



Title	Novel point mutation of the β-2-globin gene (HBA2) and a rare 2.4kb deletion of the β-1-globin gene (HBA1), identified in two chinese patients with Hb H disease
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NOVEL POINT MUTATION of α 2-GLOBIN GENE (*HBA2*) AND A RARE 2.4-KB DELETION OF α 1-GLOBIN GENE (*HBA1*) IDENTIFIED IN TWO CHINESE PATIENTS WITH HEMOGLOBIN H DISEASE

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Running head: Novel and rare α -globin gene mutations in Chinese

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Abstract

Two Chinese patients with mild and moderate hemoglobin (Hb) H disease were investigated for rare mutations in the α -globin genes (*HBA1*, *HBA2*) in addition to --(SEA) deletion. One patient was a 41-year old man with mild anemia (Hb 11.3 g/dl). Multiplex ligation-dependent probe amplification (MLPA) revealed a rare 2392-base deletion involving the entire *HBA1*. Mapping by gap-polymerase chain reaction (PCR) defined the exact breakpoints of this deletion (*HBA1*:g36859_39252del2392) and confirmed its identity with a recently reported *HBA1* deletion found in Southern Chinese. The other patient was a 53-year old man with moderate anemia (Hb 9.5 g/dl). Automated direct nucleotide sequencing identified a novel single nucleotide deletion at codon 40 of *HBA2* (*HBA2*:c123delG). This leads to a frameshift which modifies the C-terminal sequence to (40)Lys-Pro-Thr-Ser-Arg-Thr-Ser-Thr(47)COOH and the introduction of a stop codon TGA 23 nucleotides downstream. These two cases demonstrate the power of MLPA and direct nucleotide sequencing to detect and characterize rare and novel mutations. They also highlight the differential effect of *HBA1* and *HBA2* gene mutation on α thalassemia phenotype due to their different transcriptional activity.

Hemoglobin H disease is typically caused by deletion of both *HBA1* and *HBA2* on one chromosome 16 and either a complete deletion or a point mutation in *HBA1* or *HBA2* on the other homologue. This results in the deletional and non-deletional form of Hb H disease, respectively. In the southern Chinese population, the overwhelming majority of the two-gene deletion in *cis* is --(SEA). Single-gene deletions -alpha(4.2) or -alpha(3.7) are found in deletional Hb H disease, while Hb Constant Spring (CS), Hb Quong Sze (QS) and codon 30 (Δ GAG) deletion predominate in the non-deletional form. More than 90% of Hb H disease can therefore be genotyped by a simple multiplex gap-PCR and allele-specific PCR priming approach targeted at these common mutations (1). In rare instances, these routine techniques fail to reveal the complete α -globin genotype and more empirical genotyping methods are indicated. We describe here the detection of two rare mutations in two patients with Hb H disease.

Patient A was a 53-year old Chinese man with Hb 9.5 g/dL, mean corpuscular volume (MCV) 74.7 fL, mean corpuscular Hb (MCH) 19.3 pg and red cell distribution width (RDW) 21.6%. Supravital staining with brilliant cresyl blue showed 98% of red cells bearing Hb H inclusion bodies. Patient B was a 41-year old Chinese man with Hb 11.3 g/dL, MCV 73.2 fL, MCH 20.4 pg and RDW 22.4%. Hemoglobin H inclusion bodies were seen in 90% of red cells. Routine α -globin genotyping only

detected heterozygous α -(SEA) in both patients. Rare or novel mutations were suspected and automated direct nucleotide sequencing of *HBA1* and *HBA2* was performed as previously described (1). In Patient A, a hemizygous novel single nucleotide deletion was found in codon 40 of *HBA2* (AAG to AA-; HBA2:c123delG), which causes a frameshift and creates a new TGA stop codon 23 nucleotide downstream (Figure 1). This predicts the production of a truncated polypeptide 47-amino acid in length with a carboxyl end Lys-Pro-Thr-Ser-Arg-Thr-Ser-Thr. However, the mutated mRNA is more likely to undergo nonsense-mediated decay without significant translation. No functional globin chain production is therefore expected from this novel mutation. *HBB* sequencing results were normal.

No sequence variation was detected in Patient B by Sanger sequencing of *HBA1*, *HBA2* and *HBB*. An unusual large deletion of *HBA1* or *HBA2* was suspected. Alpha MLPA was performed to screen for deletion in the α -globin gene cluster. Probes and reaction mixture for ligation and PCR were purchased from MRC-Holland (SALSA MLPA kit P140-B3 HBA, MRC-Holland, Amsterdam, the Netherlands). Testing and data analysis procedures were done as previously described (2). Apart from the heterozygous loss of probe products contributed by α -(SEA), which spans from the probe binding site between pseudogenes *HBA2P* and *HBA1P* to the probe binding site 3.7 kb downstream of *HBA1*, there was further loss of products of all probes targeting

from exon 1 of *HBA1* to a site 0.5 kb downstream of *HBA1*. This suggests a deletion of the entire *HBA1* in addition to --(SEA) (Figure 2). The exact breakpoints were mapped by a gap-PCR using the MLPA probes flanking the putative *HBA1* deleted region (alpha MLPA 256 nucleotide probe as forward primer: 5' TTCTCTGCCCAAGGCAGCTTACCC 3'; alpha MLPA 310 nucleotide probe as reverse primer: 5' AGGTGTTTCTTCAGGGCAGTGAAC 3'). A 2.2-kb PCR product was obtained (Figure 3). Direct nucleotide sequencing of this PCR product identified the 5' breakpoint at nucleotide 36859 of NG_000006.1 GenBank reference sequence, which is 684 bp upstream of the start point of *HBA1* whereas the 3' breakpoint lies at nucleotide 39252, which is 867 bp downstream of the end of *HBA1*. Due to sequence micro-homology flanking the breakpoints, alternative breakpoints at nucleotide 36853 and nucleotide 39245 can also generate the same deletion (Figure 4). Results confirmed the presence of a 2392-base deletion of *HBA1*. A search of the literature revealed three reports of this *HBA1* deletion in Chinese (3-5). Simple heterozygotes are reported to have a very mild phenotype, which is consistent with a lesser contribution to globin production by *HBA1* compared with *HBA2*. The mild deletional Hb H disease phenotype observed in our patient is also consistent with this. Notably, all three previously reported Hb H disease patients of this same genotype (compound heterozygous for --SEA and 2.4 kb *HBA1* deletion) had moderate disease severity with

Hb ranging from 8.3 to 9 g/dL. One of them was a teenage female with a very low MCV of 58 fL; the other was a pregnant woman, and the age and clinical details of the remaining patient were not stated. Other contributing factors of Hb level such as age, sex, iron status, physiological hemodilution and concurrent diseases might have affected the phenotype.

These two cases demonstrate the power of direct nucleotide sequencing and MLPA in the detection and characterization of novel and rare thalassemic mutations. Moreover, they illustrate clearly the phenotypic difference between *HBA1* and *HBA2* mutations in the setting of Hb H disease. Finally, the study confirms independently the occurrence of the rare and mild 2.4-kb *HBA1* deletion in Southern Chinese.

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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Figure Legends

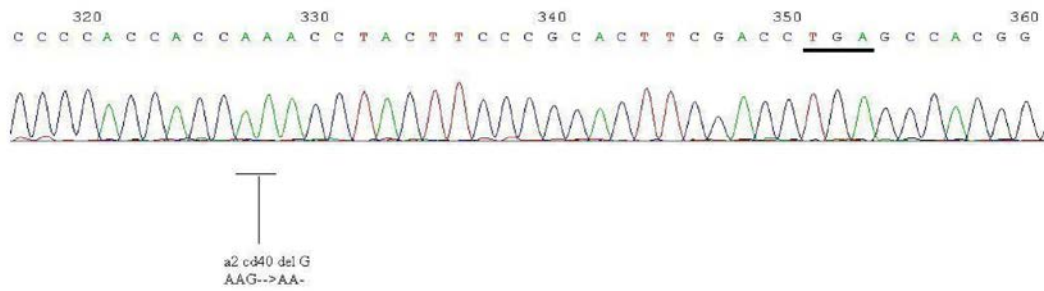


FIGURE 1 Partial **chromatogram** showing a hemizygous *HBA2* sequence tracing in Patient A. A guanine deletion at codon 40 leads to shifting of reading frame and generation of a new TGA stop codon 23 bases downstream (underlined).

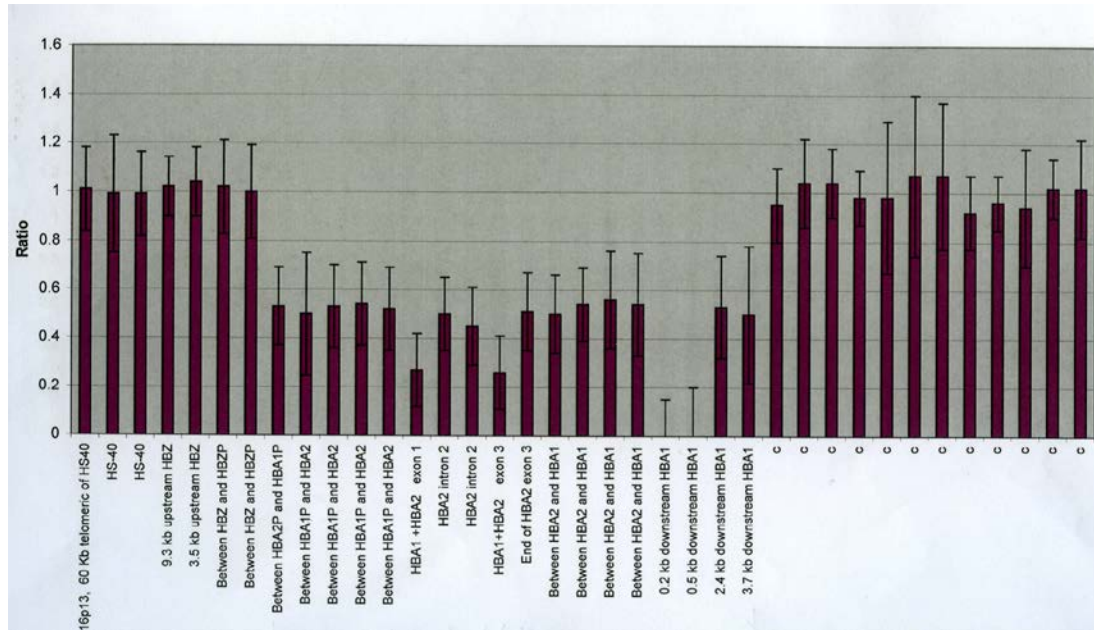


FIGURE 2 Multiplex ligation-dependent probe amplification analysis in Patient B showing a probe signal loss pattern consistent with compound heterozygous $--(SEA)$ and *HBA1* deletion. Note that probe products targeting exon 1 of *HBA1* and *HBA2* are identical and probe products targeting exon 3 of these two genes are identical. A normalized signal ratio of 0.25 for these probes in this case is consistent with a deletion of either *HBA1* or *HBA2* in addition to $--(SEA)$.

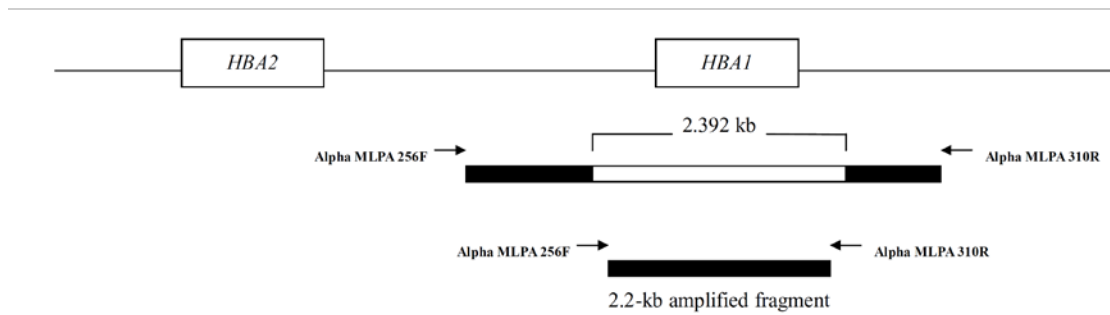


FIGURE 3 Schematic diagram showing the two gap-polymerase chain reaction primer sites (arrows). The rare *HBA1* deletion in Patient B removes 2392 bases (white bar) and allows the generation of a 2.2-kb amplified fragment (black bar) by the primer pair.

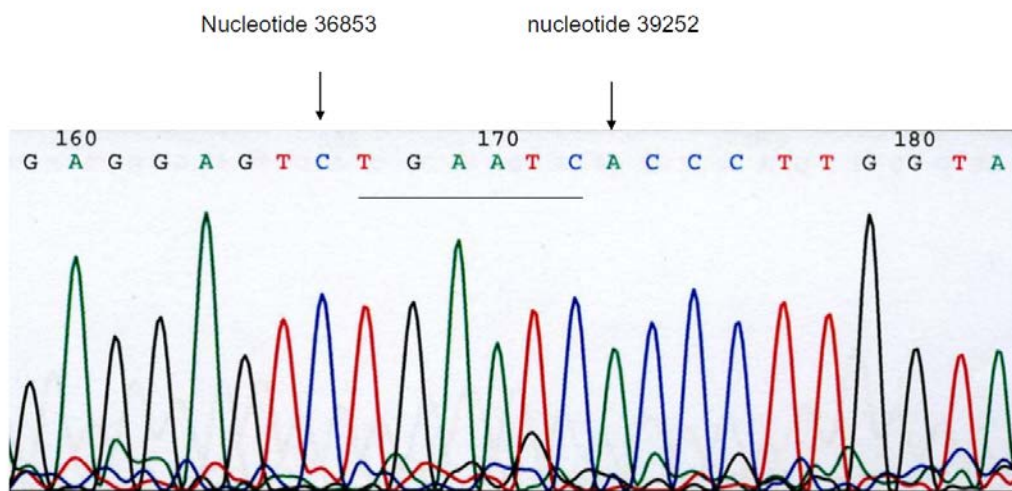


FIGURE 4 Partial chromatogram showing a hemizygous *HBA1* sequence tracing in Patient B. The same six nucleotide TGAATC sequence flanking the breakpoints (underlined) is present at nucleotide 36854 to 36859 and nucleotide 39246 to 39251. This sequence micro-homology may have facilitated interchromosomal or intrachromosomal misalignment and unequal crossing-over. Breaking and rejoining at either end of this short sequence will generate the same deletion.