



Association between Gm allotypes and asthma severity from childhood to young middle age

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Summary

Immunoglobulin constant heavy G chain (*IGHG*) gene polymorphisms are associated with atopy and can be determined by the serum Gm allotypes. We studied whether certain polymorphisms are related to asthma severity and to the extent or intensity of allergic sensitization in asthmatic subjects followed from childhood to young middle age.

Fifty-five subjects (28 males) with childhood asthma were all followed-up prospectively on six occasions from a mean age of 9 to 35 years in a study including asthma severity scoring, spirometry, skin prick, and specific serum IgE antibody testing. At the last visit, extended lung function tests and a cold air challenge were performed, and *IGHG* gene polymorphisms were identified by the alternative serum IgG subclass allotypes, employing ELISA and double immunodiffusion.

The 19 subjects with the homozygous *IGHG***bf*/**bf* genotype (originating from the *IGHG*3**b* and the *IGHG*1**f* alleles, which are in strong linkage disequilibrium), showed significantly higher asthma scores, lower airway function, and greater bronchodilator responses from childhood to adulthood, and in middle age greater airway hyperresponsiveness, compared to the subjects with the *IGHG***bf*/**ga* or *IGHG***ga*/**ga* genotypes. Among the subjects sensitized to animal danders, those with the *IGHG***bf*/**bf* genotype showed the highest specific IgE levels.

In conclusion, *IGHG* gene polymorphisms were associated with the severity and outcome of childhood asthma, and with the intensity of allergic sensitization.

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Introduction

Asthma is a heterogeneous disorder differing widely in age of onset, severity and outcome. Long-term follow-up studies indicate that growing out of childhood asthma is the exception rather than the rule, and that disease severity in childhood is an important determinant of the outcome.^{1,2} We have previously reported the course of allergic sensitization, clinical allergy, asthma severity, and airway function in 55 asthma subjects followed-up on six occasions from a mean age of 9–35 years.^{3–7} We found that allergic sensitization and reported allergies persisted in most subjects, and that the extent of sensitization to perennial allergens was associated with asthma severity and airway hyperresponsiveness.^{5–7} However, this relationship was not straightforward, suggesting that additional modifying factors are involved.

Gm allotypes (marker for IgG) refer to genetic variations or polymorphisms at loci encoding for the constant heavy chains of IgG (IGHG) and can be identified in serum.^{8–10} The genetic codes for the heavy constant chains of the IgE and IgG molecules are found on chromosome 14q32.3, with the immunoglobulin heavy constant chain (IGH) genes in the order 5'μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2, 3'.⁸ The IGHG genes display Mendelian inheritance and are expressed randomly by allelic exclusion.⁸ The alternative serum allotypes from the γ3, γ1, or γ2 genes have only minor amino acid differences,¹¹ but they are separate entities with distinct immunochemical and functional characteristics, such as fractionation rates, electrophoretic rates,¹² half-life times,¹³ and different maturation rate during childhood.¹⁴ Table 1 gives the nomenclature used in this paper for the IGHG genes, alleles, and IgG subclass allotypes. Four subsets of IGHG haplotypes (B cells) have been recognized based on the alternative expressions from the *IGHG3*, *IGHG1*, and *IGHG2* genes, and can be identified via their expressed subclass allotypes.¹⁵ The *IGHG3* and *IGHG1* genes are in absolute linkage disequilibrium (LD): *IGHG3*b* is linked to *IGHG1*f*, and *IGHG3*g* to *IGHG1*a*.

Previous studies have shown that some Gm allotypes are associated with atopy in children,¹⁶ with allergic childhood asthma,¹⁷ or with the acquisition of atopic allergy in adults.¹⁸ Polymorphisms of IGHG are also known to influence the susceptibility to several diseases, including bacterial and viral infections,^{19,20} and it is of particular interest that such polymorphisms appear to determine the strength of the antibody response to various antigens.^{20–22} Our

well-characterized asthma cohort gave us the opportunity to investigate a possible relationship between certain Gm allotypes and the extent or intensity of allergic sensitization, and to study the association between Gm allotypes and asthma severity from childhood to young middle age.

Methods

Subjects

The cohort included 55 subjects (28 males), all ethnic Swedes, with doctor verified childhood asthma. They were randomly recruited to a prospective follow-up asthma study (at a mean age of 9 years) among patients attending a pediatric outpatient asthma clinic. Three or more episodes wheezing were used for the diagnosis of asthma in accordance with Buffum.²³ This was the generally used asthma criterion in Sweden during the 1970s. The intentions were to include asthmatic children who would be at least 7 years old at the planned first follow-up (to allow spirometry) and to be half girls and half boys for gender comparison. All subjects attended all six follow-up visits (visit 2–7) at mean ages of 11.5, 13.6, 17.2, 24.3, 30.0, and 34.9 years. Detailed information about the cohort and the findings at all follow-ups has previously been reported.^{3–7}

IGHG genotyping by the IgG alternative subclass allotypes

The IgG subclass allotypes were determined by a sensitive competitive indirect ELISA, measuring both the presence and the serum concentrations of the IgG1*f, IgG1*a, IgG2*n, and IgG3*b allotypes and IgG subclass levels, as described in detail elsewhere.¹¹ The sensitivity of the ELISA was 0.0008 g/l for IgG1*a, 0.0003 g/l for IgG1*f, 0.0006 g/l for IgG2*n, and 0.0007 g/l for IgG3*b. The heterozygous IgG2*n/-n genotype was detected by murine monoclonals anti-G2m(n) 6016-10 clone SH-21 and anti-IgG2 HP 6014 (Sigma) in a double immunodiffusion assay. The *IGHG* alleles and four *IGHG* haplotypes (B cell variants) were identified, i.e. *IGHG*bfn* (B1), *IGHG*bf-n* (B2), *IGHG*gan* (B3), and *IGHG*ga-n* (B4).

Asthma scores

History was taken using a standardized interview questionnaire. Asthma severity (a composite total asthma score, 0–6) was estimated according to the frequency of wheezing episodes (symptom score, 0–3) and the extent of medication for asthma (medication score, 0–3), reported from the year prior to the visit.⁴ Symptom score 1 denoted occasional wheezing in connection with respiratory tract infections, heavy exposure to allergens, and/or hard physical exercise. Symptom score 2 denoted wheezing up to twice a week and/or after ordinary physical exercise. Symptom score 3 implied wheezing more often than twice a week and/or impairment of daily physical activities. Medication score 1 denoted occasional use of bronchodilator therapy for symptomatic relief, and/or the use of inhaled sodium cromoglycate or steroids for less than 3 months per year.

Table 1 Nomenclature and abbreviations used for the IGHG genes, alleles, and IgG subclass allotypes.

Gene	Alternative alleles	Corresponding IgG subclass allotypes
IGHG3 (γ3)	*b, *g	IgG3*b, IgG3*g
IGHG1 (γ1)	*f, *a	IgG1*f, IgG1*a
IGHG2 (γ2)	*n, *-n	IgG3*n, IgG3*-n
IGHG4 (γ4)	No allelic variations	IgG4 (no subclass allotypes)

Medication scores 2 and 3, respectively, denoted the use of bronchodilator therapy when needed and continuous use of one (score 2) or two (score 3) preventive drugs for asthma control.

Lung function tests

At visits 2–5, the forced expiratory volume in 1 s (FEV₁) was recorded using a wedge bellow spirometer before and after inhalation of salbutamol from a jet nebulizer. In a study performed in the same hospital during the 1970s of 85 healthy subjects aged 7–25 years, the mean (S.D.) increase of FEV₁ after salbutamol inhalation was 3.6 (2.3)% in males and 3.5 (2.7)% in females²⁴. All spirometric volumes were converted to body temperature, pressure and saturated air (BTPS) conditions, and expressed as percentages of predicted values. Swedish normative data obtained in children up to 18 years of age²⁵ or from adults^{26,27} were used where applicable.

At visits 6 and 7 FEV₁, vital capacity (VC), and specific airway resistance (sRaw) over five breaths were recorded at rest, 5 and 10 min after a cold dry air hyperventilation challenge (CACH), and after salbutamol inhalation, in a body plethysmograph.^{6,7} The maximum percentage fall in FEV₁ after CACH was noted.

Peripheral airway function was assessed at visit 7 using a He (helium) and SF₆ (sulfur hexafluoride) single-breath washout performed at rest, approximately 7 min after CACH and 15 min after bronchodilator therapy.²⁸ The concentration-normalized phase III slopes (Sn_{III}) were computed. A higher Sn_{III} value indicates greater inhomogeneity of ventilation distribution. In the present study only SF₆ results are reported.

Sensitization

At visit 7 total serum IgE concentrations and specific IgE antibodies to the three most important perennial inhaled allergens in Sweden, which are the danders of cat, dog, and horse, to three pollens (birch, timothy, and mugwort), and to two house dust mites (*Dermatofagoides farinae*, *Dermatofagoides pteronyssinus*) were determined using the Phadebas CAP method ("RAST"; Pharmacia Diagnostics, Uppsala, Sweden).⁷

Skin-prick tests (SPT) were performed using a panel of standardized allergen extracts (ALK Copenhagen, Denmark).⁷ A weal with a mean diameter (half of the sum of the largest diameter and its midpoint perpendicular) of more than 3 mm was regarded as positive. In the current analysis, the following allergens were included: pollens (birch, hazel, grass, and mugwort), furred animal danders (cat, dog, and horse), and house dust mites (*D. farinae* and *D. pteronyssinus*).

Ethics

The ethics committee at the Central Hospital in Skövde gave initial approval to the study. During childhood and adolescence the children and their parents gave their informed consent before every follow-up. In adulthood informed consent was received from all subjects.

Data analysis

Likelihood ratio (LR) tests were used to test for the Hardy-Weinberg equilibrium (HWE) and to compare diplotype distributions between the asthma cohort and a Caucasian reference population of 587 subjects.^{14,29} Haplotype frequencies for LD estimation were calculated using the Expectation-Maximization (EM) algorithm. Repeated measurements ANOVA was used to investigate genetic effects on bronchodilator response and baseline % predicted FEV₁ over all follow-up stations, with the allele count (0, 1, 2) as covariate, while the relationship between the mean total score taken over all follow-up visits and the genotype was tested with linear regression. At the last visit Spearman rank correlation tests were used to assess the relationship to allele counts. Proportions were compared with the Yates corrected χ^2 -test. Other two group comparisons were made using the Mann-Whitney U-test. A *p*-value <0.05 was accepted as statistically significant. No corrections for multiple testing were performed since genotypes are correlated with each other due to LD and because the phenotypes assessed by different measures and aspects of asthma are also highly correlated. A Bonferroni correction would therefore be overly conservative. Statistica 6.0 (StatSoft, Tulsa, OK, USA) was used for the statistical analyses.

Results

Genotype and allele distribution

Six *IGHG* genotypes were identified with respect to the alternative alleles for *IGHG3*, *IGHG1*, and *IGHG2*. There was a slight deviation from the HWE in the reference population (*p* = 0.004) with respect to *IGHG2* with fewer homozygotes (*IGHG2**n/*n 10.9%) than expected (22%).

The allele frequencies were similar in the asthma group and the reference population (Table 2). The three genes were all in LD. *IGHG3* and *IGHG1* were in absolute LD (*D'* = *r*² = 1), where *IGHG3**b was associated with *IGHG1**f. *IGHG1* and *IGHG2* were also in strong LD (*D'* = 0.87, *r*² = 0.30), where *IGHG**bf was associated with *IGHG**n.

Table 2 Frequencies of *IGHG1* and *IGHG2* alleles in the 55 subjects with childhood asthma and in a Caucasian reference population (*N* = 587; 157 blood donors and 430 healthy children).

	Asthma cohort		Reference population	
	<i>N</i>	%	<i>N</i>	%
<i>IGHG1</i> alleles				
*f	69	63	785	67
*a	41	37	389	33
<i>IGHG2</i> alleles				
*n	42	38	521	44
*-n	68	62	653	56

Asthma severity and sensitization in young middle age

Correlation analysis showed statistically significant associations between the number of *IGHG*bf* alleles and several aspects of asthma severity: total asthma scores, bronchial hyperresponsiveness to CACH, baseline airway resistance, and baseline ventilation inhomogeneity (SF_6 Sn_{III}), all assessed at the last follow-up (Table 3). The relationship between FEV_1/VC (%) and *IGHG*bf* alleles just failed to reach significance. There were no significant correlations between asthma scores or lung function variables and the number of *IGHG2*n* alleles (data not given). Total serum-IgE levels or the extent of sensitization, as determined by the number of positive RASTs or SPTs, did not correlate with the number of *IGHG*bf* alleles (Table 3) or *IGHG*n* alleles (data not shown).

Asthma course

At visit 7, 5 subjects had a total asthma score of 5 or 6, 10 subjects scored 3 or 4, and 28 had a score of 1 or 2. Asthma was in remission in 12 subjects at visit 7 (21%; 9 males and 3 females) who had a total asthma score of 0. Eight subjects were taking inhaled corticosteroids regularly and four of these also used long-acting β_2 agonists daily.⁷

Over the six follow-up stations as a whole, total asthma scores were significantly associated with the *IGHG*bf* allele ($p = 0.001$; Figure 1). The *IGHG*bf* allele was also associated with a greater bronchodilator response ($p = 0.005$) and a lower baseline percentage of predicted FEV_1 ($p = 0.024$). Total asthma scores or lung function measure-

ments were not significantly associated with the *IGHG2* polymorphisms (data not shown).

Asthma severity and sensitization to animal danders

At visit 7, the extent of sensitization, as measured by a positive RAST, to any of the three furred animal danders studied, did not differ significantly between the 19 *IGHG*bf* homozygotes and the 36 remainders (14/19 vs. 22/36; $p = 0.5$; Figure 2). All five subjects with severe asthma (total asthma scores 5 or 6) were, however, homozygous for the *IGHG*bf/bf* genotype and sensitized to at least one animal dander, whilst none of the similarly sensitized subjects with the *IGHG*bf/ga* or *IGHG*ga/ga* genotypes had severe asthma ($p = 0.012$; Figure 2).

Subjects with the homozygous *IGHG*bf/bf* genotype who were sensitized to any of the three animal danders had higher median serum concentrations of IgE antibodies to these allergens than the similarly sensitized subjects with the *IGHG*bf/ga* or *IGHG*ga/ga* genotypes: for cat 9.3 kU/l vs. 2.6 kU/l ($p = 0.005$; $N = 11$ and $N = 21$, respectively); for dog 11.9 kU/l vs. 3.0 kU/l ($p = 0.035$; $N = 13$ and $N = 16$, respectively); and for horse 4.1 kU/l vs. 2.4 kU/l ($p = 0.170$; $N = 13$ and $N = 9$, respectively).

Asthma course, gender, and IGHG genotypes

Total asthma scores decreased markedly (median reduction 2 score units from visit 2 to visit 7; $p = 0.018$; Figure 3) over the six follow-up visits among the 10 males who were homozygous for the *IGHG*bf/bf* genotype and decreased slightly also among the 18 males with the *IGHG*bf/ga* or

Table 3 Total asthma scores, airway function, sensitization, and total serum-IgE at visit 7 (mean age 34.9 years) in all 55 subjects with respect to *IGHG3* and *IGHG1* genotypes.

Variables	IGHG3 and IGHG1 genotypes			Statistics
	<i>*bf/bf</i> ($N = 19$)	<i>*bf/ga</i> ($N = 31$)	<i>*ga/ga</i> ($N = 5$)	
Total asthma score, median (range)	3 (0–6)	2 (0–4)	1 (0–3)	$R_s = 0.373$, $p = 0.005$
<i>Airway function, mean (SD)</i>				
Max fall in FEV_1 after CACH (%)	21.4 (17.4)	12.0 (10.5)	6.6 (5.6)	$R_s = 0.357$, $p = 0.007$
Baseline FEV_1 (% pred)	89.6 (12.0)	92.2 (11.4)	100.6 (3.6)	$R_s = -0.235$, $p = 0.085$
Baseline FEV_1/VC (%)	73.6 (7.4)	74.9 (7.0)	81.9 (3.5)	$R_s = -0.262$, $p = 0.053$
Baseline sRaw (kPa s)	1.02 (0.58)	0.77 (0.41)	0.35 (0.11)	$R_s = 0.376$, $p = 0.005$
Baseline SF_6 Sn_{III} (l^{-1})	0.255 (0.092)	0.194 (0.078)	0.178 (0.102)	$R_s = 0.323$, $p = 0.016$
<i>Overall sensitization, median (range)</i>				
RAST, no. positive tests	4 (0–7)	2 (0–5)	5 (0–7)	$R_s = 0.220$, $p = 0.107$
SPT, no. of positive tests	5 (1–8)	4 (0–7)	5 (1–8)	$R_s = 0.184$, $p = 0.178$
<i>Sensitization to three furred animals tested, median (range)</i>				
RAST, no. positive tests	3 (0–3)	1 (0–3)	3 (0–3)	$R_s = 0.187$, $p = 0.173$
SPT, no. of positive tests	3 (0–3)	2 (0–3)	3 (0–3)	$R_s = 0.196$, $p = 0.152$
Total S-IgE (kU/l), median (range)	129 (21–4125)	84 (7–860)	151 (15–378)	$R_s = 0.136$, $p = 0.321$

The statistical analysis encompasses correlation tests (R_s ; Spearman Rho) between the measured variables and the number of *IGHG*bf* alleles (2, 1, or 0). CACH = cold air hyperventilation challenge, sRaw = specific airway resistance, SF_6 Sn_{III} = normalized phase III slope for SF_6 . For remaining abbreviations, see Methods.

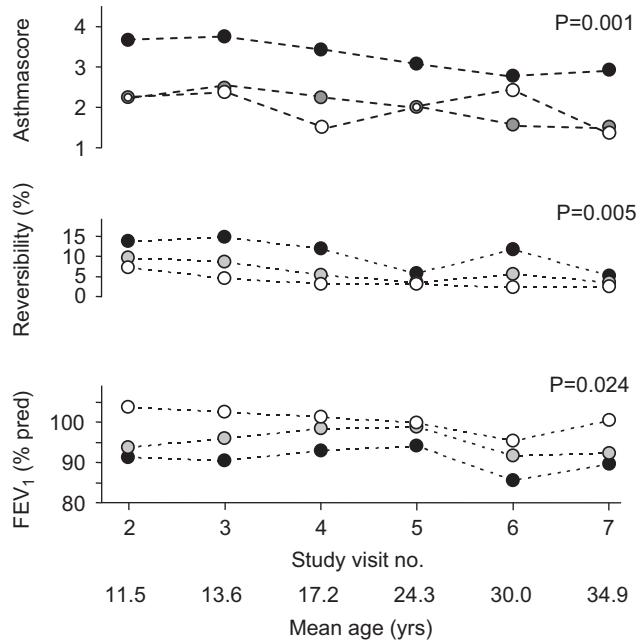


Figure 1 Mean total asthma scores, mean percentage increase in FEV₁ after bronchodilation (reversibility %), and mean baseline FEV₁ (% predicted) in all 55 subjects over visits 2–7 with respect to *IGHG* genotypes: *IGHG*bf/bf* (*N* = 19) (black filled circles), *IGHG*bf/ga* (*N* = 31) (gray filled circles), and *IGHG*ga/ga* (*N* = 5) (open circles). (*p*-values refer to comparisons between the three groups over the follow-up as a whole.)

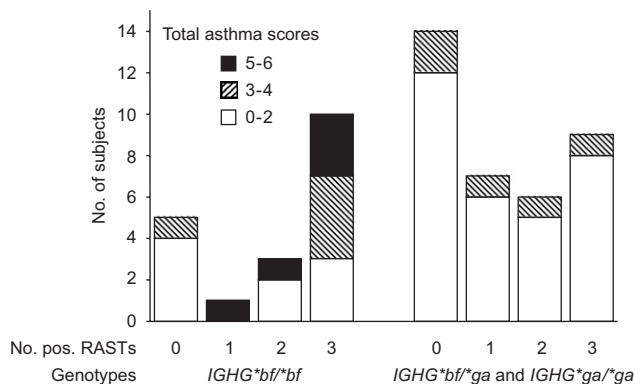


Figure 2 Asthma severity (total asthma scores) distribution at visit 7 in relation to the extent (0–3) of sensitization (limit for positive RAST >0.70 kU/l) to three furred animals danders (cat, dog, and horse) among the 19 subjects homozygous for the *IGHG*bf/bf* genotype, and among the remainders (31 *IGHG*bf/ga* and 5 *IGHG*ga/ga* subjects).

*IGHG*ga/ga* genotypes (median reduction 1 score unit; *p* = 0.028). By contrast, total asthma scores tended to increase among the females who were homozygous for the *IGHG*bf/bf* genotype (median increase 1 score unit; *p* = 0.063), and did not change among the female remainders. The difference in score change between males and females was highly significant among those with the *IGHG*bf/bf* genotype (*p* < 0.001), but not among the remainders (*p* = 0.091).

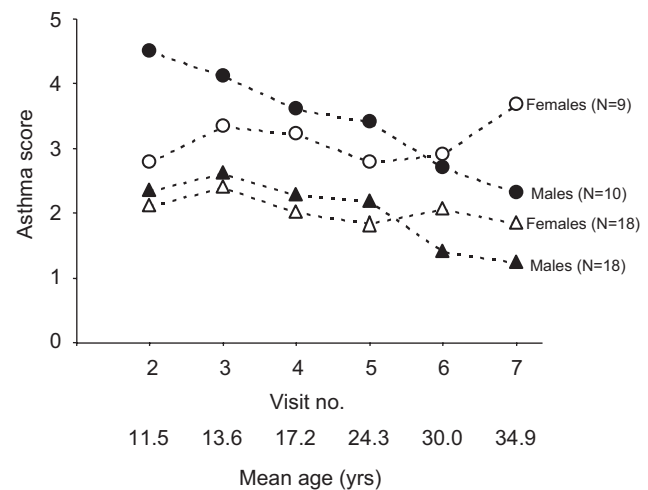


Figure 3 Mean total asthma scores over visits 2 to 7 for male and female subjects with the *IGHG*bf/bf* genotype (circles; *N* = 19), and for male and female subjects with the *IGHG*bf/ga* (*N* = 31) or the *IGHG*ga/ga* genotypes (*N* = 5) (triangles).

Discussion

The present study demonstrates a clear association between the *IGHG*bf* allele and asthma severity in young middle age, judged both clinically and with several tests of airway function. Similar results were recorded all through the follow-up period from childhood. Furthermore, the *IGHG*bf* allele was related to the intensity of sensitization to perennial allergens. The *IGHG*bf* alleles were, however, not overrepresented in the cohort compared to a reference population, indicating that they are not linked to the risk of acquiring asthma.

To our knowledge, there are no previously published studies on Gm allotypes and the course and outcome of childhood asthma. At the last follow-up, the highest asthma scores, the most pronounced airway hyperresponsiveness, and the lowest baseline central (sRaw) or peripheral (SF₆ Sn_{III}) airway function were found among the subjects with the homozygous *IGHG*bf/bf* genotype. The subjects lacking these alleles had the lowest asthma scores, were least reactive to cold air challenge and had the best baseline airway function, whilst the heterozygotes showed intermediate results. Over the whole 26-year follow-up period, the *IGHG*bf/bf* genotype was associated with the most severe asthma judged both from clinical scores and lung function findings. The extent of allergic sensitization or total serum IgE did not show a clear association with the *IGHG*bf* alleles. Interestingly, severe asthma was found only in subjects with the *IGHG*bf/bf* genotype who were sensitized to at least one animal dander. Furthermore, the intensity of sensitization to furred animal allergens was higher in the sensitized subjects who had the *IGHG*bf/bf* genotype than in the sensitized remainders.

It is well known that there are differences in asthma prevalence and airway behavior between males and females over lifetime.^{2,30} In the present study, the difference in total asthma scores between the *IGHG*bf/bf* genotype and the remainders was seen in both sexes over the whole

follow-up. The previously reported greater improvement of asthma severity among the males vs. the females in this cohort^{6,7} was most pronounced among those with the *IGHG*bf/*bf* genotype, where the males had the largest room for improvement.

The study included relatively few patients, but they all attended all follow-up visits over a period of 26 years and their asthma was carefully characterized with respect to clinical, allergological, and physiological features. The follow-up covered a period of life when major changes occur in terms of growth, maturation, and living conditions. Nevertheless, distinct differences in clinical asthma severity and lung function were found between the different *IGHG3/IGHG1* genotypes over the whole follow-up period. Furthermore, our finding of an allele dose-response with respect to airway hyperresponsiveness and lung function is characteristic for a gene influence. However, if the initial difference in asthma severity between the genotypes had occurred by chance, not involving any causal relationship, then it would probably persist over the follow-up. There is therefore a need to assess these associations in other asthma populations.

There was no significant relationship between the number of *IGHG2*n* alleles and asthma severity or allergic sensitization. In a previous study in childhood asthma, an association was found between the *IGHG*bfn/*bfn* genotype and allergic asthma.¹⁷ In another study, an overrepresentation of the *IGHG*bfn* allele was found in laboratory technicians developing allergy to furred animals.¹⁸ A recent study of subjects participating in the International Study of Asthma and Allergy in Children (ISAAC) showed an association between the *IGHG*bfn* allele and the occurrence of allergic sensitization, family history of atopy, and clinical allergy.¹⁶ In the present study there were only six subjects with the *IGHG2*n/*n* genotype, and all had the *IGHG*bfn/*bfn* genotype (Table 2). As expected in a pediatric asthma population, the great majority of the subjects in the present asthma cohort (90%) were sensitized to any allergen. Therefore, the influence of any polymorphisms on adopting allergic sensitization could not be assessed.

The study patients were recruited randomly among Swedish asthmatic school children born during the 1960s and referred from primary care physicians to a pediatric outpatient asthma clinic in a general hospital in the mid-1970s. This was prior to the "asthma epidemic", which is generally regarded to be the result of a modern Western life style. As asthma is thought to result from the interaction between several genes and environment,³¹ it can be speculated that the gene interaction with the altered environment and life style of today promotes asthma development also in other genotypes than those associated with acquiring asthma in the 1960s. It therefore remains to be seen if asthma severity and long-term asthma outcome in school children born more recently and also in those with non-Caucasian origin are similarly associated with *IGHG* polymorphisms.

Over the follow-up period, bronchodilator response was greater in the 19 subjects with the homozygous *IGHG*bf/*bf* genotype than in the remaining 36 subjects, indicating that these alleles are related to bronchial hyperresponsiveness. At the last follow-up visit, tests of large and small airway function showed that the whole airway tree was more

severely affected in the subjects with homozygous *IGHG*bf/*bf* genotype. A clear relationship has previously been shown between the degree of airway responsiveness in asthma and the intensity of airway inflammation,^{32,33} giving indirect evidence of greater airway inflammation among the subjects with *IGHG*bf/*bf* genotype during the whole follow-up period. The greater intensity of allergic sensitization to perennial allergens in this subgroup lends support to this notion.

To conclude, the present study shows a clear and consistent association between *IGHG* gene polymorphisms, identified by the alternative serum IgG subclass allotypes, and asthma severity from childhood to young middle age, and that these polymorphisms are related to the intensity of allergic sensitization to relevant perennial allergens.

Conflicts of interest

None of the authors has any interests of conflict to declare with respect to this paper.

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