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Genetics of asthma and atopy

Koppelman, Gerard Henk

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Chapter 1 | The genetics of asthma

(Asthma, 4th edition. London: Arnold, 2000: 146-174)

Gerard H. Koppelman[1,2], Gerda G. Meijer[1,2], Eugene R. Bleecker[3] and Dirkje S. Postma[2]

1. Department of Pulmonary Rehabilitation, Beatrixoord Rehabilitation Centre, Haren, the Netherlands
2. Department of Pulmonology, University Hospital Groningen, the Netherlands
3. Center for the Genetics of Asthma and Complex Diseases, University of Maryland School of Medicine, Baltimore, USA

1. Introduction

Asthma and allergies have long been recognized to have a familial basis. In a paper published in 1916, Cooke and Vanderveer studied family histories of 504 patients with allergy and concluded "that inheritance is a definite factor in human sensitisation".[1] However, the exact mechanisms that underlie this familial basis remained unknown. This situation has changed in the last two decades since new tools in molecular biology and genetic epidemiology have become available to facilitate genetic studies. The cystic fibrosis gene, discovered in 1989, follows Mendel's laws for single gene transmission. Mendelian genes show recessive or dominant patterns of inheritance in families. A current challenge is the genetic dissection of traits and diseases that do not show these Mendelian patterns. Such traits and diseases are called genetic complex diseases, which are influenced both by genes and environmental factors.[2] Examples of genetic complex diseases are multiple sclerosis, diabetes and asthma.

In general, genetic complex diseases show no simple relation of genotype and phenotype. This may be due to different genes causing the same phenotype (genetic heterogeneity) or the same genotype resulting in different phenotypes (pleiotropy). Some individuals with the mutated gene may not express the phenotype (incomplete penetrance), whereas others without the gene do show the specific phenotype (phenocopy). Furthermore, it is likely that in asthma some traits may require the presence of mutations in different genes at the same time (polygenic inheritance). These genes in turn can have different gene-gene and gene-environment interactions. Thus, mutated genes in genetic complex diseases can be regarded as risk factors for a disease comparable with other generally recognized risk factors for the development of diseases. This can be illustrated with the example of airway responsiveness. Hypothetically, genetic regulation of airway responsiveness may be influenced by two major genes. Different variants of these genes may lead to susceptibility for airway hyperresponsiveness. Environmental factors, such as smoking and allergen exposure may act as exogenous factors, resulting in airways hyperresponsiveness in susceptible individuals by different gene-environmental interactions.
The purpose of this chapter is to review the definition of asthma in genetic studies, the genetic basis of asthma and the current evidence on the localization of asthma susceptibility genes. A glossary of some genetic terms is listed in table 1.

Table 1. Explanations of genetic terms

Affected relative pair:	A set of individuals related by blood, each of whom is affected with the trait in question. The most common types of affected relative pairs include affected sibling pairs, affected cousins and affected avuncular pairs.
Affected sibling pair:	See: affected relative pair.
Allele:	Alternative variant of a gene or marker due to changes at the DNA level.
Ascertainment:	The selection of individuals for inclusion in a genetic study.
Autosome:	In humans any chromosome other than the sex chromosomes.
Candidate gene:	A gene that has been implicated in causing or contributing to the development of a particular disease.
CentiMorgan:	A measure of genetic distance, equivalent to 1% recombination.
Chromosome:	Macromolecular complex of DNA and protein. Humans have 46 chromosomes (23 pairs).
Codon:	A triplet of three bases in a DNA and RNA molecule, specifying a single amino acid.
Concordant:	A pair of relatives, mostly twins, in which both members exhibit the same phenotype or trait.
Complex trait:	A trait which has a genetic component, that is not inherited in a strictly Mendelian fashion (dominant, recessive or sex-linked).
Crossing-over:	Reciprocal breaking and rejoining of homologous chromosomes in meiosis that results in exchange of chromosomal segments.
Discordant:	A pair of relatives, mostly twins, in which both members exhibit different phenotypes or traits.
DNA:	Desoxyribonucleic acid, the molecule that encodes the genetic information in virtually all organisms.
DNA marker:	A cloned chromosomal locus with allelic variation that can be followed directly by a DNA based assay such as polymerase chain reaction.
Epistasis:	Two or more genes interacting with each other in a multiplicative fashion
Exon:	The portion of the genome that is expressed as processed mRNA.
Gamete:	Any mature germ cell.
Gene:	An individual unit of heredity. It is a specific instruction that directs the synthesis of a RNA product.
Genome:	The sum of all genetic information of an organism.
Genotype:	The observed alleles at a genetic locus for an individual.
Haploid:	The chromosome number of a normal gamete. In a gamete, only one of the two chromosomes of a chromosome pair is present.
Haplotype:	The linear, ordered arrangement of alleles on a chromosome.
Heterozygote:	A diploid organism with two distinguishable alleles at a particular locus.
Homozygote:	A diploid organism with two identical alleles at a particular locus.
Identity-by-descent:	Two alleles are identical by descent when it can be determined that they have been inherited from a common ancestor.
Imprinting:	A phenomenon in which the phenotype depends on which parent passed the disease gene.
Intron:	The non-coding regions of genes. The introns are spliced out of the mRNA following transcription.
Linkage:	Co-inheritance of two or more loci because of close proximity on the same chromosome, so that after meiosis they remain associated more often than the 50 % expected for unlinked loci.
Linkage disequilibrium	The preferential association of a particular allele, for example, a mutant allele for a disease with a specific allele at a nearby locus.
LOD score:	A statistical method that tests whether a set of linkage data indicates two loci are unlinked or linked. The LOD score is the base 10 logarithm of the odds favouring linkage.
Mapping:	The process of determining the position of a locus on the chromosome relative to other loci.
Marker:	See: DNA marker.
Meiosis:	The specialized form of a cell division that creates germ cells
Microsatellite:	A class of DNA polymorphisms arising from a short base-pair sequence that is tandemly repeated a variable number of times; microsatellites are used as genetic markers in linkage analysis.

Explanations of genetic terms (continued)

mRNA:	Messenger RNA, a type of RNA molecule that carries the information copied from a gene and serves as a template for the production of proteins.
Multifactorial:	A trait is considered to be multifactorial in origin when two or more genes, together with an environmental effect, work together to lead to a phenotype.
Mutation:	A change, deletion, or rearrangement of the DNA sequence.
Nucleotide:	The building block of RNA and DNA.
Oligogenic:	A few genes work together to produce the phenotype. Contrasted to polygenic, which implies that many genes are involved.
PCR:	Polymerase chain reaction, a technique for amplifying short stretches of DNA.
Penetrance:	The probability of expressing a phenotype given a genotype.
Phenocopy:	A trait which appears to be identical to a genetic trait, but which is caused by non-genetic factors.
Phenotype:	The observed manifestation of a genotype.
Polymorphism:	Loci at which there are two or more alleles that are each present at a frequency of at least 1% in the population.
Power:	The probability of correctly rejecting the null hypothesis.
Proband:	An individual, through which a family is ascertained for a genetic study, mostly an affected individual.
Recombination:	The formation of a new combination of genes during meiosis.
Restriction enzymes:	A group of enzymes isolated from bacteria that cut DNA molecules at specific sites characterized by specific nucleotide sequences.
RNA:	Ribonucleic acid, a ribonucleotide polymer into which DNA is transcribed.
Segregation analysis:	A method of genetic analysis that tests whether an observed pattern of phenotypes in families is compatible with an explicit model of inheritance.
Sequencing:	The process of determining the order of nucleotides in a nucleic acid or amino acids in a protein.

2. The definition of asthma in genetic studies

Asthma is a respiratory disease characterised by variable airway obstruction, airway inflammation and airway hyperresponsiveness. A major issue in genetic studies is how to define the asthma phenotype.[3] Ideally, this definition would separate 'true' asthma from other lung diseases such as chronic obstructive pulmonary disease (COPD) or healthy status. An accurate definition of asthma in genetic studies is important, as misclassification of individuals reduces the power of genetic studies to a great extent.

In defining the asthma phenotype for genetic studies, one has to recognize the marked clinical heterogeneity of the disease, with regard to its age of onset and variations in symptoms over time, its severity and the association of asthma and atopy. Furthermore, an overlap may exist between asthma and COPD. There are some clear differences between asthma and COPD, such as age of onset (asthma mainly in childhood and adolescence, COPD in older age). An example of a similarity is AH, a central feature of asthma, which can be detected in the majority of patients with COPD. Furthermore, both diseases are characterised by airway obstruction. Airway obstruction is reversible spontaneously or after the use of $ß_2$-agonists in

most patients with asthma, but not all. In contrast, airway obstruction is not reversible in most patients with COPD. However, in a 25 years follow-up study of adult asthmatic patients about 30% of these patients developed irreversible airway obstruction.[4] These patients do have asthma with irreversible airways obstruction, but without any clinical history one could easily diagnose them as patients with COPD. This example illustrates that the classification of patients with obstructive airways disease is not always easily made.

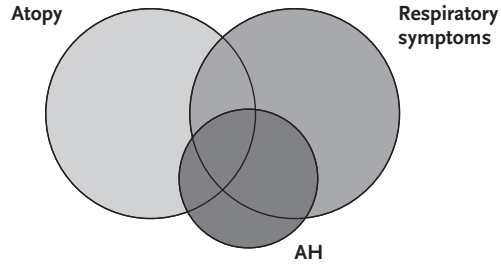
Possible approaches in defining the asthma phenotype

In genetic studies the asthma phenotype can be studied by a questionnaire that assesses self-reported wheeze or asthma, or a doctor's diagnosis of asthma. A clear advantage is that this is an easy and feasible approach which can be used in large scale studies. However, a disadvantage is that self-reported wheeze has a high population frequency and may overestimate the asthma prevalence, as some children wheeze during the course of a viral upper airway infection, but do not develop asthma.[5] Furthermore, wheeze is also present in a considerable proportion of patients with COPD. The use of a doctor's diagnosis of asthma or the use of asthma medication for classifying asthmatics has been criticized, because some evidence suggests that doctors are more likely to diagnose asthma in females, non-smokers and children who have a positive family history of allergy.[6] Furthermore, one may misclassify individuals with mild, intermittent disease because they do not attend a doctor. Thus, questionnaire-based approaches appear to have their limitations. Therefore, current studies are now directed at measuring clinical and objective characteristics, which constitute a marker of, or are associated with asthma. Examples of these traits are airway hyperresponsiveness, reversibility of airway obstruction after inhaling a ß-agonist, and markers of atopy such as total serum IgE or allergen-specific IgE levels, the number of blood eosinophils in peripheral blood and skin prick tests for common aeroallergens.

Airway hyperresponsiveness (AH) to inhaled bronchoconstrictor agents is a central feature in asthma, which can be detected in virtually all symptomatic patients. Longitudinal studies have indicated that AH is a risk factor for the development of asthma and respiratory symptoms. It can be studied by inhalation provocation either using a direct stimulus (histamine or methacholine) or indirect stimulus (adenosine-5-monophosphate, cold air, exercise or hypertonic saline).

Reversibility of airway obstruction after inhalation of a $ß_2$-agonist is often taken as a surrogate marker for AH in patients who cannot perform this test due to low lung function. However, a recent study showed that these two phenotypes are not interchangeable in the general population.[7] Variability of airway obstruction can also be assessed with serial peak expiratory flow (PEF) measurements. International guidelines have advised to use increased variability of PEF over the day as a diagnostic tool for asthma and to use it in clinical management for adjusting therapy. Although AH and variability of PEF are correlated, their different associations to allergy markers in the general popu-

Figure 1 Interrelation of airway hyperresponsiveness, atopy and respiratory symptoms



Venn diagram showing the interrelation of airway hyperresponsiveness (AH), atopy and respiratory symptoms in asthma, such as wheeze, dyspnea, cough and nocturnal asthma. This figure shows the interrelationship of these three phenotypes, which are often found together in individuals with asthma. However, these phenotypes can occur separately in individuals who do not have asthma

lation may indicate that they cannot be used interchangeably.[8] Furthermore, the genetic component of peak flow variability and reversibility has not been formally studied, and the value of this phenotype remains to be established.

Asthma has a close relation with atopy, especially in children and adolescents. Atopy can be defined as a prolonged increased production of IgE as a reaction on exposure to common antigens. Its clinical expression includes asthma, allergic rhinitis (hay fever) and atopic dermatitis (eczema). Atopy is reflected in elevated levels of serum total IgE, allergen-specific IgE levels and positive skin test to common allergens. Atopy is often accompanied by raised numbers of eosinophils in peripheral blood. The phenotypes of asthma and atopy are often interrelated (figure 1).[9,10] Therefore, in this chapter attention will be given to the complex asthma phenotype, as well as the distinct intermediate phenotypes of asthma, such as AH, reversibility, serum total IgE, allergen specific IgE, positive skin-prick tests and the total number of eosinophils in peripheral blood.

3. Asthma as a genetic disease

Asthma clusters in families. The risk that a first-degree family member of a patient with asthma will develop asthma has been calculated to be less than two to almost six times higher than the risk for individuals in the general population.[11,12,13] Both shared genes and shared environment could account for such an excess risk. Two approaches, twin studies and segregation analyses, can separate the relative contribution of genes and environment to a certain trait and are discussed below.

Twin studies

The main goal in the study of twins is to estimate the genetic and environmental contribution to a specific trait or disease. Similarities or differences are compared in monozygotic (MZ) and dizygotic (DZ) twins. Since MZ twins share 100 % of their genetic information and DZ twins 50 %, higher similarity in MZ co-twins is explained by their greater genetic similarity. The main assumptions of twin studies are: that the environment for both MZ and DZ twins is similar; that they are representative of the general population; and, in questionnaire-based studies, that self reported zygosity is correct.

The first large twin study published on asthma was a Swedish population-based study of 6996 twin pairs.[14] In this study MZ concordance for self-reported asthma was 19.0% and DZ concordance was 4.8%. This indicates that both genetic and environmental factors are important in asthma. The genetic influence is illustrated by the higher concordance in MZ twins compared to DZ twins. In contrast, environmental influences are evidenced by the finding that in genetic similar MZ twins, sometimes one of a twin pair has asthma and the other not. Since then, several other twin studies in different populations have confirmed this finding (table 2).[15-20] These twin studies provide strong evidence for the hereditary basis of asthma. Furthermore, from these twin studies one can conclude that both airway hyperresponsiveness and serum total IgE are under significant genetic control. Data of twin studies on the genetic regulation of allergen specific IgE and skin test sensitivity are, at the moment, scanty. The currently available evidence suggests that whereas the ability to produce IgE is regulated genetically, the specificity of the IgE response is governed mainly by environment (table 2).[15,21,22]

Segregation analysis

Segregation analysis tests the hypothesis that the aggregation of a trait in families is the result of the action of a major gene. It does not include molecular biological techniques or DNA analyses. This analysis compares the number of individuals with a certain trait under study in a family with the expected numbers using different genetic models of inheritance. Examples of these models are models with a Mendelian component (a dominant gene model, a recessive gene model), or non-Mendelian models such as a polygenic model (multiple genes with small effect), a mixed model (a single major gene on a polygenic background) or a non-genetic, environmental model (no evidence for genetic factors). The result of segregation analysis is the genetic model with the highest likelihood, i.e. the model that gives the best description of the segregation of the trait under study in the family data. From this model, one can estimate the mode of inheritance and parameters such as the penetrance, the heritability and allele frequencies.[23]

Segregation of the asthma phenotype has been studied in several large, questionnaire-based studies (table 3). The self-reported family history of 13 963 asthma patients participating in the European Community Respiratory Health Survey was analyzed with a complex segregation analysis. This study reveals further support for genetic regulation of asthma and provides evidence for a two-allele gene with codominant inheritance.[24] Four other

Table 2 Twin studies of asthma and asthma-associated phenotypes

Phenotype	First author, year of publication	Population	Number of twin pairs	MZ correlation+	MZ concordance#	DZ correlation+	DZ concordance#	Definition of phenotype / Comments
Asthma	Edfors-Lubs, 1971	Swedish	6996		0.19~		0.05~*	Questionnaire / population based study.
	Hopp, 1984	US	107		0.50~		0.33~	"History of asthma" by questionnaire.
	Duffy, 1990	Australian	3808	0.65		0.24*		Questionnaire.
	Nieminen, 1991	Finnish	13.888	0.43		0.25*		Hospitalization, medication or cause of death / population based study.
	Sarafino, 1995	US	94		0.59		0.24*	Questionnaire.
	Lichtenstein, 1997	Swedish	434 ♂ 456 ♀		0.62 ♂ 0.41 ♀		0.26 ♂* 0.18 ♀*	Questionnaire (ever wheezing with shortness of breath, wheezing without a cold, or parental reported asthma) / twins aged 7-9 years.
	Harris, 1997	Norwegian	2559		0.45		0.12*	Questionnaire / population based study of twins aged 18 - 25 years.
	Laitinen, 1998	Finnish	1713		0.42		0.17*	Questionnaire / population based study of twins aged 16 years.
AH	Hopp, 1984	US	107	0.67		0.34*		AH to methacholine.
Total IgE	Hopp, 1984	US	107	0.82		0.52*		
	Hanson, 1991	US apart	70	0.64		0.49*		Twins reared apart.
		US together	61	0.42		0.26		Twins reared together.
		Finnish	158	0.56		0.37*		
Specific IgE	Wütrich, 1981	German	50		0.60		0.23	At least one allergen RAST positive.
	Hanson, 1990	US apart	26		0.50		0	Specific IgE antibodies for Ambrosia artemisiifolia,
		US together	14		0.50		0.33	P. pratense and Alternaria teni were measured by RAST / apart: reared apart; together: reared together.
Skin test	Hopp, 1984	US	107	0.82		0.46*		Sum of positive intracutaneous (skin) tests.
	Hanson, 1990	US apart	39		0.55		0.50	≥1 Intracutaneous (skin) test with wheal size > 5mm
		US together	41		0.70		0.28	

* Statistically significant differences between monozygous (MZ) and dizygous (DZ) pairs. + Correlation: intrapair correlation.
# Concordance: probandwise concordance. ~ pairwise concordance. AH: airway hyperresponsiveness. Rast: radio allergo sorbent test

Table 3 Segregation analyses of asthma and asthma-associated phenotypes

Phenotype	First author, year	Number of families	Genetic model	Definition of phenotype / Comments
Asthma	Lawrence, 1994	131	Common genes of small effect	Questionnaire/ population based sample
	Holberg, 1996	906	Polygenic or oligogenic model, not a single two-allele gene	Questionnaire, physician diagnosed asthma
	ECRHSG, 1997	13.963	Two-allele gene with co-dominant inheritance could not be rejected	Questionnaire, family history of asthma was reported by proband
	Jenkins, 1997	7.394	Oligogenic model	Questionnaire/ population of school children
	Chen, 1998	309	Single locus explains a portion of wheeze that is related to respiratory allergy. Also contribution of environmental factors and/or polygenes	Questionnaire defined self-reported wheeze
AH	Townley, 1986	83	Environmental hypothesis rejected, no single autosomal locus	AH to methacholine, families with and without asthma
	Longo, 1987	40	Autosomal dominant pattern of inheritance	AH to carbachol, no formal segregation analysis performed
	Lawrence, 1994	131	Common dominant genes of small effect	AH to histamine, random population sample
Total IgE	Gerrard, 1978	173	Dominant model, dominant allele suppresses high levels of IgE	In Caucasian Americans
	Meyers, 1982	23	Mendelian co-dominant model	In US-Amish population not selected for allergy
	Hasstedt, 1983	5	No major gene, polygenic inheritance	Families selected through breast cancer probands
	Meyers, 1987	42	Mixed model with recessive inheritance of high IgE levels	In families not selected for allergic disease
	Martinez, 1994	291	Co-dominant inheritance of a major gene for high IgE levels	In hispanic and non-hispanic families
	Lawrence, 1994	131	Polygenic model	Random population sample
	Xu, 1995	92	Two locus recessive model with epistasis	Families ascertained through a proband with asthma
	Dizier, 1995	234	Recessive major gene controlling high IgE levels	Independent from specific response to allergens

ECRHS: European Community Respiratory Health Survey Group. AH: airway hyperresponsiveness

studies, each with less participants, have also shown the familial aggregation of asthma and wheeze. However, the segregation of asthma in these families was consistent with the action of multiple genes with a small effect.[25-28]

Few family studies on AH have been published. Longo *et al*. studied AH to carbachol in nonasthmatic parents of patients with asthma and a sample of healthy controls. Ten percent of the normal population showed AH, whereas 50% of the nonasthmatic parents of asthmatic children had AH. These different distributions indicate a familial clustering of AH.[29] Complex segregation analysis of AH was performed by Townley *et al*. in 83 families from the USA and by Lawrence *et al*. in 131 randomly selected families from the United Kingdom (table 3). These analyses illustrate the genetic contribution to AH, but no evidence for a single major gene for AH was found.[25,30,]

Segregation of serum total IgE has been studied most extensively (table 3). Firstly, segregation analyses of serum total IgE confirmed the results of twin studies indicating major genetic regulation of serum total IgE levels. Secondly, the mode of inheritance was assessed in several studies. These studies provide evidence for different genetic models. Using a single locus approach, best fitting models were models for a major Mendelian gene, either co-dominant,[31,32] recessive,[33] mixed model of recessive inheritance,[34] dominant[35] or, in two other studies, for polygenic inheritance.[25,36] Dizier *et al*. studied serum total IgE levels in 234 Australian nuclear families. Evidence for recessive inheritance of serum total IgE levels and significant residual familial correlations were found. However, these correlations were no longer significant when the presence of the specific immune response was accounted for in the analysis. This study suggested that regulation of serum total IgE is independent from the regulation of allergen specific IgE.[33] Xu *et al*. were the first to perform a two locus approach to the fit the serum total IgE data in 92 Dutch families ascertained through a proband with asthma. This resulted in a significantly better fit of the data than a one-locus model, thereby providing evidence for two unlinked loci regulating serum total IgE in these families. The first locus alone explained 50.6% of the variance of the level of serum total IgE, the second 19.0%. Considered jointly, the two loci account for 78.4% of the variability of serum total IgE levels in serum.[37]

To date, there are no data on the segregation of other asthma-associated phenotypes in families.

In summary, segregation analyses of asthma and airway hyperresponsiveness confirm their genetic background, but are not conclusive on the mode of inheritance and the number of genes involved. Evidence for a major gene regulating serum total IgE was provided by studies in different countries, and evidence for different genetic models was obtained. Several explanations may be given for these contradictory results. A first explanation may be the definition of the phenotype. The definition of asthma and BHR varies between studies (table 3). A second explanation may be the ascertainment of families for segregation studies. In families ascertained for asthma, estimates on allele fre-

quencies of alleles regulating serum total IgE may be higher than in families sampled randomly from the general population. A final explanation may be genetic heterogeneity. This means that in different populations, different genes act in the regulation of these phenotypes. To date, this cannot be investigated since the exact localisations of these genes are still unknown.

4. Finding genes for asthma

The human genome

The haploid human genome consists of approximately 3×10^9 basepairs. Generally, genetic distances are expressed in centiMorgans (cM), one cM corresponding to 1% recombination and approximately 1 000 000 base pairs on a physical map. One percent recombination means that a crossing-over between two loci occurs every one in hundred meioses. Roughly, every one cM contains 50 genes. DNA is organised into 22 pairs of autosomes and two sex-specific chromosomes. Each chromosome comprises two arms, the short arm denoted as 'p' and the long arm as 'q'. Every region of a chromosome has been assigned a number, for example for chromosome 1, the regions are called 1q21, 1q22, 1q23, etc.

The total number of genes is estimated as many as 65.000 to 80.000.[38] At a given place in the genome, called a locus, different variants are called alleles. Many genes have a number of alleles in the population and are therefore said to be polymorphic. The majority of the DNA is not coding for any biological product. In these non-coding regions, polymorphisms can also be detected. These polymorphisms are typically used as markers for genetic studies. Thus, in this respect the words 'markers' and 'polymorphisms' are used interchangeably. In general, two different strategies have been used to identify susceptibility genes for asthma and atopy. The first strategy is positional cloning, the second is the candidate gene approach.

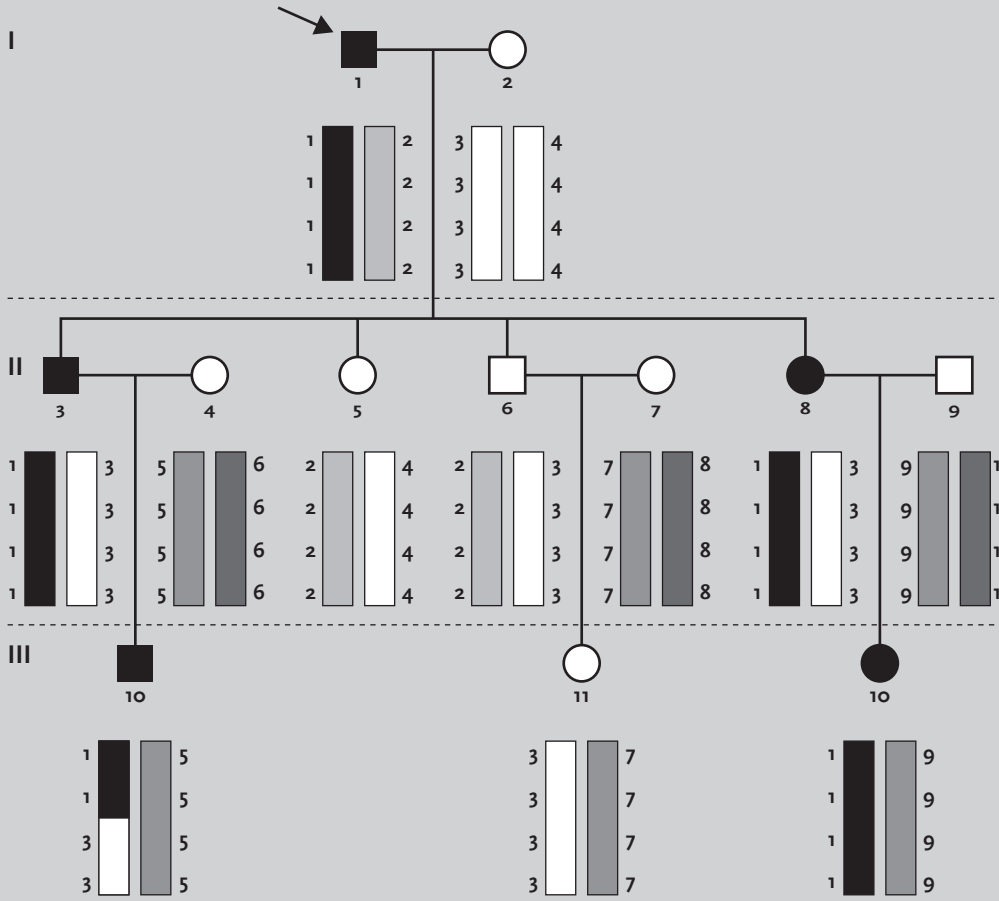
Positional cloning

The first step is to identify chromosomal regions of interest that may harbour disease genes by linkage analysis. The second step in positional cloning is to narrow down the region of interest as far as possible. Finally, in the last step the genes in this specified region are checked for mutations associated with the disease.

The principles of linkage analysis are shown in figure 2. Finding linkage is to determine a chromosomal region (sometimes millions of base pairs in length) which cosegregates with a certain trait within families. The likelihood that a trait cosegregates with a marker is expressed as a LOD score. The LOD score is the logarithm of the likelihood ratio of linkage versus no linkage. To study linkage using a LOD score approach, a model has to be specified for different genetic parameters such as mode of inheritance, penetrance, and allele frequencies. These parameters can sometimes be estimated from segregation analyses. However, given that in most studies these parameters are unknown, most investigators prefer non-parametric approaches.

A non-parametric approach is a method that does not need specification of a genetic model. Examples are affected sibling pair analysis and affected relative pair analysis. These non-parametric approaches test whether the inheritance of a chromosomal region is not consistent with random segregation. If this is the case, affected relatives inherit identical copies of alleles in this region more often than would be expected by chance.[2] The observed and expected distributions of alleles can be tested with a χ^2-test.

Figure 2 Pedigree of a family with asthma



A fictive family with asthma. Affected family members are shown as black boxes (males) or black circles (females); unaffected individuals are represented by the open boxes (males) and open circles (females). The proband is indicated by an arrow (individual 1). This family consists of three generations, as indicated by the roman capitals I, II, and III. The grandfather (individual 1), the oldest son (individual 3) and the youngest daughter (individual 8) and two grandchildren (individuals 10 and 12) are affected. On a chromosome, four subsequent markers are typed. The different alleles are coded by different numbers. In this example the trait asthma cosegregates with the haplotype of four markers with allele 1 (black chromosome). It is said that this trait is linked to the marker. Furthermore, a crossing over is observed in individual 10. From the fact, that this person is still affected, one can deduce that the gene causing this trait is located upstream of the third marker.

Candidate gene approach

Candidate genes can be detected in the process of positional cloning. In addition, investigators may choose a certain, known, gene as a plausible candidate gene for asthma. In general, candidate genes are tested with the use of association analysis in which alleles of candidate genes are tested using a case-control design. The frequency of an allele in a gene or a marker is compared between affected individuals and unaffected individuals. The finding of a positive association of an allele and a trait can be interpreted in three ways:[2]

1. the allele of interest is the relevant mutation in the disease gene;
2. the allele is in linkage disequilibrium, that means it is physically very close to the disease gene;
3. the association is a result of population admixture. This occurs if a certain trait has a higher prevalence in an ethnic subgroup within a mixed population. Any allele with a higher frequency within this subgroup will show association with the trait.

A method of testing for linkage and association is the transmission disequilibrium test (TDT).[39] Alleles of heterozygote parents are divided in transmitted and non-transmitted alleles, and the preferential transmission of a certain allele to an affected child is tested.

Results of linkage studies in asthma and atopy

The most frequently studied chromosomal regions that may harbour asthma and/or atopy susceptibility genes are chromosomes 11q, 5q and 12q (table 4).

Chromosome 11q

In 1989, Cookson *et al*. were the first to report linkage of atopy on chromosome 11q.[40] In this study, atopy was defined as one of either elevated serum total IgE, raised allergen specific IgE or the presence of one or more positive skin prick tests. Seven families were studied, whereas most of the LOD score was contributed by a single family using an autosomal dominant mode of inheritance. These authors replicated this finding in other samples, one of which was an Australian sample.[41-43] In addition, in other studies from the Netherlands, Germany, Japan and Australia, evidence for linkage was found between different asthmatic and/or atopic phenotype and markers on chromosome 11q. In a Dutch sample of 26 sib-pairs linkage was found between 11q and asthma and atopy defined as the presence of two respiratory symptoms and elevated specific or serum total IgE levels.[44] In a German study linkage was found between 11q and a clinical history of atopy and an elevated serum total IgE level[45] and in a Japanese study linkage was found between 11q and severe atopy (total serum IgE >400 IU/ml; three or more positive intradermal skin tests > 9mm or three or more positive RAST scores) in four selected families.[46] In an Australian study no linkage between chromosome 11q and atopy was

Table 4 Linkage analyses of asthma and airway hyperresponsiveness

Phenotype	Chromosome, + or – result	First author, year	Number	Genetic analysis	Definition of phenotype / Comments
Asthma	5q +	Noguchi, 1997	41 s	Sib-pair	Intermittent episodes of wheeze and dyspnea.
		CSGA, 1997	79 f	Affected relative pair	Two of three symptoms (cough, wheeze, dyspnea) and AH to methacholine or reversibility / Modest evidence of linkage.
		Ober, 1998	361 n	TDT, LR test	Bronchial hyperresponsiveness to methacholine and/or symptoms of asthma.
	5q -	Kamitani, 1997	45 s	Sib-pair	Wheeze or use of asthma medication in past year/ Random population sample.
		Laitinen, 1997	157 f	Affected relative pair, association	History of asthma, wheezing by auscultation, reversibility and/or AH.
	11q +	Herwerden, 1995	123 s	Sib-pair	Episode of asthma in the past 12 months, nocturnal shortness of breath or use of asthma medication.
	11q -	Noguchi, 1997	44 s	Sib-pair	Intermittent episodes of wheeze and dyspnea.
	12q +	Barnes, 1996	29 f	Sib-, relative pair, TDT	Reported history of asthma, confirmed by a doctor diagnosis.
		CSGA, 1997	79 f	Relative pair	Two of three symptoms (cough, wheeze, dyspnea) and AH to methacholine or reversibility after bronchodilator use.
		Ober, 1998	361 n	TDT, LR test	Bronchial hyperresponsiveness to methacholine and/or symptoms of asthma.
		Wilkinson, 1998	240 f	Sib-pair	Wheeze and asthma, defined as a quantitative asthma score
		Wjst, 1999	156 s	Sib-pair	Clinical history of asthma and ≥ 3 years of recurrent wheezing
AH	5q +	Postma, 1995	35 s	Sib-pair	AH to histamine / families ascertained through a proband with asthma.
	5q –	Doull, 1996	131 f	Association	AH to histamine / random population.
		Kamitani, 1997	51 s	Sib-pair	AH to methacholine / random population.
		Mansur, 1998	181 n	Association	AH to methacholine / random population, weak association.
	11q +	Doull, 1996	131 f	Association	AH to histamine / random population.
		Herwerden, 1995	123 s	Sib-pair	AH to methacholine / linkage even in absence of atopy.
	11q -	Lympany, 1992	9 f	LOD	AH to methacholine / in children aged 2-8 years exercise challenge test.
		Amelung, 1998	83 f	Sib-pair	AH to histamine / one marker at 11q had a modest significance level.

–: negative or not confirmative results; +: positive results.
F: number of families; s: number of sibpairs; n: number of individuals.
Sib-pair: affected sibling pair analysis. Association: association analysis. TDT: Transmission disequilibrium analysis; LOD - LOD score analysis.
LR test:likelihood ratio test, a semiparametric test for linkage. AH: airway hyperresponsiveness. CSGA: Collaborative Study on the Genetics of Asthma.

found. However, AH to methacholine appeared to be linked to 11q.[47] Linkage of chromosome 11q to atopy and asthma is still controversial due to multiple failures to replicate this finding in several other populations (table 4).[48-55] In 1992, Cookson *et al*. suggested that maternal inheritance of atopy may have obscured linkage in other studies. Excess sharing of maternal, not paternal alleles on chromosome 11q was shown in atopic children.[56] Possible explanations for maternal inheritance of atopy are paternal imprinting or maternal modification of the developing immune response. A candidate gene for atopy in this chromosomal region, the ß-chain of the high affinity IgE receptor, will be discussed in the next section of this chapter.[57]

Chromosome 5q

Chromosome 5q31-q33 contains numerous candidate genes for asthma and atopy, such as a cluster of cytokine genes (interleukine-3 (IL-3), IL-4, IL-5, IL-9, IL-13, the ß-chain of IL-12) and the genes coding for the $ß_2$-adrenergic receptor, CD-14, the corticosteroid receptor and the granulocyte-macrophage-colony stimulating factor.

In 1994, linkage between total serum IgE levels and chromosome 5q was first reported in a US Amish population.[58] This finding was replicated by Meyers *et al*. in the same year in a study of Dutch families who where ascertained through a proband with asthma.[59] In 1995, Postma *et al*. showed in the latter population that AH to histamine was linked between the same region of chromosome 5q as serum total IgE (figure 3). These findings indicated that a gene governing AH is located near a gene regulating serum total IgE.[60] Studies of asthma in Japan[54], the United Kingdom[61] and the USA[62,63] also implicated chromosome 5q as a region containing one or more susceptibility genes for asthma. However, in Australian,[64] Finnish,[65] British[66] and German populations[67] and in four US families,[68] chromosome 5q did not appear to be linked to asthma or atopy.

Table 3 Linkage analysis for total IgE and airway hyperresponsiveness (AH). Results of affected sib-pair analysis of chromosome 5q in a Dutch sample.

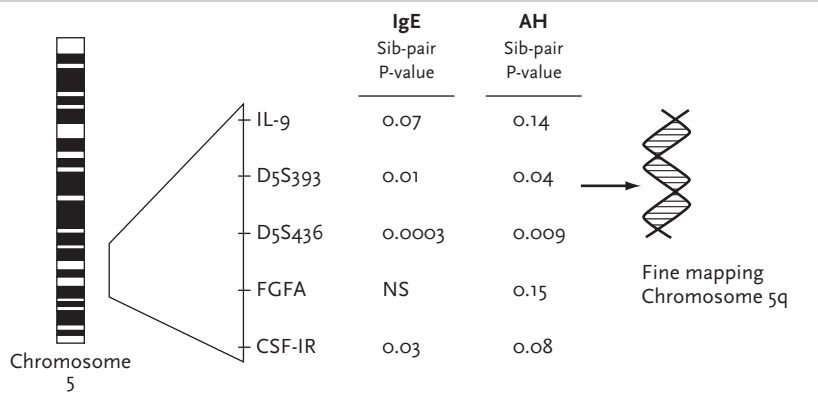


Table 5 Results of linkage analyses of atopy and total IgE

Phenotype	Chromosome, + or – result	First author, year	Number, type	Genetic analysis	Definition of phenotype / Comments
Atopy	5q +	Noguchi, 1997	71 s	Sib-pair	Total IgE >1 SD of the Japanese mean and/or elevated allergen specific IgE.
	5q -	Kamitani, 1997	103 s	Sib-pair	Skin prick test positivity to common aeroallergens.
		Laitinen, 1997	157 f	Affected relative pair, association	
	11q +	Cookson, 1989	7 f	LOD	Either of ≥1 positive skin prick test, elevated specific or total IgE.
		Cookson, 1992	70 f	Sib-pair	idem / Increased sharing of maternal, not paternal alleles.
		Young, 1992	64 f	LOD	≥1 positive skin prick test, elevated specific or total IgE.
		Collee, 1993	26 s	Sib-pair	≥ 2 respiratory symptoms and elevated total serum IgE or elevated specific IgE.
		Shirakawa,1994	4 f	LOD	Serum IgE > 400 IU/ml, ≥3 positive intradermal skin tests or ≥ 3 positive RAST scores.
		Daniels, 1996	80 f	Sib-pair	Sum of the skin-prick tests to grasses and house dust mite / Genome-wide search.
		Fölster, 1998	12 f	Affected relative pair, LOD	Clinical diagnosis of atopy and elevated total IgE levels / Families with atopic dermatitis, two- locus analysis with recessive-dominant model showed linkage in 2 of 12 families.
	11q -	Lympany, 1992	9 f	LOD	Positive skin prick test and or positive allergen specific IgE.
		Rich, 1992	3 f	Sib-pair, LOD	Either of ≥1 positive skin prick test, elevated specific or total IgE .
		Hizawa, 1992	4 f	LOD	Either of ≥1 positive skin prick test, elevated specific or total IgE, other definitions tested.
		Coleman, 1993	95 f	Sib-pair, LOD	idem / Family ascertainment through two first degree family members with atopic eczema.
		Brereton, 1994	12 f	Sib-pair, LOD	≥1 positive skin prick test.
		Martinati, 1996	45 f	Sib-pair	Either of ≥1 positive skin prick test, elevated specific or total IgE.
		Noguchi, 1997	70 s	Sib-pair	Total IgE >1 SD of the mean of the Japanese population and/or elevated allergen specific IgE.
		Amelung, 1998	83 f	Sib-pair, LOD	Elevated total IgE or number of positive skin tests.

Table 5 Results of linkage analyses of atopy and total IgE (continued)

Phenotype	Chromosome, + or – result	First author, year	Number, type	Genetic analysis	Definition of phenotype / Comments
Total IgE	5q +	Marsh, 1994	11 f	Sib pair, LOD	Eleven Amish extended families.
		Xu, 1995	92 f	Two locus LOD	First locus at 5q, second locus not mapped. (Also Meyers, 1994)
		Doull, 1996	131 f	Association	Random population sample.
		Noguchi, 1997	71 s	Sib-pair	Families ascertained through asthmatic children.
	5q -	Blumenthal,1996	4 f	Sib-pair, LOD	
		Ulbrecht, 1997	395 n	Association	Population sample.
		Mansur, 1998	181 n	Association	Population sample.
	11q +	Daniels, 1996	80 f	Sib-pair	Genome-wide search.
	11q -	Watson, 1995	131 f	LOD	Random selected families with a minimum of three children.
		Amelung, 1998	83 f	Two locus LOD	Families ascertained through a proband with asthma.
	12q +	Barnes, 1996	29 f	Sib-pair, TDT	Afro-Caribbean families ascertained through a proband with asthma.
		Barnes, 1996	24 f	Sib-pair, TDT	Amish families ascertained through one child with detectable allergen specific IgE.
		Nickel, 1997	52 n	TDT	German children ascertained from population study for high total IgE levels.

–: negative or not confirmative results; +: positive results.
f: number of families; s: number of sibpairs; n: number of individuals.
Sib-pair: affected sibling pair analysis. Association: association analysis. TDT: Transmission disequilibrium analysis; LOD - LOD score analysis.
SD: standard deviation

Chromosome 12q

Chromosome 12q is an interesting region for both asthma and atopy, because of several candidate genes, including interferon-γ (an inhibitor of IL-4 production by Th2 lymphocytes), a mast cell growth factor, and the ß subunit of nuclear factor-Y which possibly upregulates transcription of both IL-4 and the human leucocyte antigen class D-genes.

Barnes *et al*. studied individuals with a doctor diagnosed asthma and individuals with elevated total serum IgE levels in two different populations: an Afro-Caribbean population from Barbados and Caucasian Amish kindreds from Pennsylvania, USA. Evidence for linkage and association was found to this chromosomal region for both elevated total serum IgE (Barbados and Amish) and for doctor diagnosed asthma (Barbados).[69] Linkage of high serum IgE levels to 12q15-q24.1 was replicated in a German population sample of 52 children selected for high serum IgE levels.[70] Finally, evidence for linkage of asthma and 12q was shown in 240 families from the United Kingdom [71] and a study in the Hutterites in the USA.[63]

An interesting finding is that the chromosomal regions on 12q implicated in these studies are not exactly the same. Further studies are needed to fine-map this region. These studies will have to answer the question of whether one or more regions on 12q are implicated in asthma and atopy.

Other chromosomal regions of interest detected by genome-wide searches

To date, four genome-wide searches on asthma and atopy have been published. In genome-wide searches, the whole genome is scanned with markers spaced every 10 to 20 cM. The goal of a genome-wide search is to detect regions of interest for asthma and atopy using modest criteria of significance. These criteria could lead to the detection of new regions that contain susceptibility genes, as well as some regions, that could represent false-positive results. Therefore, these regions need to be followed up by additional mapping studies before a definitive conclusion can be drawn. The results from the four genome-wide searches on asthma and atopy will be discussed in this section.

In the first published genome-wide search in an Australian and British sample, evidence for linkage was found on chromosome 4 (AH), chromosome 6 (eosinophils), chromosome 7 (AH), chromosome 11 (skin tests, serum total IgE) and chromosome 16 (serum total IgE).[43]

The second genome-wide search was a US multicenter study in 140 asthma families ascertained through two or more affected siblings with asthma. Three different racial groups, namely Hispanics, Caucasians and Afro-Americans were studied.[62] An interesting result is that different regions appeared to be linked in these different racial groups. Regions of interest for asthma were chromosome 5p and 17p in African Americans; 11p and 19q in Caucasians and 2q and 21q in Hispanics.

A third genome-wide search was performed in the Hutterites. This is a religious sect that originated in Europe. In 1870, 900 members of this population moved to the USA. The current Hutterite population originates from

less than 90 ancestors, and is therefore a homogeneous population. In this study, asthma was defined as 'strict' asthma if the subjects showed AH to methacholine and reported asthma symptoms. Asthma was defined as 'loose' asthma if subjects had either AH to methacholine or reported asthma symptoms. Regions of interest for 'strict' asthma were chromosome 19q and 21q. In addition, regions of interest for 'loose' asthma were 5q and 12q. Finally, a region of interest for 'loose' asthma, not reported in other studies, was chromosome 3p. In conclusion, even in a homogeneous population such as the Hutterites, multiple susceptibility genes may influence asthma phenotypes.[63]

Finally, a fourth genome screen was performed in German families with asthma. Asthma was defined by clinical history and supported by questionnaire data of a history of at least 3 years of recurrent wheezing in children over age 3. For asthma, four possible linkages were reported at chromosome 2p, 6p, 9q and 12q. These linkage results for asthma were repeated with the study of intermediate phenotypes of asthma and atopy. For chromosome 2p, evidence for linkage was found for AR to methacholine, specific and total IgE; for chromosome 6p for total and specific IgE and eosinophils; for chromosome 9q for total and specific IgE and finally for chromosome 12q for specific IgE.[13]

Having reviewed the linkage results for asthma and atopy, some conclusions can be drawn. First, chromosome 5q, 11q and 12q are the most cited regions of interest for asthma and atopy. These findings of linkage are an important step towards the actual identification of susceptibility genes for asthma and atopy in these regions. Second, replication of linkages in other populations has proven to be difficult. Several possible reasons may explain this difficulty. One explanation may be that the definition of the phenotypes and genotypes under study are often different between studies (table 4). For example, atopy has been defined in one study as a positive history of atopic disease, in another study as a positive skin prick test and finally, in some other studies as a combination of elevated serum total IgE, allergen specific IgE or positive skin prick tests in different studies. Another explanation may be a high degree of genetic heterogeneity. This means that different genes are important in different populations; and each of these genes is sufficient to express the phenotype. In addition, several major and minor genes may interact in order to express the phenotype (oligogenic inheritance). One gene may be more prevalent in one population, whereas the second or third gene is more prevalent in other populations. Nevertheless, they provide the same phenotype. Yet another explanation may be that some of the published linkage results represent false-positive results. A final explanation is that some studies do not have a sufficient sample size to detect linkage, which may have led to false-negative results. Therefore, it is crucial that linkage results are replicated by different investigators in different populations of sufficient size. Thereafter, confirmed regions can be studied in detail and candidate genes can be detected.

5. Candidate genes for asthma

After determining linkage between asthma and a chromosomal region, the next challenge is to screen this region for candidate genes. A candidate gene for asthma has to meet four criteria:

(i) the gene product must be functionally relevant to asthma;
(ii) mutations within the gene must alter the function of the gene;
(iii) asthma needs to be linked to the chromosomal region harbouring the candidate gene and
(iv) asthma has to show association with different alleles of this candidate gene.

To date, a number of candidate genes for asthma and atopy have been studied. These include the gene encoding the $ß_2$-adrenergic receptor and genes from the cytokine cluster at chromosome 5q31-q33; the gene encoding the ß chain of the high affinity IgE receptor at chromosome 11q13, the gene encoding the interleukin 4 receptor α chain at chromosome 16p, and the major histocompability complex and the gene encoding interferon-γ at chromosome 6. Moreover, some other candidate genes for asthma and atopy will be discussed in this section.

The $ß_2$-adrenergic receptor

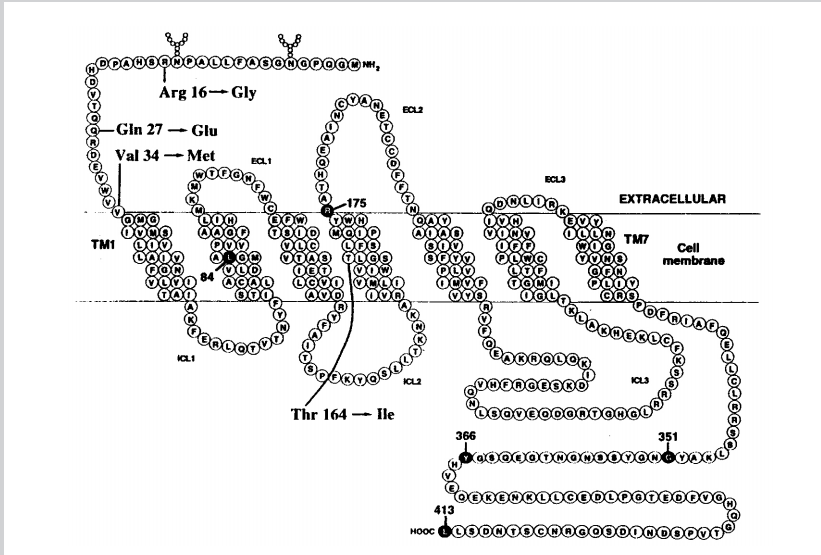
The $ß_2$-adrenergic receptor was hypothesised to play a role in the pathogenesis of asthma by Szentivanyi in 1968.[72] $ß_2$-Adrenergic receptors are localized in several human tissues and cells, including lung tissue (for instance, in airway smooth muscle and epithelium) and inflammatory cells (mast cells, macrophages, eosinophils and T-lymphocytes). $ß_2$-Adrenergic receptor function is mainly regulated by circulating epinephrine and mediates most of the effects of $ß_2$-agonists on airway function.[73]

The gene coding for the $ß_2$-adrenergic receptor is situated on chromosome 5q31. This receptor is a protein of 413 amino acids. In this gene, nine polymorphisms were identified, of which four lead to altered amino-acid sequences at positions 16, 27, 34 and 164 (figure 4). In most studies on polymorphisms of the $ß_2$-adrenergic receptor, none of these polymorphisms contributes to the risk of developing asthma. [74-76] Current evidence suggests that the polymorphisms at position 16 and 27 may play an important role in modifying the clinical severity of asthma.

Amino-acid 16 of the $ß_2$-adrenergic receptor can either be glycine (Gly) or arginine (Arg). The Gly-16 variant of the receptor might be associated with nocturnal asthma and with more severe asthma, as evidenced by the finding that patients with the Gly-16 variant were more likely to use corticosteroids and immunotherapy.[75,77] Furthermore, in a population study of children, individuals with the Gly-16 variant showed a decreased short-term bronchodilation after the use of a short-acting ß-agonist compared to individuals with the Arg-16 allele. The investigators suggest that the low response of the Gly-16 variant following ß-agonist stimulation could be a

reason for an increased use of inhaled corticosteroids.[76] The Gly-16 and Arg-16 variants of the ß$_2$-adrenergic receptor may also regulate receptor downregulation after long term exposure. Studies in airway smooth muscle cell cultures showed that long-term ß-agonists exposure downregulates the Gly 16 variant of the ß$_2$-adrenergic receptor to a greater extent than the Arg-16 variant.[78] Another study in patients with asthma showed a greater degree of agonist promoted receptor downregulation to be associated with the Gly 16 variant, not with the Arg 16 variant.[79]

Figure 4 Primary amino-acid sequence of the human ß$_2$-adrenergic receptor



Primary amino-acid sequence and proposed membrane topography of the human ß$_2$-adrenergic receptor. The darkened circles indicate codons where degenerate polymorphisms of the receptor gene were found. The four polymorphisms which result in changes in the amino-acid sequence are indicated.

The amino-acid 27 of the ß$_2$-adrenergic receptor can either be a glutamine (Gln) or a glutamate (Glu). The Gln-27 variant was associated with elevated levels of serum total IgE in a family study of 60 families with a proband with asthma. This variant was associated with more severe airway responsiveness compared to the Glu-27 variant as well.[74,80] The Glu-27 variant of the ß$_2$-adrenergic receptor showed an attenuated downregulation after the use of a long-acting ß-agonist in another study; however, this "protective" effect seemed less important than the effects of downregulation of the Gly-16 allele.[79]

In general, it is difficult to study these variants in human populations separately, since in most populations these alleles are in linkage disequilibrium. This means that the alleles at the 16 and 27 position of the ß$_2$-adrenergic

receptor are not distributed randomly in the population.
Since new polymorphisms have been recently detected in a regulatory region of the ß$_2$-adrenergic receptor, in the future more studies may be expected on the role of polymorphisms of this gene in asthma.[81]

The ß chain of the high affinity IgE receptor

The high affinity IgE receptor (FceRI) is composed of three subunits: one α, one ß and two γ-subunits. This αßγ$_2$-complex is found on the surface of mast cells, basophils, eosinophils and Langerhans cells. The binding of allergen to receptor-bound IgE on mast cells leads to activation and excretion of cytokines such as IL-4, thus upregulating IgE production by B-lymphocytes. The α-subunit is responsible for ligand binding and the γ dimer mediates for both the assembly of the receptor as well as signal transduction. The ß-subunit amplificates the signal strength mediated by the γ-subunit.[82]
Whereas the α and γ chains did not appear to be associated to asthma or atopy,[83] the ß-chain has received considerable interest.[57] The gene encoding the ß-chain is situated on chromosome 11q. As we have reported in the section on linkage, this chromosomal region was linked to asthma and/or atopy in several studies. In 1994, Shirakawa *et al*. reported that in a random British population sample an isoleucine (Ile) to leucine (Leu) change at position 181 in this protein was significantly associated with atopy if the Leu-181 variant had been inherited maternally. Of the 60 families of allergic asthmatic probands under study, this variant was detected in 10 families. In addition, at position 183 a valine (Val) to leucine (Leu) change was found.[84]
The combination of Leu-181/Leu-183 was found in 4.5 % of 1004 members of 230 two generation families in Western Australia. When inherited maternally, the Leu-181/Leu-183 variant was associated with atopy.[85] However, the Leu- 181/Leu-183 variants were not detected in other populations from Japan[86], the UK[87], Italy[53] and the Netherlands.[55]
Other polymorphisms in this gene result in two restriction sites for the restriction enzyme *Rsa* I. One of these variants was associated with atopic disease in a Japanese population[88] and atopic dermatitis in a British population[89]. However, in another Japanese study the association between these variants and atopy could not be confirmed.[90]
The most recent mutation reported is a substitution of Glu for Gly at amino acid 237 (E237G). The population frequency of this mutation is about 5% in Australian and Japanese populations. In two studies this mutation was strongly associated with asthma, and in the Australian study with AH as well.[91,86] In summary, the question remains if the Ile-181 and Ile-183 variants in the gene that codes for the ß-chain of the high affinity IgE receptor can account for the linkage reported by several groups, as it is detected in a subset of families or not detected at all in some populations. In addition, little is known on altered function of one of these variants in relation with atopy. It is therefore plausible, that other variants of this gene, such as the E237G variant, or other genes on chromosome 11q, are more important in atopy and atopic asthma.

The interleukin 4-receptor α chain

Both interleukins 4 and 13 and their receptors are candidate genes for asthma and atopy, given that IL-4 and IL-13 are central in the switch of B-cells to produce IgE and IL-4 stimulates the maturation of TH-0 to TH-2 type lymphocytes. The IL-4 receptor and the IL-13 receptor share the IL-4 receptor α-chain. The interleukin 4-receptor α gene resides at chromosome 16p. In a study from Germany, this chromosomal region was linked to markers of atopy. Only alleles inherited from the mother appeared to increase the risk on atopy in children.[92] The interleukin 4-receptor α chain has 13 known polymorphisms. Most data are available of one extracellular variant (Ile50Val), and two intracellular variants (Pro478Ser and Arg551Gln).[93] The Ile50 allele was associated with atopic asthma in one Japanese population, [94] but this could not be confirmed in another Japanese population.[95] The Arg551 allele was associated with high total serum IgE levels in a study of subjects with hyper-IgE syndrome and eczema.[96] In these studies, other polymorphisms of the IL-4 receptor α chain were not investigated. In a German population, the combination of Pro478 and the Arg551 allele was associated with lowered total serum IgE levels.[97] Arg551Gly and Pro478Ser are in linkage disequilibrium in most populations; therefore, the association of one allele with total IgE or asthma can not be studied separately. Given the multiple polymorphisms in this gene, and the contradictory association results, several groups have attempted to study the functional role of these polymorphims. First, from in vitro studies with transfected cell lines, Mitsuyashu and coworkers provided evidence that Ile50 allele, but not Arg551 allele, is involved in increased STAT-6 activation and proliferation and transcription of the IgE promoter by IL-4.[98] Second, from in-vivo immunoassays using T cells of individuals with different alleles of the Pro468Ser and the Arg551Gly, Kruse and coworkers suggested that the phosphorylation status of transcription factors IRS-1, IRS-2 and STAT6 was changed in the presence of these polymorphisms.[97] In conclusion, although the genetic associations and the available functional data indicate the importance of the IL-4R gene, more research is needed to clarify the role and the interaction of these polymorphisms in the regulation of IgE levels.

The human leucocyte antigen region

At chromosome 6p resides the human leucocyte antigen (HLA) region and the gene for tumor necrosis factor-α (TNF-α) as well. The HLA molecules are membrane-bound glycoproteins which bind processed antigenic peptides and present them to T-cells. Two HLA classes can be distinguished; class I is expressed on virtually every somatic cell; class II is merely expressed on B-cells, activated T-cells and monocytes/macrophages.

Polymorphisms in genes encoding the HLA class II molecules are associated with the specific IgE responses to several small allergens, such as ragweed pollen.[99] Other studies have failed to extend this finding to common major allergens, such as house dust mite.[100,101] Certain HLA class II alleles may be

important in susceptibility to isocyanate-induced asthma, the most common cause of industrial asthma.[102] In a collaborative US study, in Caucasian pairs of siblings with asthma, an increased sharing of alleles was found at chromosome 6p.[62] However, it is questionable whether the HLA region is implicated in asthma, considering that several other studies could not identify significant associations between asthma and the HLA region.[103,104] Thus, as atopy was present in over 75% of the US sample, the finding of linkage of asthma on 6p may, in fact, reflect the known association of the specific IgE response and the HLA region on 6p.

Tumor necrosis factor-α

TNF-α is a potent modulator of the immune inflammatory response and elevated levels can be detected in sputum and bronchoalveolar lavage fluid of patients with asthma during asthmatic attacks. Therefore, polymorphisms in this gene that may upregulate TNF-α production have been studied by Albuquerque *et al*.[105] In the promoter region of TNF-α on chromosome 6p, a polymorphism was detected at position 308 (G to A substitution), called the TNF1 allele. This polymorphisms could be associated with a six- to sevenfold upregulation in transcription of TNF-α. In a sample of 124 Australian schoolchildren, aged 6 - 12 years, this polymorphism resulted in a fivefold increased risk to asthma, defined as physician diagnosed asthma requiring the use of prophylactic medication. Moreover, all patients had positive skin prick to one or more common aeroallergens and a family history of asthma and/or atopic disease in first degree relatives.[105]
Within 7 cM of the TNF-α gene, a polymorphism in the first intron of the lymphotoxin-α gene (LTα*2 allele) showed a similar association. Furthermore, at chromosome 6p, the HLA region may also be involved in these atopic individuals. On the contrary, Moffat and Cookson found positive associations between asthma (questionnaire defined) and TNF2 and LTα*1 alleles.[106] This illustrates, that a positive association needs to be supported by linkage studies and functional studies, before definitive conclusions can be drawn regarding the role of these polymorphisms in the pathogenesis of asthma.

The cytokine gene cluster and other candidate genes

The cytokine gene cluster at chromosome 5q31-33 contains several pro-inflammatory cytokines (IL-3, IL-4, IL-9, IL-13), the glucocorticoid receptor, leukotriene C4 synthase (LTC4 syntase), CD-14 and several other candidate genes. Much interest has been given to cytokines that upregulate Th2-like lymphocytes, and therefore promote IgE production and airway inflammation. In general, studies in patients with asthma showed elevated IL4, IL-5, IL-9 and IL-13 production, and reduced IFN-γ production. These elevations may be due to polymorphisms that upregulate regulation of cytokine production. It is also possible that changes in other cytokine-genes or transcription factors that regulate these cytokines are responsible for these elevations. To date, there is some evidence for a possible role of a change in the promoter region of the IL-4 gene[107,108], the IL-9 gene[61,109], and the IL-13 gene[110], but not the IL-5 gene.[111]

The CD14 gene resides in the vicinity of the cytokine gene cluster on chromosome 5q and encodes for a high-affinity lipopolysaccheride receptor. This receptor is present as membrane bound CD14 on monocytes, macrophages and neutrophils, and in a soluble form (sCD14) in serum. Baldini and coworkers studied this CD14 gene based on the hypothesis that bacterial antigens could influence the Th1-Th2 balance and thus the development of atopy, through a CD14 dependent pathway. In a population study of children in the USA, a promoter polymorphisms in the CD14 gene was associated with levels of sCD14 in serum, total IgE levels and number of positive skin tests.[112] This interesting finding merits further study.

Other candidate genes for asthma and atopy include the IFN-γ gene at chromosome 12, T-cell-receptor genes at chromosome 7 and 14. A screening of the IFN-γ gene on chromosome 12 revealed no sequence variants in patients with asthma and controls.[113] Other groups have studied the genetics of the T-cell receptor. In most individuals, this receptor is made up of α-chains (gene at chromosome 14) and ß-chains (gene at chromosome 7). Around the α-chain, increased sharing of allele was found for the specific immune response in a UK and Australian population.[114] Around the ß-chain gene, increased sharing of alleles was found for childhood asthma and serum total IgE in a Japanese study [115] This study could not confirm the linkage to the region of the α-chain of the T-cell receptor gene. Further studies are needed to confirm these findings.

In summary, association studies of candidate genes have lead to interesting new insights into the genetics of asthma. Two polymorphisms of the $ß_2$-adrenergic receptor most likely do not cause asthma, but modify the severity of asthma. An interesting feature is that these polymorphisms might be involved in the response to medication. The high affinity IgE receptor could play a role in atopy and asthma in some populations. The E237G polymorphism of the high affinity IgE receptor especially needs to be studied in other populations. The IL-4 receptor a chain is a strong candidate for atopy, based on different association and functional studies. Finally, numerous other candidate genes have been studied. None of these candidate genes meet all four criteria as stated in the first paragraph of this section on candidate genes. It is clear that in the near future more genetic and functional studies are needed to clarify the role of these candidate genes in asthma and atopy.

Summary and future developments

The genetics of asthma has become a promising new field of research. In the pathogenesis of asthma multiple genes interact with each other and the environment. In different populations, different genes may have a major effect in the clinical manifestation of asthma. To date, several candidate genes

have been identified. Polymorphisms in the ß$_2$-adrenergic receptor do not cause asthma, but modify asthma into a more severe phenotype. Furthermore, polymorphisms in the ß-chain of the high affinity IgE receptor could play a role in a subset of the patients with atopy. However, most of the susceptibility genes for asthma and atopy remain to be determined. The recent identification of multiple linkages between asthma and different chromosomal regions represents a first step towards the identification of these asthma genes. However, in recent years, the procession from linkage to the actual identification of the gene has proved to be difficult.

In the coming years, future developments in molecular biology and genetic epidemiology may accelerate the process of the identification of genes for asthma and atopy. In the field of molecular biology, the Human Genome Project has the ultimate goal to sequence the human genome by 2003, and to identify single nucleotide polymorphisms throughout the genome. This project will aid genetic studies to a great extent.[116] Furthermore, one may anticipate new observations from animal studies, leading to further understanding of the genetics of human asthma. A genome-wide search for AH in mice has been completed, and has resulted in two possible linked regions on the mouse genome.[117] This approach could identify genes important in the regulation of AH in mice, and possible in men by searching for their homologous genes in human DNA.

In the field of genetic epidemiology, an interesting new method is the mapping of genes through the systematic analysis of shared haplotypes of affected individuals in founder populations. This approach is based on the idea that current asthmatics have identical copies of parts of chromosomes from a common ancestor. If one is able to identify such a chromosomal region which is identical by descent, it is likely that this chromosomal region contains an asthma susceptibility gene.[118]

The potential benefits of the identification of susceptibility or modifier genes for asthma are numerous. First of all, identification of persons at risk for asthma gives opportunity to early prevention, such as allergen avoidance or early introduction of medication. Secondly, protein products of these genes are potential drug targets, opening the way to causative rather than symptomatic treatment. Another clinical application may be that certain polymorphisms could result in a more severe asthmatic phenotype or predict resistance to therapy (pharmacogenetics). The latter is illustrated by the recent finding in a clinical trial of an experimental 5-lipoxygenase inhibitor in 325 patients with asthma. In this study, variants in the promoter region of the 5-lipoxygenase gene were associated with response to this anti-asthma treatment.[119]

In conclusion, although considerable progress regarding the genetics of asthma has been made, important questions remain to be answered. Which genes are the susceptibility genes for asthma? Are the genes for airway hyperresponsiveness and atopy the same or different? What is the biological function of asthma susceptibility genes? How do these genes interact with each other and with the environment? It will be a major challenge to unravel this complex genetic disease in the coming years.

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