

## Phenotype Definition, Age, and Gender in the Genetics of Asthma and Atopy

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Asthma and atopy are complex genetic diseases. The development of disease results from an interplay between multiple genes and environmental factors. The application of genetics has its origin in the tremendous progress in genetic research over the last decades. This started in 1953 with the seminal paper of Watson and Crick on the structure of DNA [1]. It was in 1956 that the correct chromosome number in humans was announced in Copenhagen at the First World Congress of Human Genetics: a diploid number of 46, not 48 as previously thought [2]. It was not merely getting the count right that was of importance; it showed that human chromosomes could be investigated with relative facility. This led to the discovery of genetic origins of many human diseases with Mendelian genetic disorders and proceeded with the publication of the working draft of the sequence of the human genome in 2001 [3,4]. Since then, large steps have been made in the hunt for genes and in understanding the complexity involved in the development of complex diseases such as asthma and atopy. The results of genome screens in 11 different populations identified at least 18 regions

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of potential linkage to asthma and atopy [5]. Hundreds of genetic association studies on asthma and atopy have been conducted, and these have reported variants in more than 64 candidate genes that may contribute to the development of asthma and atopy [6]. Despite the successes of genetic research, there are great difficulties in replicating findings between study groups. Various reasons for the discrepant results of studies have been described, such as (1) the different ethnic backgrounds of the populations studied, (2) the variable influence of environmental factors in different countries with different lifestyles of the studied populations, and (3) insufficient power of studies to detect minor genetic effects.

Another equally important reason for the contradicting results are the widespread differences in phenotype definitions. For example, when asthma was defined as a doctor's diagnosis it was shown to reflect less severe asthma than when it was also confirmed with a measurement of airway hyper-responsiveness (AHR) [7]. When studying atopy, one can imagine that atopic individuals defined by self-reported atopy constitute a heterogeneous group compared with an atopic population defined by a positive reaction to skin-prick testing.

Finally, genetic studies may have different results because important determinants of the asthma phenotype, such as age and gender of the studied populations, differ. Because asthma and atopy are heterogeneous diseases that show large differences between age groups in disease incidence and prevalence, the influence of certain genes may be different in childhood compared with adulthood. Also, different genetic mechanisms or gene-environment interactions may be involved in the development of asthma and atopy in men or women. Findings of genetic studies could thus be dependent on the window of time in which the study subjects are examined and on the gender of the subjects studied. Few genetic studies deal with age and gender issues, and frequently studies do not even describe the age and gender of the study population.

This article discusses the importance and influence of phenotype definition. The influence of age and gender on asthma and atopy phenotypes is addressed as an epidemiologic issue. Finally, the influence of age and gender on the results of genetic studies is discussed, and examples from the literature are provided.

### **Asthma and atopy phenotype definitions**

To find the genes contributing to the asthma and atopy phenotypes, it is important to have a clear definition of the phenotype. Atopy can be defined as "a hereditary predisposition to produce IgE antibodies against environmental allergens that is associated with one or more atopic diseases such as bronchial asthma, urticaria, eczema and allergic rhinitis" [8]. Asthma has been defined by the Global Initiative for Asthma [9] as "a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in AHR that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with

**Box 1. Possible definitions of asthma and atopy in genetic studies***Asthma*

## Questionnaire data

- Symptoms occurring once or several times at follow-up (wheeze, dyspnea, cough, nocturnal symptoms)
- Self-reported (doctor-diagnosed) asthma
- Use of asthma treatment
- Video questionnaire
- Doctor diagnosis

## Intermediate phenotypes of asthma

- Airway hyper-responsiveness
  - Direct (methacholine, histamine)
  - Indirect (exercise, mannitol, AMP, cold-air challenge)
- Reversibility on  $\beta$ 2-agonist
- Variability of peak expiratory flow
- Lung function (eg, FEV<sub>1</sub>, VC, micro Rint)

## Combination of questionnaires and intermediate phenotypes

- Asthma score
- Asthma algorithm

*Atopy*

## Questionnaire data

- Symptoms of atopic asthma, rhinitis, and dermatitis
- Self-reported (doctor-diagnosed) atopic asthma, rhinitis, or dermatitis
- Use of atopy treatment
- Doctor diagnosis

## Intermediate phenotypes of atopy

- Serum total IgE
- Serum allergen-specific IgE
- Allergy skin tests
- Number of eosinophils in peripheral blood

widespread but variable airway obstruction that is often reversible either spontaneously or with treatment.” These definitions show that atopy and asthma have many features and that there is not a single measurement that confirms or excludes either disease. To circumvent this problem in studies on the genetics of asthma and atopy, several strategies have been used (Box 1): use of intermediate phenotypes, an asthma algorithm or asthma score, and (video) questionnaires with questions regarding doctor’s diagnosis and symptoms. The success of these strategies depends greatly on the population under study. For example, in a child under 6 years of age, it is not possible to perform a spirometry and a bronchial provocation with methacholine. Other methods to measure lung function and bronchial hyper-responsiveness in small children have been developed, but these methods have not been standardized and validated, and a gold standard is lacking at that age. Moreover, the methods differ from the standard methods used in older children and adults. Therefore, comparison of these methods for small children with the standard methods cannot be justified. One more example is the difficulty to distinguish asthma from chronic obstructive pulmonary disease in elderly populations because both groups include subjects with respiratory symptoms, bronchial hyper-responsiveness, and airway obstruction that is partly reversible. To assess the importance and suitability of an intermediate phenotype in genetic research of complex diseases, the following criteria can be used: (1) a high genetic component, (2) regulation by major genes, (3) objective measurement, (4) quantification, (5) feasibility in every individual, (6) diagnostic value for asthma or atopy (sensitive and specific), and (7) enabling replication between studies.

This article focuses on the following phenotypes: asthma assessed by doctor’s diagnosis or symptoms, total serum IgE, and sensitization defined as a positive reaction to skin prick tests or the measurement of specific IgE, and AHR. These phenotypes have proven their usefulness in genetic studies, and the influence of age and gender on their expression has been extensively studied [10].

## **The influence of age and gender: epidemiology**

### *Asthma and age*

Asthma is a heterogeneous condition with variability between patients and within each patient over time. There is a wide variation in how the disease presents itself and how it is being diagnosed. As a consequence, several different asthma phenotypes have been described.

### *Childhood asthma*

According to Bel [11], childhood asthma can be divided into four different phenotypes based on age of onset, remission, (genetic) risk factors, pathogenetic mechanisms, prognosis, and treatment approaches (Fig. 1). The first description of different asthma phenotypes in childhood was provided by Martinez and

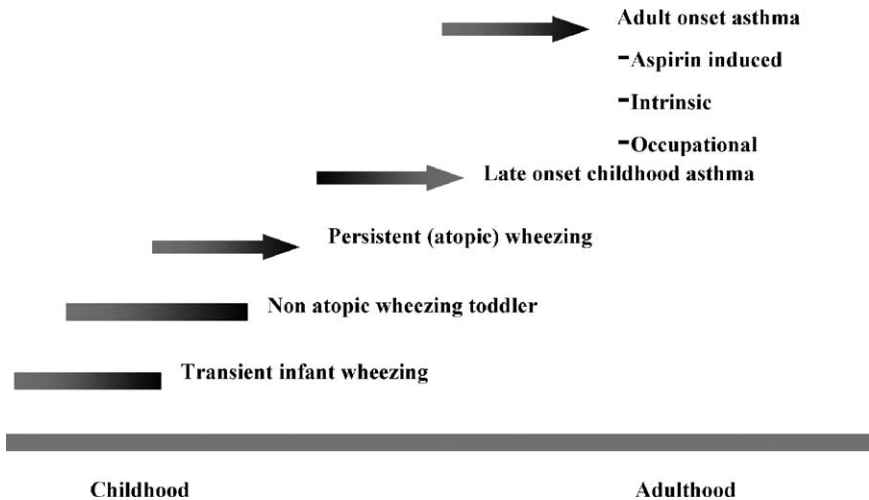


Fig. 1. Clinical phenotypes of asthma in childhood and adulthood. (Data from Bel EH. Clinical phenotypes of asthma. *Curr Opin Pulm Med* 2004;10:44–50.)

colleagues [12] based on the results of a large prospective study of 1246 newborns in Arizona (the Tucson Children's Respiratory Study) [13]: (1) the "transient infant wheezers" includes children who show nonatopic wheezing until 3 years of age and have a favorable prognosis. (2) The "nonatopic wheezing toddlers" include children who continue to wheeze beyond the third year of life and seem to develop airway obstruction in relation to viral infection. These two wheezing phenotypes in childhood are self-limiting and do not reflect asthma. (3) The persistent IgE-mediated wheezers develop persistent, chronic asthma. (4) A fourth childhood asthma phenotype was derived from a retrospective study by De Marco and colleagues [14]. This asthma phenotype occurs during or after puberty, affects mainly girls, and has a low remission rate. Most children with mild intermittent asthma outgrow their asthma as adults, but the more troublesome their asthma in childhood, the less likely they are to outgrow the disease [15].

#### *Adult asthma*

Asthma starting in adulthood seems to be different from childhood asthma. Adult-onset asthma can be subdivided in several subtypes with distinct underlying pathophysiologic mechanisms, such as aspirin-induced asthma or severe asthma [16] and steroid-resistant asthma [17].

#### *Asthma and gender*

Notwithstanding the variation in phenotype definition used to assess asthma, epidemiologic studies of questionnaire defined asthma find apparent gender differences in the development and outcome of asthma. During childhood and

adolescence, boys are nearly twice as likely as girls to develop asthma. The higher male incidence [18–21] and male prevalence of asthma continues until 16 years of age [22–25]. This is reflected by the hospitalization rates from asthma in early childhood. For instance, the hospital admission rate at 1 year of age is 5.3/1000 for boys and 2.9/1000 for girls in Finland [26]. The higher incidence and prevalence of asthma in boys reverses around the age of 16 years. In young adulthood, female gender becomes an important risk factor for the development of asthma [27], and throughout adulthood incidence and prevalence of asthma are greater in women [20,23,28–32]. Additionally, the subtype severe asthma seems to affect mainly adult women [16].

### *Total IgE and age*

At birth, IgE concentrations in cord blood are generally low. Johnson and colleagues [33] found a geometric mean IgE of 0.20 IU/mL in 538 healthy newborns recruited from a general population. IgE levels seem to rise during childhood and reach a peak value between 8 and 12 years of age [33–39]. During adolescence, the mean total IgE level of asthmatic and general populations declines steeply and continues to decline at a slower pace after 35 years of age [35,38,39] (Fig. 2A). Epidemiologic studies have shown that IgE levels of an individual track with age; thus, high IgE levels in infancy are highly correlated to high IgE levels later in life [35,40]. These data are largely based on cross-sectional studies and thus may be biased by the rising incidence of allergy observed in the last decades, which specifically affects younger individuals, thereby suggesting a reduction with age. This does not seem to offer the full explanation of the cross-sectional observations because one longitudinal study finds a pattern of IgE changes with age similar to the description above [39].

### *Total IgE and gender*

Although boys and girls have similar IgE levels measured from cord blood at birth [33,41] and IgE levels increase with age in both genders, IgE rises more rapidly in boys. Serum IgE levels are consistently found to be significantly higher in boys compared with girls above the age of 6 months, and the levels remain to be higher in boys throughout childhood [33,37,42,43]. In adulthood, men seem to have a higher total IgE than women in a general and an asthmatic population [44–47]. This observation remains significant when corrected for smoking habits. It is intriguing that men have higher IgE levels than women because in this age group asthma prevalence and incidence—which is known to be highly correlated to the atopic status of an individual—are higher among women.

### *Sensitization and age*

Sensitization is defined as one or more positive skin prick test(s) or the presence of specific IgE antibodies in serum. During childhood, the time course

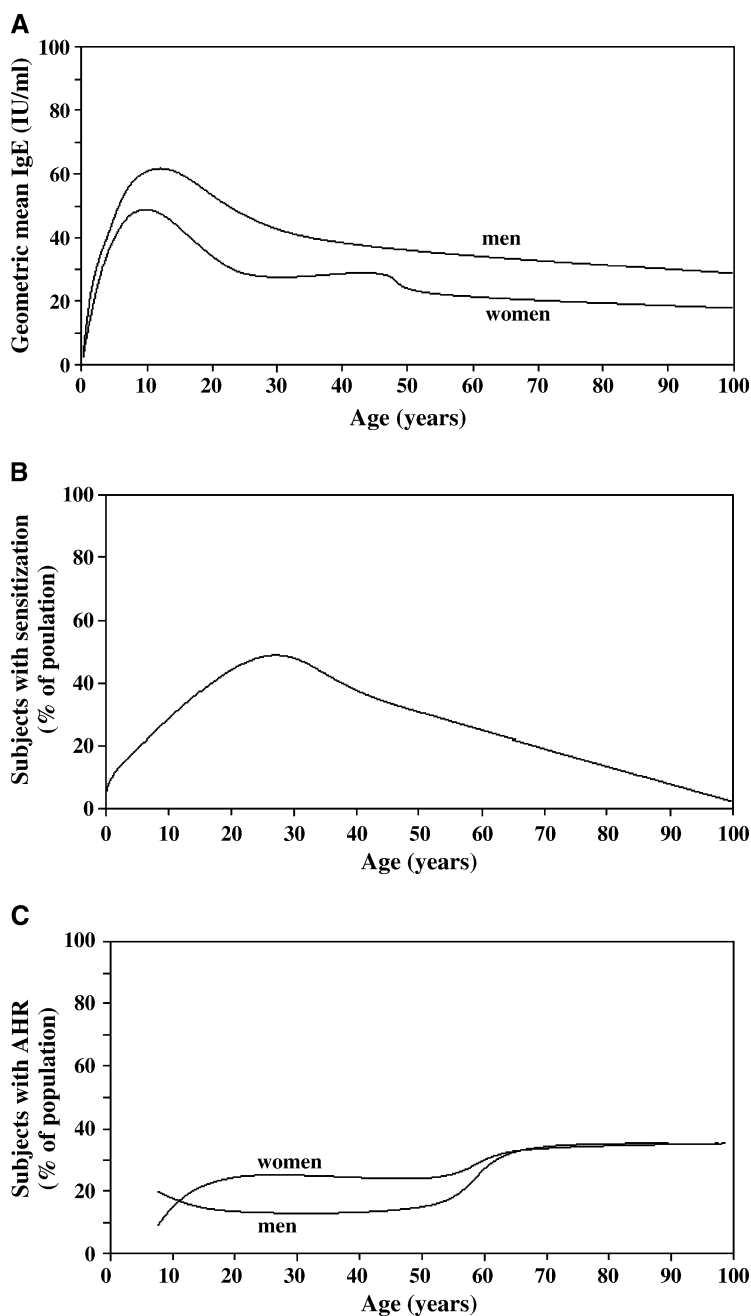


Fig. 2. Hypothetical figures on the relationship between age, gender, and phenotype based on data from the literature. (A) The geometric mean total IgE level and age in men and women in Caucasian populations. (B) Sensitization to inhalant allergens and age (no gender difference). (C) Bronchial hyper-responsiveness and age in men and women.

of sensitization to food and sensitization to inhalant allergens differs. IgE synthesis starts during fetal life, and specific IgE to food and inhalant allergens can be measured early in life in cord blood at birth, albeit in low concentrations [48,49]. Sensitization to food allergens occurs mainly in infancy and tends to be transient, whereas sensitization to aeroallergens increases throughout childhood and tends to be persistent [50,51]. In general, sensitization seems to increase during childhood and adolescence [36,50–55] and reaches a peak in the third decade of life. The prevalence of sensitization and the size of skin prick test reactions decline in persons older than 30 years of age [35,44,56–59] (Fig. 2B). This time course in sensitization to common allergens was observed in cross-sectional studies and may reflect a rising incidence and prevalence of allergic diseases generally observed during the last decades. In one longitudinal study [39], skin prick tests were performed at baseline and after a follow-up of 8 years in 1333 subjects 3 to 65 years of age at baseline. This study shows an increase in sensitization among all age groups, which has been confirmed by Broadfield and colleagues [60]. The greatest increase in prevalence occurred among children and teenagers, with only minimal increases above the age of 65 years. The strength of the reaction, estimated by skin test index, peaked between 25 and 35 years of age.

### *Sensitization and gender*

Studies on sensitization in relation to gender do not show consistent results. The results may vary with age of the population studied. The number and species of the allergens tested also seems to be of importance. Some studies mention a difference in the type of sensitization between males and females in childhood and in adulthood [33,44,52,55]. This may reflect a difference in exposure to environmental influences between males and females. However, in infancy this explanation is not plausible.

### *Airway hyper-responsiveness and age*

AHR is defined as an exaggerated response to contractile, nonallergic stimuli such as methacholine, histamine, or hypertonic saline [61]. Measurement of AHR in early childhood is not feasible due to the cooperation and coordination needed from the child to perform accurate lung function measurements. There is no standardized measurement available for children younger than 6 years of age. Because of these difficulties, studies of AHR in childhood are few and have relatively small numbers of subjects. Furthermore, accurate dosing of the challenging agents in children continues to be a matter of debate. As a result, differences in study methods make comparison of studies for AHR in early childhood difficult to perform. Despite these difficulties, studies in children above 5 years of age have shown that AHR changes with age (Fig. 2C). In childhood, airway responsiveness seems to be relatively high. There is a decrease

in AHR during adolescence, and this stabilizes in adulthood [62–68]. Some studies mention an increase of airway responsiveness above approximately 55 years of age [63,65,69]. These changes in airway responsiveness with age occur in symptomatic and in asymptomatic individuals.

### *Airway hyper-responsiveness and gender*

Despite difficulties in comparing studies of AHR at very early age, studies show evidence for a gender difference in AHR that is similar to the pattern of incidence and prevalence of asthma. In childhood, le Souef and colleagues [70] and Paoletti and colleagues [63] describe a greater responsiveness in boys than in girls, a finding that reverts during adolescence, where girls have a higher prevalence of hyper-responsiveness. Boys exposed to environmental tobacco smoke show significantly greater peak expiratory flow variability, reflecting larger airway lability [41]. A higher prevalence of AHR in women compared with men [63,65,69,71–73] has been identified in large epidemiologic studies in adult populations. Several explanations for this gender difference at adult age have been proposed. Correction for smaller airway size, lung volume, or baseline lung function parameters in women does not explain the gender difference in some studies [63,69,72,73], but it does in others [69,71]. These discrepancies may be explained by differences in statistical analysis (eg, the use of a quantitative measure [slope of dose-response curve] or an absolute measure of AHR [AHR: yes or no]). Another explanation mentioned for the gender difference is a greater susceptibility to the effects of smoking in females.

### *The influence of age and gender: genetics*

Theoretically, age and gender effects could be explained by the following characteristics:

1. Similar disease susceptibility genes but different disease expression modified by age or gender (modifying effect)
2. Different susceptibility genes in childhood asthma compared with adult-onset asthma and in males compared with females (genetic heterogeneity). This could be exemplified by asthma-related genes on the sex chromosomes.
3. Different environmental effects depending on age and gender, for example, the use of oral contraceptives (gene-by-environment interaction)
4. Epigenetic effects that may be dependent on age and gender

Few genetic studies have addressed the influence of age and gender on the development of atopy and asthma. Notwithstanding the fact that the available studies are small, they do provide the first evidence for age- and gender-related effects in the genetics of asthma and atopy.

*Age-related genetic studies*

One of the first attempts to identify whether genetic associations vary with age was performed by O'Donnell and colleagues [74]. They performed a genetic association study on longitudinal data on atopy and a polymorphism of CD14, a membrane receptor, involved in binding of lipopolysaccharide and thereby possibly influencing the postnatal Th2/Th1 shift. Failure in this immune system shift may be associated with atopic development, which makes it plausible that polymorphisms in the CD14 gene are especially associated with early atopic development. The CD14/–159 polymorphism has been associated with altered soluble CD14 and IgE serum levels in several cross-sectional studies. In an attempt to identify a possible age effect of CD14/–159 polymorphism, O'Donnell and colleagues collected longitudinal data from age 8 to 25 years on atopy and AHR from 305 subjects and genotyped these individuals. For atopy, AHR, and wheeze, these individuals were classified as having early persistent (present at age 8 or 10 years, then consistently present up to age 18 or 25 years), early remittent (present at age 8 or 10 years and at the next visit but then consistently absent up to age 18 or 25 years), late onset (absent at age 8 or 10 years, then present on at least two subsequent visits, with no visits after the age of 10 years showing absent), or no disease during follow-up. They discovered that individuals with –159CC were at higher risk for developing early-onset atopy and early-onset AHR (OR 2.2 and 2.6) compared with individuals carrying –159CT and –159TT. Cross-sectional analysis showed that the CD14/–159 CC genotype was associated with atopy and AHR in childhood but not in adulthood. Thus, it seems possible that the influence of the CD14/–159 polymorphism may cause an effect during childhood that fades away in adulthood. This suggests that other genetic or environmental factors play a role in the atopic development in late childhood or early adulthood. Because these findings do not explain why in other studies on CD14 associations are being found in adulthood [75,76], further studies need to confirm their observations.

Child and colleagues [77] studied glutathione S-transferase 1 (GSTP-1) because it may be of importance in the development of AHR. The enzyme is involved in detoxification of many environmental toxins, drugs, and by-products of oxidative stress that may otherwise cause inflammation of the airways [78]. Furthermore, variants of the GSTP-1 gene have shown association with risk of AHR and atopy in adults [79,80]. They indicated that it might be important to adjust for age in genetic association studies using the phenotype AHR. The interpretation of the measurement of AHR at young age is difficult because body size, breathing pattern, and baseline lung function may influence the measurement. In an attempt to solve these issues, they describe a method to correct dose-response slopes and PC20 values for the baseline parameters age, lung function, atopy, and height in 145 children 7 to 18 years of age [77]. The corrections made in this study had a marked effect on the AHR status in 70 of 122 children, 5 of 122 to a more severe and 40 of 122 to a less severe PC20 category. The corrections also resulted in a significant reduction of the mean dose-response

slope. The authors showed that this correction influenced the result of a genetic association study in these children. A previously unidentified association between the GSTP-1 genotype and AHR was found. This association would not have been found in this population if uncorrected AHR was tested for association with the GSTP-1 genotype [77].

One of the most extensively studied candidate genes for asthma and atopy is interleukin (IL)-13. IL-13, a cytokine primarily produced by T-helper type 2 (Th2) cells, may be important in the development of asthma and atopy because it promotes B-cell differentiation and is capable of inducing isotype class-switching of B-cells to produce IgE and IgG4 [81]. In mice, IL-13 contributes to AHR by inducing contractions of smooth muscle cells and stimulating overproduction of mucus [82]. Many studies describe one or more associations between various polymorphisms of the IL-13 gene with asthma and atopy phenotypes. We took a closer look at the results of studies investigating IL-13 polymorphisms to evaluate age and gender of the populations studied. To avoid bias by differences in ethnicity, we considered only white and Caucasian populations. Table 1 shows that there seems to be a difference between the associations found in childhood and associations found in adulthood. In childhood, consistent associations of single-nucleotide polymorphisms (SNPs) in the IL-13 gene with total serum IgE are found [83–87]. Given the difficulty to establish asthma in young children, in this age group the asthma phenotype was evaluated in only a few studies, and no associations with IL-13 SNPs were found [83,88]. In contrast, the studies performed in adulthood do find associations with asthma or AHR [89–91], but they do not find association with total serum IgE [89,90,92,93]. It is not clear why IL-13 is associated with IgE in childhood and not in adulthood. Oryszczyn and colleagues [94] showed that IgE levels seem to be stable in mid-adulthood, which suggests that in adulthood the environment may have a small effect on IgE level. In contrast, environmental factors in childhood may have a larger influence, which may bring about a gene–environment interaction that is not apparent in adulthood. Moreover, IgE levels are somewhat lower at adult age, compared with more variable and higher levels at childhood age, which may affect the outcome as well.

Finally, Ruse and colleagues [95] showed that serum IgE levels, but not the high-affinity IgE receptor polymorphisms, were associated with late-onset airway obstruction. They interpreted their findings as follows: interaction between environmental and genetic factors control serum IgE levels and disease pathogenesis may differ between early- and late-onset airway obstruction phenotypes. Thus, IgE may have different roles in childhood asthma and late-onset asthma.

Animal studies provide evidence for the existence of age-related genetics. Garret and colleagues [96] conducted time-course genetic analysis in selectively bred rats to evaluate the genetic causes of albuminuria and proteinuria as early markers of renal disease. Genome scans performed at 8, 12, and 16 weeks of age identified quantitative trait loci (QTL) on nine rat chromosomes. The QTL identified were variable with time and age of the rats. A locus for proteinuria on

Table 1  
Association studies on IL-13 polymorphisms in white and/or Caucasian populations

| Author                  | Number of subjects | Age                                                                                            | Association                               |                                                                                            |                                                                               | IL-13 SNPs tested <sup>a</sup>                                    |
|-------------------------|--------------------|------------------------------------------------------------------------------------------------|-------------------------------------------|--------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------|
|                         |                    |                                                                                                | Total IgE                                 | Sensitization                                                                              | Asthma/BHR                                                                    |                                                                   |
| Hoffjan [85]            | 207                | 1 yr                                                                                           | <i>P</i> = .0026                          | n.a.                                                                                       | n.t.                                                                          | Arg130Gln                                                         |
| He [88]                 | 329                | 2 yr                                                                                           | n.t.                                      | OR 2.5, <i>P</i> = .014                                                                    | n.a.                                                                          | Arg130Gln<br>– 1111C/T                                            |
| Liu [86,87]             | 482                | Cohort; 1–7 yr                                                                                 | OR 2.38, 95%CI 1.35–4.21, <i>P</i> = .003 | OR 3.49, 95%CI 1.52–8.02 (food allergens)/<br>OR 2.27, 95%CI 1.04–4.94 (outdoor allergens) | n.t.                                                                          | Arg130Gln<br>– 1111C/T                                            |
| Graves [84]             | 1399               | 9–11 yr                                                                                        | <i>P</i> = .000002                        | n.t.                                                                                       | n.t.                                                                          | Arg130Gln<br>– 1111C/T<br>– 1512A/C<br>Arg130Gln<br>– 1111C/T     |
| DeMeo [83]              | 666                | Mean 8.16, SD 2.11 yr                                                                          | <i>P</i> = .04                            | <i>P</i> = .0069                                                                           | n.a.                                                                          | Arg130Gln                                                         |
| Hummelshoj [92]         | 342                | Mean 27 (allergic subjects);<br>31 (controls); range 17–66 yr                                  | n.t.                                      | OR 2.1, <i>P</i> = .0053                                                                   | n.t.                                                                          | Arg130Gln<br>– 1111C/T                                            |
| Heinzmann [89]          | 300                | Young adults; no further information                                                           | n.a.                                      | n.a.                                                                                       | OR 1.83, 95%CI 1.13–2.99, <i>P</i> = .014<br><i>P</i> = .008, <i>P</i> = .007 | Arg130Gln<br>– 1111C/T                                            |
| Howard [90]             | 368                | Mean 52, range 34–76 yr                                                                        | n.a.                                      | <i>P</i> = .02                                                                             |                                                                               | Arg130Gln<br>– 1111C/T<br>3' untranslated region G/A<br>– 1111C/T |
| Van der Pouw Kraan [91] | 208                | Not mentioned<br>Recruitment from outpatient department of pulmonology; thus, probably adults. | n.a.                                      | n.a.                                                                                       | OR 7.8, <i>P</i> = .002                                                       |                                                                   |
| Nieters [93]            | 640                | 35–65 yr; no further information                                                               | n.a.                                      | n.a.                                                                                       | n.t.                                                                          | Arg130Gln                                                         |

Abbreviations: BHR, bronchial hyperresponsiveness; CI, confidence interval; n.a., not associated; n.t., not tested; OR, odds ratio; SNP, single-nucleotide polymorphism.  
<sup>a</sup> Synonyms of SNPs: Arg130Gln = Arg110Gln = Gln110Arg; – 1111C/T = C-1112T = – 1055C/T = – 1024C/T = – 1112C/T.

chromosome 10 was present at age 12 weeks and not at age 8 and 16 weeks. This suggests that the genes underlying these disease loci have a variable influence on the phenotype that changes with age. This indicates that the complex genetic mechanisms causing renal disease differ between early onset and late onset or progression of the disease. Future studies have to elucidate whether this is also the case for atopy or asthma.

### *Gender-related genetic studies*

The cytotoxic T lymphocyte-associated 4 receptor (CTLA-4) may be important in the development of atopy and asthma because it is involved in a costimulatory pathway regulating T-cell activation and subsequent IgE production. Chang and colleagues [97] found an association between the CTLA-4 genotype and cord blood IgE in a population of 644 Chinese newborns. This association with the CTLA-4 (+49 A/G) polymorphism was found only in females. In an adult Chinese population, Yang and colleagues [98] found an association of the CTLA-4 (+49 A/G) genotype and serum IgE levels, again only in females. It had been previously shown that these same CTLA-4 polymorphisms are associated with atopy or asthma [99,100]. However, these studies did not stratify for gender in their analysis. Thus, the results on a putative role of CTLA-4 SNPs in the development of atopy and the specific gender effects have been replicated in a second population. Furthermore, the CTLA-4 (+49 A/G) polymorphism has been shown to alter T-cell activation in human cells [101]. To our knowledge, there is no biologic mechanism described to explain the gender differences observed in this genetic association.

Szczeklik and colleagues [102] have described a polymorphism that shows a genetic association in women with asthma, but not in men: the prostaglandin endoperoxide H synthase COX-2 (−165 G/C). This COX-2 enzyme may be involved in asthma development as a mediator of bronchial inflammation. The COX-2 (−165 CC) homozygotes were over-represented in female but not in male asthmatics (odds ratio 3.08, 95% confidence interval 1.35–6.63,  $P = .01$ ). A functional effect of this polymorphism was confirmed by investigating prostaglandin production by peripheral blood monocytes in vitro as related to the genotype of patients. Peripheral blood monocytes obtained from CC homozygous women produced increased quantities of prostaglandins compared with monocytes from female GG homozygotes. The researchers investigated only monocytes from female patients; thus the question of whether this functional effect was present only in female monocytes remains unanswered by the authors [102]. An explanation for the gender difference in this association study is not available. COX-2 has been studied extensively in relation to heart disease, and this may provide a clue to the gender differences. One COX-2 inhibitor (Rofecoxib) was recently discovered to enhance cardiovascular risks and was taken off the market. This effect on cardiovascular risks was seen particularly in females and especially in younger women who produce estrogen [103]. COX-2 is known to produce prostacyclin, and production of this fatty acid is blocked by the COX-2 inhibitors.

Prostacyclin acts through the prostacyclin receptor. Egan and colleagues [104] studied mice lacking this prostacyclin receptor. These mice were highly susceptible to atherosclerosis. In these mice, there was no gender gap in heart disease—a divergence long observed in people and in mice in which younger males are at higher risk for heart disease than younger females. Female mice lacking this prostacyclin receptor were highly susceptible to atherosclerosis through susceptibility to oxidative stress from free radicals, which boost plaque formation in arteries. The authors studied the effect of estrogen in relation to prostacyclin production and found that in mice, estrogen increased prostacyclin biosynthesis and depressed oxidative stress. This suggests that in premenopausal females, the relative protection for atherosclerosis may be induced by their endogenous estrogen that, acting through one of its receptors, stimulates COX-2 production and prostacyclin production. This also explains why COX-2 inhibitors enhance cardiovascular risks by extinguishing the beneficial effect of estrogen in premenopausal women. The presumed estrogen dependent biosynthesis of COX-2 also explains why the genetic association of the COX-2 (−165 G/C) polymorphism with asthma before was only found in females. In males, who have low levels of estrogen, a polymorphism in the COX-2 gene has small effects.

A polymorphism of the proinflammatory cytokine IL-1 $\beta$  was found to be associated with asthma only in men in a cohort of 245 asthma patients and 405 controls [105]. The difference in genotype between asthmatics and controls was seen comparing heterozygote men with homozygote men, irrespective of the alleles. This is difficult to explain biologically, and the authors could not solve this problem. There was also no biologic explanation for the gender difference observed. These results need to be replicated before conclusions are drawn.

## Summary

When studying genetics of complex diseases it is important to have a clearly described and objective phenotype. When drawing conclusions in association studies, age and gender of the population studied should be considered. Until we know what causes phenotypic differences between males and females and between children and adults, we should try to study longitudinal cohorts with phenotype assessment at different time points and stratify our analyses for gender. To acquire sufficient power for these types of analyses, international collaboration may be the only way to elucidate the intricate gene–environmental interactions in atopy and asthma in an age- and gender-dependent manner.

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