Volume 213, number 2, 231-237 FEB 04536 March 1987

Review Letter

Human immunoglobulin heavy-chain multigene deletions in healthy individuals

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Received 29 December 1986

Extensive multigene deletions have been described in the human immunoglobulin heavy-chain constant region genes, some of them encompassing perhaps more than 100 kilobases. These deletions have all been observed in healthy individuals although these individuals lacked several immunoglobulin class or subclasses, being either homozygous for one deletion or heterozygous for two different deletions. The high frequency of consanguinity in the Tunisian population accounts for the high frequency of individuals displaying one or the orther of these deletions in a homozygous state.

Multigene deletion; Immunoglobulin; CH gene; Immunoglobulin deficiency; (Human)

1. INTRODUCTION

In the human immunoglobulin system, there are given classes (IgM, IgD, IgG, IgE and IgA) which are defined by the isotypic antigenic determinants', physico-chemical and biological activities of their heavy chains (μ, δ, γ, ϵ and α respectively). Furthermore, subclasses are known for IgG (IgG1, IgG2, IgG3 and IgG4) and IgA (IgA1 and IgA2) [1]. The constant region of the γ_1, γ_2, γ_3, α_2 and ϵ heavy chains can also be identified by allotypic antigenic determinants located on one or the other of the heavy chain constant (CH) domains [2,3]. These antigens provide genetic markers for IgG1, IgG2, IgG3, IgA2 and IgE and are, consequently, called G1m, G2m, G3m, A2m and Em allotypes, respectively. These allotypes are useful tools in the characterization of populations [4,5] and genetics of immunoglobulins [6-9]. The inheritance of unexpected and unusual sets of allotypes is particularly interesting since they reveal genetic events (point mutations, duplications, deletions, exchanges of CH exons, gene conversion, hybrid genes ...) which have occurred at the level of the coding sequences [6-9].

Due to the availability of specific CH gene probes, these genetic events affecting the locus of the human immunoglobulins which has been mapped on chromosome 14 [10] at band q32 [11] are now also studied at the DNA level. Here, we review the multigene deletions which have recently been observed in the immunoglobulin heavy-chain constant region genes of healthy individuals.

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2. DETECTION OF THE HUMAN CH GENES WITH SPECIFIC PROBES

2.1. *Cμ and Cδ genes*

Human DNA samples digested with *Bam*HI enzyme and hybridized with a Cμ-specific probe [12] show a unique 17 kb hybridizing *Bam*HI fragment

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00145793/87/$3.50

[13] whereas hybridization with a Cδ-specific probe detects an 11 kb hybridizing *Bam*HI fragment [13].

2.2. *Cγ genes*

Cγ probes containing the coding sequences of the constant regions of the γ_3 or γ_4 genes [14] detect all the Cγ genes due to the high degree of homology between the human Cγ genes [15]. Moreover, restriction fragment length polymorphism has been observed for the γ genes and the Cγ probes detect between five and eight hybridizing *Bam*HI fragments [13] (fig.1, lane N).

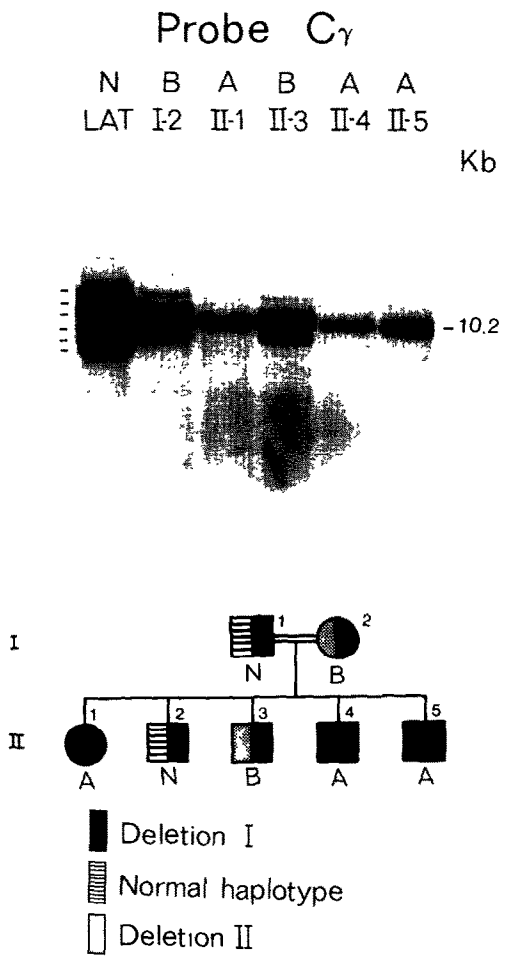

Fig.1. Filter hybridization of genomic DNA of the TOU family with a γ constant region probe [22]. Genomic DNA was digested with *Bam*HI and fractionated on 0.8% agarose. The filter was hybridized with a Cγ probe [14] which detects all Cγ subclass genes. The lines on the left of the panel signify the location of restriction fragments found in a control DNA (lane N). N, control DNA; A, individuals homozygous for the deletion I (see test and fig.4); B, individuals carrying deletion I on one chromosome 14 and deletion II on the other chromosome 14 (see fig.4).

2.3. *Cε genes*

Human DNAs analyzed using a Cε-specific probe [16] show the presence of three *Bam*HI fragments (2.7, 6 and 9 kb) [16–18] (fig.2, lanes N). It has been demonstrated that the 2.7 kb fragment carries the active Cε gene [16–18] whereas the 6 and 9 kb fragments represent pseudogenes called $\psi\epsilon_1$ and $\psi\epsilon_2$, respectively [17–19]. The pseudogene $\psi\epsilon_1$ is located within the CH-gene cluster between $C\gamma_1$ and $C\alpha_1$ [19] on chromosome 14 whereas $\psi\epsilon_2$ is a processed-type pseudogene localized on chromosome 9 [20].

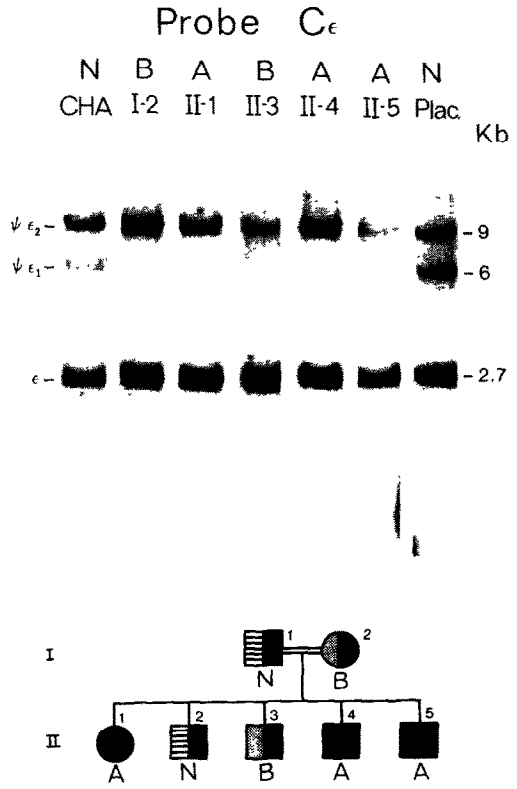

Fig.2. Filter hybridization of genomic DNA of the TOU family [22] using an ε constant region probe [16]. Genomic DNA was digested with *Bam*HI and fractionated on 0.8% agarose. N, A and B as in fig.1.

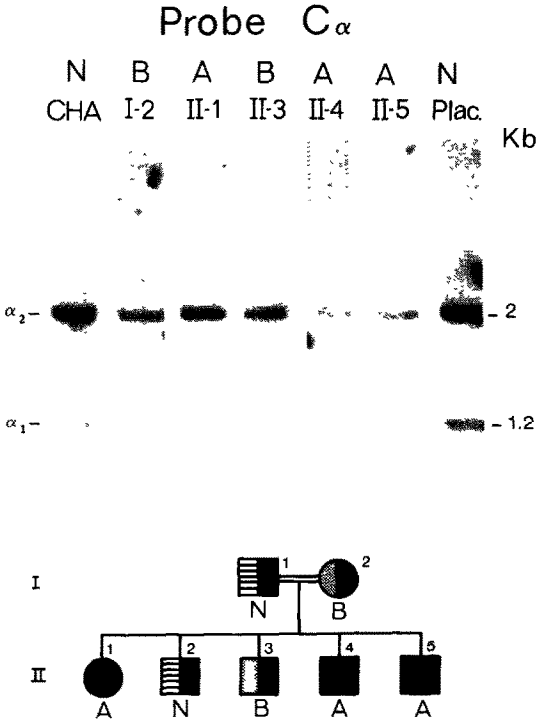

Fig.3. Southern filter hybridization of genomic DNA of the TOU family with an α constant region probe [22]. Genomic DNA was digested with *PstI* and fractionated on 1.4% agarose. The filter was hybridised with a Cα probe (α 2XP8 [19]). N, A and B as in fig.1

2.4. *Cα genes*

The restriction enzyme *PstI* yields two fragments containing α_1 (1.2 kb) and α_2 (2 kb) genes [13,19] (fig.3, lanes N) when probed with the clone $\alpha 2 \times$ P8 (which encompasses most of the CH2 exon, the beginning of the CH3 exon plus the intervening sequence between them) [19]. The advantage of this subclone is that it does not overlap the *Pst* site and therefore gives only one fragment per gene. Moreover, the same probe can be used for the determination of the A2m allotypes by restriction fragment length polymorphism [21].

3. A LARGE DELETION INCLUDING THE $C\gamma_1$, $C\gamma_2$, $C\gamma_4$, $\psi\gamma$, $\psi\epsilon_1$ AND $C\alpha_1$ GENES

The simultaneous absence of the IgG1, IgG2,

Table 1

Serological markers of haplotypes

Probands	G3m	G1m	α_1	G2m	A2m	Type of deletion	References
TAK3 (family HASS*)	b	–	–	–	2	I/I	13
TOU II-1 TOU II-4 (EZZ) TOU II-5	b	–	–	–	2	I/I	13,23
TOU I-2	b	–	–	–	2	I/II	23
TOU II-3	g	za	–	..	2		
SAF I-2	b	f	–	–	1	III/III	25
FRO I-2	b	f	–	–	1	III/IV	25
	g	za	–	–	1		
T 17	b	za	–	–	2	IV/IV	26

* TAK3's two grandsons display the same type of deletion (M.P.L. and G.L., unpublished)

Allotypic markers of γ_1 (G1m) and γ_3 (G3m) heavy chains are indicated in Arabic nomenclature. The absence of G1m allotypes due to the absence of the IgG1 subclass is indicated by a dash (-). Only one allotype is defined on the γ_2 chain and may or may not be present; two dots indicate the absence of this allotype. Nonetheless, the IgG2 subclass is present. The absence of the γ_2 allotypic marker (G2m) due to the absence of IgG2 is indicated by a dash (-). Two allotypes have been described for the γ_2 chain, A2m(1) and A2m(2). No allotype being known for the α_1 chain, the – signs indicate the absence of isotypic markers for the IgA1 subclass. b = b0, b1, b3, b4, b5, u, v; g = g1, g5, u, v

Table 2

Different types of IgCH multigene deletions in healthy individuals

Origin	Number of cases	Absent immunoglobulins	Type of deletion (see fig.4)	References
Family HASS, Tunisia	3	IgG1,IgG2,IgG4,IgA1	I/I	13
Family TOU, Tunisia	3	IgG1,IgG2,IgG4,IgA1	I/I	13,23A[a]
	2	IgA1	I/II	23B[a]
Southern Italy	1	IgG2,IgG4,IgA1,IgE	III/III	25
Sardinia	1	IgG2,IgG4,IgA1	III/IV	25
Tunisia	1	IgG2,IgG4,IgA1	IV/IV	26

[a] A and B correspond to the hybrizations in figs 1–3

The genes implicated in the different types of deletion, as indicated in fig.4, are the following: deletion I: $C\gamma_1$, $\psi\epsilon_1$, $C\alpha_1$, $\psi\epsilon$, $C\gamma_2$, $C\gamma_4$; deletion II: $\psi\epsilon_1$, $C\alpha_1$, $\psi\gamma$; deletion III: $C\alpha_1$, $\psi\gamma$, $C\alpha_2$, $C\gamma_4$, $C\epsilon$; deletion IV: $\psi\epsilon_1$, $C\alpha_1$, $\psi\gamma$, $C\gamma_2$, $C\gamma_4$

IgG4 and IgA1 immunoglobulins has been unambiguously demonstrated in a healthy 75-year-old Tunisian woman (designated TAK3, family HASS) by testing for allotypes, isoallotypes and for isotypes of these four subclasses [22] (table 1). Only IgM, IgD, IgG3, IgE and IgA2 were present. The patterns of hybridization of peripheral blood leucocyte DNA with $C\mu$, $C\delta$, $C\gamma$, $C\epsilon$ and $C\alpha$ probes, using Southern blot analysis, revealed the absence of the $C\gamma_1$, $C\gamma_2$, $C\gamma_4$ and $C\alpha_1$ genes as well as the absence of the $\psi\gamma$ and $\psi\epsilon_1$ genes [13]. An identical deletion was found in three individuals belonging to a second Tunisian family (family TOU) [23], and more recently in two TAK3's grandsons (M.P.L. and G.L., unpublished) (tables 1 and 2). For these 6 individuals the hybridization pattern with a $C\gamma$ probe showed a unique 10.2 kb *Bam*HI band representing the $C\gamma_3$ gene and, conse-

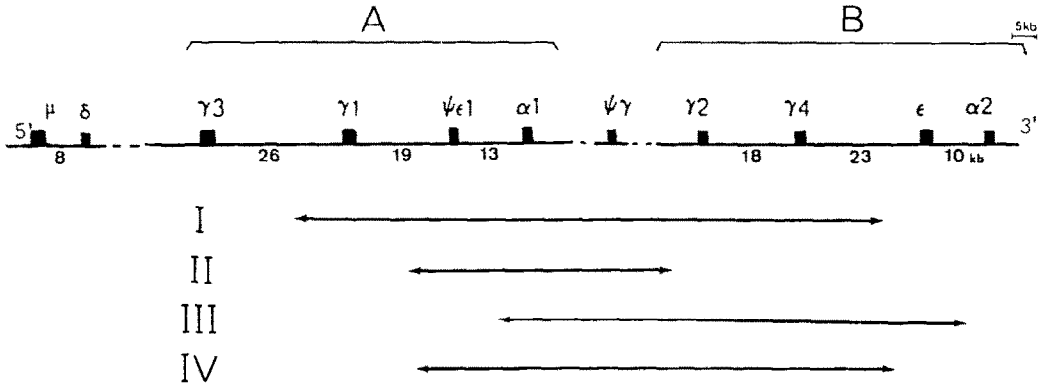

Fig.4. Multigene deletions in the human immunoglobulin heavy-constant region genes. These deletions have all been described in healthy individuals, either homozygous for these deletions or heterozygous for two different deletions (see table 1). Deletions I [13,23], II [23], III [25], and IV [25,26] are described in the text. We need to await gene cloning to establish the precise length and junction points of these different deletions.

quently, the absence of the $C\gamma_1$, $C\gamma_2$, $C\gamma_4$ and $\psi\gamma$ genes [13,23] (fig.1, lanes A). The $C\epsilon$ probe showed the 2.7 kb and 9 kb *Bam*HI fragments representing, respectively, the $C\epsilon$ and $\psi\epsilon_2$ genes, the $\psi\epsilon_1$ being deleted [13,23] (fig.2, lanes A), whereas hybridization with the $C\alpha$-specific probe revealed a unique 2 kb *Pst* fragment representing the $C\alpha_2$ gene, the $C\alpha_1$ gene (1.2 kb *Pst* fragment) being deleted (fig.3, lanes A).

4. ORDER OF THE Ig CH GENES IN HUMAN

When these deletions including γ_1, γ_2, γ_4, $\psi\epsilon_1$ and α_1 were first described [13] (deletion I, fig.4), two groups of cosmid clones had just been identified, which seemed to encompass γ_3- γ_1- $\psi\epsilon_1$- α_1 (region A) and γ_2- γ_4- ϵ- α_2 (region B) [19] (fig.4). The patterns of the deletions enabled us to predict an order for the groups of cosmid clones with region A in 5′ of region B, as the deletions start downstream of γ_3 (region A) and end upstream of the active ϵ gene (region B) [13,19].

Moreover, the absence of the $\psi\gamma$ gene in our samples showed that the $\psi\gamma$ gene was also included in the deletion and therefore must be located between the α_1 and γ_2 genes on chromosome 14 [23]. Human $C\mu$ and $C\delta$ genes have been shown to be 8 kb apart [24]. The order of the human CH genes is, therefore, the following [13,19,23,24]:

$$5'\text{-}\mu \xrightarrow{8\text{ kb}} \delta\text{-...-}\gamma_3 \xrightarrow{26\text{ kb}} \gamma_1 \xrightarrow{19\text{ kb}} \psi\epsilon_1 \xrightarrow{13\text{ kb}} \alpha_1\text{-...-}\psi\gamma\text{-...-}\gamma_2 \xrightarrow{18\text{ kb}} \gamma_4 \xrightarrow{23\text{ kb}} \epsilon \xrightarrow{10\text{ kb}} \alpha_2\text{-}3'$$

5. A SHORTER DELETION ENCOMPASSING $\psi\epsilon_1$-$C\alpha_1$-$\psi\gamma$ GENES

Heterozygous individuals for the previous deletions should give a normal pattern of hybridization using CH-specific probes due to the presence of a normal chromosome 14. Unexpectedly, DNA from two heterozygous members of the TOU family showed α and ϵ hybridization patterns identical to those of the homozygotes, e.g. characterized by the absence of the $\psi\epsilon_1$ and $C\alpha_1$ genes (figs 2,3, lanes B), whereas the $C\gamma$ hybridization showed a four-band pattern due to the absence of the $\psi\gamma$ gene [23] (fig.1, lanes B). These results raised the extremely intriguing possibility that two different types of chromosomal aberration existed in the TOU family, one carrying a small deletion including the α_1 gene as well as the $\psi\epsilon_1$ and $\psi\gamma$ genes (deletion II, fig.4), and the other carrying the previously described deletion which encompasses γ_1, γ_2, γ_4, $\psi\gamma$, $\psi\epsilon_1$ and α_1 genes (deletion I, fig.4).

6. OTHER CH MULTIGENE DELETIONS

More recently two other multiple gene deletions have been described in the heavy-chain cluster, both of them in healthy individuals. The first one, found in the homozygous state in a southern Italian, encompasses α_1, $\psi\gamma$, γ_2, γ_4 and ϵ genes [25]. This deletion (deletion III, fig.4) is different from the deletion I in that it does not include the $C\gamma_1$ gene but the $C\epsilon$ sequence, and therefore, the absence of IgE is observed (table 2).

A Sardinian was heterozygous for this deletion and for a fourth type of deletion involving $\psi\epsilon_1$, α_1, $\psi\gamma$, γ_2 and γ_4 genes [25] (deletion IV, fig.4). A similar deletion has recently been described in the homozygous state in a Tunisian but associated with a different haplotype [26] (table 1).

7. IMMUNOLOGICAL IMPLICATIONS

Deletion I [13,23] shows that a human being can survive without four immunoglobulin subclasses even including IgG1 and IgA1, quantitatively the most important IgG and IgA subclasses in normal sera (table 2). Moreover, both the women TAK3 and TOU II-1 (table 1) have had five pregnancies that were completed to five live births [22,23].

The high level of IgG3 [22], which is the IgG subclass most easily transported across the placenta, has permitted the fetus and the newborns to survive until their own immunoglobulin production had started. Probably the IgA2 levels in the colostrum and maternal milk were also higher than the normal values and may have permitted sufficient immunological protection in the gastrointestinal tracts of their nursing infants. In the homozygous state for deletion III [25], the absence of IgE has apparently no deleterious effects.

None of the abnormal patterns of immunoglobulins mentioned here have clinical consequences and all probands appear healthy. This could be due to the unrestricted use of the available VH gene repertoire by the remaining $C\gamma$ and $C\alpha$

subclasses. Anti-carbohydrate antibodies are almost exclusively confined to the IgG2 subclass in adults. In individuals with immunoglobulin heavy-chain constant region gene deletions encompassing the γ_2 gene, antibodies against polysaccharide antigens were however present and, as expected, restricted to the remaining subclass (IgG1 and/or IgG3) [27]. These results contrast with those found in IgG2-deficient individuals with a retained γ_2 gene where antibodies against most polysaccharide antigens are absent [27,28].

8. SELECTIVE IMMUNOGLOBULIN SUBCLASS DEFICIENCIES

The heterozygous individuals of the TOU family (deletions I/II), were shown to be selectively deficient in IgA1 (table 2). It is noteworthy that this selective IgA1 deficiency is a consequence of multigene deletions on both chromosomes and contrasts with IgA deficiencies where both IgA1 and IgA2 subclasses are lacking. In these latter cases, we observed no Cα gene deletions (M.P.L. and Rabbitts, T.H., unpublished). Similarly, the Cα_2 gene was also present in a selective IgA2 deficiency [29]. Recently, using a specific Cα_3 probe [30], we showed that a selective IgG3 deficiency [6,31] was not due to a Cγ_3 gene deletion [30]. Further studies, including cloning and sequencing, should be carried out in order to elucidate the mechanism of these selective immunoglobulin subclass deficiencies, which are not due to gene deletion.

9. UNEQUAL CROSSING-OVER OR LOOPING-OUT MODELS

The frequency of the IgCH multigene deletions can be estimated to be about 0.01. Since these deletions are harmless, they are not detected when present in a single dose, but are unmasked in homozygous individuals. In populations where consanguinity occurs as a rule, there is a greater chance of finding homozygosity for uncommon chromosomes.

It is very possible that many different types of gene deletions can occur without deleterious effects and that the human CH locus, which seems to possess a remarkable evolutionary flexibility, may be undergoing continual expansion and contraction [32]. It is conceivable that CH gene deletions could be mediated either by unequal crossing-over between sister chromatids or by internal looping-out models.

At present, further speculation on the mechanism needs to await gene cloning and examination of the junction points of these deletions.

ACKNOWLEDGEMENTS

We are very indebted to Dr T.H. Rabbitts, Medical Research Council, Cambridge (England) for this work. M.-P.L. was a recipient of an EMBO fellowship during part of this work in his laboratory. We would like to thank P. Flavier for typing the manuscript. This research was supported in part by Centre National de la Recherche Scientifique, Association pour le Développement de la Recherche sur le Cancer, Fondation pour la Recherche Médicale, Ministère de la Recherche et de la Technologie (N84L0922) and by the Medical Research Council.

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