BRIEF REPORT

Identification of the Molecular Genetic Defect of Patients With Methemoglobin M Kankakee (M-Iwate), α87 (F8) His → Tyr: Evidence for an Electrostatic Model of αM Hemoglobin Assembly


We determined that the molecular defect of 2 patients with hemoglobin (Hb) M-Kankakee [Hb M-Iwate, α87 (F8) His → Tyr] resides in the α1-globin gene. The proportion of Hb M observed is higher than that predicted for an α1-globin variant. Our evidence suggests that the greater-than-expected proportion of Hb M-Kankakee results from preferential association of the electronegative β-globin chains with the αM-globin chains that are more electropositive than normal α-globin chains.

© 1999 by The American Society of Hematology.

Hemoglobin (Hb) M-KANKAKEE (Iwate) is a variant Hb that presents clinically as congenital cyanosis due to methemoglobinemia.1 Hb M-Kankakee is identical to Hb M-Iwate, described in a Japanese kindred in which the proximal histidine in the patients’ α-globin chain was replaced by tyrosine (α87 His → Tyr), and with Hb M-Oldenburg and Hb M-Sendai.2

Hb M-Iwate has been well characterized with respect to its abnormal functional properties in oxygen transport,3-5 whereas no conclusive molecular genetic data have been reported. The Hb that presents clinically as congenital cyanosis due to methemoglobinemia. The proportion of Hb M to be 27.2% and 22.4% in V.W. and K.W., respectively (Table 1). Stability tests of hemolysates were normal.

A 659-bp DNA fragment encompassing exon 1 and portions of the 3′UTR of the patients α2- and α1-globin genes was separately amplified by PCR. Sequencing of the amplified DNA showed replacement of CAC (His) by TAC (Tyr) in codon 87 of one α1-globin gene, while the second α1-globin gene encoded the normal histidine residue. Southern blot analysis of genomic DNA was negative for deletion of α-thalassemia.

It has been previously established that the ratio of α-globin chains derived from the α2-globin allele compared with the α1-globin allele is approximately 3:1 (range, 2.6 to 3.1).6-8 Increased synthesis of α2-globin is caused by preferential transcription of the α2-globin gene. Translation of the α2-globin and α1-globin mRNAs is equivalent.6,7 Therefore, the inheritance of a single variant α1-globin gene should be associated with a variant Hb level of 12% to 14% [α1/(2α1 + 2α2) = 1/2 = 6.2 = 1/8.2, 12%]. Our patients have Hb M levels of 22% to 28%, a value substantially higher than predicted. We excluded the possibility that coinheritance of a deletion-type α-thalassemia allele is responsible for the increased proportion of αM-globin chains contributing to the α-globin pool.

Why is the percentage of Hb M in this kindred greater than predicted for an α1-globin variant? A possible explanation for the increased proportion of Hb M may be preferential associa-

Materials and Methods

Blood was obtained by venipuncture after informed consent.

Hb M protein studies. Hb M quantification by cation exchange high-performance liquid chromatography (HPLC) and isoelectric focusing were performed as previously described.10 The isopropanol and heat methods were used to test for Hb stability.11

DNA studies. Genomic DNA was extracted from 10 mL of whole blood using a commercial kit (Boehringer Mannheim, Indianapolis, IN). Polymerase chain reaction (PCR) primers were designed to selectively amplify the human α2- and α1-globin genes.12 The 5′ primer (5′-agttaatgctggcggctgg-3′) is complementary to a conserved region in exon 1 of the α2- and α1-globin genes. The 3′ primers were complementary to a nonhomologous region in the 3′ untranslated region (UTR) of the α2- and α1-globin genes (5′-tcgacagggagaaggtc-3′ for α2-globin gene and 5′-aattggtgagttggagc-3′ for α1-globin gene). PCR was performed on 50 ng of genomic DNA using a high-fidelity PCR-kit (Boehringer Mannheim). The reaction was carried out at 95°C × 1 minute, 65°C × 2 minutes, and 72°C × 2 minutes for 35 cycles in the presence of 0.5% dimethyl sulfoxide. Southern analysis was performed as described.11

RESULTS AND DISCUSSION

The patients are 2 sisters of a previously reported kindred with Hb M-Kankakee and of Northern European descent.1 Their hematologic parameters are summarized in Table 1. On isoelectric focusing, Hb M was 9.1 mm cathodic to Hb A, consistent with Hb M-Iwate.13 The isoelectric point was calculated to be 7 (pI = 7). Hb quantification by HPLC showed the relative proportion of Hb M to be 27.2% and 22.4% in V.W. and K.W., respectively (Table 1). Stability tests of hemolysates were normal.

From the Division of Pediatric Hematology-Oncology and the Department of Internal Medicine, University of Michigan, Ann Arbor, MI; and the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Submitted February 10, 1999; accepted May 10, 1999.

Supported in part by National Institutes of Health Grant No. T32 HL07622.

Address reprint requests to A. Ameri, MD, Division of Pediatric Hematology-Oncology, University of Michigan, Ann Arbor, MI 48109; e-mail: aameri@umich.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1999 by The American Society of Hematology.

0006-4971/99/9405-0033$3.00/0

From www.bloodjournal.org by guest on September 24, 2017. For personal use only.
Localization of the genetic defect of the αM-globin with the β-globin chain caused by electrostatic protein surface interactions. An electrostatic model for Hb assembly was proposed to explain the proportion of β-globin variant observed in individuals with Hb S, Hb C, Hb D, Hb J-Baltimore, and Hb J-Iwate. Reduced levels of the variant are observed in cases wherein the amino acid substitution renders the β-globin chain more electropositive (Hb S, Hb C, Hb D). In β-globin variants in which the amino acid substitution renders the β-globin chain more electronegative, increased association with the electropositively charged α-chain occurs, resulting in elevated proportions of the variant Hb (Hb J-Baltimore, Hb J-Iwate).

This model is not restricted to β-globin variants. A potential role for an electrostatic model of Hb assembly in α-globin variants was predicted. In α-globin variants, the presence of 4 α-globin genes makes predictions of variant Hb levels more complex, mandating a precise understanding of variant α-globin gene locus assignment (α2 v α1), the number of affected genes, and knowledge of the presence of α-thalassemia or duplicated α-globin genes. In Hb M-Kankakee, replacement of the β-globin chain by an electropositive α-globin results in decreased Hb M concentrations, consistent with the observation that electronegative α-globin variants exhibit preferential assembly with α-globin, whereas electropositive αM-globin variants may exhibit preferential assembly with β-globin. Our review of the literature on αM-globin variants and the similarity in observed proportions of the other Hb M variants support this hypothesis. In patients with Hb M-Boston, the proportion of Hb M is 20% to 30%, similar to patients with Hb M-Iwate, and we predict the proportion of Hb M might exceed 75%, a level likely incompatible with fetal viability. Neonates with acquired methemoglobinemia and levels of methemoglobin above 60% may have severe vital compromise.

In conclusion, we have revisited the molecular defect in patients with methemoglobin M-Kankakee 37 years from the initial description. Correlation of in vivo levels of Hb M, locus assignment of the genetic defect to the α1-globin gene, exclusion of concomitant deletion α-thalassemia, and knowledge of relative protein charge provide in vivo evidence of preferential assembly of electropositive α-globin variants, as predicted by the electrostatic model of Hb assembly.

REFERENCES

Identification of the Molecular Genetic Defect of Patients With Methemoglobin M Kankakee (M-Iwate), 87 (F8) His → Tyr: Evidence for an Electrostatic Model of ?M Hemoglobin Assembly