Am J Hum Genet 38:617–640, 1986

Ig Gamma Restriction Fragment Length Polymorphisms Indicate an Ancient Separation of Caucasian Haplotypes

M. JACQUELINE JOHNSON,[1,2] GERDA DE LANGE,[3] AND L. L. CAVALLI-SFORZA[1]

SUMMARY

This investigation was undertaken to study genetic variation in the human immunoglobulin gamma heavy-chain (IgG) genes using Southern blot hybridization techniques to identify restriction fragment length polymorphisms (RFLPs). A genomic Ig gamma-1 clone was used as a probe, and variants were identified with two restriction enzymes (R.E.), each of which defined RFLPs at two separate IgG loci. Once alleles and haplotypes were determined, molecular localization of the alleles was made through genetic analysis of recombinant haplotypes and through the use of regional specific subclones. Linkage between the newly defined RFLPs and switch region variants as well as protein allotypic markers (Gm) was complete. This analysis included markers for Ig Mu, Alpha 1, Alpha 2, Gamma 1, Gamma 2, Gamma 3, and Pseudo Gamma. The picture that emerges from the molecular study of two common haplotypes, each with many rare variants resulting from recombination or mutation, confirms and extends the earlier immunological observations. The accumulated differences between the two major Caucasian IgG haplotypes indicate that their separation may be ancient and maintained through heterozygote advantage.

INTRODUCTION

A total of five human gamma immunoglobulin genes have been isolated from various libraries [1–7]. Four are analogous to previously defined IgG sub-

Received May 22, 1985; revised November 22, 1985.

This work was supported by grants GM-28428, GM-20457, and GM-07790 from the National Institutes of Health.

[1] Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305.

[2] Present address: Hybritech, Inc., 11096 Torreyana Rd., San Diego, CA 92121.

[3] Centraal Laboratorium van de Bloedtransfusiedienst van het Nederlandse Rode Kruis, Amsterdam, The Netherlands.

classes (Gamma 1–4), while the fifth is defined as a pseudo gene based on its lack of a 5′ switch sequence. The five gamma genes all have three coding region exons (CH1, CH2, CH3) and a smaller hinge region exon occurring between CH1 and CH2. The gamma 3 gene has a quadruplicated hinge region that is postulated to have arisen by unequal crossing over between the pseudo gamma and gamma 1 genes [3, 7].

Molecular Characterization of IgH Gene Organization

Cosmid clones containing all the expressed gamma, epsilon, and alpha genes, and the pseudo epsilon 2 have been isolated and used to determine the order of the IgH genes in humans [3, 8]. Restriction mapping of these clones allowed identification of two nonoverlapping clusters of IgH genes, each approximately 100 kilobases (kb) in length. One cluster contains Gamma 3-Gamma 1-Pseudo Epsilon 2-Alpha 1, while the second contains Gamma 2-Gamma 4-Epsilon 1-Alpha 2. Subsequent studies by Bech-Hansen et al. [9] used data on linkage disequilibrium to map the pseudo gamma gene as occurring between the two gamma gene clusters. The IgH cluster has been mapped to human chromosome 14 [10, 11], band 14q32 [12], and all molecular evidence seems to agree that the gene order is that shown in figure 1.

DNA Sequence Polymorphisms in the IgH Locus

Given the variety of interests in the Ig gamma region—genetic, anthropological, and medical [13–15], current Gm typing methods do not allow for its full exploitation as a genetic system. Monoclonal methods could circumvent current Gm typing problems [16], but one still awaits the production of a large and diverse panel of reagents before their use becomes routine. An alternate method, and one to which the results of this study contribute, is to define restriction fragment length polymorphisms (RFLPs) for the Ig genes. DNA sequence polymorphisms detected with Ig probes can be used to characterize this region at the molecular level, to investigate aberrant gene expression, and to detect allotypic variation in IgH loci.

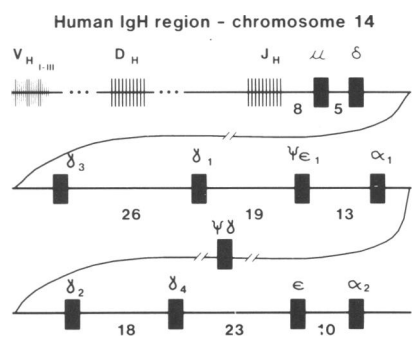

FIG. 1.—Diagram representing the human immunoglobin heavy-chain genes located on chromosome 14. The known distances in kilobases between Ig genes are noted; unknown distances are designated by *broken lines*.

Initial work by Migone et al. [17] describing RFLPs in the human Ig heavy-chain region demonstrated that a single probe can define polymorphisms at multiple loci. Of six polymorphic loci analyzed, three were located in the switch regions of IgA1, IgA2, and IgM, respectively. Altogether, a total of 33 haplotypes were identified (as compared to only five original Gm haplotypes); 28 haplotypes could be distinguished by the RFLPs alone. More recently, Lefranc and Rabbitts [18] demonstrated that it is possible to differentiate between the two Ig Alpha 2 allotypes using restriction enzyme site polymorphisms. The genetic diversity revealed by analysis of RFLPs will greatly increase our knowledge of this most interesting region of the human genome.

MATERIALS AND METHODS

Sample Collection

Blood samples were drawn from a total of 270 Caucasian individuals, most of whom live in California. These individuals are from 18 nuclear families and 10 extended families (three or more generations) representing normal individuals whose blood was previously collected in this laboratory and used in earlier studies among which that by Migone et al. [17] is the most relevant. A total of 80 unrelated individuals included in this sample were used for purposes of frequency analysis.

DNA Analysis

High molecular weight DNA was extracted from peripheral leukocytes [19], digested with the appropriate restriction enzyme (Bethesda Research, Buffalo, N.Y.), and size fractionated by electrophoresis in 0.8% agarose horizontal gels (Seakem, Rockland, Me.). DNA was transferred [20] onto nitrocellulose (Schleicher and Schuell, Keene, N.H.) or Zetabind (AMF, Meriden, Conn.) filters in 20 × SSPE (1 × = 0.18 M NaCl, 10 mM NaH_2PO_4, 1 mM Na_2EDTA, pH 7.0). DNA probes were nick-translated [21] to a specific activity of $> 1 \times 10^8$ cpm/μg using two radionuclides: [^{32}P] dATP and [^{32}P] dCTP (> 3,000 Ci/mMl; Amersham, Arlington Heights, Ill.).

Hybridizations were done with 10 ng probe/ml in 50% formamide, 1 × Denhardt's, 0.1% SDS, and 100 μg/ml yeast RNA. Filters were hybridized 48 hrs at 42°C. Washes to remove nonspecifically bound probe were done in .1 × SSPE, 0.1% SDS, for 1 hr at 65°C. Filters were placed in Pickering cassettes between two Kodak XAR-5 films with two Dupont lightning plus intensifying screens. Films were developed after 3 days, and again after a total of 8–12 days.

Protein Typing

All plasma samples were typed for the following allotypes: G1m (z,a,x,f), G2m (n), G3m (g,b0,b1,b3,b5,s,t,c3,c5), A2m (1,2), and Km [2, 17], located on Gamma-1, Gamma-2, Gamma-3, Alpha-2, and Kappa chains. Typing was carried out with the conventional hemagglutination inhibition technique in microtiter plates with reagents described [22, 23].

DNA Probes

Numerous human immunoglobulin gene clones were collected throughout the course of this study. Contributors include: Dr. T. Honjo, Osaka University, Japan, who gave us Charon 4A-Ig recombinant phage representing the Ig Gamma 2, 3, 4 and pseudo genes, a pBR322 recombinant plasmid containing Ig Gamma-1, which were isolated from a fetal liver constructed by T. Maniatis [7]. Dr. P. Leder, Harvard Medical School, contributed a Charon 28-Ig Gamma-4 phage clone. Dr. D. Perlmutter, California Institute of Tech-

nology, sent us Charon 4A clones containing Gamma 1, 2, and 4 isolated from a fetal liver constructed by T. Maniatis [2]. Dr. L. Kirsh, Naval Hospital, Bethesda, Maryland, sent a Charon 28 Ig Gamma 3 recombinant phage along with a pBR322 subclone of the Gamma 3 region sequences.

Isolation of Fragments

All recombinant Ig heavy-chain constant region sequences (IgG) were subcloned into pBR322. The subclones were digested with the appropriate restriction enzyme, and fragments were separated by electrophoresis in .8% agarose gels at .5 V/cm^2. The appropriate fragments were sliced out of the gel and isolated using a sodium iodide-sodium sulfite solution (90.8 g NaI; 1.5 g Na in 100 ml water), which was added at 10 × vol, and samples were placed at 37°C to allow gel dissolution. After 1–6 hrs, silica beads (325 mesh) stored in water slurry were added at a concentration of 1 μl/μg of DNA to be isolated, and samples were left rotating at 4°C overnight. DNA was recovered after two washes with NaI-Na_2SO_3 and three ethanol washes (50% 0.1 M NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA), by resuspending the pellet in .5 × TE, pH 7.5, for 1 hr at 37°C.

Analysis of Haplotypes

In order to identify alleles and haplotypes, all bands appearing on an autoradiogram were scored as present (1) or absent (0) for each individual in a family. For each family, those bands that were present in some members while absent in others were analyzed for Mendelian segregation. Pairwise comparisons were made between arbitrarily chosen bands in order to identify alleles as being those sets of bands that consistently appeared as (1;0), (1;1), (0;0), or (0;1). These were extended into haplotypes by combining the alleles for each of the enzymes and eventually by combining data from the two enzymes used in this study.

Genetic Analysis of RFLP Data

Once alleles and haplotypes were defined, two types of analysis were done using RFLP data. First, using data from families, analysis of linkage was done between the IgH constant region markers and the newly defined IgG RFLPs. Second, analysis of genetic association or linkage disequilibrium (LD) between the newly defined Ig Gamma RFLPs, the Gm allotype markers, and Ig Mu, Alpha 1, and Alpha 2 switch region variants [17] was done using haplotype data established by family studies for 80 unrelated individuals.

RESULTS

Identification of Restriction Enzymes Revealing Ig Gamma Polymorphisms

DNA from 10 randomly chosen individuals was digested with 12 restriction enzymes (R.E.). A recombinant pBR322 plasmid (IgG1) containing a 7.2-kb insert with the gamma 1 coding region exons as well as sequences extending 5 kb in the 3′ direction was used as a probe (fig. 2). Partial nucleotide sequencing data [3–5, 7] indicated that the Ig gamma genes are highly homologous, and use of the other four gamma genes as probes confirmed that they all hybridized to the same fragments, indicating that the use of any IgG probe is sufficient to visualize all five Ig loci (data not shown).

Seven R.E. (*Bgl*I, *Bgl*II, *Bst*EII, *Mbo*I, *Bam*HI, *Pvu*II, and *Taq*I) defined polymorphic fragments having homology to the Ig gamma probe, while five others (*Mbo*II, *Eco*RI, *Hin*dIII, *Pst*I, and *Msp*I were nonpolymorphic. A continuation of this survey with DNA from 10 sets of parents (20 unrelated individ-

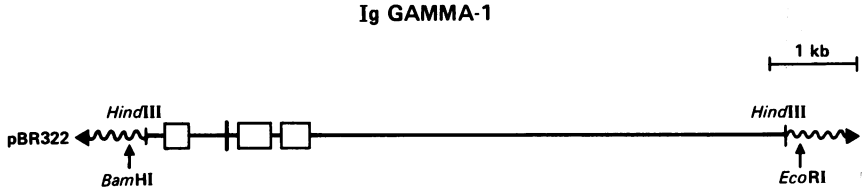

FIG. 2.—A 7.2-kb subclone of a human Ig gamma-1 sequence was used as a probe to define polymorphisms in human genomic DNA. All four coding exons (Ch1, hinge, Ch2, and Ch3) are included within the *Hin*dIII insert, as well as 5 kb of 3′ flanking sequences.

uals) determined that *Mbo*I and *Bst*EII were enzymes that best met the criteria of identifying multiple polymorphisms occurring in frequencies greater than 10%, in a fragment size range where one might expect R.E. site differences to occur within or close to the Ig gamma genes.

Identification of Genotypes

*Mbo*I. Using the restriction enzyme *Mbo*I (recognition sequence GATC), 13 distinct fragments hybridized to a 7.2-kb IgGl genomic DNA sequence. Six of these were polymorphic (3.6, 3.5, 2.8, 2.4, 1.9, and 1.6 kb) in the DNA of 80 unrelated Caucasian individuals surveyed. The 3.5-kb fragment could not be reliably scored because of its being obscured by nonpolymorphic bands of similar molecular weight; therefore, the frequencies of only five polymorphic bands were calculated. Two rare polymorphic fragments of 3.8 and 1.7 kb were seen but were not included in further analysis.

Genotypes, defined as the pattern of fragments present in an individual as visualized by autoradiography, were then ascertained. A total of six distinct patterns were generated by the five polymorphic *Mbo*I fragments analyzed.

Examples of these genotypes are shown in figure 3, and their frequencies are shown in table 1. With the exception of the two rare genotypes (.012), the other four occur in similar frequencies ranging from .175 to .300. Genotypes containing 1.7- and 3.8-kb fragments were not considered in this analysis as each generates a distinct genotype found only once in the sample.

*Bst*EII. DNA from the same 80 unrelated individuals was screened with the restriction enzyme *Bst*EII (recognition site GGTNACC). A total of 13 fragments were identified by hybridization to the 7.2-kb IgGl genomic probe. Eight bands of 5.9, 4.1, 3.7, 2.7, 2.3, 2.2, 1.7, and 1.6 kb were scored as being polymorphic.

Thirteen genotypes were identified, and examples of several of these are shown in figure 4. Frequencies of these genotypes, shown in table 2, indicate that three are found in substantial frequencies (.175–.388), while five are found with frequencies between .063 and .025. The remaining five genotypes each occurred only once in the sample.

Analysis of Haplotypes

To define the various haplotypes comprising a given genotype, segregation of the polymorphic fragments was studied in members of the 28 families partici-

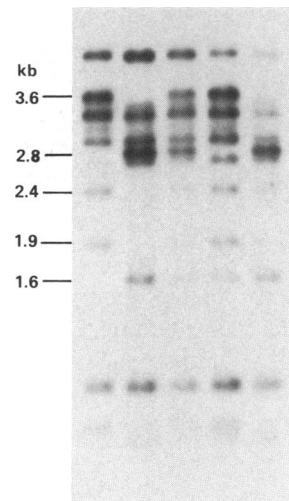

FIG. 3.—Autoradiogram showing several of the polymorphic genotypes identified with the R.E. *Mbo*I. Molecular weights indicate the sizes of the polymorphic bands that were analyzed.

pating in this study. The 80 unrelated individuals are for the most part parents in these families.

*Mbo*I. Of the six genotypes identified with *Mbo*I, segregation analysis allowed the unambiguous identification of four common and two rare haplotypes listed in table 3. Frequencies of the various haplotypes are also shown, indicating that two (*M1, M2*) are common (> .325), two (*M3, M4*) are intermediate in frequency (.081–.088), while two (*M5, M6*) are found only once in the sample.

*Bst*EII. With the 13 *Bst*EII-generated genotypes, nine haplotypes were identified by analysis of segregations in the 28 families. Two haplotypes (*B1* and

TABLE 1

*Mbo*I GENOTYPES IN THE CAUCASIAN POPULATION

*Mbo*I genotypes	No. individuals	Frequency in Caucasian population
3.6, 2.4, 1.9 kb	22	.275
2.8, 1.6 kb	14	.175
3.6, 2.8, 2.4, 1.9, 1.6 kb	24	.300
3.6, 2.4, 1.9, 1.6 kb	18	.225
2.8, 2.4, 1.9, 1.6 kb	1	.012
3.6, 2.8, 1.6 kb	1	.012
Total	80	

NOTE: The six *Mbo*I genotypes are listed in column I according to the molecular weights of the fragments that comprise each genotype. The no. individuals having a particular genotype and the frequency of their occurrence in the Caucasian population are given in columns II and III, respectively.

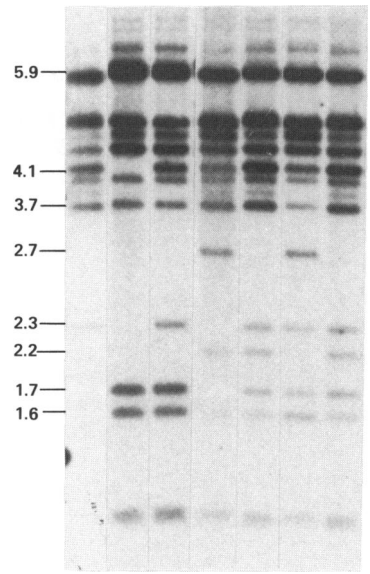

FIG. 4.—Autoradiogram showing several of the polymorphic genotypes identified with the R.E. *Bst*EII. Molecular weights indicate the sizes of the polymorphic bands that were analyzed in the study.

B2) are common, while four haplotypes, *B3–B6,* are found in lower frequencies ranging from .08 and .013. Three rare haplotypes, *B7–B9,* were found with frequencies < .01 as shown in table 4.

Hardy-Weinberg Equilibrium

Analysis of Hardy-Weinberg equilibrium was done separately for the *Bst*EII and *Mbo*I haplotypes. Both χ^2 values were in excellent agreement with all haplotypes being found in Hardy-Weinberg equilibrium (table 5).

Linkage and Genetic Associations between Ig Gamma RFLPs

All of the *Mbo*I and *Bst*EII polymorphisms segregated in complete linkage with one another. No recombinants were observed in the 117 gametes for which crossovers could be tested (i.e., those chromosomes having two or more informative markers).

As both the *Mbo*I and *Bst*EII RFLPs represent Ig gamma variants, the question of their association with each other was investigated. This analysis revealed that there was a significant deviation from the expected distribution, with a χ^2 value of 182.47, (d.f. = 20) (table 6). This very highly significant χ^2 value is due essentially to the association of the two common haplotypes (*M1* with *B1*, *M2* with *B2*) and to the association between two of the less frequent haplotypes—*M3* with *B3*.

TABLE 2

*Bst*EII GENOTYPE IN THE CAUCASIAN POPULATION

*Bst*EII genotypes	No. individuals	Frequency in Caucasian population
5.9, 4.1, 3.7, 2.3, 1.7, 1.6 kb	31	.388
4.1, 3.7, 2.3 kb	16	.200
5.9, 1.7, 1.6 kb	14	.175
4.1, 3.7, 2.3, 1.7, 1.6 kb	5	.063
4.1, 3.7, 2.3, 2.2 kb	3	.038
5.9, 4.1, 2.3, 1.7, 1.6 kb	2	.025
4.1, 3.7, 2.3, 2.2, 1.7, 1.6 kb	2	.025
5.9, 3.7, 1.7, 1.6 kb	2	.025
4.1, 2.3, 1.7, 1.6 kb	1	.013
5.9, 4.1, 3.7, 2.7, 2.2, 1.6 kb	1	.013
5.9, 4.1, 3.7, 2.2, 1.7, 1.6 kb	1	.013
5.9, 4.1, 3.7, 2.7, 2.3, 1.7, 1.6 kb	1	.013
4.1, 3.7, 2.7, 2.3, 1.6 kb	1	.013
Total	80	

NOTE: Thirteen genotypes were defined with the R.E. *Bst*EII. These are listed in column I as the combination of molecular weight fragments that comprise each genotype. The no. individuals having a particular genotype and the frequency of their occurrence in the Caucasian population are given in columns II and III, respectively.

Linkage and Genetic Association between RFLPs and Gm Allotypes

It was also of interest to examine the association between these IgG polymorphic markers and the known IgG allotypes (Gm), as it was hoped that nonrandom associations might localize the new variants to a particular IgG locus. All individuals in the survey were typed for G1m, G2m, and G3m. Again, no recombinants were found between the Gm allotypes and the *Mbo*I and *Bst*EII RFLPs.

Linkage disequilibrium was found between most RFLPs and the three gamma loci (tables 7 and 8). Calculations of linkage disequilibrium for the G2m (n) allotype may, however, be biased since discrimination between homozygosity and heterozygosity for G2m (n) could not be ascertained from the pedigrees.

TABLE 3

*Mbo*I HAPLOTYPES IN THE CAUCASIAN POPULATION

*Mbo*I haplotypes	No. chromosomes	Frequency in Caucasian population
M1: 3.6, 2.4, 1.9 kb	79	.494
M2: 2.8, 1.6 kb	52	.325
M3: 3.6, 2.4, 1.9, 1.6 kb	13	.081
M4: 1.6 kb	14	.088
M5: 2.4, 1.9 kb	1	.006
M6: 3.6 kb	1	.006
Total	160	

NOTE: Molecular weights of the various polymorphic fragments comprising the *Mbo*I haplotypes identified (*M1–M6*) are listed in the first column. One hundred and sixty chromosomes (80 unrelated individuals) were surveyed—their frequency of occurrence in the Caucasian population is indicated.

TABLE 4

*Bst*EII HAPLOTYPES IN THE CAUCASIAN POPULATION

*Bst*EII haplotypes	No. chromosomes	Frequency in Caucasian population
B1: 4.1, 3.7, 2.3 kb	69	.431
B2: 5.9, 1.7, 1.6 kb	64	.400
B3: 4.1, 2.3, 1.7, 1.6 kb	13	.081
B4: 4.1, 3.7, 2.2 kb	5	.031
B5: 5.9, 3.7 kb	4	.025
B6: 4.1, 2.3, 2.2 kb	2	.013
B7: 4.1, 3.7, 2.7, 2.3 kb	1	.006
B8: 4.1, 2.7, 2.3, 1.6 kb	1	.006
B9: 5.9, 2.7, 1.6 kb	1	.006
Total	160	

NOTE: The nine *Bst*EII haplotypes (*B1–B9*) found in this study are listed in the first column according to the molecular weights of the polymorphic fragments that comprise each haplotype. Nos. of each haplotype found in the sample of 160 chromosomes (from 80 unrelated individuals) and their frequencies in the Caucasian population are shown in the last two columns.

Linkage and Genetic Association between IgH Markers

To analyze the degree of linkage between these newly defined RFLPs and other IgH region loci, the Ig gamma haplotypes were extended to include markers representing three additional loci within the Ig heavy-chain gene cluster. These consisted of the Mu, Alpha 1, and Alpha 2 switch region RFLPs described by Migone et al. [17] for which these families had been previously typed. When extended haplotypes were constructed, segregation analysis again showed complete linkage with no incidence of recombination, even though with the number of polymorphic markers available, practically every family was informative for recombination. The variety of different haplotypic combinations (82 total), however, indicated that recombination has occurred within this region, albeit on an evolutionary time scale.

Assignment of Alleles Using Region-Specific Probes

The five gamma genes were mapped with the R.E. *Mbo*I and *Bst*EII. Specific segments of these genes were then isolated for use as regional specific probes in order to assign alleles to a particular gamma gene. Figure 5 shows the R.E. maps and indicates those gene segments that were isolated to represent either 5′, middle, or 3′ segments of the immunoglobulin genes.

*Mbo*I. Individuals homozygous for the two common haplotypes, *M1* and *M2*, were investigated using different regions of the IgG clones as probes. The first probe used was the extreme 5′ segment of the pseudo gamma (1.1 kb) as indicated in figure 5. It contains the first exon, the hinge region, and half of the second exon. When tested on *M1* and *M2* homozygous haplotypes, no differences were seen [fig. 6(*A*)]. In the figure, a 2.7-kb fragment is present in both *M1* and *M2* and therefore obscures a polymorphic band of 2.7 kb observed with the third probe of figure 6 (*C*).

TABLE 5

RESULTS OF TESTS FOR HARDY-WEINBERG EQUILIBRIUM FOR THE INHERITANCE OF THE *Mbo*I AND *Bst*EII HAPLOTYPES

	No. expected	No. observed	Contribution to χ^2
	*A. Mbo*I		
Homozygous:			
M1/M1	19.05	20	.047
M2/M2	8.45	11	.770
M3/M3	.53	1	.416
M4/M4	.62	0	.62
M5–M6/M5–M6	.03	0	.03
Heterozygous:			
M1/M2	25.38	22	.45
M1/M3	6.32	7	.073
M1/M4	6.87	8	.189
M1/M5–M6	1.41	0	1.41
M2/M3	4.20	3	.343
M2/M4	4.58	3	.545
M2/M5–M6	.94	2	1.19
M3/M4	1.14	2	.649
M3/M5–M6	.23	1	2.58
M4/M5–M6	.25	0	.25
			$\chi^2 = 9.55$ [10]
	*B. Bst*EII		
Homozygous:			
B1/B1	15.35	15	.008
B2/B2	12.80	14	.113
B3–B9/B3–B9	.69	1	.139
Heterozygous:			
B1/B2	28.03	29	.034
B1/B3	5.65	6	.018
B1/B4	1.78	1	.342
B1/B5	1.75	1	.321
B1/B6–B9	2.10	3	.386
B2/B3	5.18	3	.917
B2/B4	1.62	1	.238
B2/B5	1.62	2	.089
B2/B6–B9	1.95	1	.463
B3–B9/B4–B9	1.25	3	2.431
			$\chi^2 = 5.498$ [12]

NOTE: In both *A* and *B*, column I lists the various possible combinations of haplotypes that comprise a genotype. Columns II and III are the expected and observed numbers of these genotypes, while column IV gives the χ^2 value of significance for each observation.

TABLE 6

ASSOCIATIONS BETWEEN *Mbo*I AND *Bst*EII HAPLOTYPES

*Bst*EII HAPLOTYPES	*Mbo*I HAPLOTYPES														
	M1			*M2*			*M3*			*M4*			*M5* AND *M6*		
	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2
B1	59	34.12	18.14	5	22.75	13.85	3	5.69	1.27	2	6.13	2.78	1	1.31	.07
B2	9	31.20	15.80	44	20.08	28.49	0	5.20	5.20	10	5.60	3.46	1	1.20	.03
B3	3	6.34	1.76	0	4.23	4.23	10	1.06	75.40	0	1.14	1.14	0	0.24	0.24
B4	4	1.95	2.16	0	1.3	1.3	0	0.33	0.33	0	0.35	0.35	0	0.08	0.08
B5	0	1.95	1.95	2	1.30	0.38	0	0.33	0.33	1	0.35	1.21	1	0.35	1.21
B6–B9	3	2.44	0.13	1	1.63	.24	0	0.41	0.41	1	0.44	0.44	0	0.09	0.09

NOTE: $\chi^2_{[20]} = 182.47$. The observed (O), expected (E), and χ^2 values are given for the association between each *Mbo*I and *Bst*EII haplotype. Rare haplotypes for each enzyme were compiled into a single class to minimize their contribution to the χ^2 value.

TABLE 7

ASSOCIATIONS BETWEEN *MboI* HAPLOTYPES AND Gm ALLOTYPES IN THE CAUCASIAN POPULATION

	G1m ALLOTYPES																			
	f			fa			a			ax			za			z a x			No ALLOTYPE	TOTAL
	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2		
M1	78	52.34	12.58	0	0.49	0.49	0	0.49	0.49	0	1.48	1.48	1	12.34	10.42	0	11.36	11.36	0	79
M2	3	34.45	28.71	1	0.33	1.36	1	0.33	1.36	3	0.98	4.16	21	8.13	20.37	23	7.48	32.20	0	52
M3	12	8.61	1.33	0	0.08	0.08	0	0.08	0.08	0	0.24	0.24	1	2.03	0.52	0	1.87	1.87	0	13
M4	12	9.28	0.80	0	0.09	0.09	0	0.09	0.09	0	0.27	0.27	2	2.19	0.02	0	2.01	2.01	0	14
M5 and *6*	1	0.67	0.16	0	0.01	0.01	0	0.01	0.01	0	0.02	0.02	0	0.16	0.16	0	0.14	0.14	1	2
Total	106			1			1			3			25			23			1	160
Total χ^2 = 132.90 [20]																				

	G2m ALLOTYPES								G3m ALLOTYPES								
	n^+			n^-			NOT ASCERTAINED	TOTAL		g			b			No ALLOTYPE	TOTAL
	O	E	χ^2	O	E	χ^2				O	E	χ^2	O	E	χ^2		
M1	32	17.42	17.42	14	28.58	7.44	33	79	*M1*	1	24.31	22.35	78	54.69	9.94	0	79
M2	1	14.39	12.46	37	23.61	7.59	14	52	*M2*	43	14.77	53.96	5	33.23	23.98	4	52
M3	5	4.92	.001	6	6.83	0.10	2	13	*M3*	1	4.00	2.25	12	9.00	1.00	0	13
M4	0	2.27	2.27	6	3.73	1.38	8	14	*M4*	2	4.31	1.24	12	9.69	0.55	0	14
M5 and *6*	1	0.76	0.08	1	1.24	0.05	0	2	*M5* and *6*	1	0.62	0.23	1	1.38	0.10	0	2
Total	39			64			57	160	Total	48			108			4	160
Total χ^2 = 48.79 [4]									Total χ^2 = 115.60 [4]								

NOTE: Instances where no Gm allotypes were expressed are noted for gamma 1 and 3 and instances where chromosomal assignments were not ascertained are noted for the gamma 2 allotypes.

TABLE 8

ASSOCIATIONS BETWEEN *BstEII* HAPLOTYPES AND Gm ALLOTYPES IN THE CAUCASIAN POPULATION

	G1m ALLOTYPE																			
	f			fa			a			ax			za			z a x			No ALLOTYPE	TOTAL
	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2	O	E	χ^2		
B1	65	45.71	8.14	0	0.43	0.43	0	0.43	0.43	0	0.43	0.43	1	10.78	8.87	3	9.92	4.83	0	69
B2	18	42.40	14.04	0	0.40	0.40	1	0.40	0.40	3	0.40	0.40	21	10.00	12.10	20	9.20	12.68	1	64
B3	12	8.61	1.33	0	0.08	0.08	0	0.08	0.08	0	0.08	0.08	1	2.03	0.52	0	1.87	1.87	0	13
B4	5	3.31	0.86	0	0.03	0.03	0	0.03	0.03	0	0.31	0.31	0	0.78	0.78	0	0.72	0.72	0	5
B5	2	2.65	0.16	0	0.03	0.03	0	0.03	0.03	0	0.02	0.02	2	0.63	2.98	0	0.58	0.58	0	4
B6–9	4	3.33	0.13	1	0.03	0.03	0	0.03	0.03	0	0.09	0.09	0	0.79	0.79	0	0.72	0.72	0	5
Total	106			1			1			3			25			23			1	160
Total χ^2 = 75.43 [25]																				

	G2m ALLOTYPE								G3m ALLOTYPE								
	n^+			n^-			NOT ASCERTAINED	TOTAL		g			b			No ALLOTYPE	TOTAL
	O	E	χ^2	O	E	χ^2				O	E	χ^2	O	E	χ^2		
B1	31	16.66	12.34	13	27.34	7.52	25	69	*B1*	4	20.70	13.47	65	46.58	7.28	0	69
B2	1	16.66	14.72	43	27.34	8.97	20	64	*B2*	41	18.46	27.52	19	43.20	13.56	4	64
B3	4	3.41	0.10	5	5.59	0.06	4	13	*B3*	1	3.90	2.16	12	8.78	1.18	0	13
B4	1	0.76	0.08	1	1.24	0.05	3	5	*B4*	0	1.50	1.50	5	3.38	0.78	0	5
B5	1	0.98	0	2	1.60	0.10	2	4	*B5*	2	1.20	0.53	2	2.70	0.18	0	4
B6–9	1	0.38	1.0	0	0.62	0.62	3	5	*B6–9*	0	1.54	1.54	5	3.46	0.69	0	5
Total	39			64			57	160	Total	48			108			4	160
Total χ^2 = 45.57 [5]									Total χ^2 = 70.39 [5]								

NOTE: Instances where no Gm allotypes were expressed are noted for the gamma 1 and 3 genes, and instances where chromosomal assignments of allotypes were not ascertained are noted for gamma 2.

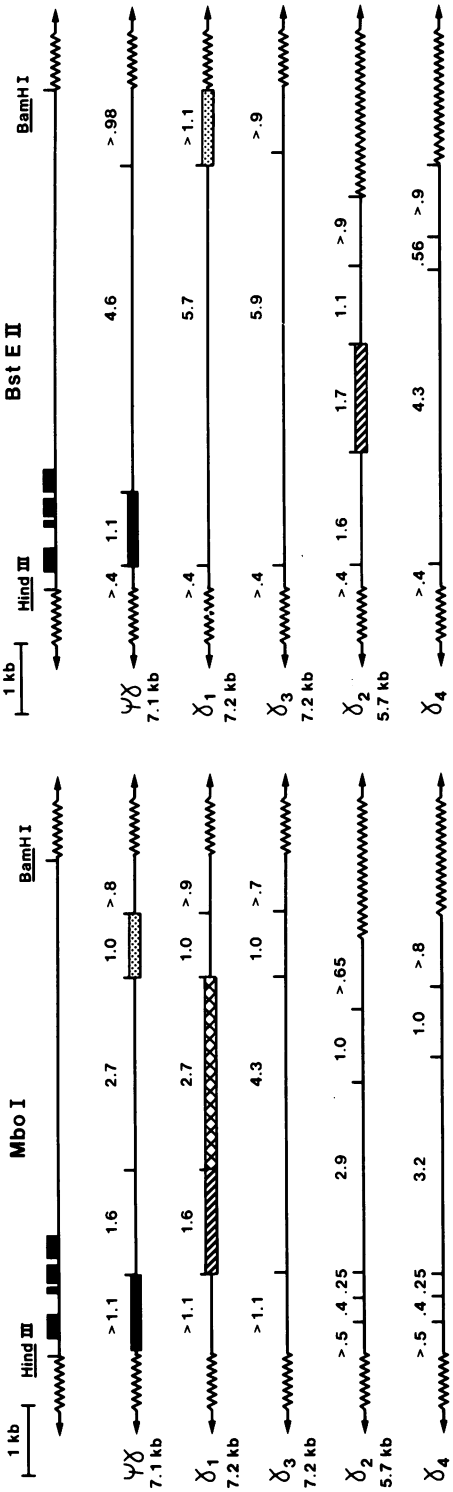

FIG. 5.—*Mbo*I and *Bst*EII restriction maps for the five Ig gamma subclones are shown under the schematic diagram of an Ig gamma genomic region sequence. The 5′ end of all subclones was determined to be a *Hin*dIII site that occurs 200 bp 5′ to exon 1. The 3′ end of each subclone was arbitrarily defined as the first *Bam*HI site; therefore the subclones vary in overall length but all have a common 5′ end. The *Mbo*I or *Bst*EII fragments isolated for use as regional specific probes are indicated as *bars* on the individual gene maps.

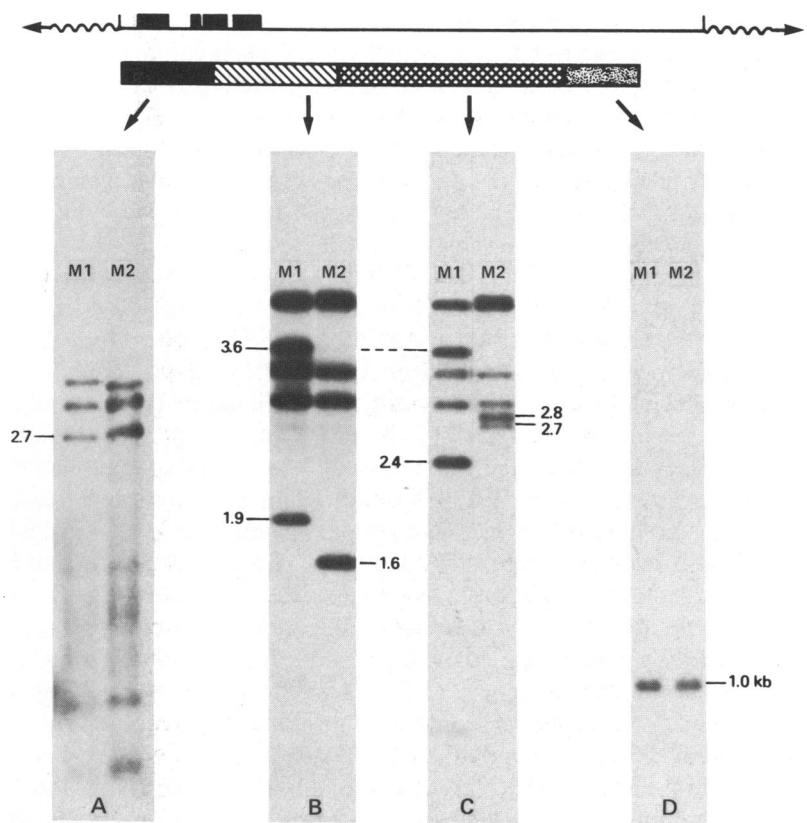

FIG. 6.—DNA was isolated from individuals homozygous for the two common *Mbo*I haplotypes (*M1* and *M2*). Probes from four different regions of the original gamma clones were used to identify polymorphisms occurring in various regions surrounding the gamma genes. Molecular weights refer to those fragments that were known to be polymorphic or, in the case of the 2.7-kb fragment, were disguised by fragments from other regions of the gamma genes that were of identical molecular weight. The probes are indicated *above the autoradiograms* for regions A–D and refer to those isolated fragments depicted in figure 5.

The second probe (1.6 kb from gamma 1) contains the remaining part of the gamma gene exons. It hybridizes to three nonpolymorphic fragments (fig. 6*B*). Mapping data from figure 5 indicate that the 2.9-kb fragment is gamma 2, the 4.3-kb fragment is gamma 3, and the 3.2-kb fragment is gamma 4. Polymorphic fragments detected with this probe consist of: (1) a 1.6-kb fragment (see fig. 6*B*) found in the *M2* haplotype, which must be a doublet as the restriction maps of both pseudo gamma and gamma 1 show a fragment of this size, and (2) a 3.6- and 1.9-kb fragment (fig. 6*B*) in the *M1* haplotype, which must be allelic to pseudo gamma and gamma 1.

When a 2.7-kb gamma 1 fragment (representing a 3′ region) was used as a probe, additional polymorphisms were revealed between the two haplotypes

(fig. 6*C*). Besides the invariant bands common to both, in haplotype *M1*, the 3.6-kb fragment and a new fragment at 2.4 kb hybridized. In haplotype *M2*, bands of 2.8 and 2.7 kb are seen. Mapping and hybridization data attribute the 2.7-kb band as belonging to both the pseudo gamma and gamma 1 genes, while the 2.8-, 3.6- and 2.4-kb fragments remained unassigned to any particular locus.

The fourth probe of 1.0 kb isolated from the pseudo gamma gene (extreme 3′ region) hybridizes to a single 1.0-kb fragment in both haplotypes (fig. 6*D*) and is likely to be a triplet containing sequences from pseudo gamma, gamma 1, and gamma 3 (see fig. 5). Whether or not G2 and G4 fragments are hybridizing to this probe is unclear. They may be too small or, more likely, the homology between the gamma genes of the G2-G4 two cluster is much less at this distance from the gene and cross-hybridization is no longer occurring.

Analysis of these Southern blot data determined that the *Mbo*I polymorphisms were the result of RFLPs at more than one Ig gamma locus. Only the polymorphic alleles of haplotype *M2* could be unambiguously identified, while assignment of the alternate alleles of G1 and pseudo gamma in haplotype *M1* could not be made. At this stage, the bands differentiating the two haplotypes were identified as being: *M1:* 3.6 kb (pseudo gamma of G1); and 1.9–2.4 kb (pseudo gamma or G1); *M2:* 1.6 – 2.7 kb (pseudo gamma); and 1.6–2.7 kb (G1).

*Bst*EII. The two prevalent *Bst*EII haplotypes (*B1* and *B2*) were also investigated in homozygous individuals, using region-specific probes (fig. 5), and the results are shown in figure 7. A probe containing most of the coding region (1.1-kb *Bst*EII pseudo gamma fragment) recognized fragments of 5.7, 4.3, 3.7, 2.3, and 1.1 kb in haplotype *B1* and 5.9-, 5.7-, 4.3-, 1.6-, and 1.1-kb fragments in haplotype *B2* (fig. 7*A*). Mapping data assigns the two nonpolymorphic fragments (4.3 and 1.1 kb) to gamma 4 and pseudo gamma, respectively. The 1.6-kb fragment could be identified as a gamma 2 polymorphism, while the 5.9-kb polymorphic fragment that occurs as a doublet (5.9- and 5.7-kb bands) in haplotype *B2* is a gamma 3 gene polymorphism (fig. 5). These results confirmed that the *Bst*EII RFLPs define two polymorphic loci, with the 3.7- and 2.3-kb fragments being allelic to the 5.9 (gamma 3) and 1.6 (gamma 2) fragments.

Using a probe representing a region immediately 3′ to the coding sequences (a 1.7-kb gamma 2 fragment), the nonpolymorphic fragments of 4.6 kb (pseudo gamma), 5.7 kb (gamma 1), and 4.3 kb (gamma 4) again hybridized in both haplotypes. Polymorphic fragments in haplotype *B1* from this region consist of the 3.7-kb, which must include both the gene and more 3′ sequences, and a 4.1-kb fragment (fig. 7*B*). In haplotype *B2*, the 5.9-kb fragment (gamma 3) hybridized to this region as well as to the gene region probe; however, the 1.6-kb fragment containing the gamma 2 gene no longer hybridized. Instead, the 1.7-kb gamma 2 fragment that is 3′ to the 1.6-kb fragment in the gamma 2 gene map (see fig. 5) hybridizes.

A probe from the extreme 3′ region of the gamma subclones (1.1 of gamma 1) revealed additional polymorphisms (fig. 7*C*). As mentioned above, the 3.7-kb fragment was not originally recognized as being polymorphic. Dissection of a larger probe into smaller segments revealed that in haplotype *B1* the 3.7-kb fragment contains sequences homologous to the gamma gene region. In con-

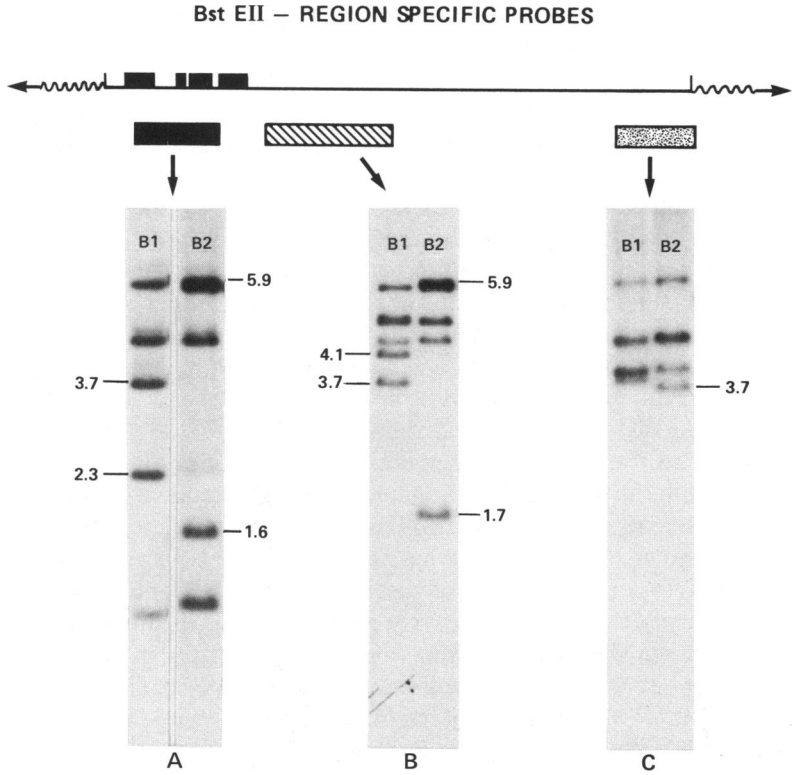

FIG. 7.—*Bst*EII delineated probes from three different regions of the original gamma clones were used to identify polymorphisms occurring in various regions surrounding the gamma genes. Molecular weights refer to those fragments that were known to be polymorphic or, in the case of the 3.7-kb fragment, which were disguised by fragments from other regions of the gamma genes that are of identical molecular weight. The specific genes from which the probe fragments were isolated are those shown in figure 5.

trast, the 3.7-kb fragment seen in haplotype *B2* is homologous to a region at least 4 kb 3′ to a gamma gene (as seen in fig. 7). These experiments again confirmed that the *Bst*EII RFLPs are also the result of variation at more than one locus, and the haplotypes were identified as being: *B1:* 3.7 kb (G2 or G3) and 2.3 kb (G3 or G2); *B2:* 5.9 kb (G3) and 1.6–1.7 kb (G2).

Assignment of Alternate Alleles through Genetic Analysis

*Mbo*I. Two rare *Mbo*I haplotypes (*M5* and *M6*) were found in the individuals surveyed and were useful in assigning the polymorphic fragments of the alternate haplotypes to a specific IgG locus. In the one individual, with the *M5* haplotype, a 3.6-kb fragment is absent in what would otherwise be the common *M1* haplotype (3.6, 2.4, 1.9). In addition, this individual expresses the G3m ^{b} allotype, but no G1m allotype on that chromosome. In most Caucasian individuals, the G1m ^{f} and G3m ^{b} allotypes are found on the same chromosome. As

molecular data indicated that the two polymorphic loci of *M1* are the 3.6-kb and the 2.4–1.9-kb fragments, one of which must be gamma 1 allele, we therefore hypothesize that the 3.6-kb fragment is the gamma 1 locus in *M1* and, by elimination, the 2.4–1.9-kb fragments represent the pseudo gamma gene of haplotype *M1*. This indicates that there has been a deletion of gamma 1 but not of pseudo gamma in this individual. Other markers 3′ to the G1 and pseudo gamma genes including the *Bst*EII markers were scored as homozygous in this individual; therefore, they did not contribute additional evidence.

The other aberrant *Mbo*I haplotype—*M6*—is the opposite configuration, having only the 3.6-kb fragment but not the 2.4- and 1.9-kb fragments. Seven individuals in a 3-generation pedigree inherited this chromosome. Allotype data indicate that this is an extremely rare haplotype, *Gm f;..;g*, which has been postulated to result from a crossover between the two common haplotypes *f;..;b* and *za;..;g* [24]. The molecular data indicate that in addition to a crossover there has been a deletion of the pseudo gamma gene. As G1m ^{f} allotypes are expressed and the 3.6-kb *Mbo*I fragment is present in these individuals, this again argues that the 3.6-kb *Mbo*I fragment is gamma 1 and the 2.4- and 1.9-kb fragments represent the pseudo gamma gene.

*Bst*EII. Analysis of segregation of the *Bst*EII RFLPs identified two haplotypes that are likely to be the result of recombination events and that were also useful in assigning alleles to specific IgG genes. These are: (1) *B3*—which contains the 4.1- and 2.3-kb fragments normally found in haplotype *B1* along with the 1.6- and 1.7-kb fragments of haplotype *B2*, and (2) *B5*—which contains a 3.7-kb and a 5.9-kb fragment. One limitation in identifying this haplotype is that the 5.9-kb fragment tends to comigrate with a nonpolymorphic 5.7-kb fragment, but longer electrophoretic runs can be used to separate them (data not shown). The Gm haplotype segregating with *B3* is *gm f;..;b*. There are three independent *B5* haplotypes from different families, one of them segregates with *Gm f;n;g*, another with *Gm za;..;b*, and a third with *Gm za;(n);g*. It is therefore clear that the recombination events leading to *B3* and *B5*, as well as to the *M5* and *M6* haplotypes, must be relatively old, as several other evolutionary events have happened since they arose.

Information from *B5* can be used to recognize the 3.7-kb fragment as a gamma 2 allele. Using a probe from the coding region to hybridize DNA from *B5/B2* heterozygotes, we find that the 3.7-kb fragment is present and cannot come from the *B2* haplotype that does not hybridize to it (fig. 8). The fragment thus contains a gamma gene, and as we have already seen when analyzing the restriction mapping data of figure 5, it must be either gamma 2 or gamma 3. As we already know that the 5.9-kb fragment contains a gamma 3 gene, unless gamma 3 is duplicated and the gamma 2 gene is missing, then the simplest hypothesis is that the 3.7-kb fragment is the gamma gene normally found in haplotype *B1*. Allotype data indicates that the gamma 2 gene is expressed by the *B5* chromosome, as some *B5* haplotypes are n^{+}. Therefore, the simplest interpretation is that *B5* results from an equal crossover as shown in figure 9. The reciprocal crossover is also found in our population and is haplotype *B3*, previously described in this paper as a recombinant.

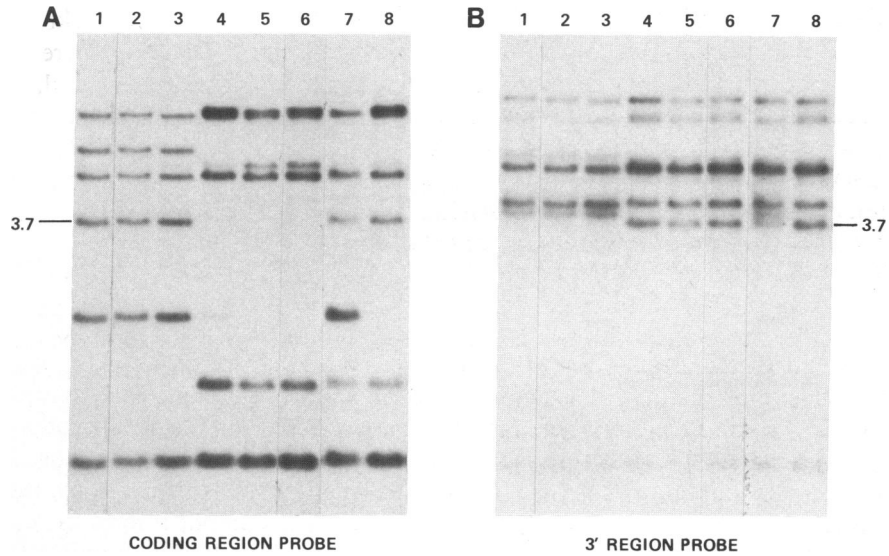

FIG. 8.—This figure illustrates the existence of a recombinant chromosome in which the 3.7-kb *Bst*EII fragment (containing gamma 2 coding regions) is found on the same chromosome as the 5.9-kb *Bst*EII fragment (containing gamma 3 coding regions). In *A*, the probe is a fragment that contains coding region sequences only. In *B*, the probe contains 3′ flanking region sequences only. *Lanes 1–3A* and *B* contain DNA from individuals homozygous for the common haplotype *B1* in which the 3.7-kb fragment is the gamma 2 gene, hybridizing to the gene containing probe but not the 3′ flanking region probe. *Lanes 4–6A* and *B* contain DNA from individuals homozygous for the alternate common haplotype *B2*, in which the 3.7-kb fragment hybridizes to the 3′ flanking region probe only (and is a region 3′ to the gamma 3 gene). *Lanes 7A* and *B* are controls showing that in a heterozygous individual (*B1/B2*) two 3.7-kb fragments hybridize, one with either probe. *Lanes 8A* and *B* illustrate that the recombinant chromosome (*B2/B5*) contains two 3.7-kb fragments, one which hybridizes to a coding region probe and another to a 3′ flanking region probe as the recombination event has brought the 3.7-kb gamma 2 fragment onto the same chromosome as the 5.9-kb gamma 3 chromosome.

In conclusion, we can identify all the polymorphic alleles of the two common haplotypes as follows:

	*Mbo*I		*Bst*EII	
	M1	M2	B1	B2
gamma 1	3.6 kb vs. 1.6 kb		5.7 kb, not polymorphic	
gamma 2	2.9 kb, not polymorphic		3.7 kb vs. 1.6 kb	
gamma 3	4.3 kb, not polymorphic		2.3 kb vs. 5.9 kb	
pseudo	2.4 kb vs. 1.6 kb		1.1 kb, not polymorphic	

DISCUSSION

This research provides a detailed analysis of genetic variation within the Ig gamma heavy-chain gene clusters in the Caucasian population. Several polymorphisms occur within the coding regions themselves (i.e., *Mbo*I gamma 1

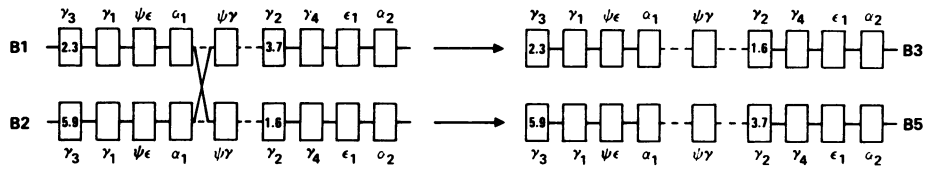

FIG. 9.—This figure illustrates that an equal, homologous crossover between the two common *Bst*EII haplotypes (*B1* and *B2*) could give rise to the two recombinant haplotypes (*B3* and *B5*) seen in the study. The crossover is shown as occurring between Alpha 1 and Pseudo Gamma for illustrative purposes only, as the data simply indicate that the crossover has occurred somewhere between the two major IgG clusters.

and pseudo gamma allele of *M2*); however, the majority of changes occur within flanking regions. Alleles in this system varied greatly in size in contrast to the IgH switch region polymorphisms that are often the result of relatively small insertion/deletion events [17]. With the switch region RFLPs, most alleles were within 500 base pairs (bp) of one another in size; therefore, they migrated in a cluster and were easily identified. This was not true of the currently described RFLPs, which, in part, explains our initial difficulties in identifying the alleles.

No recombinants were found to occur between the Ig gamma markers studied, but this was not unexpected. Altogether 117 gametes were tested that were heterozygous for at least two markers in the IgCh region. Therefore, there were approximately even chances of observing or not observing crossing over frequencies of around 1%. In a much larger investigation of immunoglobulin heavy-chain allotypes of inbred mouse strains where the progeny of 2,000 informative matings were studied, no incidence of recombination was revealed [25].

These results confirm that the Ig gamma loci are usually inherited as a single block and indicate that although as yet not physically linked to molecular methods, the four Ig constant region gene clusters must be in fairly close proximity to one another. Mimimum estimates for the length of the human IgCh region can be made based on knowledge of the murine IgC H locus, which encompasses a total of 200 kb. The human IgCh locus is certainly larger as the cluster containing gamma-epsilon-alpha has been duplicated during evolution and an additional region containing the pseudo gamma gene has been mapped as falling between the other two gamma gene clusters [9]. The region could easily be 400 kb in length, but in terms of recombination, 400 kb is only .4 centimorgans. Therefore, it is not surprising that recombinants are rarely directly observed in family studies, as the probability of witnessing such an event is rather low. The analysis of linkage disequilibrium does, however, offer the chance of studying the accumulation of recombination over generations, and from this analysis it is apparent that recombination has occurred between all the IgCh gene clusters in evolutionary time.

The methodology of dissecting RFLPs within the highly homologous Ig gamma gene region may have wider implications for the investigation of other multilocus systems. The use of long probes for the initial screening avoids a

priori predictions as to where polymorphisms will occur; therefore, coding and flanking regions can be investigated within a single experiment. In this study, enzymes were chosen that gave complex patterns and large numbers of bands, as this increased the likelihood of finding polymorphisms occurring throughout the region. Restriction enzymes such as *Bam*HI, *Hin*dIII, and *Eco*RI give much simpler hybridization patterns, as each gene and its flanking regions are present on a single fragment. However, their use in population studies and medical genetics is limited as these enzymes do not significantly increase the amount of heterogeneity that has already been observed at the protein level.

With the Ig gamma RFLPs, the number of Gm haplotypes in the Caucasian population was increased to 44 from 13 haplotypes generated by allotype data alone. By including information from the three switch region RFLPs (Mu, Alpha 1, and Alpha 2), the number of haplotypes present in this Caucasian sample was increased to 82. The advantages of this large number of alleles in population studies are immediately obvious in that this level of heterogeneity rivals that found for hemoglobins [26–30] or for mtDNA in human populations [31]. In medical studies, the increase in detectable Gm variation may elucidate new disease correlations in what were previously determined only by Gm allotype groups.

Disadvantages to this methodology were that once an initial survey was done to identify enzymes that produce many fragments, it was difficult to distinguish the different loci vs. the different alleles at a single locus. As the restriction enzyme used in this study analyzed frequently occurring sites within the Ig gamma region, probes from regions distant to the coding sequences that lack sequence homology to all five gamma genes could not be used to identify specific loci as they did not overlap with the fragments being investigated. To address this problem, methods were developed to define and isolate regional specific probes corresponding to discrete R.E. fragments. By using regional specific probes, on genomic digests of DNA from individuals homozygous for a specific haplotype, simpler hybridization patterns were attained. Clarification of the regions of the gamma genes in which polymorphisms occurred was possible with these probes, and additional heterogeneity was revealed. All fragments occurring in the subclones, whether or not polymorphic, were readily assigned to a gamma gene based on molecular weight and occurrence in a specific region.

The *Bst*EII and *Mbo*I polymorphisms confirm and extend the picture of variation in the IgCh region of the human genome [1]. They indicate that there are essentially two major haplotypes, as already seen with the G1m-G3m allotype data: 50% of the Caucasian population is haplotype *M1*, 33% is *M2*, 43% is *B1*, and 40% is *B2*. *B1* is predominantly *M1* and *Gm f;..;b*, while *B2* is predominantly *M2* and *Gm za(x);..;g*. There is then a basic polymorphism of two Caucasian haplotypes from which either by mutation, deletion/insertion, or recombination the other rare haplotypes seem to be derived. We have described examples of recombination events as well as deletions postulated to have occurred fairly early in evolution, but certainly subsequent to the separation of the two main haplotypes. It is clear that the two haplotypes are distin-

guished by a great number of mutations, as witnessed both by the immunological and the molecular data. It therefore seems reasonable to postulate that the primary event leading to the separation of the two main haplotypes was an early one in Caucasian evolution and was maintained by heterozygous advantage, so that enough time was available for the accumulation of the many mutations that differentiate the two haplotypes. Judging from immunological information, this polymorphism is present in essentially all Caucasian populations but is absent from the other major ethnic groups. Its origin is therefore likely to be subsequent to the separation of the major human ethnic groups and, perhaps, after the occupation of Europe by modern humans. If the latter is taken to correspond to the disappearance of Neanderthal in southwest Europe, then its date is between 30,000 and 35,000 years ago [32].

One may question why the long persistence of two haplotypes in a population should be indicative of heterozygous advantage. The answer is to be found in theoretical computations by Miller [33] and by Robertson [34]. In a finite population, one of two alleles is eventually fixed, but fixation can be considerably retarded if there is heterozygous advantage. For simplicity, consider the case in which selective disadvantage s_1 and s_2 of the two homozygotes with respect to the heterozygote are equal ($s = s_1 = s_2$), giving rise to an equal gene frequency for the two alleles at equilibrium. One can then calculate a "retardation factor" that increases with the effective population size N_e, and with the selective disadvantage s. If $N_e s$ is equal to 1, the retardation due to heterozygous advantage is modest. But for $N_e s = 10$, the persistence of the polymorphism is about 690 times longer than with selectively neutral alleles. $N_e s = 10$ may mean, for instance, that for $N_e = 10{,}000$, $s = 0.001$; this is a small selection coefficient that is almost impossible to measure directly from data. The persistence of a polymorphism under heterozygous advantage is longer only if the gene frequencies are between 20% and 80% [34, 35], a condition that is satisfied by our data.

With the RFLPs defined in this and other studies [1, 9, 17, 18, 36], the immunoglobulin heavy-chain region is becoming a well-characterized region of the human genome, revealing new data of our evolutionary past.

ACKNOWLEDGMENTS

We acknowledge the contributions of Drs. Vernon Oi and Howard Cann, and Juliana Hwang, Berta Young, and Lois Martine for their help with the research project and preparation of this manuscript.

REFERENCES

1. MIGONE N, DE LANGE G, PIAZZA A, CAVALLI-SFORZA L: Genetic analysis of eight linked polymorphisms within the human immunoglobulin heavy-chain region. *Am J Hum Genet* 37:1146–1163, 1985
2. ELLISON J, BUXBAUM J, HOOD L: Nucleotide sequence of a human immunoglobulin $C_{\gamma 4}$ gene. *DNA* 1:11–18, 1981
3. KRAWINKEL U, RABBITTS TH: Comparison of the hinge-coding segments in human immunoglobulin gamma heavy chain genes and the linkage of the gamma 2 and gamma 4 subclass genes. *EMBO J* 1:403–407, 1982

4. ELLISON JW, BERSON BJ, HOOD LE: The nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene. *Nucleic Acids Res* 10:4071–4079, 1982
5. ELLISON J, HOOD L: Linkage and sequence homology of two human immunoglobulin γ heavy chain constant region genes. *Proc Natl Acad Sci USA* 79:1984–1988, 1982
6. FLANAGAN JG, RABBITTS TH: Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε and α genes. *Science* 300:709–713, 1982
7. TAKAHASHI N, UEDA S, OBATA M, NIKAIDO T, NAKAI S, HONJO T: Structure of human immunoglobulin gamma genes: implications for evolution of a gene family. *Cell* 29:671–679, 1982
8. LEFRANC M-P, LEFRANC G, RABBITTS TH: Inherited deletion of immunoglobulin heavy chain constant region genes in normal human individuals. *Nature* 300:760–762, 1982
9. BECH-HANSEN NT, LINSLEY PS, COX DW: Restriction fragment length polymorphisms associated with immunoglobulin C_γ genes reveal linkage disequilibrium and genomic organization. *Proc Natl Acad Sci USA* 80:6952–6956, 1983
10. SHANDER M, MARTINIS J, CROCE CM: Genetics of human immunoglobulins: assignment of the genes for μ, α, and γ immunoglobulin chains to human chromosome 14. *Transplant Proc* XII:417–420, 1980
11. CROCE CM, SHANDER M, MARTINIS J, ET AL.: Chromosomal location of the genes for human immunoglobulin heavy chains. *Proc Natl Acad Sci USA* 76:3416–3419, 1979
12. KIRSCH IR, MORTON CC, NAKAHARA K, LEDER P: Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. *Science* 216:301–303, 1982
13. LOGHEM EV: Genetic studies on human immunoglobulins, in *Handbook of Experimental Immunology,* 3rd ed., edited by WEIR DM, Oxford, England, Blackwell, 1978, pp 11.1–11.16
14. STEINBERG AG, COOK CE: *The Distribution of the Human Immunoglobulin Allotype*. Oxford, England, Oxford Univ. Press, 1981
15. ZELASCHI D, JOHNSON MJ, NEWBY CJ, HERZENBERG LA: New methods for human allotyping, in *Handbook of Experimental Immunology,* 4th ed., edited by WEIR DM, HERZENBERG LA, BLACKWELL CC, HERZENBERG LA, Edinburgh, Blackwell, 1985
16. ZELASCHI D, NEWBY C, PARSONS M, ET AL.: Human immunoglobulin allotypes: previously unrecognized determinants and alleles defined with monoclonal antibodies. *Proc Natl Acad Sci USA* 80:3762–3766, 1983
17. MIGONE N, FEDER J, CANN H, ET AL.: Multiple DNA fragment polymorphisms associated with immunoglobulin μ chain switch-like regions in man. *Proc Natl Acad Sci USA* 80:467–471, 1983
18. LEFRANC M-P, RABBITTS TH: Human immunoglobulin heavy chain A2 gene allotype determination by restriction fragment length polymorphism. *Nucleic Acids Res* 12:1303–1311, 1984
19. FEDER J, YEN L, WIJSMAN E, ET AL.: A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. *Am J Hum Genet* 37:635–649, 1985
20. SOUTHERN EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517, 1975
21. RIGBY P, DIECKMANN M, RHODES C, BERG P: Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J Mol Biol* 113:237–251, 1977
22. SCHANFIELD MS, LOGHEM EV: Human immunoglobulin allotypes, in *Handbook of Experimental Immunology,* 4th ed., edited by WEIR DM, HERZENBERG LA, BLACKWELL CC, HERZENBERG LA, Edinburgh, Blackwell, 1985
23. LANGE GD, WRIGHT P, VAN EEDE P, VAN LEEUWEN F, LONG HT, HONG NTD: Association between leprosy and immunoglobulin allotypes: Gm-A2m and Km frequencies in Vietnamese. *J Immunogenet* 11:173–180, 1984

24. NATVIG JB, KUNKEL HG, LITWIN SD: Genetic markers of the heavy chain subgroups of human γG globulin. *Cold Spring Harbor Symp Quant Biol* 32:173–180, 1967
25. HERZENBERG LA, HERZENBERG LA: Mouse immunoglobulin allotypes: description and special methodology, in *Handbook of Experimental Immunology*, 3rd ed., edited by WEIR DM, Oxford, England, Blackwell, 1978, pp 12.1–12.23
26. HIGGS DR, GOODBOURN SEY, WAINSCOAT JS, CLEGG JB, WEATHERALL DJ: Highly variable regions of DNA flank the human α globin genes. *Nucleic Acids Res* 9:4213–4224, 1981
27. ORKIN SH, KAZAZIAN JR HH, ANTONARAKIS SE, ET AL.: Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. *Nature* 296:627–631, 1982
28. BOEHM CD, ANTONARAKIS SE, PHILLIPS JA, STETTEN G, KAZAZIAN HH: Prenatal diagnosis using DNA polymorphisms. *N Engl J Med* 17:1054–1058, 1983
29. ANTONARAKIS SE, BOEHM CD, GIARDINA PJV, KAZAZIAN HH: Nonrandom association of polymorphic restriction sites in the β-globin gene cluster. *Proc Natl Acad Sci USA* 79:137–141, 1982
30. ANTONARAKIS SE, IRKIN SH, CHENG T-C, ET AL.: β-Thalassemia in American blacks: novel mutations in the "TATA" box and an acceptor splice site. *Proc Natl Acad Sci USA* 81:1154–1158, 1984
31. JOHNSON MJ, WALLACE DC, FERRIS SD, RATTAZZI MC, CAVALLI-SFORZA LL: Radiation of human mitochondria DNA types analyzed by restriction endonuclease cleavage patterns. *J Mol Evol* 19:255–271, 1983
32. HOWELL C: *The Origins of Modern Humans* (Introduction), edited by SMITH FH, SPENCER F, New York, Alan R. Liss, 1984
33. MILLER GF: The evaluation of eigenvalues of a differential equation arising in a problem in genetics. *Proc Camb Phil Soc* 58:588–593, 1962
34. ROBERTSON A: Selection for heterozygotes in small populations. *Genetics* 47:1291–1300, 1962
35. CROW JF, KIMURA M: *An Introduction to Population Genetics Theory*. New York, Harper & Row, 1970
36. JOHNSON MJ, NATALI AM, CANN HM, HONJO T, CAVALLI-SFORZA LL: Polymorphisms of a human variable heavy chain gene show linkage with constant heavy chain genes. *Proc Natl Acad Sci USA* 81:7840–7844, 1984

MEETING: *International Congress of Human Genetics*, September 22–26, 1986, West Berlin.