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Novel human immunoglobulin heavy chain constant region gene deletion haplotypes characterized by pulsed-field electrophoresis

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SUMMARY

Fifteen patients with selective IgG1 deficiency were screened for immunoglobulin H chain C region locus (IGHC) gene deletions and three deletion haplotypes were found: *del G1*, *del G1–G4* and *del G4*. These haplotypes, together with four deletion haplotypes described by us previously (*del G1* (NY), *del G1* (VIT) *del G1–G2* (NY) and *del G2–G4* (HJE)), were further characterized using pulsed-field gel electrophoresis (PFGE) to determine the physical extent of the deletions. The *Mlu*I fragment sizes confirmed the deletions, although the deduced sizes of the most extensive deletions indicated that material had been inserted into the locus.

Keywords immunoglobulin genes gene deletion homologous recombination

INTRODUCTION

The human immunoglobulin H chain C region locus (IGHC) is roughly 300 kb in size and the genes are ordered 5′-M-D-G3-G1-EP1-A1-GP-G2-G4-E-A2-3′ [1,2]. Lack of expression of immunoglobulin isotypes has, in a few cases, been linked to homozygous deletions of the corresponding IGHC genes [3–8]. Heterozygous deletions may influence the expression of the affected isotype(s), but the expression of the residual allele(s) on the sister chromosome is often raised to compensate for the deleted gene segments [7,9–11]. To date, 10 major types of IGHC gene deletions have been described by us and others (Table 1).

We have previously described a case of homozygous deletion of G1 in an IgG1-deficient patient [7,12], and another G1 deletion in a patient with common variable immunodeficiency [11]. In this study, we have examined patients with selective IgG1 deficiency with respect to their IGHC genes, using conventional electrophoresis and pulsed-field gel electrophoresis (PFGE). We also used PFGE to characterize further the deletions we have described previously.

Table 1. Immunoglobulin H chain C region locus (IGHC) deletions

Deletion	Reference
del G1	(NY) [7] (VIT) [11] (ERI) This report
del G1-G2	(NY) [7]
del G1-G4	(TAK) [26] (TOU) [27] (LAZ) This report
del EP1-GP	(TOU) [30]
del EP1-G4	(FRO) [4] (T17) [5]
del A1-E	(SAF) [4] (DEM) [6] (SPA) [8] (D847) [10] (BAR, MON) [22]
del GP-A2	(101) [9] (CRU) [6] (ABB, MO1, ZER) [22]
del G2	(MOD) [6]
del G2-G4	(HJE) [11]
del G4	(SPA) [18] (LAZ) This report

MATERIALS AND METHODS

DNA samples for conventional electrophoresis

Genomic DNA was isolated from blood samples as described [13]. DNA samples from 15 Swedish IgG1-deficient patients

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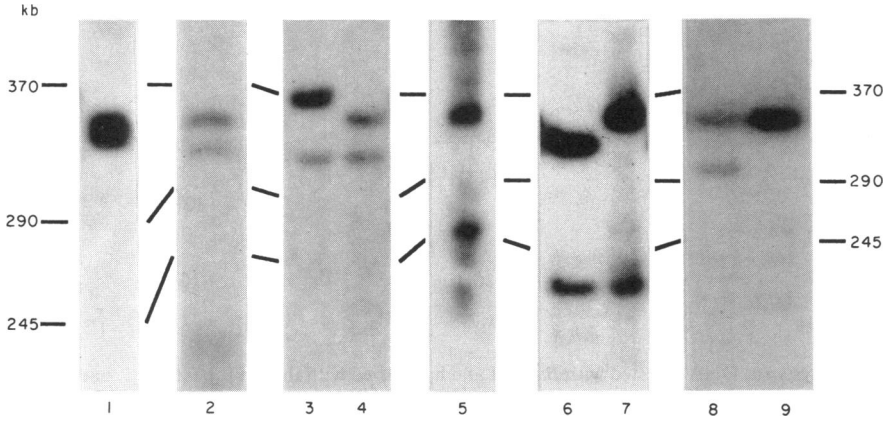

Fig. 1. Pulsed-field gel electrophoresis (PFGE) Southern blot analysis. Samples digested with *Mlu*I and probed with the G probe. Lane 1, subject carrying *del G1* (NY), this sample was electrophoresed at conditions different from the other samples (see Materials and Methods); lane 2, patient carrying *del G1* (VIT); lane 3, subject carrying *del G1* (patient 2) and a 370-kb normal band (mother of patient in lane 4); lane 4, patient carrying *del G1* (patient 2); lane 5, subject carrying *del G1-G2* (NY); lane 6, patient carrying *del G4* (patient 1) and *del G1-G4* (patient 1); lane 7, subject carrying *del G1-G4* (patient 1) (mother of patient in lane 6); lane 8, patient carrying *del G1-G2* (NY); lane 9, control, homozygous for the 350-kb band. The positions of the three smallest yeast (*Saccharomyces cerevisiae*) chromosome standards, 245, 290 and 370 kb in the gels are shown.

were used in the study. Samples from Swedish blood donors were used as controls.

Conventional Southern blot

The DNA samples were digested with restriction enzymes obtained from New England Biolabs (Beverly, MA) and Boehringer Mannheim Biochemicals (Mannheim, Germany). Incubations were carried out in the five-buffer system of Boehringer. *Bam*HI digests were used for the detection of immunoglobulin G chain genes (IGHG), *Pst*I for immunoglobulin A chain genes (IGHA) and *Bam*HI for immunoglobulin E chain genes (IGHE). DNA of each sample (4 μg) was digested with 20 U of restriction enzyme. The *Bam*HI digests for the detection of IGHG genes were separated in 0·7% agarose gels in Tris-acetate EDTA (TAE) buffer [14] at 1·0 V/cm for 50 h and the other digests (*Pst*I for IGHA and *Bam*HI for IGHE genes) were separated in 0·8% agarose in TAE buffer at 1·6 V/cm for 15 h. The gels were denatured in 0·4 M NaOH, 1·5 M NaCl, renatured in 0·5 M Tris pH 7·6, 1·5 M NaCl, and the DNA was transferred to Hybond (Amersham, Slough, UK) membranes in 2 × SSC.

PFGE DNA samples

High molecular weight DNA was prepared from peripheral blood following the strategies previously described [15]. After erythrocyte lysis in 1 mM NH_4HCO_3, 114 mM NH_4HCl, the remaining leucocytes were embedded in 0·5% low melting agarose at a final concentration of 1×10^7 cells/ml. The agarose–cell mixture was formed into 100-μl blocks using a plastic slot former (Pharmacia, Uppsala, Sweden). The solidified blocks were treated in 0·5 M EDTA pH 8, 1% sarkosyl, 1 mg/ml proteinase K for 12+24 h at 50°C and transferred to 0·5 M EDTA at 4°C for longterm storage.

PFGE

To remove EDTA, the DNA sample blocks were equilibrated three times for 30 min with the restriction enzyme incubation buffer (Boehringer Mannheim Buffer H for *Mlu*I) and digested overnight with 75 U *Mlu*I (Boehringer Mannheim, New England Biolabs, and Amersham). The digested samples were typically electrophoresed for 50 h at 225 V with a pulse time of 30 s in a 2% agarose gel, 0·25 × TAE buffer [14] using a TAFE apparatus (Beckman Instruments, Palo Alto, CA). In the attempt to separate the 345- and 350-kb fragments the pulse times were shortened to 20 s and the voltage elevated to 275 V (Fig. 1, lane 1). *Saccharomyces cerevisiae* chromosomes (Bio-Rad, Richmond, CA) and λ phage concatamers (Bio-Rad and Promega, Madison, WI) were used as markers. After ethidium bromide staining and photography, the gels were exposed to 60 mJ/cm^2 254 nm UV light and denatured in 0·5 M NaOH, 1·5 M NaCl twice for 15 min followed by transfer to nylon membrane (Hybond; Amersham) using the same solution as used for denaturation.

Probes

The following probes were used: G probe, a 2·0-kb *Hin*dIII-*Eco*RI fragment of clone 24B [16] containing the human G4; E probe, a 2·6-kb *Bam*HI fragment containing the human E[17]; A probe, a 0·6-kb *Xho*I-*Pst*I fragment of the human A2 [18]. Probes were labelled with α-^{32}P-dCTP, 3000 Ci/mM (Amersham), to a specific activity of 2×10^9 ct/min per μg using a random priming kit (Pharmacia).

Hybridizations

Hybridizations were carried out at 65°C in 0·5 M Na_2HPO_4, NaH_2PO_4 pH 7·2, 10 mM EDTA, 7% SDS, 1% bovine serum albumin (BSA) [19]. The filters were then washed at 65°C in a final concentration of 0·3 × SSC, 0·1% SDS.

Total immunoglobulin quantification

Total serum levels of IgM, IgG and IgA were measured by using nephelometry (ICS Analyzer II, Beckman Instruments).

IgG subclass analysis

Serum levels of IgG1, IgG2, IgG3 and IgG4 were determined by the single radial immunodiffusion technique (SRID) using

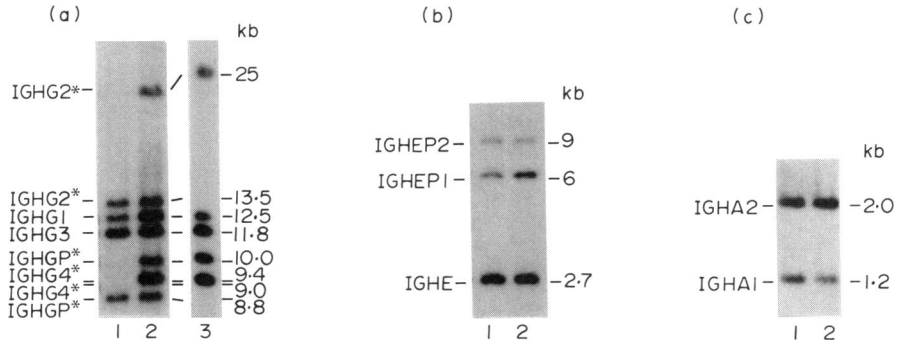

Fig. 2. Southern blot analysis. (a) DNA digested with *Bam* HI and hybridized with the G probe. Lane 1, patient 1 (*del G4; del G1-G4*); lane 2, heterozygous control; lane 3, patient 2 (*del G1*). (b) DNA digested with *BamHI* and hybridized with the E probe. Lane 1, patient 1 (*del G4; del G1-G4*); lane 2, control. (c) DNA digested with *Pst*I and hybridized with the A probe. Lane 1, control; lane 2, patient 1 (*del G4; del G1-G4*). * Polymorphic fragments.

Table 2. Immunoglobulin serum levels of subjects carrying deletions. Values underlined with a single line are subclasses expressed from a hemizygous gene, the values with double underlining are from genes that are homozygously deleted.

		Serum levels (g/*l*)					
Family	Deletion	IgG1	IgG2	IgG3	IgG4	IgA	IgM
Patient 2							
Mother	*del G1*	3·5	6·3	0·8	0·2	1·6	1·2
Father		3·9	6·5	0·8	0·2	2·8	1·2
Patient	*del G1*	2·9	2·9	1·3	0·2	1·5	2·2
Brother	*del G1*	2·6	5·8	1·4	0·0	1·8	2·6
Sister		6·3	6·1	0·9	0·5	2·3	2·3
Patient 1							
Mother	*del G1-G4*	4·9	2·7	1·2	0·4	2·6	1·3
Father	*del G4*	1·8	6·0	1·2	0·1	3·4	1·0
Patient	*del G1-G4/del G4*	1·4	8·1	2·6	0·0	1·4	1·6
Brother	*del G1-G4/del G4*	0·8	2·5	2·9	0·0	1·9	1·5
	Normal range*	4·2–13	1·2–7·5	0·4–1·3	0·0–2·9	0·4–3·1	0·3–2·0

* Normal range as determined in our laboratory.

human IgG subclass-specific MoAbs. MoAbs used were: clone NL16 (HP6012) (anti-IgG1), GOM1 (HP6008) (anti-IgG2), ZG4 (HP6010) (anti-IgG3) and GB7 (HP6013) (anti-IgG4), all purchased from Unipath (Bedford, UK).

Autoradiogram scanning

Autoradiogram band intensities were measured with a dual wavelength TLC scanner using a tungsten lamp (Shimadzu, Kyoto, Japan).

Deletion nomenclature

Deletion haplotypes were assigned using the suggested nomenclature for deletions in the IGHC [20]. Deletions were designated according to the most immunoglobulin H chain J gene segments (IGHJ) proximal and most IGHJ distal gene encompassed, i.e. a deletion encompassing G1-EP1-A1-GP-G2 was designated *del G1-G2*.

RESULTS

Patients with selective IgG1 deficiency (IgG1 < 3·6 g/*l*, normal range in our laboratory 4·2–13 g/*l*, reported mean values range between 5·9 and 8·0 g/*l* [21]) were probed with the G probe in *Bam*HI digests to screen for G1 deletions, and two of 15 patients had restriction fragment length polymorphism (RFLP) patterns indicating deletions. One patient, patient 1, lacked the G4 bands and had G1, G2 and GP restriction fragments with half the expected intensity, indicating two deletions, one involving G4 and one encompassing G1-EP1-A1-GP-G2-G4 (Fig. 2a, lane 1). Another patient, patient 2, had a G1 restriction fragment band with half the expected intensity, indicating a heterozygous deletion of G1 (Fig. 2a, lane 3). These two samples were studied further, using the E and A probes to determine the extent of the deletions. Patient 2 had EP1 and A1 restriction bands with normal band intensities (data not shown), limiting the genes deleted to G1 (*del G1*). Patient 1, carrying two deletions, had

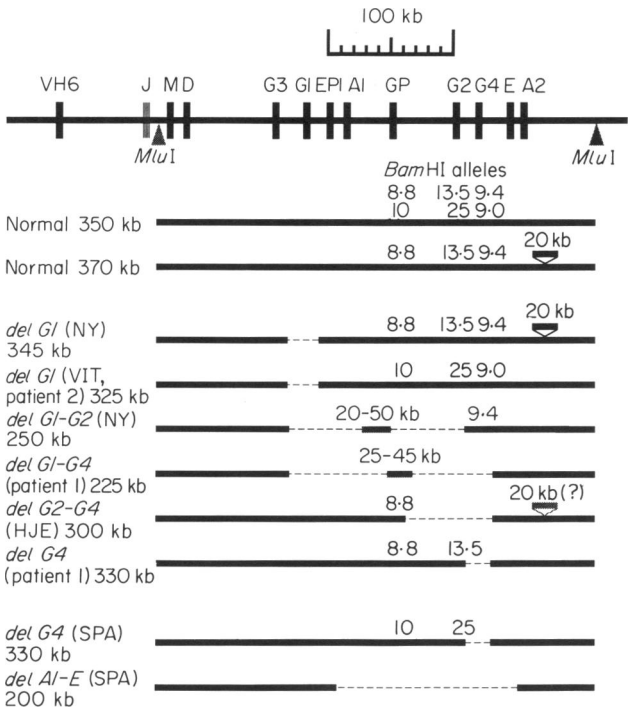

Fig. 3. Summary of pulsed-field gel electrophoresis (PFGE) results. Map derived from [1,2,18]. The *Bam*HI alleles for GP, G2 and G4 are indicated. The SPA haplotypes are from [8].

restriction bands with half the expected intensity for EP1 (Fig. 2b, lane 1) and A1 (Fig. 2c, lane 2), confirming one deletion spanning from G1 to G4 (*del G1-G4*) and restricting the other deletion to G4 only (*del G4*). The indicated half-dose bands were confirmed with dual wavelength TLC scanning, using the G3 band as internal standard for each lane (data not shown).

To determine the physical extent of the deletions, the haplotypes described above and four other deletion haplotypes described by us previously: *del G1* (NY), *del G1-G2* (NY) [7] *del G1* (VIT) and *del G2-G4* (HJE) [11] were run on PFGE, using *Mlu*I digests. *Mlu*I cuts 5′ of M and 3′ of A2, generating a 350- or 370-kb band identifiable with probes for any of the IGHC genes [1,2,22]. In this study we have used the G probe for detection of the IGHC PFGE bands.

Patient 2 had a normal 350-kb and a 325-kb band (Fig. 1, lane 4). From studying samples from patient 2's family it was concluded that the *del G1* (patient 2) haplotype was on the same background (*Bam*HI haplotype H2) as the *del G1* (VIT) haplotype [11] (data not shown), which gave an identical band of 325 kb (Fig. 1, lane 2). Conversely, the previously described *del G1* (NY) haplotype, which was found to be on a haplotype H1 background [7], had a fragment of an estimated 345 kb, hardly separable from the normal 350-kb band (Fig. 1, lane 1).

Patient 1, indicated to carry two deletion haplotypes, *del G4/del G1-G4*, had two fragments smaller than found in controls; one 330-kb band (*del G4*) and one 225-kb band (*del G1-G4*) (Fig. 1, lane 6). The deletion haplotypes previously described by us, *del G1-G2* (NY) and *del G2-G4* (HJE), generated 250-kb and 300-kb fragments respectively (Fig. 1, lanes 5 and 8).

The serum levels of the families of the IgG1-deficient patients with gene deletions are shown in Table 2.

DISCUSSION

In patients with selective IgG1 deficiency we have found a 19% (3/16) frequency of deletions (two heterozygous deletions in 15 examined in this study and one previously described homozygous deletion of G1 [7]). In the families of the IgG1-deficient patients there is not a complete correlation between heterozygous deletion and low serum levels. In three families, three of 10 subjects carrying heterozygous deletions of G1 had normal IgG1 serum levels. However, only one of five of the family members that carried two copies of the G1 had normal levels (Table 2 and [7]). The similar frequencies of low IgG1 levels indicate a defect that is not dependent on gene deletion, although the penetrance may be higher when the G1 is only present in a single copy.

It is plausible that the deletions found in the IGHC may be the result of non-equal homologous recombination (NEHR) [4,11,23,24]. The hitherto only sequenced breakpoints (*del G1-G4*) were indeed found to be in regions 15 kb 3′ to G3 and G4 that show high homology (96·4%) [24].

If NEHR has generated the G1 deletions described here, the expected size range of the deletion would be the length of the gene (i.e. the probe used, 2 kb) plus the distance to either of the flanking genes; 26 kb to G3 and 19 kb to PE1 [18] (Fig. 3, Table 3). In the two *del G1* haplotypes in this study that are on a *Bam*HI haplotype H2 we find a 325-kb *Mlu*I band indicating a 25-kb deletion of the 350-kb *Mlu*I band, in line with a deletion caused by NEHR. The *Mlu*I band size of the *del G1* haplotype on the *Bam*HI haplotype H1 background is about 345 kb, and could indicate a smaller deletion, 5 kb, not caused by NEHR. From the previous study, however, we know that the deletion involves at least 14 kb [12]. This discrepancy could be due to insertion of DNA at the event causing the deletion, or the deletion could have occurred on a 370-kb *Mlu*I haplotype background, and in this case the *del G1* haplotype would also be due to a 25-kb deletion (Fig. 3, Table 3). In two pedigrees we have studied, the 370 kb *Mlu*I allele and the *Bam*HI haplotype H1 were co-inherited (data not shown) and the 370 kb *Mlu*I band in the SPA family was also inherited in this fashion [8]. The 370-kb *Mlu*I haplotype could thus be due to a 20-kb insertion on an ancestral *Bam*HI haplotype H1, but as there have only been a limited number of 370-kb *Mlu*I alleles where the *Bam*HI haplotypes have been determined, it is still possible that there are 370-kb alleles associated with other *Bam*HI haplotypes. The 20-kb difference between the *Mlu*I 350-kb and 370-kb haplotypes resides in the 3′ half (GP-G2-G4-E-A2) of the IGHC locus, and the exact nature and location of this 20-kb insertion/deletion is not known, although there are no indications that it involves any of the known IGHC genes [22].

The *del G2-G4* haplotype *Mlu*I band was 300 kb and, as it is on a *Bam*HI haplotype H1 or H3 background, it indicates a deletion of 50 or 70 kb. The distance between G2 and G4 is 18 kb, and the genes proper (exons and introns) are about 1·6 kb each [25]. The region between GP and G2 is unfortunately poorly mapped, but the size of a deletion caused by NEHR would be predicted to be in the range of 44–71 kb (Fig. 3, Table 3).

The 330-kb *Mlu*I band for the *del G4* haplotype is the same size as the previously described *del G4* haplotype [8], and NEHR would delete 18–23 kb (Table 3). The *del G4* described here is on a *Bam*HI haplotype H1 or H3 background, whereas the

Table 3. Distinct deletion haplotypes

Haplotype	Patient	Genetic markers		PFGE size (kb)	Deletion (kb)	Theoretical deletion size (kb)		
		*Bam*HI GP-G2-G4	Other			Min.	NEHR	Max.
Normal 370		(H1)		370				
Normal 350				350				
del G1	(NY)	H1		345	5 or 25	2	21–28	47
	(Patient 2, VIT)	H2		325	25 or 45	2	21–28	47
del G1-G2	(NY)			250	100 or 120	126	144–152	170
del G1-G4	(Patient 1, TAK, TOU)			225	125 or 145	145	168–171	194
del EP1-GP	(TOU)			?		54	73–104	123
del EP1-G4	(FRO, T17)			?		125	144–148	123
del A1-E	(D847)	H4 (or H6) *de novo*		?		135	145–148	158
	(BAR, DEM, MON, SAF, SPA)			200	150 or 170	135	145–148	158
del GP-A2	(ZER, 101, CRU)		SG1 5.4	?		109	≈145	?
	(ABB, MOI, 101, CRU)		SG1 5.5	?		109	≈145	?
del G2	(MOD)	H3		?		2	20–52	70
del G2-G4	(HJE)	H1 or H3		300	50 or 70	21	44–71	94
del G4	(Patient 1)	H1 or H3		330	20 or 40	2	20–25	43
	(SPA)	H2 or H4		330	20 or 40	2	20–25	43

The deletions described have been divided into subgroups based on genetic markers. In many cases the information is sparse and deletions of the same major type are grouped together as long as no differences have been published. The two subtypes of *del G1* are distinguished by different *Bam*HI GP-G2-G4 haplotype backgrounds [11] and their different *Mlu*I band sizes (this report). The *del A1-E* haplotypes may be on the same background, but inasmuch as the D847 is a *de novo* deletion, it is distinct from the other haplotypes [10]. The two subtypes of *del GP-A2* are distinguished by different *Hin*dIII switch G1 alleles [22], 101 and CRU are listed in both subgroups as they have not been characterized with respect to their switch G1 alleles. The two subtypes of *del G4* are distinguished by different *Bam*HI GP-G2-G4 haplotype backgrounds (this report). The theoretical non-equal homologous recombination (NEHR) deletion sizes were calculated by increasing the minimum size with the size of either of the flanking regions involved. The theoretical deletion sizes are calculated assuming that the 20-kb insertion/deletion found in *Mlu*I digests is 3′ of A2.

previously described *del G4* is on an H2 or H4 background. The possibility of an H1, 370-kb, background could indicate a 40-kb deletion, but would then involve a deletion of the *Bam*HI site 3′ of G2. The size of the G2 *Bam*HI fragment is normal (13·5 kb, Fig. 2a) in the *del G4* haplotype, so it is most likely a 20-kb deletion on a 350-kb background (Fig. 3, Table 3).

The *del G1-G2* haplotype *Mlu*I band was 250 kb, indicating a deletion of 100 or 120 kb. If the estimated 98 kb distance between A1 and G2 [2] is correct, a minimum deletion of 126 kb is needed to span from G1 to G2, and about 150 kb would be lost if the deletion was caused by NEHR (Table 3). This means that the *del G1-G2* haplotype *Mlu*I fragment is larger than expected, even if the deletion occurred on a *Mlu*I 370-kb background. If the deletion was caused by NEHR it indicates that about 20–50 kb extra material has been inserted (Fig. 3).

The *del G1-G4* haplotype *Mlu*I band was 225 kb, which is the minimal size of a *del G1-G2* haplotype. Again, if the deletion was caused by NEHR, the haplotype is 25–45 kb larger than expected (Fig. 3, Table 3). The genes that constitute the *Bam*HI haplotypes (GP, G2 and G4) are all absent, and we thus do not have any indication of the haplotype background for the deleted genes, and the haplotype described here may be the same as the two *del G1-G4* haplotypes described previously [26,27].

The two larger deletions we studied thus do not comply with NEHR deletion, unless an insertion preceded/followed the deletion. These two deletions both encompass the poorly mapped A1-GP-G2 region, but we do not have any reason to believe that the two independent and unanimous estimates of the distance between A1 and G2 are incorrect [1,2].

The *del A1-E* haplotype characterized by others with PFGE gave a 200-kb band [8]. A deletion in accordance with a NEHR event would generate a 205 or 225 *Mlu*I band, depending on the original haplotype. This indicates that the *del A1-E* haplotype is the result of NEHR deletion on the 350-kb background (Fig. 3, Table 3). Contrary to the deletions we report here that also span the A1-GP-G2 region, this haplotype does not appear to include any inserted material.

The mechanism involved in the generation of deletions in the IGHC region is thus most likely NEHR. The switch region sequences, which are the location of the breakpoints in the somatically controlled deletions during isotype switch, do not seem to be the primary site of breakage, as only four of the types of deletions could have occurred 5′ of the homologous target genes. The best example of this is the generation of *del GP-A2* haplotypes, that must have involved sequences 3′ of the A1 and A2 genes, as the GP lacks the switch region. The sparse involvement of the 5′ region of the genes would also explain why no deletions have been found encompassing the G3 gene. As all

of the deletions described could have occurred 3′ of the homologous target genes, there is a possibility of homologous 'fragile' sequences 3′ of the genes. 'Fragile' sequences may, in addition to being regions of homology between the IGHC genes, contain clusters of recognition sites for DNA topoisomerases, which nick DNA [28] or the Z-DNA motif $d(TG)_n$ that induces conformational changes in the DNA [29]. The published breakpoint region contains multiple clusters of topoisomerase I recognition sites, but no Z-DNA motifs (unpublished observations). The Z-DNA motifs, however, influence the recombination frequencies in regions surrounding it (0·2–1·2 kb) and may thus lie outside the 0·9-kb region sequenced. The only requirement for enhanced recombination frequencies could, however, simply be long stretches of homologous DNA.

The 'true' frequency of deficiency of a given subclass is still not known, and for convenience any level <2 s.d. below the mean could be considered defect. This, however, assumes a Poisson distribution of serum levels of the IgG subclasses, which may not be correct. Furthermore, the definition is purely statistical and does not take into account the symptoms, if any, in the patients. In addition, the range of levels considered normal varies extensively between laboratories [30], making comparisons of data obtained by different groups difficult. A number of apparently healthy subclass-deficient [31,32] individuals have previously also been described, casting doubt on the relevance of the defect. In other patients still, the deficiency observed may be irrelevant [33] for the symptoms noted, and taken together these data suggest that the clinical status of the patient may be more important than the actual subclass levels when gammaglobulin prophylaxis is being considered.

In this study we have addressed the question whether patients with a deficiency of IgG1, with serum levels well below the normal range, carry a heterozygous deletion of the G1 gene. Only two patients exhibited such a deletion, suggesting that most deficiences are due to a regulatory dysfunction. In addition, even in individuals carrying a gene deletion, levels of IgG1 may be within the normal range (Table 2). This probably reflects that the remaining allele, being productive to a normal extent, gives rise to half of the expected serum IgG1, i.e. 2·1–6·5 g/l, and most individuals with a heterozygous gene deletion would therefore probably remain undiagnosed.

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REFERENCES

1 Bottaro A, de Marchi M, Migone N, Carbonara AO. Pulsed-field gel analysis of human immunoglobulin heavy-chain constant region gene deletions reveals the extent of unmapped regions within the locus. Genomics 1989; **4**:505–8.

2 Hofker MH, Walter MA, Cox DW. Complete physical map of the human immunoglobulin heavy chain constant region gene complex. Proc Natl Acad Sci USA 1989; **86**:5567–71.

3 Lefranc MP, Lefranc G, de Lange GG *et al.* Instability of the human immunoglobulin heavy chain constant region locus indicated by different inherited chromosomal deletions. Mol Biol Med 1983; **1**:207–17.

4 Migone N, Oliviero S, de Lange G *et al.* Multiple gene deletions within the human immunoglobulin heavy-chain cluster. Proc Natl Acad Sci USA 1984; **81**:5811–5.

5 Chaabani H, Bech-Hansen NT, Cox DW. A multigene deletion within the immunoglobulin heavy-chain region. Am J Hum Genet 1985; **37**:1164–71.

6 Bottaro A, de Marchi M, de Lange G, Boccazzi C, Caldesi F, Gallina R, Carbonara AO. New types of multiple and single gene deletions in the human IgCH locus: published erratum appears in Immunogenetics 1989; **29**:224. Immunogenetics 1989; **29**:44–48.

7 Smith CIE, Hammarström L, Henter J-I, de Lange GG. Molecular and serologic analysis of IgG1 deficiency caused by new forms of the constant region of the Ig H chain gene deletions. J Immunol 1989; **142**:4514–9.

8 Bottaro A, Cariota U, de Lange G, de Marchi M, Gallina R, Vlug A, Carbonara AO. Multiple levels of analysis of an IGHG4 deletion. Hum Genet 1990; **86**:191–7.

9 Hendriks RW, van Tol MJ, de Lange GG, Schuurman RK. Inheritance of a large deletion within the human immunoglobulin heavy chain constant region gene complex and immunological implications. Scand J Immunol 1989; **29**:535–41.

10 Walter MA, Chambers CA, Zimmerman B, Cox DW. A multigene deletion in the immunoglobulin heavy chain region in a highly atopic individual. Hum Genet 1990; **85**:643–7.

11 Olsson PG, Hofker MH, Walter MA, Smith S, Hammarström L, Smith CIE, Cox DW. Ig heavy chain variable and constant region genes in common variable immunodeficiency. J Immunol 1991; **147**:2540–6.

12 Smith CIE, Hammarström L. Mapping of two IgCH gene deletions in a patient with IgG1 deficiency. Progr Immunol 1989; **7**:547–50.

13 Smith CIE, Hammarström L. Detection of immunoglobulin genes in individuals with immunoglobulin class or subclass deficiency. Evidence for a pretranslational defect. Monogr Allergy 1986; **20**:18–25.

14 Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbour: Cold Spring Harbour Lab. 1989:6.7.

15 Poustka A, Pohl TM, Barlow DP, Frischauf AM, Lehrach H. Construction and use of human chromosome jumping libraries from *Not*I-digested DNA. Nature 1987; **325**:353–5.

16 Ellison J, Buxbaum J, Hood L. Nucleotide sequence of a human immunoglobulin Cγ4 gene. DNA 1981; **1**:11–18.

17 Max EE, Battey J, Ney R, Kirsch IR, Leder P. Duplication and deletion in the human immunoglobulin ε genes. Cell 1982; **29**:691–9.

18 Flanagan JG, Rabbitts TH. Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε and α genes. Nature 1982; **300**:709–13.

19 Church GM, Gilbert W. Genomic sequencing. Proc Natl Acad Sci USA 1984; **81**:1991–5.

20 Smith CIE, Engström PE, Olsson PG, Hammarström L. Deletions in the IgCH locus: new forms, functional consequence and suggested nomenclature. In: Chapel HM, Levinsky RJ and Webster ADB, eds. Progress in immune deficiency, III. London: Roy Soc Med Serv Int Congr Symp Ser 1991;59–60.

21 French M. Serum IgG subclasses in normal adults. Monogr Allergy 1986; **19**:100–7.

22 Bottaro A, Cariota U, DeMarchi M, Carbonara AO. Pulsed-field electrophoresis screening for immunoglobulin heavy-chain constant-region (IGHC) multigene deletions and duplications. Am J Hum Genet 1991; **48**:745–56.

23 Carbonara AO, Migone N, Oliviero S, DeMarchi M. The molecular basis of Ig deficiencies. In: Aiuti F, Rosen FS and Cooper MD, eds. Recent advances in primary and acquired immunodeficiencies. New York: Raven Press 1986;219–25.

24 Keyeux G, Lefranc G, Lefranc MP. A multigene deletion in the human IGH constant region locus involves highly homologous hot spots of recombination. Genomics 1989; **5**:431–41.
25 Takahashi N, Ueda S, Obata M, Nikaido T, Nakai S, Honjo T. Structure of human immunoglobulin γ genes: implications for evolution of a gene family. Cell 1982; **29**:671–9.
26 Lefranc MP, Lefranc G, Rabbitts TH. Inherited deletion of immunoglobulin heavy chain constant region genes in normal human individuals. Nature 1982; **300**:760–2.
27 Lefranc G, Chaabani H, van Loghem E, Lefranc MP, de Lange GG, Helal AN. Simultaneous absence of the human IgG1, IgG2, IgG4 and IgA1 subclasses: immunological and immunogenetical considerations. Eur J Immunol 1983; **13**:240–4.
28 Hu X, Ray PN, Worton RG. Mechanisms of tandem duplication in the Duchenne muscular dystrophy gene include both homologous and nonhomologous intrachromosomal recombination. Embo J 1991; **10**:2471–7.
29 Wahls WP, Wallace LJ, Moore PD. The Z-DNA motif d(TG)30 promotes reception of information during gene conversion events while stimulating homologous recombination in human cells in culture. Mol Cell Biol 1990; **10**:785–93.
30 Hammarström L, Smith CIE. Critical aspects on diagnosing IgG subclass deficiency and its clinical consequences. In: Poulik MD, ed. Protides of the biological fluids, Vol. 36. Oxford: Pergamon Press, 1989: 133–8.
31 Lefranc MP, Lefranc G. Human immunoglobulin heavy-chain multigene deletions in healthy individuals. Febs Lett 1987; **213**:231–7.
32 Lefranc MP, Hammarström L, Smith CIE, Lefranc G. Gene deletions in the human immunoglobulin heavy chain constant region locus: molecular and immunological analysis. Immunodef Rev 1990; **2**:265–81.
33 Linde A, Söderström R, Sällberg M *et al.* Herpesvirus serology and aberrant specific immunoglobulin G2 and G3 subclass patterns and Gm allotypes in individuals with low levels of IgG3. Clin Exp Immunol 1992; **90**:199–203.