Innate IgG Molecules and Innate B Cells Expressed by Immunoglobulin Constant Heavy G Chain (Fcγ) Genetic Marker Genes Are Involved in the 'Allergic March' of IgE Sensitization in Children.

Oxelius, Vivi-Anne; Krueger, Renate; Ahlstedt, Staffan; Keil, Thomas; Lau, Susanne; Wahn, Ulrich

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Title:

Innate IgG molecules and innate B cells expressed by immunoglobulin constant heavy G chain (*IGHG*)(Fcγ)(GM) genes, are involved in the "allergic march" of IgE sensitization in children.

Authors:

Vivi-Anne Oxelius MD PhD[1], Renate Krueger MD[2], Staffan Ahlstedt PhD[3], Thomas Keil MD MSc[4], Susanne Lau MD[2], Ulrich Wahn MD[2]

[1]Department of Pediatrics and Institute of Laboratory Medicine, Department of Clinical Immunology, University Hospital, Lund University, Lund, Sweden

[2]Department of Pediatric Pneumology and Immunology, Charité University Medical Centre, Berlin, Germany [3] Center of Allergy Research, National Institute of Environmental Medicine, Karolinska Insitute, Stockholm, Sweden [4]Institute of Social Medicine, Epidemiology and Health Economics, Charité University Medical Centre, Berlin, Germany

Running Title:

IGHG (GM)genes and IgE sensitisation at different ages

Address for correspondence:

Dr Vivi-Anne Oxelius

Department of Pediatrics, University Hospital, Lund University, Se-221 85 Lund Sweden

Phone: +46-46-2114440

Fax: +46-46-177051

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

Abstract

***Background*:** Inter individual variation of immunoglobulin constant heavy G chain (*IGHG*) genes on chromosome 14q32.3, are identified by alternative GM markers of IgG3, IgG1 and IgG2, respectively. They express structurally and functionally innate IgG molecules and B cells, associated with allergic disease, replicated in several studies.

***Materials and Methods*:** 1-year-old and 10-year-old, IgE sensitized and non-sensitized children from the German MAS birth cohort, were assessed by new serological methods for the Mendelian *IGHG* (Fcγ) (GM) genes, as innate IgG molecules and innate B cells.

***Results*:** Food allergy sensitization in thirty-five 1 year-old children (124 not sensitized) was associated with the *IGHG*bfn* haplotype, B^{*bfn} cells (OR 1.9, 95% CI 1.2-3.1; P=0.010). Aeroallergen sensitization in ninety-nine 10 year-old children (95 not sensitized) was associated with the same genes (OR 1.4, 95% CI 1.02-1.9; P=0.034). The IgE sensitization, was most prominent in the restrictive homozygous *IGHG*bfn/*bfn* diplotype, 34% at age 1, increasing to 60% at age 10, rating the highest numbers of positive IgE tests, expressing increased levels of IgE and innate IgG2*n.

***Conclusions*:** The *IGHG*bfn* haplotype (B^{*bfn} cells) and increased innate IgG2*n levels are predictive factors for IgE sensitization in childhood. *IGHG* genes can be assessed for prognostic and preventive purpose in clinical care.

Key words: Immunoglobulin constant heavy G chain (*IGHG*)(Fcγ)(GM) genes;

innate IgG subclass molecules, innate B cells, IgE sensitization at 1 and 10 years of age

Introduction

Several candidate genes have been found associated with the inflammatory response of IgE mediated allergy [1-3]. Asthma and allergy are linked to the *IGHG* (Fcγ) (GM) genes, replicated in several studies by serological methods [4-7]. Serological *IGHG* gene mapping, based on alternative Mendelian GM allotypes of γ3, γ1 and γ2 on chromosome 14q32.3 (5´μ, δ, **γ3, γ1**, ψε, α1, **γ2, γ4,** ε, α2 3´), have identified new innate IgG subclasses and new B cells. There is no genetic marker for IgE but IgG genes are informative. IGHG(Fc)(GM) genes are associated with allergen IgE sensitization [8-14]. Genome wide association studies (GWAS) have not included GM genes in their genotyping platform [15-17] and have missed *IGHG* as candidate genes.

The alternative GM allotypes are for γ3 chains:*b/5 and/or *g/21, for γ1 chains:*f/3 and/or *a/1 and for γ2 chains: *n/23 and/or *-n/-23. There are 10 homozygous and heterozygous individual *IGHG* diplotypes, made up by combinations of four fixed *IGHG* haplotypes: *IGHG*bfn*, *IGHG*bf-n*, *IGHG*gan* and *IGHG*ga-n*, markers of B cell variants: B^{*bfn} (B1), B^{*bf-n} (B2), B^{*gan} (B3) and B^{*ga-n} (B4) (14). The *IGHG* gene complex, dissected in alleles (allotypes), genotypes, haplotypes and diplotypes and innate IgG subclass levels are registered [18,19]. A gene map and reference serum levels of healthy Caucasians are given. See Addendum.

The innate alternative IgG subclass variants differ by structure and function, by amino acids [5], by electrophoretic and chromatographic rates [20], by developmental rate during childhood [19], by half-life times [21]. by different results on vaccination [22, 23], and treatment with monoclonal humanized antibodies [24]. Allergens, bacteria [22, 23] and virus

[25-27] affect *IGHG* genes of individuals differently and relate to the diseases as allergy [7-12] primary immunodeficiency [28], autoimmunity [29,30] and malignancy [31, 32].

Restrictive *IGHG* genes are found in patients with clinical allergy, increased IgE levels, increased IgG4 levels, with a family history of allergy and in technicians exposed to laboratory animals developing laboratory animal allergy [7-10]. The *IGHG* genes are linked to different phenotypes of childhood asthma: the *IGHG*bfn* to IgE mediated and the *IGHG*bf-n* and *IGHG*ga-n* to infectious prone asthma, combined with low levels of IgG [7].

The aim of the study was to investigate, if *IGHG* genes can predict the development of atopy in children. The rate of food and aeroallergen IgE sensitisation was investigated in *IGHG* diplotypes, *IGHG* haplotypic B cells, *IGHG2* genes and IgG subclass levels of children of age 1- and 10-years, from the MAS study. The serum levels of innate IgG subclass molecules were assessed [7] in 10 year old IgE sensitized children .

Materials and Methods

Study population

The study population comprised 194 Caucasian children from the German Multicenter Allergy Study (MAS) birth cohort, with case and controls from the same group of patients. Details of recruitment and the study subjects have been reported previously [33]. For the present analyses, we used data on allergic sensitization from the 1-year and 10-year follow-up assessments. The research protocol was approved by the ethical review board of the coordinating center (Charité University Medical Center, Berlin, Germany).

Assessment of allergic sensitization

Specific sensitization to 4 food allergens (hen's egg, cow's milk, soy and wheat) and 5 common indoor and outdoor aero-allergens (house dust mite, cat, dog, birch pollen and timothy grass pollen) was assessed as specific IgE antibodies in blood of 1 and 10 years-old children, respectively, by utilizing ImmunoCAP (Phadia,AB Uppsala, Sweden). Children were regarded as sensitized if the specific IgE to at least 1 allergen was ≥ 0.35 kU/L. The analysis were performed by Phadia Laboratories in Berlin.

IGHG genotyping and quantification of innate IgG subclass levels

The *IGHG* genes were assessed by serological *IGHG* (Fcγ) (GM) gene mapping based on alternative GM allotypes for quality and quantity. The GM allotypes are genetic markers on the constant heavy G chains the Fc part of the molecule. The alternative allotypes of γ3, γ1 and γ2 are distinctive unique entities with different structures and functions. IGHG genes and innate IgG subclasses were investigated for quality and quantity with new serological methods: Competitive ELISA, Double Immunodiffusion, Mancini technique [6,13].

Sera from 194 10 year-old and 159 1 year-old children from the MAS study were genotyped defining individual *IGHG* diplotypes, *IGHG* haplotypes (B cell variants) *IGHG* subclass genotypes and alternative *IGHG* allotypes (alleles). The very rare diplotypes (totally <1-2% of Caucasians) were left out. By the tests the quantitative expressions of *IGHG* genes are available. Total IgG subclass levels were measured with the Mancini technique. Innate IgG subclass levels were assessed in IgE sensitized patients with homozygous *IGHG*bfn/*bfn* diplotypes and heterozygous *IGHG*bfn/*bf-n* and compared to not sensitized age- and *IGHG*-matched children from the MAS study.

Statistical analysis

The data were analysed by the SPSS statistical package , SPSS 11.5. χ^2- test and 2 by 2 tables employing the "Wald" approximation of the variance of the Odds ratios, similar to simple logistic regression with an estimate of the odds ratios (OR) for the final model variables and associated 95% confidence intervals (CI). A P-value < 0.05 was considered statistically significant.The serum levels of allelic innate IgG subclasses of IgE sensitized patients with the *IGHG*bfn/*bfn* and *IGHG*bfn/*bf-n* diplotypes were compared to non-sensitized age- and *IGHG* - matched controls with the z-test.

Results

Table 1-4 are referred to the on line supplement

The "atopic march" in IGHG diplotypes, IGHG haplotypes and IGHG2 genotypes

The IgE sensitization rate for children at 1 and 10 years of age, the "atopic march", was recorded. The atopic march was most prominent within the homozygous *IGHG*bfn/*bfn* (B1/B1) diplotype, with increase from 12/35=34% to 27/45=60% sensitized (Table 1). The various specific allergen sensitization within homozygous *IGHG*bfn/*bfn* rated from 38-60% in 1 year-old and 25-39% in 10 year-old, respectively. The remaining two homozygous diplotypes showed lower rates of sensitization, *IGHG*bf-n/*bf-n* (B2/B2) from 0/7=0% to 6/9=67% and in *IGHG*ga-n/*ga-n* (B4/B4) from 1/19=5% to 9/21=43%, respectively. The specific allergen sensitization within *IGHG*bf-n/*bf-n was* 0% in 1 year-old and 0-7% in 10 year-old and within *IGHG*ga-n/*ga-n,* 0-4% in 1 year-old and 8-15% in 10 year-old, respectively. Thus, the *IGHG2*-n/*-n* was not involved in the food sensitization but to some degree in aeroallergen sensitization. IgE sensitization was increased within the homozygous *IGHG2*n/*n* (*IGHG*bfn/*bfn*) genotype compared to the opposite *IGHG2*-n/*-n*, containing *IGHG*bf-n* and *IGHG*ga-n* haplotypes (OR 2.9, 95% CI 1.1-7.2; P= 0.022). The atopic march for cat allergy was most prominent for *IGHG*bfn/*bfn*: 2 in 1 year old and rising to 15 in 10 year old; compared to the other homozygous diplotypes for *IGHG*bf-n/*bf-n*: 0 to 1 and for *IGHG*ga-n/*ga-n*: 0 to 3 in 1 and 10 year old, respectively (Table 1A,1B).

*IgE sensitization in IGHG haplotypes (innate B cells) and IGHG2 alleles (innate IgG2*n and IgG2*-n molecules)*

Of 35 sensitized (70 *IGHG* haplotypes) 1 year-old children, the *IGHG*bfn* haplotype, B^{*bfn} (B1) cells, and the *IGHG2*n* allele frequencies were significantly increased, in all sensitized (N= 41, OR 1.9, 95% CI 1.2-3.1; P=0.010), and in specific hen´s egg sensitized (N= 32 OR 2.7, 95% CI 1.4-4.9; P=0.001), in milk sensitized (N= 23, OR 2.0, 95%CI 1.1-4.0; P=0.031) and in wheat sensitized children (N= 16, OR 5.3, 95% CI 1.8-16.0; P<0.001) compared to 124 not sensitized (Table 2A, 3A). The opposite *IGHG*ga-n* haplotype and B^{*ga-n} (B4) cells with the *IGHG2*-n* allele were instead significantly decreased in all sensitized (N= 14, OR 0.5, 95% CI 0.3-0.9; P= 0.030),

Of 99 sensitized 10 year-old children (198 *IGHG* haplotypes), the *IGHG*bfn* haplotype B^{*bfn} (B1) cells and the *IGHG2*n* allele frequencies were significantly increased in all sensitized (N= 101, OR 1.4, 95% CI 1.02-1.9; P=0.034) (2B,3B) and in specific birch sensitized (N= 61, OR 2.0, 95% CI 1.3-3.0; P<0.001), in grass sensitized (N= 77, OR 1.4, 95%CI 1.0-2.0; P=0.050), in cat sensitized (N= 45, OR 1.9, 95% CI 1.2-3.1; P=0.006) and in dog sensitized (N= 46, 1.4, 95%CI 1.02-1.9; P=0.034) compared to 95 not sensitized children (Table 2B,3B). In this age group, the frequency of the opposite *IGHG*ga-n* haplotype, B^{*ga-n} (B4) cells did not differ when compared to not sensitized. The *IGHG* gene frequencies of 124 1 year-old and of 95 non IgE sensitized 10 year-old children did not deviate from those of a healthy Caucasian population (Hardy-Weinberg equilibrium P >0.5) (Addendum, Gene Map).

*Positive IgE tests associated with IGHG2*n alleles*

Next, we investigated the quality of IgE sensitization by the numbers of positive IgE tests per person, at different ages. We found that carriers of the homozygous *IGHG*bfn/*bfn* including homozygous *IGHG2*n/*n* had higher numbers of positive IgE tests than carriers of

the alternative *IGHG2*-n/*-n* 2.3 vs 1.0 positive tests per patient in 1 year old and 4.5 vs 3.2 positive tests per patient in 10 year old.

*Increased innate IgG2*n subclass levels in IgE sensitized individuals with the homozygous IGHG*bfn/*bfn and heterozygous IGHG*bfn/*bf-n diplotypes*

27 IgE sensitized 10 year-old children compared to18 non sensitized, with homozygous *IGHG*bfn/*bfn* demonstrated significantly increased amounts of the allelic innate IgG2*n subclass 1.21 ±0.43 g/l and IgE 567.29 ± 630.31 kU/l levels compared to non sensitized IgG2*n 0.92 ± 0.44 g/l and IgE 25.20 ± 20.48 kU/l, respectively (Table 4). Also 26 IgE sensitized compared to 26 non sensitized heterozygous *IGHG*bfn/*bf-n* with only one *IGHG*bfn* haplotype and one *IGHG2*n* allele demonstrated significantly increased IgG2*n 0.70 ± 0.24 g/l and IgE 332.97 ± 314,37 kU/l compared IgG2*n 0.55 ± 0.24 g/l and IgE 45.80 ± 52.95 kU/l, respectively, The level of IgG2*-n was left out.

Discussion

IGHG (γ) genes are in linkage disequilibrium with the *IGHE* (ε) gene on chromosome 14q32.3 (5´μ, δ, γ3, γ1, ψε α1, γ2, γ4, ε, α2 3´). In absence of genetic markers for *IGHE*, the *IGHG* genes are informative. The alternative GM allotypes of γ3-, γ1- and γ2- chains, respectively, were identified with a new sensitive competitive ELISA, defining the inter individual variability of innate IgG subclasses and innate B cells [13, 14]. The alternative GM allotypes are markers of the Fcγ part of the IgG molecules differ by structures and functions [5]. Specific *IGHG* genes are involved in IgE sensitization of allergy patients [7-12]. GWAS and HapMap do not include GM allotypes in their genetic platforms. This have underestimated *IGHG* as candidate genes in immunobiology and allergy [15-17].

We demonstrate a significant association of *IGHG*bfn* haplotypes with innate IgG3*b, IgG1*f and IgG2*n expressed from the innate B^{*bfn} (B1) cells, in 1 year old IgE sensitized with food allergens and in10 year-old children IgE sensitized with aero allergens (Table 2,3). IgE sensitization was most prominent in the homozygous *IGHG*bfn/*bfn* with *IGHG*bfn* inherited from both parents. The number of IgE sensitized in homozygous *IGHG*bfn/*bfn*, rose from 12/35, 34% in 1 year old, to 27/45, 60 % in 10 year old, demonstrating the "atopic march". Homozygous *IGHG2*n/*n* contained the dominating numbers of positive IgE tests/ individual, compared to the opposite homozygous *IGHG2*-n/*-n*. The innate IgG2*n levels were significantly increased in IgE sensitized carriers, in homozygous *IGHG*bfn/*bfn* and heterozygous *IGHG*bfn/*bf-n* , compared to age- and *IGHG*- matched non sensitized children (Table 4). Increased innate IgG2*n levels have been recorded in IgE mediated childhood asthma [7]. The activated *IGHG2*n* allele is probably

taking part in the IgE sensitization process. The γ2 genes are located,18kb from the γ4 and another 23 kb to the ε genes on chromosome 14q32.3, which part is activated by IL-4 and IL-13 cytokines from Th2 lymphocytes [35, 36]. GWAS has been rather insufficient to capture causal allergy gene variants. However, recently a fine-mapping study of IgE associated loci confirmed and identified SNPs of chromosome 5q31 regulating the Th2 cytokines IL-4 and IL-13 [34]. The finding of significantly increased IgG2*n levels in IgE sensitized children, is highlighted and must be compared with the slow rate of increasing IgG2*n levels during childhood in healthy children [7, 20]. The stimulation by allergens of both the *IGHG2*n* allele and *IGHE* genes, avoiding activation of *IGHG3*b* and *IGHG1*f* alleles upstreams, known to be activated by infections in healthy controls, might be in agreement with the hygiene hypothesis. The present paradigm dictates that atopy is accomplished mainly by the effect of *IGHE*, but the whole *IGH* haplotype, with both *IGHG* and *IGHE* genes are involved, which gives the particular innate IgG molecules IgG3*b, IgG1*f and IgG2*n a role in allergy, probably in the early sensitization process as the *IGHG* genes are activated before the downstream located *IGHE*. *IGHG*bfn* haplotype including the *IGHG2*n* allele and increased IgG2*n levels are most prominent risk factors for IgE sensitisation.

In 1 year-old children, food allergens had affected individuals with the *IGHG*bfn* haplotype (B1 cells) more often than the other haplotypes. A negative association of the opposite *IGHG2*-n* allele with allergen sensitization was observed. The homozygous *IGHG2*-n/*-n* genotype demonstrated a weaker sensitisation rate (Table 1-3). In 10 year-old aeroallergens affected mainly the same IGHG*bfn, but the IgE sensitization was found to some degree also in *IGHG*bf-n* and *IGHG*ga-n* haplotypes, with the *IGHG2*-n* allele (Table 1B, 2B). But when equal numbers of the six most common *IGHG* diplotypes of a childhood asthma cohort were investigated, the homozygous *IGHG*bfn/*bfn* was linked to atopy and the different homozygous *IGHG*bf-n/*bf-n* and *IGHG*ga-n/*ga-n* were linked to low levels of

IgE low IgE sensitization and low levels of innate IgG subclasses as in IgG subclass deficiencies [7].

B cells are the only cells producing antibodies and B cells can indirectly be defined by *IGHG* genes [14]. *IGHG* genes of B cells are found in all human somatic cells, also stem cells [35]. All genes constituting the *IGHG* haplotype have impact on the expressed levels of innate IgG subclasses, as it has been demonstrated in both immunodeficient and healthy individuals [19, 20, 28]. The function of the immunoglobulin G molecule is related to the constant part of the heavy G chains, the Fcγ part of the molecule, in parallel to the function of the variable adaptive antibody binding site. The *IGHG*(Fcγ)(GM) allotypes with epitope differences in the Fc part of the molecule, have influence on the outcome of both bacterial and viral infections. Individuals with the *IGHG*bfn* haplotype are high producers of antibodies against encapsulated bacteria and in vaccination with bacterial polysaccharides [22, 23]. The influence of viral particles acting as FcγRs with preference of binding to different *IGHG* (Fcγ) allotypes [25-27] is important and can be applied also in allergic disease, possibly related to exacerbations. Severe infections in infants with respiratory syncytial virus (RSV) are associated with the *IGHG*bf-n/*bf-n* diplotype and the *IGHG2*-n* alleles [36].

IGHG genes can predict different phenotypes in childhood asthma and must be investigated in other forms of allergy. Active and passive immunotherapy as allergy vaccination and treatment with humanized recombinant monoclonal antibodies affect individuals with different *IGHG* diplotypes with different results. *IGHG* genes must be investigated in relation to other genes and biomarkers with known association to IgE mediated allergy.

Conflict of interest statement

None declared

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Table 1-4 are referred to on line suppl.
Table 1.

A/ *IGHG* genes in 1-year-old children, IgE sensitized against food allergens. Diplotypes, haplotypes (B cell variants) and *IGHG2* genotypes are registered as numbers and % of total. The various homozygous genes are marked.

IGHG diplotypes	*IGHG* haplotypes B cells	*IGHG2* genotypes	1 year-old-children	IgE sensi-tized	Not Sensi-tized	Different allergen sensitization Egg	Milk	Soya	Wheat	Cat
bfn/*bfn	**B1/B1**	***n/*n***	**35 (22)**	**12(34)**	**23(19)**	**10(42)**	**7(37)**	**3(38)**	**6(60)**	**2(40)**
**bfn/*bf-n*	B1/B2	**n/*-n*	44(28)	10(29)	34(27)	6(25)	6(32)	3(38)	2(20)	3(60)
**bfn/*ga-n*	B1/B4	**n/*-n*	33(21)	7(20)	26(21)	6(25)	3(16)	1(13)	2(20)	0
bf-n/*bf-n	**B2/B2**	***-n/*-n***	**7(4)**	**0**	**7(6)**	**0**	**0**	**0**	**0**	**0**
**bf-n/*ga-n*	B2/B4	**-n/*-n*	21(13)	5(14)	16(13)	1(4)	3(16)	1(13)	0	0
ga-n/*ga-n	**B4/B4**	***-n/*-n**	**19(12)**	**1(3)**	**18(15)**	**1(4)**	**0**	**0**	**0**	**0**
Total number			**159**	**35**	**124**	**24**	**19**	**8**	**10**	**5**

1 year old children 35 IgE sensitized and 124 not sensitized, from the MAS study.

B/ *IGHG* genes in 10-year-old children, IgE sensitized against aeroallergens Diplotypes, haplotypes (B cell variants) and *IGHG2* genotypes are registered as numbers and % of total. The various homozygous genes are marked.

IGHG diplotypes	*IGHG* haplotypes B cells	*IGHG2* geno-types	10-year-old children	IgE sensi-tized	Not Sensi-tized	Different allergen sensitization Birch	Grass	Cat	Dog	Mites	Latex
bfn/*bfn	**B1/B1**	***n/*n***	**45(23)**	**27(27)**	**18(19)**	**20(39)**	**21(28)**	**15(39)**	**13(31)**	**13(25)**	**6(29)**
**bfn/*bf-n*	B1/B2	**n/*-n*	52(27)	26(26)	26(27)	11(22)	19(25)	9(24)	12(29)	14(27)	6(29)
**bfn/*ga-n*	B1/B4	**n/*-n*	41(21)	21(21)	20(21)	10(20)	16(21)	6(16)	8(19)	13(25)	4(20)
bf-n/*bf-n	**B2/B2**	***-n/*-n***	**9(5)**	**6(6)**	**3(3)**	**2(4)**	**5(7)**	**1(3)**	**1(2)**	**1(2)**	**0(0)**
**bf-n/*ga-n*	B2/B4	**-n/*-n*	26(13)	10(10)	16(17)	4(8)	7(9)	4(11)	4(10)	5(10)	2(10)
ga-n/*ga-n	**B4/B4**	***-n/*-n**	**21(11)**	**9(9)**	**12(13)**	**4(8)**	**7(9)**	**3(8)**	**4(10)**	**6(12)**	**3(15)**
Total number			**194**	**99**	**95**	**51**	**75**	**38**	**42**	**52**	**21**

10 year old children 99 IgE sensitized and 95 not sensitized, from the MAS study.

Table 2.

A/ IgE sensitized at 1-year of age registered as *IGHG* haplotypes (innate B cells): B1 (B^{*bfn}), B2 ($B^{*bf\text{-}n}$) and B4 ($B^{*ga\text{-}n}$)

(35 1-year-old children sensitized against food allergens (hen´s egg, cow's milk, wheat) and 124 not sensitized from the MAS study)

IgE sensitization	B cell variants (*IGHG* haplotypes) Numbers(% of total)					
		Yes	No	P value	OR	95% CI
Egg Sensitized		48	248			
(24)	B1 (B^{*bfn})	32(67) ↑	106(42)	0.001	2.7	1.4-4.9
	B2 ($B^{*bf\text{-}n}$)	7(15)	64(26)			
	B4 ($B^{*ga\text{-}n}$)	9(19) ↓	78(31)	0.047	0.48	0.23-1.0
Milk sensitized		38	248			
(19)	B1 (B^{*bfn})	23(61) ↑	106(42)	0.031	2.0	1.1-4.0
	B2 ($B^{*bf\text{-}n}$)	9(24)	64(26)			
	B4 ($B^{*ga\text{-}n}$)	6(16) ↓	78(31)	0.031	0.4	0.16-0.94
Wheat sensitized		20	248			
(10)	B1 (B^{*bfn})	16(80) ↑	106(42)	<0.001	5.3	1.8-16.0
	B2 ($B^{*bf\text{-}n}$)	2(10)	64(26)			
	B4 ($B^{*ga\text{-}n}$)	2(10) ↓	78(31)	0.033	0.23	0.08-1.0
All Allergens		70	248			
(35)	B1 (B^{*bfn})	41(59) ↑	106(42)	0.010	1.9	1.2-3.1
	B2 ($B^{*bf\text{-}n}$)	15(21)	64(26)			
	B4 ($B^{*ga\text{-}n}$)	14(20)↓	78(31)	0.030	0.5	0.3-0.9

B/ IgE sensitized at 10-years of age, registered as *IGHG* haplotypes (innate B cells): B1 (B^{*bfn}), B2 ($B^{*bf\text{-}n}$) and B4 ($B^{*ga\text{-}n}$)

(97 children sensitized against aero allergens (birch, grass, cat, dog) and 95 not sensitized 10 year-old children from the MAS study).

IgE sensitized	B cell variants (*IGHG* haplotypes) Numbers (%of total)					
		Yes	No	P value	OR	95% CI
Birch Sensitized		102	190			
(51)	B1 (B^{*bfn})	61(60) ↑	82(43)	<0.001	2.0	1.3-3.0
	B2 ($B^{*bf\text{-}n}$)	19(19)	48(25)			
	B4 ($B^{*ga\text{-}n}$)	22(22)	60(32)			
Grass Sensitized		150	190			
(75)	B1 (B^{*bfn})	77(51) ↑	82(43)	0.050	1.4	1.0-2.0
	B2 ($B^{*bf\text{-}n}$)	36(24)	48(25)			
	B4 ($B^{*ga\text{-}n}$)	37(25)	60(32)			
Cat Sensitized		76	190			
(38)	B1 (B^{*bfn})	45(59) ↑	82(43)	0.006	1.9	1.2-3.1
	B2 ($B^{*bf\text{-}n}$)	15(20)	48(25)			
	B4 ($B^{*ga\text{-}n}$)	16(21)	60(32)			

Dog Sensitized		84	190			
(42)	B1 (B*bfn)	46(55) ↑	82(43)	0.035	1.6	1.03-2.5
	B2 (B*bf-n)	18(21)	48(25)			
	B4 (B*ga-n)	20(24)	60(32)			
All Allergens		194	190			
(97)	B1 (B*bfn)	101(51) ↑	82(43)	0.034	1.4	1.02-1.9
	B2 (B*bf-n)	48(24)	48(25)			
	B4 (B*ga-n)	49(25)	60(32)			

Table 3.
A/ IgE sensitisated at 1 year of age children registered as *IGHG2* genotypes (*IGHG2*n* and *IGHG2*-n* alleles) (number and % of total) of 35 sensitized (hen´segg, cow¨s milk, wheat) and 124 not sensitized 1 year-old children from the MAS study

IgE sensitisation	***IGHG2* genotypes, N(%)**			***IGHG2*n and IGHG2*-n* alleles, N(%)**						
		Yes	**No**	**Yes**		**No**		**P value**	**OR**	**95% CI**
Egg Sensitized		24	124	48		248				
(24)	*n/*n	10(42)↑	23(19)					0.014	2.7	1.2-6.3
	n/-n	12(50)	60(48)							
	-n/-n	2(8)↓	41(33)					0.007	0.2	0.04-0.73
				*n	*-n	*n	*-n			
				37(56) ↑	29(44)↓	106(43)	142(57)	0.001	2.7	1.4-4.9
Milk Sensitized		19	124	38		248				
(19)	*n/*n	7(37)	23(19)							
	n/-n	9(47)	60(48)							
	-n/-n	3(16)	41(33)							
				*n	*-n	*n	*-n			
				23(61) ↑	15(39) ↓	106(43)	142(57)	0.031	2.0	1.1-3.9
Wheat Sensitized		10	124	20		248				
(10)	*n/*n	6(60)	23(19)							
	n/-n	4(40)	60(48)							
	-n/-n	0(0)	41(33)							
				*n	*-n	*n	*-n			
				16(80) ↑	4(20) ↓	106(43)	142(57)	<0.001	5.3	1.8-16.0
All allergens		35	124	70		248				
(35)	*n/*n	12(34)	23(19)							
	n/-n	17(49)	60(48)							
	-n/-n	6(17)	41(33)							
				*n	*-n	*n	*-n			
				41(59) ↑	29(41) ↓	106(43)	142(57)	0.010	1.9	1.2-3.1

Table 3 cont.

B/ IgE sensitized at 10 years of age
Registered as *IGHG2* genotypes (*IGHG2*n* and *IGHG2*-n* alleles) (number and % of total) of 99 sensitized against aeroallergens (birch, grass, cat, dog) and 95 not sensitized 10-old children from the MAS study

IgE sensitization	***IGHG2* genotypes, N(%)**			***IGHG2**n and IGHG*-n alleles N(%)**						
		Yes	**No**	**Yes**		**No**		**P value**	**OR**	**95% CI**
Birch Sensitized		51	95	102		190				
(51)	**n/*n*	20(39)↑	18(19)					0.003	2.3	1.3-4.3
	**n/*-n*	21(41)	46(48)							
	**-n/*-n*	10(20)↓	31(33)					0.022	0.4	0.21-0.91
				**n*	**-n*	**n*	**-n*			
				61(60) ↑	41(40) ↓	82(43)	108(57)	<0.001	2.0	1.3-3.0
Grass Sensitized		75	95	150		190				
(75)	**n/*n*	21(28)	18(19)							
	**n/*-n*	35(47)	46(48)							
	**-n/*-n*	19(25)	31(33)							
				**n*	**-n*	**n*	**-n*			
				77(51) ↑	73(49) ↓	82(43)	108(57)	0.050	1.4	1.0-2.0
Cat Sensitized		38	95	76		190				
(38)	**n/*n*	15(39)	18(19)							
	**n/*-n*	15(39)	46(48)							
	**-n/*-n*	8(21)	31(33)							
				**n*	**-n*	**n*	**-n*			
				45(59) ↑	31(41) ↓	82(43)	108(57)	0.006	1.9	1.2-3.1
Dog Sensitized		42	95	84		190				
(42)	**n/*n*	13(31)	18(19)							
	**n/*-n*	20(48)	46(48)							
	**-n/*-n*	9(21)	31(33)							
				**n*	**-n*	**n*	**-n*			
				46(55) ↑	38(45) ↓	82(43)	108(57)	0.035	1.6	1.0-2.5
All allergens		99	95	198		190				
(99)	**n/*n*	27(27)	18(19)							
	**n/*-n*	47(47)	46(48)							
	**-n/*-n*	25(25)	31(33)							
				**n*	**-n*	**n*	**-n*			
				101(51) ↑	97(49) ↓	82(43)	108 (57)	0.034	1.4	1.02-1.8

Table 4.
Innate IgG subclasses levels (and IgE levels), in A) homozygous *IGHG*bfn/*bfn* and B) heterozygous *IGHG*bfn/*bf-n,* in IgE sensitized 10 year old children, compared to not sensitized age-and *IGHG* gene-matched controls.

A/

Homozygous *IGHG*bfn/*bfn* 10 years-old		
Innate IgG Subclass levels IgE levels M(SD)	**27 IgE sensitized**	**18 not sensitized**
IgG3*b	0.48(0.14)	0.49(0.14)
IgG1*f	8.25(1.14)	7.95(1.50)
IgG2*n	1.21(0.43)[a)]	0.92(0.44)[a)]
IgE	567.29(630.31)[b)]	25.20(20.48)[b)]

B/

Heterozygous *IGHG*bfn/*bf-n* 10 years old		
Innate IgG subclass levels IgE levels M(SD)	**26 sensitized**	**26 not sensitized**
IgG3*b	0.45(0.14)	0.44(0.16)
IgG1*f	7.88(1.00)	7.38(1.00)
IgG2*n	0.70(0.24)[a)]	0.55(0.24)[a)]
IgG2*-n not given		
IgE	332.97(314.37)[b)]	45.80(52.95)[b)]

a),b) significantly increased in sensitized

ADDENDUM

Dissecting the *IGHG* gene complex in a healthy Caucasian population

(A)

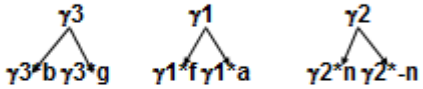
Chromosome 14q32.3 (5´μ,δ,γ3,γ1,ψε, α1,γ2,γ4,ε,α2 3´)

Constant heavy γ chains: γ3, γ1, γ2, γ4

IgG subclasses: IgG3 IgG1 IgG2 IgG4

(B)

Alternative allotypes of heavy γ chains:



(C)

Allotypic (allelic) innate IgG subclasses (6):

IgG3*b IgG3*g; IgG1*f IgG1*a; IgG2*n IgG2*-n

(D)

Homozygous and heterozygous *IGHG* subclass genes (9):

*IGHG3*b/*b, IGHG3*g/*g, IGHG3*b/*g; IGHG1*f/*f, IGHG1*a/*a, IGHG1*f/*a; IGHG2*n/*n, IGHG2*-n/*-n, IGHG2*n/*-n*

(E)

Homozygous and heterozygous innate IgG subclasses (9):

IgG3*b/*b, IgG3*g/*g, IgG3*b/*g; IgG1*f/*f, IgG1*a/*a, IgG1*f/*a; IgG2*n/*n, IgG2*-n/*-n, IgG2*n/*-n

(F)

***4 IGHG* haplotypes (fixed combinations of γ3-, γ1- and γ2- genes) B cell variants**

*IGHG*bfn* (B1)	*IGHG*bf-n* (B2)	*IGHG*gan* (B3)	*IGHG*ga-n* (B4)
(42.8%)	(24.0%)	(1.5%)	(31.6%)
503	282	18	371

(Frequency figures from 587 healthy Caucasians both children and adults, 1174 haplotypes)

(G)

10 Individual *IGHG* (Fcγ) (GM) diplotypes. Gene Map

*bfn/*bfn*	**bfn/*bf-n*	**bfn/*ga-n*	**bf-n/*bf-n*	**bf-n/*ga-n*	**ga-n/*ga-n*	**bfn/*gan*	**bf-n/*gan*	**gan/*gan*	**gan/*ga-n*
(20.1%)	(18.2%)	(26.0%)	(6.8%)	(16.2%)	(10.9%)	(2.2%)	(<1%)	(0.3%)	(0.2%)
118	107	147	40	95	64	13	0	2	1

(Frequency figures from 587 healthy Caucasians both children and adults, 1174 haplotypes)

(H)

Individual innate B cells and innate IgG subclasses. Gene Map

*bfn/*bfn*	**bfn/*bf-n*	**bfn/*ga-n*	**bf-n/*bf-n*	**bf-n/*ga-n*	**ga-n/*ga-n*	**bfn/*gan*	**bf-n/*gan*	**gan/*gan*	**gan/*ga-n*
B1/B1	B1/B2	B1/B4	B2/B2	B2/B4	B4/B4	B1/B3	B2/B3	B3/B3	B3/B4
IgG3*b	IgG3*b	IgG3*b	IgG3*b	IgG3*b	IgG3*g	IgG3*b	IgG3*b	IgG3*g	IgG3*g
IgG1*f	IgG1*f	IgG3*g	IgG1*f	IgG3*g	IgG1*a	IgG3*g	IgG3*g	IgG1*a	IgG1*a
IgG2*n	IgG2*n	IgG1*f	IgG2*-n	IgG1*f	IgG2-n	IgG1*f	IgG1*f	IgG2*n	IgG2*n
	IgG2*-n	IgG1*a		IgG1*a		IgG1*a	IgG1*a		IgG2*-n
		IgG2*n		IgG2*-n		IgG2*n	IgG2*n		
		IgG2*-n					IgG2*-n		

Fixed combination γ3-γ1-γ2 genes = *IGHG* haplotypes, also markers of 4 B cell variants

Frequencies of *IGHG* haplotypes in a healthy Caucasian population (587)

IGHG*bfn 5′ μ,δ **γ3*b,γ1*f,** ε,α1 **γ2*n,** γ4,ε,α2 3′ **Innate IgG subclasses:** **IgG3*b** **IgG1*f** **IgG2*n**	B*bfn **(B1)** 44.8%	***IGHG*gan*** 5′ μ,δ **γ3*g,γ1*a,** ε,α1 **γ2*n,** γ4,ε,α2 3′ **Innate IgG subclasses:** **IgG3*b** **IgG1*f** **IgG2*n**	B*gan **(B3)** 0.9%
IGHG*bf-n 5′ μ,δ **γ3*b,γ1*f,** ε,α1 **γ2*-n,** γ4,ε,α2 3′ **Innate IgG subclasses:** **IgG3*b** **IgG1*f** **IgG2*-n**	B*bf-n **(B2)** 22.9%	***IGHG*ga-n*** 5′ μ,δ **γ3*g,γ1*a,** ε,α1 **γ2*-n,** γ4,ε,α2 3′ **Innate IgG subclasses:** **IgG3*b** **IgG1*f** **IgG2*n**	B*ga-n **(B4)** 31.4%

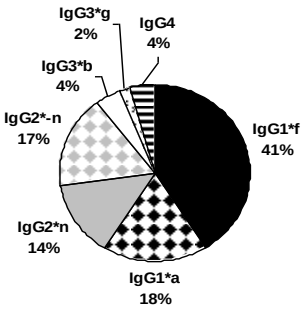
Gene map - Individual diversity of *IGHG* (GM) genes

Mendelian inheritance & allelic exclusion

10 *IGHG* diplotypes and B cell combinations (% in 587 healthy Caucasians)

IGHG*bfn/*bfn **γ3*b,γ1*f……γ2*n** **γ3*b,γ1*f……γ2*n**	**B1/B1** **20.1%**	***IGHG*ga-n/*ga-n*** **γ3*g,γ1*a……γ2*-n** **γ3*g,γ1*a……γ2*-n**	**B4/B4** **10.9%**
IGHG*bfn/*bf-n **γ3*b,γ1*f ……γ2*n** **γ3*b,γ1*f ……γ2*-n**	**B1/B2** **18.2%**	***IGHG*gan/*bfn*** **γ3*g,γ1*a……γ2*n** **γ3*b,γ1*f……γ2*n**	**B3/B1** **2.2%**
IGHG*bfn/*ga-n **γ3*b,γ1*f……γ2*n** **γ3*g,γ1*a ……γ2*-n**	**B1/B4** **25.0%**	***IGHG*gan/*bf-n*** **γ3*g,γ1*a……γ2*n** **γ3*b,γ1*f……γ2*-n**	**B3/B2** **<1%**
IGHG*bf-n/*bf-n **γ3*b,γ1*f ……γ2*-n** **γ3*b,γ1*f ……γ2*-n**	**B2/B2** **6.8%**	***IGHG*gan/*gan*** **γ3*g,γ1*a……γ2*n** **γ3*g,γ1*a……γ2*n**	**B3/B3** **0.3%**
IGHG*bf-n/*ga-n **γ3*b,γ1*f …… γ2*-n** **γ3*g,γ1*a ……γ2*-n**	**B2/B4** **16.8%**	***IGHG*gan/*ga-n*** **γ3*g,γ1*a……γ2*n** **γ3*g,γ1*a……γ2*-n**	**B3/B4** **0.2%**

Innate allelic IgG subclass levels (% of total IgG) in a nomal serum pool (about 2000 sera)

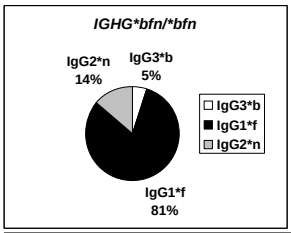


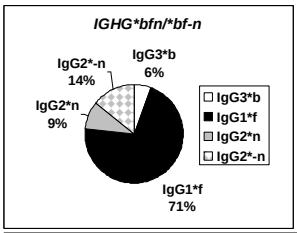
Individual diversity of *IGHG* genes with different qualities of IgG molecules

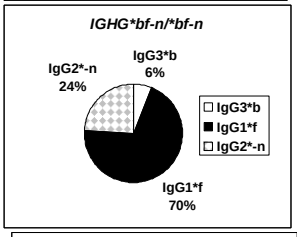
Homozygous diplotypes contain with 3 variants and heterozygous diplotypes contain 4-6 variants. Mean levels of innate IgG subclass variants, in % of total, in the 6 common (4 are very rare) *IGHG* diplotypes of 225 healthy 10-year-old Swedish Caucasian children Ref .

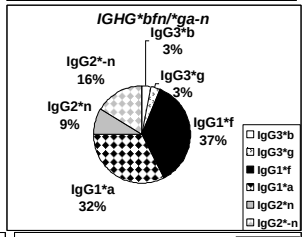
In homozygous diplotypes

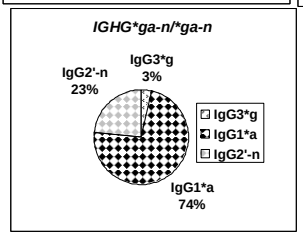
(3 innate allelic IgG subclass variants)

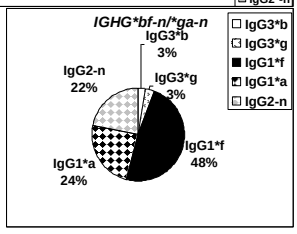












In heterozygous diplotypes

(4-6 innate allelic IgG subclass variants)