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Molecular analysis of the T17 immunoglobulin CH multigene deletion (del A1-GP-G2-G4-E)

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Abstract. In the human, the order of the immunoglobulin heavy chain constant region (Ig CH) genes is the following: 5′-M-D-G3-G1-EP1-A1-GP-G2-G4-E-A2-3′. Extensive multigene deletions have been described in the Ig CH locus, some of these encompassing up to 160 kb. To date six different multigene deletion haplotypes have been identified, designated I to VI according to the chronological order of their being found: deletion I (del G1-EP1-A1-GP-G2-G4), II (del EP1-A1-GP), III (del A1-GP-G2-G4-E), IV (del EP1-A1-GP-G2-G4), V (del GP-G2-G4-E-A2), VI (del G1-EP1-A1-GP-G2). Individuals were found either homozygous for one type of deletion or heterozygous for two different deletions, mainly (17 cases out of 18) in the Mediterranean area. So far, deletions I and II have been found in Tunisia, deletions III, IV and V in Italy, and deletion VI in Sweden. In this paper, we show that a Tunisian, T17, previously reported as being homozygous for a deletion of type IV, is, in fact, homozygous for a deletion that encompasses A1-GP-G2-G4-E. Therefore T17 is the first case of a deletion of type III reported in the Tunisian population. Molecular analysis demonstrates that the T17 deletion occurred between highly homologous regions located downstream of IGHEP1 and IGHE, respectively. In contrast to the EZZ deletion, the recombination occurred near or in the switch regions, since the homologous regions involved in the deletion extend over 4.5 kb of DNA and encompass the Iα1-Sα1 and Iα2-Sα2 regions, respectively.

Introduction

In the human, the order of the immunoglobulin heavy chain constant region (IgCH) genes, on chromosome 14, is the following: 5′-M$^{8\ kb}$D-G3$^{26\ kb}$G1$^{19\ kb}$EP1$^{13\ kb}$A1-GP-G2$^{18\ kb}$G4$^{23\ kb}$E$^{11.5\ kb}$A2-3′ (Flanagan and Rabbitts 1982a; Lefranc M-P et al. 1982, 1983; Milstein et al. 1984). The first simultaneous absence of several subclasses of immunoglobulins (IgG1, IgG2, IgG4 and IgA1) was demonstrated, in 1982, in a healthy 75-year-old Tunisian woman (designated TAK3, family HASS) by testing allotypes, isoallotypes and isotypes of these four subclasses (Lefranc M-P et al. 1982; Lefranc G et al. 1983) (Tables 1 and 2). Only IgM, IgD, IgG3, IgE and IgA2 were present. Molecular analysis revealed the deletion of the G1, G2, G4 and A1 functional genes as well as that of the GP and EP1 pseudogenes (Lefranc M-P et al. 1982; Fig. 1). An identical deletion (del G1-EP1-A1-GP-G2-G4, or deletion I) was found in three individuals belonging to a second Tunisian family (family TOU) (Lefranc M-P et al. 1983), and in two grandsons of TAK3 (Lefranc M-P and Lefranc G 1987) (Tables 1 and 2). A smaller deletion (del EP1-A1-GP or deletion II, Fig. 1) was also described in family TOU. These results were the first demonstration of a selective IgA1 deficiency detected at the DNA level prior to the availability of any serological data (Lefranc M-P et al. 1983). Four other types of multigene deletions (designated III to VI) were later described in the heavy chain gene cluster (for review see Lefranc M-P et al. 1991). Three of them were observed, as deletions I and II, in healthy individuals of the Mediterranean area. Deletion III (del A1-GP-G2-G4-E (Fig. 1), first found in the homozygous state in a southern Italian (Migone et al. 1984), is different from deletion I in that it includes not the G1 gene but the E gene. Therefore, the IgE class is absent (Table 1). Two other Italian people carrying deletion III were later identified: one had deletion III on each chromosome 14 but associated with different Gm haplotypes; the other was heterozygous for this deletion, the second chromosome being characterized by a deletion of the G2 gene (del G2, Table 1) (Bottaro et al. 1989). Two Italian siblings (6 and 13 years old) homozygous for deletion III were recently described (Plebani et al. 1993). A Sardinian was found heterozygous for deletion III and for a fourth type of deletion involving the EP1, A1, GP, G2 and G4 genes (deletion IV, Fig. 1) (Migone et al. 1984). A similar

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Table 1. Different types of Ig CH multigene deletions. In 17 cases out of 18, these deletions have been observed in healthy individuals

Origin	Number of cases	Proband	Absent immunoglobulins	Genotypes with reference to the type of deletion[a] (see Fig. 1)	References
Family HASS Tunisia	3	TAK3[b]	IgG1, IgA1, IgG2, IgG4	I/I	Lefranc M-P et al. (1982); Lefranc G et al. (1983)
Family TOU Tunisia	3	TOU II-1 TOU II-4 (EZZ) TOU II-5	IgG1, IgA1, IgG2, IgG4	I/I	Lefranc M-P et al. (1982, 1983)
	2	TOU I-2 TOU II-3	IgA1	I/II	Lefranc M-P et al. (1983)
Italy	4	SAF I-2 DEM R.B. D.B.	IgA1, IgG2, IgG4, IgE	III/III	Migone et al. (1984) Bottaro et al. (1989) Plebani et al. (1993)
Tunisia	1	T17	IgA1, IgG2, IgG4, IgE	III/III[d]	This paper
Sardinia	1	FRO I-2	IgA1, IgG2, IgG4	III/IV	Migone et al. (1984)
Italy	2	TIM MOD	IgG2[e]	III/del G2	Bottaro et al. (1989)
Italy	1	CRU	IgG2, IgG4, IgE, IgA2	V/V	Bottaro et al. (1989)
Sweden	1	NY[c]	IgG1	VI/del G1	Smith et al. (1989)

[a] Six different multigene deletion haplotypes have been identified, designed I to VI according to the chronological order in which they were found: deletion I (del G1-EP1-A1-GP-G2-G4), II (del EP1-A1-GP), III (del A1-GP-G2-G4-E), IV (del EP1-A1-GP-G2-G4), V (del GP-G2-G4-A2), VI (del G1-EP1-A1-GP-G2)

[b] The two grandsons of TAK3 display the same type of deletion, also in the homozygous state (Lefranc M-P and Lefranc G 1987)

[c] This individual has an increased susceptibility to infections

[d] The T17 deletion was erroneously interpretated as of type IV in Chaabani et al. (1985). Data presented in this paper demonstrate the deletion of the IGHE gene and the absence of IgE, and characterize the deletion as of type III

[e] These individuals also show a lack (TIM) or a low level (MOD) of serum IgG4 although they possess an apparently normal IGHG4 gene

type IV deletion, as described in a previous paper (Chaabani et al. 1985), was found in the homozygous state in a Tunisian (T17) but associated with a different Gm-Am haplotype. Deletion V (del GP-G2-G4-E-A2) was found in the homozygous state in another Italian who, as a consequence of this deletion, was deficient in the IgE class and IgG2, IgG4 and IgA2 subclasses (Bottaro et al. 1989). Finally, deletion VI (del G1-EP1-A1-GP-G2) was observed in a 5-year-old Swedish girl with a selective IgG1 deficiency (Smith et al. 1989). Such a deficiency resulted from the inheritance of this deletion on the maternal chromosome 14, whereas a single G1 gene deletion (del G1) was transmitted on the paternal one (Tables 1 and 2).

So far, molecular analysis of the deleted-recombined Ig CH locus has only been carried out for the individual EZZ from the TOU family (Lefranc M-P et al. 1982, 1983a). The EZZ deletion (deletion I), which encompasses 160 kb, involves two highly homologous regions located 13 kb downstream of IGHG3 and 13 kb downstream of IGHG4, designated as *hsg3* and *hsg4*, respectively, as they are hot spots of recombination (Keyeux et al. 1989a). These homologous *hsg* regions (Keyeux et al. 1989a) were subsequently shown to belong to a family of repetitive elements (or MER 4) (Jurka 1990). From these data, we made the hypothesis that the deletion/recombinational events that took place (i) downstream of the G1 gene and downstream of the GP or G4 gene for deletions II and IV, (ii) downstream of the EP1 and E genes for deletion III, and (iii) downstream of the A1 and A2 genes in deletion V (Fig. 1), also involved homologous regions (*hsg1/gp, hsg1/g4, hsepl/e* and *hsa1/a2*, respectively) located downstream of these duplicated genes (Keyeux et al. 1989a). In this study, DNA from the Tunisian T17 was analyzed in order to characterize the type of deletion, to draw a restriction map of the deleted/recombined chromosome 14, and to identify the breakpoint regions on the original chromosomes. Unexpectedly, this molecular analysis revealed that T17 was, in fact, homozygous for the type III deletion (del A1-GP-G2-G4-E).

Materials and methods

DNA was isolated from white blood cells from the T17 proband. Conditions for DNA extraction, Southern hybridization, washing and monitoring were as previously described (Ghanem et al. 1988a), except that probes were labeled by random priming (Feinberg and Vogelstein 1983). The following probes were used: the Cγ probe, pSH3Cγ3, is a 2-kb *Sac*I-*Sph*I fragment cloned in pUC19 (Huck et al. 1986a, b); the Cε probe, ε1.2BP25, is a 2.1-kb *Bam*HI-*Pst*I fragment cloned in M13mp701 (Flanagan and Rabbitts 1982b); the Cα probe, α2XP8, is a 600 bp *Xho*I-*Pst*I fragment cloned in M13mp8 (Flanagan and Rabbitts 1982a); the Sα probe, pSαHS2.1, is a 2.1-kb *Hin*dIII-*Sac*I fragment from λTOU ε-α, cloned in pUC19 (Keyeux et al. 1989b).

Results

Deletion of the IGHGP, G2 and G4 genes

The T17 proband DNA was digested with different enzymes, and hybridized to the Cγ probe pSH3Cγ3. As

Table 2. Serological markers of haplotypes in the individuals with homozygous or heterozygous Ig CH multigene deletions

Proband	G3m	G1m	α1	G2m	A2m	Genotypes with reference to the type of deletion[a] (see Fig. 1)	References
TAK3 (family HASS[b])	b	–	–	–	2	I/I	Lefranc M-P et al. (1982); Lefranc G et al. (1983)
TOU II-1 TOU II-4 (EZZ) TOU II-5	b	–	–	–	2	I/I	Lefranc M-P et al. (1982, 1983)
TOU-I-2	b	–	–	–	2	I/	Lefranc M-P et al. (1983)
TOU-II-3	g	za	–	..	2	II	
SAF I-2	b	f	–	–	1	III/III	Migone et al. (1984)
DEM	b	f	–	–	n.d.	III/	
	g	zax	–	–		III	Bottaro et al. (1989)
T17	b	za	–	–	2	III/III[d]	This paper
FRO I-2	b	f	–	–	1	III/	Migone et al. (1984)
	g	za	–	–	1	IV	
TIM	b	f	–	–	n.d.	III/	Bottaro et al. (1989)
	g	za	+	–		del G2	
MOD	b	f	–	–	n.d.	III/	Bottaro et al. (1989)
	b	f	+	–		del G2	
CRU	b	f	+	–	–	V/V	Bottaro et al. (1989)
NY[c]	g	–	–	–	n.d.	VI/	Smith et al. (1989)
	b	–	+	n		del G1	

[a] Six different multigene deletion haplotypes have been identified, designated I to VI according to the chronological order in which they were found: deletion I (del G1-EP1-A1-GP-G2-G4), II (del EP1-A1-GP), III (del A1-GP-G2-G4-E), IV (del EP1-A1-GP-G2-G4), V (del GP-G2-G4-A2), VI (del G1-EP1-A1-GP-G2). Allotype markers of γ1 (G1m) and γ3 (G3m) heavy chains are indicated in alphabetical nomenclature. The absence of G1m allotypes due to the absence of IgG1 subclass is indicated by a dash (–). Only one allotype is defined on the γ2 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 γ2 allotypic marker (G2m) due to the absence of IgG2 is indicated by a dash (–). Two allotypes have been described for the α2 chain, A2m(1) and A2m(2). No allotype being known for the α1 chain, the dash indicates the absence of isotypic markers for the IgA1 subclass; n.d., not determined; b = b0, b1, b3, b4, b5, u, v; g = g1, g5, u, v

[b] The two grandsons of TAK3 display the same type of deletion and are also homozygous for this deletion (Lefranc M-P and Lefranc G 1987)

[c] This individual has an increase susceptibility to infections

[d] This T17 deletion was erroneously interpretated as of type IV in Chaabani et al. (1985). Data presented in this paper demonstrate the deletion of the IGHE gene and the absence of IgE, and characterize the deletion as of type III

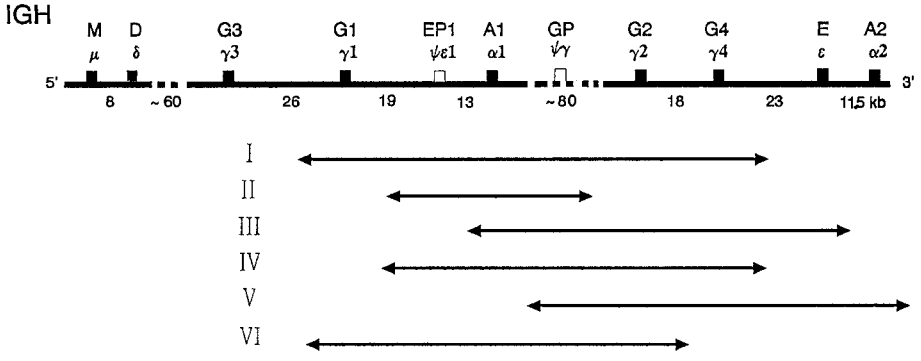


Fig. 1. Organization of the human immunoglobulin heavy chain constant region locus (Flanagan and Rabbitts 1982a; Lefranc M-P et al. 1982, 1983; Milstein et al. 1984). Nomenclature of the Ig CH genes is according to the recommendations of Human Gene Mapping HGM9 (Shows et al. 1987). Multigene deletions in the Ig CH locus have been described in healthy individuals, either homozygous for one type of identical deletion on the two chromosomes 14 or heterozygous for two different deletions. Types of Ig CH multigene deletions are designated I to VI, according to the chronological order in which they were found (Keyeux et al. 1989a). Deletions I (Lefranc M-P et al. 1982, 1983), II (Lefranc M-P et al. 1983), III (Migone et al. 1984; Bottaro et a. 1989; Plebani et al. 1993), IV (Migone et al. 1984), V (Bottaro et al. 1989) and VI (Smith et al. 1989) have been reviewed in Lefranc M-P and Lefranc G (1987), Lefranc M-P et al. (1991). We have estimated by pulsed field gel electrophoresis (PFGE) that deletion I encompasses 160 kb (Keyeux et al. 1989a). Given these data and those of Flanagan and Rabbitts (1982a), the distance between A1 and G2 may be estimated to be 85 kb. A similar estimation (80 kb) has been obtained by PFGE of normal DNAs (Hofker et al. 1989). Deletion I, del G1-EP1-A1-GP-G2-G4. Deletion II, del EP1-A1-GP. Deletion III, del A1-GP-G2-G4-E. Deletion IV; del EP1-A1-GP-G2-G4. Deletion V, del GP-G2-G4-E-A2. Deletion VI, del G1-EP1-A1-GP-G2

shown in Fig. 2, only two bands were detected, which correspond to the IGHG1 and IGHG3 genes, respectively. The size differences of the G1 and G3 genes between the T17 proband DNA and the control DNA correspond to a polymorphism of these genes, which has previously been described (Ghanem et al. 1988a, b, 1989). These data confirmed the deletion of the IGHGP, IGHG2 and IGHG4 genes on both chromosomes, and therefore the absence of the IgG2 and IgG4 subclasses in the sera of T17, as previously described (Chaabani et al. 1985).

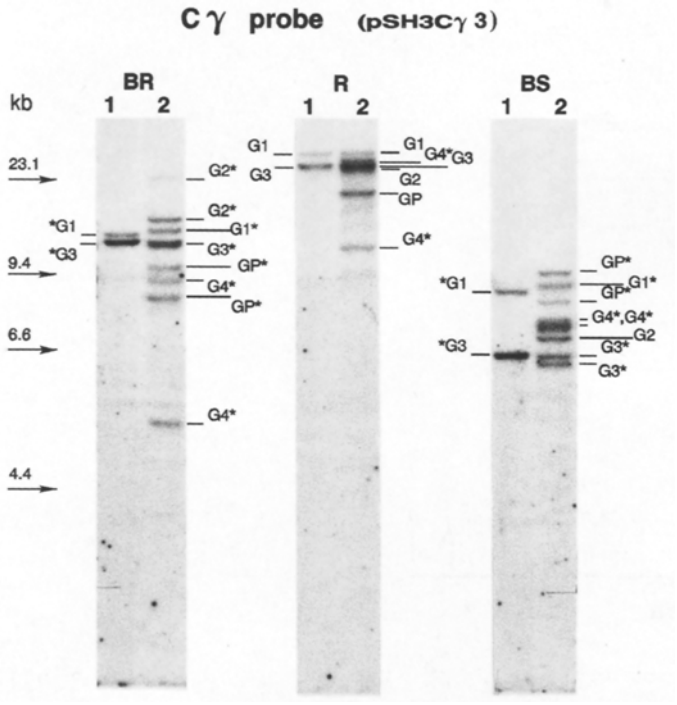


Fig. 2. Southern blot hybridization of the proband T17 DNA (*lanes 1*) and of a control DNA (*lanes 2*), to the Cγ probe, pSH3Cγ3. Restriction enzymes are indicated above the lanes. *Arrows on the left* indicate the position of the bands (size in kilobases) of the molecular weight marker. The size differences of the G1 and G3 genes between the T17 proband DNA and the control DNA correspond to polymorphism of these genes. Note that the pSH3Cγ3 probe, which contains a G3 gene (Huck et al. 1986b), gives a stronger signal with G3, compared with the other IGHG genes. *B BamHI*; *R EcoRI*; *S SacI*

Deletion of the IGHE gene

Hybridization of the *Bam*HI digested T17 DNA to the Cε probe ε1.2BP25 (Fig. 3), reveals a quite surprising and unexpected result. Indeed, it was assumed from the work by Chaabani et al. (1985), that T17 had a deletion of the EP1 gene on both chromosomes 14, but that the functional gene IGHE was present. In contrast, our data unambiguously demonstrated the deletion of the functional IGHE gene on both chromosomes as shown by the absence of the 2.7-kb *Bam*HI band (Fig. 3). The IGHEP1 pseudogene and the processed IGHEP2 pseudogene, located on chromosome 9, were present as shown by the 6- and 9-kb *Bam*HI fragments, respectively. The deletion of the IGHE gene was further confirmed by the absence of the 1.8-(5′E) and 2.8-kb (3′E) *Sac*I fragments (Fig. 3).

In DNA samples from Caucasoid populations, the EP1-A1 and E-A2 genes are contained in two large *Eco*RI bands of 30 and 38 kb, respectively (Fig. 4A and B) whereas the EP2 gene is contained in an 11-kb band. For individuals with the A2m(2) allotype, found mostly in black African populations, the presence of an *Eco*RI site in the first exon of IGHA2 (Lefranc and Rabbitts 1984) leads to a 23-kb (instead of 38-kb) *Eco*RI fragment detected with the Cε probe, whereas the 15-kb downstream fragment is only detected with the Cα probe, α2XP8 (Fig. 4B). An *Eco*RI digest of the T17 DNA hybridized to the Cε probe shows only two bands, the expected 11-kb EP2 band and an unusual 15-kb band (Fig. 3). Since the T17 DNA shows neither the 30-, 38- or 23-kb *Eco*RI band, the

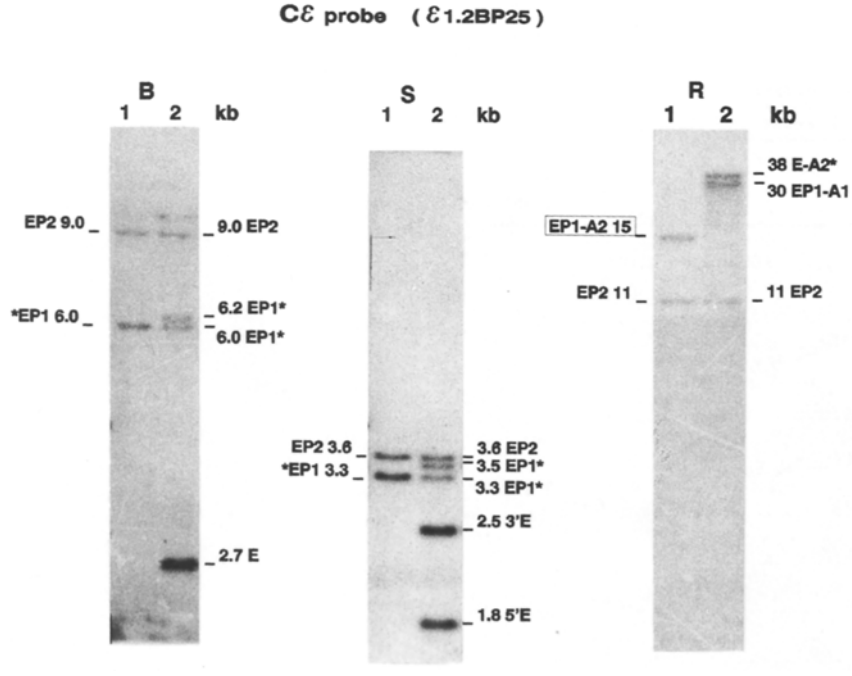
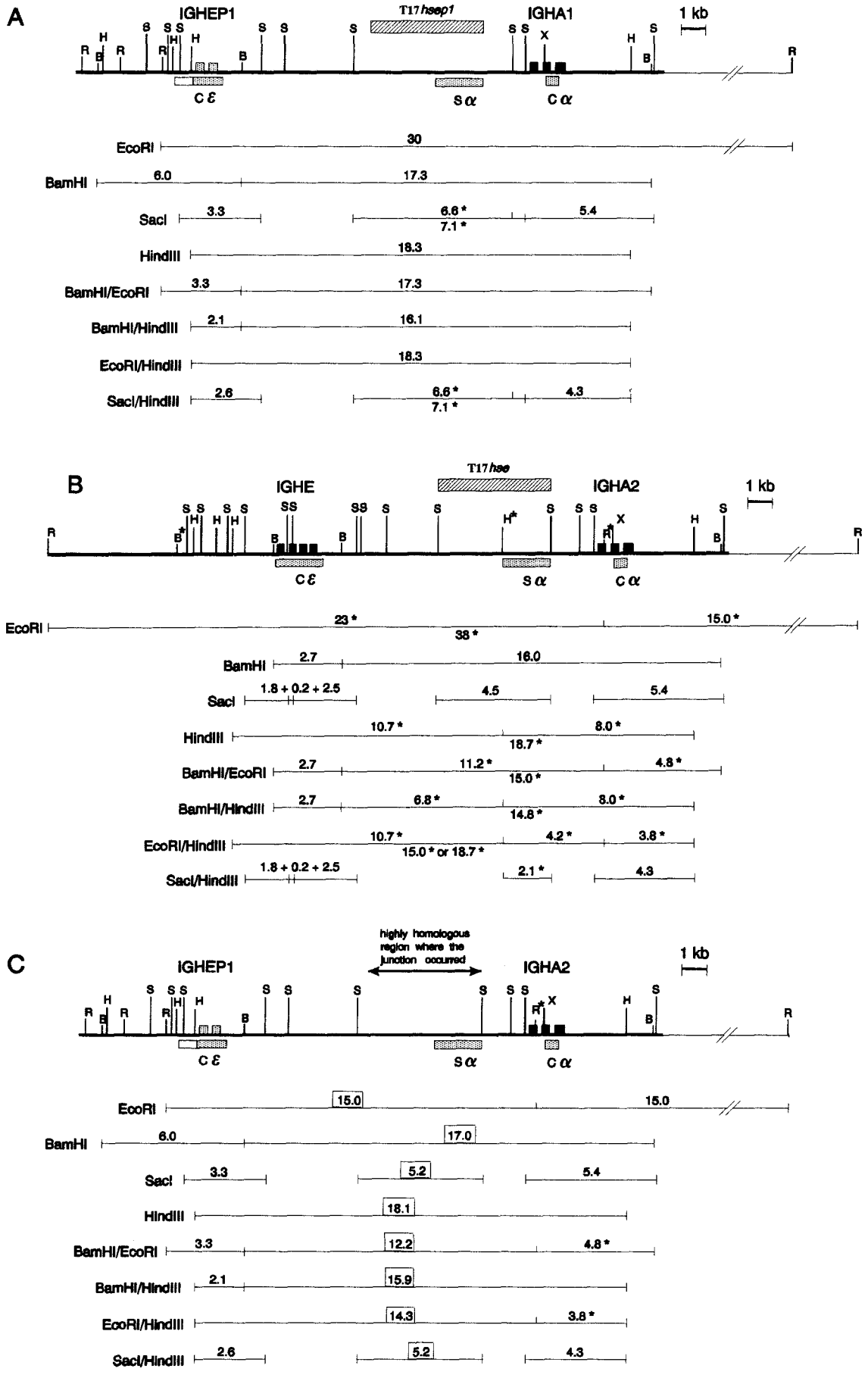


Fig. 3. Southern blot hybridization of the proband T17 DNA (*lanes 1*) and of a control DNA (*lanes 2*) to the Cε probe, ε1.2BP25. The 6.2-kb *Bam*HI and 3.5-kb *Sac*I bands observed in digests of the control DNA are due to a polymorphism of the EP1 gene that will be described elsewhere (V. Wiebe, L. Osipova, G. Lefranc and M.-P. Lefranc, in preparation). The unusual 15-kb EP1-A2 band of the T17 recombinant chromosome 14 is *boxed*. *B BamHI*; *R EcoRI*; *S SacI*


A
IGHEP1
T17 hsep1
IGHA1
1 kb
Cε
Sα
Cα
EcoRI 30
BamHI 6.0 17.3
SacI 3.3 6.6* 7.1* 5.4
HindIII 18.3
BamHI/EcoRI 3.3 17.3
BamHI/HindIII 2.1 16.1
EcoRI/HindIII 18.3
SacI/HindIII 2.6 6.6* 7.1* 4.3
B
IGHE
T17 hse
IGHA2
1 kb
Cε
Sα
Cα
EcoRI 23* 38* 15.0*
BamHI 2.7 16.0
SacI 1.8 + 0.2 + 2.5 4.5 5.4
HindIII 10.7* 18.7* 8.0*
BamHI/EcoRI 2.7 11.2* 15.0* 4.8*
BamHI/HindIII 2.7 6.8* 14.8* 8.0*
EcoRI/HindIII 10.7* 15.0* or 18.7* 4.2* 3.8*
SacI/HindIII 1.8 + 0.2 + 2.5 2.1* 4.3
C
highly homologous region where the junction occurred
IGHEP1
IGHA2
1 kb
Cε
Sα
Cα
EcoRI 15.0 15.0
BamHI 6.0 17.0
SacI 3.3 5.2 5.4
HindIII 18.1
BamHI/EcoRI 3.3 12.2 4.8*
BamHI/HindIII 2.1 15.9
EcoRI/HindIII 14.3 3.8*
SacI/HindIII 2.6 5.2 4.3

unusual 15-kb fragment that hybridizes to the Cε probe most probably results from the deletion event. T17 has been shown to be homozygous for the A2m(2) allotype both by serological analysis (Chaabani et al. 1985) and by restriction fragment length polymorphism (RFLP) analysis (this paper). Indeed, an *Eco*RI+ *Pst*I T17 DNA digest, hybridized to the α2XP8 probe, only shows the 0.9-kb band, which is due to the presence of an *Eco*RI site in exon 1 of IGHA2 and which characterizes the IGHA2*M2 allele (Lefranc and Rabbitts 1984) (data not shown). It is most likely that the *Eco*RI site at the 3′ end of the 15-kb T17 *Eco*RI fragment is the one located in exon 1 of T17 IGHA2.

Deletion of the IGHA1 gene

Hybridization of various T17 DNA digests with the Cα probe, α2XP8 (Fig. 5) led to three conclusions: (i) firstly, the deletion of IGHA1 on both chromosomes, as shown by the absence of the 1.2-kb *Pst*I band (not shown) and of the 17.3-kb *Bam*HI band (lanes 1 in Figs. 5C, 4C), and by the half intensity of the 5.4-kb *Sac*I and 4.3-kb *Sac*I/*Hin*dIII band (Fig. 5A). Indeed, T17 DNA (lanes 1) only contains the two A2 genes whereas the control DNA (lanes 2) contains the two A1 genes as well as the two A2 genes. (ii) Secondly, the presence of a normal IGHA2 A2m (2) allele on both chromosomes, as shown by the expected polymorphic 15-kb *Eco*RI, 4.8-kb *Eco*RI/*Bam*HI and 3.8-kb *Eco*RI/*Hin*dIII bands (Figs. 4B, C, 5B). (iii) Thirdly, the presence of unusual rearranged bands (17-kb *Bam*HI, 18.1-kb *Hin*dIII, 15.9-kb *Bam*HI/*Hin*dIII) that do not correspond to normal A1 or A2 bands and therefore result from the deletion event (Fig. 5C).

Restriction map of the T17 EP1-A2 region

Hybridization of the T17 DNA digests to the switch Sα probe pSαHS2.1 (Fig. 6) detects some of the bands previously observed with the Cε and Cα probes. The 18.1-kb *Hin*dIII fragment is the only one to hybridize to the three probes (Cε, Sα and Cα). The 14.3-kb *Eco*RI/*Hin*dIII fragment hybridizes to both the Cε and Sα probes whereas the 17-kb *Bam*HI and 15.9-kb *Bam*HI/*Hin*dIII fragments hybridize to the Sα and Cα probes (Fig. 4C).

Two new fragments were identified with the Sα probe, and only with that probe. They are the 5.2-kb *Sac*I (Fig. 6) and 12.2-kb *Bam*HI-*Eco*RI fragments (not shown). A precise restriction map of the T17 EP1-A2 region was constructed from these data (Fig. 4C). It shows that the recombination occurred downstream of EP1 in a 4.5-kb region, designated as T17 *hsep1* and downstream of the E gene in the homologous 4.5-kb region, T17 *hse* (Fig. 4).

Discusssion

Southern analysis (Figs. 2, 3, 5 and 6) shows that the T17 deletion is identical on both chromosomes 14 and encompasses the A1-GP-G2-G4-E genes (Fig. 4C) The T17 deletion is therefore the first deletion of type III described in the Tunisian population. The T17 deletion was previously described by Chaabani et al. (1985) as extending from EP1 to G4. The discrepancy between our data and those of Chaabani et al. regarding the type of deletion can be easily explained by the absence of *Bam*HI digestion by them for the Cε hybridization. The "levels" of IgE in the serum of T17 mentioned in their paper are probably the values of the background of the method. Indeed, T17 has no detectable plasma IgE when total plasma IgE levels determined using a radioimmunoassay (background values were observed in some tests). The complete absence of IgE due to a deletion of the IGHE genes is harmless in T17 as this already been described in four Italian individuals by Migone et al. (1984), Bottaro et al. (1989), and Plebani et al. (1993).

The distance between the IGHEP1 and IGHA2 genes on the T17 recombinant chromosome is 13 kb, that is the same distance as found between the IGHEP1 and IGHA1 genes on a normal chromosome. The T17 deletion results from a recombination that occurred in highly homologous regions downstream of IGHEP1 and IGHE. Owing to the high homology, locating the breakpoints inside these regions might be difficult, even impossible, if we do not find some special restriction sites or motifs characteristic of the A1 or A2 regions. The polymorphic *Hin*dIII site has been found to be associated with the IGHA2*M2 allele in some individuals (Lefrance M-P and Rabbitts 1984) but this is far from being the rule (Bernard et al. 1991) and the absence of the *Hin*dIII site in T17 may be just an example of such a lack of association. If that is the case, the recombination may have occurred at any point of the 4.5-kb homologous regions, designated as T17 *hsep1* and T17

Fig. 4. A Restriction map of the normal IGHEP1-IGHA1 region on chromosome 14 (from Flanagan and Rabbitts 1982a, and this paper). **B** Restriction map of the normal IGHE-IGHA2 region on chromosome 14 (from Flanagan and Rabbitts 1982a, and this paper). A polymorphic *Eco*RI restriction site in the IGHA2 gene is associated with the A2m (2) allotype (Lefranc M-P and Rabbitts 1984). Its frequency has been determined by restriction fragment length polymorphism (RFLP) analysis in several populations (Soua et al. 1989). A polymorphic *Hin*dIII site located upstream of the Sα region has also been described (Lefranc M-P and Rabbitts 1984). With *Eco*RI/*Hin*dIII digests, 10.7-, 15.0- and 18.7-kb polymorphic fragments can be detected with the Cε probe (Bernard et al. 1991). **C** Restriction map of the IGHEP1-IGHA2 region on the recombined chromosome of the proband T17. Recombination between the region downstream of IGHEP1 and the region downstream of IGHE led to a 160 kb deletion encompassing the A1-GP-G2-G4-E genes (deletion type III in Keyeux et al. 1989a; Lefranc M-P et al. 1991), and to the localization of A2 13 kb 3′ of EP1. Rearranged restriction fragments resulting from the deletion are *boxed*. Exons are shown as *black boxes* for the functional E, A1 and A2 genes, and as *dotted boxes* for the EP1 pseudogene. Probes are shown as *dotted rectangles* below the map, and in the regions to which they hybridize. The 5′ region of the Cε probe, which does not hybridize to EP1, is shown in *white*. *Asterisks* indicate polymorphic restriction fragments. Sizes are in kilobases. *Hatched rectangles* above the maps in **A** and **B** indicate the 4.5-kb T17 *hsep1* and T17 *hse* homologous regions, respectively, where the breakpoints occurred. By convention, the homologous regions involved in the multigene deletions are designated according to the genes located upstream (Keyeux et al. 1989a). *B Bam*HI; *H Hin*dIII; *R Eco*RI; *S Sac*I; *X Xho*I

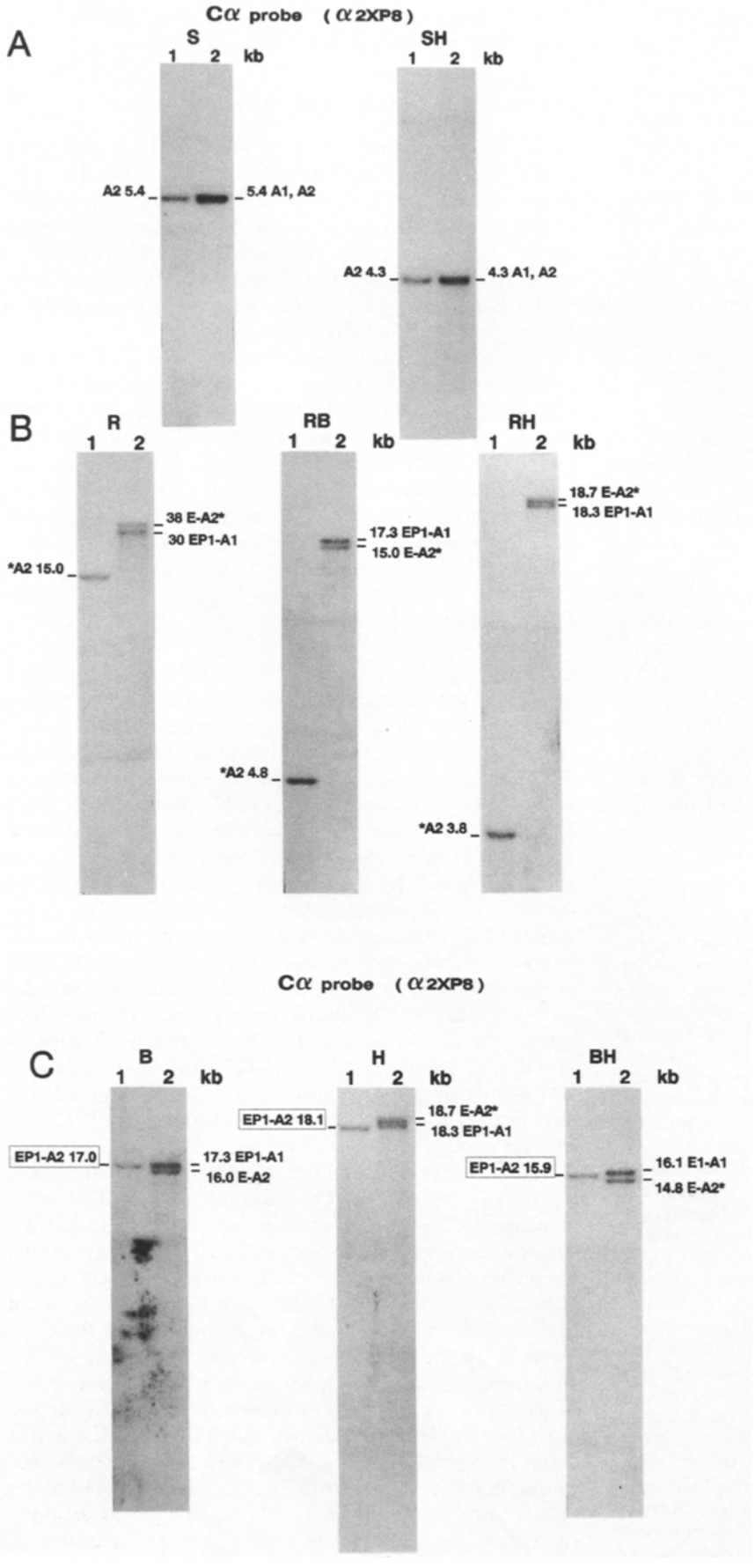


Fig. 5A–C. Southern blot hybridization of the proband T17 DNA (*lanes 1*) and of a control DNA (*lanes 2*) to the Cα probe, α2XP8. The rearranged EP1-A2 *Bam*HI, *Hin*dIII and *Bam*HI-*Hin*dIII bands of the T17 recombinant chromosome 14 are *boxed* (**C**). *B Bam*HI; *H Hin*dIII; *R Eco*RI; *S Sac*I

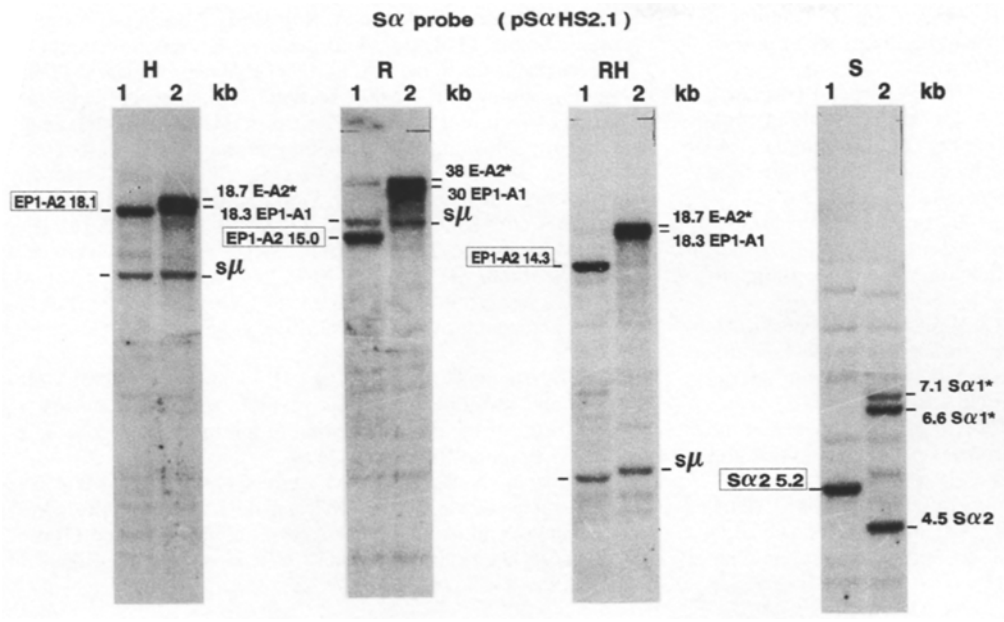


Fig. 6. Southern blot hybridization of the proband T17 DNA (*lanes 1*) and of a control DNA (*lanes 2*) to the Sα probe, pSαHS2.1. *Sμ* (only shown on the right of the lanes) indicates the switch μ bands, which are detected by cross-hybridization to the Sα probe. The polymorphic *Sac*I Sμ bands (2.2 kb in T17 DNA and 2.7 kb in control DNA) are not visible on this long run gel. *B Bam*HI; *H Hin*dIII; *R Eco*RI; *S Sac*I

hse, respectively, in Fig. 4A and B. Another hypothesis is that the *Hin*dIII site was present on the original chromosome, but was lost owing to the deletion. In that case, the recombination occurred downstream of this *Hin*dIII site, at any point of a region limited to 2.1 kb and encompassing the I-α (as defined in Nilsson et al. 1991) and switch alpha regions of the IGHA1 and IGHA2 genes, respectively. Molecular analysis of the T17 deletion confirms our hypothesis that multigene deletions in the IGH constant region locus result from recombination between highly homologous regions. These regions could be outside the switch regions as demonstrated by the EZZ deletion (Keyeux et al. 1989a) or in the switch region, or at least in its vicinity, as suggested by the T17 DNA deletion.

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