Red Cell Disorders

A new cross-over region for hemoglobin-Lepore-Hollandia

Hb Lepore-Hollandia, identified in a Thai patient, was found to be due to a new cross-over between IVS1-nt 42 and nt 56 of the δ- and β-globin genes. This differs from a previous case which crossed-over between codon 22 and IVS1-nt 16. Two independent homologous unequal cross-over events account for these two Lepore-Hollandia genes.

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The human β-globin gene cluster is organized in the order ε-^{G}γ-^{A}γ-ψβ-δ-β on chromosome 11 and since the δ- and β-globin genes share a high degree of sequence homology, unequal cross-over events due to misalignment of the δ- and β-globin genes can occur resulting in a δβ fusion gene.[1] The abnormal hemoglobins (Hb) resulting from the expression of the fusion gene are known as Hb Lepore.[2] Here we describe a new cross-over region of Hb Lepore-Hollandia. There are several different types of Hb Lepore depending on the cross-over points between the δ- and β-globin genes, but each results in an abnormal Hb with the same electrophoretic mobility. Hb Lepore-Boston-Washington, commonly found in Italy, Spain and countries of the Balkan peninsula, has a breakpoint which lies between codons 87 of the δ and 116 of the β genes.[3] Hb Lepore-Baltimore, found often in Brazil, central Portugal and Italy, has a breakpoint between δ codon 50 and β codon 86,[4] while Hb-Lepore-Hollandia, discovered in families from Papua New Guinea, Bangladesh and Thailand, has a breakpoint between δ codon 22 and β codon 50.[5,6] Direct genomic sequencing of DNA from Bangladesh subjects with Hb Lepore-Hollandia had localized the cross-over to a 40 basepair (bp) window between codon 22 and intron 1 (IVS1)-nt 16 of the δ- and β-globin genes.[7] The β-globin variant HbE is highly prevalent in Thailand and the interaction of HbE and Hb Lepore-Hollandia had been previously reported in a family from Central Thailand with the same Lepore-Hollandia breakpoint.[6] We recently encountered another Thai patient with an identical genotype (compound heterozygosity for HbE and Hb Lepore-Hollandia). This male patient had a mild clinical phenotype and was found incidentally to have microcytic anemia at his wife's antenatal screening.

The couple was referred to the antenatal diagnosis clinic for counseling and characterization of their β thalassemia (thal) mutations, prior to antenatal diagnosis for the fetus. The man's blood picture showed Hb 12.4 g/dL, MCV 65 fL, MCH 21 pg, MCHC 32.2 g/dL and reticulocytes 1.2%. Reverse dot blot analysis for the 12 β thal mutations commonly found among southern Chinese showed hemizygosity for HbE. His Hb isoelectric-focusing revealed: HbE 51%, HbF 38.5% and another Hb band (10.5%) migrating with a pI slightly less than that of HbS (compatible with Hb Lepore).

Southern blot of Sac I-digested genomic DNA and hybridization to the ^{32}P-labeled β 0.9 probe (a 0.9 kb *Bam*HI-EcoRI fragment containing IVS-2 of the human β-globin gene) showed an additional 9.0 kb fragment as well as the normal 16.4 kb fragment. This is in keeping with a 7.4 kb deletion on one of the subject's β alleles, characteristic of a δβ fusion gene of the Hb Lepore type. Polymerase chain reaction (PCR) analysis of the *Xmn*I polymorphism[8] 5' to the ^{G}γ gene revealed heterozygosity for the RFLP site. This may account in part for his elevated level of HbF. Genomic DNA of the patient was digested to completion with EcoRI and fractionated by sucrose density gradient (10-40%). The fraction containing a 2.3 kb fragment which hybridized to the ^{32}P-labeled β0.9 probe was cloned into the multiple cloning site of the pBluescript phagemid within a λ ZAPII vector (Stratagene, La Jolla, CA, USA).

The pBluescript double-stranded phagemid containing the cloned insert-DNA was subsequently excised from the λ bacteriophage according to the manufacturer's instructions. Nucleotide sequencing of the double-stranded phagemid was performed with M13 primers, δ- and β-globin gene specific primers using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, USA). The sequencing reaction mixtures were purified on Auto-Seq G-50 columns (Amersham Biosciences) and analyzed on an automated sequencer (model 3700, ABI, USA).

Sequencing results of two different clones revealed a cross-over with a 15 bp window between IVS1-nt 42 and nt 56 of the δ- and β-globin genes. This finding was further confirmed by gap PCR and sequencing of the Hb Lepore allele of the patient, using the previously described primers.[9] Figures 1 and 2 shows, respectively, the sequence and the alignment of this fusion gene with respect to δ- and β-globin genes in the cross-over region, which differs from the previous case of Hb Lepore-Hollandia from Bangladesh with a 3' cross-over region at IVS1-nt 16 of the β-globin gene.[7] However, in both cases, the cross-over occurs in the intron, which is spliced out in the mRNAs. Thus the resultant proteins would be identical. While a previous study of six Lepore-Boston mutations

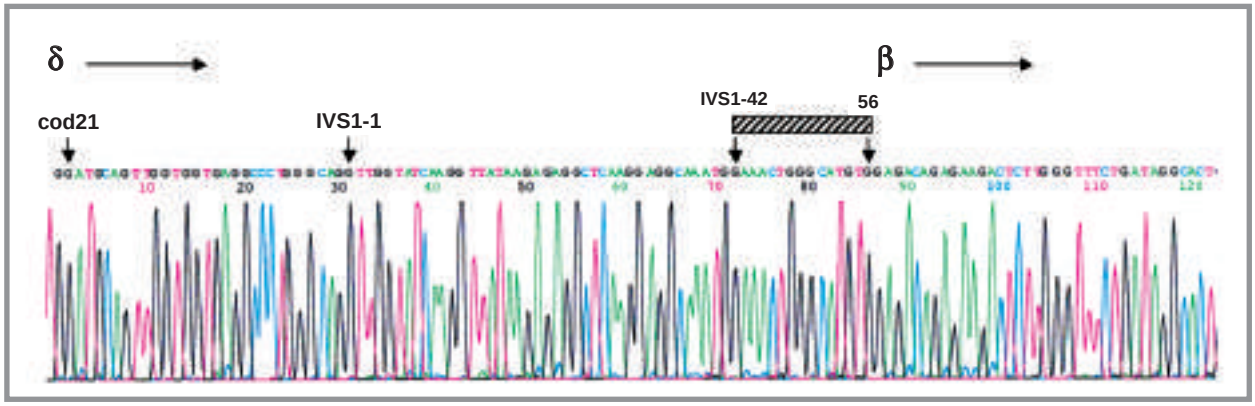

Figure 1. Sequence of the Hb Lepore-Hollandia gene using the forward primer. The cross-over region is marked by the shaded box and identity sequences of the δ- and β-globin genes are indicated by the horizontal arrows.

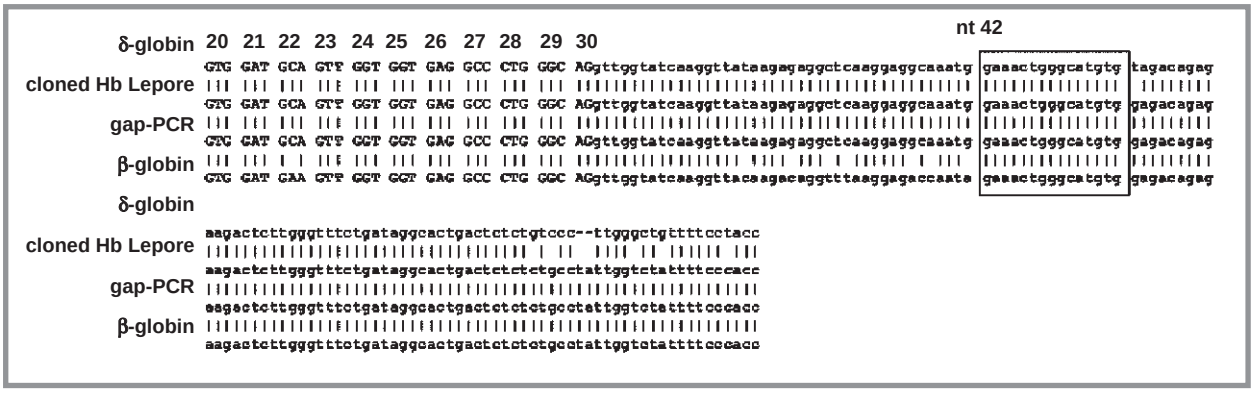

Figure 2.Comparison of the sequence of Hb Lepore-Hollandia derived by cloning and gap-PCR with that of the δ– and β- globin genes. The cross-over took place within a 15 bp window between IVS1-nt 42 – IVS1-nt 56 (boxed region). The intron sequence is given in lower case.

with different haplotype backgrounds suggested independent homologous unequal cross-over events,[10] the present finding of two Lepore-Hollandia mutations resulting from two different cross-over sites in IVS-1 is definitive proof of these being two independent events.

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Chronic Myeloid Leukemia

e6a2 BCR-ABL transcript in chronic myeloid leukemia: is it associated with aggressive disease?

We describe the fourth case of e6a2 BCR-ABL transcript in a patient with chronic myeloid leukemia (CML), using reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing analysis. The clinical and hematologic features and the aggressive course of disease in our patient and in the others reported in literature lead us to hypothesize that this atypical rearrangement may be associated with a worse prognosis.

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Chronic myeloid leukemia (CML) is characterized in 95% of patients by the expression of a large chimeric BCR-ABL fusion transcript arising from the Philadelphia (Ph) translocation t(9;22)(q34;q11). Three breakpoint cluster regions in the BCR gene have been described to date: major (M-bcr), minor (m-bcr) and micro (μ-bcr). These breakpoints result in BCR-ABL proteins that differ in size and transforming potential.

More than 90% of Ph-positive CML patients have breakpoints in the M-bcr, which typically result in b2a2 (e13a2) and/or b3a2 (e14a2) fusion mRNAs, both of which are translated into p210 BCR-ABL protein. Rarely, CML patients with the p190 BCR-ABL (m-bcr) or p230 BCR-ABL (μ-bcr) fusion genes (e1a2 and e19a2 transcripts, respectively) have been reported. Atypical BCR breakpoints outside these cluster regions have also been described. They involve splicing between whole exons, insertion of small sequences or genomic breakpoints within exons.[1-8] Despite lacking a variable number of amino acids coded for by the missing exons, BCR-ABL proteins translated from unusual transcripts are still oncogenic and can produce chronic-phase CML.

In this report we describe the fourth case of a CML patient whose chromosome 22 breakpoint was located in bcr intron 6, resulting in an unusual BCR-ABL transcript with an e6a2 junction.

A 76-years old man presented in September 2002 with massive hepatosplenomegaly and a white blood count