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Molecular Characterization of an Atypical β-Thalassemia Caused by a Large Deletion in the 5′ β-Globin Gene Region

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SUMMARY

We describe a Canadian family of Czechoslovakian descent that came to our attention because of an HbA_2 percentage approximately twice that of an average case of heterozygous β-thalassemia. This unique phenotype suggested to us the possibility of a novel genetic mechanism being responsible for their β-thalassemia. To investigate this possibility, we mapped, cloned, and sequenced the mutant β-globin allele. This molecular analysis demonstrated the presence of a unique 4,237 base pair (bp) deletion extending from 3.3 kilobases (kb) 5′ of the β-globin mRNA cap site to approximately the middle of β IVS-2. This truncated β-globin gene further extends the heterogeneity of mutations known to cause β-thalassemia and delineates new sequences involved in nonhomologous recombination events in the β-globin gene region.

INTRODUCTION

The β-thalassemias are a heterogeneous group of hereditary diseases characterized by reduced (β^+-thalassemia) or absent (β^0-thalassemia) production of normal β-globin chains from the affected allele. The molecular analysis of various

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thalassemia alleles has led to the characterization of over 30 mutations that result in β-thalassemia. The β^0-thalassemias are the result of nonsense and frameshift mutations, point mutations at intervening sequence splice junctions, and partial or complete deletions of the β-globin gene. The β^+-thalassemias result from mutations involving coding sequences adjacent to the splice junctions, the promotor region 5′ to the mRNA cap site, and the polyadenylation signal (for review, see [1, 2]). With the exception of the deletions causing β^0-thalassemia, the majority of mutations that result in β-thalassemia are single nucleotide changes.

Deletions large enough to be detectable by restriction endonuclease mapping, involving the β-globin gene but not the δ- or γ-globin genes, are rare. A 619-bp deletion found in Asian Indians includes 144 bases at the 3′ end of the second intervening sequence (IVS-2), the third exon, and 209 bases 3′ to the β-globin polyadenylation site [3, 4]. A 1.35-kb deletion extending from approximately 600 bases upstream of the mRNA cap site to roughly the middle of β-IVS-2 has been reported in an American black family [5]. A β^0-thalassemia has also been reported in a Dutch family in which a 10-kb deletion removes the entire β-globin gene [6].

This report describes a Canadian family of Czechoslovakian descent with a heterozygous β-thalassemia phenotype. An interesting feature of the β-thalassemia phenotype in this family is the presence of a greater elevation of HbA_2 than is usually seen in heterozygous β-thalassemia. Molecular characterization of the mutant allele revealed a deletion of 4,237 bp extending from approximately 3.3 kb 5′ of the β-globin mRNA cap site to approximately the middle of β-IVS-2.

MATERIALS AND METHODS

Patient Material

Blood samples were collected in vacutainers, with EDTA as anticoagulant, from all individuals indicated in figure 1. Fibroblast strains were established from individuals TS, DP, and JP, after informed consent, for use in subsequent DNA analysis. These fibroblast strains have been deposited in the Repository of Human Mutant Cell Strains of the Montreal Children's Hospital (Montreal, Quebec, Canada).

Hemoglobin Analysis

Hematologic data were obtained with a Coulter S+ Counter (Coulter Electronics, Hialea, Fla.). Quantitation of HbA_2 was by microcolumn chromatography using the commercial kit of Isolab (Akron, Ohio), and HbF, by alkali denaturation [7]. Globin chain synthesis rates [8] and $G\gamma/A\gamma$ ratios were determined by high pressure liquid chromatography [9]. Hb electrophoresis was done at alkaline pH on cellulose acetate plates (Helena Lab, Beaumont, Tex.).

Genomic DNA Analysis

High molecular weight DNAs were prepared from peripheral blood lymphocytes from individuals TS, DP, and SP as described by Poncz et al. [10]. High molecular weight DNAs were also prepared from cultured fibroblasts from individuals TS, DP, and JP as

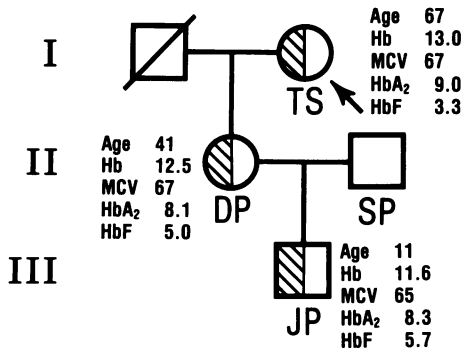

FIG. 1.—Pedigree and hematologic data on thalassemia family members. Hemoglobin (Hb) units are in g/dl, MCV in fl, HbA_2 and HbF in %. The proband is indicated with *an arrow*, and heterozygous family members with *hatch marks*.

described by Maniatis et al. [11]. The DNAs were digested with restriction endonucleases *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, and *Pst*I (as recommended by the manufacturers: Amersham Canada, Oakville, Ontario; Boehringer Mannheim, Dorval, Quebec; International Biotechnologies, New Haven, Conn.; and Pharmacia P-L Biochemicals, Dorval, Quebec) and size fractionated on horizontal 0.8%–1% agarose gels. λ DNA cut with *Hin*dIII was routinely included in all gels as a molecular weight marker. Size fractionated DNA was transferred to Gene Screen Plus (New England Nuclear, DuPont Canada, Lachine, Quebec) as recommended by the manufacturer or with the modified alkali protocol of Chomezynski and Quasba [12]. Prehybridization and hybridization to specific [^{32}P]-radiolabeled probes and subsequent washes were done according to the Gene Screen Plus protocols supplied by the manufacturer. Bound radioactivity was visualized by autoradiography on Kodak X Omat AR film using intensifying screens at −70°C [11]. Removal of bound radioactivity, for the purpose of rehybridization, was done according to the Gene Screen Plus NaOH protocol supplied by the manufacturer. The complete removal of bound radioactivity was confirmed by autoradiography.

Probes β IVS-2, δ IVS-2, and β 5′ flanking sequence (FS) were kindly provided by Dr. A. Bank (Columbia University, N.Y.). Probes β IVS-2 and δ IVS-2 contain the 0.9-kb *Bam*HI/*Eco*RI fragment that includes the respective IVS-2 of the β- or δ-globin gene. The β 5′ FS probe is a 1.9-kb *Bam*HI fragment containing the 5′ FS, Exon 1, IVS-1, and Exon II of the β-globin gene. The 0.79 intergenic (IG) probe was derived from the λHβG1 clone (supplied by Dr. T. Maniatis, Harvard University, Cambridge, Mass.), by initially subcloning the 5.5-kb *Eco*RI fragment into pUC8 (designated pβ5.5) followed by digesting this subclone with *Bam*HI, purification of the 0.79-kb fragment, and cloning this fragment into pUC8. The δβ 1.6 IG probe was constructed by double digesting the pβ5.5 clone with *Eco*RI and *Hin*dIII, followed by gel elution of the 1.6-kb fragment and subsequent cloning of this fragment into pUC8. All probes were plasmid inserts purified via gel elution prior to use in nick-translation reactions. Nick-translation reactions were done using reagents supplied by GIBCO/BRL (Burlington, Ontario) according to the recommended protocol and with [^{32}P]dCTP or [^{32}P]dATP supplied by Amersham Canada or ICN Biomedicals Canada (Saint Laurent, Quebec).

Cloning

High molecular weight DNA from individual JP was digested to completion with *Bam*HI and separated by size on a 10%–40% sucrose gradient as described by Maniatis et al. [11]. This gradient was fractionated and the DNA visualized on a 0.4% agarose gel, and the fractions containing 11–14-kb DNA were pooled. This DNA containing the

mutant 12.5-kb *Bam*HI fragment, but not the normal 22-kb *Bam*HI fragment, was then used to construct a library in λEMBL 3 (Promega Biotec, Bio/Can Scientific, Mississauga, Ontario). The λEMBL 3 arms were prepared by digesting λEMBL 3 DNA with *Bam*HI and *Eco*RI. Ligation of the size-selected DNA fragments into the λEMBL 3 arms was done according to the protocol of Maniatis et al. [11] using T4 DNA ligase (Boehringer Mannheim). The ligated DNA was packaged in vitro using packaging reactions obtained from Promega Biotec or Amersham (Amersham Canada), and the resulting λ phage library was screened on Colony/Plaque Screen (New England Nuclear, DuPont Canada) without amplification. A clone, designated λ9, was subsequently shown to contain the mutant allele and was further characterized.

Sequencing

Clone λ9 was digested with *Eco*RI, and the purified 1.3-kb fragment was cloned into pUC8 [13]. This hybrid-plasmid (designated p1.3 mutant fragment [MF]) was digested with *Hae*III, and the 402-bp fragment was cloned into pEMBL 8+ and pEMBL 8− (supplied by Dr. C. Sapienza, McGill University, Montreal, Quebec) cleaved with *Sma*I. Three hybrid-clones were obtained (p5/8+, p9/8−, and p16/8+) that contained the 402-bp *Hae*III fragment in the appropriate orientations for delineation of the deletion end points. Single-strand template DNA was prepared from each of the above plasmids according to the protocol of Dente et al. [14]. The DNA sequence was determined using the dideoxy-sequencing method of Sanger et al. [15] using Amersham M13 sequencing reagents according to recommended protocols (Amersham Canada).

RESULTS

Hematology

A 66-year-old Czechoslovakian woman (individual TS, the proband in fig. 1) was singled out for globin gene mapping studies because of a low mean cell volume (MCV) and an HbA_2 percentage almost twice that of the average case of β-thalassemia. Individual TS had an HbA_2 of 9% while her daughter (DP) and grandson (JP) had HbA_2 percentages of 8.1% and 8.3%, respectively. With the exception of this unusually high HbA_2, all other hematologic parameters were consistent with heterozygous β-thalassemia (fig. 1). Globin chain synthesis studies revealed a β/δ ratio of 0.4 in individual TS, and her Aγ/Gγ ratio was 1.4.

Deletion in the β-Globin Gene

When the DNAs from all family members were digested with restriction endonuclease *Bam*HI and analyzed by blot hybridization using the β IVS-2 probe, all family members with a thalassemic phenotype displayed an abnormal 12.5-kb fragment (fig. 2). In addition, individuals TS and DP had a normal 9.3-kb fragment, while individuals JP and SP showed a polymorphic 22-kb fragment. This 22-kb fragment has been shown to be a normal variant resulting from the loss of the *Bam*HI site 3′ to the β-globin gene without affecting β-globin gene function [16]. When this blot was stripped of the β IVS-2 probe and rehybridized with the δ IVS-2 probe (fig. 2), all family members showed the normal 4.7-kb δ-gene fragment, but in individual SP, the 4.7-kb band appeared with approximately twice the intensity of other family members. The thalassemic family members TS, DP, and JP also demonstrated the presence of an

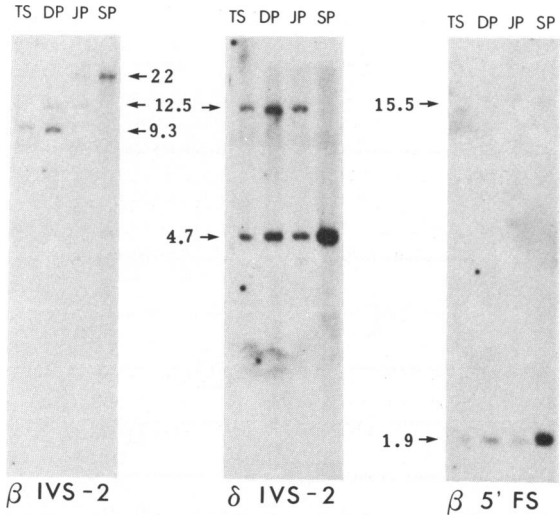

FIG. 2.—Autoradiogram of genomic DNA from heterozygous thalassemia family members (TS, DP, and JP) and normal family member (SP) digested with *Bam*HI restriction endonuclease and hybridized sequentially with the 0.92-kb β IVS-2 probe, the 0.97-kb δ IVS-2 probe, and the 1.9-kb β 5′ FS probe. Between each rehybridization, the preceding probe was stripped from the filter and complete removal was confirmed by autoradiography. The lengths of the fragments in kilobases are shown.

abnormal 12.5-kb fragment. When this blot was stripped of the δ IVS-2 probe and rehybridized with the β 5′ FS probe, all family members again demonstrated the normal 1.9-kb β-gene fragment and slight cross-hybridization to the 15.5-kb 5′ δ-gene fragment. This blot also showed individual SP's hybridization with the β 5′ FS probe to be greater than other family members. The simplest interpretation of these data is that a deletion in the 5′ β-globin gene region removes the *Bam*HI site in Exon II and the two *Bam*HI sites 5′ to the β-globin gene in the thalassemic family members TS, DP, and JP (see fig. 3). Thus, each family member with a thalassemic phenotype has one normal β-globin allele (i.e., a 9.3- or 22-kb fragment) and one truncated mutant β-globin allele (i.e., 12.5-kb fragment). These data further suggest that the 3′ end point is within β IVS-2 because the degree of hybridization of δ IVS-2 to the normal 4.6-kb fragment and the 12.5-kb fragment was comparable among the thalassemic members, indicating an intact δ IVS-2, while that of β IVS-2 to the abnormal 12.5-kb fragment is less than to the normal 9.3- or 22-kb fragments, indicating partial removal of β IVS-2 DNA. The lack of hybridization of the β 5′ FS probe with this thalassemic allele is also suggested by the differential hybridization observed in figure 2. Comparing individual SP with the other family members, the presence of a substantial deletion was suggested in the β 5′ FS DNA of the thalassemia β-globin genes.

To further define this deletion, DNA was digested with *Eco*RI, *Pst*I, *Bgl*II, or *Hin*dIII, and blots were hybridized with β IVS-2, δ IVS-2, and β 5′ FS probes. Blots of thalassemic family member DP and the normal family member SP are illustrated in figure 4*A*, *B*, *C*, and *D*.

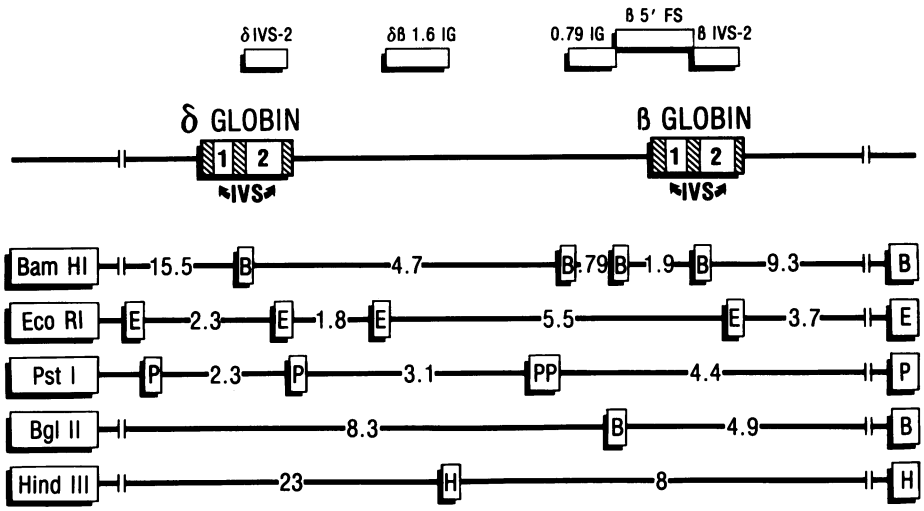

FIG. 3.—Map of the region on chromosome 11 containing the β- and δ-chain genes with the corresponding location of the probes used (*above*) and the sites of the restriction endonucleases used in the mapping of genomic DNA (*below*). The lengths of the fragments in kilobases are shown, and these correspond to nucleotide sequencing data (Poncz et al. [10]). The distance between the two *Pst*I sites flanking the 3.1- and 4.4-kb fragments is 109 bp.

When the DNAs of individuals SP and DP were digested with *Eco*RI and hybridized with the β IVS-2 probe, DP showed an abnormal 1.3-kb fragment and a less intense 5.5-kb fragment (fig. 4*A*). This indicates that a deletion of approximately 4.2-kb has occurred within this 5.5-kb *Eco*RI restriction fragment, resulting in the abnormal 1.3-kb fragment. A portion of this 4.2-kb deletion includes the DNA that normally hybridizes to the β 5′ FS probe, as seen by the disproportionate hybridization of the β 5′ FS probe to the 5.5-kb fragment in individuals SP and DP (fig. 4*A*). As judged by the intensity of hybridization of the δ IVS-2 probe with the 2.3-kb restriction fragment in individuals SP and DP, the δ-globin gene appears grossly normal (fig. 4*A*). Similar data were obtained when blot hybridizations were done with *Pst*I, *Bgl*II, and *Hin*dIII restriction endonucleases using individuals SP and DP's genomic DNA. In each case, an abnormal restriction fragment was seen when the β IVS-2 probe was used with the respective restriction enzyme (fig. 4*B, C,* and *D*). These data indicate that the two *Pst*I sites 5′ to the β-globin gene were eliminated by the deletion, as well as the *Bgl*II and *Hin*dIII sites within this same region (see fig. 3). The failure of the β 5′ FS probe to reveal the abnormal restriction fragments with each of the above restriction enzymes is also consistent with the presence of a deletion in the 5′ FS. The abnormal restriction fragment seen in individual DP when the δ IVS-2 probe was used in conjunction with *Bgl*II or *Hin*dIII restriction digests further supports both the size and location of this deletion. The 0.79 IG probe was also used with all of the restriction digests and probes discussed above, and in each case, this probe showed a complete lack of hybridization with any of the abnormal restriction fragments observed (data not shown).

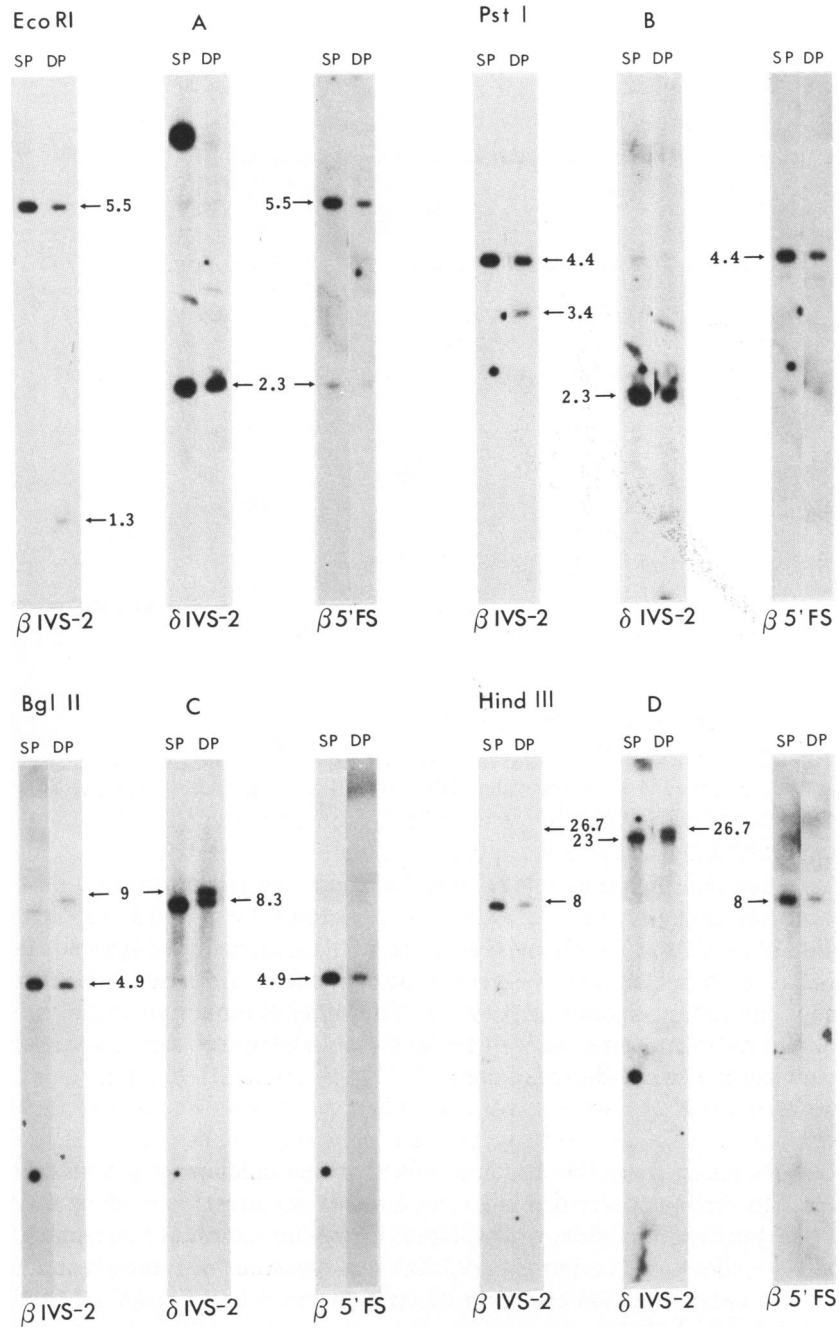

FIG. 4.—Autoradiogram of genomic DNA from normal family member (SP) and heterozygous thalassemic family member (DP) digested with *Eco*RI (*A*), *Pst*I (*B*), *Bgl*II (*C*), and *Hin*dIII (*D*) restriction endonucleases and hybridizes sequentially with β IVS-2, δ IVS-2, and β 5′ FS probes. Fragment lengths in kilobases are shown.

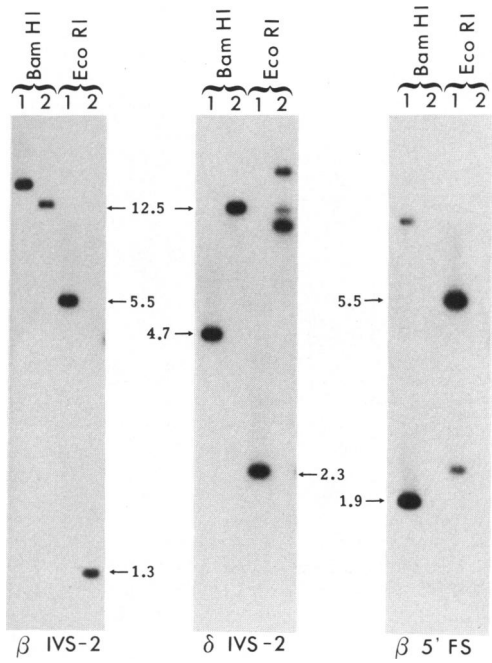

FIG. 5.—Characterization of the clone containing the mutant β-globin thalassemia allele. A control clone λHβG1 (*1*) containing a normal β- and δ-globin gene on a 13.3-kb *Eco*RI λ insert (Lawn et al. [17]) and clone λ9 (*2*) containing the mutant β-globin thalassemia allele and flanking δ-globin gene on a 12.5-kb *Bam*HI λ insert were digested with *Bam*HI and *Eco*RI restriction endonucleases and hybridized sequentially with β IVS-2, δ IVS-2, and β 5′ FS probes. Clone λ9 was isolated from a λ phage genomic library constructed in λEMBL 3. Fragment lengths in kilobases are shown.

These genomic mapping data collectively indicate that a deletion of 4.2 kb has occurred in the 5′ region of one of the β-globin alleles. The 5′ border of this deletion is within the 1.6-kb intergenic region flanked by the *Eco*RI and *Hin*dIII restriction endonuclease sites between the δ- and β-globin genes, and it extends in the 3′ direction, ending in β IVS-2. The other β-globin allele on the non-thalassemic chromosome, as well as the δ-globin genes on both chromosomes, appears normal based on these data.

Cloning

To more precisely define the end points of this deletion, a genomic library was constructed (as described in MATERIALS AND METHODS) from which a λ clone containing the mutant allele was isolated. Characterization of this clone (designated λ9) is illustrated in figure 5. λ9 DNA was digested with *Bam*HI and *Eco*RI restriction endonucleases and hybridized with the β IVS-2, δ IVS-2, and β 5′ FS probes. The λHβG1 clone prepared by Lawn et al. [17] was included as a control. The λHβG1 clone contains the δ- and β-globin genes intact and on one contiguous DNA fragment extending from the *Eco*RI site 5′ of the δ-globin gene to the *Eco*RI site within β-globin Exon 3 (see fig. 3) [17].

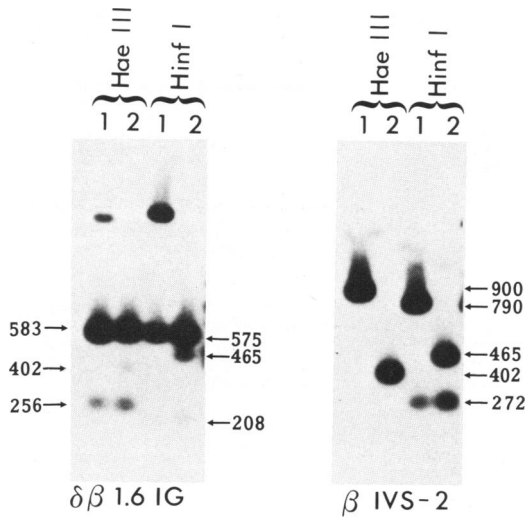

FIG. 6.—Mapping of the deletion end points in clone λ9. Autoradiogram of clones λHβG1 (*1*) and λ9 (*2*) digested with restriction endonucleases *Hae*III and *Hin*fI and hybridized to duplicate filters with the δβ 1.6 IG (an *Eco*RI/*Hin*dIII 1.6-kb fragment) or β IVS-2 probes. Fragment lengths in base pairs are shown.

When λ9 DNA was digested with *Bam*HI restriction endonuclease and hybridized with a β IVS-2 or δ IVS-2 probe, a 12.5-kb restriction fragment was seen (fig. 5). When the β 5′ FS probe was hybridized to *Bam*HI-digested λ9 DNA, no hybridization was observed. When λ9 DNA was cut with *Eco*RI restriction endonuclease and blot hybridized with β IVS-2, the abnormal 1.3-kb fragment was observed, and no hybridization was observed to this fragment with the β 5′ FS probe. These results indicate that the clone λ9 contains the mutant allele that extends from the *Bam*HI site within the δ-globin gene to the *Bam*HI site 3′ of the β-globin gene (see fig. 3).

When clone λ9 DNA and control DNA (i.e., λHβG1) were digested with *Hae*III or *Hin*fI and blot hybridized to the β IVS-2 probe, unique restriction fragments of 402 bp and 465 bp were observed in λ9 in contrast to the normal 790-bp and 900-bp fragments seen in the control clone (fig. 6). This indicates that the 3′ end of the deletion is in β IVS-2, 5′ to the 272 *Hin*fI fragment and that the 402-bp and 465-bp unique restriction fragments most likely contain both end points of the deletion (see fig. 7). To investigate this last point, probe δβ 1.6 IG was blot hybridized to λ9 and control DNA digested with *Hae*III or *Hin*fI restriction endonucleases. As illustrated in figure 6, both the 402-bp *Hae*III and 465-bp *Hin*fI restriction fragments hybridized with the δβ 1.6 IG probe, and the hybridizing sequences 5′ to these fragments all appeared normal (see fig. 7). Because both of these unique restriction fragments cross-hybridized with the δβ 1.6 IG and β IVS-2 probes, it seemed likely that these fragments contained both the 5′ and 3′ end points of the deletion in the thalassemic β-globin gene.

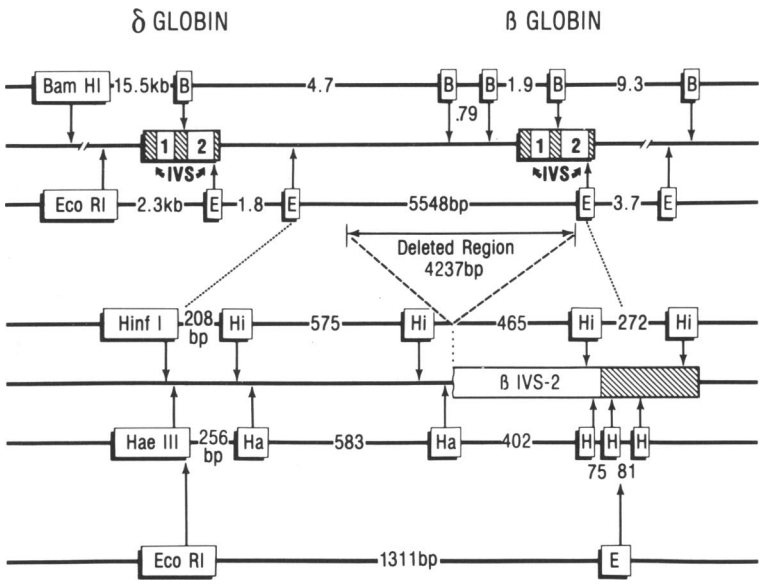

FIG. 7.—Fine structural map of the truncated β-globin gene. The mutant β-globin gene is shown with the *Hin*fI and *Hae*III restriction endonuclease sites surrounding the deletion end points, the *Eco*RI restriction endonuclease sites immediately flanking the deletion end points are shown below, and the corresponding *Bam*HI and *Eco*RI restriction endonuclease sites for the normal allele are shown to highlight the exact location of the deletion end points. Fragment lengths are shown in kilobases (kb) or base pairs (bp).

Sequencing

To precisely define the location of the deletion end points, the 402-bp *Hae*III fragment was cloned into the *Sma*I site of pEMBL 8+ and pEMBL 8− (as described in MATERIALS AND METHODS), single-stranded template DNAs were prepared, and the sequence was determined by the dideoxy chain termination method (fig. 8). By comparing the DNA sequence from this clone to the published sequence of the normal gene in the suspected deletion region [10], the 5′ and 3′ deletion end points are apparent. The 5′ end of the deletion is 57 bp 3′ to the third *Hae*III site in figure 7, and its 3′ end is 390 bp 5′ to the start of Exon III in the β-globin gene. The deletion has removed 4,237 bp consisting of intergenic DNA, the β-globin 5′ promotor region, mRNA cap site, 5′ untranslated region, Exon I, β IVS-1, Exon II, and 470 bp of β IVS-2.

DISCUSSION

This report describes a Czechoslovakian-Canadian family in which three family members, in 3 generations, all manifest the hematologic symptoms of heterozygous β-thalassemia. In most cases of heterozygous β-thalassemia, the hematologic factors include a mild anemia, red cell microcytosis, and an elevation of HbA_2, with or without elevation of HbF [18]. In this laboratory, the mean HbA_2 level in the last 675 cases of heterozygous 2 β-thalassemia was 5.08% ± 0.62% (normal 2.65% ± 0.27%). The values of 8%–9% found in the

FIG. 8.—Sequences across the deletion end points of the truncated β-globin gene and of the corresponding normal 5′ and 3′ DNAs. The normal 5′ sequence (*top line*); the sequence containing the deletion end points, deletion (*middle line*); and the normal 3′ sequence (*bottom line*) are aligned without gaps. The deletion sequence begins at the *Hae*III site immediately 5′ to the deletion end points (see fig. 7) and corresponds to the first 160 bases of this 402-bp fragment. The sequence of the deletion strand was determined by dideoxy-sequencing, and the normal 5′ and 3′ sequences correspond to those of Poncz et al. [10]. The *dots between the sequences* indicate positions at which the sequences are identical. The *vertical arrows* correspond to the two potential sites at which the deletion took place in the 5′ and 3′ normal sequences. The *horizontal line with arrows* indicates a palindromic sequence that exists in the normal 3′ sequence flanking the deletion junction.

family members reported were unusually high and suggested to us the possibility of a unique genetic mechanism being responsible for their β-thalassemia. This inference was confirmed by the complete molecular analysis of the mutant β-globin gene. We have demonstrated the presence of a 4.2-kb deletion in each family member manifesting the β-thalassemia phenotype.

Sizable deletions in the γδβ-gene cluster of several hundred to several thousand base pairs cause a variety of syndromes, including HPFH, γδβ-thalassemia, δβ-thalassemia, β-thalassemia, and the Hb Kenya and Hb Lepore conditions [1, 18]. Of these, the rare deletions involving only the β-gene and adjacent DNA result in β^0-thalassemia [3, 5, 6]. Deletions that affect only the 5′ region of the β-globin gene result in β^0-thalassemia with a higher than usual level of HbA_2.

In the black-American family reported by Padanilam et al. [5] with approximately a 1.35-kb deletion (fig. 9), the single β-thalassemic heterozygote studied had an HbA_2 of 7.2%; well above average. In the Dutch family described by Gilman et al. [6], a 10-kb deletion extends approximately 2.4-kb upstream from the β-gene (fig. 9) and the HbA_2 levels in heterozygotes were reported as 6.1% ± 1.5%; slightly higher than average. The deletion we report here extends considerably further upstream from the β gene (≃ 3.3 kb), and heterozygous family members all show significant HbA_2 elevations. Despite the fact that all of these deletions remove β-globin 5′ FS DNA and result in higher than usual elevations of hemoglobin A_2, the mechanisms responsible for their HbA_2 elevations are not well understood.

Any proposed mechanism that could account for the elevated HbA_2 in these heterozygous β-thalassemias must take into consideration both δ-globin gene loci. Several investigators [19–21] have shown that the increased output of δ-chains in heterozygous β-thalassemia is not the product of the δ-gene in cis to the β-gene carrying the thalassemia mutation, but rather is due to increased

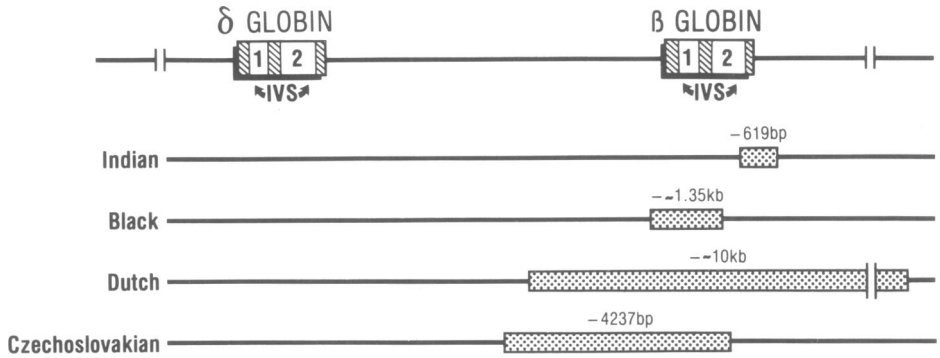

FIG. 9.—Summary of β^0-thalassemia alleles caused by major deletions described to date. Sizes of sequenced deletions are shown in base pairs (bp) and noncloned deletions in approximate kilobases (kb). All deleted regions are indicated *by boxes with stippling* and are shown with the normal δ- and β-globin genes (*above*).

output from both δ-genes in cis and trans to the mutant β-globin gene. Also, a family reported by Steinberg et al. [22] with β-thalassemia and exceptionally high HbA_2 levels (8.4%–11.4%) failed to show any abnormality in the β-globin region by restriction endonuclease mapping. Therefore, these data collectively indicate that more than one mechanism must exist that gives rise to unusually high levels of HbA_2 in association with heterozygous β-thalassemia. However, the unique form of β-thalassemia we describe appears to be particularly well suited for the investigation of unusually elevated HbA_2 levels when associated with deletions in the 5′ region of the β-globin gene. The level of HbA_2 in the family we describe is significantly elevated, and since both δ-globin genes in cis and trans to the truncated β-globin gene can be unambiguously identified and cloned, sequencing and in vitro expression studies can readily be performed. These experiments will be carried out in an attempt to delineate the mechanism giving rise to the inordinate increase in HbA_2 expression we observe.

The precise definition of the 5′ and 3′ end points of the deletion in our family is seen in figure 8. Several features should be noted: (1) The deletion took place such that the 5′ and 3′ ends were joined without additional base pairs being inserted, unlike the 619-bp β-globin deletion reported by Orkin et al. [3] where a heptanucleotide of unknown origin was found at the junction [4]. (2) A perfect 18-bp palindrome (AATATGTGTACACATATT) exists in the normal 3′ DNA strand flanking the deletion end points, and an imperfect-palindromic sequence exists (GTGGACAC) in the mutant DNA sequence at the deletion junction. The significance of these palindromic sequences is not apparent in relation to the mechanism giving rise to this deletion, although Ripley and Glickman [23] have been able to show correlations between spontaneous deletions and palindromic sequences flanking the deletion end points in the *E. coli lac I* gene. (3) A model has been proposed for the generation of β-globin deletions during DNA replication because of flanking short direct repeat sequences [24]. But no short direct repeat sequences were found flanking the deletion end points we report,

in contrast to those observed in deletion type β-globin structural variants [25, 26] or in spontaneous deletions in the *lac I* gene of *E. coli* [27]. (4) The exact end point of the deletion can be determined only to within ±1 nucleotide because of an adenine nucleotide common to both ends.

The 5′ end of the deletion we have described is located in a region known to be a recombinogenic "hot-spot" [28], and the 3′ end is in β IVS-2, another recombinogenic region [4, 5]. Yet based on sequence comparisons or restriction endonuclease mapping data of other thalassemia mutations having deletion end points in these regions, we conclude that no previously reported deletion has an end point with the same location or homologous sequences at their respective end points when compared with the deletion reported here. Therefore, the truncated β-gene found in the Czechoslovakian/Canadian family we have described further extends both the heterogeneity of mutations known to cause a β-thalassemia phenotype, and the heterogeneity of sequences in the β-globin region known to be involved in nonhomologous recombination events.

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