

From Genotypes of Immunoglobulin Constant Heavy G Chains (Fc γ) (GM) Genes (*IGHG*) to Phenotypes in Childhood Asthma

Vivi-Anne Oxelius

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

Key Words

Allelic IgG subclasses • Fc γ • GM allotypes • IgE • Immunodeficiency • Phenotypes • *IGHG* diagnostic tests • Immunoglobulin constant heavy G chain

Abstract

Background: IgE-mediated allergy is associated with immunoglobulin heavy constant G chain (Fc γ) (GM) genes (*IGHG*) on chromosome 14q32.3. Investigation of the alternative GM allotypes of γ 3, γ 1 and γ 2 chains has disclosed new structural and functional IgG subclasses and B-cell variants, with possible effects on childhood asthma. **Objective:** To investigate different *IGHG* (GM) gene complexes in a childhood asthma population for allergy parameters. **Methods:** *IGHG* alleles and correlated allotypic (allelic) IgG subclass levels were analyzed with a sensitive indirect competitive ELISA in 10-year-old children with bronchial asthma. Individual *IGHG* diplotype-, genotype- and haplotype-related B cells were compared for allelic IgG subclass levels, IgE sensitization, IgE, IgA and IgM levels, and numbers of peripheral blood eosinophils and lymphocytes. **Results:** The group with homozygous *IGHG***bfn*/**bfn* (B1/B1 cells) demonstrated low IgG1*f levels ($p < 0.001$) but increased IgG2*n levels ($p < 0.001$) together with increased IgE and *IGHG*2*n gene dose-dependent

IgE sensitization (atopic phenotype). The *IGHG***bf*-*n*/**bf*-*n* (B2/B2 cells) demonstrated low IgG1*f ($p < 0.05$) and IgG2*n ($p < 0.001$) and the *IGHG***ga*-*n*/**ga*-*n* (B4/B4 cells) low IgG1*a ($p < 0.001$) and IgG2*n ($p < 0.02$) together with low IgE sensitization (non-atopic phenotype). B**bfn* (B1) and B**bf*-*n* (B2) demonstrated increased numbers of peripheral blood eosinophils, compared to B**gan* (B4) cells, which demonstrated increased peripheral blood CD8 lymphocytes instead. **Conclusion:** *IGHG* diplotypes present different phenotypes in childhood asthma. The *IGHG*2*n dose relationship to IgE sensitization and increased IgG2*n levels in IgE sensitized are risk markers for IgE-mediated asthma. The opposite *IGHG*2*-*n* presents non-IgE-mediated asthma and IgG subclass deficiencies.

Copyright © 2012 S. Karger AG, Basel

Introduction

Childhood asthma is clinically a heterogeneous disease and the variants need to be identified already early in life, to optimize treatment. Several candidate genes have been found to be associated with the inflammatory response of IgE-mediated allergy [1–4], such as immunoglobulin constant heavy G chain (Fc γ) (GM) genes (*IGHG*) on

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2012 S. Karger AG, Basel
1018–2438/12/1591–0094\$38.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Vivi-Anne Oxelius
Department of Pediatrics, University Hospital
Lund University
SE-221 85 Lund (Sweden)
Tel. +46 46 211 4440, E-Mail vivi-anne.oxelius@med.lu.se

chromosome 14q32.3, which were reported in several studies [5, 6].

The IgG polymorphism was studied by GM allotypes found on the constant heavy G chains, the Fc part of IgG molecules [7]. GM allotypes are inherited in the Mendelian way and expressed by allelic exclusion. Using new methods, the GM allotype complex can be dissected in *IGHG* alleles, genotypes, haplotypes and diplotypes [8–11]. The two alternative GM allotypes of IgG3, IgG1 and IgG2, respectively, are unique structure and function variants [8–11]. The individually restricted IgG by *IGHG* genes is not well known (see online suppl. material, www.karger.com/doi/10.1159/000335592).

The alternative GM allotypes of IgG subclasses, allelic variants from chromosome 14q32.3: 5' μ , δ , γ 3, γ 1, α 1, γ 2, γ 4, ϵ , α 2 3' are for *IGHG3* (γ 3): *b and *g, for *IGHG1* (γ 1): *f and *a, and for *IGHG2* (γ 2): *n and *-n. The γ 3 and γ 1 alleles are in strong linkage disequilibrium and together with γ 2*n or γ 2*-n combined to four fixed *IGHG* haplotypes: *IGHG**bfn, *IGHG**bf-n, *IGHG**gan and *IGHG**ga-n [7]. The 4 *IGHG* haplotypes are indirect markers of B-cell variants: B*bfⁿ (B1), B*bf-n (B2), B*gan (B3) and B*ga-n (B4) [12]. They are found in 10 individual *IGHG* diplotypes.

Allelic IgG subclasses differ in structure and function by minor amino acids, correlating with single-base substitutions in the genes [7]. They differ by electrophoretic and chromatographic rates [8], by developmental rate during childhood [9, 10], by half-life [11] and by results of vaccination and treatment effects with intravenous immunoglobulin [11, 13, 14]. There are deviating frequencies of *IGHG* genes associated with bacterial [13, 14] and viral infections [15–17], and in primary immunodeficiencies [18] and autoimmune diseases [19, 20] compared to healthy children.

IgE-mediated allergy is associated with an increased frequency of the *IGHG**bfn haplotype. The *IGHG* association is found in patients with clinical allergy and increased IgE levels, increased IgG4 levels, a family history of allergy, and in technicians exposed to laboratory animals developing laboratory animal allergy [5, 6, 21–23]. The alternative *IGHG* haplotypes have been found with different pathways of immune regulation in patients with asthma [23].

The aim of this study was to link *IGHG* genes directly to phenotypic variants. The childhood asthma patients were *IGHG* genotyped and divided into 6 common *IGHG* diplotypes. The *IGHG* alleles and allelic IgG subclass levels were identified. The individual *IGHG* diplotypes, dissected genotypes and haplotypes (B cells) were investi-

gated for IgE sensitization, IgE, IgA and IgM levels and numbers of peripheral blood eosinophils and lymphocytes.

Methods

Study Design

The study population comprised totally 117 Caucasian *IGHG*-genotyped children with bronchial asthma; 111 were assigned to the 6 common *IGHG* diplotype groups. The patients were consecutively admitted to the Outpatient Clinic of the Department of Pediatrics, University Hospital of Lund; their age ranged from 7 to 17 years (mean 10 years) and they had recurrent obstructive asthma episodes, FEV₁/FVC <85% and positive reversibility tests after β ₂-agonist challenge. Lung function tests, blood sampling and skin prick tests (SPT) were routinely performed at the first visit and before treatment with local corticosteroids was started. To be able to compare the direct effects of the different *IGHG* diplotypes, about 20 patients were investigated in each *IGHG* diplotype group, which took a long time, particularly for the diplotypes containing the *IGHG*2*-n variant. No patient was on immunotherapy. Siblings were not included in the study. Blood samples were drawn occasionally without seasonal preference. Cap RAST tests were performed, and serum IgE levels, peripheral blood eosinophils and subsets of peripheral blood lymphocytes were determined (Clinical Immunology and Clinical Chemistry Laboratories, University Hospital of Lund).

IGHG Genotyping and Quantification of Allelic IgG Subclass Levels

A sensitive competitive ELISA was used to quantify serum IgG1*f, IgG1*a, IgG2*n and IgG3*b allotypes [9] with 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). Microtiter plates were coated with a predetermined concentration of purified myeloma proteins of the following IgG allotypes: IgG1*f, IgG1*a, IgG2*n and IgG3*b. A normal diluted serum pool and a panel of purified myeloma proteins served as positive and negative controls. Determination of homozygosity and heterozygosity for the IgG2*n allotype was done with a double immunodiffusion 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, beside absence of IgG3*b, was also the presence of the concordant IgG1*a allotype. In heterozygous individuals, the IgG3*g and IgG3*b amounts are about 50:50%, respectively, of the IgG3 amount [9, 10].

Sera from pediatric asthma patients were consecutively genotyped by alternative GM allotypes, designing the individual *IGHG* diplotype. The *IGHG* diplotypes express restricted qualities of IgG compared to a normal serum pool, which contains all IgG variants: IgG3*b, IgG3*g, IgG1*f, IgG1*a, IgG2*n and IgG2*-n. The 6 most common *IGHG* diplotypes were collected in comparable numbers: the homozygous *IGHG**bfn/*bfn expressing IgG3*b, IgG1*f and IgG2*n (lacking IgG3*g, IgG1*a and IgG2*-n), *IGHG**bf-n/*bf-n expressing IgG3*b, IgG1*f and IgG2*-n (lacking IgG3*g, IgG1*a and IgG2*n) and *IGHG**ga-n/*ga-n expressing IgG3*g, IgG1*a and IgG2*-n

(lacking IgG3*b, IgG1*f and IgG2*n) and the heterozygous *IGHG*bf-n/bf-n* expressing IgG3*b, IgG1*f, IgG2*n and IgG2*-n (lacking IgG3*g and IgG1*a) *IGHG*bf-n/ga-n* expressing IgG3*b, IgG3*g, IgG1*f, IgG1*a, IgG2*n and IgG2*-n and *IGHG*bf-n/*ga-n* expressing IgG3*b, IgG3*g, IgG1*f, IgG1*a, IgG2*-n (lacking IgG2*n), see online supplementary material. The very rare diplotypes (totally <1–2% of Caucasians) were left out. The tests enable quantitative expression of *IGHG* genes. Total IgG subclass levels were determined using the Mancini technique. Allelic IgG subclasses of the *IGHG* diplotypes of 117 childhood asthma patients were compared with 225 *IGHG* diplotype- and age-matched healthy children from an earlier investigation [9].

All the data collection was approved by the Ethics Committee of the University of Lund.

Statistical Analysis

The data were analyzed using the SPSS statistical package (SPSS 11.5). The IgG3-, IgG1- and IgG2-alternative allotype serum levels of *IGHG* diplotypes of the patients were compared to healthy age- and *IGHG* diplotype-matched controls with the z-test. IgE levels, IgE sensitization, peripheral blood eosinophils and lymphocytes of the different *IGHG* diplotypes were compared and analyzed by z-test, χ^2 test and 2×2 tables employing the 'Wald' approximation of the variance in odds ratios (OR), similar to simple logistic regression with an OR estimate for the final model variables and associated 95% confidence intervals (CI). A value of $p < 0.05$ was considered statistically significant.

Results

Allelic IgG Subclass Levels

Homozygous *IGHG* Diplotypes from B1/B1, B2/B2 and B4/B4 Cells

For the homozygous diplotype *IGHG*bf-n/bf-n* (B1/B1 cells), the serum IgG1*f level was decreased ($p < 0.001$), but IgG2*n was increased ($p < 0.001$) compared to the healthy control population (fig. 1a). For the homozygous *IGHG*bf-n/bf-n* diplotype (B2/B2 cells) both IgG1*f and IgG2*-n levels were significantly decreased ($p < 0.05$ and $p < 0.001$, respectively) as were IgG1*a and IgG2*-n levels for the *IGHG*ga-n/*ga-n* diplotype (B4/B4; $p < 0.001$ and $p < 0.02$, respectively; fig. 1a).

Heterozygous *IGHG* Diplotypes from B1/B2, B1/B4 and B2/B4 Cells

Of the heterozygous diplotypes, *IGHG*bf-n/bf-n* (B1/B2 cells), the IgG1*f level ($p < 0.001$) was low but the IgG2*n level ($p < 0.05$) increased, in agreement with the results from homozygous *IGHG*bf-n/bf-n* individuals and low IgG2*-n levels ($p < 0.001$) in agreement with the results from homozygous *IGHG*bf-n/bf-n* individuals (fig. 1). For the heterozygous *IGHG*bf-n/ga-n* diplotype (B1/B4 cells), the *IGHG*bf-n* haplotype expressed

IgG3*b, IgG1*f and IgG2*n levels without significant change from healthy children, but the *IGHG*ga-n* haplotype showed decreased IgG3*g ($p < 0.001$), IgG1*a ($p < 0.001$) and IgG2*-n ($p < 0.02$) levels. For the heterozygous *IGHG*bf-n/*ga-n* (B2/B4 cells), the IgG3*g was low ($p < 0.02$), but the IgG3*b did not deviate. The IgG1*f and IgG1*a levels were both significantly low ($p < 0.001$ and $p < 0.02$, respectively) and IgG2*-n levels were low compared to healthy controls ($p < 0.001$; fig. 1b).

IGHG Diplotypes and Dissected *IGHG2* Genotypes

Investigated for IgE Sensitization

The homozygous *IGHG*bf-n/bf-n* diplotype (B1/B1) was more frequent (21/24 IgE-sensitized patients; 88%) than both the homozygous *IGHG*bf-n/bf-n* diplotype (B2/B2 cells) with 2/10 patients (20%) and the homozygous *IGHG*ga-n/*ga-n* diplotype (B4/B4 cells) with 3/15 patients (20%; fig. 2a). The homozygous *IGHG2*n/*n* genotype showed the highest number of sensitized patients compared to *IGHG2*-n/*n* ($p < 0.001$; OR 31.0, CI 14.2–67.7) and compared to the heterozygous *IGHG2*n/*n* genotype ($p = 0.001$; OR 6.2, CI 3.0–12.6; fig. 2b). The same significant difference was found for the rate of IgE sensitization (fig. 2c). In total, there were 89 positive SPT/RAST in 24 patients, 3.7 positive tests per patient for the *IGHG*bf-n/bf-n* diplotype (B1/B1 cells) compared to both the *IGHG*bf-n/bf-n* diplotype (B2/B2 cells) with 10 positive tests in 10 patients or 1.0 test per patient ($p < 0.001$) and the *IGHG*ga-n/*ga-n* diplotype (B4/B4 cells) with 11 positive tests in 15 patients or 0.7 tests per patient ($p < 0.001$).

IGHG Diplotypes and *IGHG2* Genotypes Investigated for IgE Levels

The homozygous *IGHG*bf-n/bf-n* diplotype (B1/B1 cells) presented increased IgE levels compared to both the homozygous *IGHG*bf-n/bf-n* diplotype (B2/B2 cells; $p < 0.01$) and the homozygous *IGHG*ga-n/*ga-n* diplotype (B4/B4 cells; $p < 0.02$). IgE levels were significantly increased in the homozygous *IGHG2*n/*n* genotype, compared to the alternative *IGHG2*-n/*n* ($p < 0.01$; fig. 3a).

IGHG Diplotypes Investigated for IgG, IgG Subclass, IgA and IgM Levels

Serum IgG, IgA and IgM levels did not deviate between *IGHG* diplotypes. Total IgG subclass levels were also calculated from the combination of allelic IgG subclass levels. The IgG4 levels did not deviate.

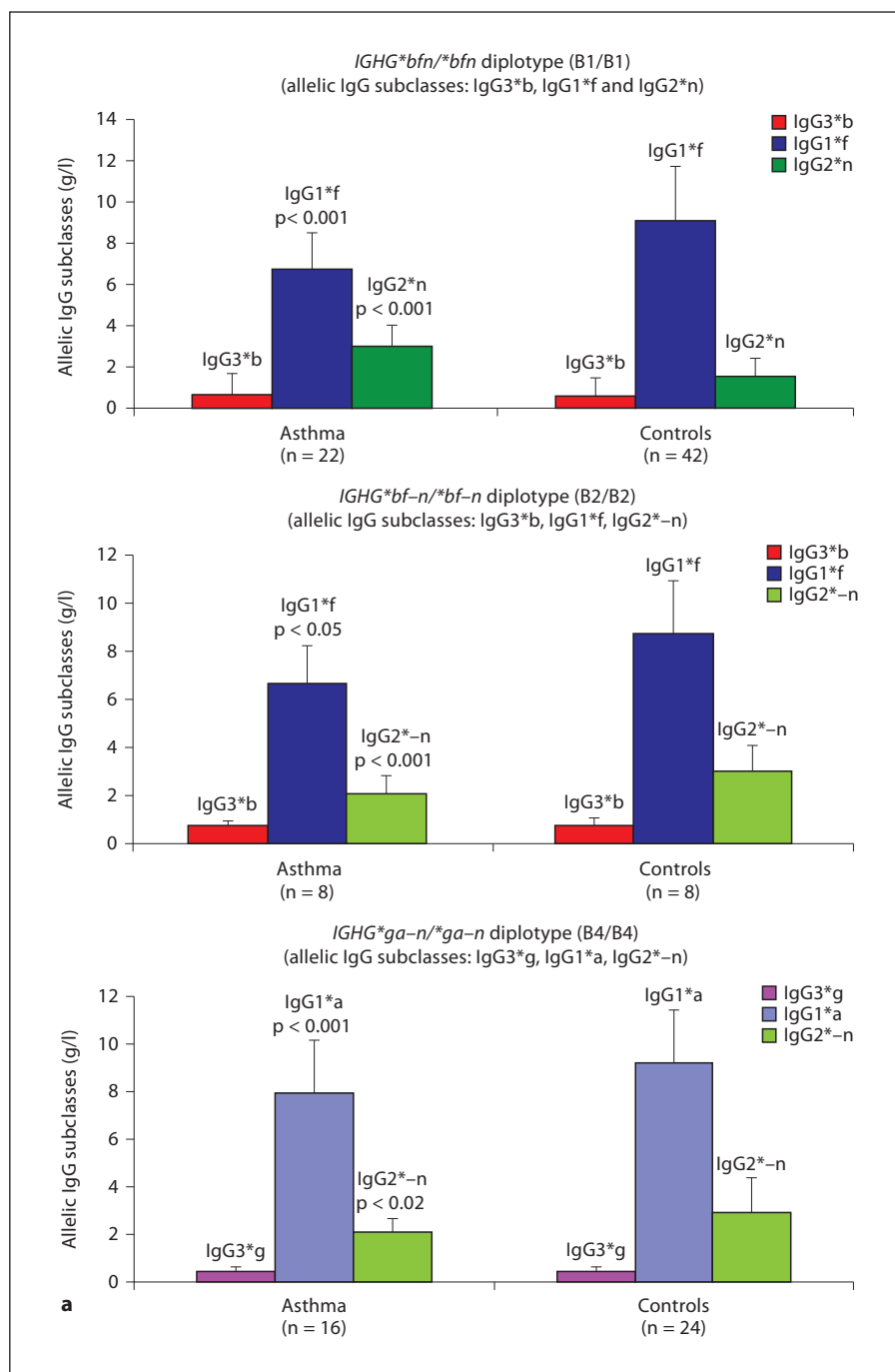


Fig. 1. a 46 homozygous *IGHG* diplotypes expressing allelic IgG subclass levels (g/l): *IGHG*bf-n/*bf-n* (B1/B1): IgG3*b, IgG1*f, IgG2*n; *IGHG*bf-n/*bf-n* (B2/B2): IgG3*b, IgG1*f, IgG2*-n and *IGHG*ga-n/*ga-n* (B4/B4): IgG3*g, IgG1*a, IgG2*-n of childhood asthma patients and age- and *IGHG* gene-matched healthy children.

Peripheral Blood Eosinophil and CD8 Lymphocyte Numbers in Homozygous *IGHG* Diplotypes

The homozygous *IGHG*bf-n/*bf-n* (B1/B1 cells; $p < 0.02$) and *IGHG*bf-n/*bf-n* diplotypes (B2/B2 cells; $p < 0.02$) demonstrated significantly increased numbers of peripheral blood eosinophils compared to *IGHG*ga-n/*ga-n* (B4/B4 cells; fig. 3b). Instead *IGHG*ga-n/*ga-n*

demonstrated increased numbers of CD8 lymphocytes compared to the homozygous *IGHG*bf-n/*bf-n* diplotype ($p < 0.01$) both with *IGHG2*-n/*-n* with a possible effect of *IGHG3-1*ga* genes compared to *IGHG3-1*bf* (fig. 3c). There was no deviation in CD4 or CD19 cell numbers comparing the different *IGHG* diplotypes of childhood asthma.

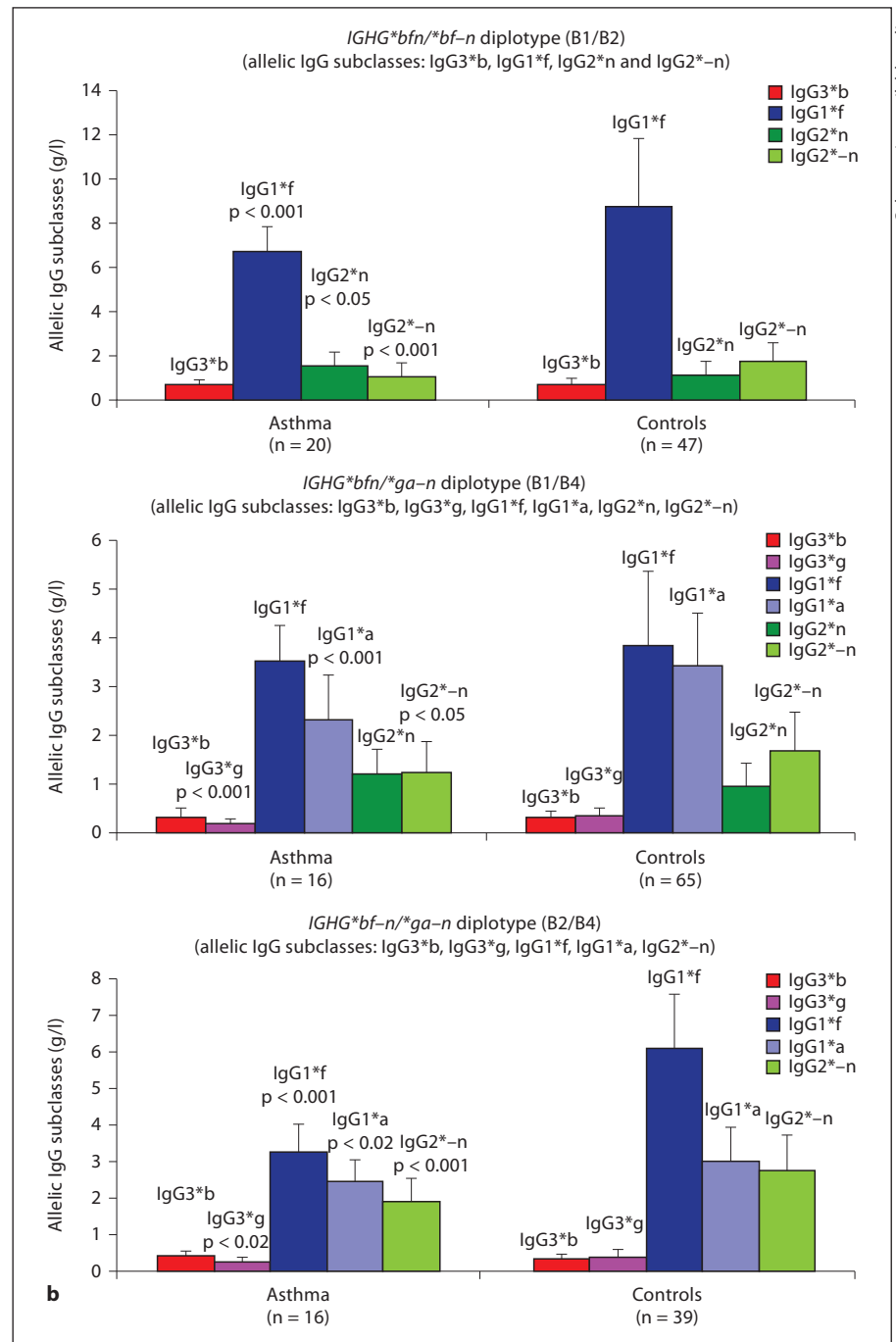


Fig. 1. b 52 heterozygous *IGHG* diplotypes expressing allelic IgG subclass levels (g/l): *IGHG*bf-n/*bf-n* (B1/B2): (IgG3*b, IgG1*f, IgG2*n, IgG2*-n); *IGHG*bf-n/*ga-n* (B1/B4): (IgG3*b, IgG1*f, IgG2*n, IgG3*g, IgG1*a, IgG2*-n, and *IGHG*bf-n/*ga-n* (B2/B4): (IgG3*b, IgG1*f, IgG2*-n, IgG3*g, IgG1*a, IgG2*-n) of childhood asthma patients and age- and *IGHG* gene-matched healthy children. Allelic variants of IgG3, IgG1 and IgG2 are shown.

Discussion

Different *IGHG* genotypes of childhood asthma are linked to different allergy parameters. New methods have made it possible to identify individual *IGHG* genes and *IGHG* gene-related B cells and their expression of IgG molecules. Allergens affect individuals with certain

IGHG genes more than others, as reported several times before. A pediatric asthma population was genotyped and the patients divided in equally sized groups for comparison. Six *IGHG* gene complexes of individual *IGHG* diplotypes, representing 98–99% of possible variants, were studied. The homozygous diplotypes with only 3 allelotypic IgG subclasses were most suitable to comment on.

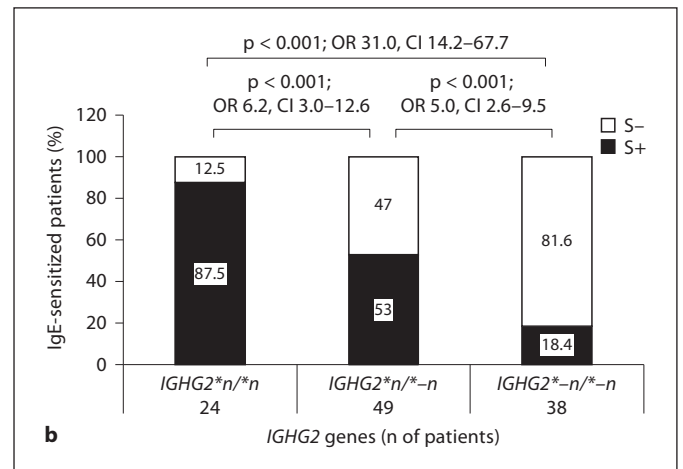
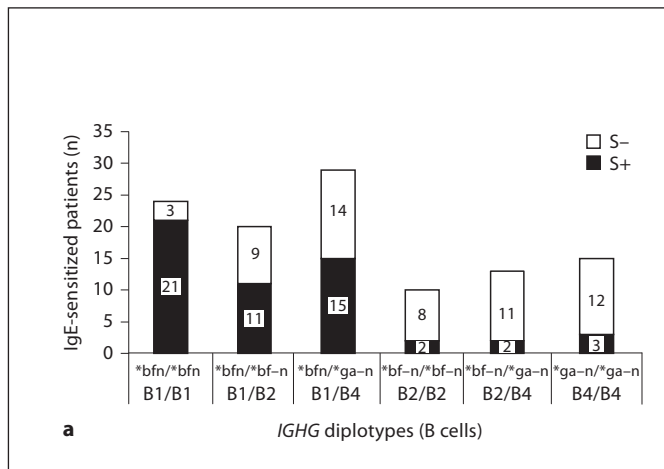
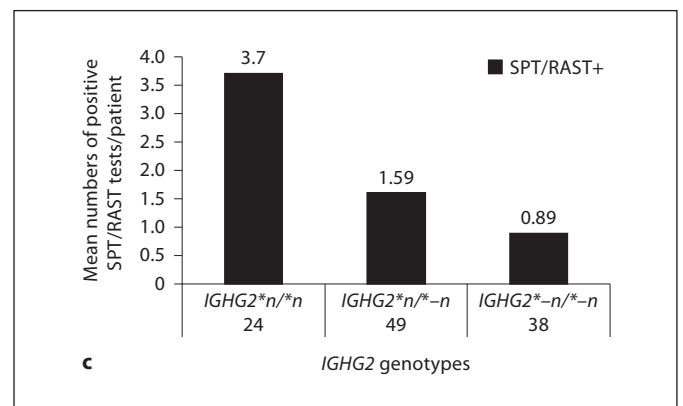


Fig. 2. **a** Numbers of IgE sensitized (S+) and non-sensitized patients (S-) in the 6 different *IGHG* diplotypes (B cells) of 111 patients with bronchial asthma. **b** *IGHG2**n gene dose dependency. IgE-sensitized (S+) and non-sensitized patients (S-) with bronchial asthma according to the *IGHG2* genotype (*n/*n, *n/*-n, *-n/*-n). **c** Numbers of positive SPT/RAST tests per patient (mean) in 111 patients with childhood asthma according to *IGHG2* (Fcγ2) genotype. *IGHG2**n gene dose dependency. n = 111.



The *IGHG**bfn/*bfn diplotype (from B1/B1 cells) demonstrated significantly increased allelic IgG2*n subclass levels (fig. 1a) and activated *IGHE* genes with increased IgE levels and increased numbers of positive RAST/SPT tests. In dissecting the *IGHG* diplotype complexes, IgE sensitization was found to be *IGHG**n dose dependent. The homozygous *IGHG2**n/*n expressed the highest degree of IgE sensitization (fig. 2a, b). The *IGHG2**n allele is located only 18 kb from *IGHG4* and another 23 kb from *IGHE*, of which the last two are induced by IL-4 and IL-13 cytokines from Th2 lymphocytes (fig. 4) [5, 24, 25]. The increased level of IgG2*n is in contrast to the rather low levels in healthy children during childhood [10].

The *IGHG**bfn/*bfn diplotype (B1/B1 cells) demonstrated also a low IgG1*f level compared to healthy controls. The down-regulated IgG1*f level in atopic asthma indicated a non-activated *IGHG1**f gene upstream of chromosome 14q32.3, usually influenced by Th1 cytokines (fig. 4), possibly in agreement with the hygiene hypothesis.

The homozygous *IGHG**bf-n/*bf-n (B2/B2) and *IGHG**ga-n/*ga-n (B4/B4) diplotypes and heterozygous *IGHG**bf-n/*ga-n (B2/B4 cells), all with *IGHG2**-n/*-n, demonstrated low IgE levels and IgE sensitization, and less response to allergens. In these patients, IgG3*g, IgG1*f and IgG1*a and IgG2*-n levels were significantly decreased compared to healthy controls, pointing to an increased susceptibility to respiratory tract infections.

Asthmatic children present with about the same clinical history with cough, obstruction and infection proneness. The non-IgE-mediated asthmatic patients are usually hard to define, but in the present study the *IGHG2**-n/*-n is protective against IgE sensitization and a marker linked to non-IgE-mediated infectious-prone asthma. Primary immunodeficiencies such as IgG2, IgG3 and IgA deficiencies and common variable immunodeficiency in which bronchial inflammation is a common part, have been found with a dominance of homozygous *IGHG2**-n/*-n [18, 26].

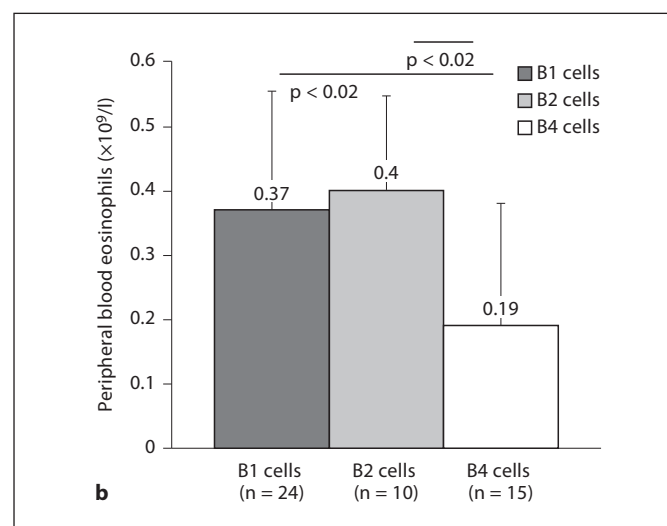
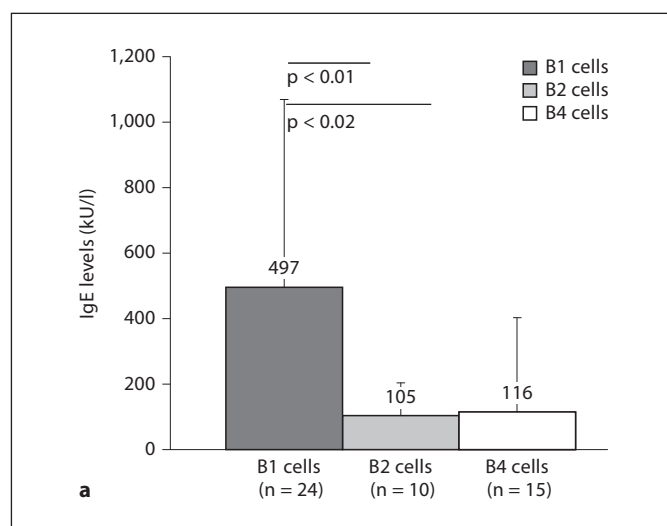


Fig. 3. Comparison of IgE levels (a), numbers of peripheral blood eosinophils (b) and percentages of CD8 of peripheral blood lymphocytes (c) in 49 homozygous diplotypes: *IGHG*bfn/*bfn* (B1 cells; n = 24), *IGHG*bf-n/*bf-n* (B2 cells; n = 10) and *IGHG*ga-n/*ga-n* (B4 cells; n = 15). Means $\pm$ SD.

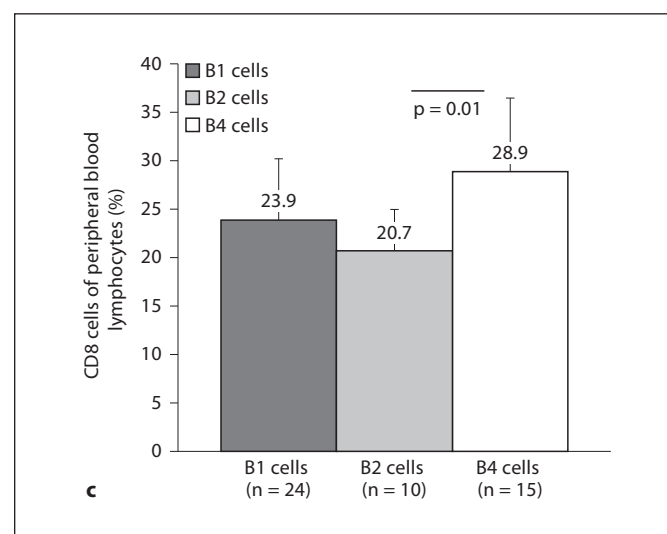


Fig. 4. The effect of cytokines on *IGH* genes, with the *IGHG*bfn* haplotype in childhood asthma as example.

IL-10	IL-10	IL-10	IL-4	IL-4	IL-10
		TGF- β	IL-13	IL-13	TGF- β
γ 3	γ 1	α 1	γ 2 γ 4	ϵ	α 2
26 kb	19	13	18	23	10
	<i>IGHG1*fl</i>		<i>IGHG2*n$\uparrow$</i>		<i>IGH$\epsilon$$\uparrow$</i>

Identification of the allelic variants of IgG3, IgG1 and IgG2 as unique entities with structural and functional differences is informative. The GM allotype determination is still the simplest way to demonstrate *IGHG* genes by 6 alleles, 9 genotypes, 4 haplotypes and 10 diplotypes. RFLP and DNA analyses have been performed with lim-

ited success [7], and SNP investigations did not detect predominance of chromosome 14q32.3 in childhood asthma [4]. The basis of alternative GM allotypes in humans is well defined at the molecular level by codon and amino acid substitutions for IgG1 on CH3 356, CH3 358 and CH3 481 and for IgG2 on CH2 281. The allelic IgG sub-

classes differ by amino acids, but uniquely also by immunochemical, functional and disease studies [8–11, 19, 20]. The method to study genes by serum protein copies is advantageous, as the serum protein quantities also disclose the activities of the genes. From earlier studies, we know all the alternative *IGHG* alleles within the haplotype [10, 16]. Allelic IgG subclass levels in childhood affecting the expression and function of allelic IgG subclasses, especially in asthma patients, were given for the first time. Knowing functionally different inherited allelic IgG subclasses, we may be able to intervene in the asthma disease process earlier. Normal reference ranges of allelic IgG subclasses have been published previously [10].

GM allotype variation influences the outcome of both bacterial and viral infections. Individuals with the *IGHG*bfⁿ* haplotype are high producers of specific antibodies against encapsulated bacteria in sepsis and meningitis, and after vaccination, which means that the Fab part of the IgG molecules is influenced by the Fc γ part [13, 14]. The FcR-like hepatitis C virus core protein has been found to bind differently to IgG of discordant GM (Fc γ) allotypes. The results provide a mechanistic explanation, protecting cells from host-mediated lysis by the involvement of GM allotypes in the outcome of HCV infection [15]. It is interesting to note that GM allotypes are also involved in immunity of other viruses, herpes simplex type 1 and cytomegalovirus, which also encode FcR-like proteins that bind to the Fc region of IgG [15, 27]. The most severe infections of respiratory syncytial virus in infants are predominantly associated with the *IGHG*bfⁿ-n/*bfⁿ-n* diplotype (B2/B2 cells) [17], referring to the non-atopic phenotype. The co-aggregation of IgG-IgE molecules at the Fc ϵ RI and Fc γ RIIb receptors blocking the activation of human mast cells has been suggested as a possible therapeutic platform for immune-based therapy of allergic disease [28]. Macrophages from individuals with HLA-DR3 and the *IGHG*bfⁿ* haplotype endocytose antigens very slowly [29].

The numbers of peripheral blood eosinophils and CD8 T cells connected with various *IGHG* gene-related

B cells differed. The *IGHG*bfⁿ/*bfⁿ* (B1/B1 cells) but also *IGHG*bfⁿ-n/*bfⁿ-n* (B2/B2 cells) diplotypes demonstrated significantly increased numbers of peripheral blood eosinophils compared to the *IGHG*gaⁿ-n/*gaⁿ-n* (B4/B4 cells). Instead, the *IGHG*gaⁿ-n/*gaⁿ-n* (B4/B4 cells) diplotype showed significantly increased numbers of CD8 peripheral blood lymphocytes, a sign of immunosuppression. Asthma severity in patients from childhood to young middle age, with decreasing lung function tests, has been found to be associated with both the atopic *IGHG*bfⁿ* and the infection-prone *IGHG*bfⁿ-n* [30].

Allergens were supposed to have triggered the *IGHG2*n* allele to increased levels after a non-activated *IGHG1*f* allele in asthmatic children with the *IGHG*bfⁿ/*bfⁿ* diplotype. The highest degree of IgE sensitization was found in individuals with homozygous *IGHG2*n/*n*. *IGHG2*-n* was found concomitantly with low IgE sensitization and low levels of allelic IgG subclasses, possible triggers of bronchial inflammation by respiratory tract infections. The *IGHG2*n* allele (IgG2*n allelic subclass) is a marker of the IgE-mediated phenotype, and the alternative *IGHG2*-n* allele (IgG2*-n allelic subclass) is a new significant marker of the non-atopic asthma phenotype. Investigation of *IGHG* genes with focus on *IGHG2* allelic variants provides insight into the pathogenesis of different asthma phenotypes and is of help in the clinical work with diagnostic, prophylactic, prognostic and therapeutic steps. Further study of *IGHG* genes in other forms of allergy and allergy vaccination are required.

Acknowledgments

Thanks go to Ann-Margreth Carlsson for skilful technical assistance, Håkan Lövkvist for statistical advice and Dr. Olöf Jonsdottir for collecting information about sensitization from the case records. The study was supported by grants from the Medical Faculty of Lund University and the University Hospital of Lund, the Swedish Asthma and Allergy Association and the Vardal Foundation, Sweden.

References

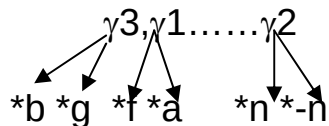
- Palmer LJ, Cookson WO: Genomic approaches to understanding asthma. *Genome Res* 2000;10:1280–1287.
- Cookson WO: Genetics and genomics of asthma and allergic diseases. *Immunol Rev* 2002;190:195–206.
- Ober C: Perspectives on the past decade of asthma genetics. *J Allergy Clin Immunol* 2005;116:274–278.
- Sleiman PMA, Flory J, Imielinski M, et al: Variants of *DENND1B* associated with asthma in children. *N Engl J Med* 2010;362:36–44.
- Oxelius VA: Immunoglobulin constant heavy G subclass genes in asthma and allergy. *Immunol Res* 2008;40:179–191.

- 6 Oxelius VA, Bråbäck L, Ahlstedt S, Björkstén B: Immunoglobulin constant heavy G chain genes as risk factors in childhood allergies. *Clin Exp Allergy* 2006;36:1616–1624.
- 7 Grubb R: Human immunoglobulin allotypes and Mendelian polymorphism of the human immunoglobulin genes; in Oss CJ, Regenmortel MHV (eds): *Immunochemistry*. New York, Dekker, 1994, pp 47–68.
- 8 Oxelius VA: Preparation of IgG subclass allotypes from polyclonal IgG. *Scand J Immunol* 1999;49:395–398.
- 9 Oxelius VA, Carlsson AM: Quantitation of Gm allotypes. *Scand J Immunol* 1993;37:143–148.
- 10 Oxelius VA, Aurivillius M, Carlsson AM, Musil K: Serum Gm allotype development during childhood. *Scand J Immunol* 1999;50:440–446.
- 11 Oxelius VA, Eibl MM: Different Gm allotype levels in human intravenous immunoglobulin (IVIG) preparations; survival of foreign Gm allotypes in immunodeficient patients. *Clin Exp Immunol* 1996;106:203–207.
- 12 Oxelius VA: Genetic B cell variation based on immunoglobulin heavy G chain (Gm) genes. *Scand J Immunol* 1999;49:345–346.
- 13 Pandey JP, Fudenberg H, Virella G, et al: Association between immunoglobulin allotypes and immune responses to *Haemophilus influenzae* and *meningococcus polysaccharides*. *Lancet* 1979;i:190–192.
- 14 Ambrosino D, Schiffman G, Gotschlich E, et al: Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. *J Clin Invest* 1985;75:1935–1942.
- 15 Namboodiri AM, Budkowska A, Nietert PJ, Pandey JP: Fc receptor-like hepatitis C virus core protein binds differentially to IgG of discordant Fc (GM) genotypes. *Mol Immunol* 2007;44:3805–3808.
- 16 Atherton A, Armour KL, Bell S, Minson AC, Clark MR: The herpes simplex virus type 1 Fc receptor discrimination between IgG1 allotypes. *Eur J Immunol* 2000;30:2540–2547.
- 17 Aurivillius M, Oymar K, Oxelius VA: Immunoglobulin heavy G2 chain (*IGHG2*) gene restriction in the development of severe respiratory syncytial virus infection. *Acta Paediatr* 2005;94:414–418.
- 18 Oxelius VA, Ochs H, Hammarström L: Restricted immunoglobulin heavy G chain genes in primary immunodeficiencies. *Clin Immunol* 2008;128:190–198.
- 19 Oxelius VA, Svantesson H, Carlsson AM: GM phenotype linkage to subsets of juvenile chronic arthritis (JCA) with influence on IgG subclass response. *Scand J Rheumatol* 1993;22:284–288.
- 20 Jönsson G, Oxelius VA, Truedsson L, Bracnieri JH, Sturfelt G, Sjöholm AG: Homozygosity for the IgG2 subclass allotype G2M(n) protects against severe infections in hereditary C2 deficiency. *J Immunol* 2006;177:722–728.
- 21 Oxelius VA: Gm allotype genes and gene dosage affecting both IgG subclasses and IgE levels in atopic patients. *Int Arch Allergy Appl Immunol* 1990;91:58–61.
- 22 Oxelius VA, Sjöstedt L, Willers S, Löw B: Development of allergy to laboratory animals is associated with particular Gm and HLA genes. *Int Arch Allergy Immunol* 1996;110:73–78.
- 23 Oxelius VA: Imbalanced switch of the *IGHG* (immunoglobulin constant heavy G chain) *Gm(bfn)* genes in atopic childhood asthma. *Allergy* 2000;55:1063–1068.
- 24 Finkelman FD, Katona IM, Urban JF, et al: IL-4 is required to generate and sustain in vivo IgE responses. *J Immunol* 1988;141:2331–2341.
- 25 Minty A, Chalon P, Derocq JM, et al: Interleukin 13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* 1993;362:248–250.
- 26 Oxelius VA, Hanson L-Å, Björkander J, et al: IgG3 deficiency: common in obstructive lung disease. *Monogr Allergy*. Basel, Karger, 1986, vol 20, pp 106–115.
- 27 Nimmerjahn F, Ravetch JV: Divergent IgG subclass activity through selective Fc receptor binding. *Science* 2005;310:1510–1512.
- 28 Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A: A chimeric human-cat fusion protein blocks cat-induced allergy. *Nat Med* 2005;11:446–449.
- 29 Legrand L, Rivat-Perran L, Hutting C, et al: HLA- and Gm-linked genes affecting the degradation of antigens (sheep red cells) endocytosed by macrophages. *Hum Immunol* 1982;4:1–13.
- 30 Gustafsson PM, Oxelius VA, Nilsson S, Kjellman B: Association between Gm allotypes and asthma severity from childhood to young middle age. *Respir Med* 2008;102:266–272.

((Online supplementary material, IAA335592))

Chromosome 14q32.3 (5'«, ¢, ¥3, ¥1, ~1, ¥2, ¥4, £, ~2 3')

ImmunoGlobulin constant Heavy G chain (*IGHG*) genes:



Alternative GM allotypes (alleles) of ¥3, ¥1, ¥2 genes

6 Allelic IgG subclasses:

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

9 *IGHG* genotypes: *IgG3*b/*b*, *IgG3*b/*g*, *IgG3*g/*g*
*IgG1*f/*f*, *IgG1*f/*a*, *IgG1*a/*a*
*IgG2*n/*n*, *IgG2*n/*-n*, *IgG2*-n/*-n*

4 Haplotypes and B cells (numbers and %) from 587 healthy Caucasians children and adults (587 x 2 =1174 haplotypes)

B ^{*bfn} or B1	B ^{*bf-n} or B2	B ^{*gan} or B3	B ^{*ga-n} or B4
<i>IGHG*bfn</i>	<i>IGHG*bf-n</i>	<i>IGHG*gan</i>	<i>IGHG*ga-n</i>
42.8%	24.0%	1.5%	31.6%
N=503	N=282	N=18	N=371

10 *IGHG* diplotypes (individual expression) (numbers and %)

(Frequency figures from 587 healthy Caucasians children and adults)

B1/B1	B1/B2	B1/B4	B2/B2	B2/B4	B4/B4	B1/B3	B2/B3	B3/B3	B3/B4
<i>*bfn/*bfn</i>	<i>*bfn/*bf-n</i>	<i>*bfn/*ga-n</i>	<i>*bf-n/*bf-n</i>	<i>*bf-n/*ga-n</i>	<i>*ga-n/*ga-n</i>	<i>*bfn/*gan</i>	<i>*bf-n/*gan</i>	<i>*gan/*gan</i>	<i>*gan/*ga-n</i>
20.1%	18.2%	25.0%	6.8%	16.2%	10.9%	2.2%	<0.1%	0.3%	0.2%
N=118	N=107	N=147	N=40	N=95	N=64	N=13	N=0	N= 2	N=1

Individual *IGHG* genes

6 common (4 rare) individual *IGHG* diplotypes, restricted allelic IgG subclasses and B cells

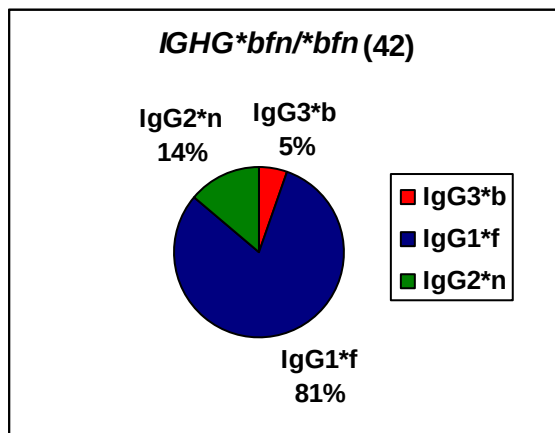
6 individual <i>IGHG</i> diplotypes	Restricted allelic IgG subclasses in serum	Restricted B cells
<i>IGHG*bfn/*bfn</i>	IgG3*b, IgG1*f, IgG2*n	B1/B1
<i>IGHG*bfn/*bf-n</i>	IgG3*b, IgG1*f, IgG2*n, IgG2*-n	B1/B2
<i>IGHG*bfn/*ga-n</i>	IgG3*b, IgG1*f, IgG2*n, IgG3*g, IgG1*a, IgG2*-n	B1/B4
<i>IGHG*bf-n/*bf-n</i>	IgG3*b, IgG1*f, IgG2*-n	B2/B2
<i>IGHG*bf-n/*ga-n</i>	IgG3*b, IgG1*f, IgG2*-n, IgG3*g, IgG1*a	B2/B4
<i>IGHG*ga-n/*ga-n</i>	IgG3*g, IgG1*a, IgG2*-n	B4/B4

Restricted allelic IgG subclasses in individual *IGHG* diplotypes

(mean levels in % of all allelic IgG subclasses) from the *IGHG* diplotypes of healthy 10-year-old children controls in the present study.

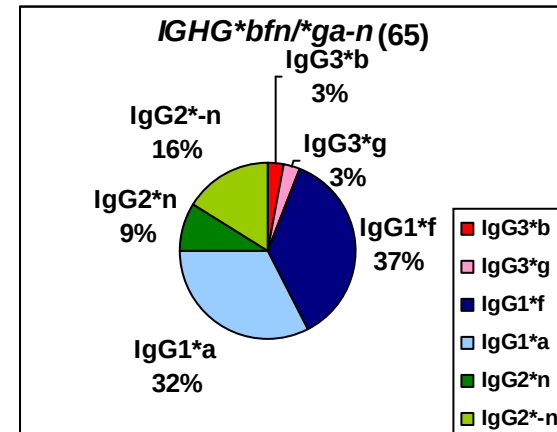
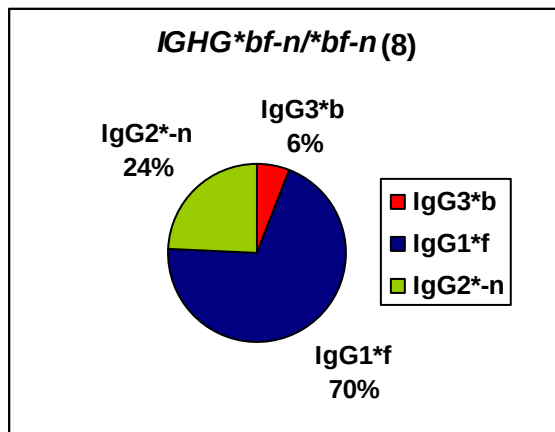
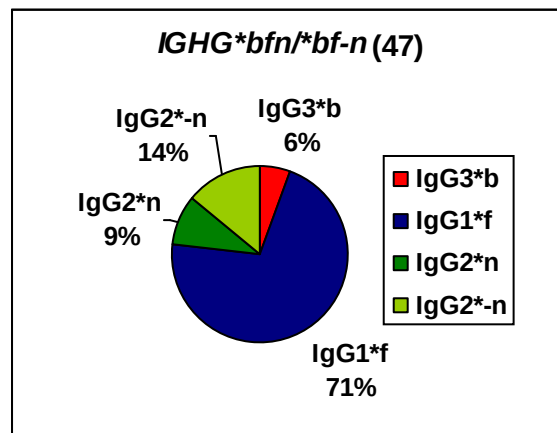
In homozygous diplotypes

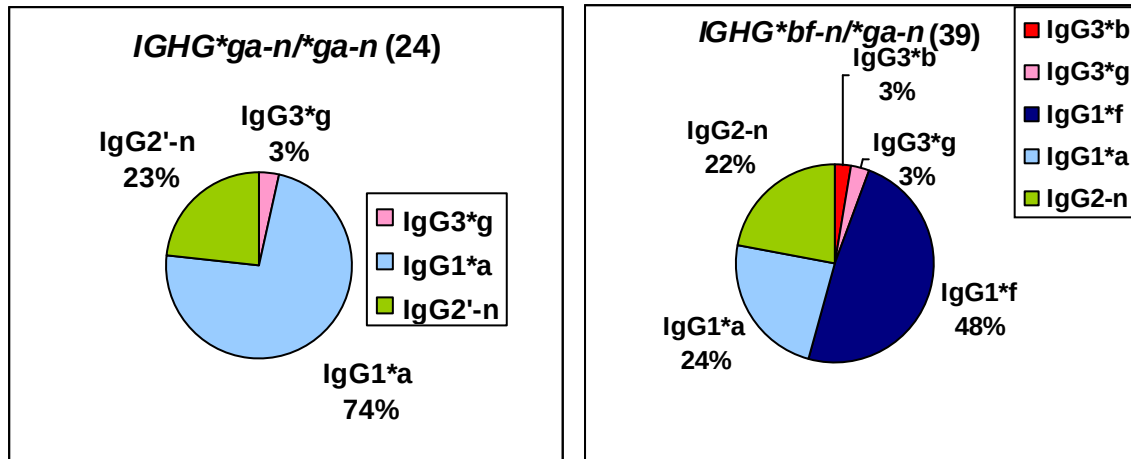
(three allelic IgG subclass proteins)



In heterozygous diplotypes

(4-6 allelic IgG subclass proteins)





Allelic IgG subclasses & IgG4 (%)
in a serum pool (about 2000 healthy adult blood donors)

