



Genetic and genomic approaches to asthma: new insights for the origins

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Purpose of review

The aim is to update current understanding of the genes identified by the recent genome-wide association studies (GWASs) of asthma and its associated traits. The review also discusses how to dissect the functional roles of novel genes in future research.

Recent findings

More than 10 GWAS aimed at identifying the genes underlying asthma and relevant traits have been published in the past 3 years. The largest of these was from the GABRIEL consortium, which discovered that the *IL18R1*, *IL33*, *SMAD3*, *ORMDL3*, *HLA-DQ* and *IL2RB* loci were all significantly associated with asthma. Many novel asthma genes, including those previously identified by positional cloning, are expressed within the respiratory epithelium, emphasizing the importance of epithelial barriers in causing asthma. The genes controlling IgE levels have surprisingly little overlap with the genes mediating asthma susceptibility, suggesting that atopy is secondary to asthma rather than a primary driver of the disease. The next challenge will be the systematic analysis of the precise functions of these genes in the pathogenesis of asthma.

Summary

GWAS have uncovered many novel genes underlying asthma and detailed functional dissection of their roles in asthma will point the way to new therapies for the disease.

Keywords

asthma, IgE, genetics, genome-wide association studies, genomics

INTRODUCTION

Asthma is a disease characterized by intermittent inflammation of the small airways of the lung with symptoms of wheeze and shortness of breath. The presence of inflammation can lead to irreversible airway scarring and intractable airflow limitation. Asthma has a high prevalence and a chronic relapsing course. Clinically, severe asthma remains difficult to treat. Environmental and genetic factors contribute to the cause of the disease. Understanding the genetic predisposition to asthma offers a means of developing new therapies and strategies for the prevention of disease. In the last 3 years, there has been a huge influx of data from genetic and genomics approaches to asthma. This review will look into the possible functional roles of the genes identified by genome-wide association studies (GWASs). We will also provide an update on the recent progress with asthma genes previously identified by positional cloning and we will discuss future systematic approaches to understand the function of novel genes.

GENOME-WIDE ASSOCIATION STUDIES ON ASTHMA

GWAS is a powerful approach for identifying the genetic causes of complex traits. It achieves this aim by examining the relationship between allele frequencies and disease status across a large number of markers spaced across the genome [1]. It is more efficient than positional cloning and can be used to investigate the entire genome simultaneously. Like positional cloning, it provides the opportunity to identify novel mechanisms of disease pathogenesis that reside in previously unsuspected genes or regulatory regions. Because of the large number of statistical comparisons in a GWAS (typically several

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KEY POINTS

- Many novel genes identified by GWAS of asthma communicate damage in the airway epithelium to the immune system.
- The loci controlling asthma are different to loci controlling IgE production.
- Functional studies of novel asthma genes are being based on gene knockdown and over-expression in cell and mouse models.

hundred thousand), the threshold for significant associations is different to that of conventional case-control studies. The stringent genome-wide significant threshold for GWAS is $P < 7.2 \times 10^{-8}$ [2] and a commonly used less stringent threshold is $P < 5 \times 10^{-7}$ [3].

The first GWAS on asthma discovered that the *ORMDL3* locus was strongly associated with childhood disease [4]. Since then, several more GWASs on asthma or asthma-relevant traits have been published. Most GWASs have focussed on the subgroups

of subjects including childhood-onset asthma, late-onset asthma and related traits such as the total serum IgE concentration and eosinophil counts in blood [5–9]. Perhaps because of limited study sizes, there was little overlapping for the loci identified amongst the GWASs on asthma or asthma relevant traits, and only few loci showed significant associations with P values $< 5 \times 10^{-7}$. There was only partial overlap between loci associated to asthma and those influencing other traits such as IgE and eosinophil counts. Interestingly, some asthma-associated loci had previously shown significant association in GWAS with other inflammatory diseases. The genes significantly associated to asthma or related traits ($P < 5 \times 10^{-7}$) are listed in Table 1.

ASTHMA GENES IDENTIFIED BY GENOME-WIDE ASSOCIATION STUDIES

In 2010, the GABRIEL consortium reported the results of the biggest GWAS so far for identifying the genes underlying asthma [10^{***}]. Twenty-three centres around the world were involved in the study which genotyped 10 365 cases of doctor-diagnosed asthma and 16 110 ancestry-matched controls with

Table 1. The most significant genes of asthma and IgE identified by GWAS ($P < 5 \times 10^{-7}$)

Genes	Chromosome location	Peak rs number	Traits	GWAS references	Possible functions
<i>FCER1A</i>	1	rs242783	IgE	[7, 10 ^{***} , 11]	Inducing type I hypersensitivity reactions; inducing NFκB activation
<i>DENND1B</i>	1	rs1775456	Childhood asthma	[12]	Clathrin-mediated endocytosis; internalization of proteins and lipids
<i>CHI3L1</i>	1	rs4950928	YKL-40, asthma	[13]	Tissue remodelling
<i>IL18R1</i>	2	rs3771166	Childhood asthma	[10 ^{***}]	Activation of NFκB
<i>IL1RL1</i>	2	rs1420101	Asthma, eosinophil	[14]	Receptor for interleukin-33
<i>RAD50</i>	5	rs270346	IgE	[7, 11]	Double-strand break (DSB) repairing
<i>PDE4D</i>	5	rs1588265	Asthma	[15]	Signal transduction pathways
<i>HLA-DRB1</i>	6	rs9271300	IgE	[10 ^{***}]	MHC class II molecule
<i>HLA-DQ</i>	6	rs9273349	Late-onset asthma	[5, 10 ^{***}]	MHC class II molecule
<i>IL33</i>	9	rs1342326	Childhood asthma	[10 ^{***}]	Inducing T helper type 2-associated cytokines
<i>SMAD3</i>	15	rs744910	Childhood asthma	[10 ^{***}]	Transcriptional modulator
<i>ORMDL3</i>	17	rs7216389	Childhood asthma	[4, 10 ^{***}]	Negative regulator of sphingolipid synthesis; calcium signalling
<i>GSDMB</i>	17	rs230548	Childhood asthma	[4, 6, 10 ^{***}]	Maintaining the final differentiation state of epithelial cells
<i>GSDMA</i>	17	rs3894194	Childhood asthma	[4, 10 ^{***}]	
<i>IL2RB</i>	22	rs2284033	Asthma	[10 ^{***}]	Endocytosis and transducer mitogenic signals

GWAS, genome-wide association studies.

Illumina single-nucleotide polymorphism (SNP) arrays. The GWAS replicated the association of SNPs in *ORMDL3* to childhood asthma ($P = 6 \times 10^{-23}$) and found novel associations within *IL1RL1/IL18R1* ($P = 3 \times 10^{-9}$), *HLA-DQ* ($P = 7 \times 10^{-14}$), *IL33* ($P = 9 \times 10^{-10}$), *SMAD3* ($P = 4 \times 10^{-9}$) and *IL2RB* ($P = 1.1 \times 10^{-8}$).

An important insight was that several asthma genes may modify the inflammatory response to epithelial damage or regulate homeostatic and healing pathways [10[■]]. The results highlight the importance of epithelial events in asthma pathogenesis. To date, such mechanisms have been little studied.

IL18R1, IL1RL1 and IL33

IL18, IL1 and IL33 belong to the IL1 family of cytokines. This family of cytokines are best known for their ability to alter the host response to an inflammatory, infectious or immunological challenge. The cytokines exert their function through a family of receptors that belong to the Toll-like receptor–IL1 receptor (TLR–IL1R) super family. An important feature of IL1 receptors signalling is the activation of transcription factor NFκB and mitogen-activated protein (MAP) kinases p38, JNK and ERK1/2 [16]. The *IL18R1* gene along with four other members of the interleukin 1 receptor family, including *IL1R2*, *IL1R1*, *ILRL2* (*IL-1Rrp2*) and *IL1RL1* (*T1/ST2*), form a cluster on chromosome 2q. *IL18R1* and *IL1RL1* flank each other with the same orientation of translation. IL18 is closely related to IL33 [17] and synergizes with IL12 to induce the production of interferon gamma (IFN-γ) and to promote Th1 responses [18]. *IL1RL1* encodes the receptor of IL33. The *IL1RL1* locus had been implicated with genome-wide significance in a previous GWAS [14], highlighting the importance of the pathway.

IL33 was originally identified in endothelial cells of high endothelial venules (HEVs) as a nuclear factor [19]. It was classified as a member of the IL1 super family in 2005 [17]. IL33 was also detected in epithelial cells from the airways [17,20]. The gene encoding IL33 is located on human chromosome 9. The activities of IL33 as a nuclear factor remain unclear [21]. Human IL33 is expressed as a pro-domain-containing polypeptide, which after treatment with caspase-1 leads to the production of mature hIL33. IL33 activates a heterodimeric receptor complex containing IL1RL1 (ST2) and IL1 receptor accessory protein (IL1RAP), leading to activation of NFκB and MAP kinases, and drives the production of T helper type 2 (Th2)-associated cytokines such as IL4, IL5 and IL13 [17]. In contrast to inducible

cytokines, IL33 is constitutively expressed in endothelial cells and epithelial cells and is thought to function as an endogenous danger signal or alarmin to alert the immune system after endothelial or epithelial cell damage during trauma or infection [22]. The well documented expression pattern of ST2 has identified a role for the IL33/ST2 axis in the classical Th2 cell and mast cell driven pathogenesis of asthma and anaphylaxis. A recent mouse gene knockout showed IL33 worked as a crucial amplifier of innate immunity [23[■]]. The induction of IL33 expression by environmental or endogenous triggers now suggests a wider role for the pathway during infection, inflammation and tissue damage [24[■]].

SMAD3

SMAD3 encodes SMAD (mothers against decapentaplegic homologue) family member 3. It is located on human chromosome 15. *SMAD3* has been shown to have a tumour suppressive function [25] as well as tumour-promoting activities [26]. A recent study showed *SMAD3* protein levels were modulated by Ras activity. High levels of *SMAD3* are required for the tumour suppressor activities of transforming growth factor-beta (TGFβ), whereas lower levels are sufficient for the tumour promoting function [27]. *SMAD3* protein functions as a transcriptional modulator activated by TGFβ. TGFβ is also critically involved in the control of T cell function, particularly in development of T regulatory cells, as well as being vital for wound healing. TGFβ family members exert fundamental effects on the maintenance of immune function in the lung [28] and TGFβ signalling pathways are activated after allergen challenge in mild asthma [29]. Mice lacking *Smad3* exhibit increased levels of proinflammatory cytokines in their lungs [30]. A conventional knockout of *Smad3* was generated in 1998 and show accelerated wound healing and an impaired local inflammatory response [31]. *Smad3* signalling is required for satellite cell function and myogenic differentiation of myoblasts [32]. The roles of *SMAD3* in the TGFβ signalling pathway in asthma need further investigation.

ORMDL3, GSDMB and GSDMA

ORMDL3/GSDMB/GSDMA locus showed the strongest known effect on asthma in the GABRIEL consortium GWAS, and the effects of this locus were confined to cases of childhood-onset disease. SNPs at this locus were strongly associated with variation in the expression of *ORMDL3*, with weaker effects

on the neighbouring GSDMB. The association signals on human chromosome 17 with asthma peaked with a block of linkage disequilibrium containing *ORMDL3* and *GSDMB*. The extended linkage disequilibrium block means that not only *ORMDL3* but also the nearby genes *GSDMB* and *GSDMA* are relevant to the function of the locus in asthma. It is feasible that the locus contributes to asthma through the differential regulation of these genes. *ORMDL3* is expressed on the membrane of the endoplasmic reticulum (ER). ER stress is linked to cellular responses to inflammation [33]. Enforced X-box-binding protein 1 (XBP1S) is a transcriptional activator that is a key element to the development of the ER. XBP1S is activated by inositol-requiring transmembrane kinase/endonuclease 1 (IRE1) which is also expressed on the ER. *ORMDL3* has been found to be upregulated in XBP-1(S) transduced NIH-3T3 fibroblasts [34]. It has been shown that changes in ORM gene expression cause dysregulation of sphingolipid metabolism [35^{***}]. Sphingolipids are amphiphatic molecules formally derived from sphingosine. Sphingolipids phosphorylation leads to sphingosin-1-phosphate and acylation to ceramide. Sphingolipids mediate cell survival, proliferation, apoptosis, differentiation and cell-cycle arrest [36]. Clinical observation shows that sphingosines and ceramide are increased in asthmatic airways [37].

It has also been suggested that *ORMDL3* modifies SERCA in the ER resulting in an unfolded-protein response and inducing inflammation [38^{*}]. Recent study showed mononuclear cells significantly increased IL17 secretion in 17q21 risk allele carrier children [39^{*}]. All these results indicated *ORMDL3* may influence multiple pathways in the ER that influence inflammation during asthma.

The *GSDM* family genes were first identified in mouse, where they showed unique expression patterns. They are expressed predominantly in the gastrointestinal tract and in the skin at a lower level [40]. The mouse syntenic homology areas to human *ORMDL3/GSDMB/GSDM1* are on chromosome 11 including mouse *Gsdm1*, *Gsdm2* and *Gsdm3*. There is no orthologue gene in mouse genome for *GSDMB*. Mouse *Gsdm* proteins contain DFNA5 domain of Pfam. Extensive expression analysis has confirmed exclusive expression of *Gsdm* family genes in the epithelium of the skin and gastrointestinal tract in a highly tissue-specific manner [41]. *GSDMA* is expressed in the differentiated tissues of the gastrointestinal tract and *GSDMA* has been known to mediate cell-growth inhibition. In contrast, *GSDMB* is expressed in stem cell-resided regions, indicating that it could have a role in stem cell proliferation [42]. Further research is needed to elucidate more

precisely the functional roles of *ORMDL3*, *GSDMA* and *GSDMB* in asthma.

IL2RB

IL2RB is located on human chromosome 22 and encodes the beta receptor of IL2. IL2 is a cytokine secreted by antigen-activated T cells and regulates different functions of various lymphoid cell subsets. IL2 controls the survival and proliferation of regulatory T cells, and is implicated in the differentiation and homeostasis of effector T-cell subsets, including Th1, Th2, Th17 and memory CD8⁺ T cells [43]. These are mediated by its binding to the IL2 receptor. The IL2 receptor is composed of α (CD25), β (CD122) and γ chains [43]. The β chain (*IL2RB*) is a signal transduction element that is also present in the IL15 receptor. It belongs to the type I cytokine receptor family and is devoid of intrinsic kinase activity [44]. The receptor modulates T-cell-mediated immune responses through endocytosis and transduction of mitogenic signals from IL2. Ectodomain shedding of IL2R β generates an intracellular fragment with biologic functions [45^{*}]. In a murine model, local blockade of the β -chain of the IL2R restored an immunosuppressive cytokine milieu in the lung that ameliorated both inflammation and airway hyper-responsiveness in experimental asthma [46].

OTHER GENES

DENND1B is located on chromosome 1q31. The polymorphisms in this gene have been highly associated with asthma from children of European and African ancestry [12]. *DENND1B* functions as guanine nucleotide exchange factor (GEF) for the early endosomal small GTPase RAB35 and binds to clathrin and clathrin adaptor protein-2 (AP2). Thus, connecdenns like *DENND1B* link RAB35 activation with the clathrin machinery [47]. Clathrin-mediated endocytosis is a major mechanism for internalization of proteins and lipids.

PDED4 is on chromosome 5q21, and encodes several isoforms that play a role in tailoring cyclic adenosine monophosphate (cAMP) signaling [48]. It was found to have an association to asthma in non-Hispanic white individuals [15]. *PDED4* ablation significantly prolongs cAMP-response element-binding protein-mediated transcription in mouse embryonic fibroblasts [49]. *CHI3L1* encodes YKL-40, a chitinase like protein that was found to be associated with asthma in the Hutterites, a founder population of European descent [13]. YKL-40 is strongly upregulated in the airway epithelium and alveolar macrophages of

patients of asthma [50]. *HLA-DQ* was the first asthma-susceptibility locus to be identified [51]. The GABRIEL GWAS results indicated it may be fundamental to late-onset asthma, perhaps by restricting the immune response to particular bacterial or other antigens that do not act as classic allergens [10¹¹].

GENES UNDERLYING IgE LEVELS

It is notable that loci showing strong association to IgE had minimal effects on risk of asthma in GWAS [10¹¹]. An exception to this may be the *IL4/IL13* locus on chromosome 5, which showed significant association to the total serum IgE concentration as well as to doctor-diagnosed asthma [10¹¹]. This finding suggests that elevation of IgE is likely to be an inconstant secondary effect of asthma rather than its cause [52], but it does not exclude a significant role for atopic mechanisms in modifying the severity of the inflammation in asthmatic airways.

FCER1A was the first gene identified by GWAS that influences IgE level in German populations [7,10¹¹]. The gene is located on chromosome 1 and encodes the alpha chain of the high-affinity receptor for IgE. The high-affinity receptor is the control receptor of IgE-induced type I hypersensitivity reactions. It also induces the immune responses through the activation of NFκB and downstream genes [53]. SNPs near *RAD50* were also associated with IgE levels in the same study [7]. The gene is located on human chromosome 5q31 and encodes a protein responsible for DNA double-stranded break repair. The 3' end of *RAD50* contains several conserved noncoding sequences and enhancer elements that act as a locus control region for regulation of the neighbouring *IL4* and *IL13* genes [54], and it is quite possible that these genes are responsible for the effects of the locus.

GENES IDENTIFIED BY POSITIONAL CLONING STUDIES

Positional cloning describes the process of identifying a gene by progressive dissection of linkage regions that were consistently co-inherited with disease. Positional cloning underlayed many of the early breakthroughs in single-gene disorders such as muscular dystrophy and cystic fibrosis. Its application to complex disease such as asthma was technically very demanding, but still resulted in some successes. Many previously implicated candidate genes and genes identified by positional cloning genes have not been picked up by GWAS at a statistically significant level. This may be because the initial results were wrong or perhaps more plausibly because of

incomplete coverage of the genome by commercial SNP chips or to population and phenotype heterogeneity [55]. A recent study that specifically combined SNPs from 14 previously identified genes with GWAS found most of the positional clone genes still significantly influenced asthma [9].

ADAM33 was the first asthma susceptibility gene to be positionally cloned [56]. It is thought to be a key molecule in the development and progression of asthma by contributing to smooth muscle and vascular modelling and remodelling.

PHF11 was positionally cloned by mapping a region of linkage to the total serum IgE [57]. Recent functional studies have shown that PHF11 expression in Th1 cells was associated with increased production of IFN-γ and IL2, possibly through a physical and functional interaction with the p65 subunit of NFκB [58]. Conversely, reduced PHF11 expression was associated with enhanced Th2 responses, and low PHF11 expression may account for the high Th2 cytokine response seen in atopic dermatitis [59,60]. PHF11 may be responsible for T cell viability and activation [61]. The G allele of rs1046295 that is located on 3UTL of *PHF11* preferentially transmitted to children with atopic dermatitis and has been associated with decreased *PHF11* RNA in Th1 cells [59]. Electrophoretic mobility shift assays have shown that rs1046295 modulated allele-specific binding by the transcription factor Oct-1 [62].

DPP10 on chromosome 2 is another positionally cloned gene that was associated with asthma [63]. In animal models of asthma, DPP10 is localized to the airway epithelium, and its expression is increased following ovalbumin challenge [64]. In neurons, DPP10 forms part of the A-type K⁺ (Kv4) ion channel complex [65]. In these cells, it can perform a role as a general modulator of voltage-gated K⁺ channel inactivation.

GPRA on chromosome 7 showed distinct distribution of protein isoforms between bronchial biopsies from healthy and asthmatic individuals and the SNP-tagged haplotypes of *GPRA* associated with high serum IgE or asthma [66]. The associations of *GPRA* haplotypes have been also replicated in different populations [67]. However, *GPRA*-deficient mice showed no difference in airway resistance in response to methacholine compared to wild-type mice [68]. The functional roles of *GPRA* in asthma remain unclear.

FUTURE STUDIES

Genetic and genomic studies of asthma have brought fruitful results, but only a small component of the overall genetic contribution to asthma has been so far identified. This might be because of

Table 2. Systematic approaches for the functional roles of asthma novel genes

Methods	Material	Tools	Characterizes
Gene knockdown	Cell line or primary cells	siRNA, shRNA	Candidate gene silenced in cell system
Gene overexpression	Cell line or primary cells	Plasmid, virus induced	Candidate gene overexpression in cell system
Gene mutagenesis	Animals (mice)	Chemical induced	Candidate gene either loss or gain of function
Gene knockin	Animals (mice)	Viral mediated	Candidate gene overexpression in the model
Gene knockout	Animals (mice)	Gene targeting	Candidate gene deleted in the genome and shows loss of the function

multiple small effects, rare highly penetrant mutations or epigenetic effects [52]. It is also influenced by the variation in the genetic background of different ethnic populations [69]. It is likely that further meta-analysis of asthma GWAS data from existing international studies will discover more novel susceptible genes [70].

It is also interesting to note that some of the susceptibility genes identified for asthma have also been found to have associations with other diseases, so that *SMAD3* and *DENND1* have been implicated in Crohn's disease [71[■]] and *ORMDL3* with ulcerative colitis [72[■]]. These results suggest an overlap in some of the inflammatory mechanisms influencing both these diseases.

Epigenetic effects, mediated through mechanisms other than sequence variation, are another possible cause of asthma familial clustering. The patterns of gene expression that determine cellular type and function become stably restricted during development, partly through methylation of CpG sequences and gene silencing. Age, sex, genetic polymorphism and environmental factors will have to be taken into account if methylation changes at individual loci are to help in understanding complex diseases [52,73,74]. Other factors such as airway microbiota may also play important roles in causing asthma [75].

Dissection of the function of novel genes identified by GWAS is of increasing importance. These studies may be on the cellular level or involve animal models (Table 2). Gene silencing or gene overexpression in cell lines or primary cells are powerful systems for the initial study of a novel gene's functions. Gene-expression profiling analyses with the knockdown or overexpression is likely to be a powerful tool for decoding the networks and pathways that contain the gene of interest.

Animal models, particularly mouse models, are widely used for the study of gene function. ENU is a chemical mutagen that randomly induces germ-line point mutations into DNA. Panels of mutagenized mice may be screened for mutations in a gene of interest or for the appearance of particular phenotypes. Both gene-driven and phenotype-

driven approaches have been successfully carried out using ENU mutagenesis [76].

Mouse gene knockout is a very useful tool to dissect gene function, exemplified by the successful knockout of t-bet [77] and GATA3 [78] which brought fundamental insights into their function in respiratory diseases. The targeting process to generate a knockout line is usually time-consuming and difficult. The use of novel techniques such as the recombineering system in the manipulation of mouse genome is leading to more rapid generation of knockout or conditional knockout strains for genes of interest [79].

CONCLUSION

In general, recent robust genetic and genomic approaches have identified several genes that increase the risk of developing asthma. Several genes act in pathways that communicate the presence of epithelial damage to the native and adaptive immune systems, providing a new focus for effective therapies. A next phase of endeavour is to understand the function of these genes and how they interact with the environment. The progressive and systematic use of cellular and animal models is an important bridge to developing new therapies that will improve clinical asthma management in the future.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 90).

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