

## Immunoglobulin constant heavy G subclass chain genes in asthma and allergy

Vivi-Anne Oxelius

Published online: 15 May 2007  
© Humana Press Inc. 2007

**Abstract** The *IGHG* (ImmunoGlobulin constant Heavy G chain) genes are situated close to the *IGHE* gene on chromosome 14q32, 5'μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2, 3', in linkage disequilibrium. The polymorphism of γ3, γ1 and γ2 genes, is investigated as alternative allotypes. They are inherited in a Mendelian fashion and are 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: *IGHG\*bf<sub>n</sub>* (=B1-cells), *IGHG\*bf<sub>n</sub>* (=B2-cells), *IGHG\*ga<sub>n</sub>* (=B3-cells) and *IGHG\*ga<sub>n</sub>* (=B4-cells). The dominance of the *IGHG2\*<sub>n</sub>* allele from the *IGHG\*bf<sub>n</sub>* haplotype (=B1-cells) has been shown in repeated investigations, namely in patients with asthma and allergy with increased serum levels of IgE > 600 ku/l and more often so in those with IgE > 1,000 ku/l or IgG4>1 g/l, in childhood asthma patients with mean level of IgE = 1,762 ku/l and in allergen exposed individuals developing laboratory animal allergy. In children with non-atopy and mean IgE level = 9.5 ku/l there is instead a dominance of the alternative allotypes from the *IGHG\*ga<sub>n</sub>* (=B4-cells) with *IGHG2\*<sub>n</sub>* alleles. In a case-control study allergic children with a family history of allergy, clinically manifest allergy and/or positive SPT, the *IGHG\*bf<sub>n</sub>* haplotype (=B1-cells) with the *IGHG2\*<sub>n</sub>* allele dominates, with increased risk of atopy and the *IGHG\*bf<sub>n</sub>* haplotype (=B2-cells) with the *IGHG2\*<sub>n</sub>* allele is infrequent with low risk, probably protective against atopy. The phenotypic expressions of the *IGHG\*bf<sub>n</sub>* haplotype (=B1 cells) and *IGHG\*bf<sub>n</sub>/\*bf<sub>n</sub>* diplotypes (B1/B1-cells) are increased IgG2\*<sub>n</sub> allotype together with increased IgE serum levels and IgE sensitisation in agreement with atopy. The alternative *IGHG\*ga<sub>n</sub>/\*ga<sub>n</sub>* diplotype (B4/B4-cells) express low IgG1\*a- and IgG2\*<sub>n</sub> allotypes, together with low IgE and non-IgE sensitisation, in agreement with non-atopy. Together these studies have given us a greater understanding of the involvement of *IGHG* genes, *IGHG* coded B-cells and immunochemical and functional variants of IgG molecules describing different forms of asthma and allergy, which will improve diagnoses and treatment.

---

Presented at the First Robert A Good Society Symposium, St. Petersburg, FL 2006.

---

V.-A. Oxelius (✉)

Department of Pediatrics, University Hospital, Lund University, 221 85 Lund, Sweden  
e-mail: vivi-anne.oxelius@med.lu.se

**Keywords** IgG subclass allotypes · *IGHG* alleles · *IGHG* genes · *IGHG* haplotypes · *IGHG* diplotypes · *IGHG* gene coded B cells · Atopy · Asthma and allergy

## Introduction

Several candidate genes have been found associated to the inflammatory response of IgE mediated allergy [1], so also the *IGHG* (ImmunoGlobulin Heavy constant G chain) genes [2–5]. The *IGHG* genes are located close to the *IGHG* gene on chromosome 14q32 (5'μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2 3') (Fig. 1).

The polymorphism of *IGHG* genes are assessed by the alternative serum allotypes from the *IGHG3* (γ3): IgG3\*b and IgG3\*g, *IGHG1* (γ1): IgG1\*f and IgG1\*a and *IGHG2* (γ2): IgG2\*n and IgG2\*-n (Table 1A). The alternative allotypes of IgG subclass molecules (Table 1B) are characterised by immunochemical and functional differences [6–8]. The polymorphism of *IGHG* genes discloses a variation of B-cells (Table 1C–E) [9], which are unique producers of immunoglobulins. The production of immunoglobulins by B-cells is highly adaptive to environmental factors. The aim of this review is to focus on the participation of the *IGHG* gene and IgG molecules, qualitatively and quantitatively, in the inflammatory reaction of different forms of asthma and allergy.

## *IGHG* genes and B cell subsets

### *IGHG* genes assessed 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 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 [2]. 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 IgG allotypes are inherited in the Mendelian way and expressed randomly in allelic exclusion [2]. The secreted alternative 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 1A).

The γ3 and γ1 genes are found in linkage disequilibrium because of their close localisation (only 26 kb in between) (Fig. 2): *IGHG3\*b* together with *IGHG1\*f*, in the gene complex\*bf and *IGHG3\*g* together with *IGHG1\*a*, \*ga [2]. Additional variation on

5' μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2 3'

**Fig. 1** The configuration of the end of the long arm q at band 32 of chromosome 14, containing the *IGH* (ImmunoGlobulin constant Heavy chain) genes of the immunoglobulins IgM, IgD, IgG3, IgG1, IgA1, IgG2, IgG4, IgE and IgA2

**Table 1** Abbreviations and descriptions of the chromosome 14q32 with alternative *IGHG* alleles (*IGHG* genes, *IGHG* haplotypes, *IGHG* diplotypes) assessed by alternative IgG subclass allotypes

|                                                                                                                    |                                                      |                                                                         |                          |                           |
|--------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------------------|--------------------------|---------------------------|
| (A) Alternative alleles of <i>IGHG</i> subclass genes assessed by IgG subclass allotypes                           |                                                      |                                                                         |                          |                           |
| <i>IGHG</i> genes                                                                                                  | <i>IGHG</i> alleles                                  | Assessment by alternative Serum IgG subclass allotypes                  |                          |                           |
| <i>IGHG3</i> (γ3)                                                                                                  | <i>IGHG3</i> * <i>b</i> and <i>IGHG3</i> * <i>g</i>  | IgG3* <i>b</i> and IgG3* <i>g</i>                                       |                          |                           |
| <i>IGHG1</i> (γ1)                                                                                                  | <i>IGHG1</i> * <i>f</i> and <i>IGHG1</i> * <i>a</i>  | IgG1* <i>f</i> and IgG1* <i>a</i>                                       |                          |                           |
| <i>IGHG2</i> (γ2)                                                                                                  | <i>IGHG2</i> * <i>n</i> and <i>IGHG2</i> *- <i>n</i> | IgG2* <i>n</i> and IgG2*- <i>n</i>                                      |                          |                           |
| (B) Alternative IgG1-, IgG2- and IgG3-allotypes in serum                                                           |                                                      |                                                                         |                          |                           |
|                                                                                                                    | IgG subclasses                                       | Alternative IgG subclass allotypes                                      |                          |                           |
| IgG                                                                                                                | IgG1                                                 | IgG1* <i>f</i>                                                          |                          |                           |
|                                                                                                                    |                                                      | IgG1* <i>a</i>                                                          |                          |                           |
|                                                                                                                    | IgG2                                                 | IgG2* <i>n</i>                                                          |                          |                           |
|                                                                                                                    |                                                      | IgG2*- <i>n</i>                                                         |                          |                           |
|                                                                                                                    | IgG3                                                 | IgG3* <i>b</i>                                                          |                          |                           |
|                                                                                                                    |                                                      | IgG3* <i>g</i>                                                          |                          |                           |
|                                                                                                                    | IgG4                                                 |                                                                         |                          |                           |
| (C) <i>IGH</i> genes of chromosome 14q32 and <i>IGHG</i> haplotypes assessed by alternative IgG subclass allotypes |                                                      |                                                                         |                          |                           |
| <i>IGH</i> genes of chromosome 14q32 5' μ, δ, γ3, γ1, α1, γ2, γ4, ε, α2 3'                                         | <i>IGHG</i> haplotypes                               | IgG allotypes in serum                                                  |                          |                           |
| Alternative alleles inserted for <i>IGHG3</i> , <i>IGHG1</i> and <i>IGHG2</i>                                      |                                                      | Alternative allotypes of heavy G subclass chains of IgG3, IgG1 and IgG2 |                          |                           |
| 5' μ, δ, <u>b</u> , <u>f</u> , α1, <u>n</u> , γ4, ε, α2 3'                                                         | <i>IGHG</i> * <i>bfn</i>                             | IgG3* <i>b</i> , IgG1* <i>f</i> , IgG2* <i>n</i>                        |                          |                           |
| 5' μ, δ, <u>b</u> , <u>f</u> , α1, <u>-n</u> , γ4, ε, α2 3'                                                        | <i>IGHG</i> * <i>bfn</i>                             | IgG3* <i>b</i> , IgG1* <i>f</i> , IgG2*- <i>n</i>                       |                          |                           |
| 5' μ, δ, <u>g</u> , <u>a</u> , α1, <u>n</u> , γ4, ε, α2 3'                                                         | <i>IGHG</i> * <i>gan</i>                             | IgG3* <i>g</i> , IgG1* <i>a</i> , IgG2* <i>n</i>                        |                          |                           |
| 5' μ, δ, <u>g</u> , <u>a</u> , α1, <u>-n</u> , γ4, ε, α2 3'                                                        | <i>IGHG</i> * <i>gan</i>                             | IgG3* <i>g</i> , IgG1* <i>a</i> , IgG2*- <i>n</i>                       |                          |                           |
| (D) Four different <i>IGHG</i> haplotypes coding four different B-cells                                            |                                                      |                                                                         |                          |                           |
| B-cells                                                                                                            | B1-cells                                             | B2-cells                                                                | B3-cells                 | B4-cells                  |
| <i>IGHG</i> haplotypes                                                                                             | <i>IGHG</i> * <i>bfn</i>                             | <i>IGHG</i> * <i>bf-n</i>                                               | <i>IGHG</i> * <i>gan</i> | <i>IGHG</i> * <i>ga-n</i> |
| % Of a healthy Caucasian population                                                                                | 44.8%                                                | 22.9%                                                                   | 0.9%                     | 31.4%                     |

**Table 1** Abbreviations and descriptions of the chromosome 14q32 with alternative *IGHG* alleles (*IGHG* genes, *IGHG* haplotypes, *IGHG* diplotypes) assessed by alternative IgG subclass allotypes

|                                                                                               |                                            |                                             |                                             |                                              |                                              |                                              |
|-----------------------------------------------------------------------------------------------|--------------------------------------------|---------------------------------------------|---------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|
| (E) The six most common B-cell combination, <i>IGHG</i> diplotypes and <i>IGHG2</i> genotypes |                                            |                                             |                                             |                                              |                                              |                                              |
| B-cells (individual)                                                                          | B1/B1-cells                                | B1/B2-cells                                 | B1/B4-cells                                 | B2/B2-cells                                  | B2/B4-cells                                  | B4/B4-cells                                  |
| <i>IGHG</i> diplotypes                                                                        | <i>IGHG</i> * <i>bfn</i> /<br>* <i>bfn</i> | <i>IGHG</i> * <i>bfn</i> /<br>* <i>bf-n</i> | <i>IGHG</i> * <i>bfn</i> /<br>* <i>ga-n</i> | <i>IGHG</i> * <i>bf-n</i> /<br>* <i>bf-n</i> | <i>IGHG</i> * <i>bf-n</i> /<br>* <i>ga-n</i> | <i>IGHG</i> * <i>ga-n</i> /<br>* <i>ga-n</i> |
| <i>IGHG2</i> genotypes                                                                        | <i>IGHG2</i> * <i>n</i> /<br>* <i>n</i>    | <i>IGHG2</i> * <i>n</i> /<br>* <i>-n</i>    | <i>IGHG2</i> * <i>n</i> /<br>* <i>-n</i>    | <i>IGHG2</i> * <i>-n</i> /<br>* <i>-n</i>    | <i>IGHG2</i> * <i>-n</i> /<br>* <i>-n</i>    | <i>IGHG2</i> * <i>-n</i> /<br>* <i>-n</i>    |
| % Of a healthy Caucasian population                                                           | 22.3%                                      | 20.4%                                       | 19.1%                                       | 7.0%                                         | 12.1%                                        | 14.0%                                        |

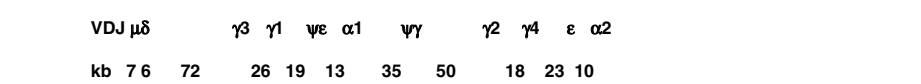


**Fig. 2** The close relationship between genes of the heavy constant chains of immunoglobulins is visualized by the number of kilo base pair. Chromosome 14 q32 (number of kb between *IGH* genes)

*IGHG2* with the \**n* or \**-n* genes, respectively, makes four different haplotypes with the following frequency in a Caucasian population namely: \**bfn* 44.8%; \**bf-n* 22.9%; \**gan* 0.9%; \**ga-n* 31.4% (Table 1D). The combinations from these four haplotypes constitute 10 diplotypes, of which six are common and four very rare. The diplotypes are found in the following frequencies in the Caucasian population: \**bfn*/\**bfn* 22.3%, \**bfn*/\**bf-n* 20.4%, \**bfn*/\**ga-n* 19.1%, \**bf-n*/\**bf-n* 7.0%, \**bf-n*/\**ga-n* 12.1%, \**ga-n*/\**ga-n* 14%, \**bfn*/\**gan* 3.8%, \**bf-n*/\**gan* 0%, and \**gan*/\**gan* 1.3 %, \**gan*/\**ga-n* 0%, (Table 1E) [10].

Functional differences in vitro of the alternative allotypes of  $\gamma 3$ ,  $\gamma 1$  and  $\gamma 2$  genes

The alternative serum allotypes (=alleles) from the  $\gamma 3$ ,  $\gamma 1$  and  $\gamma 2$  genes, respectively, are assessed by quality and quantity with a specific competitive ELISA method [6] and fractionation studies [7]. The material from pooled normal human serum from more than 500 blood donors, investigated by preparative electrophoresis revealed the alternative allotypes within the same IgG subclass to have different electrophoretic rates: IgG3\**b* is more anodal than IgG3\**g*, IgG1\**f* more anodal than IgG1\**a* and IgG2\**n* more anodal than IgG2\**-n*. Different fractionation rates are found with DEAE ion-exchange chromatography and protein-A-Sepharose chromatography, according to molarity and acidity (pH) [7]. A method is described how to separate \**f* from \**a* of IgG1 molecules, \**n* from \**-n* of IgG2 molecules and \**b* from \**g* of IgG3 molecules [7]. The alternative allotypes from  $\gamma 3$ ,  $\gamma 1$  and  $\gamma 2$ , are found as separate molecular entities, to possess different physicochemical qualities, indicating deviating functions.

The commercial IVIG (IntraVenous ImmunoGlobulin) products contain variable quantities of the alternative IgG3, IgG1 and IgG2 subclass allotypes with half to double the amounts of the IgG subclass allotype [8]. All commercial IVIGs are initially prepared by the Cohn cold ethanol fractionation method. To avoid problems with spontaneous aggregation, the purified IgG is acidified to pH 4 and additionally treated with traces of pepsin

which anyhow leaves a preparation similar to an unfractionated serum pool but with degraded IgG2\*-n. One IVIG preparation is treated with ion-exchangers to reduce the content of IgA. This step removes most of the IgG4 subclass antibodies, which as acidic proteins have electrostatic properties similar to IgA. Most of the IgG2\*n allotype is also removed by this preparation step. Still another IVIG is treated with trypsin which degrades IgG1\*f-, IgG2\*n- and IgG3\*b-allotypes. The allotypes within the same IgG subclass are thus proved to react differently on manufacturing processes [8].

#### Functional differences in vivo of the alternative allotypes of $\gamma 3$ , $\gamma 1$ and $\gamma 2$ genes

IgG subclass allotype quantification could be used in tracing foreign allotypes in intravenously immunoglobulin (IVIG) treated patients [8]. With IgG subclass allotype assessment, one can easily follow serum levels of foreign IgG allotypes originating from the infused IVIG. Quantification of IgG subclass allotypes can easily give information about the fate of infused allotypes day by day. In treatment of hypogammaglobulinemic patients with IVIG, a prolonged survival is found for the serum IgG2\*n allotype compared to the IgG2\*-n of IgG2. Different half-life times were also detected for the alternative IgG1\*a and IgG1\*f allotypes of IgG1. The findings indicate different functional qualities also in vivo of the alternative IgG subclass allotypes.

#### Developmental rates of the alternative IgG subclass allotypes during childhood

The developmental rates of the alternative serum IgG subclass allotypes in the six most common *IGHG* diplotypes, by analysing sera from 430 healthy children aged 1–15 years are described. The competitive enzyme-linked immunosorbant assay and radial immunodiffusion methods were used [6, 11]. The whole haplotype of *IGHG* genes rather than particular single *IGHG* subclass genes, influence the levels of IgG subclasses. The *IGHG*\*bf-n/\*bf-n diplotype predispose for low IgG2 levels and IgG2 deficiency [10], while the *IGHG*\*ga-n/\*ga-n diplotype, also with the \*-n/\*-n genes on  $\gamma 2$  locus expose normal IgG2 levels but low IgG3 levels and IgG3 deficiency [12].

Different maturation rates during childhood of the alternative IgG subclass allotypes were shown. The IgG2\*n development was strikingly retarded compared to IgG2\*-n from corresponding  $\gamma 2$  locus. Serum levels of IgG3\*b of homozygous *IGHG*3\*b/\*b individuals were significantly increased compared to IgG3\*g levels from homozygous *IGHG*3\*g/\*g children, which had been shown before for adults. Serum IgG1\*f levels were enhanced compared to IgG1\*a levels from  $\gamma 1$  locus [11]. For a more accurate judgement of IgG subclass deficiency, the IgG subclass determination should be accompanied by *IGHG* gene matching.

#### 4 B-cell variants coded by *IGHG* genes

B-cells are unique producers of immunoglobulins. The individual B-cell produces only one type of immunoglobulin molecules, but in a membrane bound and a secreted form. Different expressions of immunochemical and functional qualities of the alternative IgG subclass allotypes support the hypothesis of a significant variance of B-cells. 4 B cells coded from four different *IGHG* haplotypes are introduced [9]. The B-cell is expressing

genes from one *IGHG* haplotype B1-cells from *IGHG\*bf-n*, B2-cells from *IGHG\*bf-n*, B3-cells from *IGHG\*gan* and B4-cells from *IGHG\*ga-n* haplotypic variants (Table 1C, D). The six most common B-cell combinations are entitled B1/B1-, B1/B2-, B1/B4-, B2/B2-, B2/B4- and B4/B4-cells (Table 1E).

### ***IGHG* gene coded B-cells and allergy**

#### Association between atopy and the B1 cell

Asthma and allergy are strongly familial and have a genetic basis as reviewed by Cookson [1, 13]. Concordance rates for both asthma and atopic dermatitis are significantly higher in monozygotic twins than in dizygotic twins [14, 15] strongly indicating a genetic effect on the disease. Inheritance of the total IgE, as the principal marker of the atopic state, has also suggested the presence of a strong genetic effect and is used in most genetic studies.

The heavy constant chain of IgE, the  $\epsilon$  gene, is situated next to the genes for the IgG subclasses  $\gamma 4$  within 23 kb and  $\gamma 2$  within still another 18 kb (Fig. 2). A common disease such as asthma and allergy, changing appearance in short time, is likely to be due to common variants of genes, as IGH genes with additional rapid adaptable expressions. Examination of the serum protein variants from  $\gamma 3$ ,  $\gamma 1$  and  $\gamma 2$  genes on chromosome 14q32, of children with bronchial asthma revealed first the presence of one *IGHG2\*n* allele linked to atopy and the alternative *IGHG2\*n* allele linked to non-atopy [3, 4]. In Caucasian patients with bronchial asthma, atopic eczema or hay fever with increased IgE (>600 KU/l) the frequency of B1-cells was significantly increased [3]. The frequency of B1-cells with the *IGHG2\*n* allele from the  $\gamma 2$  gene locus was even more prominent in patients with IgE > 1,000 KU/l and in patients with IgG4 > 1 g/l. The *n* allele from the  $\gamma 2$  locus gene was in association with a high responding  $\epsilon$  gene in strong linkage disequilibrium. This distinctive *IGHG\*bf-n* haplotype (=B1-cells) was supposed to be a gene marker for atopy. The *IGHG2\*n* allele was associated with both a high producing *IGHE* gene and a high producing *IGHG4* gene.

The association of atopy and the *IGHG\*bf-n* haplotype (=B1-cells) was confirmed in allergen exposed 26 laboratory technicians, who had developed laboratory animal allergy (out of 102), compared to a healthy population (75% instead of 44.6%  $P < 0.001$ ) [16]. The *IGHG2\*n* gene dosages had influence on serum IgG subclass and IgE levels in atopic patients [5].

#### Association between non-IgE mediated allergy to *IGHG\*bf-n* haplotypes (=B2-cells) and *IGHG\*ga-n* haplotypes(=B4-cells)

Also non-atopic bronchial asthma is known to have a genetic predisposition even stronger than the atopic bronchial asthma. A total of 34 children with non-atopic bronchial asthma, low IgE levels (mean level 9.5 ku/l) and negative skin prick tests showed instead a highly significant increased frequency of the B4-cells, with expression of the alternative IgG subclass allotypes IgG3\*g from  $\gamma 3$ , IgG1\*a from  $\gamma 1$  and IgG2\*n from  $\gamma 2$  loci [4]. Additional 35 age-matched controls with atopic bronchial asthma demonstrated as found before, a significantly increased frequency of B1-cells. Patients with non-atopic asthma showed an increased frequency of *IGHG\*bf-n/\*bf-n* (=B2/B2-cells) *IGHG\*bf-n/\*ga-n*

(=B2/B4-cells) and *IGHG*\**ga-n*/\**ga-n* (=B4/B4-cells) diplotypes containing homozygous *IGHG2*\*-*n*/*n* genes. Homozygous B4/B4-cells are associated with low levels of IgG3 in healthy and IgG3 deficiency and homozygous B2/B2-cells associated with low levels of IgG2 in healthy and IgG2 deficiency [12]. In infants with severe respiratory syncytial virus (RSV) infection there was a dominance of the *IGHG2*\*-*n* haplotype (=B2-cells) with *IGHG*\*-*n* alleles [17]. The results emphasized the presence of different IgG subclass allotypes both qualitatively and quantitatively, in non-atopic and atopic bronchial asthma. They showed the interest in studying alternative IgG subclass allotypes (*IGHG* alleles) as markers in the pathogenesis of bronchial asthma.

#### Different pathways of immune regulation expressed by alternative *IGHG* genes in childhood asthma

Two groups of children with bronchial asthma and alternative *IGHG* genes on all three  $\gamma 3$ ,  $\gamma 1$  and  $\gamma 2$  loci, were investigated: 24 children with the homozygous expression in the *IGHG*\**bfn*/\**bfn* diplotype (=B1/B1-cells) and 16 with homozygous expression in the alternative *IGHG*\**ga-n*/\**ga-n* (=B4/B4-cells) [18]. The B1/B1-cells presented increased serum levels of IgE, increased frequency of SPT or RAST:s, increased number of peripheral blood eosinophils, constituting the atopic phenotype. Instead the B4/B4-cells presented with low serum IgE, practically no allergen specific IgE antibodies and normal number of peripheral blood eosinophils, but increased INF- $\gamma$  levels and increased number of CD 8 cells. The two groups presented qualitatively quite different IgG, and also different quantities of IgG subclasses and IgE (Fig. 3A, B). The alternative *IGHG* genes demonstrated different pathways of immune regulation, one associated with the atopic and the other with the non-atopic phenotypes of bronchial asthma [18].

#### Imbalanced activation of the expression from *IGHG* genes: low serum IgG1\*f and increased serum IgG2\*n allotypes in atopic childhood asthma

The serum allotype quantities of the B1/B1-cells, were assessed in children with atopic bronchial asthma and age- and *IGHG* genotype-matched healthy children [19]. The B1/B1-cells but also B1/B2- and B1/B4-cells with only one *IGHG*\**bfn* haplotype (=B1-cell) showed increased serum IgE. The serum levels of IgG3\*b allotype from *IGHG3* locus were unchanged but the level of IgG1\*f from *IGHG1* locus was down regulated to 3/4 of the levels of healthy, while the IgG2\*n levels from *IGHG2* locus was up regulated to double the value of healthy children. The heterozygous B1-cell variants exposed the same pattern of low IgG1\*f and increased IgG2\*n. Normal levels of IgG3 allotypes but low levels of both IgG1\*a and IgG2\*-n in the heterozygous variants indicated hypogammaglobuliemic levels (4 B). The activation was found downstream the IGH gene, known to be influenced by IL-4 and IL-13 cytokines (Fig. 4). In healthy children the development of the IgG2\*n allotype during ages is retarded [11]. But the increased IgG2\*n allotype levels in atopic children might reflect a persistent stimulation, probably by allergens, activated on this spot of the *IGH* genes by the Th2 cytokines IL-4 and IL-13 [20, 21]. There was a relative silence from the IgG1 locus otherwise responsible for the main antibody response to infections. The adaptability of the *IGH* genes on changes in the environment with an imbalanced activation of the *IGH* genes as shown by a silent *IGHG1* gene in more hygienic

environments and instead activation of the *IGH* genes downstream, may be one possible explanation of the increased prevalence of atopy in children in western countries, the last decades.

### *IGHG* genes and allergy inflammation

Different qualities and quantities of IgG antibodies [5, 6] in atopic and non-atopic bronchial asthma play a role in the modulation and maintenance of inflammation with or

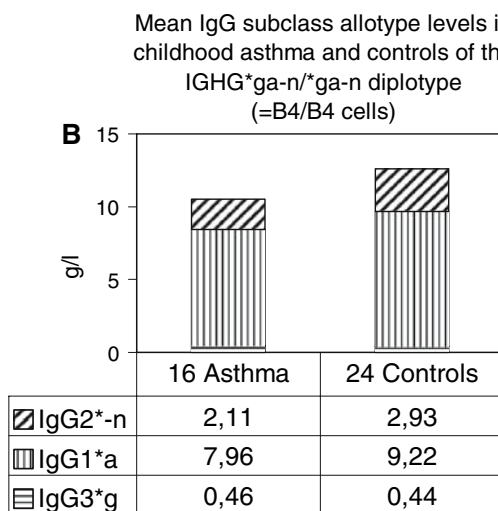
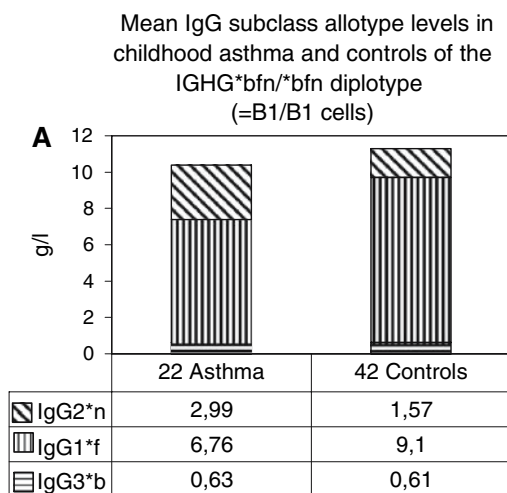


**Fig. 3** Ten-year-old asthmatic children with mean IgG subclass allotype levels in childhood asthma and controls of the (A) the *IGHG*\**bfn*/\**bfn* diplotypes (=B1/B1-cells) and (B) the *IGHG*\**ga-n*/\**ga-n* diplotypes (=B4/B4-cells), respectively, compared to age matched and *IGHG* diplotype matched controls, demonstrating qualitatively and quantitatively different IgG



**Fig. 4** Cytokines influence activation of IGH genes

without the additional IgE/mast cell axis recently described. In spite of only minor amino acid epitope differences in the IgG molecules of the two groups, they express major differences by immunochemical quality and biological function [7].

In rearrangement of genes for immunoglobulins the first step is the heavy chain rearrangement of the antigen V(variable)-region. Once the V region is determined it remain essentially unaltered and is then recombined with the constant heavy chain gene. Usually the selection of heavy constant chains for the immunoglobulin molecule is started upstream the *IGH* allele. In the defence against bacterial and viral agents there is a need of, on the first hand, IgM and IgG3 and then IgG1.

There is a linkage of IgE sensitisation to B1-cells with normal IgG3\*b, decreased IgG1\*f and increased IgG2\*n together with increased IgG4 and IgE levels. The particular qualities of IgG molecules from the B1-cells in atopy must be involved before the production of specific IgE antibodies as the *IGHG* genes and finally the *IGHE* genes are activated downstream by environmental allergens, with IgE starting the allergic cascade of inflammation. It is known that individuals with the IgG2\*n allotypes produce quantitatively more antibodies to polysaccharide antigens than individuals with the genetic marker IgG2\*-n [22, 23]. It has been reported that macrophages of individuals with the *IGHG\*bf*n haplotype (=B1-cells), degrade antigens very slowly [24]. The role of IgG has been rather neglected in the allergic inflammation, but the expression of a highly allergen responding *IGHE* gene is seen in the presence of *IGHG2\*n* gene of the *IGHG\*bf*n haplotype.

There is also a linkage of non-atopy to B4/B4-cells producing IgG3\*g, IgG1\*a and IgG2\*-n antibodies. B4/B4-cells mainly found in the non-atopic phenotype, are often found in IgG3 deficiency, also reported with increased frequency of obstructive lung disease [25]. Of children with asthma, those with the B4/B4-cells, low IgG1\*a, low IgG2\*-n and low IgE, actually reveal a diminished ability to conquer bacterial and viral invaders [18].

#### The *IGHG* genes as risk factors in childhood asthma

In a case–control study, 198 children with and without allergy heredity, symptoms and positive SPT, from the origin study of 1,390 12-year-old children participating in the International Study of Asthma and Allergy in Children (ISAAC) [26], were examined. There was a significantly dominant effect of *IGHG\*bf*n haplotype (=B1-cells) in children with a family history of allergy, allergy symptoms and positive SPT [27]. In the same group, there was also a significant deficit of *IGHG\*bf*-n haplotype (=B2-cells). No preference of *IGHG\*ga*n haplotypes (very rare in the population) or *IGHG\*ga*-n haplotypes was found. The number of individuals with all sorts of allergy phenotypes and combinations were compared to non-allergy phenotypes, respectively, and also compared to a healthy group of children without allergy heredity and with negative SPT. B1-cells dominated with similar influence from both paternal and maternal inheritance. B1-cells dominated in wheezing as allergy symptom, as well as in eczema and rhinitis and in children with positive SPT for all inhalant allergens tested. The number of children with

serum IgG2\**n* allotypes (*IGHG2\**n**) dominated significantly over IgG2\*-*n* allotypes (*IGHG2\*-*n**) in the allergy phenotypes [27] (Tables 2, 3)

Our results demonstrate that there are significant effects of the polymorphisms of the *IGHG* genes on allergy heredity originating both from the mother and the father, on allergy symptoms, beside wheezing, also on eczema and rhinitis and on positive SPT for various inhalation allergens. There was a significant dominance of B1-cells and significant deficit of B2-cells with the only difference \**n* instead of \*-*n* on *IGHG2* gene locus. The results also demonstrate significant involvement of IgG2\**n* allotype antibodies in atopic phenotypes.

**Table 2** From allergy phenotypes to *IGHG* genotypes

| Allergy phenotypes                                                                           | <i>IGHG</i> alleles<br><i>IGHG</i> haplotypes(=B-cells)                                                                                          | References |
|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Increased IgE > 600 ku/l, IgE > 1,000 ku/l, IgG4 > 1 g/l in children with asthma and allergy | <i>IGHG*bf<sub>n</sub></i> haplotype↑ (=B1-cell↑)<br><i>IGHG2*n</i> allele↑                                                                      | [3, 5]     |
| Atopic childhood asthma, mean level IgE 1,762 ku/l                                           | <i>IGHG*bf<sub>n</sub></i> haplotype↑ (=B1-cell↑)<br><i>IGHG2*n</i> allele↑                                                                      | [4]        |
| Non-atopic childhood asthma, mean level IgE 9.5 ku/l                                         | <i>IGHG*ga-n</i> haplotype↑ (=B4-cell↑)<br><i>IGHG2*-n</i> allele↑                                                                               |            |
| Confirmed allergen exposure: patients developing laboratory animal allergy                   | <i>IGHG*bf<sub>n</sub></i> haplotype↑ (=B1-cell↑)<br><i>IGHG2*n</i> allele↑                                                                      | [16]       |
| ISAAC Patients (cases): Heredity+, Symptoms+, SPT pos                                        | <i>IGHG*bf<sub>n</sub></i> haplotype↑ (=B1-cell↑)<br><i>IGHG2*n</i> allele↑ & <i>IGHG*bf-n</i> haplotype↓ (=B2-cell↓)<br><i>IGHG2*-n</i> allele↓ | [27]       |
| ISAAC controls: Heredity-, Symptoms-, SPT neg                                                | <i>IGHG*bf<sub>n</sub></i> haplotype↓ (=B1-cell↓)<br><i>IGHG2*n</i> allele↓ & <i>IGHG*bf-n</i> haplotype↑ (=B2-cell↑)<br><i>IGHG2*-n</i> allele↑ |            |
| Severe RSV in infants                                                                        | <i>IGHG*bf-n</i> haplotype↑ (=B2-cell↑)<br><i>IGHG2*-n</i> allele↑                                                                               | [17]       |

↑ or ↓ significantly increased or decreased

**Table 3** From *IGHG* genotypes to allergy phenotypes

| <i>IGHG</i> haplotype (=B-cells)<br><i>IGHG</i> diplotypes                          | Phenotypes: IgG subclass allotype levels, IgE levels, IgE sensitised RAST or SPT, eos, T-cells, IFNγ | References |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------|
| <i>IGHG*bf<sub>n</sub></i> haplotypes (=B1 cell)                                    | IgG1*f ↓, IgG2*n↑                                                                                    | [19]       |
| <i>IGHG*bf<sub>n</sub>/*bf<sub>n</sub></i> diplotype (=B1/B1-cells)                 | IgG1*f ↓, IgG2*n↑, IgE↑, Eos↑                                                                        | [18]       |
| Compared to alternative <i>IGHG*ga-n</i> /<br><i>*ga-n</i> diplotype (=B4/B4-cells) | Compared to IgG2*-n↓, IgE↓, Eos↓, IFNγ↑, CD8↑                                                        |            |

↑ or ↓ significantly increased or decreased

## Candidate genes and environmental factors

Allergy diseases are the most frequent diseases in populations of civilized countries [28]. In the past three decades an increasing prevalence was observed especially in industrial populations. This cannot be explained on the basis of genes alone, but reveals a multifactorial pathogenetic concept characteristic for complex diseases depending on both environmental and genetic factors [29]. Environmental allergen levels are probably the major determinant of whether sensitisation of genetically predisposed individuals occurs. Increased exposure to sensitising allergens and reduced stimulation of the immune system during critical periods of development are seen as the main factors driving the rising prevalence of allergic disease.

The etiology of allergic diseases is based on several interacting mechanisms both genes and environmental factors. Allergy diseases are seen as genetically complex and have much higher population prevalence than the rare monogenic diseases. Linkage of allergic asthma, allergic rhinoconjunctivitis and atopic eczema and total IgE levels to several different chromosomal regions have been described extensively but little is known about the contributing gene loci and their variants and only a few could be reproduced [1, 30].

A number of genes indirectly related to B-cells have been replicated. The major histocompatibility complex shows the strongest evidence for linkage in most studies. It is well-known that HLA-DR alleles restrict the IgE response to particular allergens [31]. The chromosome 11q13 was originally linked to atopy and was subsequently shown to contain the  $\beta$  chain of the high affinity Fc receptor for IgE [32]. Polymorphisms in Fc $\epsilon$ RI $\beta$  chain are associated with asthma and other forms of the disease and modify non-specifically the strength of the response to allergens [33, 34].

The chromosome region 5q31–q35, a region containing multiple candidate genes for allergy and asthma, including a cluster family of cytokine genes with IL-4 and IL-13 which play important interactive roles in allergic inflammatory response [35]. These genetic factors are also B-cell associated as IL-4 and IL-13 are preferably activating the *IGHG* genes downstream, resulting in increased expression from the *IGHG2*\**n*, but also increased expressions from *IGHG4* and *IGHE* with allergen specific IgE antibodies.

What we have learned about influences of *IGHG* genes on asthma and allergy

The polymorphisms of *IGHG* genes from chromosome 14 q32, expressing different IgG molecules, qualitatively and quantitatively, are associated to and express different phenotypes of asthma and allergy. The alternative *IGHG* alleles of *IGHG* haplotypes disclose a variation of 4 B-cells: B1-, B2-, B3- and B4-cells. Of the four B-cells, the *IGHG*\**bf**n* haplotype (=B1-cells), has a dominant effect on atopy, namely in patients with increased IgE, a family history of allergy, IgE sensitisation, allergy symptoms, as well as in individuals after allergen exposure. The *IGHG*\**bf*-*n* haplotype (=B2-cells) with the only difference \*-*n* instead of\**n* on *IGHG2* locus was significantly rare in atopy, probably protective. In childhood asthma, those with low IgE, mainly infectious problems and unspecific hypersensitivity, are found with *IGHG*\**bf*-*n* haplotypes (=B2-cells) and *IGHG*\**ga*-*n* haplotypes (=B4-cells) with\*-*n* on *IGHG2* locus.

The *IGHG2*\**n* allele is important for atopy, activated with increased IgG2\**n* allotype level, localised downstream the *IGH* genes next to *IGHG4* and next to an allergen responding *IGHE* gene. There is a close localization of the  $\gamma$ 2,  $\gamma$ 4 and  $\epsilon$  genes and they are inherited in strong linkage disequilibrium. An allergen activated *IGHE* gene is influenced

by the upstream located *IGHG\*bfh* haplotype. As there is no genetic marker for *IGHE* the IgG2\*n allotype can be used in children with heredity and allergy symptoms to support the atopy diagnosis. The findings of *IGHG2\*n* gene dose dependency in IgE sensitisation is another strong evidence of the *IGHG2\*n* allele linkage to atopy and allergy.

The alternative allotypes of the *IGHG3*, *IGHG1* and *IGHG2* genes demonstrates different immunochemical and functional properties. Different pathways of immune regulation are demonstrated by B1/B1-cells of childhood asthma compared to the alternative B4/B4-cells. Imbalanced activation of the *IGHG* genes of B1-cells is shown in children with atopic bronchial asthma with low levels of IgG1\*f and increased serum levels of IgG2\*n. The development of the latter is normally retarded in healthy, during childhood. In children with atopy the expression from the  $\gamma 1$  gene is low, but the activation is found downstream the *IGH* gene, at the spot  $\gamma 2$ ,  $\gamma 4$  and  $\epsilon$ , known to be influenced by the Th2 cell cytokines IL-4 and IL-13. The low expression from the  $\gamma 1$  may be in agreement with the hygiene hypothesis and demonstrate the rapid adaptability of *IGH* genes in more hygienic environments and might be one explanation for the increased prevalence of atopy in children, in western countries, the recent decades. Further studies on functional *IGHG* genetics will lead to a better understanding of the significance of different IgG molecules in the inflammatory response of different phenotypes of asthma and allergy, of importance for diagnoses and treatment.

**Acknowledgments** I thank Ann-Margreth Carlsson for skilful technical assistance. The studies was supported by grants from The Medical Faculty of the University of Lund and the University Hospital of Lund, The Swedish Asthma and Allergy Association and the Vardal Foundation, Sweden.

## References

1. Cookson WO. Genetic and genomics of asthma and allergic diseases. *Immunol Rev* 2002;190(1):195–206
2. Grubb R. Human immunoglobulin allotypes and Mendelian polymorphism of the human immunoglobulin genes. In: Oss CJ, Regenmortel MHV, editors. *Immunochemistry*. New York: Dekker; 1994. p 47–68
3. Oxelius V-A. Correlation between atopy and Gm allotypes. *Int Arch Allergy Appl Immunol* 1990;91:54–7
4. Oxelius V-A, Hultquist C, Husby S. Gm allotypes as indicators of non-atopic and atopic bronchial asthma. *Int Arch Allergy Appl Immunol* 1993;101:66–71
5. Oxelius V-A. Gm allotype genes and gene dosage affecting both IgG subclass and IgE levels in atopic patients. *Int Arch Allergy Appl Immunol* 1990;91:58–61
6. Oxelius V-A, Carlsson A-M. Quantitation of Gm allotypes. *Scand J Immunol* 1993;37:143–8
7. Oxelius V-A. Preparation of IgG subclass allotypes from polyclonal IgG. *Scand J Immunol* 1999;49:395–8
8. Oxelius V-A, Eibl MM. Different Gm allotype levels in human intravenous immunoglobulin (IVIG) preparations, survival of foreign Gm allotypes in immunodeficient patients. *Clin Exp Immunol* 1996;106:203–7
9. Oxelius V-A. Genetic B cell variation based on immunoglobulin heavy G chain (Gm) genes. *Scand J Immunol* 1999;49:345–6
10. Oxelius V-A. Serum IgG and IgG subclass contents in different Gm phenotypes. *Scand J Immunol* 1993;37:149–53
11. Oxelius V-A, Aurivillius M, Carlsson A-M, Musil K. Serum Gm allotype development during childhood. *Scand J Immunol* 1999;50:440–6
12. Oxelius V-A. Lack of the G2m(n) allotype in IgG subclass deficiency, in IgG2 deficiency together with lack of G1m(a) and G3m(g) and IgG3 deficiency together with lack of G1m(f) and G3m(b). *Scand J Immunol* 1990;31:243–7

13. Cookson WO. The alliance of genes and environment in asthma and allergy. *Nature* 1999;402 Suppl:B5–11
14. Spaich D, Ostertag M. Untersuchungen über allergische Erkrankungen bei Zwillingen. *Z Mensch Vererb-Konstitutionsl* 1936;19:731–51
15. Schultz-Larsen F, Holm NV, Henigsen K. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *J Am Acad Dermatol* 1986;15:487–94
16. Oxelius V-A, 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;110:73–8
17. Aurivillius M, Oymar K, Oxelius V-A. Immunoglobulin heavy G2 chain (IGHG2) gene restriction in the development of severe respiratory syncytial virus infection. *Acta Paediatr* 2005;94:414–8
18. Oxelius V-A, Carlsson A-M, Aurivillius M. Alternative G1m, G2m and G3m allotypes of IGHG genes correlate with atopic and non atopic pathways of immune regulation in children with bronchial asthma. *Int Arch Allergy Immunol* 1998;115:215–9
19. Oxelius V-A. Imbalanced switch of the IGHG (Immunoglobulin constant Heavy G chain) Gm(bfn) genes in atopic childhood asthma. *Allergy* 2000;55:1063–8
20. Walley AJ, Cookson WO. Investigation of an interleukin-4 promoter polymorphism for association with asthma and atopy. *J Med Genetic* 1996;33:689–92
21. Willis-Karp M, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258–61
22. Pandey J, Fudenberg H, Virella G, et al. Association between immunoglobulin allotypes and immune responses to *Haemophilus influenzae* and *meningococcus polysaccharides*. *Lancet* 1979;i:190–2
23. 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;75:1935–42
24. Legrand L, Rivat-Perran L, Hutting C, Dausset J. HLA and Gm linked genes affecting the degradation of antigens (sheep red cells) endocytosed by macrophages. *Hum Immunol* 1982;4:1–13
25. Oxelius V-A, Hanson L-Å, Björkander J, Hammarström L, Sjöholm A. IgG3 deficiency: common in obstructive lung disease. *Monogr Allergy* 1986;20:106–15
26. ISAAC. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema. ISAAC The international study of asthma and allergies in childhood (ISAAC) steering committee. *Lancet* 1998;351:1225–32
27. Oxelius V-A, Bråbäck L, Ahlstedt S, Björkstén B. Immunoglobulin constant heavy G chain genes as risk factors in childhood allergies. *Clin Exp Allergy* 2006;36:1616–24
28. Jarvis D, Burney P. ABC of allergies. The epidemiology of allergic diseases. *BMJ* 1998;316:607–9
29. Holgate ST. The epidemic of allergy and asthma. *Nature* 1999;402:B2–4
30. Marsch DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. *New Engl J Med* 1981;305:1551–9
31. Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E-responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989;i:1292–6
32. Sandford AJ, Shirakawa T, Moffatt MF, et al. Localisation of atopy and  $\beta$  subunit of high-affinity IgE receptor (Fc $\epsilon$ RI) on chromosome 11q. *Lancet* 1993;341:332–4
33. Hill MR, James AL, Faux JA, et al. Fc $\epsilon$ RI $\beta$  polymorphism and risk of atopy in a general population sample. *Br Med J* 1995;311:776–9
34. Van Der Pouw Kraan TC, Van Veen A, Boeije LC, et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun* 1999;1:61–5
35. Noguchin E, Nukaga-Nishio Y, Yokouchi Y, et al. Haplotypes of the 5' region of the IL-4 gene and SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. *Human Immunol* 2001;62:1251–7