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Multiple DNA fragment polymorphisms associated with immunoglobulin μ chain switch-like regions in man

(restriction analysis/human immunoglobulin genes/switch regions)

N. MIGONE*†, J. FEDER*, H. CANN*, B. VAN WEST*, J. HWANG*, N. TAKAHASHI‡, T. HONJO‡, A. PIAZZA*†, AND L. L. CAVALLI-SFORZA*

*Department of Genetics, Stanford University, Stanford, California 94305; and ‡Department of Genetics, Osaka University Medical School 3-57, Nakanoshima 4-Chome, Kita-Ku, Osaka 530, Japan

Contributed by L. L. Cavalli-Sforza, September 28, 1982

ABSTRACT **DNA probes containing the switch region (*S*) associated with the human immunoglobulin heavy chain μ gene were used to investigate polymorphisms in the germ-line human DNA. Six polymorphisms, detected by a single restriction enzyme (*Sst* I) are described. Linkage studies in 29 families show that five of the six polymorphisms, although relatively unassociated in random individuals, segregate in complete linkage one to the other and to *Gm* allotypes (markers on the heavy chain of IgG), while the sixth segregates independently. Altogether, when one considers the DNA markers at the five closely linked loci and the IgG1 and IgG3 heavy chain allotypes, 33 different haplotypes have been described; of these, 28 are detected by the DNA polymorphism alone. Study of 158–187 random haplotypes showed strong linkage disequilibrium only between one DNA polymorphism (*Sst A*) and *Gm*. Of the polymorphic *Sst* I loci, one, *Sst E* [associated with 2.2- to 2.7-kilobase (kb) fragments], is included in the μ chain *S* region (S_μ); another, *Sst A* (6.8–7.4 kb), must be very close to the $\gamma 1$–$\gamma 3$ chain gene cluster. Based on studies of an IgE human myeloma, a third polymorphism, *Sst C* (4.8–5.5 kb), should map 3′ of the active ε chain gene. An *Sst* I restriction enzyme map of phage clones carrying the two α chain genes indicates that *Sst A* and *Sst C* loci probably overlap with the $\alpha 1$ and $\alpha 2$ *S* regions, respectively. Both deletion/duplications and point mutations were detected.**

Polymorphisms described in human immunoglobulin heavy chains are thus far limited to variation detected in protein antigenicity and sequence (1). Observations in mice indicate the existence of individual heterogeneity at the DNA germ-line level (2, 3). The possibilities offered by the study of individual variation at the DNA level by restriction enzyme analysis (4, 5) have prompted us to initiate a search for germ-line variation in the immunoglobulin heavy chain gene regions. We show that a single probe tested with a single enzyme can demonstrate polymorphisms at many loci. This is due to the fact that DNA sequences contained in the probe are repeated (with some variation) before several immunoglobulin heavy chain genes within the so-called "switch" (*S*) regions. The *S* region derives its name from its putative function in the somatic chromosome rearrangements involved in the "heavy chain switch." This phenomenon involves a transition from production of IgM to that of IgG, IgE, or IgA during the life of a B lymphocyte without changing the antigen specificity of the molecule (6, 7). This is believed to be the result of DNA deletions removing the DNA segment between the heavy chain variable region gene and the constant region gene actually used for antibody production (8, 9), after somatic recombination between the homologous *S* regions located 5′ of most immunoglobulin genes. These *S* regions include multiple tandem repeats of sequences like G-A-G-C-T, G-G-G-G-T (10–14), and Y-A-G-G-T-T-C (15). The numerous polymorphisms we find allow us to analyze the genetic recombination in the heavy chain gene region, contribute to the study of its evolution, and provide material of potential importance for the study of association with disease (e.g., see refs. 16 and 17).

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MATERIALS AND METHODS

Blood samples (30 ml) were obtained from 256 Caucasian individuals, almost all belonging to 29 families from the San Francisco Bay area selected for linkage studies. DNA was extracted from buffy coats (18). *G1m* (*a,f,x,z*) and *G3m* (*b0, b1, b3, b5, g*) allotypes were tested in plasma by hemagglutination-inhibition tests (19), and an additional 23 genetic markers known to be polymorphic were tested by using standard procedures. Program LIPED was used for linkage analysis (20).

Restriction enzymes were purchased from Bethesda Research Laboratories. DNA (6–8 μg) was digested and electrophoresed in 0.8% agarose and transferred to nitrocellulose by the method of Southern (21). DNA probes were nick-translated with ^{32}P to a specific activity of 1–3 $\times$ 10^8 dpm/μg (22), and 50–80 ng of labeled probe was used per filter hybridization (average filter size, 160–170 cm^2). Hybridization was done in 50% formamide/0.9 M NaCl/50 mM NaH_2PO_4/5 mM Na_2EDTA, pH 7.0 at 42°C for 24 hr and followed by high-stringency washes (twice for 40 min in 0.018 M NaCl/1 mM NaH_2PO_4/0.1 mM Na_2EDTA, pH 7/0.1% $NaDodSO_4$ at 60°C).

Phage Charon 4A-H24 was isolated (10) from Maniatis' human fetal DNA library (Fig. 1). An *Eco*RI 1.2-kb fragment from this phage was cloned in pBR325 (pHμ.30). An *Eco*RI–*Hin*dIII 3.3-kb fragment was cloned in pBR322 (pHJ). *Sst* I fragments of size 4.4 and 2.2 kb were isolated by electroelution (24) from 0.8% agarose gel and subsequently purified (25) through a DEAE-cellulose column (Pharmacia). Recombinant DNA was handled under P2-EK2 conditions as described by the National Institutes of Health.

RESULTS AND DISCUSSION

At Least 13 *Sst* I Fragments Share Homology with the S_μ Probe. Restriction enzymes *Sst* I and *Pvu* II generated a much greater number of human DNA fragments detectable with phage H24 as probe, when compared with eight other enzymes. *Sst* I and *Pvu* II recognition sites differ by one single base from

Abbreviations: *J* and *S*, joining and switch region genes; S_μ, $S_{\alpha 1}$, $S_{\alpha 2}$, S_γ, S_ε, *S* region genes of μ, $\alpha 1$, $\alpha 2$, γ, and ε chains; kb, kilobase(s); lod score, logarithm of odds for genetic linkage.

†Permanent address: Medical Genetics Institute, University of Turin, 10126 Turin, Italy.

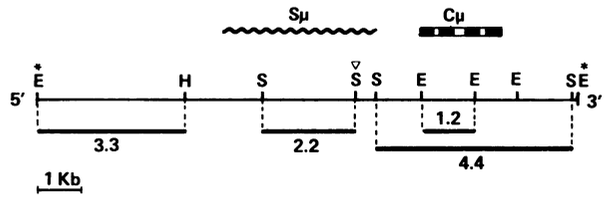

FIG. 1. Restriction endonuclease cleavage sites in the 12-kb human DNA insert in phage H24 (H, *Hin*dIII; E, *Eco*RI; *, artificial *Eco*RI sites introduced for cloning in Charon 4A vector; S, *Sst* I; ▽, polymorphic *Sst* I site). Sizes are given in kilobases (kb). The wavy line corresponds approximately to the length of the S_μ region. This is shown by the homology with the 2.2-kb *Sst* I probe after a series of single and double digestion analyses (including *Xba* I, *Pvu* II and *Pst* I, whose restriction sites are not indicated in the map). To the right is the gene for the constant region of the μ chain (C_μ), showing coding regions (closed boxes) and introns (open boxes). The solid bars below the restriction enzyme map represent the probes used. The *Eco*RI–*Hin*dIII 3.3-kb fragment, where part of the *J* sequences are located (23), was used as probe on *Sst* I-digested total human DNA and detected an 11.5-kb germ-line fragment. The 4.4-kb fragment includes the μ chain gene; it hybridizes with recombinant plasmid pHμ.30 containing the 1.2-kb *Eco*RI fragment previously shown to carry part of the μ chain gene (10).

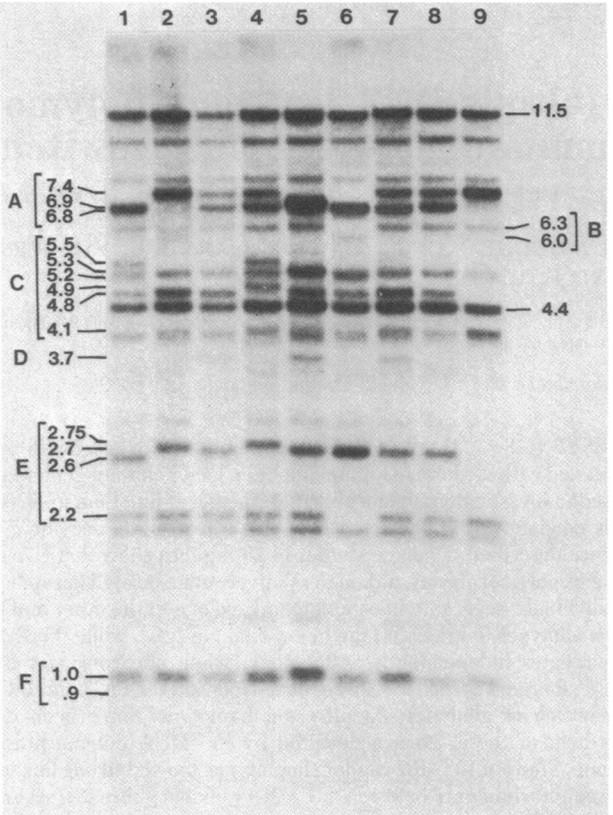

FIG. 2. Autoradiogram after hybridization to H24 probe of *Sst* I-digested DNA from nine unrelated individuals (all Caucasians, except one Oriental in lane 5), showing most alleles of six *Sst*-I polymorphisms. All the genotypes assigned were confirmed by family segregations. Molecular weights are given in kilobases. Polymorphisms: *Sst A*, 7.4 homozygotes (homo.) (lanes 2 and 9), 6.9–6.8 heterozygotes (hetero.) (lanes 1 and 6), 7.4–6.9 hetero. (lanes 3, 4, and 8), 7.4–6.8 hetero. (lane 7), and a probable duplication of the 6.9 band to be described elsewhere (lane 5); *Sst B*, 6.3 homo. (lanes 1, 3–5, and 7–9), 6.3–6.0 hetero. (lane 2), 6.0 homo. (lane 6); *Sst C*, 5.5–4.8 hetero. (lane 1), 5.5–4.9 hetero. (lane 4), 5.3–4.8 hetero. (lane 5), 5.2–4.8 homo. (lane 6), 4.8 homo. (lanes 2, 3, and 7), 4.8–"null" (lane 8), and 4.1 homo. (lane 9); *Sst D*, 3.7–(*) hetero. (lanes 3, 5, and 7), (*) homo. (lanes 1, 2, 4, 6, 8, and 9) [The explanation of (*) is given in the legend to Table 1.]; *Sst E*, 2.7 homo. (lane 6), 2.75–2.2 hetero. (lanes 2 and 4), 2.7–2.2 hetero. (lanes 3, 5, 7, and 8), 2.6–2.2 hetero. (lane 1), 2.2 homo. (lane 9). A 0.5-kb band is present (but migrated outside this gel) for all individuals except that shown in lane 6. The *Sst F* polymorphism is not clearly visible in this autoradiogram; on longer exposure a 0.9-kb band appeared in lane 3.

the six-nucleotide G-A-G-C-T-G sequence, which is known to occur very frequently within all the S regions associated with immunoglobulin genes in mouse and man (10–14). For this reason these two enzymes should be particularly efficient in detecting single-nucleotide substitutions in these specific regions. *Sst* I enzyme was chosen for screening: it gives the highest number of nonoverlapping bands within an optimal range for size measurement. At least 14 fragments were detected (Fig. 2); 4 (11.5, 2.2, 0.5, and 4.4 kb in the 5′–3′ order) were found to be contiguous in the genome as shown by mapping the phage insert (see the legend to Fig. 1).

With the exception of the 4.4-kb *Sst* I μ chain gene-containing fragment, the same bands were found by using the 2.2-kb *Sst* I fragment as the probe instead of phage H24. According to our map of phage H24 and other reports (13, 23), this fragment is believed to be entirely included within the S_μ region. Therefore, we conclude that 13 of the observed bands have sequence homology with the S_μ region.

Six Restriction Fragment-Length Polymorphisms Described. Seven of the 14 *Sst* I fragments appear to be polymorphic in 92 random individuals—all Caucasians—screened to date. One of these, involving the 11.5-kb fragment, was not considered because of difficulties in discriminating between the two major variants (about 11.5 and 12 kb, respectively). Segregation analysis of the other polymorphic fragments in 29 families with a total of 242 relatives allowed the identification of six polymorphic loci with 8, 3, 6, 2, 4, and 2 alleles per locus. (We use provisionally, for lack of a better term, the word locus to define a set of DNA fragments behaving as alleles in Mendelian segregations.) The gene frequencies and the size of the variants are listed in Table 1. Each locus was assigned a symbol, *Sst A–F*, in descending order according to the size of the most representative allele in each locus. The heterozygosities per locus, calculated from the gene frequencies, are: *Sst A*, 52.2%; *Sst B*, 17.1%; *Sst C*, 17.2%; *Sst D*, 10.4%; *Sst E*, 52.0%; *Sst F*, 1.0%. All genotypes for alleles present in numbers sufficient for statistical testing were in Hardy–Weinberg proportions (data not shown). *Sst F* locus is rarely polymorphic in Caucasians but is probably quite informative in Orientals because 4 out of 10 random Orientals so far tested appeared to be heterozygous at this locus. In one of two Oriental families, a 0.9-kb homozygote was found in the progeny of two heterozygous parents. In this individual, a 1.0-kb band was still present, although fainter, indicating that at least two different fragments usually comigrate in this position.

Five of the Six DNA Polymorphisms Show Complete Linkage to Each Other and to *Gm* Haplotypes. Of the six polymorphisms, all except *Sst B* segregate in families in complete linkage with each other. To date, no recombination has been observed. Highly significant lod scores (logarithm of odds for genetic linkage) were obtained, and all showed maxima at a recombination value θ near 0. For instance, in testing *Sst A*, the most informative polymorphism (i.e., having the highest heterozygosity), the lod score near $\theta = 0$ was 8.02 versus *Sst C*, 5.71 versus *Sst D*, and 12.98 versus *Sst E*. In the only two families informative for recombination between *Sst A* and *Sst F* (including one Oriental), the lod scores did not reach the threshold of significance, but no recombinants were observed. In other words, the segregation of these five loci occurs as a single block, indicating very close proximity.

Table 1. Gene frequencies of polymorphisms observed in 92 random Caucasians

Polymorphic loci	Fragment size of observed alleles, kb	Corresponding gene frequency
Sst A	7.5	0.005
	7.4	0.658
	6.9	0.163
	6.8	0.130
	D1–4	0.044
Sst B	6.5	0.011
	6.3	0.907
	6.0	0.082
Sst C	5.5	0.049
	5.2	0.005
	4.9	0.022
	4.8	0.908
	4.1	0.011
	null	0.005
Sst D	3.7	0.055
	(*)	0.945
Sst E	2.75	0.016
	2.7	0.468
	2.6	0.005
	2.2	0.511
Sst F	1.0	0.995
	0.9	0.005

Six fragments were found to be polymorphic. All variant fragments behaving in inheritance as allelic to each other were grouped together in six "loci" labeled from *A* to *F* according to the average size of fragments. The gene frequency observed is given in the last column. *D1–D4* are four different variants characterized by duplication to be described elsewhere. The "null" allele at locus *C* segregates in a large family; it cannot be associated to any visible fragment. Also allele (*) at locus *D* cannot be associated to a fragment but the 3.7-kb allele at this locus is faint enough that a hypothetical band corresponding to the (*) allele would easily hide under other bands.

Tested for linkage with *Gm* markers of γ1 and γ3 heavy chain immunoglobulin genes, these polymorphisms did not show any recombinations, again with highly significant lod scores (from 4.8 to 26.2 at θ near 0). This close linkage is expected, given that regions homologous to S_μ are closely associated with most immunoglobulin heavy chain genes and that there is a tightly linked cluster of these genes in humans, rabbits, and mice (26). *In situ* hybridizations (27) suggest that μ and γ chain genes are in the same band (q32) of chromosome 14.

In contrast, the *Sst B* polymorphism segregates independently of the other five *Sst* polymorphisms and of the *Gm* markers, indicating that it is either at some distance on chromosome 14 or on another chromosome. However, lod scores against the total haplotype, *Sst A, C, D, E* and *Gm* combined did not exclude loose linkage significantly. Linkage of *Sst B* with 23 standard genetic markers has so far given negative results.

This finding is in agreement with the observation that, in the mouse, some of the regions homologous to S_μ map outside the DNA interval that extends from the μ to the α chain genes (28).

Linkage Disequilibrium Between Markers of the Immunoglobulin Heavy Chain Gene Cluster. Crossing over inside the immunoglobulin heavy chain gene regions in humans, although very rare, has been reported in a few families (29). In mice, where many more genetic markers are known, no new recombinations have been found in more than 3,000 informative progeny studied (30–32). Both in humans and mice, however, there is evidence of earlier crossovers from certain haplotypic combinations. Altogether, by considering the DNA markers at the five closely linked loci and those for the two *Gm* loci, 33 haplotypes were observed (Table 2). Of these, 28 were detected

Table 2. Distribution of 158 DNA and *Gm* haplotypes identified in 29 Caucasian families

Gm haplotypes	DNA haplotypes *A*	*C*	*D*	*E*	*F*	Number observed
f;b	7.4	4.8	(*)	2.7	1.0	55
	7.4	4.8	(*)	2.2	1.0	47
	7.4	4.9	(*)	2.2	1.0	4
	7.4	4.8	(*)	2.75	1.0	2
	D2	4.8	(*)	2.2	1.0	2
	7.4	4.1	(*)	2.2	1.0	2
	7.4	5.5	(*)	2.2	1.0	1
	6.9	4.8	(*)	2.2	1.0	1
	6.8	4.8	3.7	2.2	1.0	1
az;g	6.9	4.8	(*)	2.7	1.0	6
	6.8	4.8	(*)	2.7	1.0	3
	6.8	4.8	(*)	2.2	1.0	3
	6.8	4.8	3.7	2.2	1.0	3
	6.9	4.8	(*)	2.2	1.0	2
	6.8	4.8	3.7	2.7	1.0	1
	6.9	4.8	3.7	2.2	0.9	1
	6.8	5.2	(*)	2.7	1.0	1
	7.5	4.8	(*)	2.7	1.0	1
	D4	4.8	(*)	2.2	1.0	1
azx;g	6.9	4.8	(*)	2.7	1.0	5
	6.9	4.8	(*)	2.2	1.0	2
	7.4	4.8	(*)	2.2	1.0	2
	D3	4.8	(*)	2.2	1.0	2
	6.8	4.8	(*)	2.2	1.0	1
	6.8	4.8	3.7	2.2	1.0	1
	6.9	5.5	(*)	2.2	1.0	1
	6.9	5.5	(*)	2.75	1.0	1
	D3	5.5	(*)	2.2	1.0	1
	D1	4.8	(*)	2.2	1.0	1
	6.9	4.8	(*)	2.6	1.0	1
	6.9	null	(*)	2.2	1.0	1
f;g	7.4	4.8	(*)	2.2	1.0	1
az;b	6.8	5.5	(*)	2.2	1.0	1
					Total	158

DNA fragments showing polymorphisms (polymorphic "loci") are named from *A* to *F*. Alleles at each locus are described in Table 1. *D1–D4* are duplications which can be distinguished one from the other and behave as alleles at the A locus. (*), Alleles not allocated to a specific band. The *b* symbol in both *f;b* and *az;b* haplotypes stands for the antigen complex b0,b1,b3,b4,b5.

by the DNA polymorphisms alone, whereas with five loci with 8, 6, 2, 4, and 2 alleles, respectively, a maximum of 768 haplotypes is expected. Our data were examined for linkage disequilibrium—i.e., discrepancy from random association of alleles at linked loci. The correlation coefficient, *r*, has been used to measure linkage disequilibrium between loci. It is a simple algebraic transformation of the parameter *D* usually used (33) to describe disequilibria between pairs of alleles at two loci, but it has the advantage of normalizing *D* for different values of haplotype frequencies. For simplicity, the presentation in Table 3 is limited to the most frequent allele at each locus, but a full analysis of the data (to appear elsewhere) did not show any major discrepancy from the simplified picture presented in the table. Present knowledge of the localization of DNA markers within the immunoglobulin heavy chain gene cluster follows.

The *Sst E* Polymorphic Fragments Are Within the S_μ Region. The most common *Sst E* allele is most probably the result of a point mutation because a 0.5-kb fragment was always present with the 2.2-kb variant, the other major allele being 2.7 kb. The S_μ region sequence carried by our H24 phage does not show any substantial deletion (due to propagation of the vector), and it includes a 2.2- and 0.5-kb contiguous fragment. Thus, ac-

Table 3. Association between loci in the population

	Sst A (7.4)	*Sst C* (4.8)	*Sst D* (*)	*Sst E* (2.2)	*G1m* (*f*)
Sst C (4.8)	0.15*				
Sst D (*)	0.32‡	−0.07†			
Sst E (2.2)	−0.04†	−0.16*	−0.15*		
G1m (*f*)	0.91§	0.16*	0.29‡	−0.08†	
G3m (*b*)	0.88§	0.12†	0.29‡	−0.08†	0.97§

Correlations r measuring linkage disequilibrium for four DNA loci, as defined in Table 1, and two *Gm* loci, based on n haplotypes varying from 158 to 187. Representation of each locus is limited to the most frequent allele (indicated by kilobase size in parentheses below each locus). *G3m*(*b*) indicates the complex b0, b1, b3, b4, b5. The statistical significance of each correlation coefficient was calculated by $\chi^2 = nr^2$ with one degree of freedom.

*$P < 5\%$.
†$P < 1\%$.
‡$P < 10^{-3}$.
§$P < 10^{-4}$.

cording to the restriction map, the polymorphic site should be 1.5 kb 5′ to the μ chain gene.

The *Sst C* Polymorphism Is Due to Deletions/Duplications and Probably Overlaps with the S Region of the $\alpha 2$ Chain Gene ($S_{\alpha 2}$). DNA samples representing all of the alleles at the *Sst C* locus were digested with *Pvu* II. This enzyme revealed a polymorphism that correlated with the *Sst C* variants, in that the *Sst* I fragments of size 4.8, 4.9, 5.2, and 5.5 kb corresponded to *Pvu* II fragments of 7.0, 7.1, 7.4, and 7.7 kb in the same individuals. The constant difference of 2.2 kb indicates that all mutants were generated by deletions or insertions. Double digestion confirmed that both *Sst* I restriction sites are internal to the *Pvu* II fragments. The 4.1-kb *Sst* I variant did not follow this rule but gave rise to a 7.0-kb, not a 6.3-kb, *Pvu* II fragment; therefore, it is likely that this rare variant is due to point mutation.

Studies of the DNA deletions and rearrangements within the murine immunoglobulin heavy chain genes in myelomas have proven helpful in establishing the 5′–3′ order of these genes. The analysis of an IgE-producing human myeloma, U266 (34), shows that this cell line still retains *Sst C*-related fragments after *Sst* I or *Pvu* II digestion. It has been shown (35) that the active $\varepsilon 2$ chain gene on the "Ig producer" chromosome has been rearranged close to the joining region (J) sequences, the intervening DNA being deleted. On the other chromosome, a broad deletion including J region, μ chain, $\psi\varepsilon$, and functional ε chain genes seems to have occurred. If other subtle rearrangements did not complicate the picture, the *Sst C* polymorphism should map downstream to the only functional ε chain gene. Given (36) that there is one α chain gene ($\alpha 2$) 3′ to this ε chain gene, its S region is likely to be responsible for the *Sst C* polymorphism.

In agreement with this is the evidence from the restriction map of human DNA phage clones carrying the $\alpha 2$ chain gene (unpublished data): approximately 2.4 kb upstream of this heavy chain gene, a S region-containing *Sst* I fragment appears whose size (4.8 kb) corresponds to that of the major allele at the *Sst C* locus.

The *Sst A* Fragments Probably Overlap with the *S* Region Associated with the $\alpha 1$ Chain Gene ($S_{\alpha 1}$). The *Sst A* fragments cannot be related to the S regions associated with the γ or ε chain genes (S_γ and S_ε) for the following reasons. In humans and mice, the S_μ region does not hybridize strongly with the S_γ regions (13, 30, 37, 38). In fact, in our hybridization conditions, we were unable to detect any of the S_γ region containing the *Bam*HI and *Hin*dIII fragments expected on the basis of the restriction enzyme maps of the γ chain gene clones (39–41). No γ chain gene is included in the *Sst A* fragments, as no band of that size was observed with a $\gamma 1$ probe known to hybridize to all γ chain genes (data not shown). Finally, the S region of the functional ε chain gene has different *Sst* I fragment sizes (35, 36) and that of the $\psi\varepsilon$ chain gene was not detectable with this probe in our stringent condition of hybridization. A third ε chain gene is a processed gene, unlinked to the immunoglobulin gene cluster, and lacks the S region (36, 42).

By contrast, the *Sst A* fragment is most probably associated with the $S_{\alpha 1}$ region. The S_μ and S_α regions cross-hybridize strongly in man and mice (13), and the *Sst A* fragments gave the strongest hybridization signals in Southern blots. The connection with the $\alpha 1$ chain gene is strongly suggested by the existence of a 6.8-kb *Sst* I fragment in the S region of that gene, as shown by the restriction map (unpublished data) of the $\psi\varepsilon 1$–$\alpha 1$ chain gene region reconstructed from human DNA phage clones. The length of this fragment corresponds precisely to one of the common alleles at the *Sst A* locus.

The two α chain genes are in the order 5′–$\alpha 1$–$\alpha 2$–3′ on the basis of evidence from myelomas (35, 36), but their distance is not known; it is possible that it is large, given the absence of a marked linkage disequilibrium between the DNA polymorphisms *Sst A* and *Sst C* related to the two genes (Table 3).

The relative position of *Sst A* with respect to the $\gamma 1$–$\gamma 3$ chain gene cluster, with which it shows very high linkage disequilibrium, can be conjectured upon the assumption that the observed rare haplotypes arose by crossing over (and not by gene conversion) among the common ones. As will be explained in detail elsewhere, this analysis suggests that the *Sst A* locus is external to the $\gamma 1$–$\gamma 3$ gene cluster and closer to the $\gamma 1$ chain gene. The relative position of *Sst D*, which shows association both with *Sst A* and $\gamma 1$–$\gamma 3$ chain genes, will require further research.

CONCLUSIONS

Probes that identify multiple segregating loci, like the present one, are especially useful for genetic research as they provide simultaneous information on a relatively long stretch of DNA. The total length of the human heavy chain region spanned by homologies with the S-region probe is still unknown, although the number of ψ genes and of duplicated regions so far identified indicates a larger size than in the mouse, i.e., >200 kb.

The relatively low linkage disequilibrium between most of the DNA markers and between most of these and the *Gm* markers is perhaps unexpected. No recombinants were detected in our family studies, but the number of gametes tested is still limited. Immunoglobulin heavy chain S-region probes, like the present one, may prove very useful for further testing the associations of heavy chain genes and diseases already detected and finding new ones. In addition to greatly increasing the number of haplotypes distinguishable near the $\gamma 1$–$\gamma 3$ chain gene region, it gives information on genes like those for μ and $\alpha 2$ chains, which are, as far as we now know, at the extremes of the immunoglobulin heavy chain gene cluster and are only loosely associated at the population level with the genetically better known $\gamma 1$ and $\gamma 3$ chain gene markers, *Gm*.

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