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CONCISE REPORT

The Entire β-Globin Gene Cluster Is Deleted in a Form of γδβ-Thalassemia

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We have used restriction endonuclease mapping to study a deletion involving the β-globin gene cluster in a Mexican-American family with γδβ-thalassemia. Analysis of DNA polymorphisms demonstrated deletion of the β-globin gene from the affected chromosome. Using a DNA fragment that maps greater than 40 kilobases (kb) 5′ to the ϵ-gene as a probe, reduced amounts of normal fragments were found in the DNA of affected family members. Similar analysis using radiolabeled DNA fragments located 3′ to the β-globin cluster has shown that the deletion extends more than 17 kb 3′ to the β-gene, but terminates before the 3′ endpoint of the Ghanian HPFH deletion. Hence, this γδβ-thalassemia deletion eliminates over 105 kb of DNA and is the first report of a deletion of the entire β-globin gene cluster.

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THE β-GLOBIN GENE cluster is located on the short arm of chromosome 11 and spans over 50 kilobases (kb) of DNA. Within the cluster, the orientation of the genes as well as the order of their expression in development is 5′-ϵ, $^{G}\gamma$, $^{A}\gamma$, δ, β-3′ (Fig. 1). Epsilon is the β-like embryonic gene, $^{G}\gamma$ and $^{A}\gamma$ are expressed during fetal life, and δ and β are the minor and major adult β-like globin genes, respectively.[1] Lesions within and around the β-globin gene produce the β-thalassemia syndromes, an extremely heterogeneous class of disorders characterized by alterations in the level of expression of this gene. A number of mutations that interfere with β-globin mRNA transcription, processing, and translation have been detected.[2]

Several other rare forms of thalassemia result from deletions of different parts of the β-globin gene cluster. One of these forms, γδβ-thalassemia, was originally described by Kan et al.[3] in an Anglo-Saxon child in whom γ-, δ-, and β-globin production was greatly reduced. The disorder was characterized by severe anemia in the neonatal period, caused by reduced γ-globin production, which ameliorated during the course of development to become a mild β-thalassemia. Furthermore, HbA_2 ($\alpha_2 \delta_2$) levels were normal, rather than elevated as in classical β-thalassemia. To account for these hematologic findings, Kan et al. proposed that a genetic lesion, such as a deletion, inactivated the entire γδβ-region on the affected chromosome. A Dutch family with a similar thalassemic phenotype (hereafter referred to as $\gamma\delta\beta_1$) was studied by restriction endonuclease analysis by Van der Ploeg et al.[4] They found a deletion of the ϵ-, γ-, and δ-structural genes. Although the 3′ end of this deletion was located 5′ to the β-globin gene, the β-gene on the affected chromosome was apparently inactive. Subsequent DNA analysis[5] of the original γδβ-thalassemic patient[3] (hereafter referred to as $\gamma\delta\beta_2$) demonstrated a deletion of the ϵ-, γ-, and δ-globin structural genes, which terminated in the second exon of the β-globin gene.

Because the molecular mechanism that produced these deletions is unknown, and the findings of Van der Ploeg et al.[4] raise the question of whether specific regulatory sequences near the β-globin gene cluster activate or repress gene expression, we have studied the genetic defect of a third family suspected of γδβ-thalassemia. By restriction endonuclease mapping using DNA probes from the β-globin gene cluster and regions 5′ and 3′ to the cluster, we have demonstrated a deletion of at least 105 kb, which involves the entire β-globin gene cluster and results in γδβ-thalassemia in this family.

MATERIALS AND METHODS

High molecular weight DNA was prepared from peripheral blood leukocytes of the normal mother, the affected father and child, and controls as described.[6] Five micrograms of genomic DNA from each individual were digested overnight with the restriction enzyme of interest, using the reaction conditions suggested by the manufactur-

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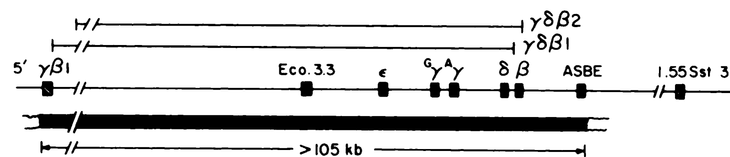

Fig. 1. The β-globin gene cluster. The arrangement of genes in the β-globin gene cluster is shown above. The extent of the two previously reported $\gamma\delta\beta$-thalassemia deletions, $\gamma\delta\beta_1$, and $\gamma\delta\beta_2$, is indicated by the narrow lines above,[13] while the minimum extent of the DNA deletion in the $\gamma\delta\beta$-thalassemia family reported here is represented by the solid bar below. The location of DNA fragments used to establish the extent of this deletion is indicated by the hatched boxes.

er. The resulting DNA fragments were separated by electrophoresis in 1.0% agarose gels, transferred to nitrocellulose, fixed, and hybridized with ^{32}P-labeled probes.[7-9] Washing of filters and autoradiography were carried out as described.[9]

Nine different DNA fragments were used for restriction enzyme analysis: (1) a β-cDNA fragment of plasmid JW 102[10]; (2) a γ-cDNA fragment of plasmid JW 151[10]; (3) a $\psi\beta_1$ genomic DNA fragment[11]; (4) a 1.8-kb genomic BamHI fragment (denoted 5'-β) derived from the region 5' to the β gene; (5) an 0.7-kb genomic Bgl II-EcoRI fragment (denoted ASBE) located 17 kb 3' to the β-gene; (6) a 3.3-kb genomic EcoRI fragment (denoted Eco 3.3) located 18 kb 5' to the ϵ gene; and (7) a 1.8-kb genomic fragment (denoted BS2) derived from an autosome.[12] This latter fragment was kindly provided by David Housman of M.I.T. In addition, two other fragments, $\gamma\beta_1$ and 1.55 Sst, were employed as probes. The fragment $\gamma\beta_1$ was originally isolated from cloned DNA overlapping the endpoints of the $\gamma\delta\beta_1$ deletion. $\gamma\beta_1$ is a 525 base pair (bp) EcoRI-BamHI genomic DNA fragment that contains a 125 bp region homologous to sequences 2.5 kb 5' to the β-gene and a 400 bp region homologous to sequences 5' to the deletion.[13] Since Grosveld[14] has

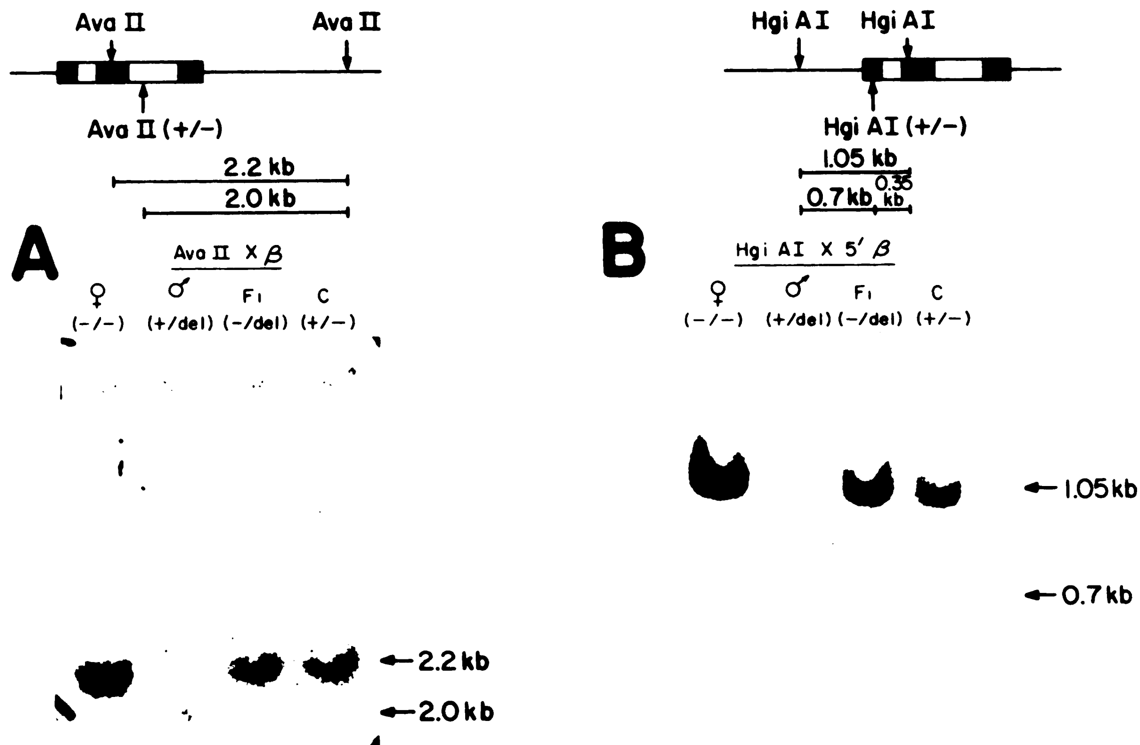

Fig. 2. Deletion of the β-globin gene. (A) Deletion of the 3' region of the β-globin gene in affected family members. Five micrograms of genomic DNA were digested with AvaII and hybridized to ^{32}P-labeled β-globin cDNA probe. The presence of the polymorphic AvaII site in IVSII of the β-gene (+ allele) is associated with a 2.0-kb fragment, while the absence of the polymorphic site (− allele) is associated with a 2.2-kb fragment. Genotypes with respect to the AvaII polymorphism are shown above each lane. From left to right, lanes are mother, father, affected son, and a +/− control. Since the son receives no AvaII fragment from his father, the 3' region of the β-gene must be deleted in both father and son. (B) Deletion of the 5' region of the β-globin gene in affected family members. Five micrograms of genomic DNA were digested with HgiAI and hybridized to a ^{32}P-labeled probe (5'-β) containing the 5' region of the β-gene. The presence of the polymorphic HgiAI site at codon 2 of the β-gene (+ allele) is associated with a 0.7-kb fragment and a 0.35-kb fragment. The absence of the polymorphic site (− allele) is associated with a 1.05-kb fragment. Note that the 0.35-kb fragment is not shown in the autoradiogram. The genotypes with respect to the HgiAI polymorphism are shown above each lane. From left to right, the lanes are mother, father, affected son, and +/− control. The affected son has inherited no HgiAI fragment from his father.

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isolated overlapping DNA fragments extending 40 kb 5′ to the ε-gene and has not observed the 400 bp sequence of $\gamma\beta_1$, this sequence is located greater than 40 kb 5′ to the ε-gene (see Fig. 3). The fragment 1.55 Sst was isolated from cloned DNA[13] overlapping the endpoints of the Ghanian HPFH deletion.[15,16] The 1.55 Sst fragment contains only sequences 3′ to the deletion.[13] All fragments were radiolabeled with ^{32}P-dATP and ^{32}P-dCTP by the nick-translation function of *Escherichia coli* DNA polymerase I as described.[17]

RESULTS

A Mexican-American male infant with microcytic anemia at birth was suspected of heterozygosity for $\gamma\delta\beta$-thalassemia when his initially severe anemia ameliorated to a mild β-thalassemia. The abnormality was inherited from the father who has hematologic findings (low MCV, slightly elevated levels of HbA_2, normal levels of HbF, and an abnormal peripheral blood smear) indicative of a β-thalassemia trait.

Deletion of the β-Globin Gene

Analysis of digested DNA from the father and the affected son with β and $\psi\beta_1$-gene probes did not reveal any abnormal DNA fragments (data not shown). However, the DNA fragments obtained after HgiAI digestion and hybridization to a DNA fragment from the 5′ region of the β-gene indicated that this region is deleted from the affected chromosome (Fig. 2B). The unaffected mother lacked the polymorphic HgiAI site at codon 2 of the β-gene[18] on both her chromosome 11 homologues (−/− genotype), while the affected father had only the fragment associated with the presence of the polymorphic site. Thus, the father could have either a +/+ genotype or a +/deletion genotype. Since the affected son had only the fragment associated with the absence of the polymorphic site and did not inherit any 5′ β-gene fragment from the father, he and his father must have −/deletion and +/deletion genotypes, respectively. Similar experiments involving the polymorphic AvaII site in IVS II[11,19] demonstrated that the affected child inherited only the Ava II-derived β-gene fragment of the mother (Fig. 2A). These data suggest that this deletion involves the entire β-globin gene.

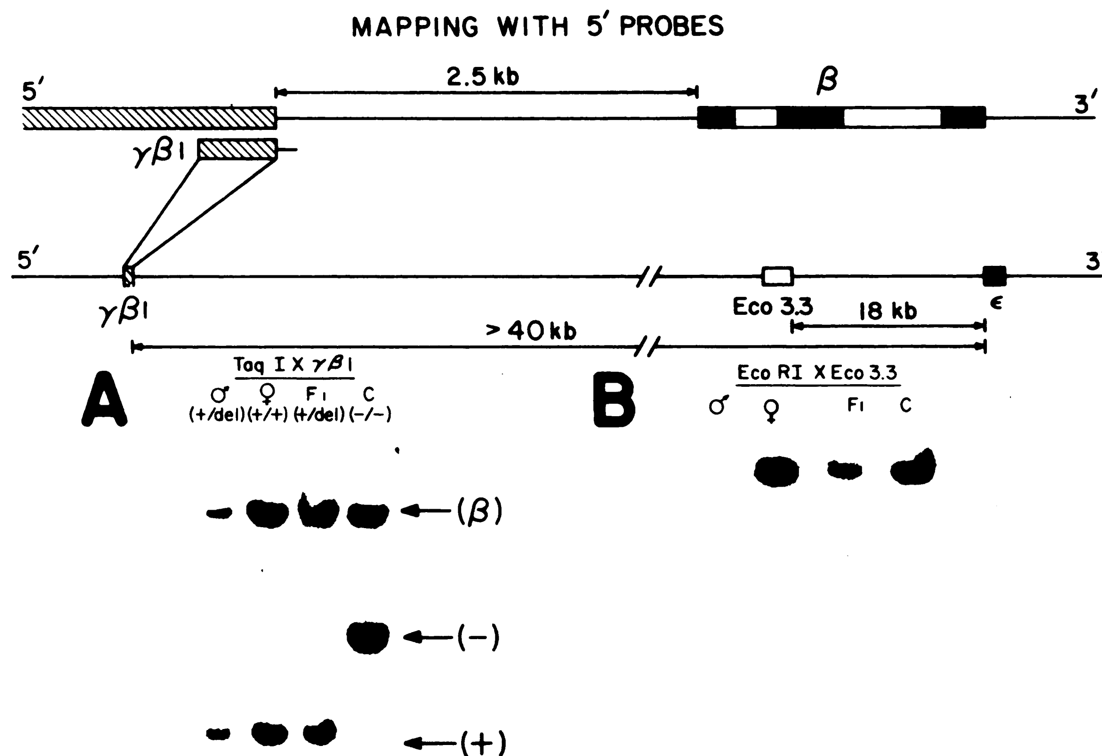

Fig. 3. Restriction endonuclease analysis with probes 5′ to the ε-gene. (A) Analysis with the $\gamma\beta_1$ probe. Five micrograms of genomic DNA were digested with TaqI and hybridized to the ^{32}P-labeled $\gamma\beta_1$ fragment. This fragment recognizes sequences 2.5 kb 5′ to the β-gene in addition to sequences greater than 40 kb 5′ to the ε-gene (see Materials and Methods). Using the $\gamma\beta_1$ fragment as a probe, a TaqI polymorphism can be detected in the region upstream to the ε-gene. The TaqI fragment containing the β-gene is indicated to the right of the gel by (β), and the TaqI fragment from the upstream region is indicated either by a (−) for the absence of the polymorphic site or a (+) for the presence of the site. From left to right, the lanes are marker DNA, father, mother, affected son, and control. Father, mother, and affected son have only the band associated with the + allele and the control is homozygous for the − allele. (B) Analysis with the Eco 3.3 probe. Five micrograms of genomic DNA were digested with EcoRI and hybridized to the ^{32}P-labeled Eco 3.3 fragment. From left to right, the lanes are father, mother, affected son, and control.

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Mapping the 5′ Endpoint of the Deletion

In order to determine the 5′ endpoint of the deletion, restriction endonuclease analysis was performed on the family members using two DNA fragments located 5′ to the ε-gene. The location of these two DNA probes, Eco 3.3 and $\gamma\beta_1$, is shown in Fig. 3. No abnormal fragments were detected when either the Eco 3.3 or $\gamma\beta_1$ probe was hybridized to digested DNA from the father and affected son (Fig. 3). Hybridization with the Eco 3.3 probe did yield bands of reduced intensity in the affected family members as compared to the bands of the mother and a control (Fig. 3B). Therefore, the sequences homologous to this probe are deleted from the affected chromosome.

The 525-bp fragment $\gamma\beta_1$ (Fig. 3A) was originally isolated from the endpoints of the $\gamma\beta_1$ deletion. A 125-bp region of the probe is homologous to sequences 2.5 kb 5′ to the β-gene and hybridizes to an 8.0-kb TaqI-derived DNA fragment containing the β-gene. The remaining 400 bp of the probe hybridizes to sequences 5′ to the endpoint of the $\gamma\beta_1$ deletion and these sequences are known to be greater than 40 kb 5′ to the ε-gene.[14] This part of the probe can detect a sequence variation that alters a TaqI restriction site in normal populations. This TaqI polymorphism is present in all ethnic groups studied and the incidence of the presence of the site is 0.24 in Mediterraneans, 0.30 in Asian Indians, and 0.67 in black Americans. In this particular family, all members have only the fragment associated with the presence of the polymorphic site (+ allele) (Fig. 3A). The reduced intensity of both the β-specific band and the upstream band in DNA of the father and son compared to the mother and control indicates that both of these regions are deleted from the affected chromosome. Furthermore, since polymorphic site analysis has established that the father and affected son have only one copy of the β-gene, the β-specific band can be used as an internal control. The ratio of the intensity of the upstream-specific band to the β-specific band is nearly 1:1 in father, mother, affected son, and control, rather than 2:1 in father and affected son as would be expected if the upstream sequences were present on the affected chromosome.

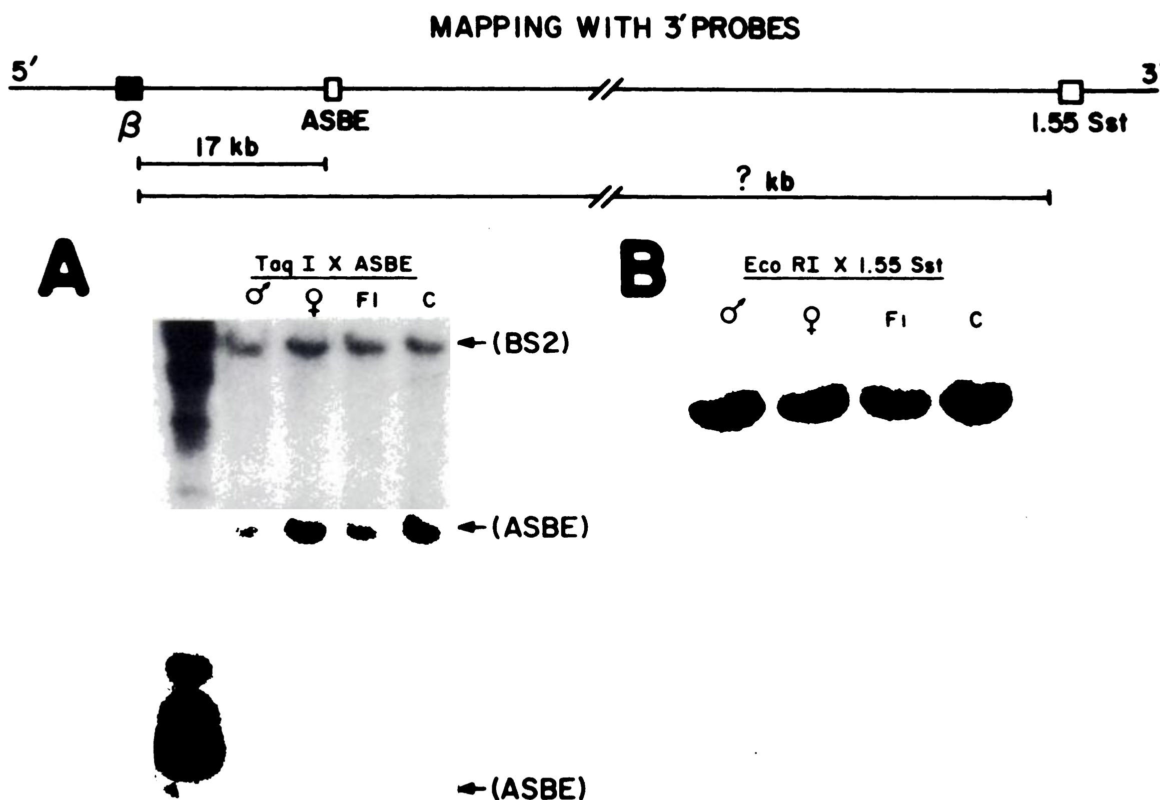

Fig. 4. Restriction enzyme analysis with probes 3′ to the β-gene. (A) Analysis with the ASBE probe. Five micrograms of genomic DNA were digested with TaqI and hybridized to the ^{32}P-labeled fragments ASBE and BS2. The ASBE fragment recognizes sequences 17 kb 3′ to the β-gene, while the BS2 fragment has been mapped distal to LDH A on chromosome 11p.[12] The BS2-specific fragments and the ASBE-specific fragments are indicated. From left to right, lanes are marker DNA, father, mother, affected son, and control. (B) Analysis with the 1.55 Sst probe. Five micrograms of genomic DNA were digested with EcoRI and hybridized to the ^{32}P-labeled 1.55 Sst fragment. This fragment was isolated from a region 3′ to the endpoint of the Ghanian HPFH deletion and contains sequences located an unknown distance 3′ to the β-gene.[15,16]

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We conclude that the 5′ terminus of this deletion maps beyond the upstream sequences of the $\gamma\beta_1$ fragment. Moreover, since (1) $\gamma\beta_1$ is derived from the 5′ endpoint of the $\gamma\delta\beta_1$-thalassemia deletion reported by Van der Ploeg et al.[4] and (2) Vanin et al.[13] have found that the 5′ terminus of the $\gamma\delta\beta_1$ deletion maps about 6 kb upstream of the 5′ terminus of the $\gamma\delta\beta_2$ deletion, the 5′ endpoint of this deletion lies beyond the 5′ endpoints of both $\gamma\delta\beta_1$ and $\gamma\delta\beta_2$.

Mapping the 3′ Endpoint of the Deletion

Restriction endonuclease analysis was performed with two DNA fragments 3′ to the β-gene, ASBE and 1.55 Sst, to determine the extent of the deletion in the 3′ direction. The location of these two probes is shown in Fig. 4. Hybridization of these probes to digested DNA from the affected family members failed to reveal any abnormal DNA fragments (Fig. 4). Using the ASBE probe, we observed reduced intensity of the ASBE-specific bands in the father and affected son compared to those of the mother and control, indicating that the sequences homologous to this probe are deleted from the affected chromosome. Furthermore, this filter was also hybridized to an autosomal probe, BS2, and the BS2-specific band was similar in intensity in father, mother, affected son, and control. The ratio of the intensity of this internal control band to the larger ASBE band was 2:1 in the father and affected son but 1:1 in the mother and control (Fig. 4A).

Using the 1.55 Sst probe, we observed that the fragment generated by EcoRI digestion was roughly equal in intensity in father, mother, affected son, and control, suggesting that the sequences represented by the 1.55 Sst probe are present on the affected chromosome (Fig. 4B). Separate analyses using this probe and the restriction endonucleases BamHI and HindIII also gave bands of roughly equal intensity in family members and control. Therefore, we conclude that the deletion extends more than 17 kb 3′ to the β-gene, but terminates 5′ to the end of the Ghanian HPFH deletion.[15,16] In addition, the 3′ endpoint of this $\gamma\delta\beta$-thalassemia deletion lies beyond the 3′ endpoints of $\gamma\delta\beta_1$ and $\gamma\delta\beta_2$ (Fig. 1).

DISCUSSION

We have demonstrated by restriction endonuclease analysis that a deletion of the entire β-globin gene cluster produces $\gamma\delta\beta$-thalassemia in a Mexican-American family. Since both the 5′ and 3′ endpoints of this deletion lie beyond the endpoints of the two previously reported $\gamma\delta\beta$-thalassemia deletions, it is the largest known deletion producing this condition. Although more than 105 kb of DNA are removed, affected family members have no abnormality other than a microcytic anemia. Thus, it can be concluded that there are no sequences close to the β-globin cluster that cause other phenotypic changes when only one gene copy is present. Karyotypic analysis at the 500 band level failed to demonstrate a visible deletion in the short arm of chromosome 11 of the father and affected son.

The large deletion we have observed might have been generated by an unequal homologous crossing-over between repeated sequences, by accidental DNA breakage and reunion, or by some other mechanism.[13] When probes become available for isolating the endpoints of this deletion, we may be able to distinguish between these several possibilities. Such probes would also be useful for the study of genetic polymorphisms and recombination around the β-globin cluster.

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