Immunoglobulin constant heavy G chain genes as risk factors in childhood allergies.

Oxelius, Vivi-Anne; Braback, L; Ahlstedt, S; Bjorkstén, B

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Title: Immunoglobulin constant heavy G chain genes as risk factors in childhood allergies

Running title: Allergy phenotypes and IGHG genes

Authors: Vivi-Anne Oxelius*,**), Lennart Bråbäck***), Staffan Ahlstedt****,*****), Bengt Björkstén*****)

Departments where the work was done:

*) Department of Pediatrics, **) Institute of Clinical Immunology, University Hospital, Lund University, Lund

***) Department of Public Health and Research, Sundsvall Hospital, Sundsvall

****) Pharmacia Diagnostics AB, Uppsala

*****) National Institute of Environmental Medicine, Karolinska Institutet, Stockholm

Corresponding author: Dr Vivi-Anne Oxelius, Department of Pediatrics, University Hospital, Lund University, Se-221 85 Lund, Sweden

Tel. +46-46-178295

Fax: +46-46-177051

E-mail: vivi-anne.oxelius@med.lu.se

Abstract

Background Several candidate genes have been found associated to the inflammatory response of IgE mediated allergy, so also the immunoglobulin constant heavy G chain (*IGHG*) genes. The *IGHG* genes are situated close to the *IGHE* gene on chromosome 14q32, 5´ μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2, 3´. They are inherited in a Mendelian fashion and expressed randomly in allelic exclusion. The alternative and functionally different γ3, γ1 and γ2 gene variants, are found in four *IGHG* haplotypes, coding 4 B-cell variants.

Objective The aim of this study was to assess the frequency of different *IGHG* genes in relation to phenotypes associated with allergy, in a case-control study.

Methods We identified the constant heavy chain genes of IgG in 198 allergic and non-allergic children participating in the Phase II of the International Study of Asthma and Allergy in Children (ISAAC). The *IGHG* genes were assessed by the alternative serum IgG subclass allotypes expressing the alternative alleles of γ3, γ1 and γ2 genes, employing ELISA and double immunodiffusion.

Results The *IGHG*bfn* haplotype (=B1-cells) and *IGHG2*n* allele dominated (51% versus 24%, P=0.002) and the *IGHG*bf-n* haplotype(=B2-cells) was infrequent (16% versus 52%, P<0.001) in allergic children with a family history of allergy, clinical manifest allergy and positive SPT. The frequency of *IGHG* genes was similar in children with maternal and paternal heredity and in children with wheezing, eczema or rhinitis, as well as in children with different positive SPT. The *IGHG*bfn* haplotype with the *IGHG2*n* allele was strongly

associated with heredity for allergy. The *IGHG*bf-n* haplotype was inversely related to allergy.

Conclusions IgG allotypes, immunochemical and functional variants of IgG molecules from *IGHG* genes are associated with atopy. The *IGHG*bfn* haplotype (=B1 cells) with the *IGHG2*n* allele dominates, associated with an increased risk for atopy. In contrast, the *IGHG*bf-n* haplotype (=B2-cells) with the *IGHG2*-n* allele is associated with low risk.

Keywords

Alternative IgG subclass allotypes, *IGHG* alleles, *IGHG* genotypes, *IGHG* haplotypes (=B-cells), *IGHG* diplotypes, allergy heredity, wheezing, eczema, allergic rhinitis, SPT.

Introduction

Asthma and allergies may affect as many as one in four urban children (1), it is associated with substantial morbidity and economic costs (2) and the factors that confer susceptibility are not well understood. Identifying genetic pathways that allow asthma and allergies to be expressed is fundamental for developing diagnostic tools and designing therapies (3).

Asthma, eczema and hay fever are all manifestations of the atopic state and they are strongly familial on a multifactorial genetic basis (4). The genetic codes for the heavy constant chains of both 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´ (5). The IgG allotypes are genetic markers of IgG proteins and therefore products of structural IgG genes. The alternative IgG allotypes from the γ3, γ1 and γ2 genes, have minor amino acid differences, which correlate with single base substitutions in the genes (5). The IgG allotypes are inherited in a Mendelian fashion and expressed randomly in allelic exclusion. The IgG allotypes are separate entities with distinct immunochemical and functional characteristics, such as fractionation rates, electrophoretic rates (7), half-life times (8) and maturation rate during childhood (9). Four subsets of B cells have been identified, based on the alternative protein expressions from the *IGHG3*, *IGHG1* and *IGHG2* genes (10), the *IGHG*bfn* haplotype (=B1-cells) is typed by IgG3*b, IgG1*f and IgG2*n;

the *IGHG*bf-n* haplotype (=B2 cells) is typed by IgG3*b, IgG1*f and IgG2*-n, the *IGHG*gan* haplotype (=B3-cells) is typed by IgG3*g, IgG1*a and IgG2*n and the *IGHG*ga-n* haplotype (=B4-cells) is typed by IgG3*g, IgG1*a and IgG2*-n allotypes in serum (6) (Table 1).

Restricted *IGHG* genes are associated with both atopic and non atopic asthma, replicated in several studies (11,12). In childhood asthma, the *IGHG*bfn/*bfn* diplotype (=B1/B1-cells) with the *IGHG2*n/*n* genotype is associated with an *IGHE* gene, inducing increased levels of serum IgE and allergen specific IgE antibodies, while the alternative *IGHG*ga-n/*ga-n* diplotype (=B4/B4) with the alternative *IGHG2*-n/*-n* is linked to a low responding *IGHE* gene. Two different pathways of immune regulation are associated with the B1/B1-cells and B4/B4-cells, respectively, in asthmatic children with possible influences on pathogenesis (13). Those with the B1/B1-cells have an imbalanced class switch in the rearrangement of the *IGHG* genes, expressed as low serum IgG1*f level and high levels of serum IgG2*n and IgE (14) and those with B4/B4 express low levels of both IgG1*a, IgG2*-n, IgG3*g and IgE.

We tested the hypothesis that various expressions of allergy are influenced by different *IGHG* gene coded B cells and thereby add diagnostic power to other procedures.

Materials and methods

Study subjects

The study group (198 children) were selected among 1390 twelve-year-old children from 25 schools in Östersund, Sweden, participating in the Phase 2 of the International Study of Asthma and Allergy in Children (ISAAC). All children with current wheezing and a random sample of non-wheezers were invited to a case-control questionaire study (15). Skin prick tests were done with 5 inhalant allergens (tree pollen, grass pollen, cat, dog and horse), strictly adhering to the ISAAC Phase 2 manual. 82 were SPT positive and 116 SPT negative. Based on the questionnaire responses the children were divided into those with and without clinical symptoms (n=130 and 68, respectively) and those with and without a family history of allergy (n=144 and 54, respectively) (Table 1). Sixty-one children had both clinical symptoms of allergy, a family history of allergy and positive SPT, while 21 children had none of these. The *IGHG* genotypes of the study population were in Hardy-Weinberg equilibrium P>0.05, compared with Caucasian populations from Sweden (16), Finland and the Netherlands (17).

Methods

IGHG genotyping by serum IgG allotypes

The IGHG genes are situated on the long arm q at band 32 on chromosome 14. They are found in order γ3, γ1, γ2, as used in the following, and together with all other immunoglobulin constant heavy chains, also ε for IgE, in the IGH gene 5´ μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2 3´. Two alternative gene variants, are reported by studies of allotypes, from the γ3, γ1 and γ2 gene loci, producing IgG subclass molecules with only minor amino acid epitope differences of the constant heavy G chains (5) (Table 1). The alternative protein variations from the γ3 (*IGHG3*), γ1 (*IGHG1*) and γ2 (*IGHG2*) genes can easily be studied qualitatively and quantitatively in serum (6). The alternative *IGHG* genes are inherited in the Mendelian way and expressed randomly in allelic exclusion. The secreted alternative IgG subclass proteins are for *IGHG3*: IgG3*b and IgG3*g, for *IGHG1*: IgG1*f and IgG1*a and for *IGHG2*: IgG2*n and IgG2*-n allotypes (Table 1).

The γ3 and γ1 genes are concordant because of their close localisation (only 26kb in between): *IGHG3*b* together with *IGHG1*f*, in the gene complex **bf* and *IGHG3*g* together with *IGHG1*a*, **ga* (5). Additional variation on *IGHG2* with the **n* or **-n* genes, respectively, makes four different haplotypes(5) which also code four different B-cells (Fig. 1)(10). The combinations from

these four haplotypes constitute 10 diplotypes, of which six are common and four very rare in a Caucasian population.

A competitive ELISA was used to quantitate serum IgG1*f, IgG1*a, IgG2*n and IgG3*b allotypes (6). The following monoclonal antibodies: anti IgG1*f clone 5F10, anti IgG1*a clone 5E7, anti IgG3*b1/u clone clone 12D9 (Janssen Biochimica, Beerse, Belgium) and anti IgG2*n clone SH21 (Sigma, St. Louis, MO, USA) and predetermined concentration of purified myeloma proteins of the following IgG allotypes: IgG1*f, IgG1*a, IgG2*n and IgG3*b were used. The sensitivity of the ELISA assay was for IgG1*a 0.0008 g/l, for IgG1*f 0.0003 g/l, for IgG2*n 0.0006 and for IgG3m*b 0.0007 g/l.

Determination of homozygosity and heterozygosity for the IgG2*n allotype was done with a double immuno diffusion assay with the monoclonal reagents anti IgG2*n clone SH21 and anti HP 6014 (Sigma, St. Louis, MO, USA). The control test for IgG3*g was, beside absence of IgG3*b, also the presence of the concordant IgG1*a allotype. In heterozygous individuals the IgG3*g and IgG3*b are about 50:50%, respectively, of the IgG3 amount. The method is described in detail elsewhere (6,9).

Frequencies of the *IGHG* alleles, and the four *IGHG* (B cell) haplotypes were identified, i.e. *IGHG*bfn* (B1), *IGHG*bf-n* (B2), *IGHG*gan* (B3) and *IGHG*ga-n* (B4), making up 10 diplotypes: B1/B1, B1/B2, B1/B3, B1/B4, B2/B2, B2/B3,

B2/B4, B3/B3, B3/B4 and B4/B4, of which 6 are common and 4 rare(<1-3 %) in the Caucasian population (5,9).

The data were analysed by the statistical package, - SPSS 11.5, calculating the *IGHG* allele, *IGHG* genotype, *IGHG* haplotype (=B-cell) and *IGHG* diplotype frequencies for different allergic and non-allergic phenotypes. An analysis of 2 by 2 tables employing the "Wald" approximation of the variance of the Odds ratios was used similar to simple logistic regression. An estimate of the odds ratios (OR) for the final model variables and associated 95% confidence intervals (CI) are presented in the tables and results. In addition, stepwise logistic regression was performed in order to assess multivariate associations between different B cell haplotypes and heredity, clinical symptoms and positive SPT.

The study was approved by the Institutional Review Board at Umeå University, Sweden.

Results

Of the 198 children in the study, 177 had one or more of either a family history of allergy, clinical allergy symptoms and/or a positive SPT, while 21 had not (Table 2). In children with any of these allergy indicators, the *IGHG2*n* allele and the *IGHG*bfn* haplotype (=B1-cells) were more and the frequencies of the *IGHG2*-n* allele and the *IGHG*bf-n* haplotype (=B2-cells) less common than in children with neither a positive family history, nor clinical symptoms of allergy, nor a positive SPT (Table 2,3). The findings were most prominent in children with a family history of allergy. The presence of two or three of the allergy variables strengthened the relation to *IGHG* genes namely, for heredity and symptoms (B1-cells: OR 3.3, 95% CI 1.6-6.9; P<0.001; B2-cells: OR 0.2, 95% CI 0.1-0.3 p<0.001) for heredity and positive SPT (B1-cells: OR 2.5, 95% CI 1.4-4.6; P=0.002; B2-cells: OR 0.3, 95% CI 0.1-0.5; P<0.001) and for allergy symptoms and positive SPT (B1-cells: OR 1.7, 95% CI 1.0-2.7; P=0.048; B2-cells: OR 0.4, 95% CI 0.2-0.7; P<0.001). The B1-cells were particularly common in sensitised children combined with a positive family history of allergy (OR 4.4, 95% CI 1.7-11.6, P=0.001) whereas the B4-cells were uncommon (OR 0.4, 95% CI 0.2-0.8; P=0.013).

In 61 atopic children with a positive family history, clinical allergy and positive SPT, the *IGHG2*n* allele and the B1-cells dominated (OR 3.3, 95% CI 1.5-7.3; P=0.002) and the *IGHG2*-n* allele and the B2-cells (OR 0.4, 95% CI 0.2-0.7;

P<0.001) were infrequent, as compared to the 21 children with none of these traits (Table 1,2). The relationship between allergy and the *IGHG*bfn* haplotype (=B1-cells) and *IGHG2*n* allele and inverse relationship with the *IGHG*bf-n* haplotype (=B2-cells) and *IGHG2*-n* allele were confirmed in subgroups of children with various allergic manifestations, such as either paternal or maternal heredity, wheezing, rhinitis and eczema, and positive SPT to various inhalant allergens (Table 3,4).

At the diplotypic level, a high frequency of the *IGHG*bfn/*bfn* diplotype (=B1/B1 cells) was found in children with a positive family history of allergy (OR 3.9, 95% CI 1.3-11.5; P=0.001) while the *IGHG*bf-n/*bf-n* diplotype (=B2/B2-cells) was uncommon (OR 0.3, 95% CI 0.1-0.7; P=0.007).

The frequencies of the *IGHG*gan* haplotype (B3-cells) (rare in the Caucasian population) and the IGHG*ga-n haplotype (B4-cells) (Table 1), as well as *IGHG1* and *IGHG3* genotypes (data not shown) were similar in allergic and non-allergic children, with the exception of a low number of the *IGHG*ga-n* haplotype in sensitised children with a family history of allergy.

Discussion

The frequencies of the *IGHG2*n* allele, the *IGHG*bfn* haplotype and homozygous *IGHG*bfn/*bfn* diplotype were high in children with either a family history of allergy, clinical symptoms or a positive SPT, while the alternative *IGHG2*-n* allele on *IGHG2* locus, the *IGHG*bf-n* haplotype and the *IGHG*bf-n/*bf-n* diplotype were uncommon. This is in agreement with earlier findings that the IgG2*n allotype is associated with an allergen responding *IGHE* gene (11-13).

The strongest association was seen in children with a positive family history of allergy while the association was less obvious for clinical symptoms and sensitisation. The high frequency of the *IGHG2*n* allele and the *IGHG*bfn* haplotype (=B1-cells) and low frequency of the *IGHG2*-n* allele and the *IGHG*bf-n* haplotype(=B2-cells) in atopic children were similar in maternal and paternal heredity, and in wheezing children, as compared to those with eczema and rhinitis, as well as in children sensitised against different inhalant allergens. Our findings underline the importance of *IGHG2*n*, but not the *IGHG2*-n*, as a risk factor with a possible role of serum IgG2*n allotype antibody molecules in the pathogenesis of allergy.

IGHG genotyping by alternative serum IgG subclass allotypes is the best way to demonstrate both *IGHG* -alleles, -genotypes, -haplotypes and -diplotypes. RFLP

and DNA analyses have been done, but with only limited success (5). The IgG allotypes are products of structural genes. They have been extensively studied in humans, rabbits, rats and in the mink (5). The basis of IgG allotypes in humans is precisely understood at the molecular level by amino acid and codon substitutions IgG1*a with Asp 356, Leu 358 and IgG1*f with Lys 214 (5). The substitutions of the alternative IgG2 allotypes have not yet been elucidated, however. We have previously shown that the alternative IgG2 allotypes have different electrophoretic-and fractionation- rates, different half-life-times and developmental rates during childhood (7,8). There is a an advantage to directly study gene derived proteins quantitatively, as this describes the activity also from the allelic variant of the gene. A serum sample is used for determination of the gene products from 6 *IGHG* alleles*,* 9 *IGHG* genotypes, 4 *IGHG* haplotypes (B-cell variants) and 10 *IGHG* diplotypes (individual expressions). The advantage, of the technique used is its simplicity, allowing both patient studies and studies of large populations.

Children with atopic manifestations and especially those with a family history of allergy presented particular IgG molecules IgG3*b, IgG1*f and especially IgG2*n from the *IGHG***bfn* haplotype, indicating that also IgG antibodies are involved, besides IgE. The precise role of IgG antibodies in allergic inflammation is unknown. During the inflammatory process the variable antigen binding part of the antibody molecule, is combined with different constant functional heavy chains both γ and ε chains. In childhood asthma the IgG1*f is

depressed but the IgG2*n levels doubled compared to healthy age-matched and *IGHG* gene-matched controls (14). During childhood there is a slow developmental rate of IgG2*n antibodies (9) while in IgE mediated asthma the opposite seems to be the case.

The antibody production from *IGHG4* and *IGHE*, next to *IGHG2* on chromosome 14q32.3, is activated by IL-4 and IL-13 (18,19), connecting TH2 cell activation to B cell activation. Increased serum level of the IgG2*n allotype in IgE mediated childhood asthma indicates a linkage between an activated *IGHG2*n* gene and sensitisation. As the *IGHG2* gene, which is situated within 18kb from *IGHG4* and another 23 kb from *IGHE* on the long arm of chromosome 14q32, is supposed to be in strong linkage disequilibrium (5), it is likely that the *IGHE* gene is influenced by the *IGHG2*n* gene located upstream. Both the *IGHG* genes and the *IGHE* gene on the chromosome 14q32 are found within a range of 250 kb in linkage disequilibrium. The *IGHG* haplotype influences the amounts expressed by single *IGHG* alleles (23).

The serum IgG2*n allotype is a marker of the *IGHG2*n* allele, either homozygous or heterozygous *IGHG2*n/-n* (B1/B2-, B1/B4-cells). The *IGHG2*n* allele is also found in the *IGHG*gan* (B3) haplotype, but only very infrequently so (<1-2%). (5, 9). Thus, the IgG2*n allotype is mainly a marker for the *IGHG*bfn* haplotype (=B1-cells).

Both the *IGHG*bfn* haplotype and the homozygous *IGHG*bfn/'bfn* diplotype were frequent in a family history of allergy, but less so in environmental dependant sensitisation and sensitisation dependant clinical symptoms.

In infectious diseases, patients with the *IGHG*bfn/*bfn* diplotype(=B1/B1-cells) have a better outcome of bacterial infections as a consequence of a high production of specific bacterial antibodies especially to polysaccharide antigens (20,21) and a more favourable outcome in autoimmune juvenile chronic arthritis (22). Furthermore the *IGHG*bf-n/*bf-n* diplotype (=B2/B2-cells), which was infrequent in children with a family history of allergy and atopy, is common in IgG2 deficiency and in common variable immunodeficiency (23, 24). Infants hospitalised with the most severe RSV (Respiratory Syncytial Virus) exhibited increased frequency of the B2-cells (25). The *IGHG*ga-n/*ga-n* diplotype (=B4/B4-cells), is associated with symptoms as bronchial reactivity to non-specific stimuli and to a history of recurrent upper and lower respiratory tract infections. It is also found in most patients with IgG3 deficiency, in whom recurrent or chronic bronchitis was the most common finding (13, 23, 26).

There is no single gene in allergy genetics but it is interesting to note that the most replicated genes *IL-4* , *IL-13, IL-4RA and FCERB1* in asthma genetics are associated with the activation of *IGH* genes, both *IGHG* and *IGHE* (27).

In conclusion, a family history, sensitisation and clinical manifestations of allergy are associated with *IGHG* genes, from the *IGHG*bfn* haplotype, expressing IgG3*b, IgG1*f and especially IgG2*n molecules. The strongest association was observed for a family history of allergy. In contrast there was an inverse relationship between allergy manifestations and presence of the *IGHG2*-n* allele and the *IGHG*bf-n* haplotype. The *IGHG2*n* is associated with an increased risk, while the *IGHG2*-n* is associated with a low risk of atopy. As there is no genetic marker for an activated or allergen responding *IGHE* gene, the serum IgG2*n allotype, from the closely located *IGHG2*n* allele, is a possible genetic marker.

Contributors

V-A Oxelius had the idea for the project, designed the study, did the laboratory work, and wrote the first version of the report. L Bråbäck was responsible for

the clinical part of the study including diagnosis and presenting serum samples from the children. V-A Oxelius, B Björkstén and S Ahlstedt jointly interpreted the data and wrote the report.

Conflict of interest statement : None

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Table 1

Abbreviations and description of the *IGHG* (ImmunoGlobulin Heavy G chain) genes on chromosome 14q32, alternative IgG subclass allotypes, *IGHG* alleles, *IGHG* haplotypes = B-cells and *IGHG* diplotypes (individual)

A)

IGH (ImmunoGlobulin Heavy chain) genes of chromosome 14q32:

5´ μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2 3´

B)

Alternative alleles of *IGHG* subclass genes:

IGHG gene	Alternative Alleles
IGHG3 (γ3)	**b* and **g*
IGHG1 (γ1)	**f* and **a*
IGHG2 (γ2)	**n* and **-n*

C)

IGH genes of chromosome 14q32.
Four different *IGHG* haplotypes coding four different B-cells

IGH genes of chromosome 14q32 5´ μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2 3´ with alternative alleles inserted for IGHG3, IGHG1 and IGHG2	*IGHG* haplotypes	IgG allotypes in serum Alternative protein variants =Genetic markers for heavy G subclass chains of IgG3, IgG1 and IgG2	B-cell variation
5´ μ, δ, b, f, α1, n, γ4, ε, α2 3´	*IGHG*bfn*	IgG3*b, IgG1*f, IgG2*n	B1-cells
5´ μ, δ, b, f, α1, -n, γ4, ε, α2 3´	*IGHG*bf-n*	IgG3*b, IgG1*f, IgG2*-n	B2-cells
5´ μ, δ, g, a, α1, n, γ4, ε, α2 3´	*IGHG*gan*	IgG3*g, IgG1*a, IgG2*n	B3-cells
5´ μ, δ, g, a, α1, -n, γ4, ε, α2 3´	*IGHG*ga-n*	IgG3*g, IgG1*a, IgG2*-n	B4-cells

D)

Serum IgG, IgG subclasses and alternative IgG subclass allotypes

IgG	IgG1	IgG1*f
		IgG1*a
	IgG2	IgG2*n
		IgG2*-n
	IgG3	IgG3*b
		IgG3*g
	IgG4	

E)

The 6 most common B-cell combination, *IGHG* diplotypes and *IGHG2* genotypes

B1/B1-cells	B1/B2-cells	B1/B4-cells	B2/B2-cells	B2/B4-cells	B4/B4-cells
*IGHG*bfn/*bfn*	*IGHG*bfn/ *bf-n*	*IGHG*bfn/ *ga-n*	*IGHG*bf-n*/ *bf-n*	*IGHG*bf-n/ *ga-n*	*IGHG*ga-n/ *ga-n*
*IGHG2*n/*n*	*IGHG2*n/*-n*	*IGHG2*n/*-n*	*IGHG2*-n/*-n*	*IGHG2*-n/*-n*	*IGHG2*-n/*-n*

Table 2
Frequency of *IGHG2* genotypes, *IGHG2*n* and *IGHG2*-n* alleles in 198 children with and without allergy variables.

Allergy variables	*IGHG2* Genotypes, N(%)			*IGHG2* alleles, N(%)						
		Yes	No	Yes		No		P value	OR	95% CI
Family History of Allergy		144	54	288		108				
	**n/*n*	35(24)	5(9)							
	**n/*-n*	65(45)	20(37)							
	**-n/*-n*	44(31)	29(54)							
				**n*	**-n*	**n*	**-n*			
				135(47)	153(53)	30(28)	78(72)	<0.001	2.3	1.4-3.7
Clinical Symptoms		130	68	260		136				
	**n/*n*	28(22)	12(18)							
	**n/*-n*	61(47)	24(35)							
	**-n/*-n*	41(32)	32(47)							
				**n*	**-n*	**n*	**-n*			
				117(45)	143(55)	48(35)	88(65)	ns	1.5	1.0-2.3
Sensitised (SPT+)		82	116	164		232				
	**n/*n*	19(23)	21(18)							
	**n/*-n*	38(46)	47(41)							
	**-n/*-n*	25(30)	48(41)							
				**n*	**-n*	**n*	**-n*			
				76(46)	88(54)	89(38)	143(62)	ns	1.4	0.9-2.1
Any of above		177	21	354		42				
	**n/*n*	38(21)	2(10)							
	**n/*-n*	79(45)	6(29)							
	**-n/*-n*	60(34)	13(62)							
				**n*	**-n*	**n*	**-n*			
				155(44)	199(56)	10(24)	32(76)	0.013	2.4	1.2-5.2
All of the above		61	21	122		42				
	**n/*n*	17(28)	2(10)							
	**n/*-n*	28(46)	6(29)							
	**-n/*-n*	16(26)	13(62)							
				**n*	**-n*	**n*	**-n*			
				62(51)	60(49)	10(24)	32(76)	0.002	3.3	1.5-7.3

Table 3
Frequency of B-cells (*IGHG* haplotypes), B1 (B^{*bfn}), B2 ($B^{*bf\text{-}n}$), B3 (B^{*gan}) and B4 ($B^{*ga\text{-}n}$) in relation to a family history of allergy, clinical symptoms and sensitisation of 198 children (396 haplotypes)

Allergy variables	B-cells coded by *IGHG* genes, n(%)					
		Yes	No	P value	OR	95% CI
Family History of Allergy		288	108			
	B1 (B^{*bfn})	133(46)	28(26)	<0.001	2.5	1.5-4.0
	B2 ($B^{*bf\text{-}n}$)	48(17)	38(35)	<0.001	0.4	0.2-0.6
	B3 (B^{*gan})	2(1)	2(2)	ns		
	B4 ($B^{*ga\text{-}n}$)	105(37)	40(37)	ns		
Clinical Symptoms		260	136			
	B1 (B^{*bfn})	115(44)	46(34)	0.045	1.6	1.0-2.4
	B2 ($B^{*bf\text{-}n}$)	43(17)	43(32)	<0.001	0.4	0.3-0.7
	B3 (B^{*gan})	2(1)	2(2)	ns		
	B4 ($B^{*ga\text{-}n}$)	100(39)	45(33)	ns		
Sensitised (SPT+)		164	232			
	B1 (B^{*bfn})	73(45)	88(38)	ns	1.3	0.9-2.0
	B2 ($B^{*bf\text{-}n}$)	27(17)	59(25)	0.033	0.6	0.4-1.0
	B3 (B^{*gan})	3(2)	1(<1)	ns		
	B4 ($B^{*ga\text{-}n}$)	61(37)	84(36)	ns		
Any of above		354	42			
	B1 (B^{*bfn})	151(43)	10(24)	0.019	2.4	1.1-5.0
	B2 ($B^{*bf\text{-}n}$)	64(18)	22(52)	<0.001	0.2	0.1-0.4
	B3 (B^{*gan})	3(1)	1(2)	ns		
	B4 ($B^{*ga\text{-}n}$)	136(38)	9(21)	0.031	2.3	1.1-4.9
All of above		122	42			
	B1 (B^{*bfn})	62(51)	10(24)	0.002	3.3	1.5-7.3
	B2 ($B^{*bf\text{-}n}$)	20(16)	22(52)	<0.001	0.4	0.2-0.7
	B3 (B^{*gan})	1(1)	1(2)	ns		
	B4 ($B^{*ga\text{-}n}$)	39(32)	9(21)	ns		

Tabell 4
Frequency of *IGHG2* genotypes and *IGHG2* alleles as risk factors in atopy subgroups from 61 children with a family history of allergy, clinical allergy and positive SPT compared to 21 controls without.

Atopy subgroups	*IGHG2* Genotypes		*IGHG2* Alleles				
	Number(%)		Number(%)		p value	OR	95% CI
Controls	21		42				
	*n/*n	2(10)					
	n/-n	6(29)					
	-n/-n	13(62)					
			*n	*-n			
			10(24)	32(76)			
Family history							
Paternal heredity	34		68				
	*n/*n	10(29)					
	n/-n	15(44)					
	-n/-n	9(26)					
			*n	*-n			
			35(51)	33((49)	0.004	3.4	1.4-8.0
Maternal heredity	34		68				
	*n/*n	7(21)					
	n/-n	16(47)					
	-n/-n	11(32)					
			30(44)	38(56)	0.031	2.5	1.0-5.9
Clinical allergy							
Wheezing	47		94				
	*n/*n	14(30)					
	n/-n	23(49)					
	-n/-n	10(21)					
			*n	*-n			
			51(54)	43(46)	<0.001	3.7	1.7-8.6
Eczema	40		80				
	*n/*n	15(38)					
	n/-n	16(40)					
	-n/-n	9(23)					
			*n	*-n			
			46(58)	34(43)	<0.001	4.3	1.9-10.0
Rhinit	30		60				
	*n/*n	10(33)					
	n/-n	14(47)					
	-n/-n	6(20)					
			*n	*-n			
			34(57)	26(43)	<0.001	4.2	1.7-10.0

Table 5
Frequency of B-cells (*IGHG* haplotypes) B1 (B^{*bfn}), B2 ($B^{*bf\text{-}n}$), B3 (B^{*gan}) and B4 ($B^{*ga\text{-}n}$) as risk factors in atopy subgroups from 61 children (122 haplotypes) with a family history of allergy, clinical allergy and positive SPT compared to 21 controls without (42 haplotypes).

Atopy subgroups	B cell (*IGHG*) haplotypes N(%)	P value	OR	95%CI
Controls (21)	42			
	B1 (B^{*bfn}) 10(24)			

	B2 (B^{*bf-n})	22(52)			
	B3 (B^{*gan})	1(2)			
	B4 (B^{*ga-n})	9(21)			
Family history of allergy					
Paternal heredity (34)		68			
	B1 (B^{*bfn})	35(52)	0.004	3.4	1.4-8.0
	B2 (B^{*bf-n})	13(19)	<0.001	0.2	0.1-0.5
	B3 (B^{*gan})	0	ns		
	B4 (B^{*ga-n})	20(29)	ns		
Maternal heredity (34)		68			
	B1 (B^{*bfn})	30(44)	0.03	2.5	1.1-5.9
	B2 (B^{*bf-n})	14(21)	<0.001	0.2	0.1-0.5
	B3 (B^{*gan})	0	ns		
	B4 (B^{*ga-n})	24(35)	ns		
Clinical allergy					
Wheezing		94			
	B1 (B^{*bfn})	51(54)	<0.001	3.8	1.7-8.6
	B2 (B^{*bf-n})	12(12)	<0.001	0.1	0.05-0.3
	B3 (B^{*gan})	0	ns		
	B4 (B^{*ga-n})	31(33)	ns		
Eczema		80			
	B1 (B^{*bfn})	46(58)	<0.001	4.3	1.9-10.0
	B2 (B^{*bf-n})	12(15)	<0.001	0.2	0.1-0.4
	B3 (B^{*gan})	0	ns		
	B4 (B^{*ga-n})	22(28)	ns		
Rhinit		60			
	B1 (B^{*bfn})	34(57)	<0.001	4.2	1.7-10.0
	B2 (B^{*bf-n})	7(12)	<0.001	0.1	0.04-0.3
	B3 (B^{*gan})	0	ns		
	B4 (B^{*ga-n})	19(32)	ns		
Sensitised (SPT+)					
Tree		42			
	B1 (B^{*bfn})	19(45)	0.039	2.6	1.0-6.7
	B2 (B^{*bf-n})	8(19)	0.001	0.2	0.1-0.6
	B3 (B^{*gan})	0	ns		
	B4 (B^{*ga-n})	15(36)	ns		
Grass		52			
	B1 (B^{*bfn})	29(47)	0.018	2.8	1.2-6.7
	B2 (B^{*bf-n})	11(18)	<0.001	0.2	0.1-0.4
	B3 (B^{*gan})	1(2)	ns		
	B4 (B^{*ga-n})	21(34)	ns		
Cat		84			
	B1 (B^{*bfn})	45(54)	0.001	3.7	1.6-8.5
	B2 (B^{*bf-n})	13(16)	<0.001	0.2	0.1-0.4
	B3 (B^{*gan})	1(2)	ns		
	B4 (B^{*ga-n})	25(30)	ns		
Dog		44			
	B1 (B^{*bfn})	20(46)	0.035	2.6	1.1-6.7
	B2 (B^{*bf-n})	8(18)	<0.001	0.2	0.1-0.5
	B3 (B^{*gan})	1(2)	ns		
	B4 (B^{*ga-n})	15(34)	ns		
Horse		52			
	B1 (B^{*bfn})	27(52)	0.006	3.5	1.4-8.5
	B2 (B^{*bf-n})	10(19)	<0.001	0.2	0.1-0.5
	B3 (B^{*gan})	1(2)	ns		
	B4 (B^{*ga-n})	14(27)	ns		