Unraveling the Genetic Basis of Asthma and Allergic Diseases

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Asthma and allergic diseases are believed to be complex genetic diseases which may result from the interaction of multiple genetic factors and environmental stimuli. In past decades, great efforts have been exerted in unraveling their genetic basis. The strategies in discovering genes and genetic variants, confirming their importance in pathogenesis of asthma and allergic diseases, as well as their strengths and limitations are summarized comprehensively and concisely. The current consensus about the genetic basis of asthma and allergic diseases is briefly described as well.

Key Words: Asthma; allergic diseases; genetic

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways characterized by airway obstruction, which is at least partially reversible with or without medication, and increased bronchial responsiveness to a variety of stimuli. Asthmatic patients are frequently bothered by recurring and varying symptoms of breathless, wheezing, cough and chest tightness. Asthma is often associated with allergy, an acquired potential for developing adverse reaction that are immunologically mediated. Atopy, an adverse immune reaction involving IgE antibodies, is believed to the strongest identifiable predisposing factor for the development of asthma.[1] Asthma affects more than 22 million persons, and is one of most common chronic diseases of childhood affecting more than 6 million children in the United States.

Asthma and allergic diseases are believed to be complex genetic diseases, which result from the interaction of multiple genes and environmental stimuli. Family studies indicated that the first degree relatives of individuals with asthma are about 5-6 times risk of asthma than the individuals in the general population.[2] Family aggregation and segregation of asthma and allergy showed no consistency across diverse populations suggesting the operation of complex and perhaps multiple genetic and environmental etiological factors. Heritability, the proportion of phenotypic variances that caused by genetic effects, varies from study to study in asthma. Multiple independent twin studies in diverse populations showed the heritability for asthma ranges between 0.48 and 0.79 indicating significant genetic

effects contributed to asthma development.[3] The genetic factor may account for 40% to 60% of variance in allergen specific IgE level, 30% in log eosinophil count and 30% to 60% in positive skin test to dust mite in etiology.[4] The remainder of atopy and asthma etiology is therefore attributable to environmental variables and partly interactive gene-environment influences. In past decades, great efforts have been exerted in unraveling the genetic basis of asthma and allergic diseases. This review is intended to briefly summarize what have been done and achieved in discovering, confirming genes and genetic variants and current consensus on genetics of asthma.

DISCOVERING GENE AND GENETIC VARIANTS OF ASTHMA AND ALLERGIC DISEASES

Several strategies have been applied to identify genes and genetic variants that predispose to asthma and allergic diseases. These mainly include positional cloning, candidate gene approach, genome wide association study as depicted in Fig. 1.

Positional cloning

Positional cloning is a popular approach used to identify genes

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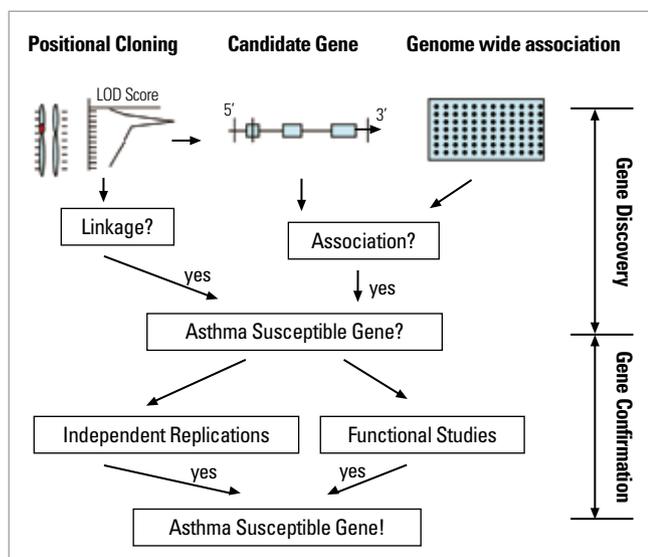
Fig. 1. Flowchart for unraveling genetic basis of asthma and allergic diseases. Susceptible genes are identified through positional cloning, candidate gene approach or genome wide association studies. Independent replications in diverse populations and functional studies for understanding the biological pathways and molecular mechanisms involved provide convincing evidences in the confirmation of a susceptible gene and the related genetic variants for asthma and allergic diseases.

for genetic diseases through a set of genetic markers on suspected chromosomes. It is also termed genome scan or genome wide linkage study when the entire genome is screened using dense panels of polymorphic DNA markers spaced across the genome to identify specific regions for phenotypes. Positional cloning is very useful to identify a disease gene or disease susceptibility gene location in genome especially when unknown genetic information is available for the pathological mechanism of a disease. The genome screening is followed by identification of the gene (or genes) within this region, as well as polymorphisms within the gene(s) that contribute to the development of a disease. The big advantage for positional cloning in complex genetic diseases such as asthma and allergy is that it can identify a previously unknown gene or a gene not considered as candidate gene.

Positional cloning is based on family study and linkage analysis.[5] Collecting phenotypic data in pedigrees of multiple families is a prior requirement for positional cloning. Polymorphic markers are DNA segments with multiple alleles that can be easily traced through multiple generations, and are chosen to cover the whole genome over certain physical distance (1-10 cM) or on suspected linkage regions. The cosegregation of the polymorphic markers and a disease trait is examined within families to determine whether a marker is linked to a specific disease trait. Due to recombination events during meiosis, the closer two loci: polymorphic marker and disease trait are to each other, the smaller the chance of recombination is, and the

greater the chance that the markers segregate with the disease is. A statistical indicator, lod score that refers to the log of the ratio of the likelihood that the disease is genetically linked versus no linkage, has been widely adopted for evidence of linkage.

Positional cloning studies linked multiple regions of chromosomes to asthma and atopy related phenotypes in diverse populations although most of them have largely focused on Caucasian population. The followed studies identify genes and genetic variants in these regions associated with asthma and allergy. The most recently found genes include ADAM33 on Chromosome 20p,[6] SPRINK5 on 5q31-35,[7] IRAKM on 12q,[8] DPP10 on 2q14-32,[9] GPRA on 7p,[10] HLA-G on 6p21[11] and PHF11 on 13q14.[12] ADAM33 is one member of metalloproteinase family preferentially expressed in smooth muscle, myofibroblasts and fibroblasts rather than epithelial cells, T cells or inflammatory leukocytes, and is believed to play an important role in epithelial-mesenchymal tropic unit in lung development.[13] The importance of its genetic variants in asthma has been testified in replicated association studies across diverse populations.[14] SPRINK5 encodes a multi domain serine protease inhibitor which protects the integrity of epidermal layer from allergen or inflammatory related protease. Functional changes associated with genetic variant may account for its importance in asthma and allergy.[7,15] IRAKM encodes a kinase, a master regulator of NFκB and inflammation, negatively regulating TLR and IL1R pathway. IRAKM is highly expressed in epithelial cells. Genetic variants including SNPs and haplotypes have been associated with early onset of asthma.[8] DPP10 was identified as an asthma related gene after resequencing the IL1 cluster region on 2q14. DPP10 functions in cleaving the terminal of dipeptides from cytokines and chemokines as its homologous family members.[9] Adopting hierarchical genotyping design leads to the identification of GPRA which is associated with both IgE and asthma, suggesting a possible role in pathogenesis of asthma and atopy.[10] HLA-G is a non-classic, class I HLA molecule on chromosome 6p21. Expression of HLA-G was detected in bronchial epithelial cells of asthmatic patients rather than unaffected individual.[11] A SNP in the 3' untranslated region of HLA-G was shown to influence the targeting of three microRNAs to this gene, suggesting that microRNA mediated mechanisms may contribute to the impact of HLA-G on asthma risk.[16] PHF11 encodes two PHD zinc fingers which can interact with the trimethylated lysine residue 4 on histone 3, a modification that marks transcriptionally active regions of the human genome. Quantitative trait locus for total serum IgE was localized around chromosome 13q14 using a comprehensive SNP map that leads to several alleles in PHF11.[12] PHF11 has been demonstrated to be an important regulator of Th1 differentiation for IFN and IL2 expression. The reduction of PHF11 expression associated with genetic variants may contribute to increased Th2 differentiation.

The application of genetic linkage analysis in asthma and al-

lergy faced more difficulties than its application in simple Mendelian disorders likely because of the possible genetic complexity for asthma and allergic diseases. Different chromosome regions have been linked to asthma and atopy related phenotypes across diverse populations suggesting the genetic heterogeneity for these phenotypes which means many genetic backgrounds may be involved. It is very common that more than one susceptibility locus were found in many of the linkage regions. The linked region on chromosome 5q31-35 has been found to harbor multiple asthma and atopy genes which play various roles in the pathological mechanisms.[17] These include genes regulating Th1 and Th2 differentiation and IgE production e.g. IL4, IL13, TIM1 and TIM3, genes modulating innate immunity e.g. CD14, genes controlling smooth muscle relaxation e.g. ADRB2, genes improving T cell adhesion e.g. CYFIP2, genes mediating inflammation process e.g. LTC4S, and genes influencing epidermal differentiation e.g. SPINK5. The effect of each linkage is relatively small compared to simple Mendel genetic disorder, and the development of asthma and atopy in any individual seems to derive from genetic effects at a number of loci. Multiple genetic factors are believed to contribute to asthma and allergy therefore suggesting polygenetic effect.

Candidate gene approach

The candidate gene approach is based on selection of a gene which is potentially involved in pathology of diseases and consequently testing the linkage or association of their genetic variants with diseases. Genetic association analysis is the most popular design in candidate gene studies in which the co-incidence of a disease and a genetic variant in a population is evaluated. The association study can detect genes with smaller effects in sample sizes comparable to those used in linkage studies, and statistically more powerful.[18] As the gene function is generally known, the association study is limited by its power to find a new gene or unidentified pathway for diseases.

Candidate genes in asthma studies were selected based on hypothesis for asthma related functions either because these genes are localized in a linked chromosome region or there are evidences to support that they may be involved in important signaling pathways for asthma. The genetic variants to be tested for association with asthma are either derived from the known databases e.g. dbSNP or are identified through gene sequencing. The tested genetic variants were genotypes or alleles on SNPs (single nucleotide polymorphisms) around the candidate gene and haplotypes which are combination of alleles at nearby variants. Recently, structural genetic variation especially copy number variation which refers to variable copy number of 1 kb or larger DNA segment compared to a reference genome has attracted much attention in the genetic study for complex diseases too.[19]

Case control design is preferable to family design in association studies because recruiting unrelated cases and controls is easier and less expensive than recruiting families.[20] Association studies were performed to compare the frequency of specific polymorphisms mostly genotypes and haplotypes in the candidate genes in unrelated asthmatic (and/or allergic disease) patients and unrelated control samples, or to test whether the alleles at one or more SNPs explain the variability of a disease-related quantitative trait such as IgE level. The odds ratio (OR) value was usually used to estimate the asthma risk associated with specific genotype or haplotype while the regression coefficient in a regression model was estimated for the quantitative traits. The statistical significance, in which inflated type error I in multiple comparisons was overcome by Bonferroni correction and permutation test, suggested an association of specific genetic polymorphism with asthma and related phenotype.[21] However, the observed association may be caused by linkage disequilibrium because the observed polymorphism is physically close to the DNA sequence that is actually important in causing the disease and therefore coherent together.

Over last decades, many genes have been examined for an association with asthma and atopy related phenotypes in diverse ethnic populations. Several excellent reviews have been published in this filed.[20-23] More than 100 genes involved in pathogenesis of asthma have been found significant association. More than 30 genes have been associated with asthma or atopy related phenotypes in five or more independent studies. Over 14 genes including ADRB2, IL4R, HLA-DRB1, IL13, CD14, IL4, TNFα, FceRIB, ADAM33, IL10, STAT6, LTA, GSTP1 (Glutathione S-transferase P1), CCL5 (CC-chemokine ligand 5, RANTES) have been replicated in more than ten independent studies. These genes are mainly involved either in environment interaction (CD14, GSTP1), antigen presentation (HLA-DRB1), Th2 cell differentiation (IL4, IL13, IL4R, STAT6), immunoregulatory (IL10, LTA, CCL5), tissue remodeling (ADAM33) or for respiratory smooth muscle relaxation (ADRB2). A recent comprehensive analysis of candidate genes examined in past through a GWAS database suggested that candidate genes including TGFb1, IL1RL1, IL18R1, and DPP10 were the most significantly associated with asthma.[24]

It has been observed that no one single gene showed significant association in all studies suggesting the complex genetic etiology of asthma and atopic diseases. This may mean that no one single gene will be the asthma gene in all populations. It is therefore reasonable to deduce that the number of genes involved in each individual will be fewer than all number of susceptible genes for asthma derived from population studies.

Genome wide association study

With the development of genotyping technology, many hundreds of thousands even millions of SNPs across the whole genome can be tested in a single gene chip.[25] The most common genetic variants associated with disease are expected to be identified in genome wide association study (GWAS). GWAS

studies offer an opportunity to explore the genetic basis of a complex disease in a comprehensive way, and not only have advantages over traditional candidate association studies in that they can discover truly novel disease candidate genes and pathways, but also have advantages over linkage studies in that it will identify genes and genetic variants with small effects.[26] It is expected that GWAS study may become a standard approach to gene discovery over the next decade when the financial costs and computational limits are overcome.

The first GWAS study about asthma was published in 2007.[27] More than 317K SNPs were scanned in genomic DNA from 994 patients with childhood onset asthma and 1,243 non asthmatics, using both family and case-control panels. The strongest association was located to 112 kb interval on chromosome 17q21 and significantly replicated in several different populations. The trait-associated markers fall in the region containing three consecutive haplotype blocks. Multiple SNPs seem to jointly contribute to disease risk in a forward stepwise regression analysis indicating more than one functional SNP underlie the locus or less likely the presence of a single functional SNP in complete linkage disequilibrium with the typed markers. The study in EBV transformed B cell lines showed that an identified SNP was associated with gene expression level of ORMDL3 (ORM 1 like 3) in the region, a novel transmembrane protein anchored in the endoplasmic reticulum with unknown function. The ORML3 transcript expression was also simultaneously associated with asthma. The association of the genetic variants in gene ORMDL3 with childhood onset asthma has been successfully replicated in multiple populations with various ethnic backgrounds.[28-30] The common genetic variants seem to be associated with chromatin remodeling such as nucleosome distribution, binding with the insulator protein CTCF (11 zinc finger transcriptional repressor) as well as promoter activity leading to altered domain-wide cis-regulation in a recent functional study.[31]

Another GWAS study in the Hutterites,[32] a founder population of European descents, found that CHI3L1 variants were associated with asthma and related phenotypes. CHI3L1 (the chitinase 3-like 1) encodes a chitinase like protein YKL-40 which may be involved in inflammation and tissue remodeling due to its ability to bind ubiquitously expressed chitin. Its findings remain to be replicated in more independent studies although it has not been replicated in a recently published GWAS study.[33] A GWAS study conducted by Himes[33] in CAMP (childhood asthma management program) and matched control indicated that the strongest region of association was located to chromosome 5q12 in PDE4D (phosphodiesterase 4D, cAMP-specific phosphodiesterase E3 dunce homolog). PDE4D is a regulator of airway smooth muscle contractility associated with cAMP.[33] PDE4D inhibitors could suppress the activation of inflammatory and resident cells in lung in response to various stimuli as in asthma, and are being developed as medications for asthma.[34]

The genetic variants of PDE4D associated with asthma could be another important mechanism accounting for the hyperresponsiveness in respiratory tract in asthmatic patients. A GWAS study conducted by Hancock et al.[35] in Mexican children with asthma using 550K Illumina HumanHap Beadchip identified novel genetic variants for childhood asthma in chromosome 9q21. The SNPs located upstream of TLE4 (transducin like enhancer of split 4 gene) were replicated to be associated with childhood asthma risk in Mexican ethnicity. TLE4 encodes a transcriptional co-repressor which interacts with the transcription factor paired box 5 for B cell activation in early B cell differentiation, and interacts with RUNX3 (runt related transcription factor 3) in dendritic cells to inhibit dendritic cell maturation.[36,37] These interactions are related to atopy and asthma development. TLE4 associated genetic variants may influence asthma through its possible impact on immune system development. Except asthma as the main phenotype, a GWAS scan on total serum IgE levels by Weidinger identified FceRIA (alpha subunit of FceRI) as a susceptible gene in birth cohorts and cross-sectional studies of European decedents.[38] The top genetic variants have also been associated with FceRIA expression on Basophils through gene expression arrays. In addition, genetic polymorphisms in RAD 50 gene, which is located close to well replicated linkage region on chromosome 5q31, were also consistently associated with IgE levels and increased risk for atopic eczema and asthma. RAD50 encodes a ubiquitously expressed DNA repair protein, and contains multiple conserved non-coding sequences with presumed regulator function for Th2 cytokine gene transcription. RAD50 was also noticed in strong linkage disequilibrium with other Th2 cytokines such as IL13, etc.[39,40] Whether genetic variants of RAD50 are directly related to IgE production or because of its linkage with Th2 cytokines remains to be elucidated in future. A recent GWAS study in African population identified 3 genes as associated with asthma including ADRA1B, PRNP and DPP10.[41] Except DPP10, ADRA1B and PRNP are the first reported to be potentially involved in asthma pathology although their possible roles in asthma remain to be explored. In a recent GWAS carried out in a combined population of European ancestry children with persistent asthma, several SNPs at a novel locus on 1q31 were found to be most significantly associated with asthma in addition to the previously reported locus on 17q21.[42] The association was also replicated with asthma in both independent series of persons of European ancestry with childhood onset asthma and in the children of African ancestry. The most strongly associated markers implicate DENND1B as a susceptible gene for asthma. DENND1B encodes a cytosolic signaling protein which is a binding partner of TNF-α receptor type I binding protein, and negatively regulate TNFR1 signaling in response to cytokine promoted stress.[43] The DENND1B protein is expressed in a subgroup of dendritic cells and in natural killer cells, and significantly upregulated in effector memory T cells

as compared with naïve T cells.[44,45] Its roles in asthma pathology remain to be delineated in future.

Overall, GWAS studies up to date have generated many interesting findings. Novel genes and pathways have been revealed including ORMDL3, DENND1B etc although the importance of these findings in asthma and allergy remains to be verified in more replicated population studies and functional genomic studies. GWAS studies are also limited in its power to discover genes for complex genetic diseases because the current GWAS arrays are biased toward common targeting variants rather than relatively rare SNPs in coding and promoter regions in their SNP genotyping panels. It has been hypothesized that numerous rare functional SNPs may be important in susceptibility to common diseases such as asthma. The design of GWAS arrays based on mostly Caucasian populations may also be biased and influence the discovery of new genes in other ethnic populations.

CONFIRMING SUSCEPTIBLE GENE AND GENETIC VARIANTS FOR ASTHMA AND ALLERGIC DISEASES

To be truly susceptible genes and genetic variants for asthma, strong evidences from both independently replicated studies for association and linkage, and related functional changes are required. However, many uncertainties are involved in replicated population studies and functional genomics investigation. These added to the complexity of asthma genetic and worth of further discussion in confirming susceptible genes and genetic variants for asthma.

Replication studies

Independent replications of genotype-phenotype association in distinct populations have been considered important convincing evidences for the identification of true disease susceptibility genes. As we know, genotype is determined by allele variants in a specific locus. Haplotype has been used more frequently in recent reports of association. Multiple genetic polymorphisms are located in most genes. It is common that an allele or haplotype, which was reported positive association in an original study, could not be found to be associated with asthma or atopy related traits even with opposite effect in replicated reports. On the other hand, the nearby alleles or different haplotypes on the same gene could be found significance in replicated studies. As diverse asthma and atopy related phenotypes have been utilized, strict or loose replication has been used to define the genotype-phenotype relationship: strict, genotype strict, phenotype strict or loose.[46] Gene itself rather than specific genetic variants or haplotypes has been recommended as a unit in defining a replication.[47]

The results from independent replications are impacted by many factors in distinct populations. These include but not exclusively phenotype heterogeneity, gene-gene interaction, gene-

environment interaction, race/ethnicity, epigenetic effect, study design such as cohort type and sample size for statistical power.

Phenotypes

Phenotype refers to genetically and environmentally determined physical appearance of diseases. Similar to other complex diseases, asthma and allergic diseases are phenotypically heterogeneous and characterized by diverse clinical aspects, varied susceptibility to stimuli and numerous intermediate phenotypic markers for physiological and histopathological changes.[48] The distinct clinical features include onset age, transient or persistent status, severity degrees, therapy response and concurrent disease status etc. The individual manifestation is derived from any combination of these features, and may be determined by specific subsets of susceptible genetic pathways. Various asthma and atopy related phenotypes have been used in published literatures including various disease statuses such as asthma, atopy, childhood asthma, atopic dermatitis, hay fever, asthma severity and intermediate or associated phenotypes such as allergic sensitization, bronchial hyper responsiveness, FEV1, IgE level, eNO and eosinophil counts.[20] The phenotype definition and criteria should be taken into account in evaluating a replication study.

Gene–gene interaction

Interaction is a term used to describe the relationship between two factors in terms of effects on an observed endpoint, in which the effect of one factor is conditional on another one. Gene-gene interaction refers to the functional interplay between genetic variants in related genes, also called epistatic interaction. Among multiple possible interaction scenarios, the synergistic effects in which the combined asthma risk of genetic variants exceeds the additive effect of two genetic variants presence alone has received greater interests in explaining the functional relevance of potential asthma related genetic variants. A good example is an interaction between different polymorphisms in the Th2 differentiation pathway significantly influencing the genetic control of serum IgE levels and the development of asthma.[49] The analysis of genotyping data in stepwise procedure from a large population of German children showed that the combined effects of three genetic variants comprised of T allele in STAT6 C2892T, T allele in IL13 C1112T, and T allele in IL4 C589T increased the risk of asthma by 16.8 fold compared to maximum effect of any individual SNP and by 5.89 fold compared to maximum effect of any combination of two SNPs. The risk of elevated serum IgE levels increased from the maximum single effect of OR 1.73 for STAT6 C289T to a maximum of 18.73 for the triplet interaction among T allele in IL13 C1112T, G allele in IL4R A148G and IL4 C589T. The interactions of IL13 and CCL17 for total IgE level,[50] IL4Rα and IL13 for asthma risk,[51] and IL13 and IL4Rα for food sensitization[52] have also been reported. It is expected that more genetic inter-

actions are involved in asthma and atopy, and remain to be explored. Therefore, the difference in allele frequency of known or unknown genetic variants with potential interaction will impact the results in replicated studies, which adds to the genetic complexity of asthma.

Gene–environment interaction

It is clear that environmental factors such as virus, allergen exposure and smoking etc are important determinants in the development of asthma. Hygiene hypothesis of asthma suggests that exposure to infections early in life influences the development of a child's immune system along a "non-allergic" pathway, leading to a reduced risk of asthma and other allergic diseases.[53,54] Gene-environment interaction is believed to contribute to a proportion of the phenotypic variance significantly in animal study and evidenced from human studies too.[55,56] Interaction of CD14 with endotoxin exposure on asthma and IgE levels provides a good example to illustrate the importance of gene-environment interaction in asthma and allergic diseases and how the genetic effect might be impacted by environmental exposure.[57] CD14 is a receptor that has specificity for LPS (Lipopolysaccharides) and other bacterial well derived components, and has also been suggested as a potential response element for the respiratory syncytial virus. The global microbial burden in life, including exposure to nonpathogenic microbes, could deviate immune responses away from those associated with allergic responses through interaction with CD14. The association of CD14C-159T with asthma related phenotypes seems to be a more complex story than expected from lots of epidemiological studies. The early study reported that atopic children with homozygous TT genotype had significantly smaller mean number of positive skin test to aeroallergens than CC homozygous suggesting that TT homozygous plays a protective role against allergy. The effects revealed by the following studies are diverse, ranging from protective to disease promoting, with some reports failing to find an association. It seems that endotoxin exposure is one of the factors determining whether it could be either protective or risk promoting. Children who were homozygous CC were associated with a decreased risk of allergic sensitization and eczema at high endotoxin exposures, but with an increased risk at low endotoxin exposures relative to the rest of the population. The individuals with TT homozygous were protected from total IgE level at low level of endotoxin exposure and at increased risk at high level. This phenomenon was also observed in the association of CD14 C-159T with asthma and asthma severity. House dust exposure is another factor that may be involved in the gene-environment interaction. TT genotype might protect against asthma for individuals with low house dust exposure, but be a risk factor of asthma with high house dust exposure although it is not clear whether there is a direct relationship between house dust level and endotoxin. Other examples include the interaction between tobacco smoke and GST and ADRB2,[58] between TLR2 and farm living[59] etc. Gene-environment interactions are believed to be a framework to reconcile the discrepancies from replicated studies. As illustrated from functional studies, binding affinity of transcriptional factors such as SP1 and SP3 differs between C and T allele of CD14-159 probably resulting in change of gene expression. Environmental exposure may activate and impact the expression of various transcription factors in different cell types. As one of the mechanisms, both changed binding affinity associated with genotypes and varied level of transcription factors associated with environmental exposure may determine the consequences in gene-environment interaction. Environment factors have been associated with epigenetic modification such as hypermethylation promoters and downregulating transcription of gene expression by ETS (environmental tobacco smoke). The epigenetic mechanisms including DNA methylation, histone acetylating and deacetylation, and other modes of chromatin remodeling associated with in utero and early life exposure to environmental factors may be involved in transgenerational phenotype transmission. Recently allele specific epigenetic modification has been identified for common genetic polymorphisms of gene ORMDL3.[31] The epigenetic effects under various innate and environment exposures may be another possible mechanisms involved in the complex interplay among gene-environment interaction.[56,60]

Race/ethnicity

The genetic studies of asthma have been conducted in diverse racial/ethnic populations in past decades. Different results in terms of importance of certain genomic region, certain genes and their genetic polymorphisms in asthma have been observed across racial/ethnic groups. The Collaborative Study on the Genetics of Asthma had identified the linkage between chromosome 5p, 17q, and 11q21 with asthma in African Americans, chromosome 11p15, 19q13, and 6p21 in Caucasians, and chromosome 2q33, 21q21, and 1p32 in Hispanics.[61,62] Multiple genetic polymorphisms in gene ADAM33, IL4, and IL4R do not show consistent associations with asthma and allergen sensitization across the ethnical diverse populations (e.g. US whites, Dutch, African Americans, and Hispanics).[63] TGFβ1 is a multifunctional cytokine which can be expressed in various inflammatory cells (e.g. T cells, B cells, Monocytes, Macrophages, eosinophils, neutrophils, mast cells, dendritic cells), epithelial cells, fibroblasts, smooth muscle cells and endothelial cells. When secreted from these cells, TGFβ1 can modulate inflammation such as cellular proliferation, T cell differentiation, cell apoptosis and cell migration, and/or impact host resistance and mediate tissue remodeling and repair in autocrine or paracrine manner through interaction with the TGFβ1 receptors. An Allele T at a SNP C-509T at promoter site of TGFβ1 has been significantly associated with higher risk of asthma in multiple Caucasian populations, but failed to show association in two East Asian popula-

tions.[64,65] The T allele frequency is almost two times in East Asian populations than in Caucasian populations. Our pilot study indicated that T allele and the associated haplotype may protect from allergy in Chinese population but increase risk of allergy in White population (not published). The significance of this finding remains to be explored in bigger populations. Significant genetic variations across racial/ethnic groups have been described in multiple studies. This can be reflected from the fact that some rare disease susceptibility alleles (frequency<=2%) may be found only in certain ethnic groups while common disease susceptibility alleles in certain ethnic group may be less common in other groups.[66,67] Multiple socioeconomic, psychological, and environmental factors have been associated with asthma in addition to genetic factors.[68] There may be important differences in those factors among diverse ethnic groups and/or subgroups within an ethnic group. Therefore, the disease susceptibility alleles that are common in most ethnic groups may have differential effects on disease risk because of gene-gene and gene-environment interaction that are unique or more common in certain ethnic groups. Furthermore, factors associated with place of birth might modify the effect of genetic and environmental exposures on asthma within or between ethnic groups. Mexican Americans born in the United States have more than three times of asthma risk than those born in Mexico no matter males or females after adjusted age, BMI, smoking history and PIR (poverty income ratio) in the recent NHANES report.[69] Failure to stratify the study population by race/ethnicity or lack of genomic control in case-control association studies will cause the interpretation of replication result difficult.

The number of independent replications used for selection of the most susceptible genes varies among reviewers. It is generally agreed that the more replications are reported, the more important will the gene and the genetic variants be in the genetic complex diseases. Applying the replication number in determining the susceptibility of genes may be limited by how long since a gene was reported and whether the original result was positive or negative and how big the sample sizes are used in the replicated studies. If an important gene is recognized recently or a negative result was reported in the original study, it is likely that there will be fewer replications in reports as expected. Many of the reported studies lack a sufficient sample size to generate a significant result especially for a modest effect. When multiple SNPs or alleles are involved, inflated type I error in multiple comparisons cannot be overcome in a small sample size study. Furthermore, the conclusion about the association from a small size study may not be generalized to a general population even though a significant effect can be seen. Due to linkage disequilibrium among nearby genetic variants, many of which have been significantly associated with asthma and atopy related phenotypes might not be the disease variants rather than in LD with the disease variants. Therefore, functional studies are necessary to clarify the roles of susceptible gene and genetic variants in asthma and allergic diseases.

Functional genomics

Disease susceptibility genes and genetic variants discovered through linkage or association study can be established through further functional characterization in in vitro, ex vivo or in vivo studies under imitated physiological environment and extraneous stimulation. The functional genomics study can identify possible pathways involved and the molecular mechanisms accounting for the roles of genetic variants, gene-gene and gene-environment interaction in diverse phenotypic expressions in asthma and allergic diseases. Many genetic variants in susceptible genes have been found to result in one or multiple functional changes such as epigenetic effects, promoter binding affinity, splicing change, mRNA stability, amino acid change or microRNA dysfunctions. To illustrate the potential role of CD14 C-159T in gene-environment interaction as described above, the functional genomic study showed that the constructs carrying the T allele have higher transcription rates than those carrying the C allele when transfected into a mononuclear cell line. Consistent results indicate that TT homozygous is associated with higher sCD14 than carriers of the other two genotypes. One hypothesis proposed by Marinez[57] was that endotoxin induced CD14 synthesis could be higher in carriers of the C allele than in carriers of the T allele especially at high endotoxin exposure making possible the protective effect for C allele, whereas higher constitutive expression of CD14 in TT homozygous than in other genotypes might be more susceptible to the protective effects of low levels of microbial products that interact with CD14. CD23 is a IgE low affinity receptor which is believed to mediate various pathogenic functions in IgE mediated immunity including antigen focusing and presentation, regulating IgE synthesis, promoting T and B cell differentiation and growth, and inducing the production of proinflammatory cytokines. A non-synonymous SNP of CD23 with Arg to Trp exchange at position 62 (R62W: rs228173) has been associated with enhanced T cell response to antigen in allergic subjects by us. The substitution of Trp for Arg seems to result in resistance of CD23 to enzymatic cleavage in transfected cell lines.[70] Therefore, the stabilized form of CD23 with Trp may be more efficient for antigen presentation than the original form accounting for the observed difference in T cell response. The changed function may be associated with diminished N-glycosylation in CD23 with Trp. More functional studies are being conducted for understanding its significance in allergic reactions in our lab.

The cytokine IL13 plays a critical role in Th2 response and induces allergic inflammation and experimental asthma. Multiple genetic variants in the promoter (C1112T: rs1800925; A-1521C: rs1881457) and coding regions (G2044A: rs20541) have been associated with atopic asthma and non-atopic asthma, increased risk of sensitization to food and outdoor allergens, and bronchial hypersensitiveness in multiple studies.[52,71] Mo-

lecular modeling suggests that the substitution of the mildly acidic acidic amino acid glutamine (Gln) for the basic arginine (Arg) at position 144 (rs20541) might promote enhanced binding of the ligand to IL13 receptor through removal of a basic-basic repulsion between Arg144 of IL-13 and His131 (histidine) of IL4Rα.[72] Subsequent functional studies on this variant suggests, as an alternative, that the Gln 144 variant of IL13 may bind less avidly to the decoy receptor IL-13Rα2, thus upregulating the ligand's availability and thus increasing the risk for asthma.[73] The functional study on SNP rs1800925 showed that -1112T allele enhanced IL13 promoter activity in primary and murine CD4+ Th2 lymphocytes. Increased expression of IL13 -1112T in Th2 cells was associated with the creation of a Yin-Yang 1 binding site that overlapped a STAT motif involved in negative regulation of IL13 expression and attenuated STAT6-mediated transcription repression.[60] Recently, IL13 A-1521C is found to be a functional polymorphism that significantly enhances HS4-dependent IL13 expression by creating a binding site for the transcription factor Oct-1. It has been demonstrated that increased activity for IL13-1521C allele was exquisitely dependent on physiological levels of Oct-1 by comparing the expression in transfected Th2 cells lines from Oct1+/+ mice and Oct1+/-.[74] These functional studies on IL13 suggest that multiple genetic variants in one gene may be involved in the functional differences among individuals who may have different combination of these genetic variants. If high linkage disequilibrium is taken into account, the individual with a haplotype with high risk alleles at multiple sites may have much higher risk of asthma due to synergistic at least additive effect. Genetic variant interaction could be another important factor to account for the complexity in confirming gene and genetic variants in asthma and allergic diseases.

MULTIFACTORIAL GENETICS OF ASTHMA AND ALLERGIC DISEASES

It is now widely accepted that genetics of asthma and allergic diseases are multifactorial. No single gene or genetic variant can explain all even most of variances in the general population for asthma and allergic diseases. The genetic basis is interacted with environmental factors in the development of asthma and allergic diseases perhaps together with development factors. Therefore, we propose that the susceptible genes for asthma may be categorized into four main groups based on their major functions: Barrier layer and defense; Antigen recognition and presentation; Immunoregulation and Th2 differentiation; and Effecter targets as showed in Fig. 2.

Barrier layer and defense

Skin, oral and bronchial epidermal are interfaces exposed to environmental agents. The integrity of the skin barrier is crucial for the interaction of host with environmental agents impacting
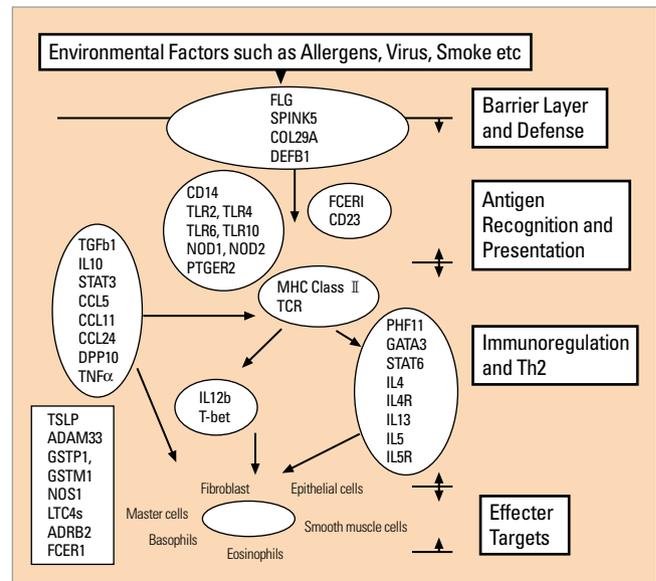

Fig. 2. Multifactorial genetics of asthma and allergic diseases. The genetic basis is interacted with environmental factors perhaps together with development factors in the development of asthma and allergic diseases. When gene-environment interaction is taken into consideration, the identified susceptible genes are categorized into four groups with major functions in barrier layer and defense, antigen recognition and presentation, immunoregulation and Th2 differentiation, and effecter targets.

the fate of future allergic reaction.[75,76] FLG (*Filaggrin*) is a member of the epidermal differentiation complex expressed in the epidermis and in oral and nasal mucosa, but not in bronchial mucosa. FLG plays a crucial role for the integrity of the epithelial barrier. Null mutation of FLG with the potential breakdown of the epidermal skin barrier may account for its high risk of atopic dermatitis and asthma reported in multiple studies.[77] SPINK5 expression is restricted to the epithelial and mucosal surfaces and in the thymus. It plays a protective role against proteases existed in many allergens or released from mast cells. A mutation of SPINK5 has been associated with asthma and eczema.[15] COL29A (collagen XXIX), an epidermal collagen, is highly expressed in the skin, lung and gastrointestinal tract and contributes to the epidermal integrity and function. The region with this gene on chromosome 3q21 was identified as a susceptibility locus for atopic dermatitis by genome wide linkage analysis.[78] DEFB1 (Defensin β1) is one of defensins that are antimicrobial peptides secreted from epithelium and may take part in airway inflammation and hyperresponsiveness. Genetic variants esp. that in 5' UTR of defensin β1 have been associated with asthma and related phenotypes in several cohorts suggesting its important roles in the development of asthma and allergy.[79,80]

Antigen recognition and presentation

When allergens and pathogens penetrate the barrier layer, a variety of receptors existing in antigen presenting cells will rec-

ognize them. Binding with specific ligands will initiate immune responses through the release of numerous inflammatory mediators and cell-cell contact, and promote antigen presentation to T cells for further cell differentiation. Genetic variants in numerous genes in this process have been associated with asthma and atopy related phenotypes suggesting their importance in allergic inflammation in the pathogenesis of asthma. This group of genes is composed of pattern recognition receptors either extracellular receptors such as CD14[57] and toll like receptors (TLRs)[81] or intracellular receptors such as NODs (nucleotide binding oligomerization domain),[82] IgE high affinity receptor (FceRI)[83] or low affinity receptor (FceRII, CD23),[70] and others such as PTGER2 (prostaglandin receptor),[84] HLA DRB1 gene,[85] and TCR (T cell receptor).[86] Recognition of pathogenic organisms by the innate immune system relies on pattern recognition receptors that detect preserved structures of bacteria, viruses, protozoa, and fungus, so-called pathogen-associated molecular patterns (PAMPs). TLR2 is involved in recognition of microbial motifs of a wide range of Gram-positive microorganisms, mycobacteria, and yeast.[59,87] Heterodimers TLR1/2 and TLR6/2 seem to be necessary for TLR1 to function for activation by lipopeptides and TLR4-MD2 for LPS.[81] TLR1, TLR6, and TLR10 are closely located on Chromosome 4p14. They have been associated with asthma and atopy related phenotypes in various studies.[88] TLR9 is a receptor for bacterial CpG DNA motifs and may interact with genetic variants of TLR2 impacting asthma risk.[87] The nod-like receptors (NLRs) are a specialized group of intracellular receptors that recognize specific bacterial and endogenous molecules, and represent a key component of the host innate immune system. Intracellular sensors NOD1 and NOD2 play a critical role in host defense when TLR signaling is reduced, such as within the intenstine due to low expression levels of TLRs or after induction of tolerization by exposure to TLR ligands.[82] Certain genetic variants of NOD1 and NOD2 were associated with an increased risk of developing asthma and atopic dermatitis or IgE level while some genetic variants of NOD1 were associated with a protective effect of exposure to a farming environment.[89,90] PTGER2 has special interests because it may interact with Pollen-associated phytoprostanes, the prostaglandin like bioactive molecules, which inhibit dendritic cell IL12 production and augment Th2 cell polarization.[91] Variants of HLA DRB1 loci have been associated with specific allergen sensitization in different DR loci such as to ragweed, cockroach allergens and to clinical syndrome of allergic bronchopulmonary aspergillosis.[85] IgE specific receptors are critical in mediating allergic inflammation. Genetic variants in both IgE high and low affinity receptor have been linked and associated with asthma and atopy related phenotypes in various studies as well.[83,92] The genetic variants in these genes might play a specific role for high risk of asthma in response to a specific allergen or an environmental factor.

Immunoregulation and Th2 differentiation

The recognition and presentation of environmental antigens will initiate regulating ether innate or adaptive immunity which will play important roles in inflammation and other pathogenic processes in asthma and allergic diseases through the release of various mediators as mentioned above. Allergic inflammation is one of the characterized features resulting from the interaction between environment and host in the pathogenesis of asthma and allergy. Th2 differentiation is a crucial event in allergic inflammation reaction.[93] Th1 cells produce IFN-γ and IL12, and promote cellular immunity. Th2 cells produce predominantly IL4, IL5, IL13 etc, which induce allergic immune response including IgE production and eosinophil activation. The differentiation of T cells into Th1 or Th2 may be dictated by a complex network of antigens, characteristic of antigen presenting cells, and cytokine environment.[94] The associations of genetic variants in Th2 related genes such as GATA3, STAT6, IL4, IL4R, IL13, IL5, IL5R, and PHF11 and in Th1 related genes such as IL12b, T-bet (T box transcription factor), HLX1 (homeobox transcription factor H.20 like homeobox 1), IRF (Interferon regulatory factor-1) with asthma and atopy related phenotypes have been reported.[22] As various genes from different cell sources are involved in regulating the development of Th2 cell differentiation, the genetic variants in genes such as TGFβ1, IL10, STAT3, CCL5, CCL11, CCL24, and DPP10 may play an important role in the pathogenesis of asthma too.[20,22]

Effecter targets

The dominant physiological event in asthma is airway narrowing and a subsequent interference with airflow. These result from bronchial smooth muscle contraction (bronchoconstriction), persistent and more progressive inflammation with airway edema, hyperemia of the airway mucosa and infiltration of mucosa with inflammatory cells, thickened airway wall with deposition of type III collagen and tenascin below the true basement membrane.[1] In more severe asthma, thickening airway wall gets worse with hypertrophy and hyperplasia of airway glands and secretary cells, hyperplasia of airway muscle, blood vessel proliferation and dilation, and the further deposition of submucosal collagen. The inflammatory mediators including chemokines, cytokines, cysteinyl-leukotrienes, IgE generated from various inflammatory cells specifically and interactively contribute to this process in asthma. The genetic variants of genes involved in this process may impact the development of asthma at different stages. These genes include ADAM33 for tissue remodeling,[6] GSTM1, GSTT1, and GSTP1 for detoxification of environmental and oxidative stress,[95] NOS1 for nitric oxide synthesis,[96] LTC4s for cysteinyl-leukotrienes generation,[97] ADRB2 for smooth muscle relaxation,[3] TNF for inflammation,[98] FceRI for allergic mediators,[83] GPRA for regulation of cell growth and neural mechanisms,[10] CCL11 and CCL5 for eosinophil chemoattractant.[99] As it is a very complicated process, the

genetic variants in more genes remain to be explored in this process. It should be kept in mind that the above gene classification is only for illustration purpose in the complexity of asthma and allergic diseases. As one gene may own multiple functions in the pathogenesis of asthma, the genetic variants in each gene may likely have multiple roles and therefore may play complicated roles in asthma and allergic diseases.

CONCLUSION AND FUTURE PERSPECTIVES

Genetics of asthma and allergic diseases are multifactorial and are conditional on other factors such as environmental exposure and race/ethnicity. In addition to what have been found, more genes and genetic variants will be identified in the pathogenesis of asthma and allergic diseases in the coming years. The development of new technology such as high through put and low cost gene chip and next generation of DNA resequencing will expedite our efforts. The combination of genome wide association study with expression microarray analysis will be helpful for illustrating the mechanism in a comprehensive way. Due to the phenotype heterogeneity in published studies, a standardized definition for phenotypes in genetic studies of asthma and allergic diseases will be useful for comparison and further personalized medicine purpose. The collection of environmental exposure information will be necessary for understanding potential gene-environment interactions involved, and for comprehensively illustrating genetic effects of susceptible genes. Functional studies are important and necessary to understand the true molecular mechanisms involved in their genetic effects in asthma and allergic diseases. Biostatistics, and bioinformatics and system biology approaches are needed to handle with vast amounts of information and identify the constellation of genes in terms of environment interaction and gene–gene interaction perhaps associated with development stages. It is expected that unraveling genetics of asthma and allergic diseases significantly improve prevention, diagnosis and treatment of asthma and allergic diseases in future.

CONFLICT OF INTEREST STATEMENT

Dr. Jianfeng Meng has no financial or other issues that might lead to conflict of interest. Dr. Rosenwasser has the following disclosure: RESEARCH STUDIES -Alcon, GlaxoSmithKline, MBBH/MacArthur Foundation, National Institutes of Health; CONSULTANT-Abbott Corp, Alcon, Alexion, Atlanta Pharmaceuticals, Biogen Idec Corp, Genentech, GlaxoSmithKline, Merck, Novartis, Sanofi-Aventis, Astra Zeneca Corp.; SPEAKERS' BUREAU-Abbott Corp, Alcon, Atlanta Pharmaceuticals, Banya Pharmaceuticals, Biogen Idec Corp, Genentech, GlaxoSmith-Kline, Merck, Novartis, Sanofi-Aventis, Schering.

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