

SHORT REPORT

Dominantly inherited β thalassaemia intermedia caused by a new single nucleotide deletion in exon 2 of the β globin gene: Hb morgantown ($\beta 91$ CTG>CG)

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J Clin Pathol 2005;58:1110–1112. doi: 10.1136/jcp.2004.023010

Family members in multiple generations of an Irish-American family were investigated for moderate to severe microcytic anaemia, inherited in an autosomal dominant fashion. A novel frameshift mutation of the β globin gene was discovered. This study highlights the importance of considering dominantly inherited β thalassaemia in the investigation of anaemia, even in patients with ethnic backgrounds not usually associated with β thalassaemia.

Beta thalassaemia major or intermedia are typically caused by homozygosity or compound heterozygosity for β globin gene mutations that greatly decrease or abolish β globin chain production.¹ These mutations are inherited as recessive traits, and have reached polymorphic frequencies in many populations because heterozygotes have increased genetic fitness against *Plasmodium falciparum* infection. An uncommon form of severe β thalassaemia intermedia is dominantly inherited, usually caused by heterozygosity of a mutation in the third exon of the β globin gene, found in people of diverse ethnicity, and not necessarily associated with increased genetic fitness against malaria.^{2,3} These types of thalassaemia have been variously termed inclusion body β thalassaemia and dominantly inherited β thalassaemia.^{4,5} We report a new mutation in the second exon of the globin gene that produces dominantly inherited severe thalassaemia intermedia found in three generations of an Irish-American family.

“An uncommon form of severe β thalassaemia intermedia is dominantly inherited, found in people of diverse ethnicity, and not necessarily associated with increased genetic fitness against malaria”

CASE REPORT

The proband is a 2½ year old girl of Irish descent who was referred for evaluation of anaemia. At birth, she had a normal haemoglobin (Hb) concentration of 195 g/litre and mild hyperbilirubinaemia, which responded to phototherapy. Microcytic anaemia was first noted at 6 months of age. Her mother, maternal grandmother, and maternal great aunt all have microcytic anaemia, pronounced splenomegaly, and facial features consistent with thalassaemia. Both the mother and the maternal great aunt had cholelithiasis that necessitated cholecystectomy. The maternal great aunt has been transfused on several occasions and the mother was transfused after cholecystectomy. The maternal grandmother and maternal great aunt have leg ulcers. The family history

indicates an autosomal dominant anaemia going back six generations from the proband and affecting both sexes.

METHODS

EDTA anticoagulated peripheral blood was sent to Boston by overnight courier. Haemoglobin analysis was done by BioRad (Hercules, California, USA) Variant II cation high performance liquid chromatography and isoelectric focusing. Genomic DNA was extracted from peripheral blood leucocytes by phenol/chloroform and ethanol precipitation.⁶ The β globin genes and promoter regions of the $\beta^G\gamma$ globin and $\beta^A\gamma$ globin genes were amplified separately by polymerase chain reaction (PCR), the amplicons were purified, and direct nucleotide sequencing was carried out.⁶ Commonly found deletions of single α globin and two α globin genes in cis and triplication of globin genes were searched for by appropriate PCR amplifications across the breakpoints of deletion (Gap-PCR).⁷

RESULTS

Table 1 summarises the haematological findings of all four family members. The mother's peripheral blood smear showed prominent anisopoikilocytosis, basophilic stippling, and nucleated red blood cells (fig 1), and her bone marrow showed hypercellularity, erythroid hyperplasia, and increased stainable iron stores but no ringed sideroblasts. Erythroblast inclusion bodies were not looked for. All four family members had raised Hb F (table 1), but no variant haemoglobin was found by either high performance liquid chromatography or isoelectric focusing.

Nucleotide sequencing of the β globin genes (*HBB*) from all four individuals showed that they were heterozygous for a novel single nucleotide (T) deletion in codon 91 (CTG>CG), as illustrated in fig 2. To account for the raised Hb F, the promoter regions of both the $\beta^G\gamma$ globin and $\beta^A\gamma$ globin genes were sequenced. No known hereditary persistence of fetal haemoglobin point mutations was found. The polymorphism C>T (*Xmn I*) at nucleotide -158, 5' to the $\beta^G\gamma$ globin gene, which has been associated with raised Hb F, was not present.⁸ The common single α globin gene deletions, the (-_{MED}) deletion, and α globin gene triplication were not detected by gap-PCR.

DISCUSSION

This novel β codon 91 (CTG>CG) mutation, Hb Morgantown, causes a frameshift in the coding DNA sequence and results in a variant β globin chain with 156 amino acid residues before it is terminated by an in frame TAA termination codon. The amino acids between codon 91 and the C-

Abbreviations: Hb, haemoglobin; PCR, polymerase chain reaction

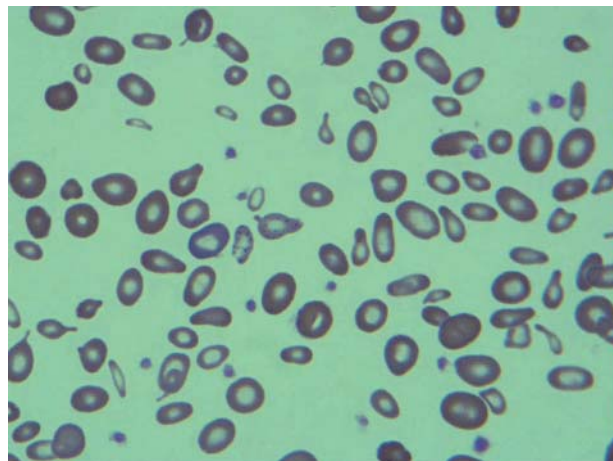


Figure 1 Mother's peripheral blood smear. Note the anisopoikilocytosis, hypochromia, basophilic stippling, and polychromasia.

terminus of the chain are entirely different from those of a normal globin chain, which has 146 amino acids. Anaemia is more severe in the maternal grandmother and great aunt than in the mother, whereas it is moderate in the proband (table 1). This is probably a reflection of the increasing splenomegaly with age. In addition, the proband has a borderline low serum ferritin concentration (13 ng/ml; table 1), indicating that she might also be iron deficient.

Interestingly, Hb Agnana, caused by insertion of two nucleotides in the β chain codon 94 (GAC>GTGAC), has 157 amino acid residues, all of which are identical to Hb Morgantown, except that Hb Agnana has 91 Leu, 92 His, and 93 Cys residues, whereas Hb Morgantown has 91 Arg and 92 Thr residues.⁹ Hb Agnana is a hyper unstable haemoglobin that imparts a severe β thalassaemia phenotype. A 3 year old Italian girl who was heterozygous for Hb Agnana had an Hb of 66 g/litre, a mean cell volume of 70 fL, hepatosplenomegaly, and required regular transfusions from age 6 years onward. No variant haemoglobin was detected in the four family members with the Hb Morgantown mutation, consistent with it also being a hyper unstable haemoglobin.

"DNA based diagnostics can readily detect and confirm these uncommon β globin gene mutations, and help implement appropriate treatment planning and genetic counselling"

There are now more than 30 known mutations that cause dominantly inherited β thalassaemia, and among these, de novo mutations are relatively common.¹⁰ These include missense, nonsense, frameshift, deletion/insertion of intact

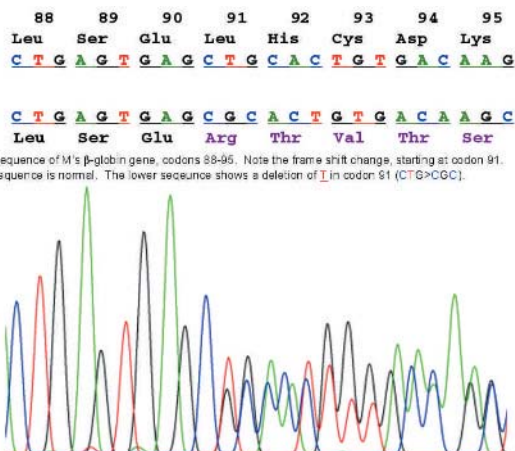


Figure 2 Nucleotide sequence of the mother's β globin gene, codons 88–95. Note the frameshift change, starting at codon 91. The upper sequence is normal. The lower sequence shows a deletion of T (red) in codon 91 (CTG>CGC).

codon, and aberrant splicing mutations, which result in elongated or truncated β globin chain variants with an abnormal C-terminus. Most of these mutations reside in the third exon of the β globin gene, although mutations in both exons 1 and 2 and intron 2 have also been reported. These mutations usually cause hyper unstable β globin chains that aggregate to form precipitates, resulting in inclusion bodies and ineffective erythropoiesis.¹¹ The excess α globin chains and their precipitation further contribute to the β thalassaemia intermedia phenotype. There are mutations that lead to apparently stable variant β globin chains, but form extremely unstable $\alpha_2\beta^{\text{variant}}_2$ tetramers.¹²

Among the four affected family members, the Hb A₂ concentration is normal, as has been reported in some families with similar syndromes. Hb F values are very high, and no genetic cause for this has yet been identified in our

Take home messages

- We report a novel frameshift mutation of the β globin gene in several generations of an Irish–American family who were investigated for moderate to severe microcytic anaemia, inherited in an autosomal dominant fashion
- This study highlights the importance of considering dominantly inherited β thalassaemia in the investigation of anaemia, even in patients with ethnic backgrounds not usually associated with β thalassaemia

Table 1 Laboratory haematological results

	Maternal grandmother	Maternal great aunt	Mother	Proband
Age (years)	53	51	25	2 ½
Hb (g/l)	60	54	70	90
MCV (fL)	62	52	66	71
Reticulocyte count	4%	6%	4%	4%
Hb A	78%	90%	86%	75%
Hb A ₂	2.6%	3.6%	3.0%	2.7%
Hb F	20.1%	7.4%	11.8%	23.5%
Serum ferritin (ng/ml)	371	505	140	13

Hb, haemoglobin; MCV, mean cell volume.

family. Both the maternal grandmother and great aunt have large leg ulcers. This is found relatively frequently in haemolytic syndromes, and in sickle cell anaemia, hereditary spherocytosis, and β thalassaemia major or intermedia. Decreased intravascular nitric oxide bioavailability, caused by the binding of nitric oxide to haemoglobin in the circulation as a result of intravascular or intramedullary haemolysis, has been proposed as an important pathophysiological cause for this clinical finding.¹³

This family study serves to underscore the importance of considering dominantly inherited β thalassaemia as a cause of moderate to severe microcytic anaemia in children and adults with pronounced anisopoikilocytosis, normal or raised iron stores, and normal or raised Hb A₂, even without a significant family history and among ethnic backgrounds not usually associated with thalassaemia. DNA based diagnostics can readily detect and confirm these uncommon β globin gene mutations, and help implement appropriate treatment planning and genetic counselling.

ACKNOWLEDGEMENT

This study was supported in part by NHLBI grant 1U54 HL070819.

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Accepted for publication 25 January 2005

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