Identification and Genomic Analysis of Hemoglobin Long Island-Marseille in a Nondiabetic Subject of Italian Origin

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We have evaluated by HPLC (Bio-Rad Diamat Analyzer, Bio-Rad, Calif., USA) [1], the hemoglobin profile of 1,250 Italian blood donors, all from Sicily, consecutively recruited at the blood bank of our Medical University. Analysis of the hemoglobin from a 22-year-old male showed a chromatographic feature with unusually high levels of HbA fraction c (56%, fig. 1). This subject was clinically and hematologically normal. The HPLC examination, extended to the other members of his family, showed the same pattern in the father and in two of three brothers, while the mother and one of the brothers did not show any hemoglobin abnormality.

DNA was isolated from white blood cells of the propos-itus by phenol-chloroform extraction, using standardized procedures.

The coding regions of the β-globin gene were amplified by the PCR technique using two sets of primers which produce two fragments: fragment A, comprising exons 1 and 2 and spanning from nucleotide (nt) position -176 5’ at the Cap site to nt 117 in the intron 2; fragment B, comprising exon 3, spanning from nt, position 585 in the intron 2 to nt, 192 3’ to the polyadenylation signal.

The sequences of the amplification primers were: fragment A, sense primer 5’.GGTTTGAAGTCCACTCCT.3’ and antisense primer 5’.AATCATTCGTCTGTGTTC.3’; fragment B, sense primer 5’.TATCATGCCTCTTGTGCCACCC.3’ and antisense primer 5’.GCACGTGACCTCCCCACATT.3’.

To evaluate the nucleotide sequence of the β-exons, the amplified DNA, after an additional step of asymmetric amplification, were directly sequenced [2] using 5’-32P-labelled primers and a dideoxy-sequencing kit (USB, Denver, Colo., USA).

The oligonucleotide primers used for sequencing fragment A were 5’.TGCTTTGACACACACTGTG.3’ (nt -45 to -27) and 5’.CCCCTCTCATGACATGAAC.3’.

Fig. 1. HPLC elution profile of the hemoglobin from blood of the propositus. a = Long Island-Marseille + ALE Hbs; b = Ao Hb; c = A2 Hb.

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Sequencing primer for fragment B was 5’-TCTGAGTCCAAACGTAGGC-3’ (nt 777 to 795 in intron 2).

Fig. 2. Nucleotide sequence of the 1st exon of the β-globin gene. The contemporary presence of the normal (adenine) and the mutated (cytosine) nucleotide at position 2 of the 3rd codon is shown by the arrow.

Sequencing analysis showed the contemporary presence of C and A nucleotides at position 2 of the third codon of the β-globin gene, indicating a heterozygous state for that gene. In fact, one allele presented the normal ‘CAC’ codon, encoding histidine, while in the other allele the codon was ‘CCC’ which encodes proline and is specific for the rare ‘Long Island-Marseille’ Hb variant. No more abnormalities were seen in the rest of the β-gene.

Hemoglobin Long Island-Marseille is a β-chain variant having an extended N-terminus, due to the lack of amino-terminal methionine cleavage, an alteration possibly caused by the inhibition of the methionine aminopeptidase which removes the methionyl residue from growing peptides. The inhibition of this enzyme seems to be due to the histidine-to-proline substitution at the second amino acid position of the β-chain [3, 4], produced by the above-described point mutation in the first exon of the β-gene.

Only three carriers of this hemoglobin variant have been identified so far; none of them of Italian origin [3-5]. In the previous studies, the hemoglobin defect had been detected through amino acid analysis of the β-chain, while the genomic alteration was also revealed by the sequencing of the reverse transcribed reticulocyte β-globin mRNA in only one of them [6]. In the present study, we have confirmed this molecular defect through the direct examination of the β-globin gene.

This paper is the first report concerning the detection of the hemoglobin Long Island-Marseille in subjects of Italian origin.

This hemoglobin variant coelutes with the HbA1c fraction c, which consequently presents a falsely elevated level on HPLC analysis. For this reason, this variant was found only in 3 diabetic patients so far, as a result of routine clinical measurements of the glycated hemoglobin. Therefore, the real prevalence of this hemoglobin has not yet been estimated in the general population. Considering that we have found it only in 1 of the 1,250 blood donors examined, we may assume that Long Island-Marseille Hb variant has a low prevalence, at least in the Sicilian population.

References

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Hb Long Island-Marseille in a Healthy Italian Subject