

Immunological and genetic aspects of asthma and allergy

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Abstract: Prevalence of allergy and allergic asthma are increasing worldwide. More than half of the US population has a positive skin prick test and approximately 10% are asthmatics. Many studies have been conducted to define immunological pathways underlying allergy and asthma development and to identify the main genetic determinants. In the effort to find missing pieces of the puzzle, new genomic approaches and more standardized ones, such as the candidate gene approach, have been used collectively. This article proposes an overview of the actual knowledge about immunological and genetic aspects of allergy and asthma. Special attention has been drawn to the challenges linked to genetic research in complex traits such as asthma and to the contribution of new genomic approaches.

Keywords: immune response, allergy, asthma, genetics, genomics

Allergy

Allergy, also called hypersensitivity, is a reaction that occurs when the immune system responds to a harmless antigen.¹ According to results published by the National Health and Nutrition Examination Surveys in 2005, more than half of the US population (54.3%) tests positive to one or more allergens (a common antigen that gives rise to an immediate hypersensitivity response)² using skin prick tests between 1988 and 1994.³ This survey also underlines the evidence of the growing prevalence of allergy in industrialized countries.

Allergies have been studied according to the different categories of allergens (indoor, outdoor, food, drug, etc) and according to the different allergic diseases (allergic rhinitis, atopic dermatitis, allergic asthma, etc). Prevalence of allergy to different types of antigen and prevalence of different allergic diseases ranges from 4% to 6% for food allergy,⁴ to 10% for allergic asthma,⁵ and up to 49% reported in one study for allergic rhinitis.⁶

Immunology of allergy

Hypersensitivity reactions have been classified by Coombs and Gell into four different types, characterized by different immunological mechanisms.⁷ These types have been well described in a review by Averbeck et al.¹ A brief description of each type is as follows. Type I hypersensitivity refers to immediate hypersensitivity responses against foreign proteins that are common (pollen, grass, animal danders, etc). It can be observed in allergic rhinitis and allergic asthma (see section “immunology of asthma” for more detail).^{1,8} This type of hypersensitivity is characterized by immunoglobulin (Ig) E production during the sensitization phase, which will bind to FcεRI receptors on mast cells and basophils. Upon renewed contact, the allergen will bind cellular IgE. Crosslinking

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of FcεRI receptors will lead to degranulation of mast cells and basophils (see Figure 1). Mediators released that way will lead to immediate and, sometimes, delayed immune responses.^{9,10}

Type II hypersensitivity is a humoral response mediated by IgG or IgM that are produced against surface antigens on body cells. An example of type II hypersensitivity is drug-induced cytopenia caused by penicillin, cephalosporin, or transfusion reactions.^{1,11} Immune response occurs within minutes after antigen contact. In drug allergic subjects, IgG or IgM will be formed in response to drugs or their metabolites which accumulate in membrane structures. This binding will allow

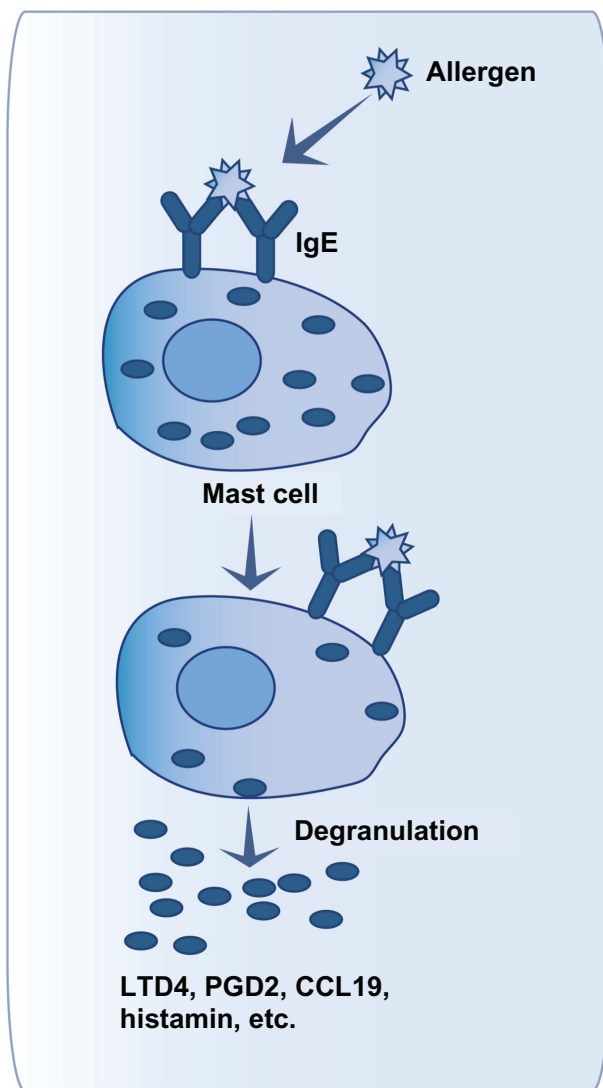


Figure 1 Mast cell activation after allergen contact.

Notes: When a first contact with allergen has already been made, immunoglobulin (Ig) E against this allergen have been produced by B cells and have bound to FcεRIβ receptors on mast cells. Another contact with the same allergen will allow its binding on cellular IgE on mast cells. This will produce a crosslinking of FcεRIβ, leading to activation and degranulation of mast cells within a few minutes. Modulators released by mast cells will promote inflammation by recruitment of new immune cells and activation of structural cells of the airways.

the killing of the target cells by three different ways.^{1,11} The first one is the activation of the classic complement pathway, which will lead to cytolysis. The second one is the antibody-dependant cell-mediated cytotoxicity, which will lead to lysis of target cells by natural killer cells. The last one is the recognition of Fc fragments of IgG and IgM by phagocytic cells, which will lead to opsonization of the target cells.

Type III hypersensitivity involves the formation of immune complexes that are not well cleared by innate immune cells as in malaria, rheumatoid arthritis, or farmer's lung.^{1,12} This response can occur within four to six hours. The accumulation of immune complexes (antigens bound to antibodies) in vessels and tissues can be caused by antigen excess for subjects with immunosuppression or insufficient antibody production. It can also be caused by repeated antigen exposure which will lead to excess IgG antibody production. The presence of those persistent immune complexes will give rise to an inflammatory response due to leukocytes' activation.^{1,12}

Finally, type IV hypersensitivity is a delayed response principally mediated by T cells.^{1,13} The best known example of type IV hypersensitivity is contact allergy. The sensitization phase lasts 10–15 days and is asymptomatic. In contact allergy, a hapten, a low molecular weight molecule, will interact with skin cells or proteins. After this contact, Langerhans and dendritic cells will present antigen to naive T cells to stimulate their differentiation into CD4⁺ and CD8⁺ T cells and to induce production of memory T cells. A renewed contact between the skin and the antigen will stimulate sensitized memory T cells via antigen-presenting cells. Early arrival of CD4⁺ and CD8⁺ T cells and activation of keratinocytes, will all contribute to promote inflammatory reaction via cytokine secretion. Finally, this reaction will be controlled by regulatory T cells.^{1,13}

This classification of allergic reactions has been widely accepted but some revised nomenclature was proposed, such as that of Johansson et al¹⁴ who are part of the European Academy of Allergy and Clinical Immunology. This revised nomenclature, based on known immune mechanisms of allergic diseases and hypersensitivity, aimed to classify the hypersensitivity reactions according to the presence of allergy, IgE production, and atopy. This classification gives a more universal definition of hypersensitivity, regardless of the targeted tissue or organ.

Genetics of allergy

Twin studies have demonstrated the strong heritable component of allergic diseases and atopy, estimated at 33%–76%.^{15,16} Twin studies with monozygous mice revealed that, even with the same controlled environment, phenotypic variability can

occur in allergic manifestations. These results demonstrated that environment explains about 30% of the phenotypic variability observed in allergy and that 70% is due to other factors, such as epigenetics.^{17,18}

However, search of the genes involved in allergy have been mostly done when studying genetic factors of allergic diseases (Table 1). Indeed, as mentioned by Hong et al,¹⁹ it is often difficult to evaluate the implication of genes on the development of allergy, using this method. However, Renkonen et al²⁰ have recently published a review of the genetics of allergic diseases and have found 39 genes associated with allergy.²⁰ In an effort to classify these genes according to their principal pathway, they concluded that 25 of them are involved in the same interaction pathway with another 70 proteins. Among all of these proteins, 20 are linked to the “host–virus interactions”. When looking to Gene Ontology categories, “response to stress” and “response to various stimuli” were also significantly enriched.²⁰ Another interesting finding of this study is the proportion of protein kinases in their results (23%). Indeed, those kinases may be involved in active allergen transport through intact epithelium.²⁰

To give a general idea of the genes identified by genetic studies on allergic diseases, here are some of the general pathways represented: the T helper type 2 (Th2) immune response and IgE switching cytokines and receptors (*IL4RA*, *IL5*, *IL13*, *FCER1A*, etc), the chemokines and chemokine receptors (*CX3CR1*, *CCXCR1*, *CCR2*, etc), the human leukocyte antigen (HLA; *HLA-DPB1*, *HLA-DQB1*, etc), and the lipoxigenase and cyclooxygenase pathways (*CYSLTR1*, *LTC4S*, etc).^{6,19–26} Table 1 shows the prevalence of allergy or some of the most studied allergic diseases and the number of genes already associated with each ones.

Asthma

One of the most studied allergic diseases is asthma. Asthmatic response is provoked by allergy in 75%–80% of all asthmatic cases.²⁹ According to the Global Initiative for Asthma,³⁰ asthma is defined as a chronic inflammatory disorder of the airways involving many cells and mediators. The principal associated symptoms are airway hyperresponsiveness and usually reversible airflow obstruction. These symptoms lead to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing.

Clinical manifestations of asthma often appear during childhood.³¹ However, some individuals show a late onset, sometimes after the age of 40 years.³² According to articles published between 1987 and 1997, the prevalence of asthma varied between 0.5% and 6% depending on the regions of the world.^{33–37} Approximately 10 years later, it is estimated that

300 million people suffer from asthma in the world and that this number will reach 400 million people by 2025.⁵ This represent a world prevalence of approximately 10% and a prevalence of 14.1% for Canada and of 10.9% for the United States.⁵

Physiopathology and immunology of asthma

Asthma is a complex trait that is influenced by several genes as well as by the environment, thus it presents heterogeneous clinical manifestations always recognized as different subphenotypes. Researchers agree that asthma is not a single disease but rather an array of disorders that share common characteristics.^{29,38,39} These characteristics are inflammation, intermittent bronchial obstruction, bronchial hyperreactivity, mucus hypersecretion, and hypertrophy and hyperplasia of smooth muscle.^{8,38}

As mentioned above, in 75%–80% of cases^{40,41} these phenotypes are caused by an allergic response, which triggers a Th2 immune response.²⁹ It is a type I hypersensitivity reaction, that is an immediate exaggerated or harmful immune reaction.^{8,42} Interestingly, only 7% of allergic people develop asthma,⁴³ which can lead us to believe that they present a unique phenotype that distinguishes them from other allergic, but nonasthmatic, individuals. The main immune cells involved in asthma are CD4⁺ T cells, mast cells, and eosinophils.⁴⁴ The different steps of the inflammatory response in asthma, from contact with the allergen to remodeling, are illustrated in Figure 2 and explained later.

Contact with the allergen

When an allergen penetrates the airways, if not expelled by the mucociliary barrier, it comes in contact with dendritic cells

Table 1 Prevalence and number of genes associated with allergy or most studied allergic diseases^{27–29}

Allergy/allergic disease	Prevalence	Number of associated genes	References
Allergy	54%	43	3, 20, 27
Allergic asthma	10%	More than 150	5, 22, 24
Allergic rhinitis	9%–42%	21	6
Atopic dermatitis	16% of children in industrialized countries	46	21, 23
Drug allergy (aspirin intolerance)	1%–2% of all admissions and 3%–5% of hospitalized patients	24	25, 26, 28
Food allergy	6% of children and up to 4% of adult	9	4, 19

which will internalize and then digest the allergen. Some dendritic cells then migrate towards the lymph nodes to present the antigen to naive T cells (Figure 2A).^{45,46} This presentation happens through the major histocompatibility complex type II conjugated to the CD80 and CD86 costimulation molecules.⁴⁷

The introduction of the antigen to naive T cells triggers the differentiation of CD4⁺ T cells into Th1 or Th2. This differentiation is modulated by the cytokines present during the introduction of the allergen, eg, interleukin (IL)-12 (Figure 2A).⁴⁸ Evidence shows, at least for mild to moderate asthma, that Th2 cells predominate.⁴⁹ Thus, after the differentiation of naive T cells into Th2 cells, the latter produce cytokines, including IL-4 which induces the production of IgE by B lymphocytes (Figure 2A).

Immediate response

When an individual has already been in contact with an allergen, its presence and the presence of IgE can activate mast cells and stimulate their degranulation just minutes after the contact with the allergen.^{2,8} The activation happens when the allergen binds with more than one IgE, which are linked to their high-affinity receptor (FcεRI; Figure 2B).^{9,10} Mast cells are key cells in immediate response. In fact, mast cell granules contain proinflammatory molecules such as histamine, tryptase and other proteases, tumor necrosis factor (TNF), and heparin.² New molecules are also produced and then released. These molecules are leukotrienes and prostaglandins, as well as cytokines, chemokines, and matrix metalloproteinases.^{2,50–53} The releasing of these mediators triggers the development of immediate response symptoms such as coughing, bronchial spasms, smooth muscle contraction, oedema, mucus secretion, and infiltration of immune cells.^{8,54} Mast cells, through these mediators, contribute to the recruitment and activation of immune cells in the lungs, such as eosinophils, T cells, macrophages, basophils, neutrophils, structure cells (fibroblasts, smooth muscle and epithelial cells), and other mast cells.^{52,55,56}

Basophils, with the presence of FcεRI receptors on their surface and the expression of Th2 cytokines, histamine and granules, can also play a role in immediate response. However, their role in asthma is less documented than that of mast cells.⁵⁷

Delayed response

Delayed, or late response, is not present in all asthmatics. It is mainly modulated by immune cells recruited by mast cells (Figure 2C).^{8,29} Among these cells, eosinophils are the main cells implicated in the development of this response.⁵⁸ Eosinophils produce Th2 cytokines,^{59–61} leukotrienes,^{62–64} and

proteins that cause damage to airway cells, such as major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase.^{65,66}

Chronic inflammation in airways

Chronic response in asthma is characterized by a persisting inflammation and the presence of structure alterations in the airways (Figure 2D).⁶⁷

Several immune cells play a role in this response. Mast cells participate in the chronic response through mediators that have an effect on bronchoconstriction and on airway remodeling causing contraction, hypertrophy and hyperplasia of smooth muscles as well as fibrogenesis.^{50–53,68,69} Dendritic cells that remain in the airways after coming in contact with the allergen repeatedly present the antigen to CD4⁺ T cells.⁷⁰ These cells, as well as the activated T cells, produce proinflammatory cytokines, maintain the chronicity of the inflammation and the eosinophilia.^{8,45,46,71} Eosinophils are indirectly involved in bronchial remodeling regarding collagen deposit, fibrogenesis, angiogenesis, and hyperplasia of smooth muscles in the peripheral airways via mediators secretion and other cells activation.^{58,72–74}

Structural cells also play an important role in maintaining the chronic response. The amount of mucous epithelial cells increases in asthmatics, thus participating in the bronchial obstruction, mainly in peripheral airways.^{75,76} Furthermore, epithelial cells, smooth muscle cells, and fibroblasts produce and/or store cytokines, chemokines, and other proinflammatory mediators.^{77–80} In asthmatics, fibroblasts also produce more collagen and other macromolecules for the extracellular matrix, and present an increased differentiation in myofibroblasts.^{81,82}

Phenotypic variability in asthma

To complete this overview of the asthmatic allergic response, the phenotypic heterogeneity has to be taken into consideration. Indeed, apart from allergic asthma, which is the most common form, other types of asthma have been described.^{8,38,83} One type is called nonallergic asthma, which develops independently from an allergic component.³² More research still has to be conducted to better define biological pathways specific to this form of asthma.^{84,85} However, as with allergic asthma, the scientific community recognizes that IgE could be implicated in an inflammatory cascade, as well as CD4⁺ and CD8⁺ T cells, eosinophils, and mast cells.^{86–91}

A second type of asthma is characterized by sensitivity to aspirin. It is estimated that approximately 10%–20% of adult asthmatics suffer from this particular type.⁹² Aspirin

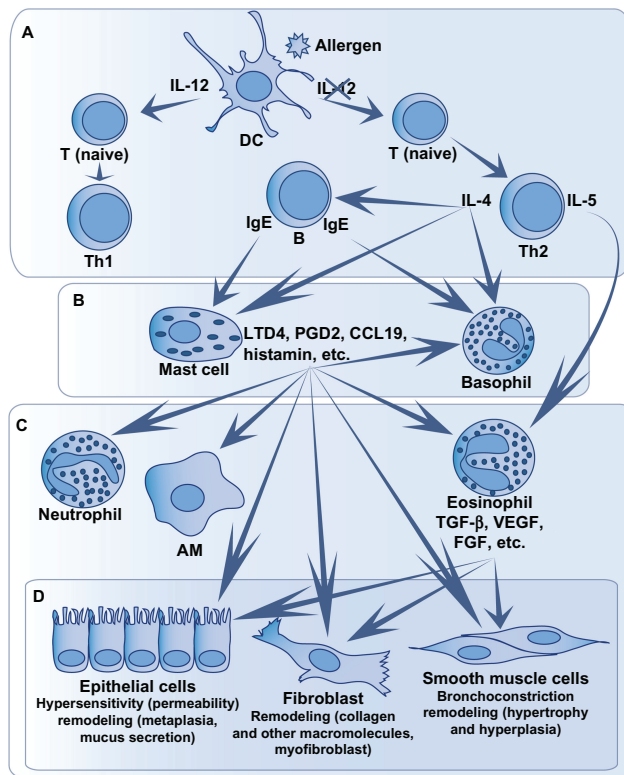


Figure 2 Immune response in asthma.

Notes: The immune response in asthma begins with (A) the contact between antigen-presenting cells (mostly dendritic cells [DC]) and the allergen. These cells stimulate naive T cell differentiation into T helper type I (Th1) or Th2 type. Interleukin (IL)-4 secretion by Th2 cell leads to the immunoglobulin (Ig) E production by B cells. IgE will bind to high-affinity receptors on mast cells and basophils and will allow immediate response to occur when another contact with the allergen will take place (B). After this new contact, mediators released by mast cells will induce recruitment and activation of other immune cell types as eosinophils, neutrophils, basophils, alveolar macrophages (AM), and structural cells (epithelial cells, smooth muscle cells, and fibroblasts). These cell types promote the delayed response that is not present in all asthmatic subjects (C). These cells not only promote the inflammatory response but also provoke hyperreactivity, bronchoconstriction, and remodeling. This will lead to asthma chronicity (D).

sensitization is thought to be non-IgE mediated so could be considered as a nonallergic subphenotype of asthma.⁹² A dysfunction of the eicosanoid metabolism is responsible for this type of asthma.^{92,93} A great number of individuals affected with this type of asthma will also resist a treatment with corticosteroids.³⁸

Asthmatics who do not respond to glucocorticoid treatments represent up to 10% of all patients affected with asthma.⁹⁴ This type of asthma can be induced by a diminution of the number of glucocorticoid receptors, by a misrecognition of the ligand by the receptors, by a decreased capacity of the receptors to link with DNA, or by an increase in the expression of certain proinflammatory transcription factors such as nuclear factor (NF)- κ B.⁹⁴

Occupational asthma is exacerbated or induced by irritants or particles present in the workplace of the affected.⁹⁵ It

is estimated that 9%–15% of all cases of asthma in adults are linked to the workplace and that up to 25% of new cases of adult asthma fall in the occupational asthma category.^{96,97}

Finally, exercise-induced asthma affects 7%–15% of the general population and 3%–14% of athletes.⁹⁸ A study indicates that up to 39% of college athletes show one of the main symptoms of asthma, that is bronchoconstriction.⁹⁹ The two major hypotheses that could explain the induction of asthma after an effort are related to the augmentation of ventilation in the lungs.^{100,101} These two hypotheses are the osmotic hypothesis (dehydration by evaporation caused by the increase in ventilation) and the thermal hypothesis (airway cooling during exercise and rewarming after exercise).^{101–103} According to Anderson and Daviskas,¹⁰¹ maybe a mix of these two hypotheses could better explain the phenotype rather than each one separately.

Asthma can also be classified according to inflammatory patterns. The three principal types are eosinophilic asthma, neutrophilic asthma, and paucigranulocytic asthma.³⁹ Paucigranulocytic asthma is defined as an asthma response without eosinophils or neutrophils.²⁹

Another way to classify asthma is according to the severity of the phenotype. This classification is used in the clinical treatment, but is also used in research in order to document the molecular and cellular biology variation related to the severity of the disease. Intermittent, mild, moderate, or severe asthma are part of this classification.¹⁰⁴ The definition criteria of these types of asthma include frequency of day and night symptoms, exacerbation, and maximum expiratory flow in one second (Table 2).³⁰

Changes in principal cells involved in the inflammatory response have been observed according to the severity of asthma. For example, inflammation is located in the main airways in individuals with mild asthma, whereas it is also present in peripheral airways and alveoli in individuals with severe asthma or during exacerbation.¹⁰⁵ It is also interesting to note that a recruitment of Th1 and CD8⁺ T cells (cytotoxic T cells) has also been observed in the case of severe and chronic asthma and during exacerbation.^{106–108} Thus, other cell types involved in the Th1 response or in immune response regulation, such as alveolar macrophages (they inactivate dendritic cells and they can be activated or inhibited through different pathways),^{109–111} could play a role in the inflammatory response observed in asthma.^{112,113}

Asthma genetics

Several studies demonstrated a strong familial structure in the prevalence of asthma.^{114–119} Studies conducted on twins on asthma and its subphenotypes (IgE, bronchial hyperreactivity,

etc) supported the idea of a genetic component in the development of asthma.¹¹⁶ Segregation analyses subsequently demonstrated that asthma does not follow a Mendelian transmission model. In fact, aggregation and segregation analyses have demonstrated that asthma is polygenic (several genes implicated), shows genetic heterogeneity (different combinations of genes appear in the families that are studied), and pleiotropy (some genes are implicated in the development of more than one phenotype). Moreover, environment plays also a role in the development of the phenotype.^{116,120} According to this, asthma is considered as a complex trait.

Many challenges appear in genetic research on asthma, such as (1) finding all the genes associated with asthma and with treatment responses (build a list of asthma genes), (2) defining the mechanisms that trigger the phenotypic heterogeneity observed in asthma (assess the contribution of each gene in the different asthma phenotypes), (3) understanding the biology of the mutated genes in the etiology of the disease (functional role of the associated genes), (4) understanding how the gene–gene and gene–environment interactions work (develop biological and mathematical analysis tools),¹²¹ and (5) defining the impact of the epigenetic modulations on the development of asthma.¹²⁰

The ultimate goal beyond these challenges is to define principal pathways involved in asthma and also pathways specifically involved in asthma subphenotypes to find targets for new treatments.¹²² According to the great phenotypic variability observed in asthma, a more precise description of pathways involved in asthma subphenotypes would increase our chance to develop more efficient treatments for people who do not respond to actual therapy. An ideal therapy should be specific enough to be taken orally without affecting the immune response in the whole body.¹²² Actually, potential targets identified (for example, mediator antagonists and inhibitors of cytokines, p38 MAP kinase inhibitors, and anti-inflammatory cytokines) are maybe too specific to be very effective.¹²² In the search for genes and pathways involved in asthma, association studies between genes or the entire genome and drug response phenotypes could also lead to pharmacogenetic therapies.¹²³ Indeed, characterizing the genetic profile of an asthmatic patient through genetic tests targeting specific genes of interest could help to select the appropriate treatment or to predict asthma development^{123,124} and allow improved prevention programs.

Genes associated with asthma

Several reviews have described the knowledge acquired on the genetics of asthma.^{22,24,121,125} Figure 3 classified the main genes associated with asthma according to their biological

function into three principal categories: inflammation, remodeling, and other pathways. Most of the associated genes could be classified into the inflammation (32/61; 52%) or remodeling categories (12/61; 20%). Indeed, this classification not only underlines the great proportion of associated genes in the inflammatory response, but also underlines the need to better define the biological function for some of the other genes (other pathways; 17/61, 28%) to better understand their implication in asthma. The complete list of genes for all categories seen in Figure 3 is available in Table 3 with ontology keywords.

Table 4 presents the same 61 genes classified according to the approach used to target them. As shown in this table, the main approach that has been used in the research of genetic determinants of asthma is the candidate gene approach. At the end of 2005, more than 100 genes had already been associated to asthma and another 54 associations have been performed in 2006 and 2007.^{22,24} Candidate gene association studies are based on the hypothesis of an association between the variants of a gene and asthma or one of its associated phenotypes. These phenotypes usually include respiratory capacity (wheezing, bronchial hyperreactivity, and pulmonary function measurements), immunological measures (total or specific blood IgE, allergy) or clinical criteria (atopic dermatitis, eczema, and rhinoconjunctivitis).¹²¹ This approach implies a literature review on genes involved in the development of the trait, or closely related to biological pathways linked with physiopathology of the trait. The choice of candidate genes can be based on several types of information: (1) gene functions, (2) documented associations with the trait in other populations or with closely related phenotypes, (3) difference of expression of the gene for the trait or a closely related phenotype, (4) at least one of these criteria documented for an animal model, or (5) association of another gene in the same biological pathway with the trait or with closely related phenotypes.^{22,127} Consequently, this approach is biased according to the literature already published for the specific trait and the possibility of discovering new potentially interesting genes could be limited.^{22,127} This limitation may be even more important considering that the underlying biology of the development of most complex traits remains to be defined.¹²⁷

In order to go past this candidate gene selection bias, several approaches have been described. One of these is to select the candidate genes according to their known functions and to crosscheck the results with data obtained from genome-wide linkage, association, or expression studies.¹²⁷ These genomic

approaches allow targeting of new genes without having to state a hypothesis regarding a specific gene or *locus* and, therefore, permit to increase the knowledge on the pathology of the trait.^{22,130}

Before going further on the contributions of these techniques on the genetics of asthma, here is a brief description of each of them. Linkage analysis and genome-wide association studies (GWAS) are two techniques used to identify chromosomal *loci* associated in a studied trait. In both cases, analyses are based on two principles: equal transmission of alleles and increase in recombination for increased distances between two markers.¹³¹ To identify those *loci*, the linkage analysis uses families to compare microsatellite markers transmission with phenotype transmission. Similarities between these two transmissions are translated into a statistical measure, the lod score. The lod score is greater when markers of a *locus* are more transmitted to sick children in studied families, and it is possible to hypothesize that genes near this *locus* are involved in the development of this disease.¹³² For GWAS, familial or case–control cohorts can be used.¹³³ Contrary to linkage analysis, different genetic variant types can be used for GWAS as coding single nucleotide polymorphisms (SNPs), tagging SNPs, or copy number variants. Figure 4 presents a brief description of the methodology used for this technique. Linkage analysis has been very efficient to target genes responsible of monogenic trait development but,^{132,134} in complex traits, one disadvantage of linkage analysis is its impossibility to detect *locus* with modest effect on phenotype compared with GWAS.^{22,135} GWAS also has the advantage of targeting more specific chromosomal regions (≤ 500 bp) because of the great number of genetic variants (more than 500,000 variants) represented on a microarray (for examples, see: <http://www.illumina.com/>).²² Moreover, new developments on genetics as HapMap international project, the development of new technologies as microarrays, and the possibility to

bring together many samples owned by different researchers opened the way to GWAS.^{121,136}

Genome-wide expression studies are another genomic approach that allow the identification of new genes and pathways involved in a target disease. Figure 4 presents the main technical steps to perform genome-wide expression studies using Affymetrix technology as example. These studies, as GWAS, used microarrays. The expression of more than 25,000 genes or transcripts can be measured in the same microarray (www.affymetrix.com). These are used to compare gene expression profiles of affected and nonaffected subjects, of treated and nontreated subjects, of measures for the same subjects at pretreatment and posttreatment, etc. Some advantages of genome-wide expression studies are accessibility of the microarray technology and analysis tools for researchers and clinicians, no sequencing step and possibility to study thousands of genes simultaneously.¹³⁷

About the contribution of these genome-wide techniques, the use of linkage studies has allowed targeting of genes involved in biological pathways that had not been studied in asthma before (Table 4). Several chromosomal regions have been associated with asthma using this method, but only a few have been replicated in several studies and populations (5q31–33, 6p21, 12q13–q24).^{22,138} GWAS and genome-wide expression studies are now also being used in order to target new genes or validate observations about already associated genes in asthma. Until now, more than 62 genome-wide expression studies have been performed on asthma or one of its subphenotypes (as found in PubMed using “asthma and microarray” and “asthma and gene chip” keywords and according to Rolph et al¹³⁹). Although most results obtained from expression microarrays are exploratory and must be validated through functional or association studies, a few genes have been targeted by these analyses (Table 4).¹²⁸ As for GWAS, only a few studies have been performed on asthma so far. The first was conducted during the summer of 2007

Table 2 The definition criteria used to classify asthma according to severity

Severity	Intermittent	Mild persistent	Moderate persistent	Severe persistent
Symptom frequency	Less than once a week	More than once per week but less than once per day	Daily	Daily
Nighttime symptoms	Less than or equal to twice per month	More than twice per month	More than once per week	Frequent
Exacerbations	Brief	Could affect activities and sleep	Could affect activities and sleep	Frequent
FEV ₁ percent of predicted value	$\geq 80\%$	$\geq 80\%$	60%–80%	$< 60\%$
Variability of FEV ₁	$< 20\%$	20%–30%	$> 30\%$	$> 30\%$

Note: Adapted from the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA).³⁰

Abbreviation: FEV₁, forced expiratory volume in the first second.

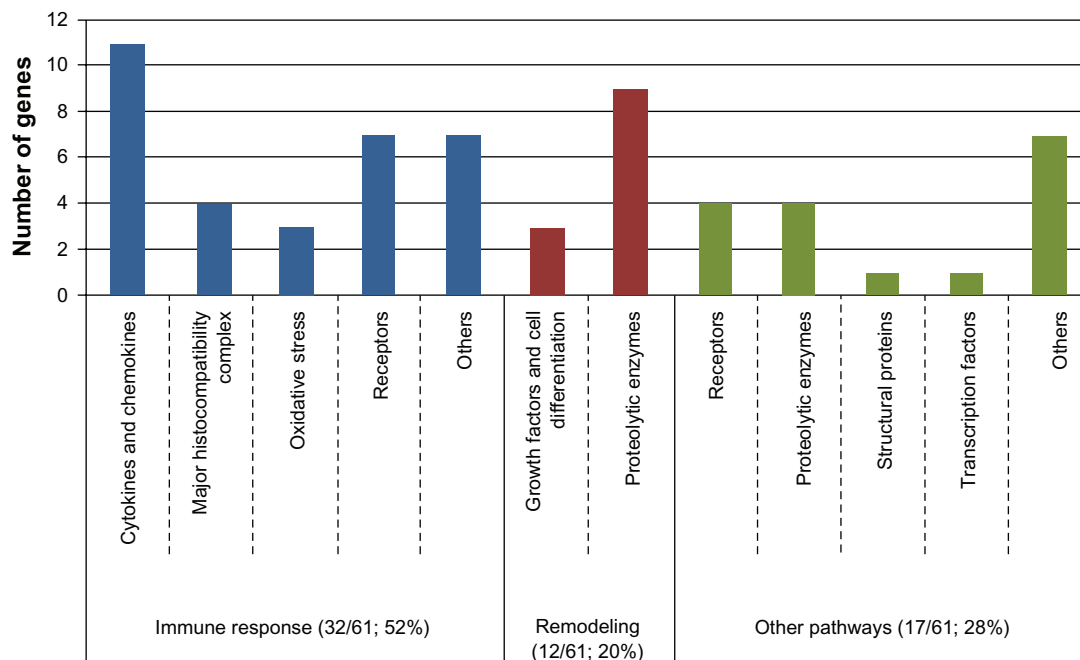


Figure 3 Classification of the 61 main associated genes with asthma into biological functions.

Notes: This figure illustrates the two levels of classification of the 61 main associated genes with asthma. The first level of classification is made according to their known principal functions. From these known functions, a possible implication in the immune response or the remodeling response has been deduced. It is interesting to note that more than half (52%) of the main associated genes are in direct link with the immune response and that 20% may be involved in the remodeling process observed in asthma lung tissues. For the 17 genes classified in the “other pathways” category, their principal known function should help us orientate future research to better document their role in asthma. The complete list of genes for all categories seen in Figure 3 is available in Table 3 with ontology keywords.

and allowed targeting of a chromosomal region that regulates the expression of the *ORMDL3*, *GSDMB*, and *ZPBP2* genes.^{140,141} However, GWAS approach is becoming more popular and many studies are underway in the field of asthma research. For example, to increase statistical power of the studies, researchers on asthma or other lung diseases group their samples together. Indeed, two major consortia have been made and will soon publish GWAS results. They are called GABRIEL Project (<http://www.gabriel-fp6.org/>) and EVE Asthma Genetics Consortium (http://arrafunding.uchicago.edu/investigators/ober_c.shtml). GABRIEL includes independent asthma genetics studies of European ancestry and EVE includes independent ones from the United States. The scientific community, including these two consortia and others, actually work to plane a meta-global GWAS analysis. The great number of data researchers obtain from these studies will allow them to target new genes and pathways involved in asthma development or its subphenotypes. These may become new therapeutic candidates or may be part of a predictive genetic profile.¹²⁴

Replication discrepancies

However, no matter what kind of approach is used, researchers are confronted with the same question: why is there an

absence of validation in several cases? These nonreplications can be linked to the statistical power of the samples used, or to other phenotypic, genetic, environmental, or epigenetic concerns.¹²¹

To increase the chance to find polymorphisms associated with asthma and also replicate anterior results, statistical power of the sample is important. Genetic association studies are more powerful than genomic studies regarding number of subjects to number of genetic variants ratio. Indeed, with the great number of genetic variants or genes tested, the multiple testing problems are much more important in GWAS and genome-wide expression studies.^{142,143} It is known that for a GWAS, a sample of approximately 15,000 individuals would be necessary to obtain a statistical power of approximately 90% with a type I error of 5%.¹⁴⁴ This objective can be reached by grouping together all asthma genetic research studies. For expression studies, analyses showed that at least three to five samples per phenotypic group could be sufficient to find a difference of expression, depending on the objective of the study (ie, to look at greater or smaller differences of expression).¹⁴⁵

Another cause of replication discrepancies is the great phenotypic heterogeneity observed in asthma, as described in the section “Phenotypic variability in

Table 3 Classification of the 61 main genes associated with asthma according to their respective ontology keywords

Categories	Gene symbol	Gene ID	Molecular function ^a	Biological process ^a
Inflammation				
Cytokines and chemokines				
	<i>CCL11</i>	6356	Chemokine	Cytokine and chemokine-mediated signaling pathway
	<i>CCL5</i>	6352	Chemokine	Cytokine and chemokine-mediated signaling pathway
	<i>CTLA4</i>	1493	Other cytokine	T-cell mediated immunity
	<i>IL18</i>	3606	Interleukin	Cytokine and chemokine-mediated signaling pathway
	<i>IL4</i>	3565	Interleukin	Cytokine and chemokine-mediated signaling pathway
	<i>IL13</i>	3596	Interleukin	Cytokine and chemokine-mediated signaling pathway
	<i>IL10</i>	3586	Interleukin	Cytokine and chemokine-mediated signaling pathway
	<i>IL4R</i>	3566	Interleukin receptor	Cytokine and chemokine-mediated signaling pathway
	<i>LTA</i>	4049	Tumor necrosis factor family member	Cytokine and chemokine-mediated signaling pathway
	<i>SCGB1A1 (CC16)</i>	7356	Other cytokine	Other immune and defense
	<i>TNF</i>	7124	Tumor necrosis factor family member	Cytokine and chemokine-mediated signaling pathway
Major histocompatibility complex				
	<i>HLA-DRB1</i>	3123	Major histocompatibility complex antigen	MHCII-mediated immunity
	<i>HLA-DQB1</i>	3119	Major histocompatibility complex antigen	MHCII-mediated immunity
	<i>HLA-DPB1</i>	3115	Major histocompatibility complex antigen	MHCII-mediated immunity
	<i>HLA-G</i>	3135	Major histocompatibility complex antigen	MHCII-mediated immunity
Oxidative stress				
	<i>GSTM1</i>	2944	Other transferase	Detoxification
	<i>GSTP1</i>	2950	Other transferase	Detoxification
	<i>GSTT1</i>	2952	Other transferase; epimerase/racemase	Detoxification
Receptors				
	<i>CD14</i>	929	Other receptor	Immunity and defense
	<i>CX3CR1</i>	1524	G-protein coupled receptor	Cytokine and chemokine-mediated signaling pathway
	<i>CYSLTR1</i>	10800	G-protein coupled receptor	Immunity and defense
	<i>HAVCR1</i>	26762	Immunoglobulin receptor family member; defense/immunity protein	Other receptor-mediated signaling pathway
	<i>IRAK3</i>	11213	Serine/threonine protein kinase receptor; protein kinase	Protein phosphorylation; other immune and defense
	<i>MS4A2 (FCER1B)</i>	2206	Immunoglobulin receptor family member; defense/immunity protein	B-cell mediated and antibody-mediated immunity; other immune and defense
	<i>TRG@^b</i>		T cell receptor gamma locus	B-cell mediated and antibody-mediated immunity; other immune and defense
Other pathways				
	<i>ALOX15</i>	246	Oxygenase	Other lipid, fatty acid and steroid metabolism; immunity and defense
	<i>CMA1</i>	1215	Serine protease	Proteolysis; granulocyte-mediated immunity
	<i>HSPD1^b</i>	3329	Chaperonin	Protein folding
	<i>LTC4S</i>	4056	Other transferase	Other lipid, fatty acid and steroid metabolism; T-cell mediated immunity; B-cell mediated and antibody-6 + mediated immunity
	<i>PRNP^b</i>	5621	Molecular function unclassified	Biological process unclassified
	<i>STAT6</i>	6778	Other transcription factor; nucleic acid binding	mRNA transcription regulation; JAK-STAT cascade
	<i>TBX21</i>	30009	Other transcription factor; nucleic acid binding	mRNA transcription regulation; T-cell mediated immunity

(Continued)

Table 3 (Continued)

Categories	Gene symbol	Gene ID	Molecular function ^a	Biological process ^a
Remodeling				
Growth factors and cell differentiation				
	<i>AREG</i>	374	Growth factor	Ligand-mediated signaling
	<i>GSDMB</i>	55876	Molecular function unclassified	Developmental processes
	<i>TGFB1</i>	7040	Growth factor	Other receptor-mediated signaling pathway
Proteolytic enzymes				
	<i>ACE</i>	1636	Metalloprotease	Proteolysis
	<i>ADAM33</i>	80332	Metalloprotease	
	<i>CHI3L1</i>	1116	Glycosidase	Other polysaccharide metabolism
	<i>chi3l3 (ym1)^b</i>	12655	Molecular function unclassified	Biological process unclassified
		(<i>Mus musculus</i>)		
	<i>NOS1</i>	4842	Synthase; oxidoreductase; calmodulin-related protein	Electron transport
	<i>PLAU</i>	5328	Serine protease	Proteolysis
	<i>SERPINE1 (PAI1)</i>	5054	Serine protease inhibitor	
	<i>SERPINH1</i>	871	Serine protease inhibitor	
	<i>SERPINB4</i>	6318	Serine protease inhibitor	
Other pathways				
Proteolytic enzymes				
	<i>ARG1</i>	383	Other hydrolase	Amino acid catabolism
	<i>ARG2</i>	384	Other hydrolase	
	<i>NAT2</i>	10	Acetyltransferase	Other metabolism
	<i>PDE4D</i>	5144	Phosphodiesterase	Metabolism of cyclic nucleotides
Receptors				
	<i>ADRB2</i>	154	G-protein coupled receptor	G-protein mediated signaling
	<i>NPSR1 (GPRA)</i>	387129	G-protein coupled receptor	
	<i>OPN3</i>	23596	G-protein coupled receptor	
	<i>TBXA2R</i>	6915	G-protein coupled receptor	G-protein mediated signaling
Structural proteins				
	<i>FLG</i>	2312	Other cytoskeletal proteins	Protein complex assembly
Transcription factors				
	<i>VDR</i>	7421	Nuclear hormone receptor; transcription factor	mRNA transcription regulation
Others				
	<i>COL29A1^b</i>	256076	Molecular function unclassified	Cell adhesion
	<i>CYFIP2</i>	26999	Other G-protein modulator	Signal transduction
	<i>DPP10</i>	57628	Select regulatory molecule	Protein targeting and localization
	<i>ORMDL3</i>	94103	Other miscellaneous function protein	Miscellaneous
	<i>PHF11</i>	51131	Double-stranded DNA binding protein	General mRNA transcription activities
	<i>POSTN</i>	10631	Other cell adhesion molecule	Cell adhesion
	<i>SPINK5</i>	11005	Select regulatory molecule	Homeostasis

Notes: ^aOntology keywords from the Panther Classification System (<http://www.pantherdb.org/>). ^bGenes not recognized by the Panther Classification System. Those have been classified according to description available in Entrez Gene by NCBI (<http://www.ncbi.nlm.nih.gov/gene>). See also Madore et al.¹²⁶

asthma". This phenotypic heterogeneity can reflect genotypic heterogeneity, thus implying that the severity and onset of asthma could be influenced by the asthmatics' genetic imprint.^{146,147} Thus, a precise characterization of the subjects remains essential. This precise phenotypic description of the sample helps to better select similar independent samples to replicate genetic association results and so helps to reduce the impact of differences in recruitment criteria and in population stratification

between samples.¹⁴⁶ According to the fact that asthma is rather a collection of several diseases than a unique trait, the key of replication success may lie in association studies with asthma subphenotypes. The review article written by Wenzel³⁹ presents several ways to categorize asthma subphenotypes that can be used for association and expression studies.

Gene–environment interactions are another source of variability since the environment can modulate the effect of

Table 4 List of the 61 main associated genes in genetic and genomic studies on asthma and classified according to their identification method

Genes identification methods				
Candidate genes ^a		Linkage analysis ^b	GWAS	Genomic expression study ^c
<i>ADRB2</i>	<i>HLA-DQB1</i>	<i>ADAM33</i> ^o	<i>CHI3L1</i>	<u>ALOX15</u>
<i>ACE</i>	<i>HLA-DRB1</i>	<i>COL29A1</i>	<u><i>GSDMB</i></u>	<i>AREG</i>
<i>SCGB1A1 (CC16)</i>	<i>IL4</i>	<i>CYFIP2</i>	<i>ORMDL3</i>	<i>ARG1</i>
<i>CCL5</i>	<u><i>IL4R</i></u>	<u><i>CYSLTR1</i></u>	<i>PDE4D</i>	<i>ARG2</i>
<i>CCL11</i>	<i>IL10</i>	<i>DPPI10</i>	<i>TCRγou</i>	<u>CX3CR1</u>
<i>CD14</i>	<i>IL13</i>	<i>HLA-G</i>	<i>TARP (BC072396)</i>	<u>HSPD1</u>
<i>CMA1</i>	<i>IL18</i>	<i>IRAKM</i>		<u>PLAU</u>
<i>CTLA4</i>	<i>LTA</i>	<i>NPSR1 (GPRA)</i> ^o		<i>POSTN</i>
<i>MS4A2 (FCER1B)</i>	<i>LTC4S</i>	<i>OPN3</i>		<u>PRNP</u>
<i>FLG</i>	<i>NAT2</i>	<i>PHF11</i>		<i>SERPINE1 (PAI-1)</i>
<i>GSTM1</i>	<i>NOS1</i>	<u><i>VDR</i></u>		<i>SERPINB4</i>
<i>GSTP1</i>	<i>SPINK5</i>			<u>SERPINH1</u>
<i>GSTT1</i>	<i>STAT6</i>			<i>Yml (Chi313)</i>
<i>HAVCR1</i>	<u><i>TBX21</i></u>			
<i>HLA-DPB1</i>	<i>TBXA2R</i>			
	<i>TGFB1</i>			
	<i>TNF</i>			

Abbreviation: GWAS, genome-wide association study.

Notes: ^aAssociated genes listed are those that have been replicated in almost five different studies according to Vercelli.¹²¹ Underlined genes are those that have been associated in more than 30 different studies.¹²¹ ^bAssociated genes are those targeted by linkage analysis according to Ober and Hoffman²² and Moffatt.¹²⁵ ^cPrincipal genes identified using genomic expression studies in asthma according to Izuhara and Saito.¹²⁸ Genes in bold are those identified by Laprise et al¹²⁹ and were positively associated with asthma. Genes in bold and underlined are those identified by Madore et al¹²⁶ and that were validated by semi-quantitative real-time polymerase chain reaction (PCR). Double-underlined genes are newly five (or more) times associated genes in the 2009–2010 periods (keywords used in PubMed: gene association study).

a gene on the resulting phenotype.¹⁴⁸ Studies showed that gene–environment interactions had a greater effect on the phenotype than the environment and genes separately.¹⁴⁹ For example, some studies have demonstrated that a high exposure to endotoxin seems to switch the susceptibility effect of the *CD14*–159C allele to a protective effect compared with the *CD14*–159T allele.^{150–152} Another example of gene–environment interaction is the interaction between polymorphisms in the regulating regions of a gene and the cell differentiation cycle, leading to differences on the expression of a specific allele.¹⁵³

A part of the etiology of asthma could be explained by epigenetic changes. Epigenetics is the study of changes that are heritable and that modulate gene expression without directly altering gene sequence.¹²⁰ Those changes can be done through DNA methylation or posttranslational modification of histones: eg, acetylation, methylation, phosphorylation, and ubiquitylation.^{154,155} Studies have demonstrated that epigenetic changes are heritable for almost two subsequent generations (eg, methylation of a coat-color gene in mice).¹⁵⁶ Many studies in asthma underline the possible role of epigenetics in its development mostly for early onset asthma (for a review, see Miller and Ho¹²⁰). For example, exposition to environmental tobacco smoke during prenatal development or during the first years of life could influence

the development of asthma and may be transmitted across two generations.^{157–159}

Gene–gene interactions are another source of variability. In complex traits, a single mutation often has a minor impact, but the combination of several mutations should increase the influences on a phenotype. In asthma, a synergy between a mutation in the *IL4* (rs2243250), the *IL13* (rs1800925), the *IL4R* (rs1805010) and in the signal transducer and activator of transcription 6 (*STAT6*) (rs324011) genes was documented and increases by 10.8 times the risk of having a high level of IgE and by 16.8 times the risk of developing asthma.¹⁶⁰

The solution to these replication problems might partly lie in new genomic methods. In fact, new developments in the field of genetic and genomic research (for example, microarrays for GWAS and genome-wide expression studies), as well as knowledge acquired on the genome and its polymorphisms (as the HapMap project) allow the implementation of new analysis tools. Indeed, although there is still no consensus regarding the analysis methods that should be employed, new tools have been developed to overcome these problems. For example, many new software packages are developed or optimized regularly in Bioconductor, an open source software for the analysis

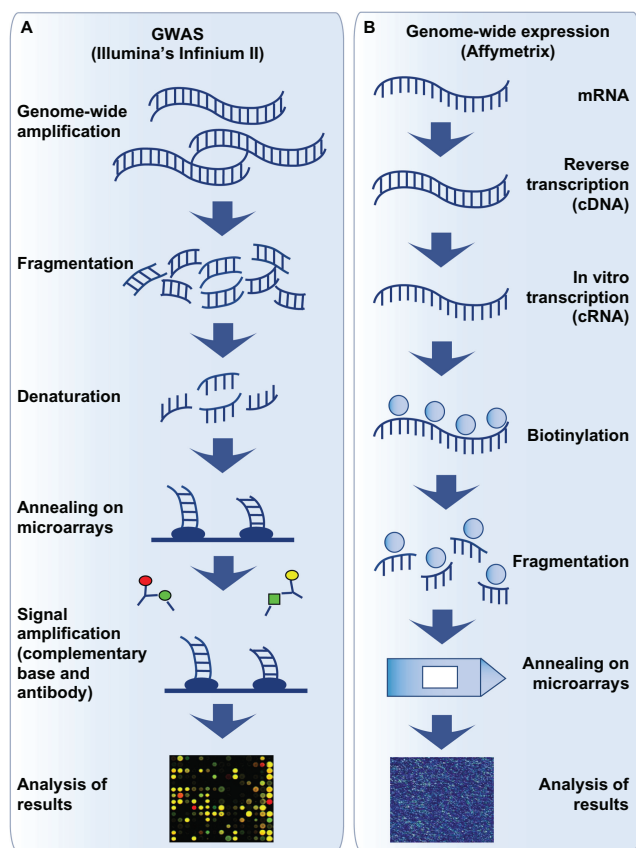


Figure 4 Illustration of the methodological steps for genome-wide association studies and genome-wide expression studies.

Notes: Part A of this figure illustrates methodological steps of genome-wide association studies (GWAS) done using Illumina technology. Briefly, the first step is the amplification of the complete genome. Second, amplified DNA is fragmented using digestion enzymes. Denaturalized fragments are then hybridized to short oligonucleotide sequences on microarrays. Finally, amplification with a fluorescent complementary base pair will allow reading and analyzing the results (Illumina <http://www.illumina.com/pages.ilmn?ID=40>). Part B of the figure illustrates methodological steps of genome-wide expression study performed using Affymetrix technology. The first step is the reverse transcription of mRNA into cDNA, followed by transcription of cDNA into cRNA. Afterward, cRNA is biotinylated, fragmented, and annealed with primers synthesized on the microarray. The signal intensity is proportional to the number of copies present in the sample.

and comprehension of genomic data, to better analyze and characterize data obtained from genome-wide studies (www.bioconductor.org). New genome-wide analysis tools are promising avenues to find new biological pathways, gene families or chromosomal *loci* involved in asthma and complex diseases, but candidate gene approach will remain a useful and powerful tool to refine our search for associated genes and to help better define the pathways involved in asthma development.

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Disclosure

The authors report no conflicts of interest in this work.

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