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Polymorphisms of a human variable heavy chain gene show linkage with constant heavy chain genes

(immunoglobulins/recombination/restriction fragment length polymorphisms)

M. J. JOHNSON*, A. M. NATALI*, H. M. CANN*, T. HONJO†, AND L. L. CAVALLI-SFORZA*

*Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305; and †Department of Genetics, Osaka University Medical School, Osaka 530, Japan

Contributed by L. L. Cavalli-Sforza, August 6, 1984

ABSTRACT **In this study, restriction fragment length polymorphisms (RFLPs) were identified with an immunoglobulin variable heavy chain region (Ig V_H) probe and the inheritance of the polymorphisms was analyzed in families. Linkage within the V_HII gene cluster and between the V_HII and Ig C_H genes was investigated by lod (logarithm of odds) score analysis. In addition, the position of the V_HII genes was determined in relation to another polymorphic locus—*D14S1*, which is tightly linked and centromeric to the C_H genes. Genetic associations between genes in the C_H and V_H clusters were analyzed. These RFLPs represent genetically characterized V_H region polymorphisms and it is hoped that they will facilitate the study of disease correlations as well as further the understanding of the genetics of the immunoglobulin heavy chain genes in humans.**

The immunoglobulins consist of four polypeptide chains, two heavy (H) and two light (L), each of which contains a variable (V) region-encoded amino terminus covalently attached to a constant (C) region-encoded carboxyl terminus. The V region polypeptides can be divided into functional segments categorized as framework regions (FR1–4) and hypervariable or complementarity-determining regions (CDR1–3). Each V_H polypeptide is composed of sequences encoded by three different genetic elements. These are (*i*) *V* region sequences coding for amino acids 1–98, which form FR1–3 and CDR1 and CDR2; (*ii*) diversity (*D*) region sequences coding for amino acids 99–103, which form CDR3; and (*iii*) joining (*J*) region sequences coding for amino acids 103–110, forming FR4 (1–3). A rearranged V_H sequence can be recombined subsequently to the 5′ end of any C_H subclass region sequence by a process known as class switching (4), which illustrates that a single V_H gene can be combined with any of the C_H genes (5, 6).

Antibody specificity is determined by genetic differences in the *V* region genes (idiotypes) (6–9). This idiotypic variation has been studied extensively in mice and rabbits. As early as 1963, antigenic determinants located on V regions of rabbit IgH chains were found (10). Pedigree analysis of rabbits homozygous for two different V_H region allotypes demonstrated that V_H and C_H region allotypes were linked, although recombinants were observed (11).

Mendelian studies involving idiotypic determinants of mice have determined that the Ig C_H and Ig V_H loci are also linked (9, 12–15), although separated by a substantial distance (2). Mouse strains of the same allotype have identical V_H gene families; however, in comparisons between inbred strains, each V_H gene family is comparable in number but not in the composition of the individual V_H genes (16).

Utilizing molecular data, it was shown that polymorphisms in V_H coding sequences are directly responsible for two major anti-arsonate idiotypes in A/J mice (17) as well as for different responses to proteins bearing the hapten (4-hydroxy-3-nitrophenyl)acetyl (18). Furthermore, a molecular genetic study of germline variation in six inbred mouse strains demonstrated that V_H gene restriction fragment length polymorphisms (RFLPs) are associated with particular allotypic variants (19).

Genetic studies involving *V* genes in humans have been hampered by the lack of any bonafide genetic markers for the Ig V_H loci. A purported antigenic determinant, Hv-1, was identified by using a hemagglutination inhibition assay system (20), but it did not show any association when tested for linkage with the C_H region (21). These results are at odds with current knowledge concerning the rabbit and mouse immunoglobulin genes.

In humans, three V_H gene families (I–III) have been defined on the basis of protein polymorphisms (22). The V_H genes have been localized to chromosome 14 by somatic cell hybrids (23) and several have been cloned and sequenced. Matthyssens and Rabbitts (24) found several instances in which two Ig V_HIII genes were found within a single phage, separated by 12–16 kilobases (kb) of spacer DNA (25). Rechavi *et al.* (26) isolated human Ig V_H genes representing family I protein sequences, several of which were also physically linked. Furthermore, a gene representing an Ig V_HII family sequence has been isolated (27) and was used in this study.

As with mice, there seem to be numerous V_H pseudogenes in humans. These genes do not show evidence of increased divergence, but rather one or two deleterious mutations define them as being pseudogenes (18, 28–32).

Molecular studies with these human V_H genes revealed that their structure is almost identical to the murine genes in that a 19 amino acid leader sequence is interrupted by an 84- to 103-base-pair (bp)-long intervening sequence at bp −5 to −4, followed by a 98 amino acid long V_H coding region. Again, the genes within a single family seem to be clustered together with an average of 14 kb between them, and genes of one family are not interspersed with members of other families (24, 26). An estimate of ≈20 genes per family was made based on Southern blot analysis of the V_HIII family (24). To date, the only evidence of polymorphisms in the human Ig V_H genes was a 7-kb *Bst*EII fragment found in one individual, but absent in two others (24).

The characterization of individual variation within the human Ig V_H gene clusters, utilizing restriction enzyme-generated polymorphisms, would therefore be important for (*i*) understanding the inheritance of the Ig V_H genes; (*ii*) clarifying the linkage relationship to the Ig C_H genes; (*iii*) examining the contribution of germline variation to the generation of

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Abbreviations: RFLP, restriction fragment length polymorphism; FR, framework region(s); CDR, complementarity-determining region(s); V, variable; C, constant; J, joining; D, diversity; H, heavy chain; L, light chain; kb, kilobase(s); bp, base pair(s).

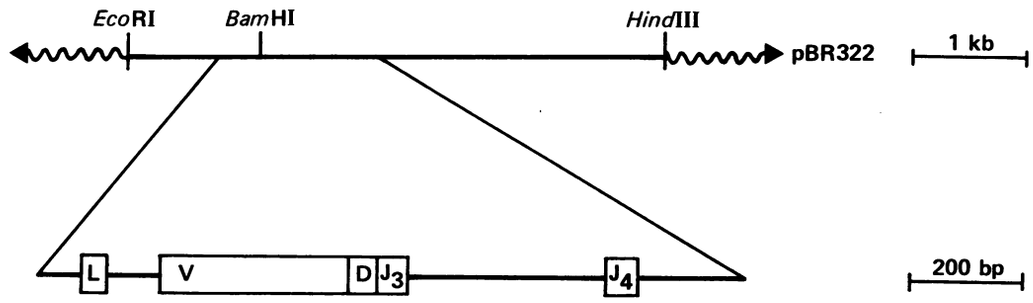

FIG. 1. Immunoglobulin V_HII gene.

antibody diversity; and (*iv*) elucidating possible correlations between V_H gene repertoires and certain autoimmune diseases.

MATERIALS AND METHODS

Sample Collection. Blood samples of 30 ml were drawn from a total of 160 Caucasian individuals representing 10 nuclear and 5 extended families (three or more generations). Cellular DNA was extracted from the buffy coats (33). All families had been typed previously for (*i*) *Sst* I switch region loci (35), (*ii*) *Mbo* I and *Bst*EII Ig G polymorphisms, and (*iii*) heavy chain allotypes (ref. 36; unpublished data).

DNA Probes. Probes used were Ig V_HII gene (27); pAW101, or *D14S1* in the Yale Human Gene Mapping Library (37, 38); and a J_H region sequence from a Charon 4A-Ig μ phage (39).

Restriction Analysis of Genomic DNA. Total cellular DNA (2–5 μg) was digested with restriction enzymes by using buffers recommended by the enzyme suppliers [Bethesda Research Laboratories and International Biotechnologies. Samples were analyzed by the Southern method (41) under conditions described previously (35).

Hybridization. Hybridizations were done with 10 ng of probe per ml of hybridization fluid, which contained 50% formamide, 5× concentrated SSPE buffer, 1× concentrated Denhardt's solution, 0.1% $NaDodSO_4$, and 100 μg of yeast RNA per ml. Filters were hybridized 36–48 hr at 42°C. Washes to remove nonspecifically bound probe were done in 0.1× concentrated SSPE buffer/0.1% $NaDodSO_4$ (two times for a total of 1 hr at 65°C). Autoradiography was done at −70°C using Kodak XAR-5 films and DuPont Lightning Plus intensifying screens.

Linkage Analysis. The computer program LIPED (42) was used to calculate lod (logarithm of odds) scores (43), except for those pedigrees involving more than four alleles. These were analyzed by hand.

RESULTS

RFLPs Identified With a *V–D–J* Probe. RFLPs for the V region were identified by screening normal individuals with a rearranged V_H gene clone from a subgroup II gene family (see Fig. 1). From a number of restriction enzymes tested, *Bgl* II was chosen, as multiple polymorphisms could be detected in a single experiment.

Four polymorphic fragments representing three distinct "loci" were detected. These are locus 1, a 12-kb fragment (scored as present or absent); locus 2, allelic fragments of 7.5 and 7.0 kb; and locus 3, a 3.4-kb fragment (scored as present or absent). The word "locus" should be considered as meaning segregating restriction fragment differences—synonymous with RFLP. Different combinations of these polymorphic loci resulted in six observed haplotypes out of the eight possible ones. Examples of several are shown in Fig. 2.

The gene frequencies were calculated from 50 unrelated individuals (parents of the families). For locus 1, the 12-kb allele was considered as dominant and its absence recessive, a supposition confirmed by the frequency of segregation in the progeny of matings between two dominant parents and between a dominant and a recessive. The gene frequency of the 12-kb allele was 0.27 ± 0.048. For locus 2, both alleles of 7.5 and 7.0 kb can be scored and the frequencies of the genotypes 7.5, 7.5/7.0, and 7.0 satisfy Hardy–Weinberg expectations, with gene frequencies of 0.1 ± 0.03 and 0.9 ± 0.03, respectively. For locus 3, calculations were made as for locus 1 and the dominant allele (3.4 kb) has a frequency of 0.83 ± 0.07. As with locus 1, segregation analysis in the families confirmed the mechanism of inheritance.

Identification of Segregating Loci as V_HII Polymorphisms. The *V* probe used in the initial survey was not in germline configuration and included both *D* and *J* region sequences. To determine the region(s) homologous to the polymorphic fragments, additional probes were constructed with sequences representing only V_H or only J_H sequences (Fig. 3). DNA from individuals with the various combinations of polymorphic fragments was electrophoresed and transferred to triplicate nitrocellulose filters. These were then hybridized with three probes: (*i*) the original *V–D–J* probe used to identify the polymorphisms (27); (*ii*) a V_H probe that contained 1.2 kb of only *V* region sequences; and (*iii*) a sequence containing J_H3 through J_H4 subcloned from a recombinant Ig μ phage (39).

Hybridization with these three probes revealed that all of the polymorphic fragments (12, 7.5, 7.0, 3.4 kb) were homologous to the V_H region segment, whereas a non-polymorphic fragment of 4.0 kb was homologous to the J_H region probe. All fragments could be accounted for with these two probes, and thus no distinct sequences hybridized to the D region. This is explained by the fact that only 14 bp of D_H region existed in the original probe, probably not enough to hybridize given the stringency of hybridization and washing

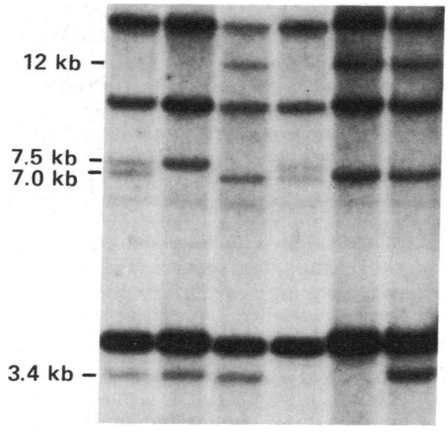

FIG. 2. Some of the different genotypes observed with the Ig V_HII probe. Molecular sizes indicated refer to polymorphic fragments defining the three loci described in text.

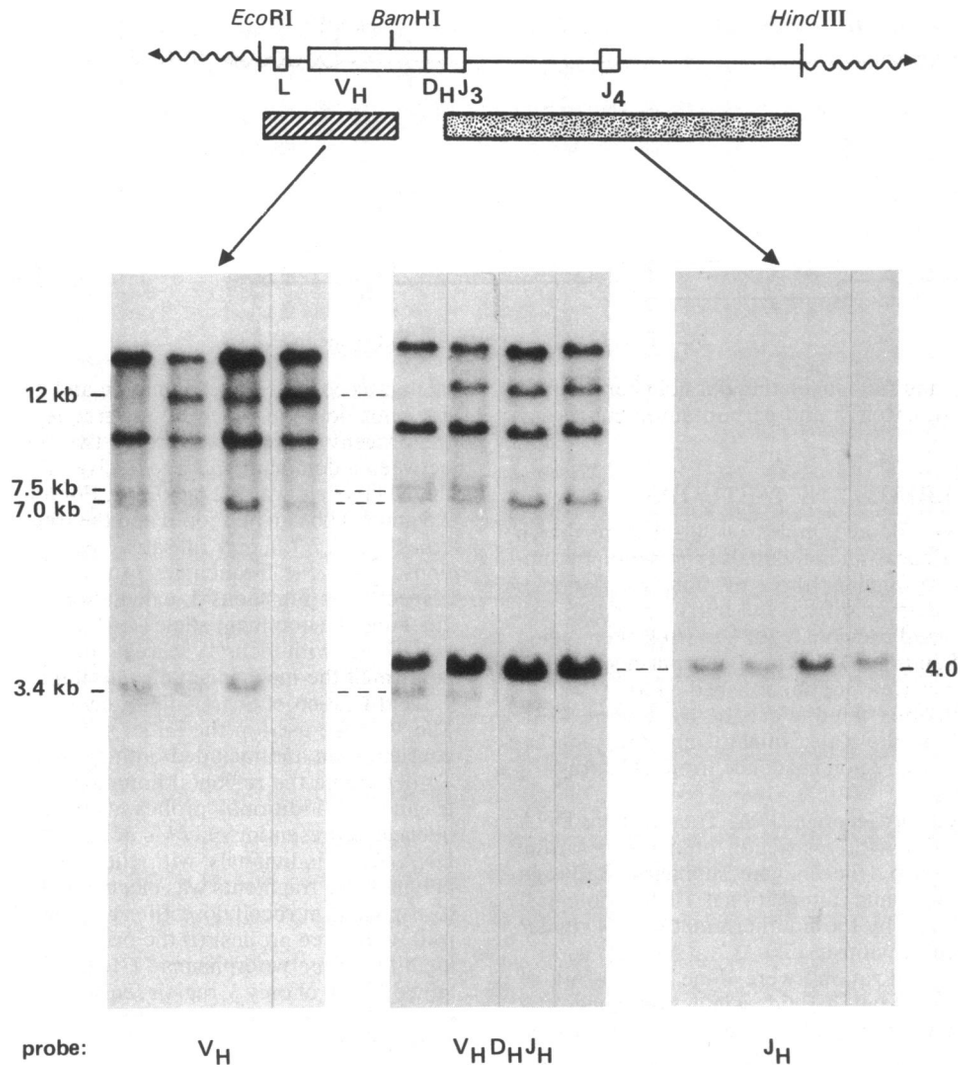

FIG. 3. Subcloning the Ig *V–D–J* probe of Fig. 1. The four individuals were tested with a subclone containing only V_H sequences (four lanes at left) and show the same polymorphic bands as the same individuals tested with the full *V–D–J* probe (center). Tests with the J_H subclone (four lanes at right) show only the 4.0-kb nonpolymorphic band.

conditions used throughout the study.

Linkage Analysis. Once it was determined that the *Bgl* II polymorphisms were due to V_H region sequence changes, segregation analysis was done in members of informative families to test whether or not V_H region genes are linked to one another and whether or not they are linked to the C_H gene cluster.

Linkage Between V_HII Loci. By testing linkage between the V_H polymorphic loci, we hoped to determine whether or not this gene family is clustered or widely dispersed along chromosome 14. Only 2 families in the sample were informative for more than one V_H locus (Table 1); no crossovers were detected. The peak lod score (1.78 at 0.0001 of 1%) does not reach a significant value. The small sample size precludes a real test of linkage: thus, this result in isolation is suggestive of linkage between V_H markers but is not conclusive proof of it.

Linkage Between V_HII and C_H Loci. We next wanted to test for linkage between the V_H polymorphic loci and the C_H loci also present on chromosome 14 in humans (23). All of the informative families for V_H region loci had been typed previously for C_H region markers and all were informative at the latter locus for testing linkage between the two clusters. As the evidence for linkage between the three V_H loci was inconclusive, analysis was first done separately between the Ig C_H region and each of the three polymorphic V_H loci and second by using a cumulative lod score.

Recombination between V_H and C_H region was observed in 2 of the 11 families analyzed (Fig. 4). For locus 1, six matings were informative, with no recombinants detected. This analysis gave a highly significant lod score with a peak value of 5.925 at $\theta = 0\%$ (Fig. 3). At locus 2, again six matings were informative; however, one putative recombinant was found. The peak lod score for this locus was 2.838 at $\theta = 5\%$. In contrast, locus 3 had only three informative matings, one of which contained a recombinant. Because of the small sample size, the highest lod score attained was 0.824 at $\theta = 7.5\%$.

Table 1. lod scores for the V_HII polymorphisms (12 kb and 7.5/7.0 kb)

	Recombination value (θ)						
Family	0.01	0.03	0.05	0.075	0.1	0.125	0.15
31	1.187	1.151	1.115	1.069	1.021	0.972	0.922
29	0.593	0.576	0.558	0.534	0.511	0.486	0.461
lod score	1.780	1.727	1.673	1.603	1.532	1.458	1.383

The overall peak lod score attained if all loci are cumulated was 9.214 at $\theta = 4\%$. The genetic distance unit (1 centimorgan) equals 1,000,000 bp. These results could then be interpreted to mean that the V_H loci, although linked to C_H genes, could be separated by ≈4000 kb; however, this computation assumes that crossing-over is equally frequent over the whole chromosome.

Analysis of Genetic Association Between V_H and C_H Regions. Analysis of linkage disequilibrium was done between each of the three V_H loci and the polymorphic C_H region markers defining μ, α 1, α 2, γ 1, γ 2, γ 3 and pseudo-γ. Correlation coefficients were then computed for each set of markers allowing for a test of signficance of the genetic associations (Table 2). This analysis indicates that no significant linkage disequilibrium is found not only between the V_H and C_H regions but also among the V_H loci as well.

Localization of the V_HII Gene Family. As the V_HII and C_H genes are linked to one another, we next wanted to determine the position of the V_H gene family with respect to the C_H genes. The C region genes have been mapped by *in situ* techniques to 14q32 (45). A second highly polymorphic locus—*D14S1*—(38) is closely linked to the γ genes (3%) (46) and maps proximally to the centromere with respect to the γ genes (47).

Of the 11 families that were analyzed for V_H–C_H linkage, 9 were informative for the *D14S1* locus. Two crossovers were found in these families and in both cases the recombination was between *D14S1* and the (V_H–C_H) region considered as a whole. Conversely, in the 2 families in which recombination occurred between the V_H and C_H regions, *D14S1* remains linked to the C_H genes; therefore, the crossovers occur between V_H and (C_H–*D14S1*). As *D14S1* is centromeric to the C_H region genes, these results locate the V_HII genes in a position telomeric to C_H and *D14S1* (Fig. 5).

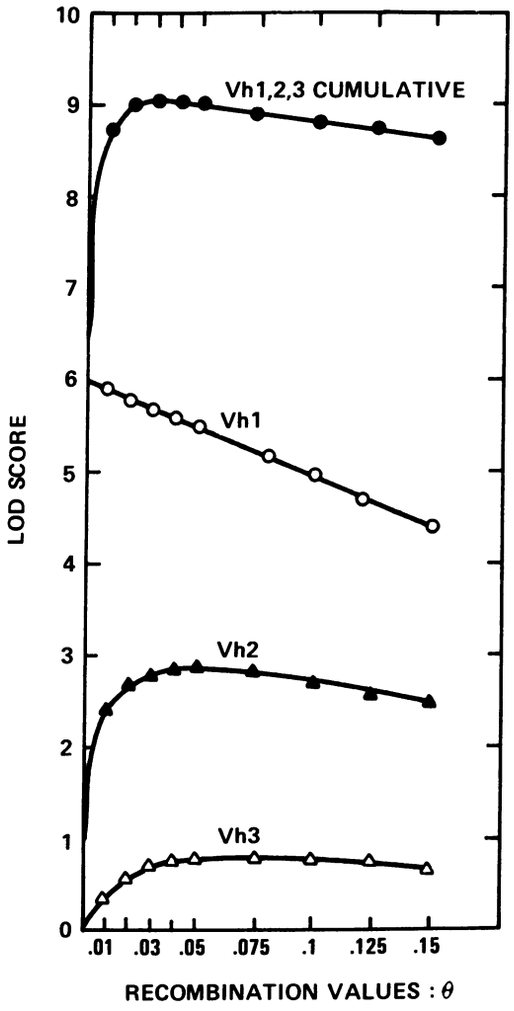

FIG. 4. lod scores (ordinates) as a function of recombination frequency (θ) for the three V_HII *Bgl* II polymorphisms and their cumulative results.

Table 2. Association among V_HII polymorphisms (upper) and between V_HII and Ig C_H loci (lower)

	V_H1	V_H2	V_H3
V_H1		−0.033	−0.143
V_H2			0.189

	A1	G1m	G2m	G3m	*Bst*EII	*Mbo* I
V_H1	0.790	0.166	−0.137	−0.164	0.244	0.342
V_H2	0.193	0.234	−0.120	−0.043	0.301	0.200
V_H3	0.181	0.151	−0.092	−0.038	0.371	0.372

Association is tested for significancy by χ^2 of the contingency $m \times n$ table formed by distributing haplotypes among the combinations of the m alleles at one locus and the n alleles at the other. The values shown in the table are coefficients calculated as $r = (\chi^2/N)$ when N is the total number of haplotypes. N was 31 for V_H3 and 72–82 for V_H1 or V_H2 (excluding 2 for which N was smaller). Only in 2×2 tables can the values be given a sign. The numbers of degrees of freedom (df) of χ^2s are $(m - 1)(n - 1)$, being 1 for 2×2 tables (upper) and 1–6 for the lower set of data. In no case is the significance level of 5% reached and there is therefore no significant evidence of linkage disequilibrium. For the meaning of r with >1 df, see ref. 44.

DISCUSSION

The *Bgl* II RFLPs described in this work represent heretofore unreported genetic markers for the human immunoglobulin V_H genes. These will be useful in clarifying the molecular organization of the region as polymorphisms identified for other V_H loci can be mapped in relation to this gene family. In addition, it will now be possible to address the questions of correlation between certain autoimmune diseases and a specific repertoire of V_H genes.

It was rather surprising that the V_HII probe recognized relatively few bands in the *Bgl* II digests. These ranged in size from 3.4 to ≈25 kb (for a total of slightly over 50 kb). If genes are spaced at 12-kb intervals, as are the human V_HIII genes and mouse V_H genes, this would imply a total of 4 or 5 V_HII genes represented by these hybridizing fragments. In contrast, the V_HIII gene probe of Matthyssens and Rabbitts (24) recognized 22 or 23 fragments, depending on the enzyme used. However, protein data suggest that the V_HII family is

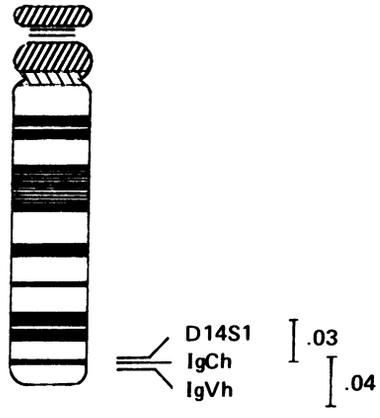

FIG. 5. The order and genetic distance of three genes on chromosome 14.

much smaller than either the V_HI or V_HIII family, having only 13 defined variants as compared to 32 and 91 (for gene families I and III, respectively) (22). As somatic mutation increases the number of protein variants, this is likely to be an overestimate of the actual number of different germline genes. Notice that also for V_HIII there is a higher number of different proteins (91) compared with DNA fragments (22, 23).

Studies involving translocations in Burkitt lymphoma cells (48–50) have shown that some V_H genes are translocated to the 8q chromosome when the c-*myc* gene is rearranged to within the μ switch region of chromosome 14. Our analysis of linkage between the V_HII, C_H, and *D14S1* loci confirms the telomeric position of these V_HII genes. This segment of chromosome 14 containing V_H–C_H–*D14S1* can be estimated to be ≈7000 kb in length (assuming constancy of the crossing-over frequency) given the recombination values of 4% between V_H–C_H (see above) and 3% between C_H–*D14S1* (46). Polymorphic markers for other V_H families will help to further clarify the genetic organization of this region and furnish an opportunity to investigate recombination in human genetics within a relatively large, well-characterized chromosomal region.

When analyses of genetic association were done on this data set, it was determined that the V_H loci are not in linkage disequilibrium with the C_H loci, a finding that was not unexpected given the rather large genetic distance that separates them. However, it was perhaps surprising that the V_HII family loci did not exhibit appreciable linkage disequilibrium within their own gene family. Further investigation of the region will be necessary to increase our understanding of this phenomenon. Based on this analysis, however, we would interpret this finding to indicate that recombination is frequent within *V* gene families of the Ig *H* region. It has been shown in the murine Ig *H* region (34) that crossovers occur within gene families rather than between them. The linkage disequilibrium within the *V* regions will have to be kept in mind in the analysis of associations of diseases and *V* genes.

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