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Identification of a novel frameshift mutation at codon 53 (−T) in the β-globin gene causing dominantly inherited β-thalassemia in a Chinese Miao family

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ABSTRACT

β-thalassemia, one of the most common inherited disorders of hemoglobin synthesis in the world, is genetically heterogeneous with over 200 different β-globin mutations worldwide. In this study, we describe a novel frameshift β-thalassemia mutation at codon (cd) 53 (−T) in exon 2 of the β-globin gene in a Chinese Miao family. In this family, all seven heterozygotes with this mutation presented with moderate anemia, jaundice, splenomegaly and elevated hemoglobin A2 levels. None of them had been transfused or carried any other known α/β-globin mutation. Pedigree analysis indicated an autosomal dominant inheritance pattern in this family. Two new haplotypes "−−−−+−+" and "−−+++−+" were identified by restriction fragment length polymorphism (RFLP) haplotype analysis. The former was associated with the cd53 (−T) mutation and the latter only existed in one family member. Thus, a novel frameshift cd53 (−T) mutation may lead to mild thalassemia intermedia even though there is no statistically significant difference in β-globin messenger RNA (mRNA) level between six heterozygotes and six normal subjects.

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Introduction

β-thalassemia is one of the most common monogenic diseases worldwide. It is generally considered as an autosomal recessive disorder because inheritance of two mutant β-globin genes is necessary to produce significant clinical feature. However, dominantly inherited β-thalassemia caused by a mutant β-globin gene has been found in different ethnic groups such as English, Italian, French, Czech, Spanish, Korean and Japanese [1]. The molecular basis of dominantly inherited β-thalassaemia is usually due to frameshift mutations, missense mutations and nonsense mutations, with a total of 40 causing-mutations previously reported. Among them, 29 are located in exon 3 and the remaining mutations occur at other sites including 7 in exon 2 of the β-globin gene [2–7]. Severity of the disease within this form of thalassemia ranges from a just detectable clinical phenotype to transfusion dependency [2]. β-thalassemia is very common in south China [8], but the mutations in different regions and different ethnic groups have not been elucidated completely, especially in the minor ethnic groups living in remote areas. As a result of population screening for heterozygous traits aimed at prevention of thalassemia major, we identified a novel frameshift mutation at cd53 (−T) in exon 2 of the β-globin gene resulting in dominantly inherited β-thalassemia and two novel RFLP haplotypes in a Chinese Miao family from Guizhou Province in southwestern China. The functional effect of this mutation was accessed at the mRNA level by real-time quantitative reverse-transcript PCR (RT-PCR) technology.

Materials and methods

Subjects and hematological analysis

The proband was a 22-year-old woman who had never been transfused. Splenomegaly and jaundice were noted. The woman and all her family members (53 subjects in three generations) belonged to the Miao population (a minor ethnic group in China) and were aborigines who lived in Guizhou Province in southwestern China. (Fig. 1). The hematological findings are shown in Table 1. β-globin gene mutation analysis of the proband was performed by using PCR-based reverse dot blot (RDB) hybridization technique [9]. None of the 24 known mutations in the Chinese population was found. Therefore, this patient and her 17 family members were referred to our laboratory for further investigation; all gave informed consent. Among the 17 family members, 6 were first and 11 second degree relatives of the proband (Fig. 1 and Table 1). The remaining members were not available for investigation. Blood counts and red cell indices were determined by automated cell counting (Model Sysmex F-820; Sysmex Co Ltd, Kobe, Japan) and the concentration of hemoglobins A, A2, F and any abnormal hemoglobin were assessed by a REP system (Helena Laboratory, Beaumout, Texas, USA).

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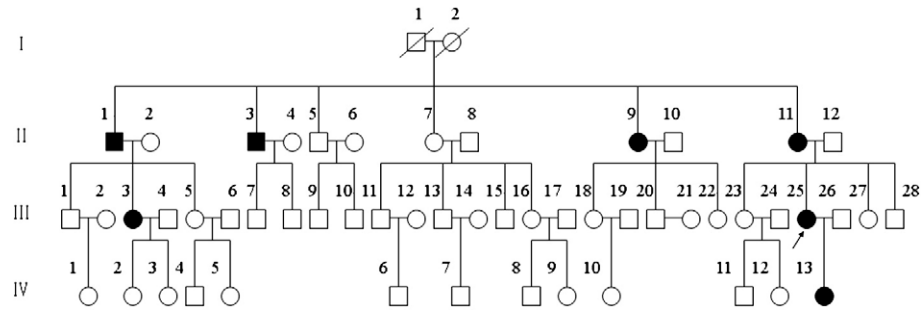

Fig. 1. Family pedigree of the Miao family with dominantly inherited β-thalassemia. The proband III25 is indicated by the arrow. The 18 members investigated are listed in Table 1.

DNA analysis

Genomic DNA was extracted from blood by standard phenol/chloroform method. The 24 known β-globin mutations in Chinese were scanned by RDB hybridization technique [9]. Direct DNA sequencing of the entire human β-globin gene (GenBank Accession NG_000007.3) was performed for identifying the unknown mutation, which is −164 bp upstream of the transcriptional start site to 115 bp downstream of stop codon. The sequences of the primers for amplifying human β-globin gene were: FP1 5′-AACTCCTAAGCCAGTGCCAGAAGAGC-3′ (nt70381–70406, forward) and RP1 5′-ATGCACTGACCTCCCACATTCCCT-3′ (nt71190–72213, reverse). These two primers were also used as sequencing primers. The other two sequencing primers were: FP2 5′-TTGGGGATCT GTCCACTCCTGAT-3′ (nt70861–70883, forward) and RP2 5′-TCAAGCGTCCCATAGACT CAC-3′ (nt71040–71060, reverse). The common α-thalassemia deletional mutations (– –^{SEA}/, –$\alpha^{3.7}$/ and –$\alpha^{4.2}$/) were typed by gap PCR [10], and 6 non-deletional mutations ($\alpha^{cd30}\alpha$/, $\alpha^{cd31}\alpha$/, $\alpha^{cd59}\alpha$/, $\alpha^{QS}\alpha$/, $\alpha^{CS}\alpha$/ and $\alpha^{WS}\alpha$/) were scanned by RDB assay [11] in all 18 samples tested. These nine mutations account for >98% of all α-thalassemia chromosomes in the Chinese population [12].

RNA analysis

QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) was used to isolate total cellular RNA from fresh whole blood. Complementary DNA was synthesized by using ExScript RT reagent kit (TaKaRa Biotechnology, Dalian, China). The gene expression at the mRNA level of both the mutant and wild-type β-globin alleles were measured by using SYBR Green-based relative quantitative RT-PCR method. PCR reactions were run on a Rotor-Gene 3000 quantitative thermal cycler (Corbett Research, Sydney, New South Wales, Australia). Two standard curves' method was used to assess the disparity among all subjects. Signal intensity of selected β-globin gene was normalized to that of β-actin gene. The primers used to amplify these two genes were described previously [13]. Six heterozygous subjects carrying cd53 (−T) mutation were selected as patient group while six normal subjects served as the control group. The standard curve was the Ct value plotted against the log-value of the input mRNA concentration at five tenfold serial dilutions.

Table 1
Hematological data and α/β-globin genotype of the proband and her 17 family members

Family members	Sex	Age (Y)	RBC 10^{12}/l	Hb (g/dl)	MCV (fl)	MCH (pg)	HbA2 %	Genotype	
								β	α
II1	M	57	4.94	9.9	67.8	20.0	5.37	53/N	N/N
II3	M	44	4.70	10.5	75.9	22.3	5.55	53/N	N/N
II5	M	42	4.86	12.4	85.7	25.5	2.78	N/N	N/N
II9	F	52	4.93	9.8	69.8	19.8	4.54	53/N	N/N
II11	F	50	5.54	10.1	63.0	20.0	5.00	53/N	N/N
II12	M	54	5.44	14.6	90.2	26.8	2.50	N/N	N/N
III3	F	30	4.18	8.2	65.0	19.6	5.65	53/N	N/N
III8	M	17	5.13	12.9	84.7	25.0	2.33	N/N	N/N
III9	M	15	4.95	12.0	80.4	24.2	2.39	N/N	N/N
III10	M	10	5.02	12.6	76.3	23.3	2.60	N/N	N/N
III13	M	24	5.08	12.0	85.9	24.8	2.17	N/N	N/N
III18	F	25	5.15	12.2	82.0	23.6	2.17	N/N	N/N
III20	M	23	6.14	11.6	67.0	18.8	2.63	N/N	SEA/N
III25	F	22	5.26	9.9	65.3	18.8	5.19	53/N	N/N
III26	M	22	5.13	13.0	86.2	25.3	2.78	N/N	N/N
III27	F	16	4.89	10.3	71.7	21.0	2.23	N/N	N/N
III28	M	25	5.06	13.6	86.9	26.8	2.56	N/N	N/N
IV13	F	6	6.00	9.7	55.1	16.1	5.48	53/N	N/N

Y years, RBC red blood cell, Hb hemoglobin, MCV mean cell volume, MCH mean cell hemoglobin, M male, F female, N normal, 53 cd53(−T), SEA (– –^{SEA}/) deletional mutation.

β-globin haplotype analysis

The β-globin haplotype associated with the novel mutation in this family was ascertained by PCR-RFLP technique and family linkage analysis. Seven classical polymorphic restriction enzyme sites selected for haplotype analysis were Hinc II-5′ε, Hind III-Gγ, Hind III-Aγ, Hinc II-ψβ, Hinc II-3′ψβ, Ava II-β and Bam HI-3′β. Oligonucleotide primers used for amplification of the regions encompassing seven polymorphic sites were previously described [14].

Statistical analysis

The difference of mean mRNA relative concentration between mutation carriers and normal individuals were analyzed by the independent samples *t* test using statistical software SPSS, version 13.0 (SPSS inc, Chicago, USA).

Results

Table 1 showed the hematological data and α/β-globin genotype of the proband and her family members. The proband (β25) and other 6 family members (II1, II3, II9, II11, III3 and IV13) all corresponded to the β-thalassemia intermedia phenotype, with moderate anemia (Hb levels 8.2–10.5 g/dl), jaundice, splenomegaly and elevated HbA2 levels (4.54–5.65%). Direct DNA sequencing analysis indicated these seven members were all carriers of cd53 (−T) mutation (the representative results showing in Fig. 2). Both β25 and χ13 only had one affected parent, and β-thalassemia intermedia phenotype appeared in every generation. Thus, the disorder was transmitted as an autosomal dominant inheritance in this family. Both β10 and β27 were considered as iron deficiency anemia because of microcytic hypochromic anemia and normal α/β-globin genotype. α-thalassemia deletion of type (– –^{SEA}/) was identified in a family member (β20), which was inherited from his father.

In two step RT-PCR analysis, two standard curves used for the linear quantitation range of mRNA were generated, with $y=-3.804x+27.671$ ($R^2=0.999$) for β-globin mRNA and $y=-3.6x+23.621$

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