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Human immunoglobulin constant heavy G chain (IGHG) (Fcγ) (GM) genes, defining innate variants of IgG molecules and B cells, have impact on disease and therapy.

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REVIEW ARTICLE

Title:

Human immunoglobulin constant heavy G chain (*IGHG*)(Fcγ)(GM) genes, defining innate variants of IgG molecules and B cells, have impact on disease and therapy

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KEYWORDS

Human immunoglobulin heavy constant G chain (*IGHG*)(Fcγ)(GM) genes (Chromosome 14q32.3),

Alternative GM allotypes of γ3, γ1 and γ2 chain genes,

Dissection of the *IGHG* gene complex in *IGHG* alleles (allotypes), homozygous or heterozygous *IGHG* genes, *IGHG* haplotypes(combinations of γ3, γ1 and γ2 chain genes) indirect markers of B cells and homozygous or heterozygous individual *IGHG* diplotypes,

*IGHG* genes, respond differently to bacteria, virus and allergens, to active and passive immunotherapy,

Mendelian *IGHG* genes are associated with or linked to diseases and phenotypes of diseases in primary immunodeficiency, allergy, autoimmunity and malignancy,

# Abstract

The distinguished alternative GM allotypes localized in immunoglobulin constant heavy G chains, *IGHG* (Fcγ) (GM) genes on chromosome 14q32.3 define two unique variants of respectively IgG3, IgG1 and IgG2 subclass, with different structures and functions. The *IGHG* allele (allotypes), expressed in homozygous or heterozygous forms, are assessed by new serological methods. Fixed combinations of γ3, γ1 and γ2 allotypes constitute the haplotypes, which are indirect markers of B cells. We highlight the role of homozygous *IGHG* genes with restricted qualities of IgG subclass molecules and B cells. These common Mendelian *IGHG* genes, respond differently to allergens and infections, both bacterial and viral, and to active and passive immunotherapy. *IGHG* genes have impact on diseases as allergy, immunodeficiency, autoimmunity and malignancy. Association/linkage of different *IGHG* genes give information about risk /protection, good or bad prognosis, for improvement of clinical care. The *IGHG* gene map of healthy Caucasians is registered.

Contents

# 1. Introduction

Human antibodies or immunoglobulins were originally defined as gamma (γ) globulins because of their mobility to the gamma region of paper electrophoresis. Subsequently, isotypes were defined serologically and structurally by the unique amino acid sequences of their constant heavy chain regions encoding the chromosome 14q32.3 (5´μ, δ, γ3, γ1,ψε, α1, γ2, γ4, ε, α2 3´). IgG was registered by 4 IgG subclasses (Table 1A). The polymorphisms of IgG are studied by GM allotypes as genetic markers of the immunoglobulin constant heavy G chains (*IGHG*) (Fcγ) (GM) genes (Table 1B), inherited in Mendelian fashion and allelic exclusion [1-3]. The distinguished alternative allotypes of γ3-, γ1- and γ2- chain genes express allelic IgG subclasses IgG3*b, IgG3*g, IgG1*f, IgG1*a, IgG2*n and IgG2*-n (Table 1C), which are unique entities with different structures and functions. Both *IGHG* gene mapping and quantities of allelic IgG subclasses are assessed by a sensitive competitive ELISA [4-6], at individual and population levels. The genome-wide association studies (GWAS) and HapMap catalogue do not include GM immunoglobulin genes in their genotyping platform [7-9].  By serological methods the *IGHG* genes are registered as alleles (allotypes), genes, haplotypes and diplotypes (Table 1-4). The haplotypes are markers of B cells [10] (Table 3).

The new variants of allelic IgG3, IgG1 and IgG2 subclasses were investigated for structural and functional differences. Various infectious antigens and allergens affect particular *IGHG* genes, responding differently to viral and bacterial infections, in allergy, immunodeficiency, autoimmunity and malignancy and responding differently on active immunotherapy (vaccines) and passive immunotherapy (IVIG, recombinant monoclonals). Individuals are mapped according to the *IGHG* gene map (Table 2) and the gene activity by allelic IgG subclass quantities in comparison to reference values of healthy Caucasian adults and children (Fig. 1, 2, Table 5) [9].

Different properties of *IGHG* genes suggest that the Darwinian selection over many generations have played a role in the maintenance of polymorphisms of *IGHG* genes, of which some are common others rare. In this review we summarize the current understanding of the influence of *IGHG* genes on human immunity. We discuss the results from dissecting the *IGHG* (Fcγ) (GM) gene complex, to present the individual IgG immunity. We highlight the role of restrictive *IGHG* genes defining the individual allelic IgG subclass molecules and individual B cells in various diseases and therapy

## 2. Dissection of the human immunoglobulin heavy constant G chain (*IGHG*) (Fcγ) (GM) gene complex from chromosome14q32.3 (5´μ, δ, γ3, γ1,ψε, α1, γ2, γ4, ε, α2 3´) by GM allotypes

The immunoglobulin heavy constant G chain (*IGHG*) genes are found on chromosome14q32.3 (5´μ, δ, γ3, γ1, ψε, α1, γ2, γ4, ε, α2 3´). The IgG3, IgG1 and IgG2 subclasses can be differentiated by the distinguished alternative GM allotypes of γ3 chains:*b/5 and/or *g/21, of γ1 chains :*f/3 and/or *a/1 and of γ2 chains: *n/23 and/or *-n/-23 (Table 1B) They have unique structures by minor amino acid differences (Table 6), correlating with single-base substitutions in the genes. The 6 alternative IgG subclasses are new unique entities of IgG3: IgG3*b or IgG3*g; of IgG1: IgG1*f or IgG1*a; of IgG2: IgG2*n or IgG2*-n (Table 1C). 9 homozygous or heterozygous *IGHG* (Fcγ) (GM) genes. 3 variants of each γ3, γ1 and γ2, (Table 1D) express the individual molecules of IgG3: IgG3*b/*b, IgG3*b/*g, IgG3*g/*g; of IgG1: IgG1*f/*f, IgG1*f/*a, IgG1*a/*a; and of IgG2: IgG2*n/*n, IgG2*n/*-n, IgG2*-n/*-n (Table 1E). 4 haplotypes are fixed combinations of γ3-, γ1- and γ2- alleles; *IGHG*bfn*, *IGHG*bf-n*, *IGHG*gan* and *IGHG*ga-n* (Table 3).They are found in

homozygous and heterozygous forms, of parental origin, and constitute 10 individual

diplotypes, six common and 4 rare (Table 2,4). Homozygous alleles and haplotypes are

highlighted as they express restricted qualities of IgG subclass molecules. They are often

associated with the extreme disease phenotypes. A B cell variation is described from the

expression of *IGHG* haplotypes [10].

## 3. Mode of inheritance of alternative *IGHG* (Fcγ) (GM) genes

The IgG class is divided in 4 subclasses. All four IgG subclasses are simultaneously present

in single individuals, but the expressions of γ3, γ1 and γ2 chains are defined by the inherited

alternative *IGHG* (GM) allotypes [1-4]. The distinguished alternative *IGHG*(GM) allotypes

(alleles) are expressed as homozygous or heterozygous *IGHG* genes constituting the IgG

subclass gene. *IGHG* gene mapping and corresponding levels of serum allotypic (allelic) IgG

subclasses were analysed with a new ELISA method [4]. The 6 allotypes(alleles) define 9

homozygous or heterozygous genotypes (Table 1D). There is a strong linkage disequilibrium

between γ3 and γ1 alleles as *bf and *ga. The γ2 *n or γ2*-n are added in known fixed

combinations of allotypes, in 4 haplotypes [3]: *IGHG*bfn*, *IGHG*bf-n*, *IGHG*gan* and

*IGHG*ga-n* (Table 3).The 4 haplotypes define 10 individual *IGHG* diplotypes, in

homozygous or heterozygous forms. Of the 10 diplotypes 6 are common, while 4 containing

the *IGHG*gan* are very rare (Table 4). The γ2 *n allele is found nearly exclusively in the

*IGHG*bfn* haplotype.

The 3 common homozygous individual diplotypes are highlighted, expressing only

3 variants of IgG molecules. The 3 heterozygous diplotypes express 4-6 IgG variants

compared to a normal serum pool (Fig. 2). The very rare diplotypes (totally <1-2% of

Caucasians) were left out (Table 2). The *IGHG* gene frequencies and allelic IgG subclass

levels of a healthy Caucasian population were given and used as references when investigating different diseases (Table 2, 5). Thus the individual homozygous *IGHG* diplotypes contain restricted qualities of IgG, compared to the normal serum pool, which contains all 6 IgG variants: IgG3*b, IgG3*g, IgG1*f, IgG1*a, IgG2*n and IgG2*-n (Fig. 1). The Mendelian inheritance was confirmed in a study of 200 children and their parents.

## 4. *IGHG* haplotypes indirect markers of B cells
## B*$^{bfn}$* or B1, B*$^{bf-n}$* or B2, B*$^{ga-n}$* or B4 and one rare B*$^{gan}$* or B3 subsets

B cells are the only cells producing immunoglobulins and they could be mapped according to the 4 *IGHG* haplotypes found in the population. The expression of *IGHG* haplotypes are indirect markers of at least 4 inherited B cells variants, 3 common B$^{bfn}$ or B1, B$^{bf-n}$ or B2, B$^{ga-n}$ or B4 and one rare B$^{gan}$ or B3 (Table 3) [10]. The $\gamma 3$, $\gamma 1$ genes are situated very close, in strong linkage disequilibrium, on chromosome 14q32.3, the combination of the alleles *bf and *ga, are most common in the Caucasian population. The combination of *ba is extremely rare. The individual *IGHG* diplotype is constituted by 2 haplotypes (alias 2 B cells), of maternal and paternal origins (Table 2). *IGHG* haplotypes and B cells are inherited according to the Mendelian law.

The B cells are involved in production of different amounts of IgG subclasses, in IgG2 deficiency associated with homozygous B$^{bf-n}$ or B2 cells and in IgG3 deficiency with homozygous B$^{ga-n}$ or B4 cells. B2 and B4 cells respond with low specific antibodies to polysaccharide antigens compared to B1 cells. The *IGHG* genetic code of B cells is present in all human somatic cells, also stem cells. The effect of B cells is on antibody production. Autologous stem cell transplantation has proven safe,-which includes also autologous antibody production. In allogeneic stem cell transplantation there may be an effect

of variable "incompatable" *IGHG* genes. The influence of individual *IGHG* genes on other

immune mechanisms should be investigated further.

## 5. The *IGHG* (Fcγ) (GM) gene map

## Reference levels of allelic IgG subclasses

## Immunological/serological methods

A sensitive competitive ELISA (sensitivity1000ng/ml) was used to register *IGHG* genes as allotypes and

quantify serum IgG1*f, IgG1*a, IgG2*n and IgG3*b allotypes[6] 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). Microtitre 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 was used and a panel of purified myeloma proteins as positive

and negative controls. Determination of homozygosity and heterozygosity for the IgG2*n allotype was done with

a double immuno diffusion assay with the monoclonal reagents anti IgG2*n clone SH21 and anti HP 6014

(Sigma, St. Louis, MO, USA) [11]. 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 [4].

In addition the quantities of allelic IgG subclasses were calculated from knowledge of the gene

frequencies in a large Caucasian population of more than 2000 serum samples, with given proportions of the GM

allotypes within the IgG subclasses for IgG3*b/ IgG3*g, 0.51/0.22 g/l, 70/30, for IgG1*f/IgG1*a 4.76/2.04 g/l,

70/30 and for IgG2*n/IgG2*-n, 1.5/1.83 g/l, 45/55 [4] (Fig.1). By the ELISA tests the quantitative expressions of

*IGHG* genes are available. Total IgG subclass levels were measured with the Mancini technique. The allelic IgG

subclass levels of the *IGHG* diplotypes of healthy adults and developmental rate during childhood have been

given (Mean +/- SD) as references [4,9]. The method to study genes by serum protein copies is advantageous, as

the protein quantities in serum also disclose the rate of gene activity.

The ELISA method is the simplest way to demonstrate *IGHG* (GM) genes, their frequencies and quantities of allelic IgG subclasses. Investigations by RFLP, DNA and SNPs have had limited success. At present, the commonly used genome-wide association studies (GWAS) and HapMAP project do not include testable GM immunoglobulin genes in their genotyping platforms. This underscores the necessity of a candidate gene approach to investigate the role played by the GM gene complex in immunobiology [7-9]. The frequency figures for *IGHG2* variants in Caucasians from different research groups were for the American: *IGHG2*n/*n* 34.3%, *n/*-n* 16.2%, *-n/*-n* 49.5% [12] for the Finnish: *n/*n* 35.4%, *n/*-n* 18.3%, *-n/*-n* 43.7% [11] and for the present Swedish:*n/*n* 34%, *n/*-n* 22%, *-n/*-n* 44% [9].

## 6.   Structural differences of alternative allelic IgG subclasses

The alternative GM allotypes of the heavy constant γ3, γ1 and γ2 chains are unique entities. In homozygous individuals the alternative allotypes are absent. They have different minor amino acid sequences (Table 6). It is evident that serologically defined allotypes within the IgG3 subclass are very complex. But IgG3*b is in strong linkage disequilibrium with IgG1*f and IgG3*g with IgG1*a. No alternative allotypes have been defined for IgG4.

The normal serum pool of 2000 Caucacian healthy adults was investigated for structural differences by immunochemical methods. The alternative allotypic (allelic) IgG subclass variants were found to have different rates by serum electrophoresis (preparative electrophoresis) (Fig.3A) and different pH related rates by DEAE and Protein A-Sepharose chromatography [5]. Allotypic IgG subclasses of the *IGHG*bfn* haplotype were located anodally and those of the opposite *IGHG*ga-n* haplotype were located cathodally. IgG2*-n could be separated from IgG2*n and IgG3*g from IgG3*b molecules.This confirmed the unique structures of alternative allelic IgG subclasses.

The commercial IVIG preparations contain all 6 studied allelic IgG subclass variants as found in the normal serum pool. Allotypic IgG subclass (GM) quantities differed

in various IVIG products. 7 commercial IVIG preparation, mean of five batches, respectively, were investigated for allelic IgG subclasses compared to a normal serum pool (2000 sera). (Fig. 3B). The different proportions are probably the result from different manufacturing processes.

In treated patients with homozygous *IGHG,* the levels of added structurally foreign settings of allelic IgG subclasses could easily be registered and different trough levels and half-life times recorded. Foreign IgG2*n was found to have longer half-life time than IgG1*f and IgG1*a (Fig. 3C)[13]. The commercial IVIG preparation have been thoroughly investigated for specific antibodies but not for allelic IgG subclasses. By collecting sera from blood donors of the same *IGHG* diplotypes, there is a possibility to make IgG preparations containing restrictive selective IgG molecules, without fractionation procedures. The homozygous diplotypes *IGHG*bfn/*bfn* and the structurally quite different *IGHG*ga-n/*ga-n* are most suitable in comparison for different functions in disease.

## 7. Functional differences of alternative allelic IgG subclasses

The Fc part establishes the function of the immunoglobulin molecule. By comparing the 10 individual *IGHG* diplotypes (6 common) of healthy, functional differences were disclosed. They were found with different levels of IgG subclasses. In a healthy population the homozygous *IGHG*bf-n/*bf-n* expresses the lowest IgG2 levels and *IGHG*ga-n/*ga-n* the lowest IgG3 levels [9]. These diplotypes dominate also in IgG2- and IgG3- deficiencies [14], respectively, but then with significantly low levels compared to *IGHG* matched healthy. Both contain the homozygous *IGHG2*-n/*-n* genotype but with opposite *IGHG3* and *IGHG1* alleles. It was found that all allotypes within the haplotype (B cell) have influence on the expression of the separate IgG subclass levels [9]. In childhood the allotypic IgG subclasses

demonstrate different developmental rates. IgG2*n levels are retarded compared to the opposite IgG2*-n. The retarded IgG2*n levels could be compared to the known retarded levels of IgA during childhood. The IgG2 allotypic levels continue to increase in adulthood while the IgG1 allotypic levels decrease.

The function of *IGHG* (Fcγ) (GM) genes results in different susceptibility/resistence to bacterial infections and to viral discrimination. Various *IGHG* (Fcγ)(GM) gene recipients respond differently to active immunotherapy (vaccines) and passive immunotherapy (IVIG, Therapeutic recombinant monoclonal antibodies). *IGHG* genes are associated/linked with diseases and phenotypes of diseases as immunodeficiency, allergy, autoimmunity and malignancy (for details and ref. see below).

## 8.   Infections associated with *IGHG* (Fcγ) (GM) genes

There is a genetic control of survival in epidemics. Descendants of 367 Dutch colonists (100 families) who emigrated to Surinam in 1847, survived epidemics of typhoid and yellow fever with a total morality of 60%. The survivals were tested for GM allotypes (compared to a healthy Dutch population) comprising 88% (69%) *IGHG*bfn* & *IGHG*bf-n* and significantly low numbers 11% (29%) of *IGHG*ga-n* also according to our normal references (P<0.001) [15,4].Therefore these data might indicate selection through genetic control of survival in these epidemics.Various bacterias, virus and allergens affect individuals with particular *IGHG* haplotypes (B cells) more often. In addition various haplotypes are possibly discriminated as carriers or non carriers of bacteria or viruses. This could be the selective force for the

maintenance of various frequencies of *IGHG* haplotypes, some common others very rare. By the Mendelian inheritance of IgG molecules there is a possibility that not all family members are affected by infections at the same time.

## 8.1. *IGHG* (Fcγ)(GM) gene susceptibility/resistence to bacterial infections

Neonatal septicaemia caused by streptococcus B was common in infants of mothers with the *IGHG\*ga-n/\*ga-n* diplotype, being carriers of streptococcus B [16]. The IgG2\*-n allotype was associated with increased susceptibility to Haemophilus influenzae infections compared to IgG2\*n (P<0.05) [17,18]. Caucasian children less than 18 months with the IgG2\*-n allotype have 5.1-fold higher risk of severe Hib infections than had those with IgG2\*n. Discordant GM allotypes correlate with different amounts of specific antibodies. The homozygous *IGHG\*bf-n/\*bf-n* correlate with the lowest levels of specific antibodies against polysaccharide antigens of pneumococci and meningococci [17,18]. In Moraxella catarrhalis infections there were low levels of IgG3 and low levels of M protein specific antibodies in homozygous *IGHG\*ga-n/\*ga-n* [19, 20]. Chronic bronchitis is the most common diagnosis in IgG3D patients in whom the *IGHG\*ga-n/\*ga-n* diplotype is found in 70%.

## 8.2. Viral discrimination of *IGHG* (Fcγ)(GM) genes

Herpes simples type 1 has evolved strategies for decreasing the efficacy of the host immune response and interfering with viral clearance. Several studies have shown that HSV-1-encoded FcγR binds anti-HSV-1 antibodies by bipolar bridging: the Fab part of the antibody molecule binds to its antigenic target on the virus, whereas the Fcγ part of the antibody binds

to the FcγR- like binding site on the viral protein, thus offering survival advantage to the virus by sterically hindering the access of FcγR-expressing effector cells eg. NK cells to HSV-1 infected cells. The HSV-1 encoded FcγR discriminates between IgG1 molecules. The FcγR binds IgG1*a more strongly than IgG1*f. This increased affinity to IgG1*a implies that subjects possessing IGHG1*a genes are more likely to have their Fc domains scavenged resulting in decreased immunocompetence to eliminate the virus and they would consequently be at higher risk of developing HSV-1-induced /spurred diseases [21-23].

The FcγR-like hepatitis C virus core protein has been found to bind differently to IgG of discordant GM (Fcγ) allotypes. *IGHG* genes are associated with the outcome of hepatitis C virus infection, GM modulate the viral strategy trough the different binding of the core protein. The absorbance value for binding HCV core protein is higher for IgG1*f than for IgG1*a (P=0.003) [24,25].

The Fc segment for human cytomegalovirus (HCMV) modulate the viral strategy through differential binding to the viral FcγR. HCMV has evolved a large repertoire of strategies for evading immunosurvailance avoiding the effector consequencies of antibody binding ADCC, complement-dependent neutralization and phagocytosis. HCMV TRL11/IRL11 encoded FcγR has increased binding to *IGHG1*f* compared to *IGHG1*a,* P=0.0005, with potential implications for genetic etiology of HCMV associated diseases [26].

The host control of human immunodeficiency virus (HIV) replication has been found related to both FcγR genes and Fcγ genes. In 73 Caucasian Americans who had spontaneously controlled HIV replication those with the FcγRIIa allele combined with the *IGHG3*b* allele were found more often than those combined with *IGHG3*g* (OR=7.47) and those with the FcγRIIIa allele combined with the *IGHG3*b* allele were found more often than those combined with *IGHG3*g* (OR=3.26) [27].

Infants with the most severe infections of respiratory syncytial virus are predominantly found with the *IGHG\*bf-n/\*bf-n* diplotype, which also is found in non atopic asthma phenotypes[28].

## 9. Effects of immunotherapy in various *IGHG* (Fcγ)(GM) gene recipients

### 9.1 Active immunotherapy (Vaccines)

*IGHG* (Fcγ) (GM) genes are associated with specific antibody response thought to be exclusively associated with the variable region of IgG and acquired immunity. The alternative allotypes of IgG2 have regulatory influence on the IgG2 specific antibody response to a variety of immunologically distinct bacterial polysaccharide antigens. Individuals with the homozygous *IGHG2\*n/\*n* responded with increased numbers of specific IgG2 antibodies to polysaccharide antigens of Haemophilus influenzae type b (Hib), pneumococci, meningococci and Streptococcus A, compared to *IGHG2\* -n/\* -n* with low numbers [17,18,29]. Heterozygotes *IGHG2\*n/\*-n* expressed intermediate responses disclosing a *IGHG2\*n* gene dependency. After Hib polysaccharide vaccine the IgG1 responses were not significantly different in adults who were homozygous for *IGHG2\*n/\*n* and those who were homozygous *IGHG2\*-n/\*-n*. Similar results were found for children.  The above results were obtained after vaccination of adults with unconjugated polysaccharide vaccines [12]. IgG3D patients with 70% *IGHG\*ga-n/\*ga-n* diplotypes demonstrated low prevaccination levels compared to controls but they responded well on a protein conjugated Hib vaccine [30]. The conjugate ACT-Hib vaccine overcomes the IgG3D state and the genetic predisposition for lower responsiveness [30]. The conventional Haemophilus polysaccharide is thought to be the thymic-independent type of immunogen, whereas the protein-conjugated vaccine is thymic

dependent. Thymic dependent antigens are independent of regulation by genes associated with the *IGHG2* locus. Thymic independent and thymic dependent antigens attack *IGHG* genes differently resulting in various amounts of specific antibodies. The insufficient or side effects of some vaccines may be due to the GM variation in the recipients.

## 9.2. Passive immunotherapy (IVIG, Therapeutic recombinant monoclonal antibodies)

The commercial IVIG preparations have been thoroughly investigated for numbers of specific antibodies, but not for amounts of allelic IgG subclasses. The commercial IVIG preparations contain all the allelic IgG subclasses but in slightly different proportions. (Fig. 3B) Patients with immuno deficiencies and patients with autoimmune disorders often with homozygous *IGHG* genes and restricted IgG, are supported with foreign qualities of allelic IgG subclasses. The foreign variants can be recorded directly and their half-life times assessed. In Common variable immunodeficiency (CVID) with homozygous *IGHG*bf-n/*bf-n,* the added foreign levels of IgG3*g, IgG1*a and IgG2*n could be followed (Fig. 3C)[13]. The survival of IgG2*n was prolonged compared to both IgG1*f and IgG1*a allotypic subclasses, in all four patients tested, but must be investigated further. In IVIG treatment, there is an advantage to know the *IGHG* genotype of the recipient, to calculate trough levels and survival times (Fig. 3C).

There is an expanding usage of recombinant monoclonal antibodies as therapeutics. Almost all of these are based on the whole IgG isotype format which employ the human constant region sequences, but vary in the origin of the variable regions between mouse, humanized mouse and fully human sequences. Polymorphisms (allotypes) within the IgG isotype originally demonstrated that allotypic variants can be immunogenic and provoke

antibody responses as a result of allo-immunization. Allotype differences can contribute to or potentiate immunogenicity. Humanized antibodies may neutralize, enhance clearance or precipitate severe adverse reactions, in treatment of different diseases, and depends on the genetic constitution of the recipient. In patients with anti-therapeutic antibodies there is a possibility to trace the foreign allotypic IgG1 subclasses. Administration to patients homozygous for the alternative allotypes presents added potential for immunogenicity [31]. The importance of "matched allotypes" could be speculated upon in passive immunotherapy.

Antibody-dependent cell-mediated cytotoxicity (ADCC) which links the innate and the adaptive arms of immunity, is a leading mechanism underlying the clinical efficiency of therapeutic antibodies such as trastuzumab and cetuximab. ADCC is triggered upon ligation of Fcγ receptor to the Fc region of the IgG molecules. There are differential inhibition of trastuzumab and cetuximab induced cytotoxicity of tumour antigens (human epidermal growth factor receptor antigens) of cancer cells by alternative *IGHG1* genes *IGHG1\*f* and *IGHG1\*a*. Mechanism of resistence of cetuximab therapy is registered in colorectal cancer [32]. In passive immunotherapy with humanized (IgG1) mouse monoclonals, the foreign allelic IgG subclasses could be traced and half-life times registrated.

## 10. *IGHG* gene frequencies in diseases and phenotypes of diseases

## 10.1. Immunodeficiency

IgG has a central role in primary immunodeficiency disorders (PID). Low serum IgG levels or hypogammaglobulinemia and low levels of IgG subclasses/or specific antibodies are correlated to diminished defence against pathogens in primary immunodeficiency disorders

(PID). The expression of heavy constant γ3-, γ1- and γ2- chain gene variants decide the qualities of the IgG3, IgG1 and IgG2 molecules. The fixed *IGHG* genes within the haplotypes (B cells) define also the quantities of allelic IgG subclasses molecules, registered in healthy (Table 5). In IgG2 deficiency there is a dominance of homozygous *IGHG*bf-n/*bf-n* and B2/B2 cells (IgG3*b, IgG1*f and IgG2*-n molecules) and absence of IgG3*g, IgG1*a and IgG2*n molecules. In the same way in IgG3deficiency, there is a dominance of homozygous *IGHG*ga-n/*ga-n* and B4/B4 cells (IgG3*g, IgG1*a and IgG2*-n) and absence of IgG3*b, IgG1*f and IgG2*n [14]. PID are found in family members however without known regulatory mechanisms, but often with *IGHG* genes in common.

In 235 children and adults with various PIDs, the *IGHG2*-n/*-n* (IgG2*-n) dominated [33] (Fig.4A). The homozygous *IGHG*bf-n/*bf-n* diplotype (B2/B2 cells) dominated in IgG2D (P<0.001), in CVID (P<0.001), in IgAD (P=0.028) and in AT (P=0.042). The homozygous *IGHG(*ga-n/*ga-n)* diplotype (B4/B4 cells) dominated in IgG3D (P<0.001), in IgAD (P<0.001) and in WAS (P=0.002). IgG2D and CVID are both associated with the same homozygous *IGHG*bf-n/*bf-n* diplotype (B2/B2 cells)[33]. Both low levels and restricted qualities of IgG antibodies (Fc domains) expressed from different B cells have influence on pathogenesis. The *IGHG*bfn/*bfn* diplotypes (B1/B1 cells) with IgG2*n were found infrequently and seem to be protective. Primary monogenic defects as ataxia telangiectasia and Wiscott Aldrich syndrome due to single gene mutations, were also found with a dominance of the homozygous *IGHG2*-n/*-n*. The nature and extent of interactions between rare and common gene variants is currently unknown, but common variants may act as "modifier" genes that affect the clinical penetrance of rare variants analogous to the genetic model as described in monogenic conditions as WAS and AT.  Particular *IGHG* genes may be risk factors by themselves, but combined with other factors they may add to developing disease. In these PID the *IGHG*bf-n* haplotype with the B$^{*bf-n}$ , B2 cells and the *IGHG*ga-n*

haplotype with the B$^{*ga-n}$, B4 cells dominated (Fig. 4A, 4B). Investigation of frequencies of *IGHG* genes and quantities of allelic IgG subclasses, is a better way to describe antibody deficiencies than assessment of IgG subclasses alone.

## 10.2. Allergy

Asthma, eczema and hayfever are all manifestations of the atopic state, strongly familial on a multifactorial genetic basis. The genetic codes for immunoglobulin heavy constant chains for both IgE and IgG are found on chromosome 14q32.3. In childhood asthma the IgE-mediated allergy is associated with the *IGHG\*bfn* haplotype. The highest IgE levels and highest numbers of positive skin prick tests are associated with homozygous *IGHG\*bfn/\*bfn,* B1/B1 cells. This is found in patients with clinical allergy and increased IgE levels, a family history of allergy and in technicians exposed to laboratory animals developing laboratory animal allergy [34-36]. The alternative *IGHG* haplotypes are linked to different pathways of immune regulation in patients with asthma. The *IGHG\*bfn* haplotype from B1 cells is more prone to respond to allergens than other haplotypes and B cells. Severe IgE mediated allergy and asthma in children is associated with the homozygous *IGHG\*bfn/\*bfn* diplotype. IgE sensitisation is dependent on the *IGHG2\*n* gene dose [36]. There is an increased frequency of the allelic *IGHG2\*n* in patients with increased IgE>600kU/l and IgE>1000 kU/l, 76% and 88% respectively, but also in patients with increased IgG4 >1g/l , 94% compared to 46% in healthy (Fig. 4C, 4D). Children with bronchial asthma but with IgE levels < 10 kU/l are instead associated with the opposite *IGHG\*ga-n*/*ga-n diplotype, infection proneness and IgG subclass deficiencies [34]. Children with bronchial asthma were mapped for the 6 most common IGHG diplotypes and investigated for phenotypes. The *IGHG\*bfn/\*bfn* diplotype was linked to the atopic phenotype and the opposite *IGHG\*ga-n/\*ga-n* was linked to the

infectious proneness phenotype [36]. *IGHG* genes are involved in the IgE sensitization process. Childhood asthma patients with increased IgE have increased IgG2*n levels compared to *IGHG* gene- age- matched healthy children. Infants with the most severe infections of respiratory syncytial virus are predominantly found with the *IGHG*bf-n/*bf-n* diplotype, also found in non atopic phenotypes [28]. The most severe prognosis of patients with middle age asthma is found both in *IGHG2*n/*n*, the atopic phenotype, and the *IGHG*-n/*-n* the infectious prone phenotype [37].

## 10.3. Autoimmunity

Homozygous *IGHG* diplotypes are associated with different prognoses in autoimmune disorders as in severe JCA with dominating *IGHG*ga-n/*ga*-n [38]. The best prognosis without severe infections in C2 deficiency (SLE) is found in patients with *IGHG*bfn/*bfn* [39]. Patients with increased IgG4 levels > 1g/l were occasionally found with the *IGHG*bfn/*bfn* diplotype, with the IgG2*n molecule in 94% compared to 46% in healthy (Fig.4C). Patients with autoimmune disorders as well as immunodeficiencies express homozygous diplotypes with restricted IgG and the good effect by IVIG therapy is probably the support of foreign IgG molecules.

## 10.4. Malignancy

Different amounts of protecting antibodies have been observed in breast cancer associated with discordant GM allotypes [40]. Specific antibodies to mucin 1 (MUC 1) the membrane-bound glycoprotein that is overexpressed in adenocarcinomas are associated with good prognosis in patients with breast cancer. Anti-MUC1 IgG antibody levels in subjects who

were carriers of the genetic variant *IGHG2\*n* were significantly higher, compared to those who were non carriers with *IGHG2\*-n* (P=0.003). These results could potentially devide the population into high and low responders to MUC 1, which has important implications for MUC 1-based immunotherapeutic interventions in breast cancer. It means that the specific antibody production were influenced by the *IGHG2\*n* chain genes from the *IGHG\*bfn* haplotype or B1 cells.

There is a strong rational for the involvement of GM genes in the prognosis of lung cancer by their possible involvement in antibody-dependent cell-mediated cytotoxicity (ADCC) a potent host immunosurveillance mechanism against tumors. IgG antibody-mediated ADCC is triggered upon ligation of FcγR to the Fc of IgG molecules to cause the destruction of lung cancer cells. Simultaneous genotyping of GM and Fcγ could significantly improve our ability to predict a patient´s prognosis of non-small cell lung cancer. Homozygosity of *IGHG\*ga-n/\*ga-n* (B4/B4 cells) was dominating in lung cancer (P= 0.0009) [41].

## 11. Conclusions

Two unique innate variants of IgG3, IgG1 and IgG2 molecules, respectively, were discovered by dissecting the *IGHG*) (Fcγ)(GM) gene complex on chromosome 14q32.3 by alternative allotypes. The new serological *IGHG* ELISA method has made it possible to further differentiate IgG at individual and population levels by gene mapping and quantities. They are found in homozygous and heterozygous forms as alleles, genes, haplotypes and diplotypes. Homozygous individuals express restricted qualities of IgG molecules. The *IGHG* haplotypes are markers of B cells, actually found in all human cells, also stem cells. The restricted expression of IgG molecule variants and B cell variants, in homozygous individuals,

is highlighted. The alternative allelic IgG subclasses have different structures and functions and differentiate phenotypes of disease.

Various bacterial and viral antigens and allergens affect individuals with particular *IGHG* haplotypes (B cells) more often than others, who are kept healthy. The various haplotypes are sometimes discriminated as carriers and others as non carriers. *IGHG* genes have direct active effects as in infections, immunodeficiency, allergy, autoimmunity and malignancy, but have also modifying effects in monogenic diseases. The common Mendelian *IGHG* genes have influence on the outcome of disease and therapy. The ability to predict disease susceptibility as well as classify diseases into subphenotypes from *IGHG* genotype information and registering the possible reponse to active and passive immunotherapy, will improve clinical care.

The studies of *IGHG* (Fcγ) (GM) genes have contributed to a new understanding of the role of innate IgG molecules in human immune response. Identification of the causative *IGHG* alleles, their functional conseqencies and biological mechanisms have already yielded important insight into the immune system by which they influence disease pathogenesis. *IGHG* genes are not detected by GWAS or SNPs, and therefore serological methods are most valuable. The *IGHG* (Fcγ) (GM) genes assessed by the present methods, are easily mapped, for qualities (gene frequencies) and quantities (amounts of allelic IgG subclasses) at individual and population level. The *IGHG*(Fcγ) genes present, in all human somatic cells, must be investigated for influence on other genetic mechanisms. Population studies of the *IGHG* (GM) gene complex with selective effects of these loci bring a rationale for examining the role of *IGHG* genes in the origin of human disease. IgG and *IGHG* genes have a key role in human host immunity. We focus on *IGHG* genes for improvement of clinical care.

Competing interest statement:

The authors have no potential financial conflict of interest related to this manuscript.

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**Table 1**

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

**(A)**
**Chromosome 14q32.3  (5´$\mu$,$\delta$,$\gamma$3,$\gamma$1,$\psi\epsilon$, $\alpha$1,$\gamma$2,$\gamma$4,$\epsilon$,$\alpha$2 3´)**
**Constant heavy $\gamma$ chains:  $\gamma$3, $\gamma$1, $\gamma$2, $\gamma$4**
IgG subclasses: IgG3 IgG1 IgG2 IgG4
**(B)**
**Alternative allotypes of heavy $\gamma$ chains:**

| $\gamma$3 | $\gamma$1 | $\gamma$2 |
|---|---|---|
| $\gamma$3*b $\gamma$3*g | $\gamma$1*f $\gamma$1*a | $\gamma$2*n $\gamma$2*-n |

**(C)**
**Allotypic (allelic) IgG subclass variants (6):**
**IgG3*b IgG3*g;  IgG1*f IgG1*a;   IgG2*n IgG2*-n**


**(D)**
**Homozygous and heterozygous *IGHG* 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 allelic 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*

**Table 2**

# *IGHG* gene map

Individual expressions:
*IGHG* diplotypes (B cells and allelic IgG subclasses)

| *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 | | |
| 118 | 107 | 147 | 40 | 95 | 64 | 13 | 0 | 2 | 1 |
| (20.1%) | (18.2%) | (25.0%) | (6.8%) | (16.2%) | (10.9%) | (2.2%) | (<1%) | (0.3%) | (0.2%) |

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

**Table 3**

## *IGHG* haplotypes products of
## 4 B cell variants

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

| *IGHG\*bfn* | **B**<sup>*bfn*</sup> | *IGHG\*gan* | **B**<sup>*gan*</sup> |
|---|---|---|---|
| 5´μ,δ**γ3\*b,γ1\*f,**α1 **γ2\*n,**γ4,ε,α2 3´<br>**Allelic IgG subclasses:**<br>**IgG3\*b**<br>**IgG1\*f**<br>**IgG2\*n** | **(B1)**<br>42.8% | 5´μ,δ**γ3\*g,γ1\*a,**α1 **γ2\*n,**γ4,ε,α2 3´<br>**Allelic IgG subclasses:**<br>**IgG3\*g**<br>**IgG1\*a**<br>**IgG2\*n** | **(B3)**<br>1.5% |
| *IGHG\*bf-n* | **B**<sup>*bf-n*</sup> | *IGHG\*ga-n* | **B**<sup>*ga-n*</sup> |
| 5´μ,δ**γ3\*b,γ1\*f,**α1 **γ2\*-n,**γ4,ε,α2 3´<br>**Allelic IgG subclasses:**<br>**IgG3\*b**<br>**IgG1\*f**<br>**IgG2\*-n** | **(B2)**<br>24.0% | 5´μ,δ**γ3\*g,γ1\*a,**α1 **γ2\*-n,**γ4,ε,α2 3´<br>**Allelic IgG subclasses:**<br>**IgG3\*g**<br>**IgG1\*a**<br>**IgG2\*-n** | **(B4)**<br>31.6% |

**Table 4**

## 10 individual *IGHG* diplotypes

(4 B cell variants of *IGHG* haplotypes) from chromosome 14q32.3)
(% in 587 healthy Caucasians)

| | | | |
|---|---|---|---|
| *IGHG\*bfn/\*bfn* <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*n,** γ4,ε,α2 3´ | **B1/B1** <br> 20.1% | *IGHG\*ga-n/\*ga-n* <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*-n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B4/B4** <br> 10.9% |
| *IGHG\*bfn/\*bf-n* <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B1/B2** <br> 18.2% | *IGHG\*gan/\*bfn* <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*n,** γ4,ε,α2 3´ | **B3/B1** <br> 2.2% |
| *IGHG\*bfn/\*ga-n* <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B1/B4** <br> 25.0% | *IGHG\*gan/\*bf-n* <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B3/B2** <br> <1% |
| *IGHG\*bf-n/\*bf-n* <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*-n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B2/B2** <br> 6.8% | *IGHG\*gan/\*gan* <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*n,** γ4,ε,α2 3´ | **B3/B3** <br> 0.3% |
| *IGHG\*bf-n/\*ga-n* <br> 5´μ,δ **γ3\*b,γ1\*f,** α1 **γ2\*-n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B2/B4** <br> 16.8% | *IGHG\*gan/\*ga-n* <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*n,** γ4,ε,α2 3´ <br> 5´μ,δ **γ3\*g,γ1\*a,** α1 **γ2\*-n,** γ4,ε,α2 3´ | **B3/B4** <br> 0.2% |

Table 5    Reference levels (g/l) of allotypic (allelic) IgG subclasses (Mean, SD)
in 6 common *IGHG* diplotypes from healthy adults Ref 9.

| *IGHG* diplotype | | Allelic IgG3 subclass levels | | Allelic IgG1 subclass levels | | Allelic IgG2 subclass levels | |
|---|---|---|---|---|---|---|---|
| | No 99 | IgG3*b | IgG3*g | IgG1*f | IgG1*a | IgG2*n | IgG2*-n |
| *bfn/*bfn | 22 | 0.50(0.21) | - | 5.94(2.08) | - | 2.91(0.88) | - |
| *bfn/*bf-n | 24 | 0.62(0.24) | - | 6.52(1.57) | - | 1.97(0.61) | 1.71(0.75) |
| *bfn/*ga-n | 21 | 0.33(0.18) | 0.29(0.19) | 2.94(0.81) | 2.77(0.85) | 2.00(0.58) | 1.88(0.76) |
| *bf-n/*bf-n | 7 | 0.56(0.23) | - | 7.17(1.74) | - | - | 2.43(0.57) |
| *bf-n/*ga-n | 9 | 0.37(0.10) | 0.35(0.10) | 3.32(1.14) | 2.77(0.49) | - | 3.99(0.97) |
| *ga-n/*ga-n | 16 | - | 0.53(0.18) | - | 6.26(1.43) | - | 3.80(1.02) |

Table 6       Amino acid substitutions of alternative GM allotypes (allelic IgG
subclasses) Ref. 24

| GM allotypes Allelic IgG subclasses | CH1(214) | CH2(282) | CH3(356) | CH3(358) | CH3(431) |
|---|---|---|---|---|---|
| IgG1*a | Lys | | Asp | Leu | Gly |
| IgG1*f | Arg | | Glu | Met | Ala |
| IgG2*n | | Met | | | |
| IgG2*-n | | Val | | | |

**Figure 1** **Proportions (%) of allotypic (allelic) IgG subclasses and IgG4 in pooled normal sera (2000 blood donors)**
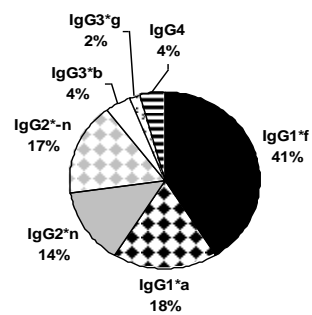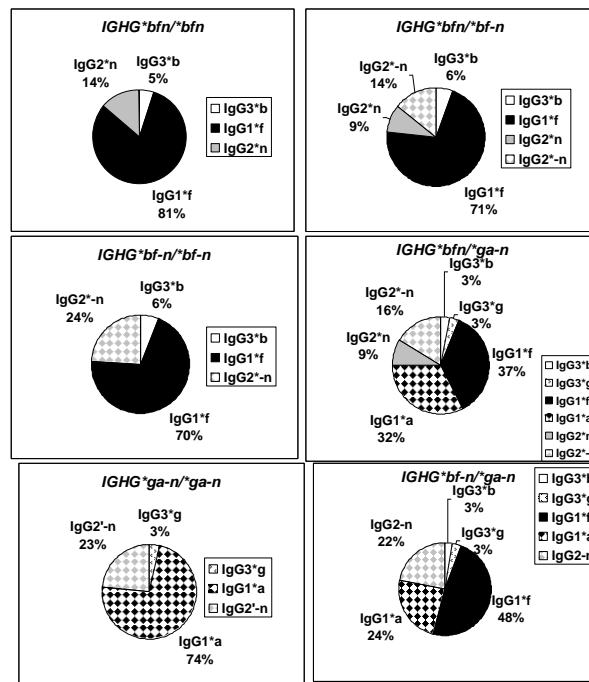
**Figure 2**　**Proportions of allelic IgG subclasses in 10 year old children in homozygous and heterozygous *IGHG* diplotypes (mean levels in %) (225 healthy 10-year-old Swedish Caucasian children).**

## In homozygous diplotypes

(three allelic IgG subclass proteins)

## In heterozygous diplotypes

(4-6 allelic IgG subclass proteins)

**Figure 3    Alternative allelic IgG subclasses  A/ fractions in preparative electrophoresis of a normal serum pool, B/ in commercial IVIG preparations,  C/ Foreign allelic IgG subclasses in individuals treated with IVIG**

Fig.3A        Distribution and amounts of alternative IgG subclass allotypes by
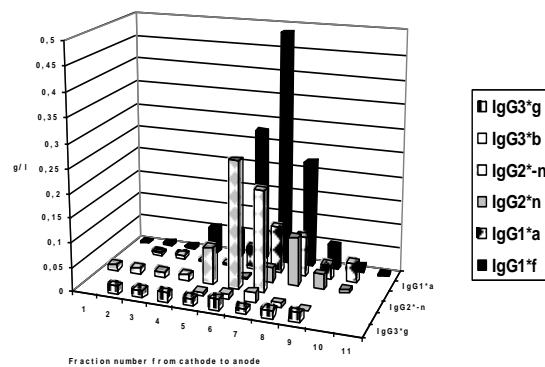preparative electrophoresis

Fig.3B    Proportions of alternative allelic IgG1*f and IgG1*a, IgG2*n and
IgG2*-n, IgG3*b and IgG3*g subclasses in 7 commercial intravenous
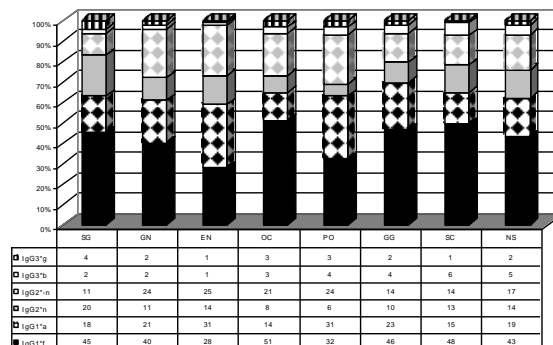preparations and a normal serum pool (2000 blood donors)



| | SG | GN | EN | OC | PO | GG | SC | NS |
|---|---|---|---|---|---|---|---|---|
| IgG3*g | 4 | 2 | 1 | 3 | 3 | 2 | 1 | 2 |
| IgG3*b | 2 | 2 | 1 | 3 | 4 | 4 | 6 | 5 |
| IgG2*-n | 11 | 24 | 25 | 21 | 24 | 14 | 14 | 17 |
| IgG2*n | 20 | 11 | 14 | 8 | 6 | 10 | 13 | 14 |
| IgG1*a | 18 | 21 | 31 | 14 | 31 | 23 | 15 | 19 |
| IgG1*f | 45 | 40 | 28 | 51 | 32 | 46 | 48 | 43 |

Fig. 3C    Foreign allelic IgG subclasses in individual patients with PID, treated
with  IVIG  (trough levels, mean of 8 determinations ). Prolonged survival of
IgG2*n compared to IgG1*f and IgG1*a

(After infusion with IVIG:IgG1*f 20,79 g/l, IgG1*a 10,51 g/l, IgG2*n 5,54 g/l, proportions 4:2:1, given intravenously every
4th week at 400 mg/kg body weight )
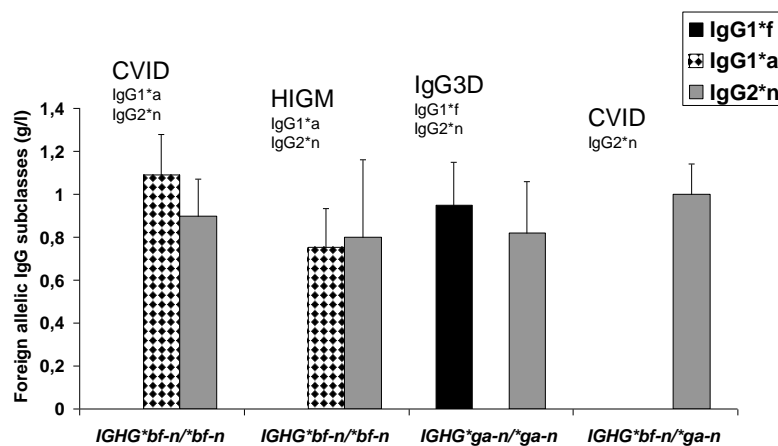
**Figure 4 Frequencies of alternative *IGHG2* alleles, dominating in disease.
In (A,B) patients with primary immunodeficiencies and in (C,D) patients with increased
IgE and IgG4 and in IgE sensitized patients**

Fig. 4A  Dominance of *IGHG2\*-n* allotypes in PID: IgG2D, IgG3D, IgAD, CVID,
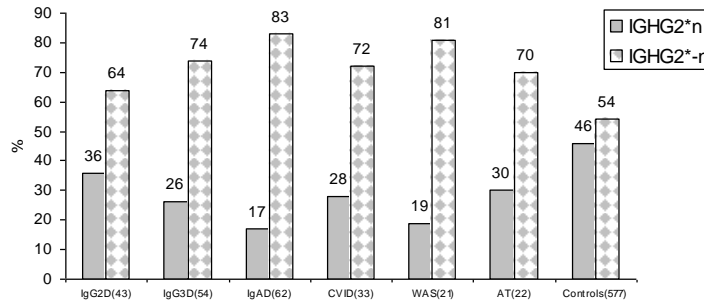WAS and AT Ref. 33



Fig. 4B        Frequency of different homozygous *IGHG2\*-n/\*-n* diplotypes,
*IGHG\*bf-n/\*ga-n* (B2/B4), *IGHG\*ga-n/\*ga-n* (B4/B4) and IGHG\*bf-n/\*bf-n*
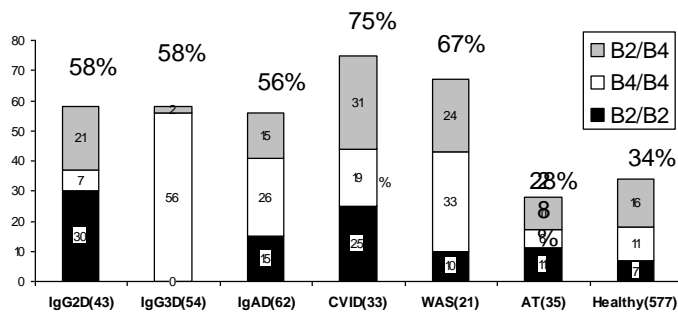(B2/B2) in PID: IgG2D, IgG3D, IgAD, CVID, WAS and AT, compared to
healthy. Ref. 33

Fig. 4C   Dominance of *IGHG2*n* alleles compared to *IGHG2*-n* in patients
with IgE>600kU/l, IgE>1000kU/l, IgG4>1g/l (number of patients and healthy)



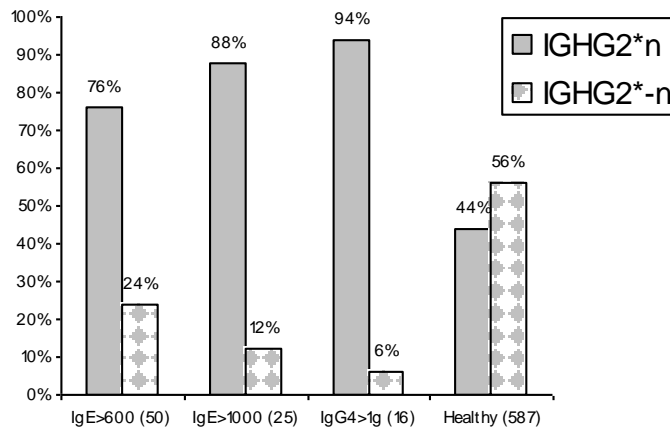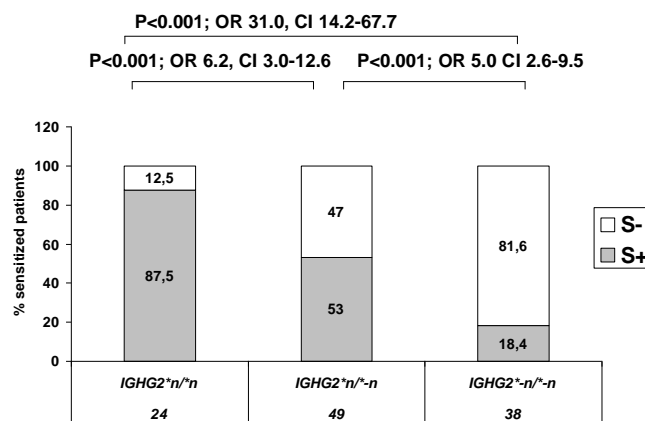Fig. 4D     *IGHG2*n* gene dose dependency in IgE sensitisation.
*IGHG2* genotypes in 111 children with bronchial asthma Ref.34

**Figure legends:**

**Table 1   Dissecting the *IGHG*(Fcγ) (GM) gene complex by the alternative allotypes of γ3, γ1 and γ2 genes in a healthy Caucasian population**

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

**with γ3, γ1, γ2, γ4 genes and**

**IgG  subclasses IgG3, IgG1, IgG2, IgG4**

**B:** Alternative alleles of the constant heavy γ chains: **γ3**: *b and *g, of **γ1:** *f and *a  and of **γ2:***n and *-n.

**C:** 6 allotypic (allelic) IgG subclasses variants IgG3*b, IgG3*g, IgG1*f, IgG1*a, IgG2*n, IgG2*-n

**D:** 9 homozygous or heterozygous *IGHG* genes

**E:** 9 homozygous or heterozygous allelic IgG subclasses

**Table 2  The *IGHG* gene map**

**Table 3  *IGHG* haplotypes – B cells**

**Table 4  Individual *IGHG* diplotypes**

**Table 5 Allelic IgG subclass levels in 99 healthy adults**

**Table 6 Amino acid substitutions of alternative GM allotypes (allelic IgG subclasses)**

**Figure 1   Proportions of allotypic IgG subclasses and IgG4 (%)** in a normal serum pool (serum samples from about 2000 Caucasian blood donors).

**Figure 2   Proportions of allotypic IgG subclasses** Allotypic (allelic) IgG subclass levels (mean, g/l) in individual with *IGHG* homozygous and heterozygous diplotypes  in 225 10 year

old, healthy Caucasian children.The homozygous express 3 variants of allotypic IgG

subclasses and the heterozygous express 4-6 variants

**Figure 3**

A: **Amounts of allelic IgG subclasses in a fractionated normal serum pool**

Quantities (g/l) of allelic IgG1*f, IgG1*a, IgG2*n, IgG2*-n, IgG3*b, IgG3*g in fractions

from anode to cathode.

**B: Comparison of the amounts of allelic IgG subclass in 5 commercial IVIG**

**preparations** and a normal serum pool in  relative g/l and %.

**C: Foreign allelic IgG subclasses in individual patients with PID treated with IVIG.**


**Figure 4  Alternative *IGHG2* alleles in disease**

**A: Dominance of the *IGHG2*-n* allele** compared to *IGHG2*n*  in patients with primary

immunodeficiencies: 43 IgG2 deficiency, 54 IgG3 deficiency, 62 IgA deficiency, 33 Common

variable immunodeficiency, 21 Wiscott Aldrich syndrome, 22 Ataxia telangiectasia compared

to 587 healthy controls.

**B: Dominance (%) of homozygous *IGHG2*-n/*-n* diplotypes in PID**

**C: Dominance of  the *IGHG2*n* alleles** in patients with IgE>600kU/l (50), IgE>1000kU/l

(25), IgG4>1g/l(16) compared to 587 healthy controls.

**D: *IGHG2*n* gene dose dependency in IgE sensitized children with bronchial asthma**