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Sequence of a human immunoglobulin gamma 3 heavy chain constant region gene: comparison with the other human C γ genes

S.Huck, P.Fort¹, D.H.Crawford², M.-P.Lefranc and G.Lefranc

Laboratoire d'Immunogénétique, Université des Sciences et Techniques du Languedoc and ¹Laboratoire de Biologie Moléculaire, U.A. CNRS 541, U.S.T.L., 34060 Montpellier, Cedex, France and ²I.R.C.F. University College, London, UK

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ABSTRACT

We report the first and complete nucleotide sequence of a human gamma 3 heavy chain constant region gene (C γ 3). This gene displays the same organization than the others C γ genes and exhibits normal RNA splice and polyadenylation sites. A comparison of its primary sequence with those of C γ 1, C γ 2 and C γ 4 genes confirms the high degree of homology (95%) of the human family in both coding and non-coding regions, and the divergence of the hinge region. The C γ 3 gene we sequenced codes for a Gm(b) γ 3 chain (EZZ). Comparison with other known protein sequences reveals that only two specific aminoacids are involved in the Gm(b) and Gm(g) allotypes, which suggests an important part of the spatial configuration in the allotypic specificities.

INTRODUCTION

The immunoglobulin heavy (H) chain genes consist of four linked families of gene segments : the variable (VH), diversity (D), joining (JH) and constant (CH) gene segments. The human heavy chain locus has been mapped on chromosome 14 (1,2) and the order of the CH genes has been determined as 5'- μ -4.5kb- δ -...- γ 3-26kb- γ 1-19kb- $\psi\epsilon$ 1-13kb- α 1-...- $\psi\gamma$ -...- γ 2-18kb- γ 4-23kb- ϵ -10kb- α 2 (3-8). Most of the human genomic CH genes have been completely sequenced : γ 1 (9), γ 2 (5), γ 4 (10), ϵ (11,12), $\psi\epsilon$ 1 (12), α 1 and α 2 (13). Only the hinge region of C γ 3 has been determined (4,14).

Of all the immunoglobulin CH genes, the most polymorphic is the γ 3 gene. In the present study, we describe the first protein and nucleotide complete sequence of a γ 3 heavy chain constant region gene expressing the G3m(b) allotype. We compare the four human gamma nucleotide sequences and discuss our results in term of evolution and gene correction in the human multigene family.

MATERIALS AND METHODSConstruction and screening of a phage library from EZZ DNA

EZZ is a Tunisian (individual II-4 of the TOU family (7,8)) shown to be

homozygous for the G3m(b) allotypes (8) by testing of IgG allotypic markers by the haemagglutination inhibition method. In this individual both chromosomes 14 display a large deletion encompassing the C γ 1, ψ c1, C α 1, ψ γ , C γ 2 and C γ 4 genes (7,8). Therefore, the only C γ present is the C γ 3 gene.

An Epstein-Barr (EB) virus transformed B cell line was obtained by infection of peripheral blood B lymphocytes (15) with EB virus derived from the B95-8 cell line (16). The EZZ DNA was partially digested with MboI and size-fractionated on a sucrose gradient. Restriction fragments of 15-20 kb were ligated into BamHI digested λ 2001 (17) and packaged in vitro.

Recombinant phages were screened according to the in-situ plaque hybridization procedure (18). The probe, radioactively labelled with α -³²P-dATP and α -³²P-dCTP by nick-translation (19), was the C γ 4 probe pBRH 4.1 (4) which detects all C γ subclass genes. All the recombinant positive clones isolated from the EZZ phage library were shown to contain the C γ 3 gene as predicted.

Subcloning and sequencing strategies

Among the λ recombinant clones hybridising with the C γ probe pBRH4.1, one clone containing a 19 kb piece of genomic DNA (λ EZZ γ 3) (figure 1) was selected for shotgun subcloning into pBR322. From these subclones, two fragments were isolated, a 900 bp HindIII-SstI and a 2 kb BglII-SphI which contain the C γ 3 gene (figure 1). These inserts were shortened using the exonuclease III (Genofit) and nuclease S1 (BRL, Boehringer) method as described (20), then subcloned in M13 (mp 10, mp 11, mp 19). The C γ 3 hinge region consists in a tandem repeat of four highly homologous intron-exon blocks. Therefore, in order to assess rapidly each reading to its genomic location, M13 ssDNA carrying shortened inserts were hybridised with M13 ssDNA carrying the full length fragment in reverse orientation, then treated with S1 nuclease (21). The S1 nuclease resistant DNAs were sized on 1% agarose slab gel. The nucleotide sequences were derived using the dideoxy chain termination procedures (22).

Data were treated using the BISANCE system from the CITI2 (Centre interuniversitaire d'informatique à orientation biomédicale, PARIS).

RESULTS AND DISCUSSIONS

Organisation and sequence of a human C γ 3 gene

One clone (λ EZZ γ 3) containing a 19 kilobases piece of genomic DNA was isolated and selected for characterisation. A restriction map of this clone is shown in figure 1. The complete nucleotide sequence of the C γ 3 gene is

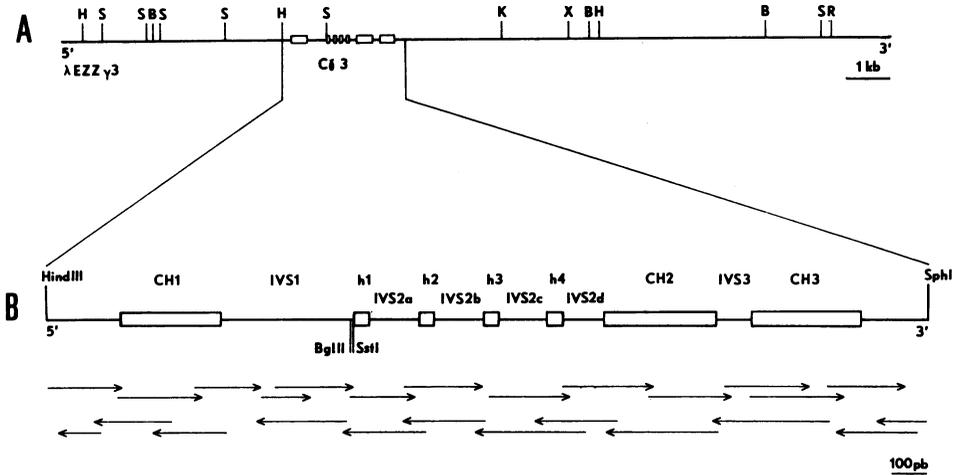


FIGURE 1 - Structure of a human $\gamma 3$ gene and DNA sequencing strategies

A - Restriction map of a cloned human DNA fragment (λ EZZ $\gamma 3$) containing a $C\gamma 3$ gene. Letters on the top refer to cleavage sites for the following restriction enzymes : B, BamHI ; H, HindIII ; K, KpnI ; R, EcoRI ; S, SstI.

B - Sequencing strategies for the $C\gamma 3$ gene. Arrows indicate the location and extent of each sequence reading.

Exons are shown as boxes. Introns (or intervening sequences) correspond to IVS1, IVS2a, b, c, d, IVS 3.

presented in figure 2. The sequence (2590 bp) begins 215 nucleotides 5' to the CH1 coding region and continues 207 residues past the termination codon. The organization of the gene is typical of the other gamma genes in that each domain of the protein (CH1, CH2, CH3) and its hinge (h) are coded on separate exons. However, the hinge coding region of the $\gamma 3$ is made of four distinct exons separated from each other by short introns. This arrangement is distinct from the other genes in that these genes possess only one hinge exon. This has been proposed, first, from the aminoacid sequences (23) and later demonstrated from the nucleotide sequences (4,14).

The CH domain boundaries were assigned by analogy with the other gamma genes. The splice junction sequences (24) are present at the expected positions and match perfectly to the consensus AG/GUPu (5' donor) and (Py)_n XCAG/X (3'acceptor), except the 5' donor of the CH1 exon which is UG/GUPu. Table I compares the length of the exons and introns of the four human $C\gamma$ genes. In figure 2, the predicted aminoacid sequence of the $\gamma 3$ heavy chain is presented beneath the coding sequence. Before the stop codon, the

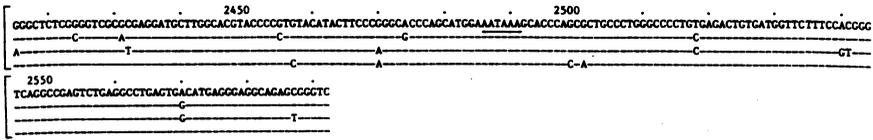


FIGURE 2 - Sequence of a human immunoglobulin $\gamma 3$ constant region gene and alignment with the $C\gamma 1$, $C\gamma 2$ and $C\gamma 4$ genes

The sequence of the $\gamma 3$ EZZ gene is given in full. Deduced aminoacid sequence from the $\gamma 3$ gene are shown above the nucleotide sequence (the letter is above the first nucleotide of a codon). The $\gamma 3$ gene sequence was aligned with the $\gamma 1$, $\gamma 2$, and $\gamma 4$ sequences, except in the hinge region.

Dashes represent identity of the $C\gamma 3$ gene to the other $C\gamma$ sequences ; where differences occur, the $C\gamma 1$, $C\gamma 2$ and $C\gamma 4$ sequences are indicated. A total of 2590 nucleotide positions are compared, including gaps introduced into the genes to maintain sequence homology. These gaps are indicated by asterisks. The presumptive poly(A) addition signal sequence is underlined. An asterisk at the end of the deduced protein sequence indicates the terminal codon.

nucleotide sequence of the human $\gamma 3$ encodes an additional lysine not present in any of the published γ aminoacid sequences. This COOH-terminal lysine has been found in the nucleotide sequences of the human (5,9) and mouse γ genes (25-28) and in the cDNA of a γ heavy chain disease (OMM) (29). All the $\gamma 3$ protein sequences were performed using Edman's procedure , from tryptic fragments. Thus, this terminal lysine is the last aminoacid to be sequenced and might have been missed because of modifications that alter its behaviour during the chromatographic step. Alternatively, this lysine might be removed

TABLE I - Exon and intron lengths in human $C\gamma$ genes

Cygenes	References	CH1	IVS1	Hinge exon(s)	IVS2	CH2	IVS3	CH3
$C\gamma 1$	9	294	388	45	118	330	96	321
$C\gamma 2$	5	294	392	36	118	327	97	321
$C\gamma 3$	Present study	294	391	51 45 45 45	143 143 143 118	330	97	321
$C\gamma 4$	10	294	390	36	118	330	97	321

The exons are represented by CH1, CH2, CH3 and the hinge exons.

The four numbers for the hinge exons of the $C\gamma 3$ gene correspond respectively to h1, h2, h3 and h4 in the figure 1.

The introns or intervening sequences are represented by IVS1 (CH1-hinge intron), IVS2 (hinge-CH2 intron), IVS3 (CH2-CH3 intron).

For the $C\gamma 3$ gene, the four numbers in the IVS2 column correspond respectively to IVS2a - IVS2b - IVS2c - IVS2d in the figure 1.

from the IgH chains post-synthetically, although no such feature was hitherto reported. The hexanucleotide A-A-T-A-A-A thought to be a signal for polyadenylation (30) is identically positioned in all sequences (figure 2), 103 nucleotides 3' to the translation stop codon.

Nucleotide sequence comparison between the C γ genes

In figure 2, the nucleotide sequences of the human C γ 3 (present study), C γ 1 (9), C γ 2 (5) and C γ 4 (10) are aligned for direct comparison. Only the C γ 3 hinge is shown since the hinges of the different C γ have already been compared (14).

Table II lists the percentage of nucleotide differences in the various coding and non-coding segments of the four human C γ genes. First, this confirms the high degree of homology of the four human C γ genes. The percentage of homology at the nucleotide level for the coding regions is respectively 97% (γ 3/ γ 1), 96.4% (γ 3/ γ 2), 95.3% (γ 3/ γ 4), 96% (γ 1/ γ 2), 95.5% (γ 1/ γ 4) and 95.8% (γ 2/ γ 4). Mouse C γ genes share significantly less homology (75-85%) (31,32). Secondly, the number of substitutions which alter the aminoacid sequence (replacement site substitutions) exceeds the number of silent substitutions. This could be the result of a pressure of natural selection in favor of divergence of the very closely related polypeptides encoded by these genes. Thirdly, the percentage of homology in the non-coding areas is quite high (93.3-95%) and very close from that observed in coding regions (95.3-97%). At last, the hinge exons are only about 70% to 85% homologous, thus are far more divergent than any of the other exons or introns; up to 85% of the total differences are replacement site substitutions. Several explanations may be proposed: non-conservative changes in the hinge region could be fixed rapidly throughout the populations as a result of a positive selection. Indeed, it has been previously suggested that changes in hinge sequences may be responsible for the differences in effector functions of the IgG subclasses (9). Alternatively, the hinge regions could be free to diverge. In this case, the process of fixation of mutations in CH exons and introns would be lowered, either by a negative selection, either by gene correction mechanisms. A more detailed analysis of molecular evolution of the C γ family will be presently published (S.Huck, in preparation).

γ 3 EZZ predicted amino-acid sequences

The heavy chain disease γ 3 proteins (WIS (33), ZUC (34), OMM (29)) exhibit a complete deletion of the CH1 domain, while CH1 sequences from myeloma proteins (GOE (35) and JIR (36)) have not been determined (only ten aminoacids have been determined for the KUP and BRU myeloma proteins

TABLE II - Nucleotide sequence comparison of the four human γ genes

		% nucleotide differences					
		$\gamma 3/\gamma 1$	$\gamma 3/\gamma 2$	$\gamma 3/\gamma 4$	$\gamma 1/\gamma 2$	$\gamma 1/\gamma 4$	$\gamma 2/\gamma 4$
5' flanking region (215)		8.4	5.6	4.7	6.5	7.0	5.1
CH1 (294)	Silent	0.7	2.0	1.7	2.0	2.4	1.7
	Replacement	1.4	2.0	1.4	3.1	2.7	1.4
	Total	2.1	4.0	3.1	5.1	5.1	3.1
IVS1 (392)		6.9	4.8	5.9	5.9	7.7	5.1
hinge		hl : hc	hl : hc	hl : hc			
	Silent	0 : 4.4	5.3 : 5.4	2.6 : 8.1	2.7	2.7	3.0
	Replacement	13.0 : 11.1	18.4 : 16.2	21.0 : 13.5	10.8	16.2	15.1
	gaps	2.2 : 0	5.3 : 2.7	5.3 : 2.7	2.7	2.7	9.1
	Total	15.2 : 15.5	29.0 : 24.3	28.9 : 24.3	16.2	21.6	27.2
IVS2 (a:121/d:118)		a : d	a : d	a : d			
		10.7 : 5.1	10.7 : 6.8	9.9 : 6.8	0.9	2.5	3.4
CH2 (330)	Silent	2.4	1.2	3.2	2.4	3.0	3.0
	Replacement	1.2	3.0	2.7	3.0	2.1	3.3
	Total	3.6	4.2	5.9	5.4	5.1	6.3
IVS3 (97)		9.3	3.1	6.2	6.2	7.2	5.2
CH3 (321)	Silent	0.9	0.9	1.9	0.6	1.6	1.6
	Replacement	2.5	1.6	3.1	0.9	1.9	1.6
	Total	3.4	2.5	5.0	1.5	3.4	3.2
3' flanking region (210)		3.8	4.8	3.3	5.7	6.2	5.2
Total CH exons (hinge exons excluded)							
Silent		1.3	1.4	2.3	1.7	2.3	2.1
Replacement		1.7	2.2	-2.4	2.3	2.2	2.1
Total		3.0	3.6	4.7	4.0	4.5	4.2
Total non coding areas (IVS2 a-b-c-excluded)		6.7	5.0	5.4	5.0	6.1	4.8

Percentage of nucleotide differences = number of differences (substitutions and gaps) / number of residues compared x 100 ; gaps in the alignment are counted as single differences.

The number of residues compared are shown into brackets. For the coding regions, we calculated the percentage of nucleotide differences leading to synonymous amino acid (silent changes) and to different amino acid (replacement). For the hinge exons, gaps were calculated separately and were counted as single differences. We compared separately h1 (first exon of the $\gamma 3$ hinge) and hC (a consensus of h2, h3, h4) with the other γ hinge exon. The $\gamma 3$ IVS2a and IVS2d introns (see figure 1) were compared separately with the other γ IVS2 intron.

(37)). Therefore, the $\gamma 3$ EZZ amino-acid sequence deduced from the nucleotide sequence represents the first complete sequence of a $\gamma 3$ CH1 region (figure 2).

The constant region of the human $\gamma 3$ heavy chain carries antigenic determinants or G3m allotypes, which are genetic markers of this subclass, the G3m(b) and G3m(g) chains being the most common in caucasoid populations. The γ gene we sequenced code for a G3m(b) $\gamma 3$ chain EZZ, carrying the Gm (b=b0,b1,b3,b4,b5) and Gm(u,v) allotypes on the CH2 and CH3 domains (7,8). We therefore compared the deduced aminoacid of this gene with the G3m(b) $\gamma 3$ WIS (33) and ZUC (34), as well as with the G3m (g=g1,g5) and Gm(u,v) OMM (29) proteins and with the two $\gamma 3$ myeloma proteins carrying allotypic specificities found primarily in mongoloïd populations: GOE, G3m (b0,b3,b5,s,t,v) (35) and JIR, G3m (b0,b3,b5,s,t) (36). Table III shows the aminoacid differences between these $\gamma 3$ proteins as well as between the $\gamma 1$ (38,39), $\gamma 2$ (40-42) and $\gamma 4$ (43) chains. The predicted protein sequence of $\gamma 3$ EZZ agrees with the WIS sequence at all but four positions in the CH2

TABLE III - Amino-acid differences in the constant region of the human chains

Domain	EU index	γ^1 EU, NIE, CL	γ^2 ZIE, TIL, FIG, CL	γ^3			γ^4			
				EZZ	WIS	ZUC	GOE	JIR	OMM	VIN, CL
CH1 (118-215)	131	Ser*	Cys	Cys	-	-	ND	ND	-	Cys
	133	Lys*	Arg	Arg	-	-	ND	ND	-	Arg
	137	Gly	Glu	Gly	-	-	ND	ND	-	Glu
	138	Gly	Ser	Gly	-	-	ND	ND	-	Ser
	177	Ser	Ser/Ala(TIL)	Ser	-	-	ND	ND	-	Ser
	192	Ser	Asn*	Ser	-	-	ND	ND	-	Ser
	193	Leu	Phe*	Leu	-	-	ND	ND	-	Leu
	196	Gln	Gln	Gln	-	-	ND	ND	-	Lys*
	199	Ile*	Thr	Thr	-	-	ND	ND	-	Thr
	203	Asn	Asp	Asn	-	-	ND	ND	-	Asp
214	Lys/Arg(EU)	Thr	Arg	-	-	ND	ND	-	Arg	
CH2 (231-340)	233	Glu	-*	Glu	Glu	Glu	ND	ND	Glu	Glu
	234	Leu	Pro	Leu	Leu	Leu	ND	ND	Leu	Phe
	235	Leu	Val*	Leu	Leu	Leu	ND	ND	Leu	Leu
	236	Gly	Ala*	Gly	Gly	Gly	ND	ND	Gly	Gly
	268	His	His	His	His	His	His	His	His	Gln*
	274	Lys*	Gln	Gln	Glx	Glx	Gln	Gln	Gln	Gln
	276	Asn	Asn	Lys*	Lys*	Lys*	Lys*	Lys*	Lys*	Asn
	291	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Leu	Pro
	296	Tyr	Phe	Tyr	Phe	Phe	Tyr	Tyr	Tyr	Phe
	300	Tyr	Phe	Phe	Phe	Phe	Phe	Phe	Phe	Tyr
	309	Leu	Val*	Leu	Leu	Leu	Leu	Leu	Leu	Leu
	327	Ala	Gly	Ala	Ala	Ala	Ala	Ala	Ala	Gly
	330	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ser*
	331	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Ser*
339	Ala	Thr	Thr	Thr	Thr	Ala	Thr	Ala	Ala	
CH3 (341-446)	355	Arg	Arg	Arg	Arg	Arg	Arg	Arg	ND	Gln*
	356	Asp/Glu(EU)	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu
	358	Leu/Met(EU)	Met	Met	Met	Met	Met	Met	Met	Met
	379	Val	Val	Val	Val	Val	ND	Met	Val	Val
	384	Asn	Asn	Ser	Ser	ND	ND	Ser	Asn	Asn
	392	Lys	Lys	Asn	Asn	ND	ND	Lys	Asn	Lys
	397	Val	Met	Met	Met	ND	ND	Val	Met	Val
	409	Lys	Lys	Lys	Lys	Lys	ND	Lys	Lys	Arg*
	419	Gln	Gln	Gln	Glx	Glx	Glu	Gln	Gln	Glu
	422	Val	Val	Ile*	Ile*	Ile*	Ile*	Ile*	Ile*	Val
	430	Glu	Glu/Gly(FIG)	Glu	Glu	Gly	Glu	Glu	Glu	Glu
	435	His	His	Arg	Arg	Arg	His	His	Arg	His
	436	Tyr	Tyr	Phe	Phe	Phe	Tyr	Tyr	Tyr	Tyr
445	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Leu*	

- 1 : EU (38), NIE (39), γ^1 CL (9)
- 2 : ZIE (40), TIL (41), FIG (42), γ^2 CL (5)
- 3 : EZZ (this study), WIS (33), ZUC (34), GOE (35), JIR (36), OMM (29)
- 4 : VIN (43), γ^4 CL (10)

CL = deduced amino acid sequences from nucleotide sequences of clones of genomic DNA. Differences in amide assignment probably due to technical problems are not indicated. Dashes indicate that no aminoacid is present at that position, the CH1 domain being deleted in the γ^3 HCD proteins WIS, ZUC and OMM. N.D. = not determined

Only the CH3 domain of the FIG protein has been determined.

Asterisks correspond to amino acids which characterize a subclass. In the case of the γ^3 chain, they are lysine at position 276 and isoleucine at position 422.

Some G3m allotypes have been related, to aminoacids. Histidine at position 435 of a γ^3 chain (36) and methionine at position 379 (36) have been postulated for G3m(s) and (t).

Our own results suggest that the residues 291 and 384 might be involved in the Gm(b) and Gm(g) allotypes, respectively Proline-Serine for Gm(b) and Leucine-Asparagine for Gm(g).

domain. Three of these are differences in amide assignment. We found a glutamic acid codon for positions 283 and 294 (EU index) (38) and an asparagine codon for position 315 whereas in the WIS sequence, there are respectively glutamine and aspartic acid. These differences are probably due to technical problems of protein sequencing. The major difference is at position 296 where unexpectedly we found a tyrosine for $\gamma 3$ EZZ instead of a phenylalanine (WIS protein). This finding contradicts previous reports ascribing some of the G3m(b) allotypic specificities to a phenylalanine residue at position 296 (44). It also confirms that a tyrosine cannot be related to the G3m(g) allotype since this aminoacid is found on the Gm(b) EZZ IgG3 as well as on the Gm(g) OMM and Gm(g-) GOE and JIR proteins (35,36).

Comparison of $\gamma 3$ G3m(b) EZZ and $\gamma 3$ G3m(g) OMM show four aminoacid changes on the CH2 and CH3 domains : respectively Pro/Leu in 291, Thr/Ala in 339, Ser/Asn in 384 and Phe/Tyr in 436. The GOE and JIR proteins, both G3m(g-), and OMM protein, G3m(g+) show the same residue in 339 (Ala) and 436 (Tyr) suggesting that the allotypes Gm (g and b) might depend from the other two residues in 291 and 384, respectively Leu and Asn for G3m(g) and Pro and Ser for G3m(b). More $\gamma 3$ genes or protein sequences are necessary to confirm the assignment of allotypic specificities to given amino-acids. However, the large number of allotypic specificities and the relatively small number of aminoacid differences between the $\gamma 3$ chains suggest that allotypic specificities must depend of the secondary and tertiary structures of the proteins.

CONCLUSION

Comparisons of the human C γ genes show an extensive degree of homology which might be explained not only by a relatively recent duplication of the locus (6) but also by mechanisms of gene correction and transfer of DNA between these genes belonging to a same family. Gene conversion, which has been proposed to explain both the maintenance of homology between non-allelic genes and the generation of diversity at one locus of multigene families (45), must play an important part in the evolution of the human immunoglobulin family as this has already been reported for the VK (46), C α (47) and C γ (48) genes. The situation in the human C γ genes contrasts however with other systems such as the M.H.C. class I genes (49) where the conversion events occur preferentially in exons whereas the intron sequences seem to have evolved by mutational drift. In the human C γ locus, both introns and exons of non-allelic genes share a high degree of homology, the hinge being

the only region which has evolved by insertions, deletions and mutations. Concerted evolution of the C γ locus concern coding and non-coding areas as well, at the exception of the hinge region. This emphasizes the role played by this structure in the different effector functions of the IgG subclasses.

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