**Allergologia et
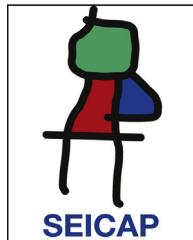immunopathologia**

Sociedad Española de Inmunología Clínica,
Alergología y Asma Pediátrica

www.elsevier.es/ai

Genome-wide association studies (GWAS) and their importance in asthma

A. García-Sánchez [a,b,*], M. Isidoro-García [b,c,d], V. García-Solaesa [b,c], C. Sanz [b,e],
L. Hernández-Hernández [a,b], J. Padrón-Morales [c], F. Lorente-Toledano [a,b,f], I. Dávila [a,b,g]

[a] Departamento de Pediatría, Universidad de Salamanca, Spain
[b] Grupo de Alergia, Instituto Biosanitario de Salamanca (IBSAL), Spain
[c] Servicio de Bioquímica Clínica, Complejo Asistencial Universitario de Salamanca, Spain
[d] Departamento de Medicina, Universidad de Salamanca, Spain
[e] Departamento de Microbiología y Genética, Universidad de Salamanca, Spain
[f] Servicio de Pediatría, Hospital Universitario de Salamanca, Spain
[g] Servicio de Alergia, Complejo Asistencial Universitario de Salamanca, Spain

Summary   Asthma is a complex disease determined by the interaction of different genes and environmental factors. The first genetic investigations in asthma were candidate gene association studies and linkage studies. In recent years research has focused on association studies that scan the entire genome without any prior conditioning hypothesis: the so-called genome-wide association studies (GWAS). The first GWAS was published in 2007, and described a new locus associated to asthma in chromosome 17q12-q21, involving the *ORMDL3*, *GSDMB* and *ZPBP2* genes (a description of the genes named in the manuscript are listed in Table 1). None of these genes would have been selected in a classical genetic association study since it was not known they could be implicated in asthma. To date, a number of GWAS studies in asthma have been made, with the identification of about 1000 candidate genes. Coordination of the different research groups in international consortiums and the application of new technologies such as new generation sequencing will help discover new implicated genes and improve our understanding of the molecular mechanisms underlying the disease.
© 2014 SEICAP. Published by Elsevier España, S.L.U. All rights reserved.

Introduction

Asthma is clinically characterised by bronchial hyperresponsiveness associated to airway obstruction episodes. Bronchial obstruction is the result of an inflammatory

* Corresponding author.
  E-mail address: chonela@usal.es (A. García-Sánchez).

process triggered by allergens, infections or other still little known factors. The prevalence of the disease is high, particularly in developed countries. In the last few decades there has been a notorious increase in the number of cases of asthma. Although it has been known for a long time that asthma has an important hereditary component, the rapidness with which the prevalence of the disease has increased suggests that not only genetic factors are involved. In this respect it has been suggested that certain environmental factors, particularly related to the industrialisation process, and their influence on the genetic component of the disease, could contribute to increasing the frequency of allergic diseases in general, and of asthma in particular. However, in recent decades the identification of specific genes associated to the risk of suffering the disease, and their validation, has been one of the challenges facing research in asthma. The Human Genome Project is an international initiative designed to identify the sequence of the human genome. Following the publication in 2001 of a draft of the sequence, investigators have focused on genomic variations among individuals. In this respect, polymorphisms are variations in the genetic sequence that are found in over 1% of the population. Single nucleotide polymorphisms (SNPs) are polymorphisms that affect a single base. It has been estimated that as of 23 July 2013, the single nucleotide polymorphism database (dbSNP) listed 62,676,337 SNPs in humans (http://www.ncbi.nlm.nih.gov/mailman/pipermail/dbsnp-announce/2013q3/000133.html). Some of these variations could affect the quantity, timing and function of a protein encoded by a gene, and contribute to the risk of suffering a disease. Most diseases are caused by an interaction between genetic and environmental factors.[1] Many case–control studies have been carried out to clarify the relationship between genetic variations and specific diseases.[2]

The present review analyses how GWAS can contribute to improving our understanding of asthma through the identification of markers specifically related to the disease (Table 1).

Genome-wide association studies (GWAS)

Genome-wide association studies (GWAS) allow the analysis of hundreds of thousands of polymorphisms with the purpose of relating them to a specific phenotype, generally a disease such as asthma, or a characteristic of the disease such as the levels of IgE. The main advantage of GWAS has been their excellent resolution, their statistical power in detecting risk variants with moderate effects, and their capacity to analyse the entire genome. GWAS has offered the opportunity to identify new genes or regulatory regions not previously related to the disease, but without the need to study families. They are able to detect loci with modest effects upon the disease, although GWAS are not useful for detecting rare disease variants.

The inconveniences of GWAS are that they need large sample sizes and tools for processing a very large body of data. Because of the great number of statistical comparisons in GWAS (hundreds of thousands), the threshold defining significant associations is different from that of conventional case-control studies. The most restrictive threshold is $p < 7.2 \times 10^{-8}$, and the least restrictive $p < 5 \times 10^{-7}$.[2] Many of the GWAS published to date have not yielded loci with the level of statistical significance required to indicate a genuine association. As a result, these studies have not fully met the initial expectations, although the information produced may be very useful.

Large international research consortiums have been founded with the aim of conducting rigorous statistical GWAS meta-analyses for common diseases. In Europe, the GABRIEL consortium was created for the study of asthma, analysing a population of 10,365 cases and 16,110 controls.[4] The European initiative was followed by the EVE consortium comprising North American investigators. The EVE consortium fundamentally reflects the ethnic diversity of North America, comprising a population of European, African/Caribbean and Hispanic origin, with the inclusion of over 5000 cases of asthma.[5] At present, the Transnational Asthma Genetics Consortium (TAGC) is conducting an international GWAS that will allow the completion of a meta-analysis of asthma with the examination of about 30,000 cases and 50,000 controls.[6]

GWAS in asthma

The first GWAS in asthma was carried out in 2007 with a population of approximately 1000 cases and 1000 controls.[7] It identified the *ORMDL3* gene in chromosome 17q21 as a contributor to the risk of developing childhood asthma. This finding has been recognised as the most consistent genetic association obtained by a GWAS in asthma to date. Genetic variants in this particular locus regulate expression of the *OMRDL3* gene and the neighbouring *GSDML* gene in asthmatic children. The variant rs7216389-T, which shows an increased association to asthma and increased expression of the *OMRDL3* gene, is located in a region with high linkage disequilibrium in chromosome 17q21, very close to the *ORMDL3* gene and within the first intron of the *GSDML* gene. This non-coding region shows notorious homology among species and contains a possible binding element for the transcription factor C/EBPβ, which is very important in regulating the genes implicated in immune and inflammatory responses.[7] Due to the important degree of linkage among the SNPs and the co-regulation of genic expression in this region, it has not been possible to distinguish between the effects of these two genes; we therefore generally speak of an association to asthma on the part of locus 17q21.[2] This association has been reproduced in several studies in different ethnic populations, though it seems that the mentioned genomic region is associated to childhood asthma, but not to adult asthma.[2,3] Sleiman et al.[8] carried out a GWAS in children with persistent asthma requiring daily inhaled glucocorticoid therapy, and a control group. In addition to confirming the association to locus 17q21, this study identified a new locus related to asthma susceptibility in chromosome 1q31, which contains the *CRB1* and *DENND1B* genes. *DENND1B* encodes for a protein with a TNFR1 binding domain. It constitutes a negative TNFR1 regulator in response to cytokine-induced stress. *DENND1B* is expressed both by dendritic cells and by activated T cells, and modulates the Th1-Th2 cytokine cascade and other inflammatory signals through the repression of TNFR1.[8]

Table 1 Description of genes by alphabetical order.

Name/gene ID	Description
AGER	Advanced glycosylation end product-specific receptor
C11orf30	Chromosome 11 open reading frame 30
CD14	CD14 molecule
CHRNA3/5	Cholinergic receptor, nicotinic, alpha 3/5
CRB1	Crumbs family member 1, photoreceptor morphogenesis associated
CRIM1	Cysteine rich transmembrane BMP regulator 1
DENND1B	DENN/MADD domain containing 1B
FAM13A	Family with sequence similarity 13, member A
FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
FOXP3	Forkhead box P3
GAB1	GRB2-associated binding protein 1
GATA3	GATA binding protein 3
GLCCI1	Glucocorticoid induced transcript 1
GSDMB	Gasdermin B
GSTCD	Glutathione S-transferase, C-terminal domain containing
HHIP	Hedgehog interacting protein
HLA-DQ	Major histocompatibility complex, class II, DQ
IKZF4	IKAROS family zinc finger 4
IL1RL1	Interleukin 1 receptor-like 1/
IL2RB	Interleukin 2 receptor beta
IL-3	Interleukin 3
IL4R	Interleukin 4 receptor
IL-6	Interleukin 6
IL6R	Interleukin 6 receptor
IL12A,	Interleukin 12A
IL12RB1	Interleukin 12 receptor, beta 1
IL13	Interleukin 13
IL18R1	Interleukin 18 receptor 1
IL33	Interleukin 33
IRF2	Interferon regulatory factor 2
LOC730217	Hypothetical LOC730217
LRRC32	Leucine rich repeat containing 32
MYB	V-myb avian myeloblastosis viral oncogene homolog
NOTCH4	Notch 4
OPRK1	Opioid receptor, kappa 1
OR7E156P	Olfactory receptor, family 7, subfamily E, member 156 pseudogene
ORMDL3	ORM1-like 3
PDCD4	Programmed Death Cell Domain 4
PDE4D	Phosphodiesterase 4D, cAMP-specific
PDE11A	Phosphodiesterase 11A
PRKG1	Protein kinase, cGMP-dependent, type I
PTGDR	Prostaglandin D2 Receptor Gene
PYHIN1	Pyrin and HIN domain family, member 1
RAD50	RAD50 homolog
RARB	Retinoic acid receptor, beta
RPL32P28	Ribosomal protein L32 pseudogene 28p
SMAD3	SMAD family member 3
STAT4	Signal transducer and activator of transcription 4
STAT6	Signal transducer and activator of transcription 6, interleukin-4 induced
SYNPO2	Synaptopodin 2
THSD4	Thrombospondin, type I, domain containing 4
TLE4	Transducin-like enhancer of split 4
TLN1	Talin 1
TNFR1	Tumour necrosis factor receptor 1
TSLP	Thymic stromal lymphopoietin
USP38	Ubiquitin-specific peptidase 38
ZNF323	Zinc finger protein 323
ZPBP2	Zona pellucida binding protein 2

A GWAS carried out in a paediatric population in North America identified a strong association to the *PDE4D* gene (5q12), which encodes for a bronchial phosphodiesterase. The association was not observed in children of African origin.[3] Belonging to the same family as *PDE4D*, the *PDE11A* gene was associated to asthma in a GWAS involving six-year-old asthmatic children. Although statistical significance was not reached, it seems that this phosphodiesterase family may play a role in the pathogenesis of asthma.[3] Another GWAS found an association to the *TLE4* gene (9q21) in a population of 492 Mexican children – although statistical significance was likewise not reached in this case. Nevertheless, this gene was replicated in a Mexican cohort. The mentioned gene, which participates in the development of B lymphocytes, had not been previously associated to asthma.[3]

The meta-analysis carried out by the GABRIEL consortium identified six susceptible loci, containing the *IL1RL1/IL18R1*, *HLA-DQ*, *IL33*, *SMAD3*, *ORMDL3/GSDMB* and *IL2RB* genes, which participate in Th1 or Th2 cell responses.[3,4]

In 2011, the EVE consortium conducted a meta-analysis of GWAS in asthma among North American populations.[5] A total of five susceptibility loci were identified: locus 17q21 (*GSDMB/ORMDL3*) and the previously described *IL1RL1*, *TSLP* and *IL33* genes.[4] In addition, a new asthma-related locus specific of individuals of African origin was identified in the *PYHIN1* gene.

A GWAS in asthma was carried out in 2011 in a Japanese population of adults, with the description of five candidate loci.[6] Association of the *HLA* locus (6p21) was confirmed, with identification of the *TSLP* gene (5q22), which participates in the maturation of Th2 lymphocytes through the activation of antigen presenting cells (APCs). This locus had already been associated by Torgerson in a North American population.[5] The locus containing the *USP38-GAB1* genes (4q31) was also described. *USP38* encodes for ubiquitin-specific peptidase 38 (USP38), the function of which remains uncertain. GAB1 in turn is a protein that participates in signalling pathways activated by the receptors of cytokines IL-3, IL-6, Interferon α and γ, and the B and T lymphocyte receptors. A region was identified in chromosome 10 that does not appear to contain a coding gene, but which is located at 1 Mb in 3′ sense from the *GATA3* gene, the main regulator of Th2 lymphocyte differentiation. In chromosome 12q13 is located *IKZF4* gene (also known as *EOS*) that is involved in the differentiation of regulatory T lymphocytes as a FOXP3 silencing co-regulator.[6]

A GWAS was carried out in an Australian population in combination with a large number of samples from the GABRIEL consortium. Significant associations were observed in the *IL6R* (1q21), *C11orf30/LRRC32* (11q13), *PRKG1* (10q11) and *RPL32P28/OR7E156P* (13q21) genes.[3]

Approximately 5% of all asthma patients suffer a serious form of the disease that is difficult to control with the existing treatments. Li et al.[6] focused on this kind of patients in their GWAS. A total of 292,443 SNPs were studied in a population of cases supplied by the Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR), versus a control population. The study confirmed the relevance of the regions corresponding to the *RAD50-IL13* (5q31 and *HLA-DR/DQ* genes (6p21), implicated in the regulation of Th2 cytokines and in antigen presentation in difficult to control severe asthma.

GWAS have also been used in pharmacogenetic studies. In this respect, a recent publication has identified genic variants associated to changes in lung function in response to inhaled corticosteroids. A functional variant of *GLCCI1* was associated to an important decrease in patient response to corticosteroid treatment.[6]

Immunoglobulin E levels are closely correlated to the clinical expression of allergic asthma. Several GWAS have found variants that may modulate IgE levels in asthmatic individuals, including *FCER1A*, *STAT6*, *IL4R*, *IL13* and *HLA*.[3] A recent GWAS in a Korean population of 877 asthmatic subjects has identified new variants in the *CRIM1*, *TLN1* and *SYNPO2* genes, associated to total IgE levels. In addition, in this group of asthmatics, an association was found in the intergenic region close to the *OPRK1* and *LOC730217* genes correlated to the levels of specific IgE against the dust mites *Dermatophagoides pteronyssinus* and *D. farinae*, respectively.[9]

A meta-analysis was carried out in 2012 using data from 19 GWAS, with a total of about 23,000 individuals mostly of European origin, of whom 8165 were asthmatics. The aim of the study was to identify genic variants associated to body mass index (BMI) in asthmatic children and adults.[10] Associations were found between variants of the *DENND1B* gene and BMI in the asthmatic children. However, this association could not be replicated in independent groups, and the effect was moreover heterogeneous depending on the geographical setting considered. The mentioned gene had already been related to the risk of suffering asthma in children,[8] and the detection of an association to another phenotype is interesting – although replication of the association is needed in order to validate the results.

Altered lung function is a phenotype associated to asthma and to chronic obstructive pulmonary disease. Variants of the *HHIP* and *CHRNA3/5* genes have been associated to this phenotype and have been replicated in several populations.[3] Table 2 enumerates other loci associated to lung function.

In recent months a number of studies have conducted in depth evaluations of previously published GWAS. Li et al. investigated the association of genic variants related to lung function in four North American populations of asthmatic Caucasians based on GWAS and meta-analyses. Four of the 32 loci associated to impaired lung function corresponded to type Th1 genes (*IL12A*, *IL12RB1*, *STAT4* and *IRF2*).[11]

Two previous meta-analyses in the general population found 28 loci associated to the regulation of lung function. The meta-analysis conducted in asthmatics identified seven of these 28 loci (*HHIP*, *FAM13A*, *THSD4*, *GSTCD*, *NOTCH4-AGER*, *RARB* and *ZNF323*), fundamentally implicated in airway structure and remodelling.[11]

On the other hand, no association of type Th2 genes (which confer susceptibility to asthma) (*IL13*, *TSLP*, *IL33* and *IL1RL1*) to lung function was found – thus suggesting that the genes associated to lung function which might influence the severity of asthma are different from the genes associated to asthma susceptibility. The authors proposed a genetic model of asthma progression in which genic variants in type Th2 genes induce a Th2 dominant atopic and asthma susceptibility response. Genetic variants in type Th1 genes or IL-12, and in airway structure and remodelling genes, together

Table 2 Published genome-wide association studies of asthma and related phenotypes.

Gene	Chromosomal locus	Association	Study
ORMDL3	17q21	Asthma	Moffat et al. (2007)
GSDMB			
CHI3L1	1q32	Asthma/YKL-40 serum levels	Ober et al. (2008)
IL1RL1	2q12	Eosinophil	Gudbjartsson
IKZF2	5q31	count	et al. (2009)
GATA2	3q21		
IL5	2q12		
SH2B3	12q24		
TLE4	9q21	Asthma	Handcock et al. (2009)
PDE4D	5q12	Asthma	Himes et al. (2009)
PDE11A	2q31	Asthma	DeWan et al. (2010)
RAD50	5q31	Asthma	Li et al.
HLA-DR/DQ	6p21		(2010)
ADRA1B	5q33	Asthma	Mathias
PRNP	20p12		et al. (2010)
DPP10	12q12		
IL1RL1/IL18R1	2q12	Asthma	Moffat el al.
HLA-DQ	6p21	Childhood-	(2010)
IL33	9p24	onset	
SMAD3	15q22	asthma	
IL2RB	22q12		
ORMDL3/GSDMB	17q12		
DENND1B	1q31	Paediatric asthma	Sleiman et al. (2010)
CRB1	1q31-q32		
HLA-DPA1/HLA-DPB1	6p21	Paediatric asthma	Noguchi et al. (2011)
IL6R	1q21	Asthma	Ferrerira
C11orf30/ILRRC32	11q13		et al. (2011)
USP38-GAB1	4q31	Asthma	Hirota et al.
TSLP/WDR36	5q22		(2011)
NOTCH1/HLA-DRA/HLA-DQA2/IKZF4	6p21		
LOC338591	10p14		
IKZF4/CDK2	12q13		
GSDMB	17q12	Asthma in	Torgerson
IL1RL1	2q12	four	et al. (2011)
TSLP	5q22	ethnically	
IL33	9p24	diverse	
PYHIN1	1q23	North	
C11orf71	11q23	American	
CRCT1	1q21	populations	
ORMDL3	17q12	Asthma	Ferrerira et al. (2011)
PDCD4	10q24	Severe asthma and IgE level	Binia et al. (2013)
C11orf30/ILRRC32	11q13	Allergic	Ramasamy
TMEM232/SLCA25A46	5q22	Rhini-	et al. (2011)
HLA region	6p22	tis/grass	
FCER1A	1q23	IgE levels	Granada
IL13	5q31		et al. (2012)
HLA-A	6p22		
STAT6/NAB2	12q13		
DARC	1q23		
HLA-DQA2	6p21		
FCER1A	1q23	Serum IgE	Weidinger
STAT6	12q13	levels	et al. (2008)
RAD50	5q31		

Table 2 (*Continued*)

Gene	Chromosomal locus	Association	Study
CRIM1	2p21	Serum IgE	Kim et al.
ZNF71	19q13	levels	(2013)
TLN1	9p13		
SYNPO2	4q26		
OPRK1	8q11		
LOC730217	14q32		
Epidermal Differentiation Complex	1q21	Asthma/Atopic	Weidinger
LRRC32	11q13	dermatitis	et al. (2013)
RAD50/IL13	5q31		
HLA	6p22		
CHRNA3/5	15q24	COPD	Pillai et al. (2009)
FAM13A	4q22	COPD	Cho et al. (2010).
RAB4B/EGLN2/MIA/ CYP2A6	19q13	COPD	Cho et al. (2012)
HHIP	4q31	FEV_1/FVC	Wilk et al.
HHIP	4q31		(2009)
GPR126	6q24		Handcok
ADAM19	5q33		et al. (2010)
AGER-PPT2	6p21		
FAM13A	4q22		
PTCH1	9q22		
PID1	2q36		
HTR4	5q33		
INTS12-GSTCD-NPNT	4q24	FEV_1	
TNS1	2q35	FEV_1	Repapi
GSTCD	4q24	FEV_1	et al. (2010)
HHIP	4q31	FEV_1/FVC	
HTR4	5q33	FEV1	
AGER	6p21	FEV_1/FVC	
THSD4	15q23	FEV_1/FVC	
MFAP2	1p36	FEV_1/FVC	Soler-
TGFB2	1q41	FEV_1/FVC	Artigas
HDAC4	2q37	FEV_1/FVC	et al. (2011)
RARB	3p24	FEV_1/FVC	
MECOM	3q26	FEV_1	
SPATA9	5q15	FEV_1/FVC	
ZKSCAN3	6p22	FEV_1	
NCR3	6p21	FEV_1/FVC	
ARMC2	6q21	FEV_1/FVC	
C10orf11	10q22	FEV_1	
LRP1	12q13	FEV_1/FVC	
CCDC38	12q23	FEV_1/FVC	
MMP15	16q21	FEV_1/FVC	
CFDP1	16q23	FEV_1/FVC	
KCNE2	21q22	FEV_1/FVC	
DLEU7	13q14	FEV_1	Imboden et al. (2012)
THSD4/UACA/TLE3	15q23	FEV_1/FVC	Yao et al. (2013)
ITGB5	3q21	Airway hyperre-	Himes et al. (2013)
AGFG1	2q36	sponsiveness	
GLCCI1	7p21	Glucocorticoid therapy in asthma	Tanisira et al. (2011)

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E.

with other mechanisms, affect lung function and increase the severity of asthma.[11]

Binia et al. analysed a GWAS previously conducted in asthmatic children[7] and focused on the group with severe asthma. They found that certain variants of the *PDCD4* gene (10q24) are significantly associated to severe childhood asthma and to total IgE levels. Gel retardation and reporter gene tests confirmed that the identified SNP alters binding of the transcription factor MYB, influencing the expression of *PDCD4* – a known target of MYB.[12]

An association has recently been described between DNA methylation and asthma susceptibility dependent upon age and gender.[13] The authors carried out a GWAS with the purpose of determining whether the association of the region of chromosome 17q12-q21 to childhood asthma was gender-specific. The study identified a regulatory region in the promoter of the *ZPBP2* gene that showed statistically significant differences among males, females and age in terms of DNA methylation. The effect of the variants in 17q12-q21 upon asthma susceptibility could be attenuated by DNA methylation. Adult males and females carrying the 17q12-q21 alleles predisposing to asthma would not be affected, due to the high methylation levels of the *ZPBP2* gene. The authors suggested that locus-specific DNA methylation could exert a modulating effect upon the development of asthma. The loci identified in the main GWAS published to date are summarised in Table 2.

The future of genetics in asthma

Genome-wide association studies have caused a genuine revolution in the study of complex diseases such as asthma. Over 1000 genes have been associated to asthma (Human Genetic Epidemiology Navigator Database, http://hugenavigator.net/HuGENavigator/home.do, search in September 2013), and may constitute potential therapeutic targets. However, despite such success, the results have fallen short of the expectations when it comes to explaining the genetic origin of the disease – a condition that has been referred to as ''missing heritability''. This may be due to technological problems that preclude detection of all the SNPs or to copy number variations (CNV). Another hypothesis is that environmental factors interact with the genetic variants, influencing the development and severity of the disease. An example of this is the interaction between a polymorphism in the *CD14* gene and exposure to a bacterial lipopolysaccharide, where the response of individuals with the same genetic burden depends on the polysaccharide levels in the environment.[3] Genome wide interaction scans (GWIS) apply genetic variant searches taking into account environmental factors. A number of such studies have found new genes not detected in a first GWAS.[2,3,14] On the other hand, GWAS in asthma have also been carried out taking into account gene–gene interactions, in which the variation of a locus alters the effect of the variation of a second locus reflecting epistasis between two or more genes. The gene–gene interaction studies are still in course, and it is not yet known whether this method will be able to explain the heritability of asthma. However, gene–environment interactions would in part help explain the mentioned ''missing heritability''.[2]

Another issue that remains to be addressed is the association of genes of unknown function or which cannot be easily related to the disease, such as the *ORMDL3/GSDM1L* or *DENND1B* genes. The phenotypic heterogeneity of a disorder such as asthma could be decisive for establishing genuine genetic associations. Efforts to obtain more homogeneous asthma patient subgroups will serve to increase the performance of future GWAS in asthma.

In the coming years, technologies for the identification of genetic variants will continue to develop, giving rise to a tremendous increase in the volume of information. The combination of genome-wide studies with next generation sequencing (exome, whole genome, methylation) and re-sequencing techniques targeted to concrete regions of the genome will allow us to detect rare or infrequent variants that cannot be detected using microarray technology, and which contribute to the complexity of asthma. In fact, it is now believed that uncommon variants, not detected by GWAS, could make a greater contribution to risk – in contrast to the previously held idea that common diseases are associated to common genic variants. Most of the genic variants described are found in non-coding regions suggesting that gene regulation plays an important role in susceptibility to developing the disease. Sequencing the complete genome will help clarify this point.[2,3]

Epigenetic regulation is crucial in mediating environmental influences upon genic expression. We have recently described variations in the DNA methylation of a specific locus in the B cell population of a subgroup of asthmatic individuals.[15] There have been descriptions of variations, including methylation, in the promoter of the *PTGDR* gene (Prostaglandin D2 Receptor) in asthmatics and atopic individuals.[16] The combination of GWAS and promoter methylation, histone modifications and the influence upon the genic regulation on non-coding RNA is an extremely interesting approach that will open up new paths of research in asthma. The great amount of information produced by GWAS should serve to address functional genetics with a view to understanding the different pathways and genes that participate in the pathogenesis of asthma.

Acknowledgements

References

1. Lorente F, Isidoro-García M, Macías E, Dávila I. Do genetic factors determine atopy or allergy? Allergol Immunopathol. 2010;38:53–5.
2. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunol Rev. 2011;242:10–30.

3. March ME, Sleiman P, Hakonarson H. Genetic polymorphisms and associated susceptibility to asthma. Int J Gen Med. 2013;6:253–65.

4. Moffat MF, Gut IG, Demenais F, Strachan D, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363:1211–21.

5. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nat Genet. 2011;43:887–92.

6. Tamari M, Tanaka S, Hirota T. Genome-wide association studies of allergic diseases. Allergol Int. 2013;62:21–8.

7. Moffat MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature. 2007;448: 470–3.

8. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SAG, et al. Variants of DENND1B associated with asthma in children. N Eng J Med. 2010;362:36–44.

9. Kim JH, Cheong HS, Park JS, Jang AS, Uh ST, Kim YH, et al. A genome-wide association study of total serum and mite-specific IgEs in asthma patients. PLOS ONE. 2013;8:e71958.

10. Melén E, Granell R, Kogevinas M, Strachan D, Gonzalez JR, Wjst M, et al. Genome wide association study of body mass index in 23,000 individuals with and without asthma. Clin Exp Allergy. 2013;43:463–74.

11. Li X, Hawkins GA, Ampleford EJ, Moore WC, Li H, Hastie AT, et al. Genome-wide association study identifies TH1 pathway genes associated with lung function in asthmatic patients. J Allergy Clin Immunol. 2013;132:313–20.

12. Binia A, Van Stiphout N, Liang L, Michel S, Bhavsar PK, Fan Chung K, et al. A polymorphism affecting MYB binding within the promoter of the PDCD4 gene is associated with severe asthma in children. Hum Mutat. 2013;34:1131–9.

13. Naumova AK, Al tuwaijri A, Morin A, Vaillancourt VT, Madore AM, Berlivet S, et al. Sex- and age-dependent DNA methylation at the 17q12-q21 locus associated with childhood asthma. Hum Genet. 2013;132:811–22.

14. Gauderman WJ, Zhang P, Morrison JL, Lewinger JP. Finding novel genes by testing gene interactions in a genome-wide association study. Genet Epidemiol. 2013;37:603–13.

15. Pascual M, Suzuki M, Isidoro-García M, Padrón J, Turner T, Lorente F, et al. Epigenetic changes in B lymphocytes associated with house dust mite allergic asthma. Epigenetics. 2011;6:1131–7.

16. Isidoro-García M, Sanz C, García-Solaesa V, Pascual M, Benito-Pescador D, Lorente F, et al. PTGDR gene in asthma: a functional, genetic, and epigenetic study. Allergy. 2011;66:1553–62.