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The Search for Genetic Variants and Epigenetics Related to Asthma

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For the past two decades, a huge number of genetic studies have been conducted to identify the genetic variants responsible for asthma risk. Several types of genetic and genomic approaches, including linkage analysis, candidate gene single nucleotide polymorphism studies, and whole genome-wide association studies have been applied. In this review article, the results of these approaches are summarized, and their limitations are discussed. Additionally, perspectives for applying upcoming new epigenetic or genomic technologies, such as copy number variation, are introduced to increase our understanding of new omic approaches to asthma genetics.

Key Words: Asthma; epigenetics; gene; genome; linkage; polymorphism; variants

INTRODUCTION

Asthma is a genetically complex disease that is associated with the familiar segregation of atopy and increased levels of total serum IgE.[1] Asthma and atopy are also closely associated with increased bronchial hyper-reactivity and elevated blood eosinophil count.[2,3] These intermediate phenotypes are highly heritable and the subject of much asthma genetics research. The occurrence of patients with an asthma cluster in their family indicates that a genetic component is likely operating. Twin studies represent a useful first step to determine whether a given trait or disease has a measurable genetic component. In a large twin study with 7,000 same-sex twins born between 1886 and 1925, the concordance rate for self-reported asthma in monozygotic twin pairs was 19%, which is four times higher than the 4.8% rate in dizygotic twins.[4] This heritability must be determined by genetic factors. However, the prevalence of childhood and adult-onset asthma has increased dramatically during the last two or three decades in both developed and developing countries.[5] These result indicate that epigenetic factors may play an important role in increased asthma prevalence. Genome-wide linkage studies, biologically plausible candidate gene approaches, and genome-wide association scans (GWAS) have been performed over the past 20 years to search for the genetic background of asthma.

WHOLE-GENOME LINKAGE, POSITIONAL CLONING, AND FINE MAPPING STUDIES

Linkage-based methods have been used for individual families in which members are highly affected by the disease. These studies have attempted to demonstrate a linkage between disease occurrence and genetic markers in a chromosomal region. Linkage analysis is performed to determine the chromosome location of a susceptibility gene or genes by demonstrating co-segregation of the trait or disease with known genetic markers, which are usually polymorphic DNA markers. Two general linkage analysis approaches have been used: 1) genome-wide and 2) candidate region searches of the genome in which candidate genes have already been mapped. These approaches have been successfully used to map and clone genes causing monogenic disorders with simple Mendelian inheritance such as cystic fibrosis.[6] They have also been used over the past 20 years by several groups to isolate susceptibility loci for asthma.[7,8] Whole-genome or candidate chromosome scans using micro-

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satellite markers and a positional cloning approach has led to the discovery of several genes for asthma and related phenotypes (Table 1). These results have increased our understanding of the genetic background of asthma and its subphenotypes. Using whole-genome linkage analysis, positional cloning, and case control studies, at least five asthma genes including disintegrin and metalloprotease 33 (*ADAM33*) 20p13,[9] dipeptidyl-peptidase 10 2q14.1 (*DPP10*),[10] plant homeodomain zinc finger protein 11 13q14.2,[11] G protein–coupled receptor for asthma susceptibility 7p15-p14,[12] and prostaglandin D2 receptor 14q24[13] have been identified as strong genetic variants for asthma (Table 1). However, replication studies including the British 1958 Birth Cohort study of 7,703 adults revealed that small increases in asthma risk were identified only with *DPP10* and *ADAM33*.[9,10] Based on recent replication results, it has been concluded that applying linkage analyses to multi-factorial complex diseases is less successful than had been previously thought. Thus, the growing recognition of the limitations of linkage analysis for investigating a complex human disease has shifted emphasis away from linkage analysis and microsatellite markers toward single-nucleotide polymorphism (SNP) genotyping and different analytical strategies based on association and haplotype analyses.[14]

CANDIDATE GENE ASSOCIATION STUDIES USING SNPS

During the past decade, genetic and genomic molecular technologies have been rapidly developed. Generating SNP maps from high-throughput sequencing projects has promoted the discovery of genes related to asthma. There are several potential advantages of using SNPs to investigate the genetic determinants of a complex human disease such as asthma. SNPs are plentiful throughout the human genome, as they are found in exons, introns, promoters, enhancers, and intergenic regions, allowing them to be used as markers in dense positional cloning investigations. Additionally, groups of adjacent SNPs exhibit linkage disequilibrium and haplotypic diversity patterns that could be used to enhance gene mapping. More than 300 genes have SNPs associated with asthma and allergy subtypes based on the NCBI website (http://www.ncbi.nlm.nih.gov). We have also genotyped 2,800 SNPs on 180 genes whose functions are related to asthma and found that SNPs on 19 genes are associated with the risk of asthma and its traits (Fig. 1).[15-30] Interestingly, almost all SNPs have an odds ratio (OR) <2.0, indicating that the candidate gene approach provides genetic variant information regarding the production of significant increases in asthma risk; however, their contribution to the development of asthma

Table 1. Candidate genes in loci linked to asthma and the intermediate phenotypes

Chr	Loci	Gene	Asthma	Ig E	ST	BHR	Eos
1	1p31-34	IL-10, IL12Rβ2	O	O			
2	2p14.1	DPP10*	O	O	O	O	
2	2q33	CD28, TGF-α	O	O			
5	5p15, 5q23-31	IL-3,4,5,9,13, GM-CSF, ADRβ2, CD14, GCR	O				
6	6p21	TNFα, HLA	O	O			O
7	7p15-p14	GPR*	O	O			
7	7q36.2	TCRα, IL-6				O	
9	9q31.3	TMOD	O	O			
11	11q13, 11p15	FCER1β, CC10/CC16	O	O	O		O
12	12q13-24	INFγ, SCF, IGF1, STAT6, NOS1,LTA4H	O			O	O
13	13q14	TPT1	O		O		O
13	13q14.2	PHF11*	O	O	O		
14	14q24	PTGDR*	O	O			O
14	14q11, q32	TCRα/δ, MCC	O				
16	16p12, p22-24	IL-4Rα		O			
17	17p11, q12-21	CC chemokine cluster	O		O		
19	19q13	TGFβ, IL-11, CD22	O			O	
20	20p13	ADAM33*	O	O		O	

Ig E, immunoglobulin E; ST, skin prick test; BHR, Bronchial hyper-reactivity; Eos, eosinophil; IL. interleukin; DPP10, dipeptidyl peptidase 10; CD, cell differentiation antigen; TGF-α, transforming growth factor-α; GM-CSF, granulocyte macrophage colony stimulating factor; ADRβ2, adrenergic receptor; GCR, glucocorticoid receptor; TNF-α, tumor necrosis factor-α; GPR, G-protein coupled receptor; TCR, T-cell receptor; TMOD, tropomyosin-binding protein; FCER1β, IgE binding receptor β; INFγ, interferon γ; SCF, stem cell factor; IGF1, insulin-like growth factor; STAT6, signal transducer and activator of transcript 6; NOS1, nitric oxide synthase 1; LTA4H, leukotriene A4 hydrolase; TPT1, tumor protein; transitional controlled 1; PHF11, PHD finger protein 11; PTGDR, prostaglandin D2 receptor; TCRα/δ, T-cell receptor α/δ; MCC, mutated in colorectal cancers; ADAM33, adhesion disintegrin and metalloprotease 33.
*Indicates asthma-related genes identified by genome linkage analysis, positional cloning, and case-control studies.

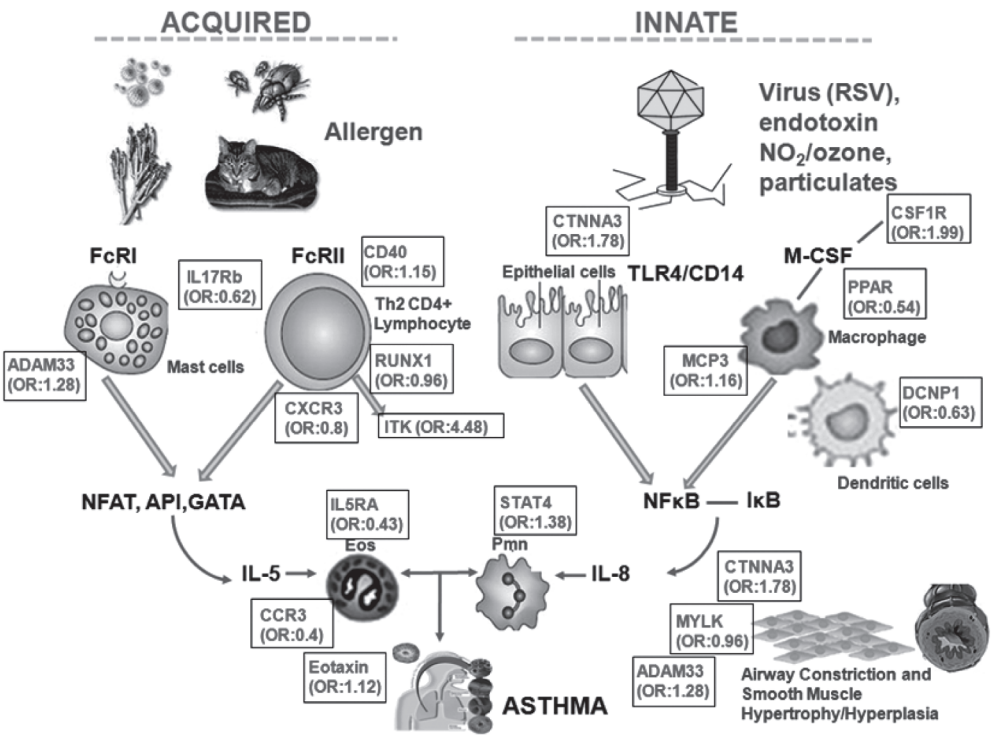

Fig. 1. Odd ratios of single nucleotide polymorphisms (SNPs) associated with asthma and asthma phenotypes including IgE and eosinophils counts (2003–2010, SCH Genome Research Center). IgE, immunoglobulin E; RSV, respiratory syncytial virus; NO_2, nitrogen dioxide; FcRI and FcRII, high-affinity IgE receptor isoforms; TLR, toll-like receptor; M-CSF, macrophage colony-stimulating factor; NFAT, nuclear factor of activated T-cells; API, activator protein 1; GATA, GATA-binding factor; NFκB, nuclear factor kappa B; IκB, inhibitor of NFκB; OR, odd ratios; ADAM33,[15] disintegrin and metalloproteinase domain-containing protein 33; CXCR3,[16] chemokine receptor 3; IL17Rβ,[17] interleukin 17 receptor β; CD40,[18] cluster of differentiation 40; RUNX1[19]; runt-related transcription factor 1; ITK,[20] IL2-inducible T-cell kinase; CTNNα3,[21] catenin α 3; CSF1R,[22] colony-stimulation factor 1 receptor; MCP3,[23] monocyte chemotactic protein 3; PPAR,[24] peroxisome proliferator-activated receptors; DCNP1,[25] dendritic cell nuclear protein 1; IL5Rα,[26] interleukin 5 receptor α; STAT4,[27] signal transducer and activator of transcription 4; CCR3,[28] C-C chemokine receptor type 3; MYLK,[29] myosin light chain kinase; eotaxin.[30]

may be smaller than expected.

GWAS

With the development of SNP sequencing technologies, the international Hap-Map Consortium has revealed nearly 4 million SNPs on the whole chromosome and demonstrated that individual SNPs predict adjacent SNPs, suggesting that genotyping 500,000 SNPs may allow for a nearly complete survey of all common genetic variability.[31] Based on this concept, whole-genome SNP genotyping arrays have been developed and applied over the past 5 years to research the genetic background behind multifactorial complex diseases. This approach is a hypothesis-free test of the association between phenotypes and SNPs across the genome and diseases, usually involving 500,000 markers that are reasonably polymorphic and are spread fairly evenly across the genome. As of June 2010, 904 statistically significant GWAS have been published for 165 traits of common allele complex diseases (NHGRI catalog at (http://www.genome.gov/gwastudies/). A GWAS on asthma has been published from 2007 until now (Table 2).[21,32-38] Among the nine studies, seven consider Caucasians, one study considers Mexicans, and two studies consider Asians, including Koreans. Eight studies focused on asthma risk, and the remaining one focused on occupational asthma. The first GWAS was created in England in 2007.[32] They applied a 30 kb SNP chip to the DNA from 994 patients with childhood-onset asthma and 1,243 non-asthmatics, using family and case-referent panels. As a result, a strong signal was found at chromosomes 17q and 2. The SNPs associated with childhood asthma were consistently and strongly associated ($P=10^{-22}$) with transcript levels of ORMDL3, a member of a gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum.[32] The second GWAS analyzed sequence variants affecting eosinophil counts in the blood of 9,392 Icelanders. The most significant 10 SNPs were further studied in 12,118 Europeans and 5,212 east Asians, including Koreans. SNPS (rs1420101) at IL1RL1 and at 2q12 were strongly associated with asthma ($P=5.5\times10^{-12}$) in a collection of ten different populations including 7,996 cases and 44,890 controls. However, the ORs were <1.5. Very recently, a large-scale, consortium-based GWAS of asthma with 10,365 asthmatic patients and 16,110 controls confirmed the associa-

Table 2. Genome-wide association scan studies on asthma (2007–2010)

rs number	Gene	Races	OR	*P* value	MAF	AF	Reference
rs7216389	ORMDL3	Caucasian	1.45	0.031	0.62	0.218	Moffatt et al.[32]
rs10762058 rs7088181	CTNNA3	Korean	1.85	0.0005	0.372	0.24	Kim et al.[21]
rs142010	IL1RL1	Caucasian/Korean/Taiwan	1.16	5.5×10^{-12}	0.405	0.061	Gudbjartsson et al.[33]
rs11778371	PDE4D	Caucasian	2.32	8.1×10^{-7}	0.09	0.106	Himes et al.[34]
rs3734083	TLE4	Mexican	1.68	1.21×10^{-5}	0.18	0.109	Hancock et al.[35]
rs1775444	DENND1B	Caucasian	1.83	9.43×10^{-7}	0.049	0.039	Bardy et al.[36]
rs2244012	RAD50-IL13	Caucasian	1.64	3.04×10^{-7}	0.212	0.119	Li et al.[37]
rs3771166 rs2305480	IL1RL1/IL18R1 ORMDL3/GSDMB	Caucasian	<1.5	3×10^{-9} 6×10^{-23}	NA NA	NA NA	Moffatt et al.[38]

OR, odds ratio; MAF, minor allele frequency; AF, attributable risk fractions.

Table 3. Estimates of heritability and number of loci for several complex traits

Disease	Number of loci	Proportion of heritability	Heritability measure
Age-related macular degeneration	5	50%	Sibling recurrence risk
Crohn's disease	32	20%	Genetic risk (liability)
Systemic lupus erythematosus	6	15%	Sibling recurrence risk
Type 2 diabetes	18	6%	Sibling recurrence risk
HDL cholesterol	7	5.20%	Residual* phenotype variance
Height	40	5%	phenotypic variance
Early onset myocardial infarction	9	2.80%	phenotypic variance
Fasting glucose	4	1.50%	phenotypic variance

*Residual is after adjusting for age, gender, and diabetes.
Adapted from Moffatt et al.[38]

tion of the previously defined SNPs, including ORMDL3 and IL1RL1.[38] However, the ORs were within the range of 0.5 to 1.5. We calculated population attributable risk fractions of these SNPs in asthma and its traits,[39] and they were between 3.9–24% (Table 2).

IDENTIFYING THE MISSING HERITABILITY OF ASTHMA

GWASs have identified hundreds of genetic variants associated with complex human diseases and traits and have provided deep insights into their genetic makeup. In the case of age-related macular degeneration, proportions of heritability explained by five SNPs are responsible for 50% of the risk. Thirty-two loci for Crohn's disease explain 20% of the heritability (Table 3). However, most variants identified to date confer a relatively small increase in risk and explain only a small proportion of heritability. Thus, this leads to the question of how the missing heritability can be explained.[40] In the GWAS results, the population attributable risk fractions of these SNPs in asthma and its traits ranged between 3.9–24% (Table 2). The population attributable risk fraction (AF) of 20% indicates the relatively strong impact of genetic variants on the development of asthma. In studies including patients with asthma, rs7216389 on ORMDL3 has a 21.8% of AF, whereas the SNPs on the other gene have an AF of <12%. These results suggest that SNPs discovered even by several GWASs only have a limited ability to explain genetic effects for the development of asthma.

Manolio et al.[40] regarded the factors related to the limitations of GWAS as an imprecise disease phenotype, use of control groups of questionable comparability, and inconsideration of environmental contributors. To overcome the missing heritability, the overall asthma strategy should be classified into specific phenotypes, and environmental factors should be introduced into the analysis of these subgroups. From a schematic view, the pathogenic mechanism of asthma can be divided into two major pathways. One is an acquired immune response pathway, and the other is an innate pathway (Fig. 1). In the former, Th2 cells, mast cells, and eosinophils participate in a antigen specific IgE- and Th2 cytokine-dependent manner. This process usually starts at a young age. In the latter, macrophages, dendritic cells, epithelial cells, and neutrophils are involved in IgE-independent adult-onset asthma. Thus, patients with asthma can be stratified into several subphenotypes (Table 4).

Based on the triggering environmental factors, asthma can be subgrouped into IgE-dependent allergic, aspirin-exacerbated respiratory disease, occupational asthma, exercise-induced

asthma, and menstruation- or obesity-associated asthma. The inflammatory patterns observed from a sputum analysis reveal eosinophilic, neutrophilic, and pauci-graniulocytic types of asthma. Patients with asthma can be clinically and physiologically stratified into several subgroups. Thus, a genetic association study should be conducted on patients with asthma in well-defined subphenotypes.

IMPROVEMENT OF AF OF GENETIC VARIANTS ACCORDING TO THE SUBPHENOTYPES OF ASTHMA AND THE ENVIRONMENTS

The SNP on ORM1-like 3 was reanalyzed in Caucasians and Koreans according to the age of asthma onset (Table 5).[41] When the subjects were stratified based on an age of 16 years, the statistical difference of rs 7216389 on 17q21 became more apparent in the age group <16 years in Caucasians and Koreans, whereas the statistical significance disappeared in the group >16 years. The ORs of the younger group ranged from 1.26 to 1.49, whereas that of the older group ranged from 0.87 to 1.11. AFs were 0.269 for early-onset asthma and 0.057 for late-onset asthma, indicating the necessity for stratifying asthma according to age, because the two types of asthma may have a different pathogenesis.

The impact of genetic variants on asthma may be enhanced when environmental factors are introduced. An interesting finding related to 17q21 variants was revealed after 1,511 subjects from 372 families were grouped by passive exposure to environmental tobacco smoke during early life.[42] The ORMDL3 gene variant on rs8076131 showed a significant association with the risk of asthma in a family sample with offspring exposed to tobacco smoke (OR=2.5), but not in those who were not exposed to tobacco smoke (OR=1.38), indicating the importance of environment factors in genetic studies of asthma. One important and easily accessible environmental factor is occupational exposure to inducers or triggers.

A GWAS study was performed on a well-defined subphenotype of asthma in 84 Korean patients with toluene diisocyanate (TDI)-induced asthma and 263 unexposed healthy normal controls.[21] Genetic catenin alpha 3, alpha-T catenin polymorphisms are significantly associated with the TDI-induced asthma phenotype (OR=5.84106 for rs10762058) and the AR increases to 24% (Table 2), indicating that the missing heritability of asthma can be compensated for by stratification into asthma sub-phenotypes and by introducing environmental factors into

Table 4. Classification of asthma phenotypes

1. Phenotypes related to triggers
Aspirin or non-steroidal anti-inflammatory drugs
Environmental allergens
Occupational allergens or irritants
Menses
Exercise
Viral infection
Obesity
2. Inflammatory phenotypes
Eosinophilic
Neutrophilic
Pauci-granulocytic
3. Clinical or physiological phenotypes
Severity-defined: from mild to severe
Exacerbation-prone: Brittle vs. non-Brittle
Defined by chronic airflow restriction
Treatment-resistant: resistance to steroids
Defined by age at onset

Table 5. Association of the T allele of rs7216389 in ORM1-like 3 with asthma

Study group	All asthma			Adults, early onset			Adults, late onset		
	na/nc	*P* value	OR (95% CI)	na/nc	*P* value	OR (95% CI)	na/nc	*P* value	OR (95% CI)
Iceland	1,648/30,898	$1.6 \cdot 10^{-7}$	1.23 (1.14, 1.33)	617/30,898	$1.4 \cdot 10^{-9}$	1.44 (1.28, 1.63)	744/30,898	0.064	1.11 (0.99, 1.23)
Australia	647/564	0.44	1.07 (0.90, 1.27)	370/564	0.016	1.26 (1.04, 1.52)	226/564	0.22	0.87 (0.70, 1.08)
The Netherlands	221/1,564	0.0073	1.32 (1.08, 1.61)	156/1,564	0.0081	1.37 (1.09, 1.73)	57/1,564	0.55	1.12 (0.77, 1.63)
Korea*	1,387/558	0.14	1.13 (0.96, 1.33)	211/558	0.0049	1.49 (1.13, 1.96)	76/558	0.35	1.08 (0.92, 1.28)
United Kingdom	292/241	0.064	1.26 (0.99, 1.61)	81/241	0.07	1.39 (0.97, 1.99)	60/241	0.63	1.10 (0.74, 1.65)
Germany I	307/560	0.014	1.28 (1.05, 1.56)	-	-	-	-	-	-
Germany II	415/204d	0.0034	1.43 (1.12, 1.81)	-	-	-	-	-	-
Combined non-Icelandic	3,269/3,691	$2.7 \cdot 10^{-6}$	1.21 (1.18, 1.31)	818/2,927	$1.6 \cdot 10^{-6}$	1.35 (1.19, 1.52)	1,519/2,927	0.74	1.02 (0.91, 1.15)
Combined all	4,917/34,589	$2.0 \cdot 10^{-12}$	1.22 (1.15, 1.29)	1,435/33,825	$1.5 \cdot 10^{-14}$	1.40 (1.28, 1.52)	2,263/33,825	0.11	1.07 (0.99, 1.16)

Shown above are the number of cases (na) and controls (nc), the OR with a 95% confidence interval, and two-sided dual and combined study populations using the Mantel-Haenzel mode.

*Attributable risk fraction values are 0.089 for all asthma, 0.269 for early-onset asthma, and 0.057 in late-onset asthma.

Adapted from Halapi et al.[41]

the genetic analysis.

IMPROVEMENT IN THE ORS OF GENETIC VARIANTS BY DISCOVERY OF SNPS WITH RARE ALLELE FREQUENCY

Much of the speculation about missing heritability from GWASs has focused on the possible contribution of rare variants (low minor allele frequency [MAF] <0.5%). To date, GWAS chips have been created to analyze common variants of >5% MAF. Sequencing of individual genomes has started to generate more information on these rare human chromosome variants.[43] The full genome sequences of 1,000 anonymous subjects have been identified. The 1,000 Genomes Project (http://www.1000genomes.org/page.php) has already identified more than 11 million new SNPs in low-depth coverage of 172 individuals. The newly discovered SNPs will be applied to genetic association studies in the near future.

STRUCTURAL VARIATION AND EPIGENESIS IN ASTHMA GENETICS

Previous epidemiological studies demonstrated that SNPs are not responsible for all phenotypical differences. The twin cohort study showed that the concordance rate for self-reported asthma in monozygotic twin pairs is 19%.[44] Asthma develops almost concurrently considering that monozygotic twins have similar genetic variants. However, the concordance rate is <20%. One of the explanations for this disconcordance is epigenesis. It was recently discovered that the mother's diet affects the risk of allergic asthma in her offspring.[45] Recently, Ho et al.[5] extensively reviewed the environmental epigenetics of asthma. Thus, we focused on the epigenesis of aspirin hypersensitivity in asthma. The methylation pattern on the DNA CpG sites was analyzed using a whole genome methylation analysis of 27,168 CpG sites using nasal polyps from patients with aspirin exacerbated respiratory disease (AERD) and aspirin tolerant asthma (ATA).[46]

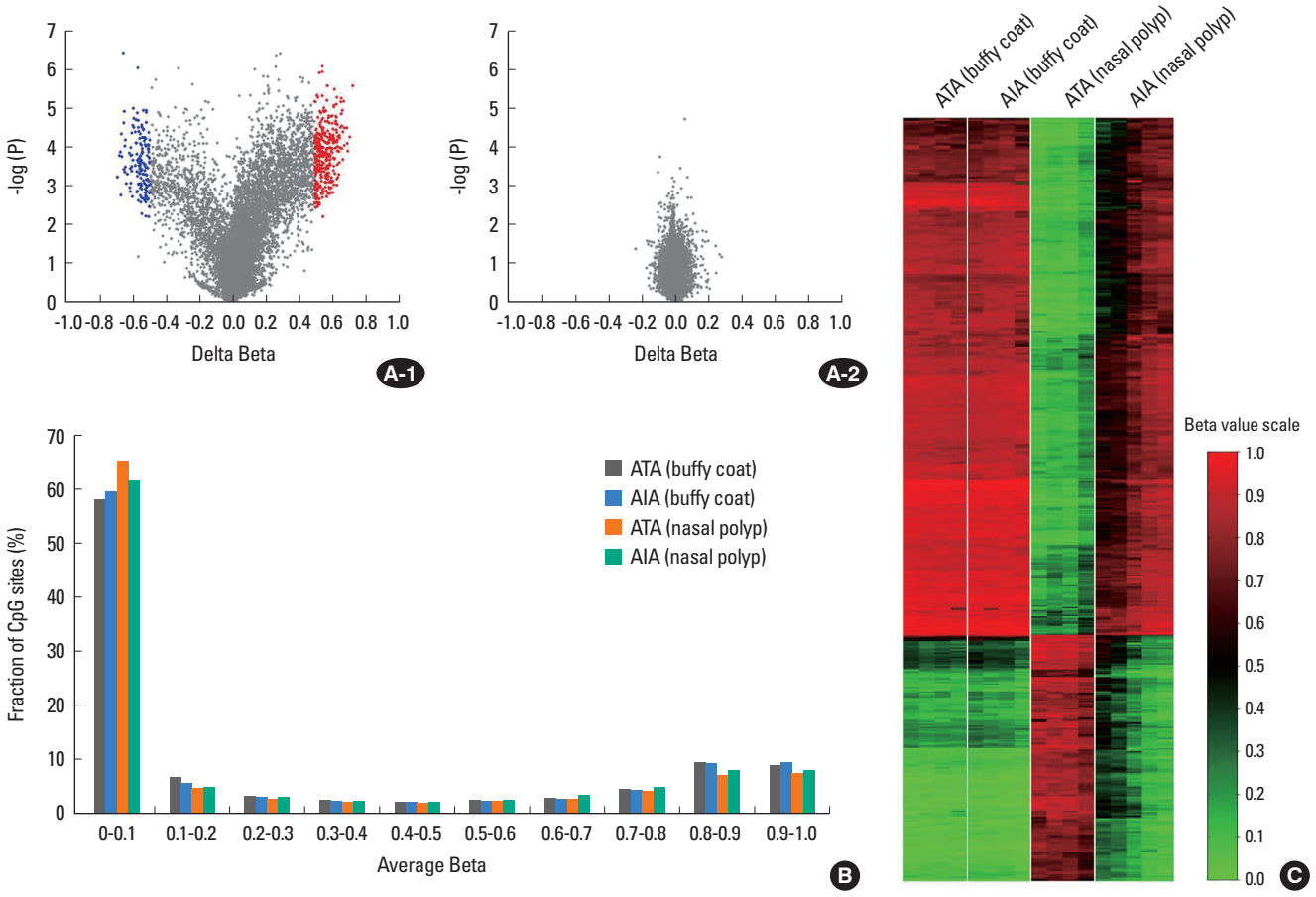

Fig. 2. DNA methylation pattern of nasal polyps from patients with aspirin-exacerbated respiratory disease and those with aspirin tolerant asthma. (A) Volcano plot of differential methylation level between aspirin intolerant asthma (AIA) and aspirin tolerant asthma (ATA) in nasal polyp tissues (A-1) and buffy coat samples (A-2). Red dots, Delta Beta ≥0.5 and *P* value ≤0.01; blue dots, Delta Beta ≤-0.5 and *P* value ≤0.01; grey dots, -0.5≤ Delta Beta ≤0.5 and *P* value >0.01. Delta Beta: difference in DNA methylation level (subtracting the DNA methylation level of ATA from AIA). -log (P): log-transformed *t*-test *P* values. (B) Distribution of the DNA methylation level of AIA and ATA in buffy coat and nasal polyps. Average Beta: DNA methylation level (0 to 1). (C) Heat map of 490 differentially methylated CpGs between AIA and ATA in buffy coat and nasal polyps.

Table 6. DNA methylation level of CpGs in arachidonic acid metabolism genes

Gene name	Gene product	Beta value of ATA (Mean±SD)	Beta value of AIA (Mean±SD)	Delta Beta (ATA-AIA)	*t*-test (*P* value)
PGDS	Prostaglandin-D synthase	0.86±0.05	0.18±0.15	-0.68	0.0002
LTB4R	Leukotriene B4 receptor	0.88±0.06	0.27±0.16	-0.61	0.0004
PTGES	Prostaglandin-E synthase	0.07±0.04	0.63±0.15	0.56	0.0006
ALOX5AP	Arachidonate 5-lipoxygenase-activating protein	0.84±0.06	0.31±0.24	-0.52	0.006
LOXHD1	Lipoxygenase homology domain 1	0.67±0.18	0.20±0.15	-0.46	0.006

*Differentially methylated CpGs using the criteria of $P \leq 0.01$ and Delta Beta of ≥ 0.50.
ATA, aspirin tolerant asthma; AIA, aspirin intolerant asthma.

As a result, the methylation patterns were significantly different in nasal polyps, but not so different in the buffy coat. A volcano plot showed different methylation levels in AERD and ATA: 332 CpG sites on 296 genes were hypomethylated and 158 sites on 141 genes were hypermethylated (Fig. 2). Hierarchical clustering of 490 differentially methylated CpGs clearly distinguished the nasal polyps between patients with AERD and ATA. The CpG-site methylation of nasal polyps was not correlated with that of the buffy coats, indicating that the difference in methylation pattern was a nasal tissue-specific finding. Among the arachidonate pathway genes, prostaglandin E synthase was hypermethylated and prostaglandin D synthase, arachidonate 5-lipoxygenase activating protein, leukotriene B4 receptor, and lipoxygenase homology domain 1 were hypomethylated (Table 6), indicating that different methylation patterns of these candidate genes in the arachidonate pathway may be responsible for the penetration of specific phenotypes such as AERD in asthma.

Genomic variability is present in many forms, including SNPs, variable number tandem repeats (e.g., mini- and microsatellites), presence/absence of transposable elements (e.g., *Alu* elements), and structural alterations (e.g., deletions, duplications, and inversions). Until recently, SNPs were thought to be the predominant form of genomic variation and to account for much of the normal phenotypic variation. However, the widespread presence of copy number variation was recently reported in normal individuals.[47] Contrary to our previous beliefs, identical twins are not genetically identical. Researchers studied 19 pairs of monozygotic, identical twins and found differences in copy number variations and found that contingent negative variation (CNV) is associated with Parkinson's disease.[48] A GWAS of CNV in 16,000 cases of eight common diseases including T1, 2D, rheumatoid arthritis, and Crohn's disease have revealed that some CNV is strongly associated with the risk for complex diseases.

FUTURE OF ASTHMA GENOME RESEARCH

Genome-wide SNP association and fine mapping studies will widen the scope of candidate genes for asthma, but only when applied to sub-phenotypes rather than all asthma types and when analyzed with increased sample sizes considering environmental contributors. An active subject of research in the postgenomic era is to identify functional regulatory elements for human gene expression. Epigenetics is a study of heritable changes in DNA structure that do not alter the underlying sequence. Well-known examples are DNA methylation and histone modification. These changes may remain after cell divisions for the life of the cell and may last for multiple generations. The most exciting idea in epigenetics is that it could be possible to intervene at the junction between the genome and the environment. Epigenetic variants and copy number variations are the starting point to obtain data from clinical samples, and it will be another powerful tool for research into the genetic heritability of asthma.

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REFERENCES

1. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. N Engl J Med 1989;320:271-7.
2. Burrows B, Sears MR, Flannery EM, Herbison GP, Holdaway MD, Silva PA. Relation of the course of bronchial responsiveness from age 9 to age 15 to allergy. Am J Respir Crit Care Med 1995;152:1302-8.
3. Bousquet J, Chanez P, Vignola AM, Lacoste JY, Michel FB. Eosinophil inflammation in asthma. Am J Respir Crit Care Med 1994;150: S33-8.
4. Edfors-Lubs ML. Allergy in 7000 twin pairs. Acta Allergol 1971;26: 249-85.
5. Ho SM. Environmental epigenetics of asthma: an update. J Allergy Clin Immunol 2010;126:453-65.
6. Zielenski J, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 1995;29:777-807.
7. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CSGA). Nat Genet 1997;15:389-92.
8. Moffatt MF, Cookson WO. Gene identification in asthma and aller-

gy. Int Arch Allergy Immunol 1998;116:247-52.
9. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, Torrey D, Pandit S, McKenny J, Braunschweiger K, Walsh A, Liu Z, Hayward B, Folz C, Manning SP, Bawa A, Saracino L, Thackston M, Benchekroun Y, Capparell N, Wang M, Adair R, Feng Y, Dubois J, FitzGerald MG, Huang H, Gibson R, Allen KM, Pedan A, Danzig MR, Umland SP, Egan RW, Cuss FM, Rorke S, Clough JB, Holloway JW, Holgate ST, Keith TP. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 2002;418:426-30.
10. Allen M, Heinzmann A, Noguchi E, Abecasis G, Broxholme J, Ponting CP, Bhattacharyya S, Tinsley J, Zhang Y, Holt R, Jones EY, Lench N, Carey A, Jones H, Dickens NJ, Dimon C, Nicholls R, Baker C, Xue L, Townsend E, Kabesch M, Weiland SK, Carr D, von Mutius E, Adcock IM, Barnes PJ, Lathrop GM, Edwards M, Moffatt MF, Cookson WO. Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nat Genet 2003;35:258-63.
11. Zhang Y, Leaves NI, Anderson GG, Ponting CP, Broxholme J, Holt R, Edser P, Bhattacharyya S, Dunham A, Adcock IM, Pulleyn L, Barnes PJ, Harper JI, Abecasis G, Cardon L, White M, Burton J, Matthews L, Mott R, Ross M, Cox R, Moffatt MF, Cookson WO. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. Nat Genet 2003;34:181-6.
12. Laitinen T, Polvi A, Rydman P, Vendelin J, Pulkkinen V, Salmikangas P, Makela S, Rehn M, Pirskanen A, Rautanen A, Zucchelli M, Gullsten H, Leino M, Alenius H, Petays T, Haahtela T, Laitinen A, Laprise C, Hudson TJ, Laitinen LA, Kere J. Characterization of a common susceptibility locus for asthma-related traits. Science 2004;304:300-4.
13. Oguma T, Palmer LJ, Birben E, Sonna LA, Asano K, Lilly CM. Role of prostanoid DP receptor variants in susceptibility to asthma. N Engl J Med 2004;351:1752-63.
14. Risch NJ. Searching for genetic determinants in the new millennium. Nature 2000;405:847-56.
15. Lee JH, Park HS, Park SW, Jang AS, Uh ST, Rhim T, Park CS, Hong SJ, Holgate ST, Holloway JW, Shin HD. ADAM33 polymorphism: association with bronchial hyper-responsiveness in Korean asthmatics. Clin Exp Allergy 2004;34:860-5.
16. Cheong HS, Park CS, Kim LH, Park BL, Uh ST, Kim YH, Lym GI, Lee JY, Lee JK, Kim HT, Ryu HJ, Han BG, Kim JW, Park C, Kimm K, Shin HD, Oh B. CXCR3 polymorphisms associated with risk of asthma. Biochem Biophys Res Commun 2005;334:1219-25.
17. Jung JS, Park BL, Cheong HS, Bae JS, Kim JH, Chang HS, Rhim T, Park JS, Jang AS, Lee YM, Kim KU, Uh ST, Na JO, Kim YH, Park CS, Shin HD. Association of IL-17RB gene polymorphism with asthma. Chest 2009;135:1173-80.
18. Park JH, Chang HS, Park CS, Jang AS, Park BL, Rhim TY, Uh ST, Kim YH, Chung IY, Shin HD. Association analysis of CD40 polymorphisms with asthma and the level of serum total IgE. Am J Respir Crit Care Med 2007;175:775-82.
19. Chae SC, Park BL, Park CS, Ryu HJ, Yang YS, Lee SO, Choi YH, Kim EM, Uh ST, Kim YH, Kim KK, Oh B, Chung HT, Kimm K, Shin HD. Putative association of RUNX1 polymorphisms with IgE levels in a Korean population. Exp Mol Med 2006;38:583-8.
20. Lee SH, Chang HS, Jang AS, Park SW, Park JS, Uh ST, Kim YH, Oh B, Lee JK, Park BL, Shin HD, Park CS, Kimm K. The association of a single-nucleotide polymorphism of the IL-2 inducible T-cell kinase gene with asthma. Ann Hum Genet 2011;75:359-69.
21. Kim SH, Cho BY, Park CS, Shin ES, Cho EY, Yang EM, Kim CW, Hong CS, Lee JE, Park HS. Alpha-T-catenin (CTNNA3) gene was identified as a risk variant for toluene diisocyanate-induced asthma by genome-wide association analysis. Clin Exp Allergy 2009;39:203-12.
22. Shin EK, Lee SH, Cho SH, Jung S, Yoon SH, Park SW, Park JS, Uh ST, Kim YK, Kim YH, Choi JS, Park BL, Shin HD, Park CS. Association between colony-stimulating factor 1 receptor gene polymorphisms and asthma risk. Hum Genet 2010;128:293-302.
23. Park BL, Kim LH, Choi YH, Cheong HS, Park HS, Hong SJ, Choi BW, Lee JH, Uh ST, Park CS, Shin HD. Association analysis of monocyte chemotactic protein-3 (MCP3) polymorphisms with asthmatic phenotypes. J Biochem Mol Biol 2005;38:77-81.
24. Oh SH, Park SM, Lee YH, Cha JY, Lee JY, Shin EK, Park JS, Park BL, Shin HD, Park CS. Association of peroxisome proliferator-activated receptor-gamma gene polymorphisms with the development of asthma. Respir Med 2009;103:1020-4.
25. Kim Y, Park CS, Shin HD, Choi JW, Cheong HS, Park BL, Choi YH, Jang AS, Park SW, Lee YM, Lee EJ, Park SG, Lee JY, Lee JK, Han BG, Oh B, Kimm K. A promoter nucleotide variant of the dendritic cell-specific DCNP1 associates with serum IgE levels specific for dust mite allergens among the Korean asthmatics. Genes Immun 2007;8:369-78.
26. Cheong HS, Kim LH, Park BL, Choi YH, Park HS, Hong SJ, Choi BW, Park CS, Shin HD. Association analysis of interleukin 5 receptor alpha subunit (IL5RA) polymorphisms and asthma. J Hum Genet 2005;50:628-34.
27. Park BL, Cheong HS, Kim LH, Choi YH, Namgoong S, Park HS, Hong SJ, Choi BW, Lee JH, Park CS, Shin HD. Association analysis of signal transducer and activator of transcription 4 (STAT4) polymorphisms with asthma. J Hum Genet 2005;50:133-8.
28. Lee JH, Chang HS, Kim JH, Park SM, Lee YM, Uh ST, Rhim T, Chung IY, Kim YH, Park BL, Park CS, Shin HD. Genetic effect of CCR3 and IL5RA gene polymorphisms on eosinophilia in asthmatic patients. J Allergy Clin Immunol 2007;120:1110-7.
29. Lee SO, Cheong HS, Park BL, Bae JS, Sim WC, Chun JY, Isbat M, Uh ST, Kim YH, Jang AS, Park CS, Shin HD. MYLK polymorphism associated with blood eosinophil level among asthmatic patients in a Korean population. Mol Cells 2009;27:175-81.
30. Lee JH, Moore JH, Park SW, Jang AS, Uh ST, Kim YH, Park CS, Park BL, Shin HD. Genetic interactions model among Eotaxin gene polymorphisms in asthma. J Hum Genet 2008;53:867-75.
31. Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med 2009;360:1759-68.
32. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007;448:470-3.
33. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, Helgadottir H, Steinthorsdottir V, Stefansson H, Williams C, Hui J, Beilby J, Warrington NM, James A, Palmer LJ, Koppelman GH, Heinzmann A, Krueger M, Boezen HM, Wheatley A, Altmuller J, Shin HD, Uh ST, Cheong HS, Jonsdottir B, Gislason D, Park CS, Rasmussen LM, Porsbjerg C, Hansen JW, Backer V, Werge T, Janson C, Jonsson UB, Ng MC, Chan J, So WY, Ma R, Shah SH, Granger CB, Quyyumi AA, Levey AI, Vaccarino V, Reilly MP, Rader DJ, Williams MJ, van Rij AM, Jones GT, Trabetti E,

Malerba G, Pignatti PF, Boner A, Pescollderungg L, Girelli D, Olivieri O, Martinelli N, Ludviksson BR, Ludviksdottir D, Eyjolfsson GI, Arnar D, Thorgeirsson G, Deichmann K, Thompson PJ, Wjst M, Hall IP, Postma DS, Gislason T, Gulcher J, Kong A, Jonsdottir I, Thorsteinsdottir U, Stefansson K. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009;41:342-7.

34. Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, Strachan DP, Wilk JB, Willis-Owen SA, Klanderman B, Lasky-Su J, Lazarus R, Murphy AJ, Soto-Quiros ME, Avila L, Beaty T, Mathias RA, Ruczinski I, Barnes KC, Celedon JC, Cookson WO, Gauderman WJ, Gilliland FD, Hakonarson H, Lange C, Moffatt MF, O'Connor GT, Raby BA, Silverman EK, Weiss ST. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am J Hum Genet 2009;84:581-93.
35. Hancock DB, Romieu I, Shi M, Sienra-Monge JJ, Wu H, Chiu GY, Li H, del Rio-Navarro BE, Willis-Owen SA, Weiss ST, Raby BA, Gao H, Eng C, Chapela R, Burchard EG, Tang H, Sullivan PF, London SJ. Genome-wide association study implicates chromosome 9q21.31 as a susceptibility locus for asthma in mexican children. PLoS Genet 2009;5:e1000623.
36. Bardy GH, Smith WM, Hood MA, Crozier IG, Melton IC, Jordaens L, Theuns D, Park RE, Wright DJ, Connelly DT, Fynn SP, Murgatroyd FD, Sperzel J, Neuzner J, Spitzer SG, Ardashev AV, Oduro A, Boersma L, Maass AH, Van Gelder IC, Wilde AA, van Dessel PF, Knops RE, Barr CS, Lupo P, Cappato R, Grace AA. An entirely subcutaneous implantable cardioverter-defibrillator. N Engl J Med 2010;363:36-44.
37. Li X, Howard TD, Zheng SL, Haselkorn T, Peters SP, Meyers DA, Bleecker ER. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. J Allergy Clin Immunol. 2010;125:328-35.e11.
38. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WO. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-21.
39. Shin HD, Park BL, Kim LH, Jung JH, Kim JY, Yoon JH, Kim YJ, Lee HS. Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. Hum Mol Genet 2003;12:901-6.
40. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. Nature 2009;461:747-53.
41. Halapi E, Gudbjartsson DF, Jonsdottir GM, Bjornsdottir US, Thorleifsson G, Helgadottir H, Williams C, Koppelman GH, Heinzmann A, Boezen HM, Jonasdottir A, Blondal T, Gudjonsson SA, Thorlacius T, Henry AP, Altmueller J, Krueger M, Shin HD, Uh ST, Cheong HS, Jonsdottir B, Ludviksson BR, Ludviksdottir D, Gislason D, Park CS, Deichmann K, Thompson PJ, Wjst M, Hall IP, Postma DS, Gislason T, Kong A, Jonsdottir I, Thorsteinsdottir U, Stefansson K. A sequence variant on 17q21 is associated with age at onset and severity of asthma. Eur J Hum Genet 2010;18:902-8.
42. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, Chateigner N, Gormand F, Just J, Le Moual N, Scheinmann P, Siroux V, Vervloet D, Zelenika D, Pin I, Kauffmann F, Lathrop M, Demenais F. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med 2008;359:1985-94.
43. Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Margulies M, Weinstock GM, Gibbs RA, Rothberg JM. The complete genome of an individual by massively parallel DNA sequencing. Nature 2008;452:872-6.
44. Thomsen SF, van der Sluis S, Kyvik KO, Skytthe A, Backer V. Estimates of asthma heritability in a large twin sample. Clin Exp Allergy 2010;40:1054-61.
45. Miller RL. Prenatal maternal diet affects asthma risk in offspring. J Clin Invest 2008;118:3265-8.
46. Cheong HS, Park SM, Kim MO, Park JS, Lee JY, Byun JY, Park BL, Shin HD, Park CS. Genome-wide methylation profile of nasal polyps: relation to aspirin hypersensitivity in asthmatics. Allergy 2011;66:637-44.
47. Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, Robson S, Vukcevic D, Barnes C, Conrad DF, Giannoulatou E, Holmes C, Marchini JL, Stirrups K, Tobin MD, Wain LV, Yau C, Aerts J, Ahmad T, Andrews TD, Arbury H, Attwood A, Auton A, Ball SG, Balmforth AJ, Barrett JC, Barroso I, Barton A, Bennett AJ, Bhaskar S, Blaszczyk K, Bowes J, Brand OJ, Braund PS, Bredin F, Breen G, Brown MJ, Bruce IN, Bull J, Burren OS, Burton J, Byrnes J, Caesar S, Clee CM, Coffey AJ, Connell JM, Cooper JD, Dominiczak AF, Downes K, Drummond HE, Dudakia D, Dunham A, Ebbs B, Eccles D, Edkins S, Edwards C, Elliot A, Emery P, Evans DM, Evans G, Eyre S, Farmer A, Ferrier IN, Feuk L, Fitzgerald T, Flynn E, Forbes A, Forty L, Franklyn JA, Freathy RM, Gibbs P, Gilbert P, Gokumen O, Gordon-Smith K, Gray E, Green E, Groves CJ, Grozeva D, Gwilliam R, Hall A, Hammond N, Hardy M, Harrison P, Hassanali N, Hebaishi H, Hines S, Hinks A, Hitman GA, Hocking L, Howard E, Howard P, Howson JM, Hughes D, Hunt S, Isaacs JD, Jain M, Jewell DP, Johnson T, Jolley JD, Jones IR, Jones LA, Kirov G, Langford CF, Lango-Allen H, Lathrop GM, Lee J, Lee KL, Lees C, Lewis K, Lindgren CM, Maisuria-Armer M, Maller J, Mansfield J, Martin P, Massey DC, McArdle WL, McGuffin P, McLay KE, Mentzer A, Mimmack ML, Morgan AE, Morris AP, Mowat C, Myers S, Newman W, Nimmo ER, O'Donovan MC, Onipinla A, Onyiah I, Ovington NR, Owen MJ, Palin K, Parnell K, Pernet D, Perry JR, Phillips A, Pinto D, Prescott NJ, Prokopenko I, Quail MA, Rafelt S, Rayner NW, Redon R, Reid DM, Renwick, Ring SM, Robertson N, Russell E, St Clair D, Sambrook JG, Sanderson JD, Schuilenburg H, Scott CE, Scott R, Seal S, Shaw-Hawkins S, Shields BM, Simmonds MJ, Smyth DJ, Somaskantharajah E, Spanova K, Steer S, Stephens J, Stevens HE, Stone MA, Su Z, Symmons DP, Thompson JR, Thomson W, Travers ME, Turnbull C, Valsesia A, Walker M, Walker NM, Wallace C, Warren-Perry M, Watkins NA, Webster J, Weedon MN, Wilson AG, Woodburn M, Wordsworth BP, Young AH, Zeggini E, Carter NP, Frayling TM, Lee C, McVean G, Munroe PB, Palotie A, Sawcer SJ, Scherer SW, Strachan DP, Tyler-Smith C, Brown MA, Burton PR, Caulfield MJ, Compston A, Farrall M, Gough SC, Hall AS, Hattersley AT, Hill AV, Mathew CG, Pembrey M, Satsangi J, Stratton MR, Worthington J, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand W, Parkes M, Rahman N, Todd JA, Samani NJ, Donnelly P. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. Nature 2010;464:713-20.
48. Toft M, Ross OA. Copy number variation in Parkinson's disease. Genome Med 2010;2:62.