Chapter 1
Genetics in Asthma

Tohru Sakamoto and Nobuyuki Hizawa

Abstract Asthma is a complex disease determined by the interaction between genetic and environmental factors. The heritability of asthma is estimated to be more than 50%. The search for genes with genomic variants associated with asthma has been greatly advanced by hypothesis-driven candidate gene association studies and hypothesis-free genome-wide association studies (GWASs). The genes identified by the hypothesis-driven approach are roughly classified into four types of functional group: (1) innate immunity and immunoregulation, (2) differentiation and regulation of T-helper 2 cells, (3) airway epithelial mucosal immunity, and (4) airway remodeling and lung function. Although the majority of the variants have been identified by the hypothesis-driven approach, novel genes and pathways associated with asthma have been successively clarified by GWASs. Nonetheless, these genomic variants explain only a small proportion of asthma heritability. This is partly due to the phenotypic heterogeneity of asthma, epistatic gene-gene interactions, gene-environment interactions, and epigenetic effects. Further elucidation of the causal variants can be achieved by GWASs that limit participants to those with distinct asthma phenotypes and by integrative applications of genome-wide epistatic and epigenetic approaches. Understanding of the genetic profiles of asthma pathogenesis contributes to individualized disease prevention as well as to development of new therapies.

Keywords Association study · Epigenetics · Epistasis · Gene-environment interaction

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1.1 Introduction

Asthma is a complex syndrome characterized by reversible airflow obstruction, airway hyperresponsiveness, and chronic airway inflammation. Although asthma has been recognized as a disease with heritable components, the increase in asthma prevalence, especially in developed countries, suggests that both genetic and environmental factors are involved in its pathogenesis. The different clinical phenotypes of asthma also reflect a variety of interactions between multiple genetic and environmental risk factors. In regard to the genetic contribution, a recent meta-analysis of 71 twin studies estimated the heritability of asthma to be 54% [1]. In the past few decades, the search for genes associated with asthma has been rigorously carried out, and almost 700 genes have been reported. A major breakthrough in this identification of the genes has been achieved by genetic association studies, i.e., candidate gene and genome-wide association (GWAS) studies. Although more than half of the identified genes have not been replicated by other studies, the numerous remaining genes with repeated validations confirm that the disease risk depends on many different genetic variants. Characterizing the genetic architecture of asthma contributes to understanding of its pathophysiological mechanisms and leads to the discovery of new biological drugs. The genetic profiles associated with asthma susceptibility are extremely useful for identifying at-risk individuals and for performing early intervention. And the genetic profiling for progression, severity, and therapeutic response of asthma can significantly contribute to modifying the disease's progression, preventing severe disease, and developing personalized medicine.

In this chapter, we first summarize the results of association studies on asthma and discuss their limitations. We then discuss the genetic approaches to asthma pathogenesis considering the presence of disease heterogeneity, epistasis (gene-gene interactions), gene-environment (GxE) interactions, and epigenetics.

1.2 Association Studies

Genetic association studies test for relationships between disease traits and genetic variations such as single-nucleotide polymorphisms (SNPs) to identify candidate genes for the disease. Such studies usually compare the allele or genotype frequencies of individuals with the disease and healthy controls (case-control association study). Genetic association studies can be further differentiated into two approaches: hypothesis-driven candidate gene association studies and hypothesis-free GWASs.

1.2.1 Candidate Gene Association Studies

In candidate gene association studies, genes are generally selected on the basis of their known function that is hypothesized to influence asthma susceptibility or on the basis of their chromosomal position identified by previous linkage

studies. Although associations identified by this method are easy to interpret, candidate gene association studies cannot discover novel genes or pathways. To validate an association, the association must be replicated in at least several independent studies. According to the DisGeNET database [2], almost 700 genes associated with asthma have been reported as a result of using this method, and around 100 genes have been replicated more than 4 times at the gene level. The gene level means that any variants associated with asthma are considered to represent a replication if they are located within the same gene. Figure 1.1 provides a summary of the top 30 genes identified first by candidate gene association studies and subsequently replicated in at least 15 independent reports. These genes are classified into four functional categories [3], as described hereafter:

(1) Genes associated with innate immunity and immunoregulation: Pattern-recognition receptors such as CD14 and TLR4 can be triggered by a variety of environmental stimuli, which leads to secretion of mediators stimulating adaptive immune responses. CTLA4, TGFB1, IL10, and IFNG are immunoregulatory cytokines. CTLA4 contributes to the suppressor function of regulatory T cells; thus, dysregulation of CTLA4 has the potential to affect the pathogenesis of asthma. TGFB1 has profound immunosuppressive actions involving both innate and adaptive responses. IL10 regulates the immune response by inhibiting antigen-presenting cells. IFNG is important for the activation of macrophages. By upregulating Th1 responses, IFNG subsequently inhibits Th2 differentiation. Each of these immunoregulatory cytokines plays an important role in orchestrating both innate and adaptive immunity. HLA class II molecules such as HLA-DRB1 and HLA-DQB1 are essential for activation of antigen-specific T cells.
(2) Genes associated with differentiation and regulation of Th2 cells: IL4, IL13, and their receptor, IL4R, are critically involved in the onset and effector phase of Th2 immune responses. MS4A2, also known as FCER1B, is a high-affinity IgE receptor and stimulates Th2 responses. Conversely, IL12B induces differentiation of Th1 cells and downregulates Th2 responses.
(3) Genes expressed in epithelial cells and involved in mucosal immunity: CCL5, also known as RANTES, is a chemoattractant for Th cells and eosinophils. SCGBA1 is a clara cell secretory protein and plays an important role in immunoregulation. FLG, an essential regulator of epidermal homeostasis, is highly associated with atopic dermatitis and influences susceptibility to asthma. Interestingly, FLG is not expressed in bronchial epithelial cells, which led to the concept of the atopic march.
(4) Genes associated with airway remodeling and lung function: This category includes genes related to inflammatory responses (*TNF*, *LTC4S*, *LTA*, *ALOX5*, *IL18*, *ACE*, *IL1RN*, *CCR5*, and *IL6*), detoxification (*GSTP1*, *GSTM1*, and *GSTT1*), and bronchoconstriction (*ADRB2* and *CYSLTR1*). TGFB1 is the master regulator of fibrosis [4] and acts as an immunoregulatory cytokine.

Gene	Chromosome	Function
ADRB2	5q32	Bronchial smooth-muscle relaxation
TNF	6p21.33	Inflammatory responses
IL13	5q31.1	Th2-cell differentiation and effector functions
IL4	5q31.1	Th2-cell differentiation and effector functions
IL4R	16p12.1	Th2-cell differentiation and effector functions
GSTP1	11q13.2	Environmental and oxidative stress — detoxification
GSTM1	1p13.3	Environmental and oxidative stress — detoxification
CD14	5q31.3	Innate immunity — microbial recognition
TGFB1	19q13.2	Immunoregulation, profibrotic effects
CCL5	17q12	Monocyte, T-cell, and eosinophil chemoattractant
LTC4S	5q35.3	Inflammatory responses
IL10	1q32.1	Immunoregulation
MS4A2	11q12.1	Th2-cell differentiation and effector functions
SCGB1A1	11q12.3	Epithelium-derived anti-inflammatory responses
TLR4	9q33.1	Innate immunity and immunoregulation
GSTT1	22q11.23	Environmental and oxidative stress — detoxification
FLG	1q21.3	Epithelial barrier integrity
LTA	6p21.33	Inflammatory responses
CYSLTR1	Xq21.1	Bronchoconstriction
HLA-DRB1	6p21.32	Antigen presentation
ALOX5	10q11.21	Inflammatory and allergic responses
HLA-DQB1	6p21.32	Antigen presentation
IL18	11q23.1	Inflammatory responses
ACE	17q23.3	Inhibition of inflammatory responses
IFNG	12q15	Innate and adaptive immune responses
CTLA4	2q33.2	T-cell-response inhibition and immunoregulation
IL12B	5q33.3	Downregulation of Th2 responses
IL1RN	2q14.1	Inhibition of inflammatory responses
CCR5	3p21.31	Inflammatory responses to infection
IL6	7p15.3	Inflammatory responses

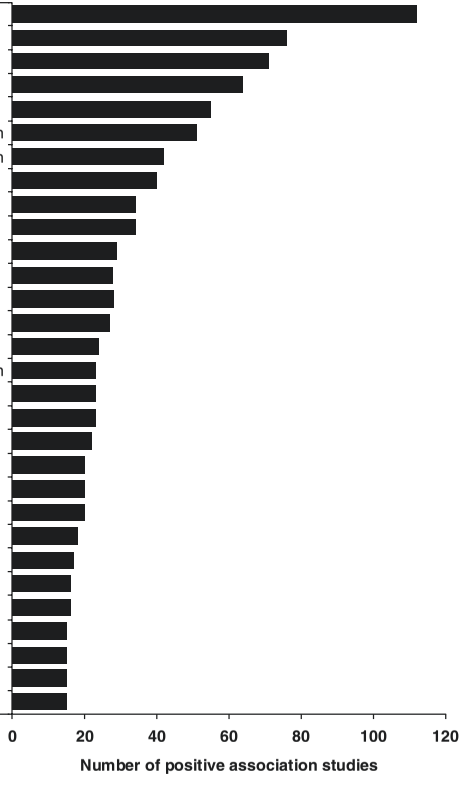

Fig. 1.1 Top 30 genes identified by candidate gene association studies on asthma. Genes associated with asthma in at least 15 independent reports were selected by searching the public database DisGeNET (http://www.disgenet.org/web/DisGeNET/menu;jsessionid=1fhetotkci0swxh27imgj7tdg). *ADAM33* and *NPSR1* were excluded because associations of these two genes with asthma were first reported by hypothesis-free linkage analysis followed by positional cloning

Although associations of *ADAM33* and *NPSR1* with asthma have been replicated in 27 and 16 independent candidate gene association studies, respectively, we have excluded these two genes from Fig. 1.1 because associations of the genes with asthma were first reported by hypothesis-free linkage analysis followed by positional cloning. ADAM 33 is preferentially expressed in mesenchymal cells such as smooth muscle cells and fibroblasts, but not in bronchial epithelium and inflammatory cells. This membrane-anchored protein is implicated in a variety of biological processes involving cell-cell and cell-matrix interactions. It is suggested that ADAM33 is a tissue remodeling protein and affects airflow obstruction and lung function [5]. The identification of ADAM33 provided an important breakthrough in understanding epithelial-mesenchymal interactions in the pathogenesis of asthma. NPSR1 is a G protein-coupled receptor for neuropeptide S and related to multiple neuroendocrine and inflammatory responses. NPSR1 is highly expressed in the bronchial epithelium and smooth muscle cells of asthma patients.

1.2.2 Genome-Wide Association Studies

GWAS allows the analysis of hundreds of thousands to millions of polymorphisms located throughout the genome and permits unbiased or hypothesis-free study. Table 1.1 shows a summary of GWASs on asthma from the GWAS Catalogue (https://www.ebi.ac.uk/gwas/). To date, 17 GWASs on asthma identified 83 SNPs with a standard genome-wide significance level ($P < 5 \times 10^{-8}$) in the discovery data.

The first asthma-associated locus identified by GWAS is located on chromosome 17q21, which includes the *ORMDL3* and *GSDMB* genes [6]. The function of ORMDL3 and GSDMB in asthma had been largely unknown. However, after this first GWAS, associations of *ORMDL3* and *GSDMB* with asthma were replicated in 17 and 16 independent candidate gene association studies, respectively. The SNPs identified by these studies are strongly associated with increased expression of both *ORMDL3* and *GSDMB* [7]. Recently, it has been shown that intrinsically elevated levels of ORMDL3 in the endoplasmic reticulum of airway smooth muscle cells upregulate their own contractility and proliferation, which might contribute to airway hyperresponsiveness (AHR) independent of extrinsic inflammation [8]. GSDMB is highly expressed in human bronchial epithelial cells in asthma. Overexpression of GSDMB increases expression of 5-lipoxygenase and TGFB1 in bronchial epithelium, which also leads to AHR and airway remodeling in the absence of inflammation [9]. The understanding of these functions of both ORMDL3 and GSDMB challenges the current paradigm that AHR and airway remodeling in asthma are secondary to airway inflammation.

Another promising GWAS locus for asthma is *IL33* on chromosome 9q24, which has been replicated in six independent asthma GWASs. IL33 is an innate epithelial cytokine and released from inflamed or injured epithelial cells. *IL1RL1*, on chromosome 2q12, encoding part of the IL33 receptor complex, has also been replicated in five asthma GWASs. IL1RL1 is expressed on Th2 cells, type 2 innate lymphoid

Table 1.1 Summary of genome-wide association studies on asthma ($P < 5 \times 10^{-8}$)

First author	Journal	Disease/ trait	Ethnicity	SNP	Gene	*P* value
Moffatt MF	Nature, 2007	Childhood asthma	European	rs7216389	*ORMDL3*	9×10^{-11}
Himes BE	Am J Hum Genet, 2009	Childhood asthma	European	rs1588265	*PDE4D*	3×10^{-8}
Sleiman PM	N Engl J Med, 2010	Childhood asthma	European	rs2786098	*DENND1B–CRB1*	9×10^{-11}
Moffatt MF	N Engl J Med, 2010	Asthma	European	rs3771166 rs744910 rs3894194 rs2284033 rs9273349 rs1342326	*IL18R1* *SMAD3* *GSDMA* *IL2RB* *HLA-DQA1–HLA-DQB1* *IL33*	3×10^{-9} 4×10^{-9} 5×10^{-9} 1×10^{-8} 7×10^{-14} 9×10^{-10}
Torgerson DG	Nat Genet, 2011	Asthma	European African Latino	rs1101999 rs3771180 rs1837253 rs2381416 rs11078927	*PYHIN1* (African) *IL1RL1* *TSLP* *IL33* *GSDMB*	4×10^{-9} 2×10^{-15} 1×10^{-14} 2×10^{-12} 2×10^{-16}
Hirota T	Nat Genet, 2011	Asthma	Japanese	rs7686660 rs1837253 rs204993 rs404860 rs3129943 rs3117098 rs3129890 rs7775228 rs9275698 rs9500927 rs10508372 rs2069408 rs1701704	*LOC729675* *TSLP* *PBX2* *NOTCH4* *C6orf10* *BTNL2* *HLA-DRA* *HLA-DQB1* *HLA-DQA2* *HLA-DOA* *LOC338591* *CDK2* *IKZF4*	2×10^{-12} 1×10^{-16} 2×10^{-15} 4×10^{-23} 3×10^{-15} 5×10^{-12} 5×10^{-13} 5×10^{-15} 5×10^{-12} 4×10^{-9} 2×10^{-15} 1×10^{-10} 2×10^{-13}
Noguchi E	PLoS Genet, 2011	Childhood asthma	Japanese	rs3019885 rs987870	*SLC30A8* *HLA-DPB1*	5×10^{-13} 2×10^{-10}
Ferreira MA	Lancet, 2011	Asthma	European	rs4129267 rs7130588	*IL6R* *LRRC32*	2×10^{-8} 2×10^{-8}
Forno E	J Allergy Clin Immunol, 2012	Childhood asthma	European	rs9815663 rs7927044	*IL5RA* *NR*	2×10^{-8} 7×10^{-9}
Wan YI	Thorax, 2012	Asthma	European	rs4794820	*ORMDL3*	1×10^{-8}
Ramasamy A	PLoS One, 2012	Asthma	European	rs13408661 rs9268516	*IL18R1–IL1RL1* *HLA-DRA–BTNL2*	1×10^{-9} 1×10^{-8}
Lasky-Su J	Clin Exp Allergy, 2012	Asthma	European	rs9272346	*HLA-DQA1*	2×10^{-8}
Ding L	Hum Genomics, 2013	Childhood asthma	European African Hispanic	rs16929097 rs17218161 rs12570188	Intergenic Intergenic *HPSE2*	8×10^{-9} 2×10^{-8} 5×10^{-8}

Table 1.1 (continued)

First author	Journal	Disease/ trait	Ethnicity	SNP	Gene	*P* value
Bonnelykke K	Nat Genet, 2013	Childhood asthma	European	rs6967330	*CDHR3*	3×10^{-14}
				rs2305480	*GSDMB*	6×10^{-23}
				rs928413	*IL33*	9×10^{-13}
				rs3894194	*GSDMA*	3×10^{-21}
Ferreira MA	J Allergy Clin Immunol, 2013	Asthma and hay fever	European	rs9273373	*HLA-DQB1*	4×10^{-14}
				rs4833095	*TLR1*	5×10^{-12}
				rs1438673	*WDR36*	3×10^{-11}
				rs10197862	*IL1RL1*	4×10^{-11}
				rs7212938	*GSDMA*	4×10^{-10}
				rs1837253	*TSLP*	1×10^{-9}
				rs72699186	*IL33*	2×10^{-9}
				rs17294280	*SMAD3*	4×10^{-9}
				rs7009110	*ZBTB10*	4×10^{-9}
				rs62026376	*CLEC16A*	1×10^{-8}
Pickrell JK	Nat Genet, 2016	Asthma	European	rs11655198	*ZPBP2*	1×10^{-63}
				rs3104367	*HLA-DRB5–*	1×10^{-40}
				rs144829310	*HLA-DQA1*	1×10^{-31}
				rs1837253	*RANBP6–*	3×10^{-31}
				rs202011557	*IL33*	5×10^{-31}
				rs56375023	*SLC25A46–*	2×10^{-21}
				rs7936323	*TSLP*	1×10^{-16}
				rs2428494	*IL1RL2–*	1×10^{-16}
				rs2244012	*IL1RL1*	2×10^{-16}
				rs34290285	*SMAD3*	2×10^{-15}
				rs7203459	*C11orf30–*	4×10^{-15}
				rs4233366	*LRRC32*	5×10^{-15}
				rs12413578	*HLA-C–MICA*	8×10^{-12}
				rs10957978	*RAD50*	1×10^{-11}
				rs10519068	*D2HGDH*	4×10^{-11}
				rs5743618	*CLEC16A*	4×10^{-11}
				rs58521088	*B4GALT3–*	7×10^{-11}
				rs3001426	*ADAMTS4*	1×10^{-10}
				rs73196739	*GATA3*	7×10^{-9}
				rs6683383	*TPD52–*	1×10^{-8}
				rs13412757	*ZBTB10*	1×10^{-8}
				rs1723018	*RORA*	1×10^{-8}
				rs3784099	*TLR1*	2×10^{-8}
				rs6959584	*BACH2*	2×10^{-8}
				rs200634877	*STAT6–LRP1*	3×10^{-8}
				rs6691738	*LPP*	3×10^{-8}
				rs662064	*ADORA1*	3×10^{-8}
					ID2	
					CD247	
					RAD51B	
					CDHR3	
					NDFIP1	
					TNFSF18–	
					TNFSF4	
					PEX14	

(continued)

Table 1.1 (continued)

First author	Journal	Disease/ trait	Ethnicity	SNP	Gene	*P* value
Almoguera B	Am J Respir Crit Care Med, 2017	Asthma Adult asthma	African European	rs11788591 rs72721168 rs1776883 rs75446656 rs36080042 rs3095318	*PTGES* *MOB3B–EQTN–TEK* *GRM4–HGMA1* *JMJD1C* *REEP3* *PSORS1C1*	4×10^{-8} 7×10^{-10} 5×10^{-9} 4×10^{-8} 5×10^{-8} 2×10^{-11}

cells (ILC2s), and innate immune cells such as mast cells and basophils. The IL33/IL1RL1 signaling pathway induces type 2 immune responses. These findings emphasize an important role of innate epithelial cytokines in promoting type 2 immune responses related to asthma pathogenesis. TSLP is also an innate epithelial cytokine, which has been replicated in five independent asthma GWASs. TSLP is released in response to environmental stimuli and induces type 2 immune responses by activating dendritic cells, ILC2s, and T cells. TLR1 also plays an important role in regulation of adaptive as well as innate immune responses. TLR1 forms a heterodimer with TLR2 and recognizes a wide variety of microbial ligands. Association of *TLR1* with asthma has been replicated in three independent GWASs.

As predicted by their function in activation of antigen-specific T cells, associations of HLA class II molecule genes (*HLA-DQ*, *HLA-DR*, *HLA-DP*, and *HLA-DO*) with asthma have been replicated in several independent GWASs.

Recently, a large GWAS involving 28,399 asthma cases and 128,843 controls demonstrated 27 SNPs, with a significance of $P < 5 \times 10^{-8}$ [10]. Six of these SNPs showed *P* values even lower than 1×10^{-20}. Each of these six SNPs is located in the locus containing *ZPBP2* ($P = 1.0 \times 10^{-63}$), *HLA-DRB5–HLA-DQA1* ($P = 1 \times 10^{-40}$), *RANBP6–IL33* ($P = 1.3 \times 10^{-31}$), *SLC25A46–TSLP* ($P = 3.3 \times 10^{-31}$), *IL1RL2–IL1RL1* ($P = 5.1 \times 10^{-31}$), and *SMAD3* ($P = 2.4 \times 10^{-21}$). The *ZPBP2* gene is located in a dense haploblock on chromosome 17q21 encompassing the *ORMDL3*, *GSDMB*, *GSDMA*, and *IKZF3* genes. Asthma-risk alleles in this locus affect the expression of *ORMDL3* and *GSDMB* [7], which may lead to AHR and airway remodeling. *HLA-DRB5* and *HLA-DQA1* are HLA class II genes responsible for adaptive immune responses. As mentioned above, TSLP and IL1RL1 play an important role in linking innate and adaptive immunity in asthma, and both have been replicated in several independent asthma GWASs. SMAD3 is a downstream mediator of TGFB1 and plays a central role in regulating airway smooth muscle cell proliferation and airway remodeling [11]. SMAD3 has been replicated in four independent asthma GWASs.

Although GWASs have identified many previously unknown candidate genes, which have led in turn to understanding of novel biological mechanisms and pathways of asthma susceptibility, the genetic variants identified so far explain only a small proportion of the heritability. This is partly due to the fact that GWAS deals primarily with common variants with minor allele frequencies >1%. Rare variants and structure variants (duplications, inversions, translocations, and copy-number

repeats) that are not included in available genotyping arrays might play an important role in the pathogenesis of common diseases such as asthma. Other potential reasons stem from the phenotypic heterogeneity of asthma, epistasis (gene-gene interactions), GxE interactions, and epigenetic effects. Despite these limitations, GWASs have still given rise to many novel concepts related to the pathogenesis of asthma.

1.3 Phenotypic Heterogeneity, Epistasis, Gene-Environment Interactions, and Epigenetics

Unbiased cluster analyses have demonstrated that asthma is a syndrome with heterogeneous phenotypic groups [12]. To increase the statistical power to detect true variants associated with asthma, several recent GWASs focused on distinct asthma phenotypes. We identified the *HCG22* gene through a GWAS focusing on late-onset asthma (age of onset ≥40 years) [13]. And a GWAS limiting participants to those with a smoking history of no more than 10 pack-years identified *HAS2* as a susceptibility gene for adult asthma [14].

Epistasis is defined as the interaction between two or more genes affecting a phenotype of interest. The combined effect of multiple loci on the phenotype is not additive but rather synergistic or antagonistic. Several epistatic effects on asthma have been studied by focusing on interactions between *IL4*- and *IL13*-related genes [15]. Other epistatic effects on asthma include interactions between innate immunity-related genes such as *CD14* and *AOAH* [16], between oxidative stress-related genes such as *NQO1*, *MPO*, and *CAT* [17], and between airway remodeling-related genes such as *PAI-1* and *MS4A2* [18]. These studies have used candidate gene approaches for analysis of the epistatic interactions. Hypothesis-free analysis of genome-wide epistatic interactions is severely hampered by a huge number of SNP-SNP combinations, which leads to significant difficulties in attaining sufficient statistical power. When all possible pairs of genes in a GWAS including 500,000 SNPs are selected, potentially 250 billion interactions must be tested. Nevertheless, several statistical methods have been developed to identify genome-wide epistatic interactions. Recently, an epistatic interaction between *ADAM33* and *GLI2* has been reported as a result of a genome-wide search [19]. As the transcriptional factor GLI2 regulates TGFB1 expression in $CD4^+$ T cells [20], GLI2 also seems to be involved in the pathogenesis of asthma.

GxE interactions are another possible reason for why the identified genetic variants explain only a small proportion of the heritability. Although the GxE interactions in asthma have been well recognized, the necessity for a large number of samples and the difficulties in accurately assessing environmental exposures mean that only a few comprehensive studies have been performed to date. The results of the genome-wide GxE interaction studies of asthma have been disappointing, with none of them meeting genome-wide criteria for statistical significance. The majority of the GxE interaction studies dealt with candidate GxE interactions, especially interactions between microbial exposures and variants in genes of the innate and

adaptive immune systems and between oxidant exposures and variants in antioxidant genes. Interaction between endotoxin exposure and a variant in endotoxin signaling pathway genes including *CD14* is associated with asthma susceptibility [21]. Interactions between oxidant air pollutants and antioxidant genes such as *CAT* and *HMOX-1* contribute to asthma susceptibility during adolescence [22].

GxE interactions can also be mediated by epigenetic modifications of the genome. Epigenetic modifications are caused by environmental stimuli and are defined as heritable changes in gene expression that do not involve changes in the underlying DNA sequence. Three types of genetic modifications are recognized to cause epigenetic effects: DNA methylation, histone modification, and noncoding RNA-associated gene silencing. Among these modifications, DNA methylation has been extensively focused on because a quantitative survey of methylation modification has recently become widely available. DNA methylation is covalent binding of a methyl group to the cytosine ring of genomic DNA. In humans, DNA methylation typically occurs at a cytosine nucleotide adjacent to a guanine residue (CpG dinucleotide) in somatic cells. Hypermethylation in the promoter region of the gene leads to gene silencing, while hypomethylation leads to active transcription. The effects of methylation in intragenic regions are complex and less clear. The majority of DNA methylation studies use genomic DNA isolated from leukocytes or mononuclear cells because these cells are readily collected from blood. Several array-based platforms for genome-wide methylation analysis are now available.

A genome-wide methylation study with blood mononuclear cells of inner-city children identified 81 differentially methylated regions in asthma patients when compared with healthy controls [23]. Several immunity-related genes involved in T cell maturation (*RUNX3*), Th2 immune responses (*IL13, IL4* and *TIGIT*), and oxidative stress (*CAT*) were hypomethylated in the asthmatic patients. Considering the functions of these genes, the results seem to be reasonable. However, whether the differentially methylated regions are causes or consequences of asthma development is difficult to determine. Methylation studies with blood samples taken before the emergence of asthma symptoms can discriminate the causes of the disease from the consequences. Recently, a genome-wide methylation study using cord blood mononuclear cells from neonates was performed to study development of childhood asthma by the age of 9 years [24]. The methylation level in the promoter region of the *SMAD3* gene was selectively associated with the development of asthma in children of asthmatic mothers. Furthermore, *SMAD3* promoter methylation in children of asthmatic mothers was significantly associated with neonatal IL1B production. These results suggest that epigenetic modification in response to the in utero environment promotes the developmental trajectory toward childhood asthma.

1.4 Conclusion

Candidate gene association studies of asthma have identified many risk alleles and loci. GWASs have further added a number of novel risk genes. Although the genetic basis for asthma is robust, each genomic variant identified by these

strategies contributes only a small proportion to the heritability of asthma. This is partly due to the disease's heterogeneity, epistatic interactions, GxE interactions, and epigenetic modifications. Integrative applications of genome-wide epistatic and epigenetic approaches using an adequate number of samples with distinct asthma phenotypes are necessary for further elucidation of the causal variants behind the single-gene association studies. Genetic profiling for susceptibility to asthma and for the subsequent progression, severity, and therapeutic response of asthma is extremely useful for identifying individuals with asthma onset risk, modifying the disease progression, and developing personalized medicine.

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