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Identification of a recent recombination event within the human β-globin gene cluster

(meiosis/genetic linkage/chromosome 11/haplotypes)

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ABSTRACT **In a detailed study of inheritance of DNA sequence polymorphism in a large reference pedigree, an individual was identified with an apparent genetic recombination event within the human β-globin gene cluster. Analysis of the haplotypes of relevant individuals within this pedigree suggested that the meiotic crossing-over event is likely to have occurred within a 19.8-kilobase-pair region of the β-globin gene cluster. Analysis of other DNA markers closely linked to the β-globin gene cluster—segment 12 of chromosome 11 (D11S12) and loci for insulin, the cellular oncogene c-Ha-*ras*, and preproparathyroid hormone—confirmed that a crossover event must have occurred within the region of chromosome 11 between D11S12 and the β-globin gene cluster. It is suggested that the event observed has occurred within a DNA region compatible with recombinational "hot spots" suggested by population studies.**

Genetic recombination in mammals is a process that has been under study for many decades. Attempts to identify the molecular basis of this process have been hindered by the high complexity of mammalian genomes. To precisely analyze meiotic recombination mechanisms, it would be extremely desirable to identify the DNA segments that recently have undergone crossing-over at meiosis. To achieve this goal, it is necessary to have available a significant number of genetic markers distributed over a chromosomal DNA segment of 100 kilobase pairs (kbp) or less. At present, however, most genetic markers are distributed at such great distances along a mammalian chromosome that precise delineation of the site of a crossover is quite difficult. One region of the human genome that is relatively favorable for a detailed analysis is the β-globin gene cluster (HBBC) of human chromosome 11. Within a 63-kbp region starting 4.2 kbp 5′ of the ε-globin gene and extending 19 kbp beyond the 3′ end of the β-globin gene, there are at least 17 polymorphic restriction enzyme recognition sites (1).

The question of whether recombination occurs with equal frequency at all sites along the chromosome is one of significance in understanding the molecular basis of meiotic recombination. Evidence suggesting that frequency of recombination is not evenly distributed within this region has been obtained through population studies of these polymorphic loci. Antonarakis, Orkin, and their collaborators (2, 3) have analyzed HBBC haplotypes in various human populations and demonstrated significant linkage disequilibrium among some alleles within the cluster. Their data suggest that recombination events are not evenly distributed within the region. These authors propose that crossovers occur with a relatively high frequency within a localized region of 11 kbp 5′ of the β-globin gene.

In the course of linkage studies of a large multigenerational pedigree, we analyzed the segregation of several restriction fragment length polymorphisms (RFLPs) on the short arm of chromosome 11, including several in the β-globin gene region. This analysis led to the identification of an individual with one chromosome that appears to be a product of a recombination within the β-globin gene region itself. Since detailed analysis of the structure of such recombinant chromosomes should give insight into the distribution of and mechanisms underlying meiotic crossing-over, we report here our initial analyses of this event.

MATERIALS AND METHODS

Pedigree Studied. The pedigree reported on here is part of the Old Order Amish pedigree no. 110 previously studied by Egeland and collaborators (4, 5). Lymphoblast and fibroblast cell lines have been established on 51 individuals in this kindred by the Institute for Medical Research (Camden, NJ).

Preparations of DNA. DNA was extracted from cultured cells by standard procedures (6).

Analysis of DNA Polymorphisms. DNAs were digested with different restriction enzymes that have been shown to identify polymorphic sites when hybridized with a given probe on a Southern blot. The digests were performed with 2–3 units/μg of DNA overnight under conditions specified by the manufacturer. DNAs were run on appropriate-percentage gel, transferred as described by Southern (7) to nylon-based filter, and probed with nick-translated probes (8).

Definitions of Loci Studied. Globin haplotypes for each individual were characterized by the use of the following restriction enzymes in the Southern hybridization procedure. The HBBC sites were: (*i*) the *Hinc*II site 5′ to the ε-globin gene (2); (*ii*) the *Hin*dIII sites present in the intervening sequence *2* (IVS*2*) of both $^{G}\gamma$- and $^{A}\gamma$-globin genes (9, 10); (*iii*) the *Hinc*II sites present in the $\psi\beta_1$-globin gene and 3′ to the $\psi\beta_1$-globin gene (2); (*iv*) the *Taq* I site 5′ to the δ-globin gene called "*E*" (11); (*v*) the *Hin*fI site 5′ to the β-globin gene (12); (*vi*) the *Rsa* I site 5′ to the β-globin gene (27); (*vii*) the *Hgi*AI site in the first exon of the β-globin gene (3); (*viii*) the *Ava* II site in the IVS*2* of the β-globin gene (2); (*ix*) the *Hin*dIII site 3′ to the β-globin gene homologous to probe pRK-29 isolated by R. Kaufman as described in ref. 13; and (*x*) the *Bam*HI site 3′ to the β-globin gene (14).

The four other marker loci used in the study are *HRAS1* (human oncogene c-Ha-*ras-1*), D11S12 (segment 12 of chromosome 11), *INS* (insulin gene), and *PTH* [gene for preproparathyroid hormone, which is enzymatically cleaved to parathyroid hormone (PTH)]. The *HRAS1* locus was typed on DNAs digested with *Bam*HI (15, 16) and is characterized

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Abbreviations: *INS*, insulin locus; *HRAS1*, designation for human locus of oncogene c-Ha-*ras-1*; *PTH*, designation for locus of preproparathyroid hormone; IVS, intervening sequence; cM, centimorgans; kbp, kilobase pairs; HBBC, β-globin gene cluster.

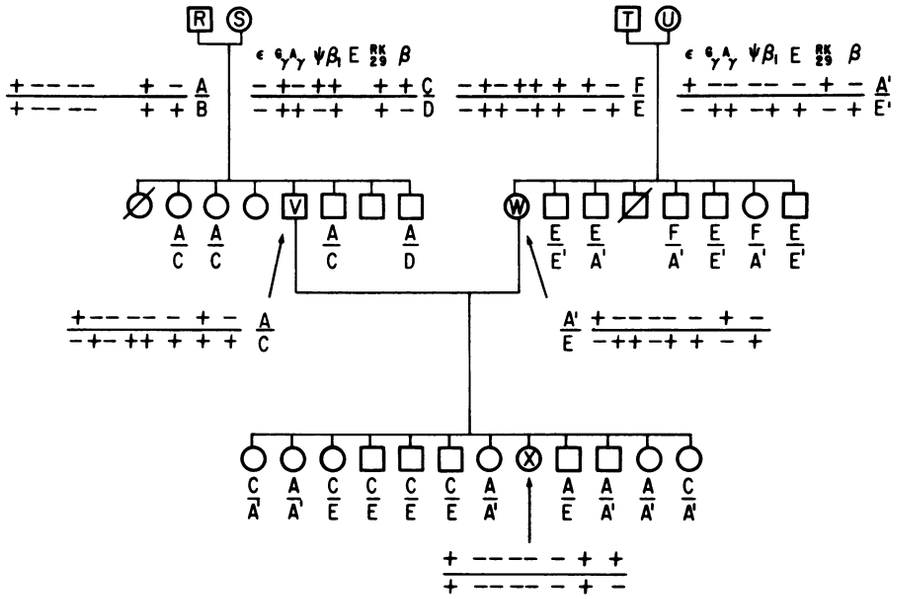

FIG. 1. Globin haplotypes of key members of the sibship. Partial globin haplotypes are shown for each relevant member. +, Presence of a restriction enzyme recognition site; −, absence of the restriction enzyme recognition site. Individual X exhibits a unique β-globin haplotype not exhibited by her parents, individuals V and W. The β-globin haplotypes of V and W can be inferred by the β-globin haplotypes of their parents, individuals R and S and T and W, respectively. Intact transmission of the β-globin gene region of the parents of X to the remaining siblings was observed and indicated by letter designation in Table 1. The designation β refers to the *Bam*HI polymorphism 3′ to the β-globin structural gene; all other sites near the β-globin gene were not segregating in the relevant individual.

by variously sized insertion sequences. Three alleles were distinguishable. The D11S12 locus was typed on *Taq* I-digested DNA; the two alleles were defined by presence or absence of the polymorphic *Taq* I site (17). *PTH* was typed on *Pst* I-digested DNA; two alleles were defined by presence or absence of the polymorphic *Pst* I site in the third exon (18). *INS* was studied with both *Sac* I and *Pvu* II. The allelic definitions for this restriction fragment length polymorphism are discussed below with the results and discussion. The estimated map distances of these markers from the globin cluster are 9 centimorgans (cM) to *INS*, 10 cM to *HRAS1*, 3 cM to D11S12, and 7–20 cM to *PTH* (18–21) (see Fig. 3).

RESULTS

Fig. 1 illustrates the family structure for the sibship in which the β-globin haplotype of interest was observed. A possible explanation for an inconsistent globin haplotype would be nonpaternity for individual X; however, 32 previously typed polymorphic marker systems, including *HLA*, (refs. 5 and 22; unpublished results) are in complete agreement with the biological relationships shown in Fig. 1. Based on the markers alone, the odds of the identified parentage to a random paternal gamete for individual X exceed 3.5×10^4. All other DNA polymorphisms studied were consistent with parental genotypes, further increasing the odds that the attributed paternity is correct. These results, which led us to reject nonpaternity as an explanation for the unexpected HBBC haplotype in individual X, are consistent with previous studies of the general nature of this community (23, 24) and personal knowledge (J.A.E.) of this cohesive family unit.

The availability of all four grandparents of the core sibship has made the establishment of linkage phase in the two parents (V and W in Fig. 1) unequivocal for the markers in the HBBC as well as for the other loci studied. Six different HBBC haplotypes are observed segregating in this pedigree. Polymorphic loci within the HBBC are depicted in Fig. 2. Eight of the 12 polymorphic sites shown in Fig. 2 were used to establish the globin haplotype shown in Fig. 1. Haplotypes *A* and *E* are each represented twice in the grandparents. The two parents (V and W in Fig. 1) have the genotypes *A/C* and *A′/E*, respectively. These genotypes predict four possible types of offspring: namely, *AA′*, *A′C*, *CE*, and *A′E*. All four combinations are represented; two of the offspring were *A′C*, four were *AA′*, one was *AE*, and four were *CE*. Individual X has a genotype that could not have arisen by transmission of the intact β-globin gene region carried by the parental chromosomes from her father or her mother.

Several explanations appeared initially compatible with the data: (*i*) a recombination within the β-globin gene region, (*ii*) a gene conversion event within the β-globin gene region, (*iii*) a new mutation at the *Bam*HI site in the germ cell of either individual X's father or mother, and (*iv*) an artifact due

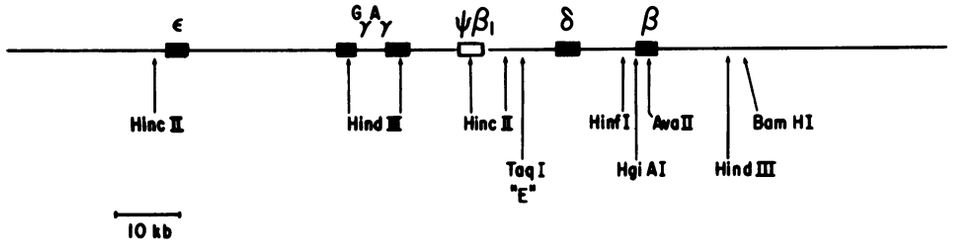

FIG. 2. Map of the HBBC. Arrows indicate the polymorphic restriction enzyme recognition sites. ▬, Coding regions; □, β-globin pseudogene.

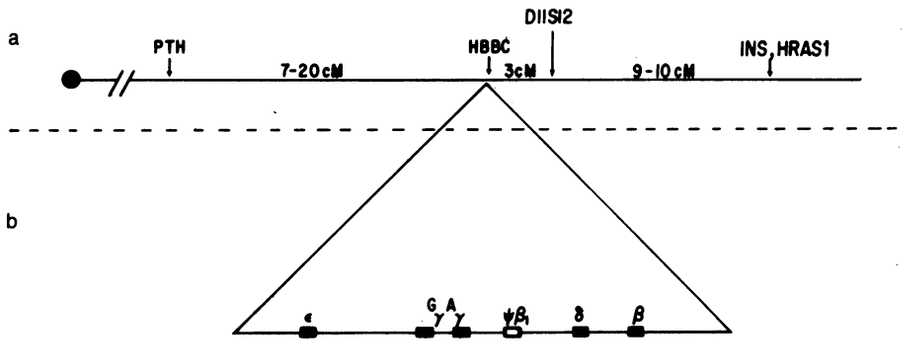

FIG. 3. (*a*) Order of the five loci studied as determined by linkage analysis. Recombination distance between markers on the short arm of chromosome 11 was estimated by two-point analysis (refs. 19 and 20; unpublished results). The heavy dot represents the centromere. (*b*) The predicted orientation of the genes within the HBBC with respect to the centromere.

to a mutation at the *Bam*HI site in the cultured lymphoblast cell line from which the DNA of individual X was obtained.

To address the fourth possibility, the typing of individual X was repeated on DNA derived from an independent source. A skin biopsy obtained from individual X was used to derive DNA from cultured fibroblasts. The results of this determination were identical to results with DNA derived from cultured lymphoblast cells of individual X; the *Bam*HI typing was heterozygous. During the course of these studies, determination of the key β-globin polymorphisms were performed two to four times to eliminate any possible typing error in the β-globin haplotypes.

To distinguish among the remaining three possibilities, we attempted to establish whether the chromosome 11 carrying the +−−−−−++ β-globin haplotype had undergone an obligate crossover within the region of 11p carrying the HBBC. Data were obtained for four markers known to be closely linked to the HBBC (18, 19, 21). These markers and their estimated map distance from the HBBC are shown in Fig. 3*a*. Table 1 gives the genotypes of the relevant individuals in the sibship for these markers. It is possible to determine linkage phase for all of the markers for the two parents in Fig. 1 (V and W, Fig. 4). These assignments are compatible with the genotypes of the 11 other children of this couple (Table 1).

Both parents of individual X are heterozygous (+/−) for the D11S12 DNA segment. The genotypes of the grandparents of individual X establish the phase of the D11S12 alleles in the mother of individual X. Individual X's maternal grandmother (individual U) transmitted HBBC haplotype *A'* to her daughter. Since U was homozygous for the presence of the *Taq* I site in D11S12, we must conclude that W carried the + allele for D11S12 and HBBC *A'* on one chromosome and the − allele for D11S12 and HBBC *E* on the other chromosome.

This assignment is consistent with all typing of the 11 siblings of individual X. Individual V, the father of individual X, was also heterozygous at D11S12. Typing of his 11 other offspring establishes that in V the HBBC haplotype *A* is associated with the absence of the *Taq* I site while haplotype *C* is associated with the presence of this *Taq* I site. Individual X is homozygous for the presence of the *Taq* I site. The genotype of individual X at each of the polymorphic sites of the β-globin gene region except the 3' *Bam*HI site and at D11S12 requires an obligate paternal crossover between the HBBC and D11S12. In contrast to the other sites in the HBBC, no crossover is required between the 3' *Bam*HI site and D11S12. Even if the *Bam*HI site had been converted from − to + by a gene conversion event, a crossover between D11S12 and the HBBC would still have been required by the data. Further evidence supporting the occurrence of a paternal crossover within this region is provided by analysis of genotypes at the *HRAS1* locus. At the *HRAS1* locus, the father was homozygous for the smallest of the three *Bam*HI fragments—*1,1*—while the mother was heterozygous, having one copy of the smallest *Bam*HI fragment and one copy of the largest *Bam*HI fragment—*1,3*. The haplotypes shown in Fig. 4 support the view that the maternal chromosome 11p was transmitted intact without crossover in the region between the HBBC and *HRAS1*. The most parsimonious ex-

Table 1. Genotypes of five loci of family members analyzed

Individual	Genotypes				
	PTH	HBBC	D11S12	*HRAS1*	*INS*
R	+/+	*A/B*	+/+	*1/1*	*3/3*
S	+/+	*C/D*	ND	*1/1*	*1/1*
T	−/−	*F/E*	+/−	*3/3*	*2/9*
U	+/−	*A'/E'*	+/−	*1/3*	*3/3*
V	+/+	*A/C*	+/−	*1/1*	*1/3*
W	−/−	*A'/E*	+/−	*1/3*	*2/3*
X	+/−		+/+	*1/1*	*3/3*
	+/−	*C/A'*	+/+	*1/1*	*3/3*
	+/−	*A/A'*	+/−	*1/1*	*1/3*
	+/−	*C/E*	+/−	*1/3*	*2/3*
	+/−	*C/E*	−/−	*1/3*	*1/2*
	ND	*C/E*	+/−	*1/3*	*2/3*
	+/−	*C/E*	+/−	*1/3*	*2/3*
	+/−	*A/A'*	+/−	*1/1*	ND
	ND	*A/E*	−/−	*1/3*	*1/2*
	+/−	*A/A'*	+/−	*1/3*	*1/2*
	+/−	*A/A'*	+/−	*1/1*	*1/3*
	+/−	*C/A'*	+/+	*1/1*	*3/3*

ND, not determined.

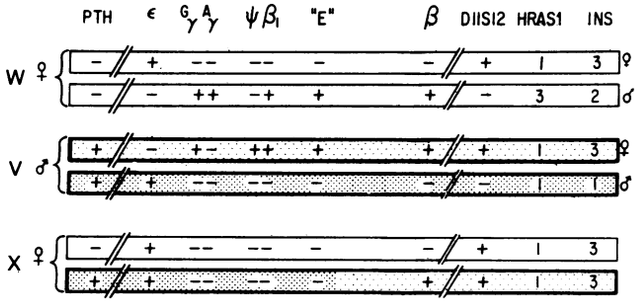

FIG. 4. Haplotypes of the parents W and V and individual X determined from typings of all kindreds in this sibship. +, Restriction enzyme site is present; −, restriction enzyme site is absent. The typings were done on DNA digested with enzymes indicated in *Materials and Methods*. *HRAS1* allele *1* is a 6.6-kbp *Bam*HI fragment, while allele *3* is 8.4 kbp. The three alleles of *INS* are: *1*, 800 bp; *2*, 830 bp; and *3*, 900 bp.

planation of the genotype of individual X is thus a single paternal crossover event occurring between the *Taq* I site at the 5′ side of the δ-globin gene ("*E*") and the *Bam*HI site at the 3′ side of the β-globin gene.

This interpretation of the data specifies the orientation of the genes within the HBBC with respect to the other markers on 11p. If a crossover has occurred within the β-globin gene region, then the D11S12 DNA sequence must be closest to the 3′ end of the HBBC. The data are thus most consistent with the gene order shown in Fig. 3*b*. If, however, a gene conversion event was responsible for the *Bam*HI genotype of individual X, then no inference can be made regarding the gene order given in Fig. 3*b*.

The polymorphic site 5′ to the *INS* gene is due to insertion of sequences that are made up of 14-bp repeats (25). In the general population, a wide range of size variation is observed in this region of the DNA. This variation is exhibited by digestion of the DNA with *Sac* I and hybridization to a probe that contains a segment of the 5′ region of *INS*. Within the segment of the Old Order Amish population that we have studied, considerably less variation in length of this DNA segment is observed than in the general population. To clearly identify sequence length variation in *INS*, we used the probe pHins310, which includes the region directly adjacent to the variable DNA segment (26). This identifies DNA fragments from this region, which vary in length between ≈800 bp and ≈900 bp. Allele *1* is ≈800 bp, allele *2* is ≈830 bp, and allele *3* is ≈900 bp. The father and the mother of individual X are heterozygous for this locus. These alleles are assigned to the paternal and maternal chromosomes as shown in Fig. 4. The fact that individual X is homozygous for allele *3* of *INS* also supports the conclusion that a paternal crossover occurred between *INS* and the 5′ cluster of sites in the HBBC but that no crossovers occurred between *INS* and D11S12 or between *INS* and the *Bam*HI site 3′ of the HBBC.

Typing of the *PTH* locus did not give additional information since the father was homozygous for the presence of the polymorphic *Pst* I site, while the mother was homozygous for the absence of this site. Individual X is, as expected, heterozygous for this site.

In an attempt to further delineate the site of the crossover, we typed relevant individuals in the sibship for a number of reported polymorphisms around and within the HBBC. The *Hgi*AI, *Hin*fI, *Rsa* I, and *Ava* II sites (Fig. 2 and *Materials and Methods*) proved to be uninformative because both the father and mother of individual X were homozygous for the same allele in each case.

DISCUSSION

The data presented here demonstrate either a crossover event within the β-globin gene cluster or a gene conversion event encompassing a segment of the cluster. Previous studies based on population genetic methods have suggested that recombination within the cluster may be relatively much more frequent between the $\psi\beta_1$- and β-globin genes than in the ≈20 kbp on either side (2, 3). This conclusion is based upon the observation of nonrandom associations of alleles at the various polymorphic sites. Alleles within the cluster of polymorphic sites stretching from 5′ of the ε-globin gene to 3′ of $\psi\beta_1$-globin pseudogene show strong linkage disequilibrium; similar linkage disequilibrium exists for the alleles in the cluster of sites from 5′ of the β-globin gene to 17 kbp downstream. No linkage disequilibrium exists between these two subclusters. Since recombination is the primary evolutionary mechanism for reducing linkage disequilibrium, the implication of the population data is that recombination rate is relatively much higher between the two subclusters than within either subcluster. Thus, on an evolutionary time scale, this region has been implicated as a "hot spot" for recombination (see ref. 1 for review). Chakravarti *et al.* (27) have used population genetic theory and the observed linkage disequilibrium in the HBBC to estimate the rate of recombination in the 5′ subcluster, in the 3′ subcluster, and in the small "hot spot" between them. Their calculations, based upon two-locus linkage disequilibrium theory for sites on opposite sides of the "hot spot," is 0.003. In a previous study, Stamatoyannopoulos *et al.* (28) estimated the recombination between the δ-globin structural gene and β-thalassemia to be 0.03. Assuming that our observations represent a single crossover event, we can estimate the recombination rate between the $\psi\beta_1$- and β-globin loci. We have typed other branches of this Old Order Amish pedigree and a large Venezuelan pedigree (29) for these same loci (19, 20). A modified version of LIPED (30, 31) gave a maximum lod score (logarithm of odds of linkage) of 21.49 at $\theta = 0.018 \pm 0.021$; the two-support-unit confidence interval (32) extends from $\theta = 0.0037$ to $\theta = 0.056$. Thus, our estimate of the recombination frequency in this interval is not significantly greater than Chakravarti's. All three of these estimates are significantly higher than that predicted on distance in nucleotides alone.

We do not have as clear an expectation for the rate of gene conversion. In no other case in our study have we observed an anomalous transmission of a multisite haplotype, but it is difficult to calculate the exact number of nonconversions definitely observed. We can say that, if the 3′ *Bam*HI site was converted, we also observed an independent crossover event between the HBBC and D11S12. This second event has a prior probability of only 3% since the map distance involved is 3 cM (19, 20). Although this probability is low, it is not sufficiently low to reject the hypothesis.

If we accept the hypothesis that a crossover within the HBBC has been observed, it is of interest to note that the crossover falls within the region suggested to be a hot spot for recombination. The identification of additional polymorphic sites in the region of interest would clearly be of utility in delineating the site of the crossover. The most direct approach to this problem would be to isolate DNA from the crossover region from the relevant individuals by recombinant DNA techniques and to identify DNA sequence polymorphism directly by DNA sequencing methods.

It would be of interest to compare the data obtained here with results in other well-mapped regions of the human genome. The most obvious candidate for such an analysis would be the human major histocompatibility complex (MHC). Crossover individuals have already been identified in this region by immunological methods. Sites of recombination also have been studied in the murine MHC. Data from this region of the mouse genome indicate that specific hot spots for recombination exist within this region (33). Comparison of DNA sequences at sites of recombination in the two species would make it possible to determine whether crossover sites within the human and mouse MHC regions exhibit sequence homology to sites of crossing-over in the HBBC.

The data presented here allow a prediction to be made on the orientation of the HBBC with respect to other markers in the adjacent region of chromosome 11. The data presented here are most consistent with the organization of this region of chromosome 11 as shown in Figs. 3*b* and 4. In this interpretation, the ε-globin gene region of the HBBC is closest to *PTH*, and the β-globin gene is closest to *INS*, *HRAS1*, and D11S12.

Note Added in Proof. We have completed the genotype analysis of the restriction fragment length polymorphism associated with the *Rsa* I site 9.1 kbp 3′ of the *Bam*HI site (27). The genotype of individual X is +/− at this *Rsa* I site . Individual V is +/− at this site, while individual W is +/+. Typing of remaining members of the sibship assigns the − allele for individual V to the *C* haplotype for HBBC. This result is consistent with the occurrence of a crossover

within the region indicated in this paper. If a gene conversion has occurred, it must have extended over a region of at least 9.1 kbp.

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