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De Novo Mutation of the β-Globin Gene Initiation Codon (ATG→AAG) in a Northern European Boy

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We present a case of β-thalassemia intermedia involving a 13-year-old boy of Northern European descent. His mother, father and older sister have normal hematologic indices. Molecular studies demonstrate that the proband carries a novel mutation of the β-globin gene initiation codon (ATG→AAG) which should give rise to β°-thalassemia trait. The possibility of non-paternity was excluded, indicating that the novel mutation was the result of a de novo event. A review of the literature indicates that mutations involving the β-globin gene initiation codon can give rise to a more severe phenotype than is generally associated with most other β⁺ or β° mutations. Am. J. Hematol. 56:179–182, 1997.

Key words: β-thalassemia; initiation codon; de novo mutation

INTRODUCTION

β-thalassemia is one of the most common single-gene disorders of man, occurring at high frequencies among individuals of Mediterranean, East Indian, Middle Eastern, African, or Southeast Asian descent [1]. More than 150 different β-thalassemia mutations have been reported, most of which are single base substitutions or small insertions or deletions within the β-globin gene [2].

The phenotypic severity of individual β-thalassemia mutations can vary considerably depending on the molecular defect [3–5]. The mildest mutations, designated β^+, are those that reduce the expression of normal β-chains. β° mutations abolish expression of normal β-chains and therefore are more severe than β^+ mutations. The most severe class of mutations is the so-called inclusion body or dominant β-thalassemia mutations that give rise to highly unstable β-chain variants [6–8]. Carriers of such mutations exhibit a relatively severe β-thalassemia intermedia phenotype.

In this report, we describe a case of β-thalassemia intermedia due to a de novo mutation of the β-globin gene initiation codon. A review of the literature confirms that this type of mutation gives rise to a more severe phenotype than is generally associated with most other β^+ or β° thalassemia mutations.

TABLE I. Hematology Results for the Family Under Study

	Father (48 years)	Mother (42 years)	Proband (13 years)	Sister (15 years)
Hb (g/dL)	14.8	12.7	9.0	12.6
Hct	0.424	0.360	0.270	0.356
RBC (10^{12}/L)	4.58	3.95	4.87	4.29
MCV (fL)	92.4	92.0	55.0	83.0
MCH (pg)	32.2	32.0	18.6	29.4
RDW	13.7	13.5	26.9	12.7
Hb A (%)	96.1	95.3	87.9	95.7
Hb A_2 (%)	3.3	3.0	5.7	3.1
Hb F (%)	0.6	1.7	6.4	1.2
α-genotype	αα/αα	αα/αα	αα/αα	αα/αα

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TABLE II. Hematologic Phenotypes of Heterozygotes for β-Globin Gene Initiation Codon Mutations

Mutation	Population	Reference	Sex-age	Hb (g/dL)	MCV (fL)	Hb A_2 (%)	Hb F (%)
ATG → GTG	Japanese	Hattori et al. [12]	M-13	11.6	53.0	6.7	1.9
			F-41	9.9	56.0	6.0	0.8
			F-51	11.0	55.0	6.2	1.5
			F-54	9.1	70.0	6.2	7.1
ATG→ACG	Swiss	Beris et al. [14]	M	11.5	59.0	4.0	3.2
			F	9.4	60.0	7.3	3.1
	Yugoslavian	Jankovic et al. [15]	M-52	11.5	57.6	6.2	0.6
			M-25	11.6	54.1	5.9	0.9
			F-23	10.0	56.1	6.0	0.7
	Belgian	Wildmann et al. [16]	F-70	10.2	55.4	5.9	1.1
			M-37	11.8	56.1	5.4	0.9
			M-34	12.5	54.0	6.1	1.1
			F-11	10.2	50.7	6.3	0.5
			M-07	10.0	53.3	6.4	1.8
			F-06	9.2	50.1	5.4	2.3
			F-05	9.4	53.4	5.9	4.4
			M-04	9.9	48.5	5.8	6.8
			F-01	9.1	51.4	4.1	22.0
ATG→AGG	Korean	Koo et al. [18]	F-53	10.4	53.0	6.1	2.6
			F-50	10.6	58.0	6.4	2.2
			F-48	11.0	62.0	5.7	4.7
			M-25	12.9	52.0	6.1	0.7
			M-16	11.3	49.0	6.6	3.8
			F-25	10.5	76.0	3.9	21.7
			F-15	9.6	67.0	4.8	11.8
			M-13	11.0	61.0	5.7	16.0
ATG→ATA	Swedish	Landin et al. [19]	M-73	10.2	54.8	5.9	0.6
			F-47	10.7	55.2	5.2	<0.5
			M-35	12.0	53.4	5.9	<0.5
			M-21	12.1	56.4	4.7	<0.5
			F-17	9.6	56.1	5.0	1.2
			M-2	9.5	48.9	5.5	4.4
			M-1	9.6	49.1	5.5	4.4
ATG→ATT	U.S. Caucasian	Rahbar and Nozari [21]	F-23	7.7	74.0	6.4	2.0
ATG→AAG	N. European	This report	M-13	9.0	55.0	5.7	6.4

TABLE III. Hematologic Phenotypes of Heterozygotes for Different β-Thalassemia Mutations*

Mutation	Severity	Hb (g/dL)	MCV (fL)	Hb A_2 (%)
−87 C→G	Mild β^+	14.1 ± 1.4 (male)	74.0 ± 5.2	5.3 ± 0.7
IVS-I-6				
T→C	Mild β^+	13.8 ± 0.7 (male)	70.4 ± 1.9	3.9 ± 0.4
IVS-I-110				
G→A	Severe β^+	13.2 ± 1.0 (male)	63.8 ± 2.7	4.5 ± 0.5
IVS-II-745				
C→G	Severe β^+	13.1 ± 0.5 (male)	62.5 ± 3.0	5.0 ± 0.5
IVS-I-1				
G→A	Severe β°	12.8 ± 0.8 (male)	63.3 ± 3.1	4.8 ± 0.4
Codon 39				
C→T	Severe β°	13.3 ± 0.9 (male)	62.9 ± 3.0	5.3 ± 0.7
Initiation	Severe β°	11.1 ± 1.1 (male)	56.4 ± 6.6	5.7 ± 0.7
codon		9.9 ± 0.8 (female)		

*The values given for initation codon mutations (mean ± SD) were derived from Table II. The values given for all other mutations were reported by Rosatelli et al. [22].

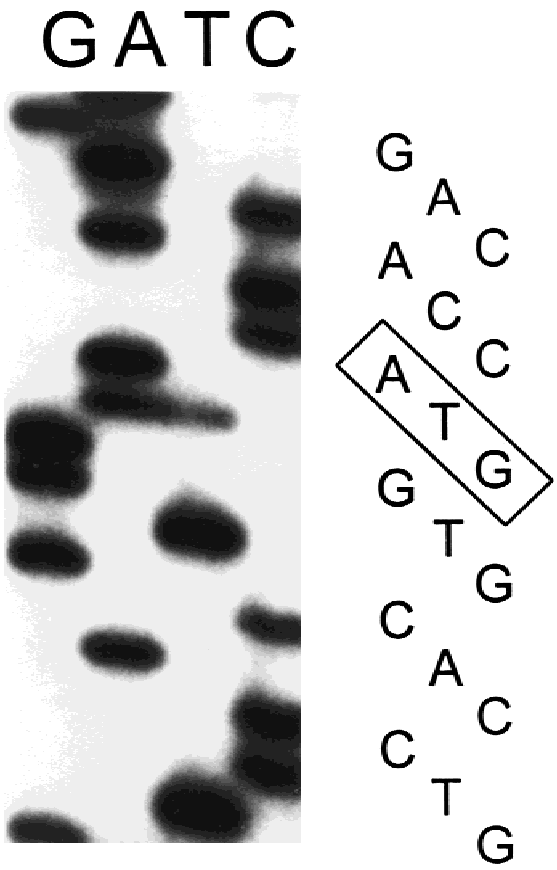

Fig. 1. Nucleotide sequence analysis of PCR amplified β-globin gene sequences from the proband. The normal initiation codon (ATG) is boxed.

CASE REPORT

The proband is a 13-year-old boy of Northern European descent who presented with the hematologic profile of β-thalassemia intermedia (Hb = 9.0 g/dL, MCV = 55.0 fL, Hb A_2 = 5.7%, Hb F = 6.4%). The blood film showed hypochromia, microcytosis, targeting, and stippling. The physical examination revealed splenomegaly, palpable at 1 cm below the left costal margin. The proband's parents and older sister have normal hematologic indices (Table I).

Molecular studies were initiated on the proband. The β-globin gene was amplified using the polymerase chain reaction (PCR) [9] and the PCR product was sequenced using the dideoxy termination method [10]. As shown in Figure 1, the proband is heterozygous for a single base substitution involving the initiation codon (ATG→AAG). This mutation abolishes the recognition site for the restriction endonuclease *Nco* I. PCR amplified DNA from the family members was digested with *Nco* I and analysed by polyacrylamide gel electrophoresis. The results demonstrate that only the proband carries the initiation codon mutation. The possibility of non-paternity was investigated by RFLP typing of VNTR loci D2S44 and D5S110 and by PCR typing of the HLA-DQα locus [11] (data not shown). The test results were fully consistent with paternity as stated, with a paternity index of 3,888 and a probability of paternity in excess of 99.97% (prior probability = 0.5). Therefore, the novel initiation codon mutation was the result of a de novo event.

DISCUSSION

Mutations of the β-globin gene initiation codon have been described in several different populations. Including the novel ATG→AAG mutation presented in this report, seven of the nine possible single-base substitutions have been reported [12–22]. It is of interest that the hematologic profile of the patient described in this report is more severe than typical β-thalassemia trait. In particular, the proband's microcytic anemia (Hb = 9.0 g/dL, MCV = 55.0 fL) is quite striking for someone who carries only one β-thalassemia allele.

As noted by Landin et al. [19], severe microcytic anemia appears to be a common feature associated with mutations of the initiation codon of the β-globin gene. A review of 35 patients from the literature, all of whom are carriers of mutations involving the initiation codon of the β-globin gene, reveals that both the hemoglobin level and MCV are markedly reduced relative to typical β-thalassemia trait (Tables II and III) [23,24]. It is thought that mutations affecting mRNA translation are associated with decreased cytosolic mRNA levels, most likely due to an intranuclear event or the nuclear-cytoplasm transport process [25,26]. In the case of initiation codon mutations, this effect may be exaggerated and thereby account for the severely abnormal hematologic indices. Another possibility may be that translation of the mutant mRNA initiates from the single in-frame initiation codon (codon 55) and produces a truncated β-chain that is highly unstable. Proteolytic degradation of the truncated β-chains would allow free α-chains to accumulate and precipitate, possibly resulting in ineffective erythropoiesis and/or peripheral hemolysis. In this manner, initiation codon mutations may resemble the exon III mutations that are associated with dominant β-thalassemia [6–8].

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