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SHORT COMMUNICATION

A Novel 6.3 kb Deletion and the Rare 27.6 kb Deletion Causing α⁺-Thalassemia in Two Chinese Patients

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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A Novel 6.3 kb Deletion and the Rare 27.6 kb Deletion Causing α+-Thalassemia in Two Chinese Patients

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Abstract

We report a novel –α⁶.³ deletion and a rare –α²⁷.⁶ deletion causing α+-thalassemia (α+-thal), in two Chinese patients. One patient was a 35-year-old Chinese man with a mild α+-thal phenotype [mean corpuscular volume (MCV) 83.6 fL] and the Hb A₂ level (2.5%) was close to borderline of the normal range. Multiplex ligation-dependent probe amplification (MLPA) revealed a novel 6344 bp deletion involving the entire HBA1 gene. Mapping by gap-polymerase chain reaction (gap-PCR) defined the exact breakpoint of this deletion to be NG_000006.1: g.31022_37366del6344. It was unique relative to other forms of α-thalassemia (α-thal) reported in the literature, and was designated as –α⁶.³ deletion. The other patient, a 41-year-old woman had Hb H (β⁴) disease [hemoglobin (Hb) level of 8.9 g/dL] with a compound heterozygosity for the –α⁵.⁹SEA deletion. The MLPA and gap-PCR methodologies confirmed the breakpoint (NG_000006.1: g.9079_36718del27640) and identified it as the rare –α²⁷.⁶ deletion.

Keywords α-Thalassemia (α-thal), Chinese, Novel deletion

α-Thalassemia (α-thal) is inherited as an autosomal recessive disorder characterized by microcytic hypochromic anemia, and a clinical phenotype varying from almost asymptomatic to a lethal hemolytic anemia. It is probably the most common monogenic gene disorder in the world, and is especially frequent in tropical and subtropical regions. Most α-thalassemias are due to deletions of one (–α) or both (––) α genes from the α-globin gene cluster (NG_000006.1) located on chromosome 16p13.3 (1). Occasionally, point mutations in critical regions of the α₂ (α⁴.⁷α) or α₁ (αα⁺) genes may cause nondeletional α-thal. The phenotype of the α-thal is directly related to the number of α-globin genes affected. If two α genes are inactive on a single chromosome, the thalassemia genotype is designated as α⁰-thal; if either of the two α genes loses activity, the thalassemia genotype is designated as α⁺-thal. Clinically, heterozygotes for an α⁰-thal mutation showed an α-thal trait phenotype, with reduced mean cell volume (MCV) and mean cell hemoglobin (Hb) (MCH); heterozygotes for an α⁺-thal mutation are usually asymptomatic, but their hematological parameters are close to borderline of the normal range.

In the Chinese population, –α⁵.⁹SEA (NG_000006.1: g.26264_45564del19301) was the most common α⁰-thal genotype, –α⁴.⁷ (rightward) (NG_000006.1: g.34164_37967del3804) and –α⁴.²
(leftward) (AF221717) were the most common α⁺-thal genotypes (2). These three deletions accounted for more than 80.0% of clinical cases identified in the southern regions of China. Until recently, only four kinds of rare α⁺-thal deletions were discovered throughout the world; they included −α²⁷.⁶ (NG_000006.1: g.9079_36718del27640), −α²¹.⁹ (NG_000006.1: g.14373_36299del21927; insGGGAAGGTTGGAATAACAGCTTTT), −α²⁷ (NG_000006.1: g.36664_39364del2701) and −α²⁴ (NG_000006.1: g.36589_39251del2392) (2,3). Here we have identified a novel 6.3 kb (−α⁶.³) (NG_000006.1: g.31022_37366del6344) α⁺-thal deletion and the rare 27.6 kb (−α²⁷.⁶) α⁺-thal deletion in two Chinese families.

Case 1

Patient A, a 35-year-old Chinese man, and his wife attended a pre pregnancy screening for thalassemia at the Shantou Central Hospital, Shantou, Guangdong Province, People’s Republic of China (PRC). He displayed a heterozygous α⁺-thal phenotype with the following hematological parameters: Hb 16.1 g/dL, mean corpuscular volume (MCV) 83.6 fL, mean corpuscular Hb (MCH) 26.4 pg and Hb A₂ 2.5%. Gap-polymerase chain reaction (gap-PCR) and reverse dot-blot assay were used to detect three common deletional α-thal genotypes (−α³.⁷ SEA, −α³.⁷ and −α⁴.²) and three nondeletional α-thal genotypes [Hb Quong Sze (Hb QS) αQS (HBA2: c.377T>C), Hb Constant Spring (Hb CS) αCS (HBA2: c.427T>C) and Hb Westmead (αWestmead) (HBA2: c.369C>G) in this subject as previous reported (2,4). None of above six genotypes was observed. However, it was confusing that the gap-PCR analysis showed a new PCR amplification product of ~2100 bp. Therefore, an unusually large deletion of either the HBA1 or HBA2 genes was suspected. Multiplex ligation-dependent probe amplification (MLPA) was performed using the SALSA MLPA kit, P140-B2 HBA (MRC-Holland, Amsterdam, The Netherlands) to screen for deletions on the α-globin gene cluster. Based on the MLPA result (Figure 1), we identified a reduction in gene copy number from probe 8488-L08410 (NG_000006.1, 30786-30785) to probe 4630-L04011 (NG_000006.1, 37628-37627), which indicates a deletion of about 6.3 kb from the α-globin cluster. Gap-PCR was used to amplify the breakpoint and sequencing with the following primers: 4.2-F (5’-GGT TTA CCC ATG TGG TGC CTC-3’) and 3.7/20.5-R (5’-AAA GCA CTC TAG GGT CCA GCG-3’) (5). The sequencing results (Figure 2) showed that the breakpoints were at 2754 bp upstream of the α1-globin gene translation initiation codon and 2903 bp downstream of the α1-globin gene translation termination codon, with a deletion total of 6344 bp (NG_000006.1: g.31022_37366del6344). In addition, a new fusion gene was detected with an insertion of nucleotide T. To the best of our knowledge, the α-globin chain production is controlled by two genes on each chromosome 16. This novel deletion removed on single α-globin gene (HBA2) and caused deficient α-globin production. Therefore, the carrier showed a mild α⁺-thal phenotype. It was unique relative to other forms of α-thal reported in the literature, and was designated as a −α⁶.³ deletion.

Case 2

Patient B, a 41-year-old Chinese woman from Xiamen City, Fujian Province, PRC, was a medical outpatient for hypochromic microcytic anemia at the Shantou Central Hospital, Shantou, Guangdong Province, PRC. No hepatosplenomegaly, jaundice, iron deficiency or other complications were observed in this patient. The patient was diagnosed with Hb H (β4) disease.
by electrophoresis on cellulose acetate (pH 8.6). The hematological data were as follows: Hb level of 8.3 g/dL, MCV 53.2 fL and MCH 13.4 pg. Hb H inclusion bodies were seen in 92.0% of red cells. According to our previous description (2,4), α-thal genotypes (−SEA, −α^{3.7} and −α^{4.2}) and three nondeletional α-thal genotypes (Hb QS, Hb CS and Hb Westmead) common in the Chinese population were investigated by gap-PCR and reverse dot-blot assay, respectively, and only the common −SEA deletion was identified in the patient, suggesting an unknown defect allele on the other chromosome 16.

The amplification and sequence analyses of the α1- and α2-globin genes were based on our previous report (4). However, only the α1-globin gene could be amplified, implying that the α2-globin gene had been deleted. Result of the MLPA assay (Figure 3) revealed a large compound deletion between probe 4926-L04017 (NG_000006.1, 4528-4529) to probe 6707-L06294 (NG_000006.1, 42032-42033). The complete deletion region is between probe 4625-L04005 (NG_000006.1, 23609-23610) to probe 8497-L08420 (NG_000006.1, 36672-36673). Based on our previous analysis and the result of the MLPA assay, one is the −SEA deletion, another unknown deletion is from probe 4926-L04017 (NG_000006.1, 4528-4529) to probe 8497-L08420 (NG_000006.1, 36672-36673). According to a previous report (6), the exact breakpoints were mapped by a gap-PCR with the primers P1 and P2. A 1.7 kb product was obtained. Compared with the GenBank NG_000006.1 reference sequence, the direct DNA sequencing of the PCR product confirmed a 27,640 bp deletion, with the 5’ breakpoint at 9079 bp and the 3’ breakpoint at 36,718 bp. A search of the literature revealed a report of this −α^{27.6} deletion (NG_000006.1: g.9079_36718del27640) in the Chinese population (6). The clinical phenotype of the case was similar to the previous report (6).

References
Figure legends

Figure 1. Multiplex ligation-dependent probe amplification analyses of patient A showing a probe signal loss pattern consistent with a heterozygous deletion from probe 8488-L08410 (NG_000006.1, 30786-30785) to probe 4630-L04011 (NG_000006.1, 37628-37627), indicating a deletion of about 6.3 kb from the α-globin cluster.

Figure 2. Characterization of the breakpoints of this novel deletion by direct sequencing. A) Sequence analysis of the 2.1 kb amplification product obtained with the gap-PCR. The breakpoints were found to be joined with an insertion sequence of one nucleotide T. B) Schematic diagram showing the two gap-PCR primer sites and the novel deletion of 6.3 kb from NG_000006.1:31022 to NG_000006.1:37366. The primers 4.2-F and 3.7/20.5-R were used for gap-PCR to detect the breakpoints.

Figure 3. Characterization of the breakpoints of the 27.6 kb deletion in Patient B. A) Multiplex ligation-dependent probe amplification analyses in patient B showing a probe signal loss pattern consistent with compound heterozygous deletions (–α^{27.6}/–SEA). B) Schematic diagram showing the compound deletion, one is the rare 27.6 kb deletion (from NG_000006.1:9079 to NG_000006.1:36718), the other is the common ––SEA deletion. The primers P1 and P2 were used for gap-PCR to detect the breakpoints of the 27.6 kb deletion.