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Duplication of the Human Immunoglobulin Heavy Chain Gamma$_2$ Gene

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SUMMARY

The five $C\gamma$ genes in the human immunoglobulin heavy chain region show nonrandom association and segregation as haplotypes. From the study of genetic variation in $C\gamma$ genes of 58 healthy Caucasian volunteers, we have identified a haplotype that involves a duplication of $C\gamma 2$. This haplotype contains both the 13.5-kilobase (kb) and 25-kb *Bam*HI fragment alleles of $C\gamma 2$. In addition, the patterns and relative intensity of *Bam*HI fragments containing $C\gamma$ genes were those expected for genomic DNA containing three copies of $C\gamma 2$ for every two copies of the four other $C\gamma$ genes. A new *Eco*RI polymorphism in $C\gamma 4$ was useful in defining the haplotype containing the duplication. Alleles of the $C\gamma$ genes in the duplication haplotype, including Gm markers of $C\gamma 1$ and $C\gamma 3$ and DNA polymorphisms of $C\psi\gamma$, $C\gamma 2$, and $C\gamma 4$, were consistent with its origin from an unequal crossover between the two common $C\gamma$ haplotypes, *H1* and *H2*. This recombinant haplotype, which has been designated *H2;1(γ2dup)* to reflect its origin, occurred with a frequency of .043 in a random sample of 116 chromosomes.

INTRODUCTION

Human immunoglobulin gamma (γ), epsilon (ε), and alpha (α) genes, determinants of the constant (C) region of the heavy chains for IgG, IgE, and IgA, respectively, are organized in two gene clusters. The $C\gamma 3$-$C\gamma 1$-$C\psi\epsilon$-$C\alpha 1$ cluster is located upstream (5′) of $C\gamma 2$-$C\gamma 4$-$C\epsilon$-$C\alpha 2$ [1] and a pseudo γ gene, $C\psi\gamma$, lies

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between these clusters in tight association with *Cγ2* [2]. This genomic arrangement within the IgH region has apparently evolved through a series of duplications [1]. Extensive serological testing has identified several *rare* duplications and deletions of IgH genes, indicating that subsequent unequal crossing-over events have occurred in this genomic region [3, 4]. The nonrandom association of alleles (linkage disequilibrium) for most IgH genes has resulted in preferred combinations of alleles referred to as IgH or *Cγ* haplotypes [2]. Two haplotypes (*Cγ-H1* and *Cγ-H2*) predominate, accounting for 90% of Caucasian *Cγ* haplotypes. We now report the identification of a haplotype that contains a duplication of *Cγ2* and occurs at an estimated frequency of .043 in the Caucasian population. The *Cγ* haplotype contains the two known DNA fragment alleles (13.5 and 25 kb) of *Cγ2* and could have arisen from unequal crossing-over between the two common *Cγ* haplotypes.

MATERIALS AND METHODS

Blood samples (10–40 ml) were obtained from healthy Caucasian volunteers including several families as described [2]. The samples were collected in EDTA or heparin and enriched for leukocytes either (1) by selectively collecting the white cell layer following centrifugation or (2) by a method of red cell lysis using 5 vol of a solution containing 0.14 M NH_4Cl and 17 mM Tris HCl, pH 8.0, and pelleting of the nonlyzed cells (D. Hoar, Calgary, personal communication, 1983). Such preparations of cells were mixed or suspended in 100 mM Tris-HCl, pH 8.0/10 mM EDTA (TE) and lyzed by sodium dodecyl sulfate (0.5%). High molecular weight DNA was extracted by a standard phenol (TE-buffered) and chloroform-isoamyl alcohol (24:1, v:v) method, as described [5].

Blot hybridization analysis of genomic DNA was performed as described [2, 5]. DNA probe 24BRH was a 2-kb *Hin*dIII-*Eco*RI fragment containing the coding sequence for *Cγ4* [6, 7].

Gm allotypes (*a, f, b, x,* and *g*) were determined by hemagglutination inhibition tests with typing reagents provided by Schanfield [8].

RESULTS

Cγ-coding sequence probes identify from five to eight *Bam*HI fragments in human genomic DNA [2, 5]. Of the eight fragments, two are invariant and six represent the polymorphic alleles of three different *Cγ* genes [2]. The DNA fragments segregate as five-fragment haplotypes, which in Caucasian samples most frequently result in two homozygote five-band patterns, *H1/H1* (13.5, 12.5, 11.8, 9.4, 8.8 kb) and *H2/H2* (25, 12.5, 11.8, 10.0, 9.0 kb) or *a/a* and *b/b* (fig. 1, lanes 1 and 2) and an eight-band heterozygote pattern, *H1/H2* or *a/b* (fig. 1, lane 3) [2]. Two less frequent *Cγ* haplotypes, *H3* (13.5, 12.5, 11.8, 9.0, 8.8 kb) and *H4* (25, 12.5, 11.8, 10.0, 9.4 kb) or *c* and *d,* have also been observed [2].

In blot hybridization analyses to investigate DNA polymorphisms and organization of the human IgH region [2], we noted a band pattern in some *Bam*HI-digested DNA samples that was apparently the combination of a five-fragment (*H2*) and a previously unrecognized haplotype. Specifically, a seven-band *Bam*HI fragment pattern having all *Cγ* bands except for the 8.8-kb *Cψγ* band (fig. 1, lane 4) was observed in one parent's DNA in each of two Caucasian families (M and Z). Although the seven-band pattern was interpretable as the combination of five-fragment haplotypes, possible haplotype combinations

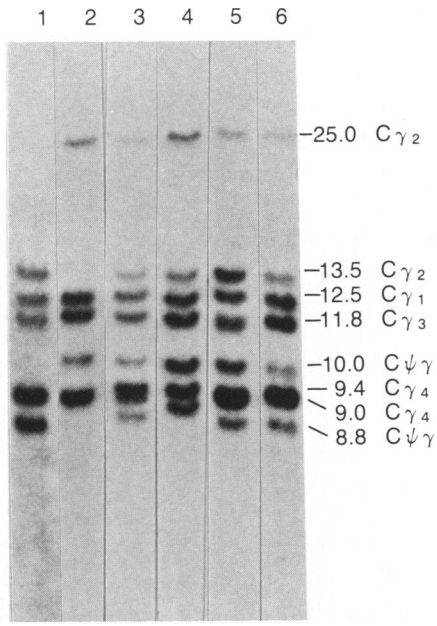

FIG. 1.—*Cγ* gene hybridization patterns. DNAs from peripheral blood leukocytes digested with *Bam*HI were analyzed by blot hybridization analysis using the probe 24BRH. *Cγ* gene assignment to specific *Bam*HI fragments was established previously [2, 5]. Examples of the following haplotype combinations are shown: *lane 1, H1/H1* (13.5-, 12.5-, 11.8-, 9.4-, and 8.8-kb bands); *lane 2, H2/H2* (25-, 12.5-, 11.8-, 10.0-, and 9-kb bands); *lane 3, H1/H2* (25-, 13.5-, 12.5-, 11.8-, 10.0-, 9.4-, 9.0-, and 8.8-kb bands); *lane 4, H2/'γ2 dup'* (25-, 13.5-, 12.5-, 11.8-, 10.0-, 9.4-, and 9.0-kb bands); *lane 5, H1/'γ2 dup'* (25-, 13.5-, 12.5-, 11.8-, 10.0-, 9.4-, and 8.8-kb bands); *lane 6, H1/H4* (25-, 13.5-, 12.5-, 11.8-, 10.0-, 9.4-, and 8.8-bands).

did not explain the band patterns for the *Cγ2* alleles of all the offspring in one of these families (family M) without multiple meiotic recombinations. However, segregation of haplotypes within family M (maternal and paternal band patterns shown in fig. 1, lanes 4 and 6, respectively) could readily be defined if the maternal genotype were interpreted as the combination of the five-fragment *Cγ-H2* haplotype, which contains the 25-kb *Cγ2* allele, and a six-fragment haplotype consisting of both *Cγ2* fragment alleles (25 and 13.5 kb) and the 12.5-, 11.8-, 10-, and 9.4-kb fragments. In support of this explanation, it was noted that both families' unusual parental band patterns showed a 13.5-kb band of half-intensity compared to the 12.5- and 11.8-kb bands and an abnormally heavy 25-kb band for a possible *Cγ2* heterozygote, which appeared more like that seen in a homozygous *H2/H2* pattern (e.g., fig. 1, lane 2). Stated parentage in both families was supported by serum protein typing.

The segregation of the putative *Cγ2* duplication haplotype in family M is illustrated in table 1. Three of the four offspring received the maternal six-fragment haplotype containing both of the *Cγ2* DNA fragment alleles. Offspring 1 showed a band pattern missing the 9.0-kb and 8.8-kb bands, reflecting the *Cγ2* duplication haplotype in combination with the *H4* haplotype. Two

TABLE 1

SEGREGATION OF *Bam*HI SIX-FRAGMENT $C\gamma$ HAPLOTYPE TO OFFSPRING IN FAMILY M

	HAPLOTYPES				
	PATERNAL		MATERNAL		$C\gamma$ DNA FRAGMENT HAPLOTYPE
OFFSPRING	GM	*Bam*HI fragments*	GM	*Bam*HI fragments	COMBINATIONS†
1	*axg*	11.8,12.5,10,25,9.4	*axg*	11.8,12.5,10.25,13.5,9.4	*H4/H2;1(γ2dup)*
2	*axg*	11.8,12.5,10,25,9.4	*fb*	11.8,12.5,10,25,9.0	*H4/H2*
3	*fb*	11.8,12.5,8.8,13.5,9.4	*axg*	11.8,12.5,10,25,13.5,9.4	*H1/H2;1(γ2dup)*
4	*fb*	11.8,12.5,8.8,13.5,9.4	*axg*	11.8.12.5,10,25,13.5,9.4	*H1/H2;1(γ2dup)*

* The fragment alleles are ordered according to the known genetic map of $C\gamma$ genes [1, 2]; duplicated $C\gamma 2$ genes are underlined.

† *BamHI* $C\gamma$ haplotype designations *H1*, *H2*,and *H4* correspond to the notation *a*, *b* and *d*, respectively, used previously [2]. *H2;1(γ2dup)* is used to indicate the composite nature of the six-fragment haplotype that contains both fragment alleles of $C\gamma 2$ (see also fig. 2).

offspring had a seven-band $C\gamma$ pattern missing only the 9.0-kb band (fig. 1, lane 5). This pattern was different from the paternal *H1/H4* haplotype combination (lane 6) only in that the intensity of the 13.5-kb band was equal to that of the two invariant 12.5-kb and 11.8-kb bands (fig. 1 and table 2), which suggested the presence of two 13.5-kb $C\gamma 2$ alleles and one 25-kb $C\gamma 2$ allele, consistent with the transmission of the $C\gamma 2$ duplication and *H1* haplotypes to these two individuals.

Our initial dosage interpretation of $C\gamma 2$ from visual inspection of band intensities was confirmed by densitometry scanning of autoradiographs (table 2). In the homozygotes *H1/H1* and *H2/H2* and heterozygote (*H1/H4*) combinations, $C\gamma 2$ was diploid (1:1), while in haplotype combinations of the putative $C\gamma 2$

TABLE 2

QUANTITATIVE ANALYSIS OF $C\gamma 2$ ALLELES AS *Bam*HI FRAGMENTS

HAPLOTYPE COMBINATIONS	No.*	NORMALIZED PEAK AREA RATIOS†	
		Expected	Observed
H1/H1	3	0:2	0:2.0
H2/H2	3	2:0	2.0:0
H1/H2	9	1:1	1:1
H1/H4	2	1:1	1.0:1.0
H1/H2;1(γ2 dup)	5 (2)	1:2	1.0:2.4
H1/H2;1(γ2 dup)	8 (5)	2:1	1.7:1.0

* Total no. samples from gel with specified diplotypes; nos. individuals from families M and Z are indicated in parentheses.

† Autoradiograms were scanned with a transmittance densitometer; peak areas were determined by weighing and were normalized for recovery relative to the equivalent of one copy of the 11.8-, 12.5-kb peaks (i.e., one-quarter of their combined area). Relative peak area ratios were calculated by setting the band intensities for the 25-kb and 13.5-kb alleles in the *H1/H2* diplotypes equal to 1 in each of the data sets and adjusting the band intensities of all other diplotypes accordingly. Ratios are shown as 25-kb:13.5-kb fragment alleles.

duplication with either *H1* or *H2*, *Cγ2* was apparently triploid (1:2.4 and 1.7:1.0, respectively). The deviation from the expected 1:2 and 2:1 ratios of relative peak areas for 25-kb and 13.5-kb alleles in the diplotypes involving *Cγ2* duplications could be attributed to the variable and reduced efficiency of transfer of the 25-kb fragment allele relative to the 13-5-kb allele.

Five independent examples of the *Cγ2* duplication haplotype were identified in a total of 58 clinically normal and unrelated individuals (116 chromosomes), giving a frequency of .043 in the Caucasian population studied. Two were found in combination with *H1* and three in combination with the *H2* haplotype.

As the *Cγ2* duplication haplotype contains the two different *Cγ2* alleles, its origin is consistent with an asymmetrical cross-over event between homologous chromosomes of haplotypes *H1* and *H2* (see fig. 2). This would, as observed, result in a composite haplotype where the region 5′ to the recombination site is of haplotype *H2* and 3′ is of haplotype *H1*. As the flanking *Cγ* alleles are consistent with this hypothesis, we have designated the new haplotype *H2;1(γ2dup)*. As there was no apparent change in the length of the *Cγ2* *Bam*HI fragments in DNA with the duplication (fig. 1), the putative recombination event must have occurred outside of the *Bam*HI fragments.

Analysis of Gm allotypes [8] (serological markers for *Cγ1* and *Cγ3*) in plasma from individuals with the *Cγ2* duplication was consistent, in four of five cases, with the *H2;1(γ2dup)* haplotype segregating with the *Glm* haplotype $Glm^{a,x}$,$G3m^{g}$. The remaining recombinant haplotype segregated with Glm^{a}, $G3m^{b}$, a *Gm* haplotype found only rarely in Caucasian populations. These results suggest that the H2 chromosome involved in the recombination event that generated the *Cγ2* duplication was of *Gm* type $Glm^{a,x}$,$G3m^{g}$. The one exception could represent either a similar but different duplication event or a subsequent recombination event leading to the exchange of Gm haplotype markers.

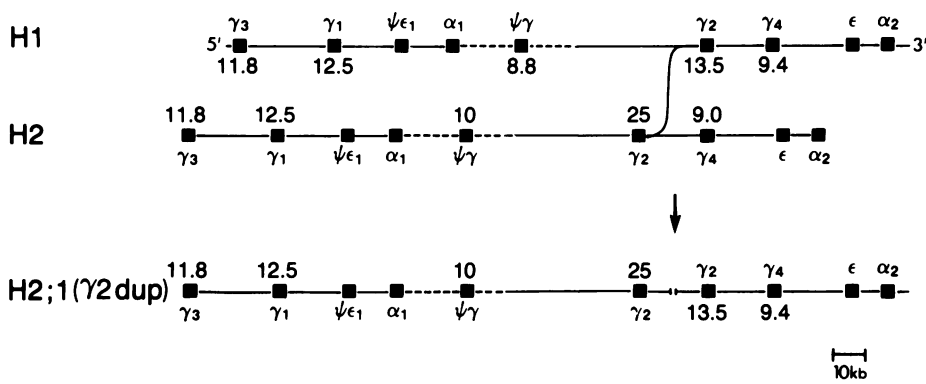

FIG. 2.—Unequal cross-over between *Cγ* haplotypes leading to *Cγ2* duplication. The organization of C_H genes is based on cloning [1, 6, 8] and analysis of linkage disequilibrium [2]. The distances between *Cα1*, *Cψγ*, and *Cγ2* are not known, although analysis of an approximately 45-kb, *Cγ2*-containing cosmid clone, provided by Dr. J. Ellison, would suggest that *Cψγ* lies at least 40 kb 5′ of *Cγ2* (our unpublished results, 1983). Haplotypes are distinguished by the polymorphic *Cγ* *Bam*HI fragments containing *Cψγ*, *Cγ2*, and *Cγ4*.

In experiments to define further the recombinant haplotype, a new *Eco*RI fragment length polymorphism specific to *Cγ4* was discovered. Allele frequencies were .75 and .25 (polymorphism information content, or PIC [9], 0.32) based on 53 of the 58 random samples originally used to determine *Cγ BamHI* haplotype frequencies [2]. *Eco*RI digestion of *H2/H2* DNA samples produced only the previously observed 17- to 26-kb *Cγ*-containing fragments [1, 10] (fig. 3, lane 3), while *Eco*RI digestion of *H1/H1* and *H1/H2* DNA samples routinely produced the 17- to 26-kb fragments along with two smaller fragments of 9 kb and 11 kb (fig. 3, lanes 1 and 2).

Assignment of this *Eco*RI polymorphism to *Cγ4* was established from the double digestion (*Eco*RI/*Bam*HI) of genomic DNAs representative of the common *Cγ* haplotype combinations. Blot hybridization analysis of such a digest with a *Cγ4* probe, 24BRH, produced *Cγ* fragment patterns that differed in two aspects from those produced by *Bam*HI digestion (e.g., fig. 1). First, the 25-kb *Cγ2* fragment allele was invariably shortened to 18 kb, and, second, the *Cγ4*-containing 9.4-kb fragment was frequently cut to appear as 5- and 4-kb fragments. However, only the *Eco*RI digestion of the 9.4-kb *Bam*HI fragment correlated with the presence of the *Eco*RI restriction site.

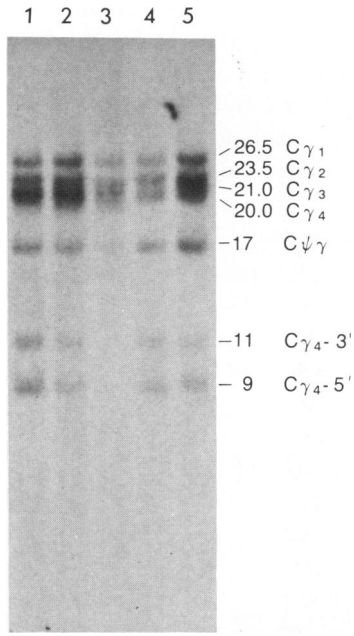

FIG. 3.—*Cγ* gene hybridization patterns. DNAs digested with *Eco*RI were analyzed by blot hybridization analysis using the probe 24BRH. Assignment of *Cγ* genes to *Eco*RI fragments was based on published restriction maps [1, 6, 7]. Furthermore, a 2.2-kb *Bam*HI-*Hin*dIII probe corresponding to the 5′ flanking ("switch") region of *Cγ4* [2, 5] failed to hybridize with the 17-kb *Eco*RI fragment which is consistent with this fragment containing *Cψγ*. The map relationship of the 9-kb and 11-kb *Eco*RI *Cγ4* fragments was deduced from the observation that the 5′ flanking region ("switch") probe recognizes the 9-kb but not the 11-kb fragment while a 3′ flanking *Cγ* probe [5] recognizes the 11-kb but not the 9-kb fragment. Examples of the following haplotype combinations are shown: *lane 1, H1/H1; lane 2, H1/H2; lane 3, H2/H2; lane 4, H1/′γ2 dup′; lane 5, H2/′γ2 dup′.*

All five $C\gamma 2$ duplication-containing DNA samples (e.g., fig. 3, lanes 4 and 5) showed the 11- and 9-kb *Eco*RI fragments, consistent with each of the *H2;1(γ2dup)* haplotypes carrying the *Eco*RI restriction site. Moreover, this observation suggests that the *H1* chromosome involved in formation of the $C\gamma 2$ duplication contained the newly identified $C\gamma 4$ *Eco*RI recognition site and that the five examples of the *H2;1(γ2dup)* haplotype likely had a common ancestry.

DISCUSSION

Our study provides direct evidence for a $C\gamma 2$ duplication in the human population. The haplotype carrying this gene duplication is noteworthy for its relatively high frequency, .043, of Caucasian $C\gamma$ haplotypes examined. As the $C\gamma 2$ duplication haplotype contains the two allelic forms of $C\gamma 2$ and flanking $C\gamma$ alleles consistent with a composite haplotype (fig. 2), an unequal cross-over between $C\gamma$ haplotypes *H1* and *H2* rather than a gene conversion could explain the origin of this gene duplication. Present information suggests that the cross-over event occurred in intergenic regions, between $C\gamma 2$ and $C\gamma 4$ on *H2* and $C\psi\gamma$ and $C\gamma 2$ on *H1* chromosomes. The specific site of this event should be identifiable as a novel fragment.

In one previously studied family [5], the segregation of $C\gamma$ *Bam*HI restriction fragments and the band intensity of $C\gamma 2$ alleles was consistent with another $C\gamma 2$ duplication in a six-fragment haplotype. The haplotype in this family, we suggest, was originally generated by an asymmetric cross-over between $C\gamma$ haplotypes *H2* and *H3*, yielding a putative recombinant haplotype, *H2;3(γ2dup)*, with the following allele arrangement: 5′-10 kb $C\psi\gamma$-25 kb $C\gamma 2$-13.5 kb $C\gamma 2$-9.0 kb $C\gamma 4$-3′. As the *Bam*HI $C\gamma 4$ allele in this haplotype was not cut by *Eco*RI and the Gm allotypes (Glm^a, $G3m^b$) were different from that of the *H2;1(γ2dup)* haplotype (our unpublished observation, 1984), the event that generated this other duplication haplotype was most likely distinct from the one detailed in the present report.

Observations using a probe for the switch (5′) region of $C\mu$ suggest that there also exists in Caucasians a haplotype that contains a $C\alpha 1$ duplication [11].

Unequal cross-over events leading to rare haplotypes have been implicated in several other locations in the human C_H gene cluster consistent with such a mechanism resulting both in expansion (duplications) and contraction (deletions) of this gene family [1, 3, 4, 12–14]. Unequal cross-overs are also known to occur in the mouse IgH region as illustrated by the presence of a duplication of $C\gamma 2b$ in the Japanese wild mouse [15]. Our present example of unequal crossing-over leading to the duplication of the human $C\gamma 2$ gene offers further evidence for the important role of recombination in facilitating the evolution of the human genome.

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