |
| IKZF2         | 5q31.1  | rs12619285                         | Eosinophil count                           |                    |
| GATA2         | 3q1.3   | rs4857855                          |                                            |                    |
| IF5           | 2q12.1  | rs4143832                          |                                            |                    |
| SH2B3         | 12q24.12| rs3184504                          |                                            |                    |
| TLE4          | 9q21.31 | rs2378383                          | Asthma                                     |                    |
| PDE4D         | 5q12.1  | rs1588265                          |                                            |                    |
| PDE1A         | 2q12.1  | rs11684634                         | Asthma                                     |                    |
| RAD50         | 5q31.1  | rs2244012                          | Asthma                                     |                    |
| HLA-DRA/DQB1  | 6p21.32 | rs3998159                          | Asthma                                     |                    |
| ADRA1B        | 5q33    | rs10515807                         |                                            |                    |
| PRNP          | 20q12   | rs6052761                          |                                            |                    |
| DPP10         | 12q12.3 | rs1435879                          |                                            |                    |
| IL1RL1/IL1B   | 2q12.1  | rs3771166                          | Asthma                                     |                    |
| HLA-DQ        | 6p21.32 | rs9273349                          | Childhood-onset asthma                     |                    |
| IL33          | 9p24.1  | rs1342326                          |                                            |                    |
| SMAD3         | 15q23.2 | rs744910                           |                                            |                    |
| IL2RB         | 22q12.3 | rs2284033                          |                                            |                    |
| ORMDL3/GSDMB  | 17q12   | rs2305480                          |                                            |                    |
| HLA-DPA1/HLA-DPB1 | 6p21.3 | rs987870                          | Pediatric asthma                           | Noguchi et al[12]  |
| DENND1B       | 1q23.1  | rs2786098                          | Pediatric asthma                           | Steim et al[22]    |
| IL6R          | 1q21.3  | rs4129267                          | Asthma                                     | Ferreira et al[23] |
| C1orf30/ILRRC32 | 11q13.5 | rs7130588                          |                                            |                    |
| USP38-GAB1    | 4q31    | rs7686660                          |                                            |                    |
| TSLP/WDR36    | 5q22    | rs1837253                          |                                            |                    |
| NOTCH4/HLA-DRA/ | 6p21.32 | rs404860                          |                                            |                    |
| HLA-DQA2/IKZF4| 6p21.32 | rs404860                          |                                            |                    |
| LOC338591     | 10p14   | rs1050372                          |                                            |                    |
| IKZF4/CDK2    | 1q23.1  | rs4845783                          |                                            |                    |
| GSDMB         | 17q12   | rs11078927                         | Asthma in four ethnically diverse North American populations |                    |
| IL1RL1        | 2q12.1  | rs10173081                         |                                            |                    |
| TSLP          | 5q22.1  | rs1837253                          |                                            |                    |
| IL33          | 9p24.1  | rs2381416                          |                                            |                    |
| PYNIN1        | 1q23.1  | rs1102000                          |                                            |                    |
| C1orf71       | 11q23.2 | rs11214966                         |                                            |                    |
| CRCT1         | 1q23.1  | rs4845783                          |                                            |                    |
| ORMDL3        | 17q12   | rs6503525                          | Asthma                                     | Ferreira et al[27] |
| C1orf30/ILRRC32 | 11q13.5 | rs2155219                          | Allergic rhinitis/grass sensitization       | Ramasamy et al[20] |
| TMEM232/SCLA25A46 | 5q22.1 | rs17513503                         |                                            |                    |
| HLA region    | 6p21    | rs7775228                          |                                            |                    |
| FCER1A        | 1q23.2  | rs2251746                          | IgE levels                                 | Granada et al[14]  |
| IL13          | 5q31.1  | rs20541                            |                                            |                    |
| HLA-A         | 6p22.1  | rs2571391                          |                                            |                    |
| STAT6/NA62    | 12q13.3 | rs1059513                          |                                            |                    |
| DARC          | 1q23.2  | rs13962                            |                                            |                    |
| HLA-DQA2      | 6p21.32 | rs2858331                          |                                            |                    |
| FCER1A        | 1q23.2  | rs2427837                          | Serum IgE levels                           | Weidinger et al[19]|
| STAT6         | 1q23.2  | rs12368672                         |                                            |                    |
| RAD50         | 5q31.1  | rs2706347                          |                                            |                    |
| CHRNA3/S      | 15q24   | rs8034191                          | COPD                                      | Pillai et al[35]   |
| FAM13A        | 4q22.1  | rs7671167                          | COPD                                      | Cho et al[34]      |
| RAB4B/EGLN2/MIA/CYP2A6 | 19q13 | rs7937                            | COPD                                      | Cho et al[37]      |
| HHIP          | 4q31.22 | rs13147758                         | FEV/FVC                                   | Wilk et al[18]     |

(Continued)
Five loci reached GWA significance, one of which, IL1RL1, was also shown to be associated with asthma in a collection of ten different populations.

Altered lung function, and airflow obstruction in particular, is associated with both asthma and chronic obstructive pulmonary disease. Two SNPs at the α-nicotinic acetylcholine receptor (CHRNA3/5) surpassed genome-wide significance in the study and replicated in two of three independent cohorts. The authors also reported that SNPs at the HHIP locus on chromosome 4 showed association and were consistently replicated across the study cohorts but did not reach genome-wide significance. In the first of the three lung-function GWA studies that included 7691 Framingham Heart Study participants, the only locus to surpass genome-wide significance for association with forced expiratory volume in 1 second/forced vital capacity ratio and replicate in an independent cohort of 835 Family Heart Study participants was HHIP. Two studies resulted in the identification of eleven novel loci associated with measures of lung function; both studies also replicated the previously reported association of the HHIP locus.

The future of asthma genetics

New technology

As the technologies that exist for the identification of genetic variants and the analysis of those variants continue to evolve, the information dealing with the effects of genetic variations on the development of and susceptibility to asthma will grow at a rapidly increasing pace. The advent of next-generation sequencing is bringing complete sequences of genomes and exomes into the public domain. High-throughput sequencing will allow the identification of rare variants with minor allele frequencies far too low to be captured with array technologies that contribute to complex common diseases like asthma. An excellent recent report used targeted sequencing of nine candidate genes to discover rare variants in those loci that

| Reported gene | Locus | Top single nucleotide polymorphism | Endpoint analyzed | Reference |
|---------------|-------|------------------------------------|-------------------|-----------|
| HHIP          | 4q31.22 | rs1980057                          | FEV₁/FVC          | Hancock et al⁹³ |
| GPR126        | 6q24.1  | rs3817928                          | FEV₁/FVC          |           |
| ADAM19        | 5q33    | rs2277027                          | FEV₁/FVC          |           |
| AGER-PPT2     | 6p21.3  | rs2070600                          | FEV₁/FVC          |           |
| FAM13A        | 4q22.1  | rs2869967                          | FEV₁/FVC          |           |
| PTCH1         | 9q22.32 | rs1690989                          | FEV₁/FVC          |           |
| PID1          | 2q36.3  | rs1435867                          | FEV₁/FVC          |           |
| HTR4          | 5q33.1  | rs11168048                         | FEV₁/FVC          |           |
| INTS12-GSTCD-NPNT | 4q24 | rs17331332                         | FEV₁               | Repapi et al⁹⁴ |
| TNS1          | 2q35    | rs2571445                          | FEV₁               |           |
| GSTCD         | 4q24    | rs10516526                         | FEV₁               |           |
| HHIP          | 4q31.22 | rs12504628                         | FEV₁/FVC          |           |
| HTR4          | 5q33.1  | rs3995090                          | FEV₁               |           |
| AGER          | 6p21.32 | rs2070600                          | FEV₁/FVC          |           |
| THSD4         | 15q23   | rs12899618                         | FEV₁               |           |
| MFAP2         | 1p36.13 | rs2284746                          | FEV₁/FVC          | Soler Artigas et al¹³⁸ |
| TGBF2         | 1q41    | rs993925                           | FEV₁               |           |
| HDAC4         | 2q37.3  | rs12477314                         | FEV₁/FVC          |           |
| RARB          | 3p24    | rs1529672                          | FEV₁               |           |
| MECOM         | 3q26    | rs1344555                          | FEV₁               |           |
| SPATA9        | 5q15    | rs153916                           | FEV₁/FVC          |           |
| ZKSCAN3       | 6p22.1  | rs6903828                          | FEV₁               |           |
| NCR3          | 6p21.3  | rs2857595                          | FEV₁/FVC          |           |
| ARMC2         | 6q21    | rs2798641                          | FEV₁               |           |
| C10orf11      | 10q22.2 | rs11001819                         | FEV₁               |           |
| LRPI          | 12q13.3 | rs11172113                         | FEV₁               |           |
| CCDC38        | 12q23.1 | rs1036429                          | FEV₁/FVC          |           |
| MMP15         | 16q21   | rs12447804                         | FEV₁/FVC          |           |
| CFDP1         | 16q23.1 | rs2865531                          | FEV₁/FVC          |           |
| KCNE2         | 21q22.1 | rs9978142                          | FEV₁/FVC          |           |
| DLEU7         | 13q14.3 | rs9316500                          | FEV₁               | Imboden et al¹⁹⁹ |

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IgE, immunoglobulin E.
associate with asthma. The authors show evidence for the probable existence of rare variants that associate with asthma and identify variants in the IL12RB1 locus that contribute to asthma susceptibility in Americans of both European and African ancestries. Many of the associated variants were unexpectedly found in noncoding regions of these genes, indicating that regulation of the genes plays a crucial role in disease susceptibility. Future efforts that include whole exome and whole genome sequencing will greatly expand this type of information, while bringing the considerable challenge of identifying which variants in an individual are relevant for the diseases being studied.

An additional factor to consider is the issue of uncharacterized genes. Many of the most recent GWA studies have identified loci associated with asthma containing genes that have either no known function or no known function that is easily correlated with the disease phenotype. Genes involved in the development of the immune system or specifically in the skewing of the immune response towards or away from an allergic phenotype have obvious implications for asthma susceptibility or severity. However, genes are now being identified with no obvious connections to asthma. The DENND1B and ORMDL3 loci are examples of genes that are difficult to connect to asthma-related phenotypes. Additionally, the list of loci in Table 3 includes several loci corresponding to completely uncharacterized genes with no known function (c11orf30/LRRC32), (c11orf71) (GPR126), (c10or11), or to pseudogenes (LOC338591) reported to be unexpressed. A sizeable effort will be required to understand how these genes contribute to asthma and it remains to be seen if researchers will undertake such challenges and if institutes and agencies will provide funding for this kind of work.

Gene–environment interactions
Asthma, as an immune-mediated disease, involves the response of the body to the environment, in the form of pollutants, allergens, viruses, and other pathogens and irritants. These environmental factors interact with genetic variation to influence the development or severity of disease. Researchers are finding that specific genetic variants affect susceptibility to, and the severity of, asthma in different ways depending on the environments of the individuals carrying those variants, a phenomenon known as “gene–environment interaction.” Several examples of gene–environment interaction exist in asthma, with perhaps the best characterized being CD14, which was originally associated with asthma in linkage studies. A polymorphism in the CD14 promoter was associated with increased CD14 protein levels in serum and reduced serum IgE levels. Several studies attempted to associate this polymorphism with asthma, with conflicting results. These conflicts were resolved when the polymorphism was considered in the context of environmental influences. Different alleles of the CD14 promoter were associated with allergic phenotypes in children, depending on the type of pets or animals to which the children were exposed. One allele correlated with higher IgE levels in children exposed to household pets such as cats and dogs, while the other allele associated with the same phenotype in children exposed to stable animals like horses. Homozygotes for one allele were found to be at lower risk for asthma if exposed to comparatively low levels of house dust endotoxin but at higher risk at higher endotoxin exposures. Other polymorphisms at the CD14 locus have been associated with different outcomes in specific populations, depending on environmental exposure. Given the large number of identified asthma susceptibility loci and the daunting number of environmental variables that may influence complex diseases, much work remains to be done before we have a reasonable understanding of the roles of gene–environment interactions in asthma.

Gene–gene interactions
A comparatively small number of studies have been published to date examining the role in asthma of gene–gene interactions, where variation at one locus alters the effects of variations at a second locus, reflecting epistasis between two or more genes. The existing literature consists mainly of studies in which researchers have chosen two or more specific genes (and occasionally specific variants of those genes) to examine in the context of asthma, looking for evidence of interactions between the two loci. Examples of gene–gene interactions that have been observed in association with asthma include IL9 and IL9R polymorphisms in Koreans, TGFBR2 and FOXP3 in specific IgE production, IL13 and IL4 in Dutch cohorts, and LTA4H in Latinos. Larger scale analysis examining 169 SNPs in 29 genes identified a number of gene–gene interactions affecting both total and antigen-specific IgE levels. Methods are actively being developed to enable large scale and unbiased analysis of gene–gene interactions and visualization of the resulting networks, but these efforts are in their relative infancy. Given the number of previously identified relevant genes and the possibilities for discovery of new loci, the combinatorial potential for interactions between gene effects is daunting. Much development of methods and tools remains to be done before we can truly grasp these vast possibilities.
Pharmacogenetics

Pharmacogenetics, in which variations in genotype are examined for their effects on the response to treatments, is of growing interest with asthma, with the hope that it will increase efficacy and reduce toxic side effects of medications. The best example at this time is provided by beta-adrenergic receptor agonists (or simply beta-agonists), which are prescribed to treat bronchoconstriction and provide long-term symptom control for asthmatics. The ARDB2 locus encodes the beta_2-adrenergic receptor, which binds to and is activated by beta-agonists. Two studies have implicated variations in ARDB2 as modulators of response to inhaled bronchodilators. However, a randomized double-blind study was performed in which subjects were genotyped before being enrolled so that they could be stratified by genotype before receiving prescriptions. This study showed no association of genotype with the response to beta-agonists. Another study showed that a polymorphism in the ARDB2 protein influences the response to regularly administered albuterol, with one genotype receiving less relief from regular long-term use of short-acting beta-agonists. Yet another group has shown that genotype at ARDB2 does not affect the response to combined beta-agonist and inhaled corticosteroid treatment.

A recent study identified variants in the promoter of the GLCCI1 gene that are associated with reduced responses to inhaled glucocorticoids. A specific promoter variant was found to possess reduced transcriptional activity in reporter assays. The same variant was associated with reduced changes in lung function following glucocorticoid treatment. The authors calculate that this variant accounts for about 6.6% of the variability in inhaled glucocorticoid responses. Another recent publication reports variants in the low affinity IgE receptor gene, FCER2, associated with severe exacerbations in children in a trial of inhaled glucocorticoids. The association was present in both European Americans and African Americans and one of the polymorphisms correlated with reduced FCER2 expression. Variants have been identified that alter the response to a 5-lipoxygenase inhibitor and that associate with variability in the response to a cysteinyl leukotriene receptor 1 antagonist. Polymorphisms in corticotrophin-releasing hormone receptor (CRHR1) and the STIP1 gene (involved in the signaling initiated by glucocorticoids) associate with variable forced expiratory volume in 1 second response after inhaled glucocorticoid treatment, as do polymorphisms in TBX21, encoding a transcription factor important in the generation of T_h1 cells.

This latter study demonstrates that variations in genes not directly involved in the metabolism or signaling cascades of a drug can be important modulators of the response to that drug. New study designs and analysis techniques will be required if the pharmacogenetics field is to be able to account for all the variables that may contribute to variable responses to therapies.

Conclusion

Considerable challenges remain in our understanding of the genetic underpinnings of asthma. The incredibly large quantity of data collected to date only explains a fraction of the heritability of asthma. This missing heritability is a common problem in the genetics of complex diseases. Future GWA studies may fill some of the gap in knowledge, although GWA studies are best suited to finding relatively common alleles of modest effect sizes. The use of next-generation sequencing in complex disease research may bring the identification of rare variants with larger effects, which will likely explain at least some of the missing heritability. Additionally, techniques for studying epigenetic phenomena, such as DNA methylation, have the power to expand our understanding of the causes of asthma. Recently, variations in DNA methylation in transformed B cells were described at a specific locus in a specific subset of asthmatics. Variations, including methylation, in the promoter of the Prostaglandin D2 receptor gene, were reported in cohorts of asthmatic and atopic individuals. It is probable that many more epigenetic variations, in a variety of cell types relevant to the development, severity, and treatment of asthma, will be reported in the near future. The expanded genetic and epigenetic information from future studies, combined with improved understanding and analysis of gene–gene and gene–environment interactions are likely to fill many of the gaps in our current understanding and allow us to improve the care we provide to asthma sufferers.

Disclosure

The authors declare no conflicts of interest in this work.

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