Missense mutation of the last nucleotide of exon 1 (G->C) of β globin gene not only leads to undetectable mutant peptide and transcript but also interferes with the expression of wild allele

Hemoglobin Monroe (*β globin* G->C, codon 30) is a missense mutation. We could not detect either the mutant peptide or transcript in reticulocyte-enriched preparation and in expanded erythroid progenitor cells. By quantitative gene expression assay β globin mRNA was found to be reduced by more than 70% in all heterozygous subjects with different haplotypes. We conclude that this mutation also interferes with expression of wild type allele.

Haematologica 2007; 92:1715-1716. DOI: 10.3324/haematol.11543

Hb Monroe results from a splice site point mutation (G->C) in the last nucleotide of β globin exon 1, which is also the penultimate nucleotide of codon 30 of the β globin peptide (AGG->ACG; Arg->Thr). The molecular basis of its thalassemic phenotype is unknown. A 29-year old, asymptomatic woman of Asian-Indian descent (Bengali extraction) was referred to us because of elevated serum ferritin (>3,000 ng/mL). She had microcytic anemia (Hb 9.5 gm %, MCV 68 fL). A Star plus MRI revealed no increase in cardiac iron but hepatic siderosis; liver biopsy revealed 4+ liver iron, significant fibrosis, and iron in both hepatocytes and macrophages. No mutations of HFE, ferroportin, and hemojuvelin genes were detected. HPLC revealed: HbF 51%, HbE 43.2%, HbA2 5.8%. A compound heterozygosity for HbE/β^0-thalassemia was suspected. β *globin* gene sequencing revealed HbE and a missense mutation (β *globin* IVS1 (-1) G->C). The asymptomatic brother of the proband was found to have anemia (Hb 11.5 gm %, microcytosis MCV 70 fL), HbF 8.5 %, HbA 87.2%, HbA_2 5.0%, and heterozygosity for Hb Monroe mutation. The aim of our study was to determine the molecular basis of the thalassemia phenotype associated with Hb Monroe mutation in the proband's brother and in an Afro-American family with two members, known to be heterozygous for Hb Monroe. Clinical and laboratory parameters of the study subjects are summarized in Table 1.

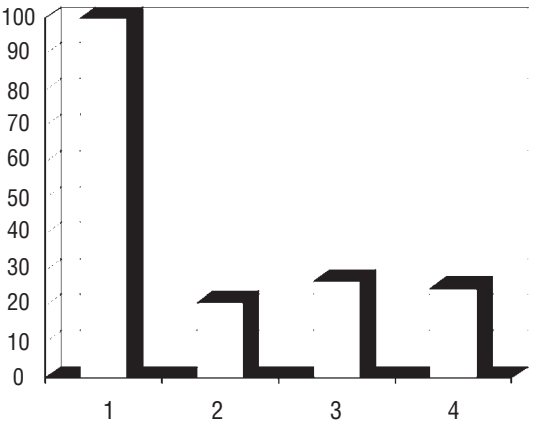

Figure 1. β *globin* quantitative gene expression profile from subjects heterozygous for Hb Monroe mutation b (relative percent expression compared with healthy controls): (1) average value of three healthy individuals (100%); (2) Asian Indian (Bengali) male (21%); (3) Afro-American female #1 (25%); (4) Afro-American female #2 (27%).

In vitro expansion of peripheral blood erythroid progenitors was performed. The cells were harvested at the stage of late normoblast differentiation. Total RNA was isolated and cDNA synthesized. β *globin* cDNA from the reticulocytes and expanded erythroid progenitors were amplified. β *globin* DNA and cDNA sequencing was performed. Genomic DNA was isolated and β *globin* haplotype analysis performed using five known restriction sites. Real-time quantitative PCR Assay for β *globin* gene expression was performed. The quantity of β *globin* gene expression (18S RNA normalized) in our subjects was compared to the average value of three healthy controls. Material and methods are available as *Supplementary Data*.

After the original reports in 1989, Hb Monroe has been reported in low frequency, mostly in compound heterozygosity with other β *globin* mutations.[1-5] The original studies suggested either unstable mutant peptide or aber-

Table 1. Clinical and laboratory parameters in our study subjects: A) Asian Indian (Bengali) female (proband), compound heterozygous for Hb Monroe and Hb E; B) Asian Indian (Bengali) male (proband's brother), heterozygous for Hb Monroe; C) African American female #1, heterozygous for Hb Monroe; D) African American female #2, heterozygous for Hb Monroe.

Subject	*Sex*	*Clinical Symtoms*	*Clinical Signs*	*Genotype*	*Hb (gm/dL)*	*Hct (%)*	*MCV (FL)*	*HbA (%)*	*HbA2(%)*	*HbF(%)*	*HbE (%)*
A	Female	Fatigue	Pallor +, no organomegaly	βE/-βMonroe	9,5	29	68	0	5,8	51	43,2
B	Male	Asymptomatic	Pallor +, no organomegaly	β/-βMonroe	11,5	34	70	87,2	5	8,5	0
C	Female	Fatigue	Pallor +, no organomegaly	β/-βMonroe	7,2	23,8	56,2	92,7	4,6	2,7	0
D	Female	Fatigue	Pallor +, no organomegaly	β/-βMonroe	8,1	26	61	95,1	2,9	2	0

No history of blood transfusion in any of the study subjects. Hb: hemoglobin; Hct: hematocrit; MCV: mean cell volume of the erythrocytes.

rantly processed mRNA to be the molecular basis of thalassemic phenotype but no study examining native mRNA has been reported. We did not detect mutant peptide in fresh hemolysate or in reticulocytes. The cultured late normoblasts revealed ~1% of unidentified peptide but its further analysis by mass spectroscopy revealed it to be glycated fetal hemoglobin. In contrast, both the original reports[6,7] described a small amount of unknown Hb thought to be Monroe. However, we could not confirm these reports using more definitive methodology. We could not detect any splice variants when full-length β globin cDNA were examined using two different primer sets for cDNA amplification. Sequencing of the amplified cDNA revealed only normal β globin mRNA transcript. Furthermore, no promoter region or stop codon mutations, deletions or splicing mutations were found from promoter - 90 region to 3' UTR including poly-A region.

Quantitative *β globin* expression assay revealed diminished β globin mRNA (70% reduction) in an Asian Indian subject with haplotype (+ + + + -) as well as in two Afro-American subjects heterozygous for Hb Monroe with, however, different β globin haplotype (- - + + -) (Figure 1). Similarly diminished β globin mRNA in all our subjects present in different β globin haplotypes also rules out the unlikely possibility of an unidentified promoter mutation present in trans.

Nonsense-mediated decay (NMD) is a cellular mechanism of mRNA surveillance which detects and degrades mRNA containing premature translation termination codon (PTC).[8] NMD has been reported to occur in β thalassemia. Heterozygous cases of β^0 and β^+ thalassemia with PTC have been found to have a reduction of β globin mRNA of 40% and 27% respectively (by quantitative gene expression assays), suggesting interference with transcription of mutant allele.[9] However, we did not find stop codon generating mutations either in the *in vitro* generated mRNA transcripts or in any theoretically formed transcripts created and analyzed in silico. Furthermore, our subjects have a more than 70% reduction of the β globin gene expression compared with the normal controls, suggesting interference with transcription of not only mutant allele but also wild type allele. We conclude that this missense mutation not only leads to undetectable mutant peptide and transcript but also interferes with the expression of wild type allele.

Neeraj Agarwal, Ferdane Kutlar,° Mariluz P. Mojica-Henshaw,* Ching N. Ou,# Amos Gaikwad,# N. Scott Reading,@ Lakeia Bailey,° Abdullah Kutlar,° Josef T. Prchal*@*

**Hematology Division, University of Utah School of Medicine, Salt Lake City, UT; °Hematology Division, Medical College of Georgia, Augusta, GA, USA; #Pediatric-Hematology-Oncology, Baylor College of Medicine, Houston, TX, USA; @ARUP Laboratories, Salt Lake City, UT, USA*

Correspondence: Josef T. Prchal, University of Utah, Hematology Division, 30 North 1900 East, Utah 4C416, Salt Lake City, USA. E-mail: josef.prchal@hsc.utah.edu

References

1. el-Kalla S, Mathews AR. A significant β-thalassemia heterogeneity in the United Arab Emirates. Hemoglobin 1997 21:237-47.
2. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of β-thalassaemia mutations on the Indian subcontinent: the basis for prenatal diagnosis. Br J Haematol 1991;78:242-7.
3. Gupta RB, Tiwary RS, Pande PL, Kutlar F, Oner C, Oner R, et al. Hemoglobinopathies among the Gond tribal groups of central India; interaction of α- and β-thalassemia with β chain variants. Hemoglobin 1991;15:441-58.
4. Fattoum S, Guemira F, Oner C, Oner R, Li HW, Kutlar F, et al. β-thalassemia, HB S-β-thalassemia and sickle cell anemia among Tunisians. Hemoglobin 1991;15:11-21.
5. Sweeting I, Serjeant BE, Serjeant GR, Kulozik AE, Vetter B. HB S-HB Monroe; a sickle cell-β-thalassemia syndrome. Hemoglobin 1998;22:153-6.
6. Gonzalez-Redondo JM, Stoming TA, Kutlar F, Kutlar A, Hu H, Wilson JB, et al. Hb Monroe or α2β230(B12) Arg----Thr, a variant associated with β-thalassemia due to A G----C substitution adjacent to the donor splice site of the first intron. Hemoglobin 1989;13:67-74.
7. Vidaud M, Gattoni R, Stevenin J, Vidaud D, Amselem S, Chibani J, et al. A 5' splice-region G----C mutation in exon 1 of the human β-globin gene inhibits pre-mRNA splicing: a mechanism for β+-thalassemia. Proc Natl Acad Sci USA 1989;86:1041-5.
8. Hentze MW, Kulozik, AE. A perfect message: RNA surveillance and nonsense-mediated decay. Cell 1999;96:307-10.
9. Mo QH, Li XR, Li CF, He YL, Xu XM. A novel frameshift mutation (+G) at codons 15/16 in a β^0 thalassaemia gene results in a significant reduction of β globin mRNA values. J Clin Pathol 2005;58:923-6.