Chapter 9
Introduction to Genetics and Genomics in Asthma: Genetics of Asthma

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Abstract While asthma is a heterogeneous disease, a strong genetic basis has been firmly established. Rather than being a single disease entity, asthma consists of related, overlapping syndromes [Barnes (Proc Am Thor Soc 8:143–148, 2011)] including three general domains: variable airway obstruction, airway hyper-responsiveness, and airway inflammation with a considerable proportion, but not all, of asthma being IgE-mediated further adding to its heterogeneity. This chapter reviews the approaches to the elucidation of genetics of asthma from the early evidence of familial clustering to the current state of knowledge with genome-wide approaches. The conclusion is that research efforts have led to a tremendous repository of genetic determinants of asthma, most of which fall into the above phenotypic domains of the syndrome. We now look to future integrative approaches of genetics, genomics (Chap. 10), and epigenetics (Chap. 11) to better understand the *causal* mechanism through which, these genetic loci act in manifesting asthma.

Keywords Genetics • Linkage analysis • Positional cloning • Genome-wide association study • Linkage disequilibrium • Population stratification • Heritability • Complex traits • Asthma

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9.1 Evidence for a Genetic Basis to Asthma

9.1.1 Familial Aggregation of Asthma

Heritability is the proportion of variation in a quantitative trait or risk of disease for a dichotomous trait that can be attributed to genetic variation. Familial aggregation or clustering of asthma was recognized early in the twentieth century (Wiener, et al. 1938; Sakula 1988). The earliest familial studies performed by Cooke (Cooke and Vander Veer 1916; Spain and Cooke 1924) in 1916 and 1924 established an increased occurrence of asthma in relatives of subjects having the disease as compared to relatives of normal controls. Numerous family studies performed in the 1920s and 1930s (Wiener, et al. 1938; Schwartz 1952) and more recently (Gerrard et al. 1976; Dold, et al. 1992; Aberg 1993) found similar results of familial aggregation of the disease. Twin studies which have a greater advantage over the above-mentioned family-based studies: easier detection of nonadditive genetic effects (dominant or epistatic effects) and matching for environmental effects have consistently shown higher concordance between monozygotic twins in contrast to dizygotic twins. A tremendous range in the heritability of asthma is seen from these studies range from 36 to 95 % (Edfors-Lubs 1971; Duffy et al. 1990; Laitinen, et al. 1998; Skadhauge et al. 1999; Koeppen-Schomerus, et al. 2001; Hallstrand et al. 2005; Nystad et al. 2005; van Beijsterveldt and Boomsma 2007; Fagnani, et al. 2008; Willemsen et al. 2008; Thomsen, et al. 2010), with higher estimates generally observed in studies implementing more objective diagnostic criteria.

This wide spectrum of heritability estimates for asthma is not unexpected as heritability is a feature of the sample at hand particularly with respect to the relative contribution of genetic and environmental variability representing the complex interplay of genes and environment. It is useful to summarize these observations in the context of the relative risk to sibs (λ_s), which for monogenic diseases or largely genetic disorders, tends to be high (e.g. $\lambda_s \sim 500$ for Cystic Fibrosis). In contrast the λ_s is only about 2.0 for asthma (Cookson and Palmer 1998) which along with the complex genetic background implicated above makes the search for genetic loci that cumulatively contribute to this risk incredibly difficult. Despite this, there has been tremendous success in identifying genetic determinants of this disease as illustrated below.

9.1.2 Inheritance Models for Asthma

Further illustration of the complex nature of asthma comes from segregation analysis where evidence in support of a wide range of inheritance models has been noted. Segregation analysis finds its roots in Mendel's Law of Segregation: every individual inherits *factors* from his/her parents, and in the formation of gametes, these factors *segregate* into separate gametes, manifested themselves as specific genotypic, and consequently phenotypic distributions in the offspring generation. The evaluation of

complex models of genetic transmission to explain the observed distribution of asthma in a sample of families (Khoury and Beaty 1993) has provided evidence for codominant models with a correlation between age of onset and number of disease alleles (Wiener et al. 1938), dominant models (Schwartz 1952), and even polygenic and recessive models (Wang et al. 2000; Holberg et al. 1996; Martinez and Holberg 1995). From these studies, it is apparent that asthma should be considered as a paradigmatic complex genetic disease, manifesting through the interaction of multiple susceptibility genes with environmental.

9.2 Linkage Studies for Asthma

The promise of genetic mapping for disease-gene identification is that it requires no prior assumptions on the candidacy of a gene or locus in the biology of asthma—a so-called hypothesis-free approach. The first such application as a genome-wide approach was linkage analysis, a family-based mapping strategy designed to detect susceptibility loci (i.e., disease-susceptibility genetic regions) with large effect sizes that co-segregate with disease in either large pedigrees or nuclear families (Box 9.1). Families are ascertained by design; typically contain multiple affected and unaffected individuals (e.g., multiplex families with affected and unaffected individuals) identified on the basis of an index case (proband) and require genotype and phenotype information on affected and unaffected individuals. The affected-only ascertainment is an alternative whereby allele sharing between affected relative pairs (e.g., affected sibling pairs) is compared against the expected allele sharing given the relative-pair kinship. By relying solely on genetic co-segregation, linkage enables the discovery of novel genes and pathways without preconceived biases regarding the underlying biology. Parametric models that explicitly specify the mode of inheritance (i.e., dominant vs. recessive vs. co-dominant) have proven particularly effective for mapping variants underlying rare Mendelian diseases like cystic fibrosis. However, for complex diseases like asthma, where the correlation between individual mutations and disease risk (that is, genetic penetrance) is relatively low, less powerful nonparametric approaches are commonly used. These latter methods that compare allele sharing given the phenotype of the relative pair against the expected sharing for the relative-pair kinship are limited in power to detect smaller effect sizes (evidenced by the observation that linkage signals discovered thus far typically fail to meet strict genome-wide linkage thresholds of LOD > 3.7 and $p < 2 \times 10^{-5}$ (Lander and Kruglyak 1995). Furthermore, identified regions of linkage are typically wide (often more than 10 million bases) and encompass numerous genes that may cumulatively explain the overall linkage signal. Nonetheless, since the first genome-wide linkage screen for asthma susceptibility loci was published in 1996 (Daniels et al. 1996), >20 independent chromosomal regions have been identified though linkage approaches, many of which are widely replicated (chromsomes 2p, 4q, 5q21-33, 6p24-21, 11q13-21, 12q21-24, 13q12-14, 16q21-23, and 19q, Fig. 9.1) (Wills-Karp and Ewart 2004). Large scale meta-analyses of individual linkage scans (Bouzigon

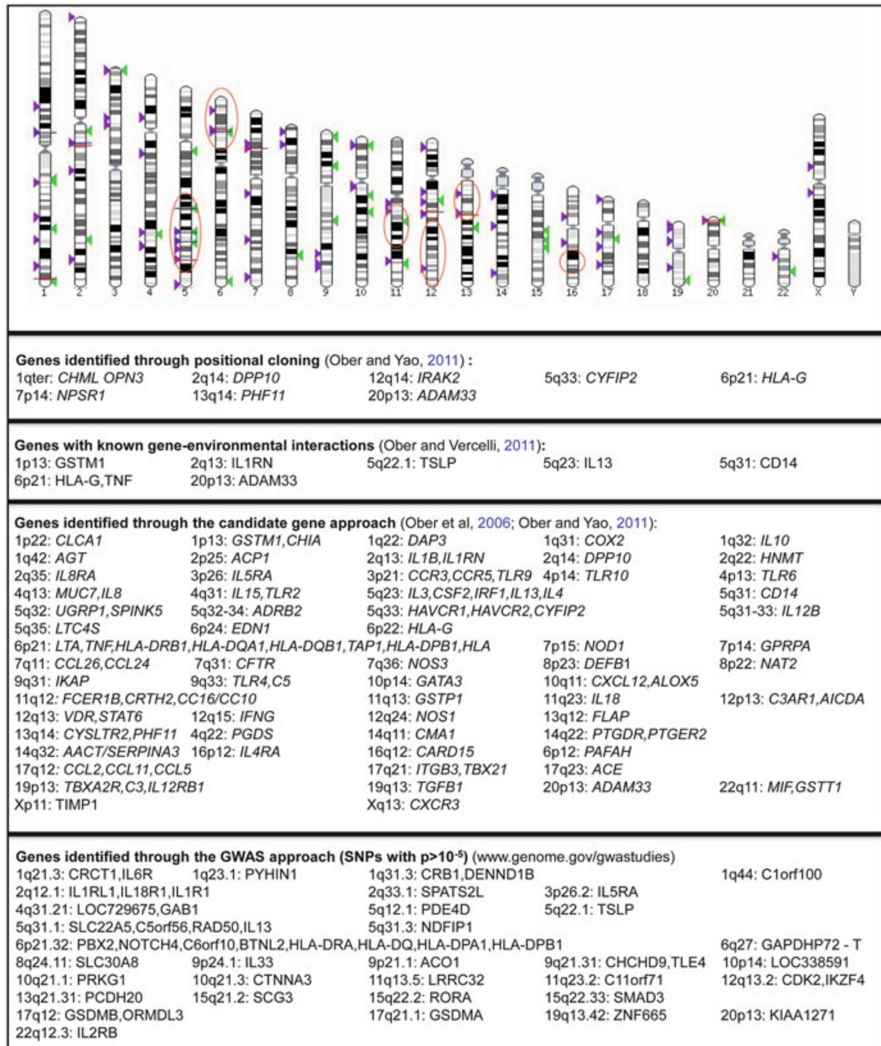


Fig. 9.1 Genetic loci discovered for the phenotype of asthma to date. Genes identified through the candidate gene approach are identified in *purple arrows* to the left of each chromosome. GWAS-identified genes with *p*-values <10^{-5} are identified in *green arrows* to the right of each chromosome genes involved in gene*environment interactions are identified in *horizontal red bars* and genes identified through positional cloning are identified by *horizontal red bars*. Regions of peak linkage evidences are represented by the *orange lasso* on chromosomes 5, 6, 11, 12, 13, and 16

et al. 2010; Denham et al. 2008) have revealed the value of a combined approach leading to the identification of a novel 2p21-p14 locus not noted in any single study. Meta-analyses have also revealed high between-study heterogeneity, which may be reflective of study design and family ascertainment differences, but probably also reflects the intrinsic complexity of the disease. While an analysis of 11 studies of

Caucasian asthma populations (1,267 pedigrees, $n = 5{,}832$) did not identify any region showing genome-wide significance with asthma, significant linkage with bronchial hyper-responsiveness (BHR) was observed with 2p12-q22.1, 6p22.3-p21.1 and 11q24.1-qter (Denham et al. 2008). A separate linkage-based meta-analysis of 20 different populations of differing ethnicities (3,024 pedigrees, $n = 10{,}027$) found genome-wide evidence for linkage with asthma on 2p21-p14 and 6p21 in the subset of European families.

Once linkage is observed, identification of the causal genes and variants first requires further narrowing of the candidate region through the process of positional cloning. Positional cloning typically consists of association testing of dense panels of single nucleotide polymorphisms (SNPs) across the linked regions to define those variants and their corresponding haplotype blocks that show strong genetic association with disease. *ADAM33* was the first report of a positionally cloned asthma gene (Van Eerdewegh, et al. 2002). A multistep approach of (1) linkage analysis in families yielding evidence for a novel locus on chromosome 20p13; (2) determination of a homologous region on mouse chromosome 2 previously linked to BHR; (3) subsequent case–control association approaches; (4) validation of association in family-based approaches; and (5) demonstration of *ADAM33* expression in lung cell types yielded the strongest evidence for associations to variants within the *ADAM33* gene identifying it as the most likely gene from a set of ~40 within the linkage peak. Additional successes using similar positional cloning approaches for asthma include *DPP10* (Allen et al. 2003) on 2q14, *PHF11* (Zhang et al. 2003) on 13q14, *NPSR1* (Laitinen et al. 2004) on 7p14, *HLA-G* (Nicolae et al. 2005) on 6p21, *CYFIP2* (Noguchi et al. 2005) on 5q33, *IRAK2* (Balaci et al. 2007) on 12q14, and *OPN3/CHML* (White et al. 2008) on 1qter.

While linkage analysis in asthma has suffered from lack of replication between studies, meta-analysis has emphasized select regions that may be robust to study-specific heterogeneity. In general, the identification of a single gene as the source of the highly replicated linkage signals has been limited. However, as summarized by Ober and Hoffjan (2006) and as illustrated in Fig. 9.1, many in the set of *most associated* genes map to regions of *most replicated linkage*. A striking illustration of this is the widely replicated linkage to chromosome 5q31-33 (Ober et al. 1998; Ober and Hoffjan 2006; CGSA 1997; Yokouchi et al. 2000; Yokouchi et al. 2002; Haagerup et al. 2002); at least 14 genes in this region have been shown to be associated with asthma and its related atopy phenotypes including some of the most replicated associations (*IL4*, *IL13*, *CD14*, *ADRB2*, *SPINK5*, *LTC4S*) (Ober and Hoffjan 2006; Ober and Yao 2011). This region also includes a positionally cloned gene (CYFIP2) (Noguchi et al. 2005), genes with documented environment interactions (Baldini et al. 2002; Zambelli-Weiner et al. 2005), and genes that influence drug response (Martinez et al. 1997). Given the complex nature of the genetic architecture of asthma, including the polygenic model established by multiple gene–gene and gene–environment interactions, the cumulative relative risk (λ_s) conferred by each locus is small. Families most probably segregate multiple loci that determine family-specific risks, with strong heterogeneity between families. It is likely that the next frontier of asthma genetics, which includes sequencing of entire genomes and therefore regions of prior linkage, will allow the direct evaluation of this hypothesis.

9.3 Association Studies for Asthma

Association testing explicitly tests for the nonrandom correlation between the observed phenotype of asthma and genotyped markers (most often SNPs) in a population and this is typically performed in a case–control design setting wherein allele frequencies at a measured SNP are compared between case and control samples from the population (Box 9.1). It is based on the concept of linkage disequilibrium; the nonrandom association of alleles at two or more loci (Box 9.1). While in linkage two or more loci on a chromosome have reduced recombination between them simply because of their physical proximity to each other, in LD combinations of specific alleles at genetic markers occur more or less frequently in a population than would be expected. If the allele under consideration is at higher frequency in cases in the population, then it is referred to as a *susceptibility* or *risk* allele. One of the major drawbacks of the case–control design is the potential for spurious associations due to population stratification: the presence of a systematic difference in allele and disease frequencies between subpopulations in a population give rise to confounding effects and false associations when cases and controls are not matched on subpopulation membership. Family-based designs such as the case-parent trio design where an affected individual and his/her parents are included rely on the transmission disequilibrium test (TDT) and are free from confounding due to population stratification. In principle the case-parent trio design tests for excess transmission of a specific allele from parents to affected offspring in a comparison of transmitted vs. untransmitted alleles.

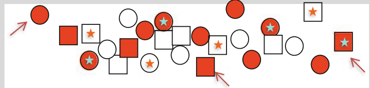
9.3.1 The Candidate Gene Approach

This approach is founded on prior knowledge; genes are selected to be tested as determinants of asthma using principles of association illustrated in Box 9.1 because they are either (1) believed to be biological candidates given their known function; (2) physically located within a region of linkage evidence; or (3) physically located within a region of prior association evidence. The main advantage to this approach is that it is narrow in hypothesis and thereby not limited by the stringent thresholds set in place with significance testing in the more unbiased (by prior knowledge) genome-wide approaches. On the other hand, the approach is limited in that it does not include novel loci that may add to the understanding of biology, each candidate gene study is generally insular only considering a gene in contrast to the pathway from which the biological candidacy is determined and often there is lack of replication between studies because of the lack of consideration of environmental effects further discussed below.

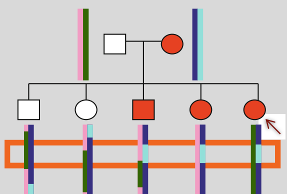
Despite these limitations, the candidate gene approach has had many successes in asthma, and these successes are elegantly summarized by a number of excellent review articles (Ober and Hoffjan 2006; Ober and Yao 2011; Vercelli 2008). Most of the >100 loci found to be harboring genetic determinants of asthma and its associated allergic phenotypes have evidence based in this approach, of which the most

Box 9.1 An overview of the concepts in linkage and association analysis for the discovery of asthma susceptibility loci

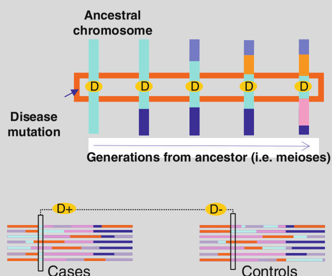
A reference population of cases (asthmatics) and controls (non-asthmatics)



Linkage reflects the tendency for chromosomal segments to be inherited intact from parent to offspring. Here, all affected offspring share a chromosome segment (blue) inherited from the mother that is not shared with the unaffected offspring which suggests a susceptibility locus within the shared chromosome segment.



Linkage disequilibrium is the nonrandom association of alleles at two or more loci. Here, association testing would identify a correlation between SNPs in the block of high LD (blue) and case status which would suggest the presence of an unobserved disease variant (D).



(continued)

Box 9.1 (continued)

- An allele-sharing approach that specifically tests for co-segregation of a genetic locus along with phenotype across individuals *within* each family. Then adds information across the families.
- Families are selected on the basis of an index individual (proband).Typically probands are a specific sample of *affected* individuals from the reference population.
- Genetic variant allele that co-segregates with phenotype has to be the same allele within a family, but does not need to be the same allele between families.
- Because closely related individuals share extensive regions of their genome, ~500 polymorphic markers are adequate to identify linkage.
- However, because relatively few recombinant events are seen within a single family, there is simultaneously difficulty in refining the genetic below the order of megabases.

- Traditionally tests for association of a specific allele of a genetic variant with phenotype across *independent, unrelated* individuals.
- Individuals are selected on the basis of affection ( ) in the reference population: cases = affected / controls = unaffected.
- An allele that is higher in frequency in cases is the susceptibility/risk allele.
- Because of LD, SNPs are correlated with each other and therefore “tag SNPs” are usually sufficient to adequately capture all variation in and around the unmeasured disease locus.
- Difficulty in tagging arises when the variant allele is rare or is common but from multiple ancestral chromosomes. If rare, then a SNP is usually not adequately tagged by other SNPs. If common but from multiple ancestral chromosomes then there may be multiple haplotype backgrounds on the variant.

frequently replicated are listed in Fig. 9.1. Asthma susceptibility genes have been argued to fall into four main categories, and candidate genes with identified associations fall into each of these categories as has been reviewed in-depth by Vercelli (2008). Briefly the asthma susceptibility loci fall into the following categories: (1) a class of genes associated with innate immunity and immunoregulation (examples include CD14, Toll-like receptors TLR2, TLR4, TLR6, and TLR10, cytokines such as IL10, TGFβ1, and HLA class II molecules); (2) genes associated with Th2-cell differentiation and effector function (including IL13 the central effector of allergic inflammation where genetic determinants of asthma are perhaps some of the best understood to date in their functional consequence) (Vladich et al. 2005; Cameron et al. 2006); (3) genes expressed in epithelial cells and involved in mucosal immunity (genes in the CC-chemokine cluster for example); and (4) the final class of genes that appear to determine lung function, airway remodeling, and asthma severity (two of the most consistent asthma loci *ADRB2* and *TNF*).

9.3.2 The Genome-Wide Association Approach

The advent of dense oligonucleotide microarrays that enable multiplex genotyping of large numbers of variants at low cost has made feasible the extension of genetic association beyond the study of candidate genes or regional positional cloning to a truly genome-wide survey. A variety of commercially available arrays enable typing of hundreds of thousands to several millions of variants simultaneously (Distefano and Taverna 2011) and rigorous statistical methods, including SNP genotype imputation methodologies have been developed to facilitate comprehensive testing of virtually all common genetic variation, including more than 35 million sequenced variants cataloged in the Thousand Genomes Project (Abecasis et al. 2012). Taking advantage of these advancements, the genome-wide association (GWA) era combines the strength of the unbiased nature of the query for genetic determinants of disease risk (similar in spirit to genome-wide linkage) along with the ability to recover most common human variation using a relatively small set of tagging genetic variants (similar in spirit to candidate gene association) (Risch and Merikangas 1996). The premise of the tagging approach is that given genetic architecture wherein SNPs are often found within blocks of LD where all SNPs within a block are highly correlated to each other, it is not necessary to genotype all variants in a single block to capture association between the disease locus within the block and phenotype; a reduced set of SNPs is sufficient to represent most of the variation contained within a block and can be used as a proxy for all remaining variants within the block.

The foundation for the GWA approach is the “common disease, common variant” hypothesis, wherein common diseases are argued to be attributable (in part) to common genetic variants (Reich and Lander 2001; Collins et al. 1997) and the leverage of the case–control design in place of the traditional family-based approaches necessary for genome-wide linkage. There are two obvious ramifications of this hypothesis: common genetic variants influencing disease are not

expected to have a large effect size (highly deleterious variants are generally recent and therefore uncommon in human populations (Tennessen et al. 2012); and, for common alleles with small effects to explain common disorders multiple loci each with small effect must cumulatively influence disease susceptibility.

The precise number of asthma GWA studies (GWAS) is hard to identify as many of the individual studies are folded into larger meta-analyses; several recent reviews are now available (Ober and Yao 2011; Akhabir and Sandford 2011). Table 9.1 is a comprehensive list of all GWAS publications relevant to asthma and highlights three points: (1) GWAS-identified loci are generally common in frequency; (2) GWAS-identified loci have modest effect sizes; and (3) although most loci are not replicated across the studies, there are several that are novel, highly replicated, and perhaps most importantly, robust to ethnicity.

The first asthma-susceptibility locus to be identified by GWAS is that on chromosome 17q21 (Moffatt et al. 2007). The associated variants reside on a common, cosmopolitan (i.e., observed in populations of diverse ancestry) haplotype that spans more than 100 kb and includes four genes: *ORMDL3*, *GSDMB*, *ZPBP2*, and IKZF3. This association with asthma has been among the most highly reproduced (Sleiman et al. 2008; Tavendale et al. 2008; Bouzigon et al. 2008; Galanter, et al. 2008; Hirota et al. 2008; Bisgaard et al. 2009; Wu et al. 2009; Leung et al. 2009; Halapi et al. 2010; Flory et al. 2009; Madore et al. 2008), observed in both children and adults, and across diverse ethnic groups (Galanter et al. 2008). The haplotype has regulatory potential, as it is associated with the expression of ORMDL3, GSDMB and ZPBP2 and functional fine-mapping studies suggest the causative variant regulates the differential binding of the insulator protein CTCF (Verlaan et al. 2009). However, due to the extensive linkage disequilibrium at this locus and it's impact on the expression on multiple genes, it remains unclear which of these genes is the culprit target. It is interesting that this locus overlaps with meta-analyses linkage regions for atopy and not asthma (Bouzigon et al. 2010; Denham et al. 2008), but it should be pointed out that these genes have never been studied under the candidate gene approach, supporting the importance of the unbiased GWAS approach in identifying novel loci for asthma. The ORMDL3 gene encodes ER-resident transmembrane protein and has high expression in cells involved in the inflammatory response (Moffatt et al. 2007). Alterations of protein folding or Ca(2+) levels within the endoplasmic reticulum (ER) result in the unfolded-protein response (UPR) which is an endogenous inducer of inflammation. ORMDL3 has been shown to alter ER-mediated Ca(2+) homeostasis and thereby facilitate the UPR (Cantero-Recasens et al. 2010). It has been shown that heterologous expression of ORMDL3 protein increased resting cytosolic Ca(2+) levels and reduced ER-mediated Ca(2+) signaling, an effect reverted by coexpression with the sarco-endoplasmic reticulum Ca(2+) pump (SERCA). Increased expression also promoted stronger activation of UPR transducing molecules and target genes. In contrast siRNA-mediated knockdown of ORMDL3 potentiated ER Ca(2+) release and attenuated the UPR adding further support for a likely biological explanation to the associations seen at this locus with asthma risk.

Another consistently replicated GWAS locus is that mapping to a region upstream of *IL33*, the gene encoding interleukin 33 (IL-33) located on chromosome 9q24.

Table 9.1 Summary of GWAS studies on asthma as the primary phenotype summarized from the Catalog of Published Genome-Wide Association Studies highlighting three regions with replication across multiple studies and ethnicities. Studies include those with a panel of >100,000 SNPs and reported *p*-values on the discovery data of $p < 10^{-5}$

Pubmedid	First author	Year	Sample size of discovery cohort(s)	Region	Hg19 chromosomal Position	Reported gene(s)	SNPs	Predicted function of peak SNP	Risk allele frequency	p-Value	OR or beta
Asthma	Moffatt MF	2007	994 cases,1,243 controls	17q12	38069949	ORMDL3	rs7216389	Intron	0.52	9.00E-11	1.45
	Himes BE	2009	422 cases, 1,533 controls	5q12.1	59369794	PDE4D	rs1588265	Intron	0.29	3.00E-08	1.18
	Mathias RA	2010	464 African American cases, 471 African American controls, 1,028 African Caribbean family members	NR		NR	NR		NR	NS	NR
	Sleiman PM	2010	793 European ancestry child cases, 1,988 European ancestry child controls	1q31.3	197325908	DENND1B, CRB1	rs2786098	Intron	0.85	2.00E-13	1.43
	Li X	2010	607 cases, 3,294 white controls	5q31.1	131901225	RAD50	rs2244012	Intron	0.21	3.00E-07	1.64
				15q21.2	51969668	SCG3	rs17525472	Intergenic	NR	2.00E-06	NR
				20p13	3827309	KIAA1271	rs4815617	nearGene-5	NR	8.00E-06	NR
	Himes BE	2010	359 non-Hispanic white cases, 846 white controls	NR		NR	NR		NR	NS	NR
	Moffatt MF	2010	10,365 cases, 16,110 controls	2q12.1	102986222	IL18R1	rs3771166	Intron	0.62	3.00E-09	1.15
				5q31.1	131723288	SLC22A5	rs2073643	Intron	0.45	2.00E-07	1.11
				5q31.1	131995843	IL13	rs1295686	Intron	0.2	1.00E-07	1.15
				6p21.32	32625869	HLA-DQ	rs9273349	Intergenic	0.58	7.00E-14	1.18
				9p24.1	6190076	IL33	rs1342326	Intergenic	0.16	9.00E-10	1.2
				15q22.2	61069988	RORA	rs11071559	Intron	0.86	1.00E-07	1.18
				15q22.33	67446785	SMAD3	rs744910	Intron	0.49	4.00E-09	1.12
				17q12	38062196	GSDMB	rs2305480	Missense	0.55	1.00E-07	1.18
				17q21.1	38121993	GSDMA	rs3894194	Missense	0.45	5.00E-09	1.17
				22q12.3	37534034	IL2RB	rs2284033	Intron	0.56	1.00E-08	1.12
	DeWan AT	2010	66 case children, 42 control children	NR		NR	NR		NR	NS	NR
	Ferreira MA	2010	986 European descent cases, 1,846 European descent controls	17q12	38095174	ORMDL3	rs6503525	Intergenic	0.43	5.00E-07	1.33
	Ege MJ	2011	850 European asthmatic children, 348 European children with atopy, 510 European child controls	NR		NR	NR		NR	NS	NR
	Schauberger EM	2011	112 European ancestry children cases, 165 European ancestry children controls	NR		NR	NR		NR	NS	NR

(continued)

Table 9.1 (continued)

Hirota T	2011	1,532 Japanese ancestry cases, 3,304 Japanese ancestry controls	4q31.21	144003159	LOC729675	rs7686660	Intergenic	0.27	2.00E-12	1.16
			4q31.21	144357737	GAB1	rs3805236	Intron	0.25	7.00E-08	1.12
			5q22.1	110401872	TSLP	rs1837253	Intergenic	0.35	1.00E-16	1.17
			6p21.32	32155581	PBX2	rs204993	Intron	0.58	2.00E-15	1.17
			6p21.32	32184345	NOTCH4	rs404860	Intron	0.5	4.00E-23	1.21
			6p21.32	32338695	C6orf10	rs3129943	Intron	0.62	3.00E-15	1.17
			6p21.32	32358513	BTNL2	rs3117098	ncRNA	0.25	5.00E-12	1.16
			6p21.32	32414273	HLA-DRA	rs3129890	Intergenic	0.61	5.00E-13	1.15
			6p21.32	32658079	HLA-DQB1	rs7775228	Intergenic	0.63	5.00E-15	1.17
			6p21.32	32687973	HLA-DQA2	rs9275698	Intergenic	0.79	5.00E-12	1.18
			6p21.32	32961361	HLA-DOA	rs9500927	Intergenic	0.26	4.00E-09	1.13
			10p14	8972018	LOC338591	rs10508372	Intergenic	0.433	2.00E-15	1.16
			12q13.2	56412487	IKZF4	rs1701704	Intergenic	0.18	2.00E-13	1.19
			12q13.2	56364321	CDK2	rs2069408	Intron	0.23	1.00E-10	1.15
Torgerson DG	2011	2,088 European American cases, 1,612 African American and African Caribbean cases, 1,688 Hispanic ancestry cases	1q21.3	152492559	CRCT1	rs4845783	Intergenic	NR	6.00E-06	NR
			1q23.1	158932555	PYHIN1	rs1101999	Intron	NR	4.00E-09	NR
			2q12.1	102953617	IL1RL1	rs3771180	Intron	NR	2.00E-15	NR
			5q22.1	110401872	TSLP	rs1837253	Intergenic	NR	1.00E-14	NR
			9p24.1	6193455	IL33	rs2381416	Intergenic	NR	2.00E-12	NR
			11q23.2	114231255	C11orf71	rs11214966	Intergenic	NR	6.00E-07	NR
			17q12	38064405	GSDMB	rs11078927	Intron	NR	2.00E-16	NR
Noguchi E	2011	938 Japanese ancestry	6p21.32	33042880	HLA, DPB1	rs987870	Intron;nearGene-5	0.14	2.00E-10	1.4
		cases, 2,376 Japanese ancestry controls	8q24.11	118025645	SLC30A8	rs3019885	Intron	0.31	5.00E-13	1.34
Ferreira MA	2011a, b	12,475 European ancestry cases, 19,967 European ancestry controls	1q21.3	154426264	IL6R	rs4129267	Intron	0.37	2.00E-08	1.09
			10q21.1	53493473	PRKG1	rs7922491	Intron	0.11	5.00E-07	1.13
			11q13.5	76270683	LRRC32	rs7130588	Intergenic	0.34	2.00E-08	1.09
			13q21.31	63638329	PCDH20	rs3119939	Intergenic	0.51	8.00E-06	1.08
Du R	2012	395 European ancestry asthmatic children	NR		NR	NR		NR	NS	NR
Anantharaman R	2011	490 Chinese ancestry cases, 490 Chinese ancestry controls	NR		NR	NR		NR	NS	NR
Karunas AS	2011	330 European ancestry cases, 348 European ancestry controls	NR		NR	NR		NR	NS	NR
Tantisira KG	2012	418 European ancestry cases	6q27	166534742	T	rs6456042	Intergenic	NR	6.00E-06	NR
Wan YI	2012	933 European ancestry cases, 3,346 European ancestry controls	2q12.1	102971200	IL18R1,IL1R1	rs9807989	Intergenic	NR	6.00E-08	1.33
			5q31.1	131796922	C5orf56	rs11745587	Intron	NR	2.00E-06	1.26
			5q31.3	141445980	NDFIP1	rs6867913	Intergenic	NR	4.00E-06	1.33
			9p21.1	32433526	ACO1	rs10970976	Intron	NR	4.00E-06	1.28
			17q12	38089344	ORMDL3	rs4794820	Intergenic	NR	1.00E-08	1.33
			19q13.42	53682042	ZNF665	rs16984547	Intron	NR	4.00E-06	1.43
Li X	2012	813 European ancestry cases, 1,011 European ancestry controls	NR		NR	NR		NR	NS	NR
Ramasamy A	2012	1,716 European ancestry cases, 16,888 European ancestry controls	2q12.1	102955082	IL1RL1, IL18R1	rs13408661	Intron	0.84	1.00E-09	1.23
			6p21.32	32379489	BTNL2, HLA-DRA	rs9268516	Intergenic	0.24	1.00E-08	1.15
Lasky-Su J	2012	Up to 1,238 European ancestry cases, up to 2,617 European ancestry controls	6p21.32	32604372	HLA-DQA1	rs9272346	nearGene-5	NR	2.00E-08	NR

Table 9.1 (continued)

Childhood onset asthma	Hancock DB	2009	492 Mexican trios	9q21.31	82039362	TLE4, CHCHD9	rs2378383	Intergenic	0.78	7.00E-07	1.64
	Ricci G	2011	135 European ancestry children with asthma, 134 European ancestry children with rhinoconjunctivitis	NR		NR	NR		NR	NS	NR
	Forno E	2012	573 European ancestry children	1q44	244511176	C1orf100	rs4658627	Intergenic	0.2418	6.00E-06	0.13
				3p26.2	3614887	IL5RA	rs9815663	Intergenic	0.182	2.00E-08	0.17
				11q24.2	127761666	NR	rs7927044	Intergenic	0.0134	7.00E-09	0.16
				13q13.3	36351766	NR	rs7328278	Intron	0.0285	3.00E-06	0.13
				17p12	13559080	NR	rs10521233	Intergenic	0.0735	3.00E-06	0.14
Asthma (toluene diisocyanate-induced)	Kim SH	2009	84 Korean ancestry cases, 263 Korean ancestry controls	9p21.3	20098711	Intergenic	rs16937883	Intergenic	0.02	7.00E-06	5.29
				10q21.3	68088508	CTNNA3	rs10762058	Intron	0.25	6.00E-06	5
				13q12.13	27415673	Intergenic	rs9319321	Intergenic	0.29	3.00E-06	5.2
Asthma (aspirin-intolerant)	Kim JH	2010	80 Korean cases, 100 Korean controls	NR		NR	NR		NR	NS	NR

A GWAS in one Asian and nine European population demonstrated association for asthma of a variant situated ~6 kb upstream of *IL33* (Gudbjartsson et al. 2009). Subsequently, another variant situated ~27 kb upstream of *IL33* was associated with asthma in the European-based GABRIEL Consortium—the largest GWAS meta-analysis to date (>26,000 subjects) (Moffatt et al. 2010). A second meta-analysis of nine North American asthma GWAS—the EVE Consortium—also replicated this association with variants ~22 kb upstream of *IL33* (Torgerson et al. 2011), demonstrating consistency across populations of diverse ethnicity (European American, African American, and Hispanic American). In contrast to the 17q21 locus, *IL33* along with its receptor *IL1RL1/ST2*, also implicated in GWAS (Gudbjartsson et al. 2009; Moffatt et al. 2010; Torgerson et al. 2011) represent extremely strong well-understood biological candidates for asthma (Wjst et al. 2013). Produced by mast cells following IgE-mediated activation (Hsu et al. 2010), IL-33, a member of the interleukin-1 (IL-1) cytokine family is directly involved in eosinophil- and basophil-mediated inflammation and IL-5 production, hallmark features of allergic disease (Schmitz et al. 2005; Cherry et al. 2008; Suzukawa et al. 2008; Smithgall et al. 2008; Pecaric-Petkovic et al. 2009; Smith 2010). Greater *IL33* expression in airway smooth muscle cells (Prefontaine et al. 2009) has been observed in airway epithelium of patients with asthma compared to healthy individuals (Prefontaine et al. 2010). Its receptor, ST2 in its soluble form (sST2) on chromosome 2q12.1, neutralizes IL-33 by acting as a decoy receptor (Sanada et al. 2007) and is another replicated GWAS signal robust to ethnicity. Serum ST2 has been associated with atopic asthma (Oshikawa et al. 2001). It is indeed striking that two genes in the interleuin-1/Toll-like receptor (TIR) superfamily pathway, what has emerged as a central pathway in asthma, have both independently been implicated as asthma determinants in GWAS approaches and points to the merits of the unbiased GWAS approach. It is notable that several additional TLRs have also been implicated as determinants of asthma in the candidate gene approach noted in Fig. 9.1.

The GWA approach is not without limitations as follows. The SNPs identified from the GWAS are not the true causal variant themselves but generally a proxy for some unmeasured disease-causing variant that needs additional follow-up for discovery. Given the vast number of statistical tests performed, stringent thresholds are set in place to control the family-wise error rate; the most commonly used approach being the Bonferroni correction given by $0.05/n$, where n is the number of tests being performed, and 0.05 is the Type I error rate typically selected. This often leads to the failure to address association signal from loci with less striking effects, now being discussed as a part of the missing/residual heritability from GWA studies of complex phenotypes. The comparison across studies can be difficult when differing set of SNPs are analyzed due to differences in GWA genotyping arrays; this, however, can be overcome using in-silico genotyping approaches such as imputations to a standard reference panel of SNPs such as the HapMap or Thousand Genomes panels. The standard for replication in a GWAS is the SNP-to-SNP replication with a consistent direction of risk effect. This last concern is an important one to consider given the tagging strategy upon which the GWA relies; especially in the comparison between ethnic groups where the same causal variant may lie on different haplotype backgrounds and thereby manifest as association to an alternate SNP in the GWA panel of SNPs or the same SNP with a different direction of risk (i.e., the risk allele in one group is the protective allele in another). Finally, GWA analysis generally ignores the presence of multiple loci, gene–gene and gene–environment interactions. Nonetheless, despite these limitations, GWASs have led to numerous successes in asthma and at least three different loci that appear to be robust to ethnicity (17q12, 9q24, 2q12.1 and 6p21.32).

9.4 Gene–Environment Interactions in Asthma

The role of environmental factors as key determinants of allergic inflammation and asthma risk has been well established (Strachan 1989; von Mutius 2004), and the potential for gene–environment interactions in asthma has been well recognized (Kauffmann and Demenais 2012). However, despite the importance of the environment in asthma, and the need to consider gene–environment (G x E) interaction in a systematic fashion, few such studies have been conducted to date. This is largely because of the complexity involved in conducting such studies, including the requisite large sample sizes, the large number of interaction models under consideration, and the difficulties in accurately measuring environmental exposures (Box 9.2A, B, C) (Kraft and Hunter 2005; Khoury et al. 1988). The design of a study that takes into account G x E interactions requires accurate assessment of both phenotype and environment, and depending on the magnitude of the G x E effect, considerably larger samples sizes than those required to detect main effects of just genetic loci. The availability of suitable replication populations is important as well, i.e., additional studies where both gene and environment are measured and the environmental exposures are similar in effect.

Despite these limitations, early (largely candidate-gene) GxE studies have reported numerous examples of gene–environment interaction in asthma (Fig. 9.1, Box 9.2C).

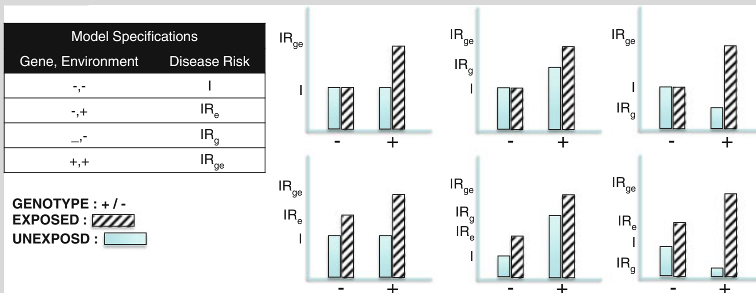
Box 9.2A Ignoring gene–environment interactions can mask genetic effects and thereby lead to the genetic heterogeneity between populations in the evaluation of a single genetic locus

COMBINED		
Disease \ Gene	+	-
+	100	100
-	200	200
OR(D|G) = 1.0		

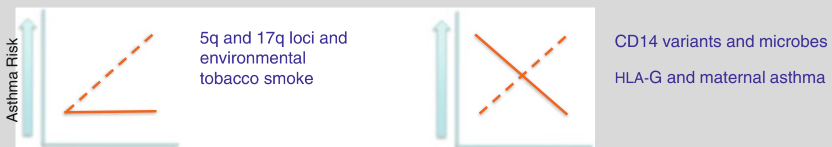
Exposed Group		
Disease \ Gene	+	-
+	25	10
-	20	50
OR(D|G) = 6.25		

Unexposed Group		
Disease \ Gene	+	-
+	75	90
-	180	150
OR(D|G) = 0.69		

Box 9.2B Complexity in models of gene–environment interaction (adapted from Khoury et al. 1988)



Box 9.2C Established gene–environment interactions in asthma (reviewed in Ober and Yao 2011)



Of these, the most extensively studied are those focused on *CD14*, a component of the toll-like receptor (TLR) signaling complex that facilitates endotoxin responsiveness. A series of association studies on a functional promoter polymorphism (*CD14*-260CT) has revealed protection against asthma (Leynaert et al. 2006), increased asthma risk (Ober et al. 2000), and a plethora of studies with no significant associations (Vercelli 2008). In subjects of African descent, the effect of *CD14*-260CT on asthma is dependent on levels of domestic endotoxin exposure; the TT genotype appears to protect against asthma in low domestic endotoxin exposure, but is a risk factor in high exposure (Zambelli-Weiner et al. 2005). These gene–environmental effects for *CD14*-260CT carry forth to a wide variety of asthma-associated phenotypes as well, including IgE (Eder et al. 2005), atopic dermatitis (Gern et al. 2004), allergic sensitization, eczema, and wheezing (Simpson et al. 2006). In all these examples, the effect of the variant differs based on environmental exposure, and in fact CD14 offers an excellent illustration of how ignoring environment can lead to the appearance of genetic heterogeneity in genetic determinants of asthma risk.

9.5 The Next Frontier of Association Studies: DNA Sequencing

Despite the early successes of GWAS in identifying novel asthma loci, there has been criticism that, for complex diseases broadly, and asthma specifically, the GWAS approach has not provided sufficient insight into the genetic contribution to disease risk. Though more than 30 asthma GWAS have been published, describing 51 genes having *p*-values $<1.0\times10^{-5}$ specifically for asthma (Hindorff et al. 2013), the cumulative genetic risk explained by the associated variants is relatively low (<15 %), precluding their use as predictive or diagnostic clinical models. This so-called missing heritability problem (Eichler et al. 2010) is a frequent occurrence in GWAS (Frazer et al. 2009), which are based on the premise of common disease/common variant hypothesis (Lander 1996; Cargill et al. 1999; Chakravarti 1999). “Missing heritability” is simply *residual* heritability or the leftover disease risk that is unaccounted for by the GWAS-identified genetic loci. The source of the “missing heritability”(Eichler et al. 2010) could include (1) common variants of smaller effects that fall far below the stringent significance thresholds applied in the GWAS approach; (2) rare variants with large effects or structural variants that are poorly tagged by commercial GWAS genotyping arrays; (3) limited power to detect interactions (Manolio et al. 2009); and (4) unmeasured epigenetic phenomena in the GWAS approach. It has even been argued that what appears to be association signal from a common variant in a GWAS approach could in fact be representative of multiple underlying rare variant association signals (Dickson et al. 2010) due to linkage disequilibrium.

Since the late 1970s Sanger termination sequencing (Sanger et al. 1977) has been the sole method of choice for sequencing studies. Key pieces of technology development in 2005 (Margulies et al. 2005; Shendure et al. 2005) heralded the current era of Next Generation Sequencing (NGS) techniques that have entailed arraying thousands of sequencing templates enabling sequences that can be analyzed in parallel, a

dramatic increase to the 96 sequencing templates on a contemporary Sanger capillary sequencer. Today, NGS systems include SOLiD/Ion Torrent PGM from Life Sciences, Genome Analyzer/HiSeq 2000/MiSeq from Illumina, and GS FLX Titanium/GS Junior from Roche (Metzker 2010) among others and enable the rapid sequencing of either predefined genomic regions (such as all the protein coding regions of the genome, i.e., the exome (Teer and Mullikin 2010; Fu et al. 2013) or entire human genomes (Abecasis et al. 2010). Despite the increasing computational complexities (Hoffmann 2011) inherent to these methods, the dramatic decreases in sequencing costs (Wolinsky 2007) make association studies using this approach highly attractive. Genome-wide sequencing studies in asthma are in their early stages, and it remains unclear what impact they will have on addressing the missing heritability problem.

With respect to asthma, an observation from Fig. 9.1 is the lack of overlap in genes identified through earlier approaches of positional cloning and candidate genes studies and the finding that most of the >100 genes established as asthma loci through these earlier approaches are not rediscovered through GWAS. An elegant argument that many of associated variants in these genes are simply not adequately captured by commercially available GWAS arrays has been demonstrated for asthma (Rogers et al. 2009). In addition to the arguments provided above, this provides compelling reason to extend asthma genetics to the new frontier of sequencing designs which ensures complete coverage of common variants, adequate coverage of rare variants (provided adequate sequencing depth), and importantly, the discovery of novel variants in sequenced cases. Using a theoretical framework that genes with molecular signatures of weak purifying selection are more likely to harbor an excess or rare/low frequency variants, resequencing has revealed that rare variants (in *AGT*, *DPP10*, *IKBKAP*) contribute to asthma susceptibility (Torgerson et al. 2012). Interestingly, the contribution of rare variants to asthma susceptibility was predominantly due to noncoding variants, and these early results of resequencing approaches offer the first promise of the value in a transition from tagging-SNP common-variant GWAS approaches over the past decade to resequencing approaches in the near future. It also provides an argument for consideration of the extensive human variation that exists outside the coding regions of the genome; exciting work by the Encyclopedia of DNA Elements (ENCODE) project (Dunham et al. 2012) has demonstrated that the vast majority of the human genome participates in at least one biochemical RNA and/or chromatin associated event in at least one cell type!

9.6 Heterogeneity in Asthma Genetics

Although a genetic basis for asthma is undeniable and >100 genes have been implicated, the elucidation of causal variants to explain this basis has been fraught with issues of between-study replication that stem from a variety of arguments including (1) heterogeneity in the asthma phenotype wherein "asthma" constitutes multiple overlapping syndromes rather than a single disease entity (Barnes 2011); (2) strong interactions with environment (Vercelli 2008); and (3) the high likelihood of true genetic heterogeneity (different sets of genes determine risk for asthma in different

populations). Large-scale sequencing of the human genome has revealed the dramatic potential for the latter (Abecasis et al. 2010): common human variation (allele frequencies ≥10 %) are almost all found in all of the populations studied, however, 17 % of low-frequency variants in the range 0.5–5 % were observed in a single ancestry group, and 53 % of rare variants at 0.5 % were observed in a single population. Genetic heterogeneity has been noted in asthma linkage signals (CSGA 1997), candidate gene studies (Ober and Hoffjan 2006; Ober and Yao 2011), and GWAS (PYH1N1 is a novel asthma susceptibility locus found only in populations of African descent (Torgerson et al. 2011). Importantly, sequencing approaches reveal rare variant determinants of asthma in four genes (*AGT*, *DPP10*, *IKBKAP*, and *IL12RB1*) among African Americans, but only rare variant determinants of asthma in *IL12RB1* among European Americans, further confirming the potential role of population heterogeneity in genetic determinants of asthma (Torgerson et al. 2012). To date, replication is typically evaluated in the strict sense—a SNP-for-SNP replication with the same direction of effect (Barnes 2011; Vercelli 2008); a transition to NGS approaches opens the window to "burden tests" is to assess association between "clusters" of rare variants within windows (e.g., a gene) and disease status (Li and Leal 2008; Morris and Zeggini 2009; Schaid and Sinnwell 2010; Zhu et al. 2010; Price et al. 2010; Cohen et al. 2004). Briefly, computationally fast tests include (1) cohort allelic sums test (CAST), where the number of individuals with one or more mutations in a window is compared between affected and unaffected individuals (Cohen et al. 2004) and (2) Combined Multivariate and Collapsing (CMC) method (Li and Leal 2008), where all rare variants (e.g., <1 %) are collapsed and treated as a single common variant analyzed along with all common variants in the region using multivariate analysis. More sophisticated approaches include those where variants are weighted according to their frequency (Madsen and Browning 2009) giving more weight to rarer alleles, tests that optimally select an allele frequency cut-off (Price et al. 2010), tests where rare variants in a gene are allowed to have both protective and risk effects (Wu et al. 2010), and tests allowing for misclassification of variant function (Liu and Leal 2010). The general spirit of these approaches is to specifically move beyond any single variant to collapsing information across multiple variants within a window of interest, thereby overcoming some of the limitations of strict replication rules that have plagued asthma genetics thus far.

In conclusion, the road to the discovery of genetic determinants of asthma has had numerous successes as study designs and technology have morphed from small linkage and family-based studies to extensive meta-analyses of GWAS data (Moffatt et al. 2010; Torgerson et al. 2012). One of the biggest successes of the GWAS approach has been the identification of a novel locus on 17q21 that is robust to population ancestry and has been highly replicated since the initial discovery. With the extensive LD and likely coregulation of multiple genes within this association peak, it is yet unclear which gene(s) in this chromosomal region are responsible for the association with asthma (*ORMDL3*, *GSDMB*, *ZPBP2*, or *IKZF3*). Integrative applications of genomic and epigenetic approaches are necessary in further elucidating causal variants behind the genetic association signals described in this chapter, and such applications specific to this 17q21 are further described in Chaps. 10 and 11.

Box 9.3 The genomewide association (GWA) approach

The GWA approach leverages the technological advancements in high-throughput genotyping along with the tagging approach to interrogate the entire genome for common variants that may be determinants of common disease. The completion of the International HapMap project provided the backbone for the design of genotyping arrays containing the smallest set of SNPs that captured the largest amount of common genetic variation given LD block structure and inter-SNP correlation, i.e., a set of tag SNPs to be genotyped as representatives of common human variation. In the design of a GWA study (GWAS), it is important consider to the wide choice of genotyping arrays available and select the array ideal for the population to be studied; for populations with high levels of African ancestry, African Americans, for example, an array that considers the smaller LD blocks that are results of the African representation in the admixed populations are important. GWAS are traditionally done by comparing allele frequencies at genotyped variants between well-phenotyped affected cases and unaffected controls, and clinical characterization is an important consideration for the study design. In asthma, the relevance of age of onset in GWA analyses has been shown to have an impact of strength of association signals observed at the chromosome 17q12 locus, wherein variation in this locus is an important determinant of childhood onset asthma. Careful consideration of population structure differences between the cases and controls is necessary as this can lead to spurious associations. Given the unbiased nature of the ~10^6 SNPs typically on an array (i.e., the vast majority of the SNPs do not in fact correlate with the disease under consideration), the genotype distributions can be used to detect cryptic population structure typically using principal components analysis/multidimensional scaling. An overall difference between cases and controls across the genome measured by the principal components (PCs) is usually indicative of population structure and the PCs can be used to correct for this structure in the single-SNP association tests.

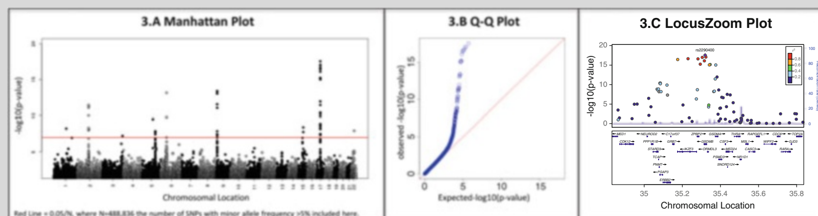
Two commonly used tools in the interpretation of the GWA results are the Manhattan Plot (**3.A**) and the Q–Q Plot (**3.B**). In these illustrations from the publicly available GABRIEL data, one sees the overall GWA p-values plotted as –log10(P-value) against chromosomal position (**3.A**) and quantile–quantile distributions of observed versus expected p-value to show deviation of observed from the expected distribution (**3.B**). In the Manhattan plot, regions of the genome that cross the stringent Bonferroni threshold of significance (red line) required for the multiple testing of SNPs are easily evident. The Q–Q plot supports (i) the lack of population stratification (the vast majority of p-values fall along the red line; they would be expected to be above the red line in the case of population stratification) and (ii) the presence of true association signal

(continued)

Box 9.3 (continued)

denoted by the upper set of strong p-values that are considerably stronger than expected for the range. One final tool is the zoom plot (**3.C**) of the peak association signal showing LD between SNPs in the region to the peak SNP, recombination fractions, and known genes in the region. Often the peak SNPs in a GWAS is a region of LD that includes multiple SNPs; the commonly used LocusZoom plot is a useful tool to identify genes that are potentially implicated by the peak GWAS SNP.

The GWAS has led to numerous discoveries and between 2005 and 2012 there have been over 1,350 publications relying on this approach. However, there are some pitfalls to the approach that must also be highlighted. A narrow and well-defined case/control definition is critical to maximize power, a well-selected GWA array suitable for the population under consideration, a robust control of false positives due to multiple testing, and an efficient way to detect and correct for population stratification are all relevant issues. A more fundamental issue at hand, given the higher degree of unexplained heritability even for some of the most successfully GWA studied phenotypes, is the robustness of the common disease common variant hypothesis. With the advent of high-throughput sequencing that has been dramatically decreasing in cost, it is anticipated that sequencing, targeted and whole genome, will be the next frontier in the tool box of asthma geneticists enabling a query of all variation, common and rare, as determinants of asthma risk.



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