REVIEW

The importance of genetic influences in asthma

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The importance of genetic influences in asthma. H. Los, G.H. Koppelman, D.S. Postma. ©ERS Journals Ltd 1999.
ABSTRACT: Asthma is a complex genetic disorder in which the mode of inheritance is not known. Many segregation studies suggest that a major gene could be involved in asthma, but until now different genetic models have been obtained. Twin studies, too, have shown evidence for genetic influences in asthma, but have also revealed substantial evidence for environmental influences, in which nonshared environmental influences appeared to be important. Linkage, association studies and genome-wide screening suggest that multiple genes are involved in the pathogenesis of asthma. At least four regions of the human genome, chromosomes 5q31–33, 6p21.3, 11q13 and 12q14.3–24.1, contain genes consistently found to be associated with asthma and associated phenotypes.

Not only genes associated with asthma but also genes which are involved in the development and outcome of asthma will be found in the future. This will probably provide greater insight into the identification of individuals at risk of asthma and early prevention and greater understanding for guiding therapeutic intervention in asthma. Exchange of information between researchers involved in the genetics of asthma is important because of mandatory agreement on phenotypes and analytical approaches. Genetics will contribute to the a better understanding and management of asthma in the future..
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It is well established that there is an important hereditary contribution to the aetiology of asthma. The inheritance of asthma and allergy does not follow the classical Mendelian patterns, which are characteristic of single- gene disorders. Asthma is a complex genetic disorder in which the mode of inheritance cannot be classified as autosomal, recessive or sex-linked. Moreover, it is clear that the development of asthma can be attributed to both genetic and environmental factors. Studies on the genetics of asthma are hampered by the fact that there are some difficulties in standardizing the diagnosis of asthma. The most current definitions of asthma characterize it as a variable airway obstruction usually associated with inflammation in the conducting airways of the lungs and eosinophilia [1–3]. These definitions do not distinguish between different clinical entities such as early- and late-onset asthma, allergic (extrinsic) asthma, asthma without evidence of allergy (intrinsic), occupational asthma and exercise-induced asthma. All of these clinical entities are called asthma, thus the accepted criteria for asthma are an oversimplification of a complex disease.

Asthma is a common disease in both low income and developed countries. Large geographical differences in asthma prevalence have been reported, varying 2–11.9% [4–5]. Asthma prevalence further differs with ethnicity. The prevalence rates of asthma in the USA are 6.9% for Caucasians and 9.2% for African-Americans [6], whereas the prevalence rates in Africans are very low, *i.e.* ~0.5% [7]. Furthermore, different prevalences have been found in urban and rural areas in that, in a population in

Zimbabwe, exercise-induced asthma was associated with urban residence and high living standards [8]. A recent study in children in a city in former West Germany (Munich) and two cities in former East Germany (Halle and Leipzig) has shown that asthma and allergy were significantly more frequent in children in former West Germany [9]. Thus, it appears that asthma is a disease of the Western lifestyle. Data suggest also that asthma increases in the Western world over the last 20 yrs [10] cannot be explained by changes in genetic make-up. A possible explanation for the increase in asthma prevalence could be differences in exposure levels to aeroallergens, such as house dust mite [11], smoking behaviour [12], dietary sodium intake [13, 14], occupation [15, 16], indoor and outdoor pollution [17] or immunization against certain infectious diseases [18]. The widely accepted paradigm is that environmental factors are important to the development of asthma, but one must be genetically predisposed to respond to environmental influences.

The purpose of this review is to provide a general overview of the genetics of asthma and the current evidence of asthma susceptibility genes and to provide some insight into the different asthma phenotypes and their relation to its genetic influences.

General considerations

In genetic studies, it is of great importance to define the phenotype of a trait correctly. In the case of asthma, this

Glossary

- **Additive genetic effects:** the effects of alleles at two different loci are additive when their combined effect is equal to the sum of their individual effects.

- **Allele:** alternative forms of a gene or locus marker due to changes at the level of the DNA.

- **Ascertainment:** the scheme by which individuals are selected, identified and recruited for participation in research study.

- **Association:** association studies frequently involve the comparison of allele frequencies of a marker locus between a diseased population and a control population. When statistically significant differences in the frequency of an allele are found between a diseased and a control population, the disease and allele are said to be in association.

- **Candidate gene:** a gene that has been implicated in causing or contributing to the development of a particular disease.

- **Centimorgan:** on a global level, a centimorgan covers roughly ~1 million base pair of DNA and is usually equivalent to ~1% recombination.

- **Complex trait:** a trait which has a genetic component that is not strictly Mendelian (dominant, recessive or sex-linked).

- **Deoxyribonucleic acid (DNA):** the molecule that encodes the genetic information in all organisms except some viruses. DNA molecules usually consists of two strands of nucleotides. DNA is a component of chromosomes.

- **DNA marker:** a cloned chromosomal locus with allelic variation that can be followed directly by a DNA-based assay such as Southern blotting or polymerase chain reaction.

- **Epistasis:** two or more genes interacting with one another in a multiplicative fashion.

- **Expression:** a description as to how a gene demonstrates a phenotype.

- **Gene:** an individual unit of heredity. It is a specific instruction that directs the synthesis of a protein or ribonuclease acid product. Each gene is located at a specific site (locus) on a chromosome.

- **Genetic model:** the overall specification of how the disease alleles act to influence the disease.

- **Genome:** the sum of all genetic information of an organism.

- **Genotype:** the observed alleles at a genetic locus for an individual. For autosomal loci, a genotype is composed of two alleles, one of which was paternally transmitted and the other of which was maternally transmitted. For X-linked loci, a genotype of a female includes two alleles, a genotype of a male includes only one allele.

- **Heritability:** in the narrow sense, heritability is defined as the proportion of the total phenotypic variance in a trait that is due to the additive effects of genes, as opposed to dominance or environmental effects. In the broad sense, heritability is the proportion of the total phenotypic variance of a trait that is due to all genetic effects, including additive and dominance effects.

- **Heterogeneity:** different genetic causes for the same disease phenotype.

- **Heterozygous:** the alleles at a genetic locus are different from one another on the two partners of a chromosome pair.

- **Homozygous:** the alleles at a genetic locus are identical on the two partners of a chromosome pair.

- **Identity-by-descent (IBD):** alleles shared by two relatives that were transmitted from the same ancestor.

- **Imprinting:** a phenomenon in which the phenotype of the disease depends on which parent passed on the disease gene.

- **Linkage:** the tendency for genes that are located close to each other on the same chromosome to be inherited together.

- **Linkage disequilibrium:** linkage disequilibrium is often termed "allelic association". When alleles at two distinctive loci occur in gametes more frequently than expected given the known allele frequencies and recombination fraction between the two loci, the alleles are said to be in linkage disequilibrium.

- **Locus:** any genomic site.

- **LOD score:** a statistic calculated in linkage analysis and used as a measure of the likelihood of linkage. The LOD score is calculated as the log of the ratio of the probability of the observed trait patterns if linkage is present to the probability of the observed patterns if no linkage is present.

- **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.

- **Mapping:** the process of determining the position of a focus on the chromosome relative to other loci.

- **Marker:** a characteristic by which a cell or molecule can be recognized or identified. Genetic markers consist of specific nucleotide patterns.

- **Phenotype:** the observed manifestation of a genotype.

- **Polygenic:** pertaining to a phenotype that results from interactions among the products of two or more genes with alternative alleles.

- **Polymorphism:** a tendency for a gene to exist in more than one form, or the specific alleles thereof.

- **Proband:** the individual who caused a family to be identified and included in a genetic analysis, usually an affected individual.

- **Recombination fraction:** the frequency of crossing over between two loci.

- **Segregation:** the principle that two partners of a chromosome pair are separated during meiosis and distributed randomly to the germ cells. Each germ cell has an equal chance of receiving either chromosome.

appears to be quite difficult, since several different clinical entities exist. Most genetic studies so far have concentrated on extrinsic (allergic) asthma. Many epidemiological and genetic studies on asthma assess the asthma phenotypes by means of a questionnaire, assessing self-reported asthma, wheeze or doctor's diagnosis of asthma. Although the validity of questionnaires is relatively good [19–22], there is a possibility of underestimation or overestimation of asthma prevalence [23, 24]. An advantage is that this method constitutes an easy and feasible approach and it can be used in large-scale studies. However, the use of this design may introduce serious diagnostic bias because misclassification with other obstructive lung diseases and respiratory viral infections in wheezing children is not uncommon [25].

Another problem in genetic studies is that asthma is not always detectable at the time individuals are being tested, especially at older age. Remission may occur for up to >20 yrs after childhood, so that earlier episodes of asthma may have been forgotten by the individual in question. Because of the intermittent nature of asthma symptoms and difficulty in standardizing the diagnosis of asthma, studies on the genetics of asthma are currently directed at measurable biological markers, called phenotypes in genetic studies. These include bronchial hyperresponsiveness, total immunoglobulin E (IgE), specific IgE directed against different allergens, skin test reactivity against common aeroallergens and eosinophilia. Other phenotypes associated with asthma are allergic rhinitis and atopic dermatitis. It is, however, questionable whether these traits have a common pathway or whether they are inherited separately from each other.

Family studies

It has long been established that genetic factors are very important in the pathogenesis of asthma. Familial aggregation of asthma was probably first described by Sennertus in 1650 [26]. At the beginning of this century, R. Cooke performed two large studies on the inheritance of atopy, one in 1916 and the other in 1924 [27, 28]. The first study examined asthma and its related phenotypes, e.g. allergy, urticaria and angioneurotic oedema, in 504 subjects. In the second study, only allergy and asthma were studied, in 462 individuals. A series of 115 nonatopic subjects were recruited as a control. The family history of atopy was determined by interviewing the proband and as many as possible of the other members of the family. This case/control study compared the relatives of a proband to those of a control. In atopic subjects, a family history of atopy was found in 48.4% of cases in the 1916 study and in 58.4% in the 1924 study. Only 7% of the 115 nonatopic subjects reported a family history of atopy. This suggested autosomal dominant inheritance of atopy.

Another extensive approach to the study of genetic factors of asthma was made by M. Schwartz in 1952. The prevalence rates of asthma in the 1,634 relatives of the 161 asthmatic subjects was 6.6%, but, in the 1,790 relatives of the control group, only 1% [29]. In 1980, SIBBALD et al. [30] described 77 asthmatic and 87 control children and their relatives. The overall prevalence of asthma in the first degree relatives of asthmatics was 13%, and that in the relatives of controls only 4%. The prevalence of asthma in the relatives of atopic asthmatics was significantly higher than that in the relatives of nonatopic asthmatics (p<0.01). In the relatives of both atopic and nonatopic asthmatics, the prevalence of asthma was higher than that in the relatives of controls, suggesting that both types of asthma are hereditary, but that the hereditary component underlying atopic asthma is of greater magnitude than that underlying nonatopic asthma [30]. In another study by the same author, the distributions of asthma, eczema and hay fever among the relatives of 512 asthmatics showed a similarity in the distributions of asthma among the relatives of clinically different groups of asthmatic patients, suggesting that all of these various types of asthma are hereditary and probably have similar modes of inheritance [31]. These and other family studies in the early 1900s have shown that there is a considerable genetic component in the pathogenesis of asthma [32–34].

Segregation analysis

The application of segregation analysis has furthered the assessment of the genetics of asthma in families. This method is used to analyse the pattern of inheritance of a disorder by observing how it is distributed within families. This analysis compares the number of affected individuals with the expected number using different analytical models. Segregation analysis can provide insight into the genetics of a trait, e.g. the number of genes involved and the genetic model: dominant or recessive, polygenic, such as mixed models, and those with environmental effects. The model which fits the data best is the model which gives the best description of the segregation of the trait in the families. Using this type of analysis, the heritability, mode of inheritance, penetrance and frequency of a trait can be estimated [35, 36] and indications of major genes found. Table 1 shows the results of available studies using segregation analysis to determine the genetics of asthma and its associated phenotypes.

Asthma

Segregation analysis of the asthma phenotype has mostly been carried out by means of questionnaires. A population study of 131 families ascertained through general practice register in Southampton, UK was undertaken in 1994. The phenotype of asthma was established by questionnaire and by measurements of bronchial hyperresponsiveness to histamine inhalation, atopic status was determined by total IgE levels and by skin-prick testing using common allergens. Correlation analysis showed an association between IgE levels and asthma score (r=0.38). Segregation analysis under the mixed model showed a heritability of ~0.61 for IgE levels and 0.28 for asthma score. Both under the mixed model and the two-locus model, segregation analysis suggested evidence of major genes acting against a polygenic background [37].

A large study performed by the European Community Respiratory Health Survey Group (ECRHS Group) analysed the pooled data from 13,963 families (consisting of 75,392 randomly selected individuals) using complex segregation analysis. The results of this study showed further

Table 1. – Segregation analysis of asthma and related phenotypes

First author [Ref.]		Year	Population	Phenotype	Genetic model	H	Comments
Symptoms							
LAWRENCE	37	1994	131 families, UK	Asthma score	Mixed, two-locus	0.28–0.63	Common genes of small effect
ECRHG	38	1997	13,963 families, Europe	Asthma	Two-allele gene with codominant inheritance		Questionnaire, self-reported asthma
JENKINS	39	1997	7,394 families		Codominant model		Questionnaire, population schoolchildren
HOLBERG	40	1996	906 families, Arizona, USA		Polygenic or oligogenic		
CHEN	41	1998	309 families, Saskatchewan, Canada	Wheeze	Single locus, contribution of polygenes and environment		Questionnaire, self-reported, wheeze
IgE							
GERRARD	42	1978	173 families, Saskatchewan, Canada	IgE	Major gene, dominant alleles suppress high IgE levels	0.43	Selected population by members with ragweed allergy
BLUMENTHAL	43	1981	3 large families Minnesota, USA	IgE	Major gene with polygenic transmission	0.43	Selected population for ragweed allergy, genetic model based on pooled data
MEYERS	44	1982	23 Amish families, Pennsylvania, USA	IgE	Mendelian codominant		No selection for allergy
MEYERS	45	1987	42 families, USA	IgE	Mixed with recessive inheritance	0.36	No selection for allergy
HASSTEDT	46	1983	5 families, USA	IgE	Polygenic inheritance, no major gene involved		Selected population for ragweed allergy
MARTINEZ	47	1994	291 Hispanic and non-Hispanic families, Arizona, USA	IgE	Major gene, codominant inheritance for high IgE levels		
DIZIER	48	1995	234 families, Busselton, Australia	IgE	Recessive major gene for high IgE levels		
PANHUYSEN	49	1996	92 families, Holland	IgE	Two-locus recessive		Families ascertained through proband with asthma
BHR							
TOWNLEY	50	1986	83 families, USA	BHR	No single autosomal locus		Families with and without asthma
LAWRENCE	37	1994	131 families, UK	BHR	Mixed, weak support for a major gene	0.27	Random families

H: heritability; ECRHG: European Community Respiratory Health Group; IgE: immunoglobulin E; BHR: bronchial hyperresponsiveness.

evidence of genetic regulation of asthma and a model with a two-allele gene with codominant inheritance fitted the data best, assuming a major gene has to be involved in the pathogenesis of asthma, but the penetrance of such a gene is low [38]. JENKINS *et al.* [39] presented a segregation analysis of 7,394 families in which 15.9% of the index individuals had asthma. A general major gene model fitted the data but as in the analysis of the ECRHS Group, the codominant model was the best fitting model.

A segregation analysis of physician-diagnosed asthma in 3,369 randomly selected individuals from 906 nuclear families in Tucson, AR, USA showed evidence of a polygenic or an oligogenic model with some evidence of a recessive gene, explaining only part of the segregation. There was no evidence of a single two-allele-locus model for asthma [40].

A questionnaire-based study of self reported wheeze in 309 nuclear families (1,053 individuals) in the town of Humboldt, Saskatchewan, Canada reported evidence for a single locus gene which explains a proportion of wheeze related to respiratory allergy. Common environmental and polygenetic effects also contribute to familial aggregation of wheeze [41]. However, it is debatable as to whether wheeze is equivalent to asthma since only a small proportion of wheezing children may actually develop asthma [25, 51].

Total immunoglobulin E

Many segregation analyses of total serum IgE-concentration have been published in recent decades. Most of these studies conclude that IgE levels are highly heritable [42–49, 52, 53]. In 1978, 173 families from Saskatoon, Saskatchewan, Canada were studied by GERRARD *et al.* [42]. Total IgE levels were analysed by means of path analysis and segregation analysis. Path analysis provided evidence of a genetic heritability of 42.5% for serum IgE levels. The mixed model with recessive inheritance of high IgE levels and evidence of polygenic effects gave the best fit to the data [42].

Another study on total IgE levels was a study of three large pedigrees. There was no single model which best fitted the data for each pedigree, thus strongly suggesting genetic heterogeneity. In the pooled data, the best fit of the data was the model with a major gene and polygenic transmission. The estimated heritability was 49.5%. Although segregation analysis is very informative in large pedigrees as in this study, there was an ascertainment bias because the families were selected by members who had ragweed respiratory disease [43].

A segregation analysis of total serum IgE levels in 208 Amish individuals from 23 nuclear families in Pennsylvania, USA, showed no evidence for a Mendelian dominant or recessive model, but the Mendelian codominant model could not be rejected. In this study, there was no selection bias for allergy [44]. The same author presented a study, in 1987, in which 278 individuals from 42 nuclear families were selected for large family size (both parents and all available children were studied). The families were not selected for the presence of allergic disease. Segregation analysis showed a mixed model with recessive inheritance of high levels of IgE to fit the data best. The estimated heritability was 36% [45]. An interesting finding in this study was the high correlation of log IgE between the parents. The authors had no explanation for this; maybe there was a selection bias in favour of atopic disease, or maybe these parents had shared their environment to a greater extent than expected.

Other studies have found strong genetic regulation of IgE levels, with different modes of inheritance: polygenic inheritance [37, 46], codominant inheritance of a major gene for high IgE levels [47], and a recessive major gene controlling high IgE levels [48]. Segregation analysis of 92 Dutch families ascertained through a proband with asthma used a two-locus approach. This model resulted in a better fit of the data than the one-locus model. The first locus explained 50.6% of the variance of the level of total IgE and the second locus 19.0%. Together, the two loci explained 78.4% of the variance in total serum IgE levels. This study provides evidence that there are at least two loci involved in the regulation of total serum IgE levels [49].

Bronchial hyperresponsiveness

In addition to atopy, bronchial hyperresponsiveness is another feature of asthma that may have a heritable component. Several studies have shown a strong association between atopy and bronchial hyperresponsiveness [54, 55], but it is clear that not all people with atopy have bronchial hyperresponsiveness, and it is also true that subjects with bronchial hyperresponsiveness may not have symptoms of asthma [56, 57]. It is interesting that this discrepancy between symptomatic asthma and bronchial hyperresponsiveness might indicate that subjects with bronchial hyperresponsiveness may have an asthma-predisposing gene, yet need a trigger to develop full-blown asthma. Although several family and twin studies have been performed on bronchial hyperresponsiveness [58–62], very few segregation analyses on bronchial hyperresponsiveness have been published. One of the largest of these studies described 83 families of which 51 were ascertained through an asthmatic proband (467 individuals) and 32 through a nonatopic control (291 individuals); 26 cases were included for a negative family history of all-

ergic disease [50]. The methacholine response in these families was found to be bimodally distributed, suggesting a major gene. However, segregation analysis showed no evidence for a single major locus model. LAWRENCE et al. [37] studied 131 families ascertained through General Practice register in Southampton. No selection for atopy or asthma occurred. The estimated heritability for bronchial hyperresponsiveness was 26.7%. Under the mixed model, there was no strong evidence for dominant genes in the pathogenesis of bronchial hyperresponsiveness [37].

In conclusion, these segregation analyses suggest that a major gene could be involved in asthma, but until now different genetic models have been obtained. Evidence for a major gene for IgE levels was found by several studies in different countries, but the exact mode of inheritance is still not known. There is also a possibility that more genes than one are involved in determining IgE levels. Most probably, many genes will finally determine the asthma phenotype.

Twin studies

An important goal in the study of twins is the estimation of the effects of family environment and the detection of the genetic contribution to complex traits. In a twin design, the separation of genetic and environmental variance is possible because monozygotic (MZ) twins share 100% of their genetic make-up and dizygotic (DZ) twins on average 50%. If a trait is influenced by genetic factors, MZ twins should resemble each other to a greater extent than DZ twins, and the correlations between MZ and DZ twin pairs may be used to obtain estimates of the relative sizes of the genetic and environmental influences. If there are DZ twins of unlike sex, the twin design provides a way of assessing the extent to which individual differences are due to the same genetic influences in males and females. When different genes account for the variance in male and female twins, the correlation between DZ unlike pairs will be smaller than that in same-sex pairs.

The main assumptions of twin studies are that the environment for both MZ and DZ twins is similar, they are representative of the general population and, in questionnaire-based studies, the self-reported zygosity is correct [63]. Table 2 presents results of asthma twin studies [64–73].

Asthma

A large twin study reported in 1971 was a questionnaire-based study of 6,996 twin pairs from the Swedish Twin Registry, born between 1886 and 1925. In this study, the MZ concordance for self-reported asthma was 19% and DZ concordance was 4.8%. Although environmental influences appeared to be important in the pathogenesis of asthma, genetic factors were also important. No distinction could be made regarding the mode of inheritance [64].

A large Finnish study investigated 13,888 twin pairs; a diagnosis of asthma was made by linking the twin registries with databases on hospital admission, usage of medication and death certificates from the central statistical office. A concordance rate of 0.13 for MZ twins and 0.7 for DZ twins was found. Under the multifactorial threshold model, the heritability of asthma combining the sexes was 36% [65].

DUFFY et al. [66] published a study of 3,808 Australian twin pairs. This questionnaire-based study showed a

Table 2. – Twin studies of asthma and related traits

First author [Ref.]		Year	Population	Phenotype	MZ cor-relation	DZ cor-relation	H	Genetic model	Comments
Symptoms									
EDFORS-LUBS	64	1971	Sweden	Asthma, allergy	0.65	0.25			Population study, self-reported asthma
NIEMIENEN	65	1991	Finland, adults	Asthma	0.43	0.25	0.36		Population study, doctor's diagnosis of asthma
DUFFY	66	1990	Australia, adults	Asthma	0.65	0.24	0.60–0.75	Data best fitted by: males: ADE; females: AE*	Population study, self-reported asthma
HARRIS	67	1997	Norway, age 18–25 yrs	Asthma	0.75	0.21	0.75	ADE and AE fit data best*	Population study, self-reported asthma
LAITINEN	68	1998	Finland, age 16 yrs	Asthma	0.76	0.45	0.79	AE fits data best in twins*	Parental/twin design
LICHTENSTEIN	69	1997	Sweden, age 7–9 yrs	Asthma, eczema, hay fever, urticaria			0.33–0.76	AE fits data best, but shared environmental effects were present for hay fever and urti-caria in both sexes	Parent-reported asthma questionnaire-based population study
SKADHAUGE	70	1999	Denmark, age 12–41 yrs	Asthma			0.73	AE fits data best*	Population study, self-reported asthma
IgE									
HOPP	71	1984	USA	IgE	0.82	0.52	0.61		No genetic modelling
HANSON	72	1991	USA	IgE	0.42	0.26			Twins reared together
HANSON	72	1991	USA	IgE	0.64	0.49			Twins reared apart
HANSON	72	1991	Finland	IgE	0.56	0.37			Twins reared together
DUFFY	73	1998	Australia	IgE					Cotwin control study
Skin test									
HOPP	71	1984	USA	Skin test	0.82	0.46	0.72		No genetic modelling
HANSON	72	1991	USA	Skin test	0.70	0.28			Twins reared together
HANSON	72	1991	USA	Skin test	0.55	0.50			Twins reared apart
DUFFY	73	1998	Australia	Skin test					Cotwin control study
Specific IgE									
HANSON	72	1991	USA	Specific IgE	0.50	0.33			Twins reared together
HANSON	72	1991	USA	Specific IgE	0.50	0			Twins reared together
BHR									
HOPP	71	1984	USA	BHR	0.67	0.34	0.66		No genetic modelling

*: no evidence for shared environment. MZ: monozygotic; DZ: dizygotic; H: heritability; A: additive genetic effects; D: dominant genetic effects; E: nonshared environment; IgE: immunoglobulin E; BHR: bronchial hyperresponsiveness.

correlation of self-reported asthma of 0.65 among MZ twins and 0.24 among DZ twins. The heritability was 60% for females and 75% for males. Genetic modelling showed that the nonadditive component made a large contribution to the variance in mates. In females, additive genetic effects made a large contribution to the observed variance, and no evidence for shared environment existed for both sexes.

5,864 Norwegian twins participated in a study on health and development in Oslo. All twins born between 1967 and 1974 were identified through the National Medical Birth Registry. The probandwise concordance for asthma was 0.45 for MZ twins and 0.25 for DZ twins. Genetic modelling showed that genetic effects (additive and non-additive) explained ~75% of the variation for both sexes, and the remaining 25% was attributable to nonshared environment [67].

A population-based twin family study in 16-yr-old Finnish twins and their parents presented combined twin/family data on the inheritance of asthma. The population under study consisted of families with asthma and families with unaffected parents. A strong impact of genes on asth-

ma morbidity in adolescence existed. The heritability of asthma was approximately 79%, whereas 21% was due to unique environmental factors (parental asthma status ignored). In families with parental asthma, additive genetic factors and unique environmental factors showed the best fit to the data, with no evidence of a shared environment. In families without parental asthma, the common environment and unique environment model could not be rejected, but, because of the low occurrence of asthma among the offspring of nonasthmatic parents, there was insufficient statistical power to detect a difference between the genetic models [68].

Other twin studies have provided comparable results. An important finding in most twin studies in different parts of the world is the strong heritability of asthma and the lack of evidence of a shared environment [69–70, 74]. Further, the estimates of heritability are consistent with those from family studies. In most of these large-scale twin studies, a diagnosis of asthma was based on self-reporting methodology. Subjects in these studies were not tested clinically; thus, recall bias could result in a decrease in the prevalence rates of the disease [23, 24].

Asthma-related phenotypes

Several twin studies have been directed at measurable clinical parameters of asthma, such as bronchial hyperresponsiveness, IgE and skin test reactivity to common allergens [71–73, 75–77]. HOPP *et al.* [71] studied 107 pairs of twins, aged 6–31 yrs, recruited from a university outpatient clinic. Bronchial responsiveness to methacholine, skin test responses and total IgE levels were investigated. The heritability of IgE levels, bronchial hyperresponsiveness and skin test reactivity were 61, 66 and 72%, respectively, suggesting greater genetic than environmental influences on these traits. No genetic modelling was carried out; therefore, no statement on the mode of inheritance could be made. A cautionary note on this study is that selection occurred in recruiting the studied population. Sixty-three US twin pairs reared together, 68 reared apart and two sets of reared-apart triplets were studied. In addition, 158 Finnish twin pairs reared together were investigated [72]. Total IgE level correlations were similar for MZ twins reared apart and MZ twins reared together, suggesting that the effects of shared environment were not very large. In contrast, specific serum IgE levels and skin test responses were not different between MZ and DZ twin pairs. Thus, environmental influences appeared to be more important for these phenotypes than genetic factors.

In summary, data from twin studies provide evidence that the ability to produce IgE is largely genetically regulated and that the specificity of the IgE response is mainly determined by environmental factors [75–77].

Linkage studies in asthma

Linkage studies involve investigations of deoxyribonucleic acid (DNA). From 1985 onwards, studies have started to identify which genes may cause asthma [78]. The complexity of the immunological network involved in the pathogenesis of asthma and atopy and its related traits (bronchial hyperresponsiveness and levels of total IgE, specific IgE against aeroallergens, different cytokines, T-cells, *etc*.) and the existence of different asthma phenotypes is consistent with the hypothesis that different genes may be involved in the pathogenesis of asthma. Therefore, some studies have started to investigate specific regions of chromosomes on which genes, which are most probably implicated in the pathophysiology of asthma, are located. This may involve genes encoding cell surface receptors (*e.g.* the β_2-adrenergic receptor), mediators (*e.g.* IL-4) and proteins derived from inflammatory cells. Another approach is to perform a genome-wide search and then investigate further to pinpoint the exact location of the gene, thereby possibly finding new genes, known as positional cloning (fig. 1).

The candidate gene approach comprises association with candidate genes. The region identified may include a number of genes whose functions suggest that they may be relevant to the aetiology of the disease. By comparing the genotypes at these candidate loci between cases with the disease and controls, it may be discovered that a candidate region may cause the disease [79].

Positional cloning investigates chromosomal regions that may harbour disease genes through linkage analysis. Genetic linkage analysis is performed by maximum like-

lihood of disease (LOD) ratio methods. This method assesses the likelihood that a trait cosegregates with a marker, which is expressed as an LOD score, *i.e.* the log of the ratio of the likelihood of linkage and the likelihood of no linkage [79, 80]. A value of +3 is traditionally taken as evidence for linkage and a value of -2 is considered evidence against linkage.

Linkage analysis requires pedigree data as opposed to association studies in which unrelated cases and controls are needed. In order to study linkage using the LOD score method, a genetic model has to be specified and different parameters such as mode of inheritance, allele frequencies and penetrance have to be defined [79, 81]. As seen in the segregation and twin studies, these parameters are not known in most cases. A more robust form of linkage analysis, in which there is no need for a specified genetic model, is the sib pair or affected relative pair method. Briefly, this method assumes that the inheritance of a chromosomal region is not consistent with random segregation and is performed by testing (standard Chi-squared test) whether affected relatives share identical copies of alleles in this region more often than would be expected by chance [79–82].

Genome search

To date several genome-wide screening studies have been published [83–88]. Table 3 shows the results for positive linkage with different phenotypes of asthma [83–86].

A population of 80 nuclear families selected from a sample of 230 families from Busselton, Western Australia was studied for linkage with asthma-associated traits. The clinical analysis consisted of asthma symptoms from a standard questionnaire (modified British Medical Research Council questionnaire), bronchial hyperresponsiveness to methacholine, total IgE levels, skin test reactivity and eosinophil numbers. Another 70 families from Oxford, UK were used to replicate the positive findings in the Busselton families. One or more asthma-related phenotypes were linked to six chromosomes: 4, 6, 7, 11, 13 and 16. Chromosomes 11 and 16 showed linkage to IgE levels. Chromosomes 4,
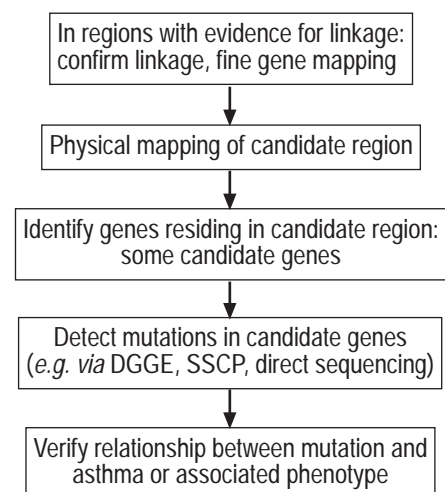

Fig. 1. – From linkage analysis, *via* positional cloning, to mutation detection. DGGE: denaturing gradient gel electrophoresis; SSCP: single-strand conformation polymorphism analysis.

Table 3. – Results of genome-wide screening regarding positive linkage with different phenotypes of asthma

Chromosome	CSGA Population	UK		Australia		Hutterite		Holland	
		Location	Phenotype	Location	Phenotype	Location	Phenotype	Location	Phenotype
1	-	-	-	-	-	-	-	1	BHR, SPT
2	Hispanic	-	-	-	-	2	Asthma, BHR, symptoms	2	BHR
3	-	-	-	-	-	3p24.2–22	Asthma, BHR, symptoms	3	BHR, Eos
4	-	4	IgE	4	IgE, BHR	-	-	4	IgE, Eos
5	African-American	-	-	-	-	-	-	5p	IgE
	Caucasian	-	-	-		5q23–31*	Asthma, BHR, symptoms	5q	IgE, BHR
6	Caucasian	-	-	6	IgE, Eos	-	-	6p	Eos
7	-	-	-	7	IgE, Eos, BHR	-	-	7q	IgE
8	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	9	Asthma, BHR, symptoms	-	-
10	-	-	-		-	-	-	-	-
11p	Caucasian	11p15	-	-	-	-	-	-	-
11q	-	11q13	Asthma	11q13	IgE, SPT	-	-	-	-
12	Caucasian, Hispanic	-	-	-		12q15–24*	Asthma, BHR, symptoms	12q	IgE
13	Caucasian	13	Atopy	13q	Atopy	13	Asthma, BHR	-	-
14	Caucasian	-	-	-	-	-	-	14	Eos
16	-	16	Asthma, atopy	16	IgE	-	-	16	Eos
17	African-American	-	-	-	-	-	-	17q	IgE
19	Caucasian	-	-	-	-	19q13*	Asthma	-	-
21	Hispanic	-	-	-	-	21q21	Asthma	-	-

*: Linkage evaluated both by likelihood ratio and transmission disequilibrium test in the primary and pooled primary and replication samples (see text for further details). CGSA: Collaborative Study on the Genetics of Asthma. BHR: bronchial hyperresponsiveness; SPT: skin-prick test; Eos: eosinophilia; IgE: immunoglobulin E.

and 7 were linked to bronchial hyperresponsiveness, and chromosome 6 and 7 to eosinophil count. Atopy, combining skin test and IgE level results, showed linkage to chromosomes 6 and 13. In the second (Oxford) group, the markers showing p<0.001 for linkage were tested for replication. Linkage of asthma to FCεKB and to D16S289 (D16, meaning a marker on chromosome 16, gives the location) was seen in the Oxford group, and linkage was found of atopy to D13S153. Interestingly, maternal linkage was stronger than paternal linkage for D4S426 (chromosome 4), FCεRB (chromosome 11) and D16S289 [83].

In 1997, a multicentre study in America was carried out to identify all important loci that could contribute to the development of asthma and its related traits. To include the possibility that different genes are responsible for the phenotypes of individuals of different racial background, three racial groups were included in the study (African-Americans, Caucasians and Hispanics). Families were ascertained through two asthmatic siblings. The sample consisted of 140 families: 43 African-American, 79 Caucasian and 18 Hispanic. The population was characterized using bronchial hyperresponsiveness to methacholine, asthma symptoms from a standard respiratory questionnaire, skin test reactivity, total IgE levels and reversibility of airway obstruction. Multipoint linkage analysis was carried out. Six new regions showed some evidence for linkage: 5p15 and 17p11.1–q11.2 in African-Americans, 11p15 and 19q13 in Caucasians, and 2q33 and 21q21 in Hispanics. Further-

more, evidence for linkage was also detected in regions previously reported to be linked to asthma-related phenotypes: 5q21–31, 6p21–23, 12q14–24, 13q21, 14q11–13 in Caucasians, and 12q14 in Hispanics [84].

A third genome-wide screen was carried out by OBER et al. [85] in the Hutterites, a religious community of European ancestry, 900 members of which migrated to South Dakota, USA in 1870. This founder population offers some advantages for mapping genetic traits, and particularly complex traits [85–87]. A total of 361 individuals were studied according to the same protocol as that used in the multicentre study in America [84], and 292 individuals from five additional communities in South Dakota were studied as a replication sample. Individuals were stratified into four groups: A: unaffected, B: asthma symptoms and equivocal bronchial hyperresponsiveness, C: bronchial hyperresponsiveness and equivocal asthma symptoms, and D: strict asthma. Evidence for linkage was evaluated by means of the semiparametric likelihood ratio (LR) Chi-squared test and the transmission-disequilibrium test (TDT). Twelve markers in 10 regions showed evidence for linkage to asthma or a related phenotype in the primary sample. Four of the 12 markers also showed evidence for linkage using the TDT in the primary sample (D3S1768, D12S375, D13S787 and D19S178). Evidence for linkage by both the LR test in the primary sample and the TDT in the pooled primary and replication samples was found for the markers D5S1480, D12S375, D19S178

and D21S1440. Moreover in the Hutterites, two markers (D3S2432 and D3S1768) showed linkage not previously reported in the Hutterites [85].

A genome screen by KOPPELMAN *et al.* [86], has been recently completed in 141 Dutch families identified through a parent who was characterized for asthma 25–35 yrs ago. Probands, spouses and their children and grandchildren (age >6 yrs) were characterized for asthma symptoms (standard respiratory questionnaire), bronchial hyperresponsiveness to histamine, allergy skin tests, total IgE levels, eosinophil numbers and bronchodilator reversibility. Multipoint LOD scores were calculated. Linkage was found for IgE on chromosome 4q, 5p, 5q, 7q, 12q and 17q. No evidence for linkage on chromosome 11 or 13 was found to either IgE levels or bronchial hyperresponsiveness. A region of interest was found on chromosome 3, which was linked to hyperresponsiveness and eosinophilia. The previous findings of linkage of IgE levels and bronchial hyperresponsiveness to chromosome 5q31–33 were confirmed [86].

These genome-wide searches show that there are several areas in the genome where linkage is consistently shown, or at least occurs in more than one population. This corroborates the importance of further fine mapping of these regions in order to establish the exact location of asthma genes.

Linkage and association studies in asthma

Chromosome 5 and the cytokine gene cluster

Chromosome 5q31–33 is a region rich in genes that are implicated in the immunological network associated with asthma. A cluster of cytokines, interleukin (IL)-3, IL-4, IL-5, IL-9, IL-13 and the β-chain of IL-12, are localized in this area as well as the genes encoding the β-adrenergic receptor, the corticosteroid receptor and granulocyte-macrophage colony-stimulating factor (GM-CSF). One of the mechanisms of asthma may be an imbalance between bronchoconstricting and bronchodilating forces [82]. There is evidence that the β-adrenergic receptor may be abnormal in asthmatics [89, 90]. The gene encoding the β-adrenergic receptor is located on chromosome 5q31 and represents a protein of 413 amino acids. Table 4 shows the available linkage and association studies of asthma in relation to chromosome 5.

In 1993, REISHAUS *et al.* [91] studied 51 moderate or severe asthmatics and 56 normal subjects. They found nine different point mutations and four of these polymorphisms were found to cause changes in the encoded amino acids at residues 16, 27, 34 and 164. The two most common mutations were not more common in asthmatics than in nonasthmatics. In the asthma group, one mutation (arginine $(Arg)^{16} \rightarrow$ glycine $(Gly)^{16}$) identified a subset of patients with a distinct profile, being more likely to be steroid dependent and following a disadvantageous clinical course [91].

TURKI *et al.* [92] found an allele frequency of 80.4% for $Arg^{16} \rightarrow Gly^{16}$) in 23 nocturnal asthmatics compared with non-nocturnal asthmatic controls in which the allele frequency was 50% [92]. Recently a study of mild/moderate asthmatics (n=86), fatal/near fatal asthmatics (n=81) and 84 controls showed no single polymorphism or haplotype to be associated with fatal or near-fatal asthma. However the Gly^{16}/glutamine $(Gln)^{27}$ haplotype was more prevalent in moderate asthmatics than in mild asthmatics. The allele frequencies in this Caucasian population were 61% for Gly^{16} and 57% for Gln^{27} [93]. These were similar to the results reported by MARTINEZ *et al.* [94] in their study of unrelated Caucasians. In this study, the Gly^{16} allele was associated with a lower response of forced expiratory volume in one second (FEV1) to albuterol compared to the Arg^{16} allele. The authors hypothesized that the low response on β-mimetics in individuals with the Gly^{16} polymorphism could be a reason for their increased use of corticosteroids [94].

HALL *et al.* [95] found that the $Gln^{27} \rightarrow$ glutamic acid $(Glu)^{27}$ polymorphism was associated with less reactive

Table 4. – Results of linkage studies on chromosome 5 with respect to asthma, immunoglobulin E (IgE) and bronchial hyperresponsiveness (BHR)

First author	[Ref.]	Year	Phenotype	Genetic analysis	Result	Comments
REIHSAUS	91	1993	Asthma	Association	+	β₂-adrenergic receptor
TURKI	92	1995	Nocturnal asthma	Association	+	β₂-adrenergic receptor
WEIR	93	1998	Mild, moderate and near fatal asthma	Association	+	β₂-adrenergic receptor
MARTINEZ	94	1997	Asthma	Association	-	Children, β₂-adrenergic receptor
HALL	95	1994	BHR	Association	+	Mild-to-moderate asthma, β₂-adrenergic receptor
DOULL	96	1996	Total IgE	Association	+	IL-9: 118 allele
MARSH	97	1994	Total IgE	Sib pair/LOD	+	11 Amish families
MEYERS	98	1994	Total IgE	Sib pair/LOD	+	92 families, Holland
POSTMA	99	1995	BHR	Sib pair/LOD	+	Families, Holland
BLUMENTHAL	100	1996	Total IgE	Sib pair/LOD	+	4 families, Minnesota, USA
NOGUCHI	101	1997	Total IgE	Sib pair/LOD	+	Population, Japan
NOGUCHI	101	1997	Asthma, wheeze and dyspnoea	Sib pair/LOD	+	
SANDFORD	102	1995	Total IgE/BHR	Sib pair/LOD	-	
KAMITANI	103	1997	BHR	Sib pair/LOD	-	Random population, Australia
LAITINEN	104	1997	Asthma, wheezing, BHR	Association	-	Random population, Finland
MANSUR	105	1998	BHR	Association	-	Random population
ULBRECHT	106	1997	Total IgE	Association	-	

IL: interleukin; LOD: likelihood of disease. +: positive; -: negative.

airways. Glu^{27} homozygotes were reported to have a four-fold higher geometric mean provocative dose of methacholine causing a 20% fall in $FEV1$ than individuals who were homozygous for Gln^{27} [95] (fig. 2).

Thus, mutations in the β_2-adrenergic receptor may not increase susceptibility to asthma, but may induce a more severe phenotype of asthma. However, whether polymorphisms in the β_2-adrenergic receptor are associated with less responsiveness is still a debatable issue, since conflicting results have been reported in relatively small populations of asthmatics [107, 108].

For an investigation of IL-9, 131 families were randomly selected from the patients of 104 general practitioners in Southampton. Six traits were examined: IgE levels, skin-prick test results for common allergens, bronchial hyperresponsiveness to histamine, history of wheezing, eczema and seasonal rhinitis. The authors found that the 118 allele at the IL-9 locus showed a significant association with high IgE levels (p<0.003) [96].

MARSH et al. [97] searched for linkage between total serum IgE levels and markers at chromosome 5q31–33. The population consisted of 170 subjects from 11 Amish families who were selected on the basis of detectable serum IgE levels to common inhalant allergens in at least one child. They used the sib pair method. The results showed significant evidence for linkage between different markers in the region on chromosome 5q31 and IgE levels [97]. Shortly after this study, MEYERS et al. [98] published data from 92 Dutch families (538 individuals), all ascertained through a parent with asthma. They showed evidence for linkage between IgE and different markers on chromosome 5. The highest LOD score was derived for D5S436, with 9% recombination (p=0.0003), using a recessive model for inheritance of serum total IgE levels [98]. In the same population, using sib pair analysis, linkage between bronchial hyperresponsiveness and several markers on chromosome 5 was found (p<0.001) [99]. In a study of four large families in Minnesota, USA consisting of 110 individuals, there was no evidence of linkage between IgE levels and markers on chromosome 5 [100]. Linkage analysis was performed under a model
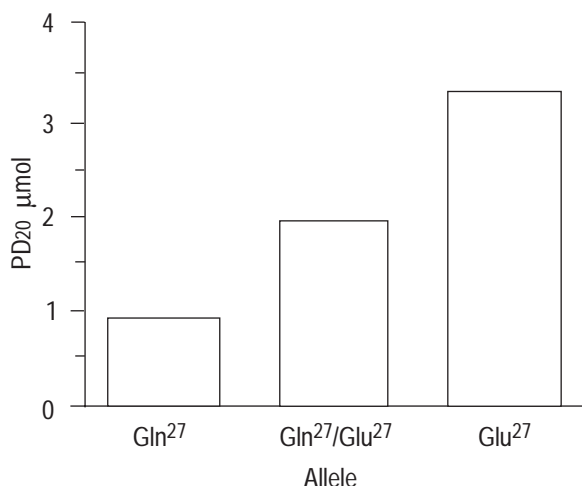
that describes the inheritance of total IgE levels as an autosomal dominant trait and using the affected sib pair method of analysis. Twelve markers were used including those used by MARSH et al. [97] and MEYERS et al. [98] (D5S393, IL-9), who found positive linkage results in their studies. It may seem surprising that no linkage was found. However, the lack of linkage may also be due to the selection of families enriched with asthma, differences in family structure and clinical differences between the studies, and, furthermore, because of the genetic heterogeneity of asthma, the power of the study was probably too low to detect linkage. Other studies have not found linkage between asthma/atopy and chromosome 5 [102–106].

Chromosome 6

The human leukocyte antigen (HLA) and the gene for tumour necrosis factor (TNF)-α are located on the short arm of chromosome 6. It is known that immune response is controlled by the major histocompatibility complex (HLA). HLA molecules are involved in binding antigenic peptides and presenting them to T cells. Two classes are distinguished: class I and class II, the latter acts merely on B cells, activated T cells and macrophages. This class may be of importance in the pathogenesis of asthma and atopy. Studies directed at finding associations between HLA and serological analysis (i.e. specific IgE responsiveness) showed no conclusive results [109–116]; several studies have found an association with IgE response to mite allergens and ragweed pollen and the HLA class II region [109–112], whereas other studies could not confirm these results [112–116].

A study of 20 Colombian nuclear families, involving 107 individuals, showed evidence of linkage between IgE responsiveness to mite allergens in patients with asthma and the HLA region, but no association with a specific allele was found [116]. The same group attempted to detect alleles involved in the genetic control of patients with asthma and mite IgE responsiveness. The results of their study showed that the allele DPB1*0401 is significantly more frequently absent in patients with asthma compared with normal controls (p<0.008), suggesting that this gene could protect against asthma or atopic diseases [118].

Certain HLA class II alleles may be involved in industrial (isocyanate) asthma [119]. Recently, SORIANO et al. [120] reported data from 26 outbreaks of asthma caused by inhalation of soybean dust in Barcelona, Spain. The presence of the allele DRB1*13 represented the greatest risk of having asthma during this epidemic: the odds ratio for low total IgE levels was 14.5, for mid range total IgE levels 1.33 and for high total IgE levels 1.93 [120].

Tumour necrosis factor-α

The cytokine TNF-α has several functions in asthma. TNF-α induces the influx of inflammatory cells via increased expression of adhesion molecules, activates several inflammatory cells, including eosinophils and T-cells, and increases bronchial hyperresponsiveness [121]. A polymorphism in this gene may upregulate TNF-α production and cause asthma. Recently, MOFFATT et al. [122] reported an association between the TNF-α gene, located on chromosome 6p21.3, and asthma [122]. ALBUQUERQUE et al.

Fig. 2. – Genetics of β-adrenergic receptor polymorphisms. Association between more severe hyperresponsiveness and a mutation of the β-adrenergic receptor. PD20: provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second; Gln: glutamine; Glu: glutamic acid. (From [95].)

[123] detected a polymorphism at position 308 in the promoter region of this gene (TNF1 allele). There is evidence that this polymorphism is associated with a six-fold upregulation of transcription of TNF-α [123].

Chromosome 11

IgE exists only in mammals and is one of the five classes of antibody recognized in humans. IgE-secreting B cells are abundantly present in skin, lungs and gut and IgE shows a range of cellular responses to antigens, including inflammation and bronchoconstriction. IgE constitutes a miniscule fraction of the total antibody concentration in human serum, and is of little help in neutralizing antigens. The action of IgE is upregulated *via* special receptors to which it binds. The high affinity receptor (FcεRI) is responsible for immediate reactions and is composed of three different subunits: one α, one β and two γ chains. This complex is found on the surface of mast cells, basophils, eosinophils and Langerhan′s cells. The binding of allergen to the receptor-bound IgE leads to degranulation of the cell and the synthesis and release of cytokines (IL-4), and activated inflammatory cells. The activation of inflammatory cells may induce a low-affinity IgE receptor (FcεRII) also known as CD23. This receptor is responsible for IgE-dependent antigen presentation to T cells [124]. Thus, IgE plays an important role in the pathophysiology of atopy and asthma.

The first study which showed linkage between IgE responses underlying asthma and atopy and chromosome 11q was presented by COOKSON *et al.* [125] (table 5). Seven pedigrees were ascertained through atopic asthmatics attending a clinic or *via* media advertizing. In this study a "broad" definition of atopy was used: elevated serum IgE concentration, raised specific IgE level against allergen or the presence of one or more positive skin-prick tests. Most of the LOD score was attributed to one of the seven families studied, using an autosomal dominant mode of inheritance [125]. The same group studied 64 nuclear families with the same markers (DIIS97). An LOD score of 3.8 was found under the assumption of equal recombination fraction in both sexes [126]. Other authors from the same group could confirm these results [127]. Besides the Oxford, UK group, several other groups reported linkage between asthma/atopy and chromosome 11q13. A small Dutch sample of 26 nuclear families showed, by means of affected sib pair analysis, linkage between atopy and the markers pMS51 and PYGM located on chromosome 11q13 [128]. An Australian sample of 123 affected sibling pairs recruited from the general population showed genetic linkage between the FCεRIβ locus on chromosome 11q13 and clinical asthma. Analysis of nonatopic sibling pairs showed a strong linkage between bronchial hyperresponsiveness and the 11q locus, and no linkage was found between atopy and chromosome 11q [129]. However, from at least nine linkage studies performed by other groups, no linkage could be confirmed between atopy and chromosome 11q13 [101, 130–137].

In 1992, COOKSON *et al.* [138] provided evidence for the transmission of atopy at the chromosome 11q locus through the maternal line. Of 723 subjects studied, 259 were recruited through a proband attending a clinic with atopic asthma or rhinitis, the remainder *via* media appeals for large families with subjects known to have asthma and/or atopy. Atopy was defined by a skin-prick test response with a diameter of ≥2 mm more than the negative control, a positive specific IgE test result or a high concentration of total IgE. Affected sib pairs were more likely to share a maternal chromosome 11 than a paternal chromosome 11. The authors explained their finding by parental imprinting with suppression of expression of the atopy locus in paternally derived alleles, or by maternal modification of the infants immunoresponse.

Table 5. – Results of linkage studies on chromosome 11 with respect to asthma, immunoglobulin E (IgE) and bronchial hyperresponsiveness (BHR)

First author	[Ref.]	Year	Phenotype	Genetic analysis	Result	Comments
COOKSON	125	1989	Total IgE, SPT	LOD score	+	Most of the LOD score contributed by one family
YOUNG	126	1992	Total IgE, SPT	LOD score	+	
				LOD score, sib pair analysis	+	
MOFFATT	127	1992	Total IgE, SPT			
COLLEE	128	1993	Total IgE, specific IgE, respiratory symptoms	Sib pair analysis	+	
VAN HEWERDEN	129	1995	Clinical asthma	Sib pair analysis	+	
LYMPANY	130	1992	Specific IgE, SPT, BHR	LOD score	-	
RICH	131	1992	Total IgE, SPT	Sib pair/LOD	-	
COLEMAN	132	1993	Total IgE, specific IgE, SPT	Sib pair/LOD	-	Family ascertainment through probands with active atopic eczema
HIZAWA	133	1992	Total IgE, specific IgE, SPT	LOD score	-	
AMELUNG	134	1992	Total IgE, specific IgE, SPT	LOD score	-	
WATSON	135	1995	Atopy	LOD score	-	
NOGUCHI	101	1997	Total IgE, specific IgE	Sib pair analysis	-	
BRERETON	136	1994	SPT	Sib pair/LOD	-	
AMELUNG	137	1998	Total IgE, SPT	Sib pair/LOD	-	
COOKSON	138	1992	Total IgE, SPT	Sib pair analysis	+	Increased sharing of maternal alleles
SANDFORD	139	1993	Total IgE, SPT	LOD score	+	FcεR1-β on chromosome 11q1 Leucine^{181}→isoleucine^{181}
SHIRAKAWA	140	1994	Total IgE, specific IgE, SPT	LOD score	+	leucine^{183}→valine^{183}

SPT: skin-prick test; LOD: likelihood of disease.

SANDFORD et al. [139] found that the b subunit of the high-affinity receptor for IgE was located on chromosome 11q13 and was strongly associated with atopy. SHIRAKAWA et al. [140] sequenced the FcεRI-β-subunit gene and found two polymorphisms, a substitution of leucine (Leu) by isoleucine at position 181 and of Leu by valine at position 183. These polymorphisms were associated with atopy [140]. In a study of 1,004 members of 230 families in Australia, the polymorphism Leu[181]/Leu[183] was found in 4.5% of the population. All 13 children who had inherited the maternal variant were atopic [141]. These polymorphisms could not be confirmed in other Dutch, Japanese or Australian populations [134, 142–144].

The most recent mutation reported is a substitution of Glu for Gly at position 237. In a Japanese population, this polymorphism was found in 18% of asthmatics, 8% of nonatopic asthmatics and 6% of controls. The frequency of this variant is 5% in the Australian population [144].

Chromosome 12

Since 1995, chromosome 12 has attracted attention in the identification of genes associated with asthma and related phenotypes. This chromosome harbours possible candidate genes, i.e. interferon gamma (IFN-γ), insulin-like growth factor-1, B subunit nuclear factor-γ (NFYB) and mast cell growth factor. BARNES et al. [145] studied IgE levels and doctor diagnosed asthma in two populations, one of 29 asthmatic probands ascertained from several clinics in Barbados, West Indies. Families were extended by recruiting all available parents, siblings and relevant family members. Another population consisted of 209 Amish subjects who had been described previously by MARSH et al. [97]. Affected sib pair analysis revealed linkage of asthma to D12S379, D12S311, D12S95 and D12S360 in the Barbadian population.

Additional sib pair analysis showed evidence for linkage between high total IgE levels and markers located in the same region. In the Amish population, sib pair analysis showed linkage between total serum IgE levels and 17 markers on chromosome 12q. In the Barbadian families, asthma was significantly associated with D12S379, D12S95 and PAH, with the strongest evidence for allelic association with D12S95 (p<0.002). In the Amish families, modest significant results were found for D12S379, D12S58, D12S806, D12S78, D12S800, D12S338 and PLA2G1B on this chromosome. Thus, there is evidence for linkage between asthma and IgE levels and chromosome 12q15–q21.1 in two different populations [145]. Linkage to high levels of IgE to 12q15–q24.1 was rapidly replicated in a German population of 52 children selected for high IgE levels [146]. Several other groups have now confirmed these results [85, 147]. Recently, WILKINSON et al. [148] studied 240 families, including 131 families recruited from the population without reference to asthma or atopy; 60 families were recruited through asthma probands with affected sibs and the remaining 49 families were ascertained through a proband without regard to family history. In this study, phenotypic variables were constructed into quantitative scores to define asthma and atopy. Evidence for linkage was found between marker D12S342 and asthma, D12S324 and asthma score and D12S366 and wheeze [148].

Thus, there is substantial evidence that chromosome 12q14–24.3 contains genes which are related to asthma phenotypes.

Chromosome 14

It is now well established that bronchial mucosal inflammation is an important factor in the pathogenesis of asthma. T-lymphocytes play a key role in orchestrating the interaction of participating cells. There are two alternative T cell antigen receptors (TcRs); most T cells exhibit the αβ-TcR and ~4% exhibit the γδ TcR. The αβ-TcR recognizes antigens that are bound to major histocompatibility complexes molecules. The α and β chain are located on chromosome 14 and the β chain is located on chromosome 7. Activation of the T cell through binding of antigen or its fragments may result in a T-helper cell (Th)1 or Th2 response in which Th1 cells are generally defined by the synthesis of IL-2, interferon (IFN)-γ and TNF-α. Th2 cells are reported to produce IL-4, IL-5, IL-9, IL-10 and IL-13. The set of cytokines produced in the Th2 cell response is central in mediating IgE, production, whereas the Th1 profile has more of an anti-inflammatory nature [149, 150].

MOFFATT et al. [151] investigated genetic linkage between specific IgE reactions to major allergens (house dust mite grass pollen, Der p I, Der p II and Fel d I) and the TCR (αβ subunit) located on chromosome 14 and 7, respectively. Two different populations were studied: 66 nuclear families and five extended pedigrees consisting of 410 British individuals and 413 Australian subjects from 88 nuclear families. Families were ascertained through family members with asthma or rhinitis. No linkage was found in the TcRβ microsatellite in either population; there was significant linkage in the whole population for each phenotype in affected sib pairs, since the affected sib pairs showed significant allele sharing of TcRα microsatellite alleles from both parents. Nonaffected sibling pairs showed no significant allele sharing [151]. Support for this finding came from the Collaborative Study on the Genetics of Asthma, in which linkage was found with chromosome 14 in the Caucasian population [84].

A Japanese study by NOGUCHI et al. [152] found linkage between markers near the β gene (chromosome 7) and both IgE levels and asthmatic phenotypes; no linkage was detected for specific IgE levels and asthma to markers near the TcRα gene [152].

To date there seems to be evidence for genetic linkage between asthma phenotypes and the TcRα gene on chromosome 14, but conflicting results exist between the different studies, which may be due to differences in selection criteria and ethnic background.

Summary and future directions

The present review shows that genetic influences are important in the pathogenesis of asthma and allergy. Asthma and its related phenotypes are considered complex since they are determined by interactions between major and minor genes and nongenetic factors (environment) are also usually important in the expression of the disease.

The mode of asthma inheritance is still not known. Many segregation studies are not unanimous in their outcome, probably due to the different populations studied, selection of the populations towards asthma, differences between

populations in environmental exposure, genetic differences between populations and differences in analytical approaches between the various investigators. In spite of these problems, data suggest that a major locus is involved in determining serum IgE levels. Compatible with the results of the segregation analysis, twin studies have also shown evidence for genetic influences in the pathogenesis of asthma. Several large-scale questionnaire-based studies in different countries have shown a heritability of 60–79%. Twin studies have revealed substantial additional evidence for environmental influences, of which nonshared environment appeared to be important. Genetic modelling in the different twin studies are in accordance with each other and evidence has been found for a major locus regulating IgE levels.

Linkage and association studies suggest that multiple genes are involved in the development or the severity of asthma. At least four regions in the human genome, chromosomes 5q31–33, 6p21.3, 11q13 and 12q14.3–24.1, contain genes consistently found to be associated with asthma and its related phenotypes, but several other regions (7, 14, 19q13 and 21q21) could also contain candidate genes involved in the pathogenesis of asthma (table 6). For complex diseases like asthma, problems arise concerning the inconsistent results of linkage and association studies. In the case of chromosome 11q and 5q31–33, positive studies have been reported from different ethnic backgrounds, yet negative results have also been published, even in the same ethnic groups. The reason for these inconsistensies may lie in differences in ascertaining the families under study, genetic heterogeneity, environmental differences between populations and gene/environment interactions [123, 146]. Moreover, published association studies frequently lack sufficient power due to the low numbers of individuals investigated.

Several genome-wide screens have been published and have confirmed the earlier positive linkage results, but different regions have been linked to asthma and its related phenotypes in different ethnic groups, suggesting that different genes have a major effect in asthma in various ethnic groups, or that possible interaction with different environmental factors plays a major role.

It can be expected that not only genes associated with the development of asthma but also genes which modulate the outcome of asthma will be found in the future. The Human Genome Project is working hard to sequence the human genome and will probably detect new candidate genes implicated in the pathogenesis and severity of asthma. The identification of asthma susceptibility genes will probably provide more insight into the identification of individuals at risk of asthma in the near future and open the way for early prevention. New genes will offer a better understanding of full or partial resistance to therapy and progression into a more severe asthma phenotype and the development of more effective medical treatment. The recent observation that a mutation in the promoter region of the 5-lipoxygenase gene is associated with a smaller or absent response to a leukotriene antagonist [153] supports the notion that pharmacogenetics may open a way for better patient management.

An important question regarding the genetics of asthma is whether the same genes are involved in asthma, bronchial hyperresponsiveness and atopy or whether different genes are of relevance and, most importantly, whether and how these genes interact with the environment. These questions remain to be answered.

Future investigations should deal with the above problems and be directed at genetically homogeneous populations for whom the environment is stable (founder populations) [154], outbred populations and twin studies. The latter will be particularly helpful in studying the relation of the genetic and environmental variances contributing to a trait. The classical twin study, the parental twin design and the cotwin control study are suitable for this purpose. More work is needed to address more precisely the different asthma phenotypes, and the impact of environmental influences which may be related to asthma. Furthermore, exchange of information between groups involved in the genetics of asthma is extremely important because of mandatory agreement on phenotypes and analytical approaches.

Finally, simply knowing which genes are associated with asthma will not reveal everything about the development of the disease. Rather, genetic and environmental

Table 6. – Candidate genes for asthma and related phenotypes

Function	Candidate genes	Region
Antigen presentation	IL-1α, IL-1β	2q21
Cytokines regulating IgE, mast cell and eosinophil function	IL-3, IL-4, IL-5, IL-9, IL-13, GM-CSF	5q31
Receptor kinetics	β-adrenergic receptor	5q32
Antigen presentation	HLA complex	6p
Inflammatory cytokines	TNF-α	6p21.3
T cell activation	TCR β receptor	7
Non-HLA antigen presentation		8p23
Signalling on mast cells, basophils and dendritic cells	FcεR1	11q13
IgE-dependent antigen presentation	FcεRII	11q13
T cell regulation, inflammatory mediators	IFN-γ, NYFB, MGF, iNOS	12q
T cell activation	Esterase-D	13q
	TcR α-δ complex	14q11.2
Induction of IgE with IL-4	IL-4 receptor	16p, 17p11–q11.2
Inflammatory mediators	STAT-5, RANTES	17q

IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor; IgE: immunoglobulin E; HLA: human leucocyte antigen; TNF-α: tumour necrosis factor-α; TcR: T cell antigen receptor; IFN-γ: interferon gamma; NFYB: B subunit nuclear factor-γ; MGF: mast cell growth factor; iNOS: inducible nitric oxide synthase; STAT-5: signal transducer and activator of transcription-5; RANTES: regulated on activation, normal T cell expressed and secreted.
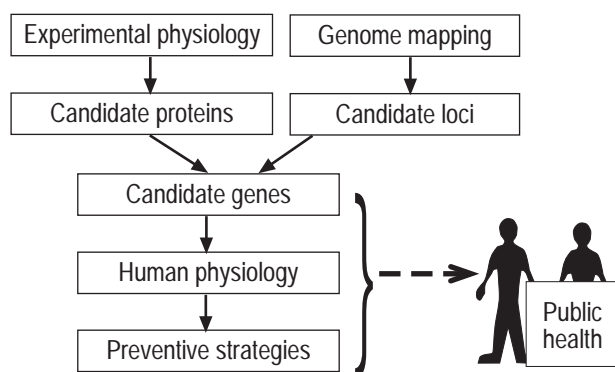
Fig. 3. – Genetic discovery: the link with public health. Implications of several approaches to unravelling the development and progression of asthma for public health.

influences which play a role in early stages of its pathogenesis, will eventually result in asthma. Are the same genetic influences expressed before and after development of irreversible airway obstruction as a consequence of airway remodelling? These questions can be solved by longitudinal studies investigating changes in gene expression in response to environmental influences [155].

Conclusions

Asthma research has made a step forward in studying the genetics of asthma. Genes relevant to the severity of asthma have been found, which may ultimately lead to better, most probably individually tailored, treatment strategies. A fruitful interaction between researchers involved in pathophysiology, epidemiology, clinical research and genetics is mandatory. Research in animal models is not addressed in this review, but hopefully will also help in discovering the function of as yet unknown but so on to be discovered genes (fig. 3). The next century holds promise for a better understanding and management of asthma. Genetics will contribute significantly in this respect.

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