Post-Graduate Course: Microbiome and omics in allergy

Genome-wide association studies in asthma

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Asthma is a heterogeneous and genetically complex respiratory disease. Finding the genetic architecture of asthma was possible owing to linkage analysis, candidate gene association study, and genome wide association study (GWAS).[1] GWAS of asthma, in particular, have provided mechanistic insights by identifying novel risk loci with no bias. For example, GWAS of asthma have identified novel risk loci, such as 17q12-21, that provide insights into mechanisms.[2]

I first summarize results from GWAS of asthma and other allergic phenotypes and then use gene sets from published GWAS to identify significant biological pathways. I also mention the current challenges faced by GWAS of complex diseases and traits including asthma and the strenuous efforts to overcome these challenges.

GWAS of asthma

Since the first GWAS of asthma in 2007,[3] many have been conducted and about 70% of them are in Europeans.[3-27] Table 1 summarizes the findings. Most of GWAS have yielded informative results, some of which have highlighted the importance of genetic variants in or near genes that were already implicated in asthma.

Biologic pathway from genes of asthma GWAS

We determine significant biological pathways using network analysis of Ingenuity Pathway Analysis (IPA, http://www.ingenuity.com/) based on gene sets of (a) reported genes from GWAS of asthma, (b) only genome-wide significant genes from GWAS of asthma, and (c) reported genes from GWAS of both asthma and one of other allergic diseases/traits including allergic rhinitis, atopic dermatitis or allergic sensitization.

Table 1. Asthma susceptibility loci that met criteria for genome-wide significance in each GWAS

	Year	Region	Reported genes	Strongest SNPs	RAF in controls	*P*-value	OR	95% CI	Ethnicity of discovery sample	Ref.
1	2007	17q21.1	*ORMDL3*	rs7216389	0.52	9.00E-11	1.45	[1.17-1.81]	European	3
2	2009	5q12.1	*PDE4D*	rs1588265	0.29	3.00E-08	1.18	[1.08-1.30]	European	5
3	2009	9q21.31	*TLE4, CHCHD9*	rs2378383	0.78	7.00E-07	1.64	[1.32-2.04]	Mexican	4
4	2009	1q31.3	*DENND1B, CRB1*	rs2786098	0.778	2.00E-13	1.18	[1.08-1.30]	European	11
5	2010	5q31.1	*RAD50, IL13*	rs2244012	0.21	3.00E-07	1.64	[1.36-1.97]	European	8
6	2010	2q12.1	*IL1RL1, IL18R1*	rs3771166	0.62	3.00E-09	1.15	[1.10-1.20]	European	10
		9p24.1	*IL33*	rs1342326	0.16	9.00E-10	1.2	[1.13-1.28]		
		15q22.33	*SMAD3*	rs744910	0.49	4.00E-09	1.12	[1.09-1.16]		
		17q21.1	*GSDMB*	rs2305480	0.55	1.00E-07	1.18	[1.11-1.23]		
		17q21.1	*GSDMA*	rs3894194	0.45	5.00E-09	1.17	[1.11-1.23]		
		22q12.3	*IL2RB*	rs2284033	0.56	1.00E-08	1.12	[1.08-1.16]		
		6p21.32	*HLA-DQ*	rs9273349	0.58	7.00E-14	1.18	[1.13-1.24]		
7	2011	17q21.1	*ORMDL3*	rs6503525	0.43	5.00E-07	1.33	NA	European	6
8	2011	8q24.11	*SLC30A8*	rs3019885	0.31	5.00E-13	1.34	[1.24-1.45]	Japanese	15
		6p21.32	*HLA-DPA1, HLA-DPB1*	rs987870	0.14	2.00E-10	1.4	[1.26-1.55]		
9	2011	2q12.1	*IL1RL1*	rs3771180	0.856	2.00E-15	1.2	[1.11–1.29]	European, African American, African Caribbean, Hispanic	17
		5q22.1	*TSLP*	rs1837253	0.741	1.00E-14	1.19	[1.12–1.27]		
		17q21.1	*GSDMB*	rs11078927	0.552	2.00E-16	1.27	[1.20–1.34]		
		3q27.3	*RTP2*	rs2017908	0.134	4.4E-09	1.63	[1.43–1.82]		
10	2011	4q31.21	*GAB1*	rs3805236	0.25	7.00E-08	1.12	[1.08-1.17]	Japanese	14
		5q22.1	*TSLP*	rs1837253	0.35	1.00E-16	1.17	[1.13-1.22]		
		10p14	*LOC338591*	rs10508372	0.433	2.00E-15	1.16	[1.12-1.21]		
		12q13.2	*CDK2*	rs2069408	0.23	1.00E-10	1.15	[1.10-1.20]		
		12q13.2	*IKZF4*	rs1701704	0.18	2.00E-13	1.19	[1.14-1.25]		
		6p21.32	*C6orf10*	rs3129943	0.62	3.00E-15	1.17	[1.12-1.21]		
		6p21.32	*BTNL2*	rs3117098	0.25	5.00E-12	1.16	[1.11-1.21]		
		6p21.32	*HLA-DRA*	rs3129890	0.61	5.00E-13	1.15	[1.11-1.20]		
		6p21.32	*HLA-DQB1*	rs7775228	0.63	5.00E-15	1.17	[1.12-1.21]		
		6p21.32	*HLA-DQA2*	rs9275698	0.79	5.00E-12	1.18	[1.12-1.24]		
		6p21.32	*HLA-DOA*	rs9500927	0.26	4.00E-09	1.13	[1.09-1.18]		
		6p21.32	*PBX2*	rs204993	0.58	2.00E-15	1.17	[1.12-1.21]		
		6p21.32	*NOTCH4*	rs404860	0.5	4.00E-23	1.21	[1.16-1.25]		
11	2011	1q21.3	*IL6R*	rs4129267	0.37	2.00E-08	1.09	[1.06-1.12]	European	13
		11q13.5	*LRRC32*	rs7130588	0.34	2.00E-08	1.09	[1.06-1.13]		
12	2012	2q12.1	*IL18R1, IL1R1*	rs9807989	NR	6.00E-08	1.33	[1.20-1.47]	European	21
		17q21.1	*ORMDL3*	rs4794820	NR	1.00E-08	1.33	[1.20-1.45]		
13	2013	7q22.3	*CDHR3*	rs6967330	0.19	3.00E-14	1.26	[1.18-1.33]	European	23
		17q21.1	*GSDMB*	rs2305480	0.6	6.00E-23	1.32	[1.23-1.39]		
		9p24.1	*IL33*	rs928413	0.28	9.00E-13	1.24	[1.17-1.32]		
		5q31.1	*RAD50*	rs6871536	0.22	8.00E-07	1.17	[1.10-1.25]		
		2q12.1	*IL1R1*	rs1558641	0.85	6.60E-09	1.56	[1.34-1.811		
14	2012	2q12.1	*IL18R1, IL1RL1*	rs13408661	0.84	1.00E-09	1.23	[1.15-1.31]	European	20
		6p21.32	*HLA-DRA, BTNL2*	rs9268516	0.24	1.00E-08	1.15	[1.10-1.21]		
15	2012	6p21.32	*HLA-DQA1*	rs9272346	NA	2.00E-08	NA	NA	European	18
16	2013	4p14	*TLR1*	rs4833095	0.74	5.00E-12	1.2	[1.14-1.26]	European	24
		5q22.1	*WDR36*	rs1438673	0.49	3.00E-11	1.16	[1.11-1.21]		
		2q12.1	*IL1RL1*	rs10197862	0.85	4.00E-11	1.24	[1.16-1.32]		
		17q21.1	*GSDMA*	rs7212938	0.46	4.00E-10	1.16	[1.11-1.20]		
		5q22.1	*TSLP*	rs1837253	0.71	1.00E-09	1.17	[1.12-1.24]		
		9p24.1	*IL33*	rs72699186	0.15	2.00E-09	1.26	[1.16-1.35]		
		15q22.33	*SMAD3*	rs17294280	0.23	4.00E-09	1.18	[1.11-1.25]		
		8q21.13	*ZBTB10*	rs7009110	0.36	4.00E-09	1.14	[1.09-1.19]		
		16p13.13	*CLEC16A*	rs62026376	0.72	1.00E-08	1.17	[1.11-1.24]		
		6p21.32	*HLA-DQB1*	rs9273373	0.54	4.00E-14	1.24	[1.17-1.30]		
		11q13.5	*LRRC32*	rs2155219	0.48	5.00E-11	1.16	[1.11-1.21]		
17	2014	17q21.1	*IKZF3*	rs907092	0.696	5.7E-13	1.49	[1.33-1.64]	Latino	25
18	2015	10q21.3	*CTNNA3*	rs7915695	0.09	2.19E-08	NA	NA	European	27

The table presents only risk alleles which reached at genome-wide significance (typically P 5x < 10^{-8}).

Exploration of biological pathway using network analysis give us more functional insight of the gene sets although the information of the analysis has been built on existing knowledge based on the literature. Therefore, many novel connections or pathways (for example with the 17q genes) will not be included in this analysis and we are limited to gene connections that are known.

We used as a gene set the 44 genes that were closest to the 54 single nucleotide polymorphisms (SNPs) that were genome-wide significant within each reported GWAS (Table 1). The only significant network (score 40; cutoff score, 30) was enriched for functions including inflammatory responses, inflammatory disease, or connective tissue disorders (Figure 1A). When these 122 genes were interrogated from GWAS of susceptible genes (N=334) of other allergic diseases/traits including allergic rhinitis, atopic dermatitis, food allergy, pulmonary functions, allergic sensitization and/or immunoglobulin (Ig) E levels, 42 asthma susceptible genes were overlapped with genes of other allergic diseases/traits. And figure 1B shows the only significant network (score 34) from the overlapped genes, which seems to harness most of hub genes shown in Fig. 1A.

The most significant canonical pathways generated from genes associated with asthma at genome-wide significance from GWAS were T helper cell differentiation (9.08×10^{-11}) and antigen presentation pathway

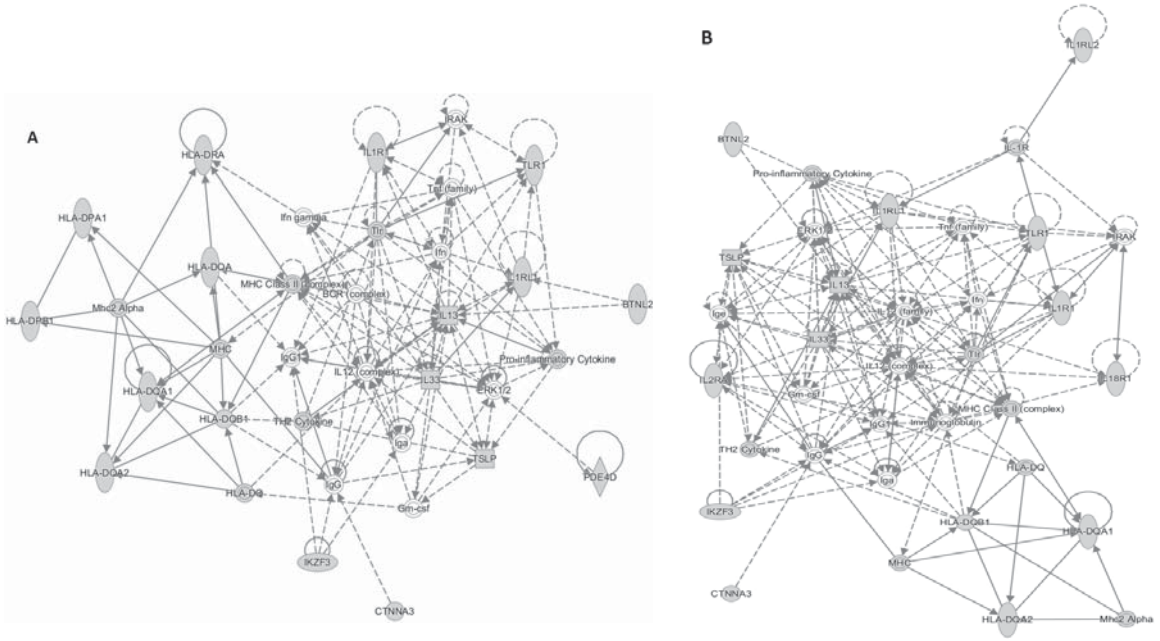

Figure 1. Ingenuity pathway analysis. A data set containing the 44 gene names was uploaded into IPA software to map and generate putative networks based on the manually curated knowledge database of pathways in IPA. The gene networks were generated using both direct and indirect relationships/connectivity. These networks were ranked by scores that measured the probability that the genes were included in the network not by chance alone. (A) The only significant network (score 40) generated from the genome-wide significant genes. (B) The only significant network (score 34) generated from both asthma and other allergic diseases/traits genes.

(9.72 x 10-11). The most significant canonical pathway generated from genes associated with both asthma and other allergic diseases/traits from GWAS was also T helper cell differentiation (2.94×10^{-7}). Not surprisingly, HLA genes are a significant portion of both networks (Fig. 1A, 1B). The HLA class II genes *HLA-DP*, *-DQ* and *-DR* are expressed on the surface of antigen-presenting cells and present peptides to T-helper cells.[28] They interact with Th2 cytokines and B cell receptor complex and would induce Th2 inflammation. This network analysis confirms the known important role of antigen presentation and Th2 cytokine genes.

Another hub gene is *IL13* in the network, which interacts with *IL33* and *TSLP*. The current understanding is that environmental stimulants including either allergens or infectious pathogens induce danger signals, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), by airway epithelial cells.[29] These three epithelial cell-derived cytokines act directly (IL-25, IL-33) or indirectly (via dendritic cells; TSLP) on helper T cell to differentiate down the Th2 pathway, and finally create a type 2 cytokine milieu including IL-4, IL-5, IL-9 and IL-13.[30,31] At the same time, the cytokine milieu as well as environmental stimulants such as virus can also induce epigenetic modification in airway epithelium, and aberrant or exaggerated cytokine milieu.[29,32] Subsequently, the vicious signals activate mast cells, innate lymphoid cell (ILC)2 and eosinophils, and clinically promote bronchospasm, decreased lung function, sputum production and daily symptoms impairment.[29]

Challenges in GWAS of asthma

GWAS is conventionally used for identifying common variants with modest effect sizes, but its impact on overall predictive power is limited for complex traits.[33,34] The common variants identified by GWAS can clarify asthma pathogenesis, on the other hand, they cannot sufficiently account for its heritability estimates, formally known as the 'missing heritability' and then the 'hidden heritability'.[35-37] A recent study investigated whether hidden heritability is due to the problem of asthma GWAS to examine common variants. However, the study showed that even associations of rare and low-frequency variants, which are predicted to have larger phenotypic effects, are not likely to explain a significant portion of the 'missingness' of asthma.[38]

Another problem is that controlling the false-discovery rate in GWAS places much emphasis on few top single nucleotide polymorphisms (SNPs) with extremely small P values (typically P <10-8), which reveals associations that have been dubbed the 'low-hanging fruit' including potential true associations.[2] This is important to note that disregarding these low-hanging fruits in GWAS would have implications in polygenic models and interactions, such as gene-gene and gene-environment interactions (GEI). They cannot be explained with overly simplistic statistical approaches used in GWAS.[39]

Furthermore, DNA sequence information, allele frequencies, and effect sizes vary in populations. Different

populations also exhibit heterogeneity in linkage disequilibrium (LD) patterns between the identified variants and the causative functional variants that underlie disease risk.[22,40-42] Studies based on gene sets have a large effect size on complex trait compared to individual SNPs and are more likely to detect functionally relevant genes, and improve the interpretability of GWAS on complex diseases, such as asthma.[43]

A recent GWAS sought to determine the genetic underpinnings of longitudinal patterns of lung function growth and decline from early childhood through early adulthood, including reduced growth and early decline, in subjects with childhood asthma.[44] They found evidence of a strong genetic association for the pattern of normal growth with early decline on the chromosome 8, rs4445257, but showed the opposite direction of effect from the discovery GWAS in the two other cohorts. From the interaction analysis using combined discovery and replication populations, they presented evidence that rs4445257 is a risk factor for early decline following normal growth of lung function, but protective of early decline following reduced growth. The interactions of two or more genomic loci including low-hanging fruits need to be deciphered in light of the polygenic and pleiotropic genomic architecture of complex diseases and traits including asthma.[45] Elaborate phenotypic categorization and longitudinal GWAS rather than case-control cross sectional one would help overcome the current limitations.

Conclusions

Heterogeneity of asthma pathogenesis comes from a combination of genetic heterogeneity and environmental heterogeneity. There is also an overwhelming number of confounding environmental factors including the age at which environment most effectively influences the genes, whether environmental factors are prolonged, and how burdensome a particular environmental factor may be to an individual, etc. Furthermore, there are the pitfalls between genetic variants and gene expression as well as genetic heterogeneity including copy number variation, deletion-insertion, gene-gene interactions, sex-specific variants, and parental inheritance, etc. The emergence of deep learning allows computational models that are composed of multiple processing layers to learn representation of data with multiple levels of abstraction. It might allow us to more understand the heterogeneity of asthma.

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