BRIEF COMMUNICATION

**Hb Wanjiang: A New β-Globin Chain Variant with Two Amino Acid Substitutions (HBB: c.255_264delinsTTTTTCTCAG)**

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**ABSTRACT**

We report a new hemoglobin (Hb) variant that we have named Hb Wanjiang (HBB: c.255_264delinsTTTTTCTCAG). We identified this variant in a Chinese man by the next-generation sequencing (NGS) method. The father of the proband also carried the same variant. This variant results from a 10 bp deletion at codons 84–87 of the β-globin chain, replaced with 10 nucleotides coming from the δ-globin gene at the same position, leading to the substitution of two amino acids in the peptide chain with no change in the β-globin chain length. The heterozygotes had a normal hematological feature with no abnormal Hb variant detectable on capillary electrophoresis (CE) and high performance liquid chromatography (HPLC). The combination of Hb Wanjiang and β-thalassemia (β-thal) was not found to aggravate anemia.

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Globin gene mutations that alter the protein composition instead of the amount of expression are common in humans and produce structurally abnormal globin proteins [1]. These types of defects are mainly composed of missense mutations that cause single amino acid substitutions on the α- or β-globin chains, resulting in abnormal hemoglobin (Hb) variants. Hemoglobin variants are rarely associated with deletions, multiple amino acid substitutions, and stop codon mutations [2]. In this study, we report a new β-globin chain variant, Hb Wanjiang, with a double amino acid substitution at codons 86/87 (HBB: c.255_264delinsTTTTTCTCAG; p.Ala87_Thr88delinsSerGln).

A Chinese couple was screened for thalassemia in the free premarital screening program. The wife had microcytic hypochromic anemia with an enhanced Hb A2 value. The husband had normal hematological parameters and a normal Hb composition detected by capillary electrophoresis (CE) (CapillaryS2: Sebia, Lisses, France) (Supplementary Figure 1). The couple was designated as low risk for giving birth to infants with severe thalassemia syndrome, and no further studies were performed. In the mother’s first trimester of pregnancy, both partners of the couple participated in expanded carrier screening using next-generation sequencing (NGS) for 11 recessive diseases including α- and β-thalassemia (α- and β-thal). Exons of the targeted genes, along with their 10-bp flanking intronic regions were captured using an Agilent Custom Target Enrichment Probe Kit (Agilent Technologies, La Jolla, CA, USA). The amplicons were sequenced on a NovaSeq 6000 instrument (Illumina Inc., San Diego, CA, USA). The resulting reads were mapped to the National Center for Biotechnology Information (NCBI) reference sequence (hg19/GRCh37), and variant calls were made using Genome Analysis Toolkit (GATK) (https://gatk.broadinstitute.org/hc/en-us). As expected, the NGS panel reported a heterozygous codons 41/42 (–TTCT) mutation (HBB: c.126_129delCTTT) in the woman, which was in accordance with her hematological findings. However, the panel reported a variant at HBB: c.255_264delinsTTTTTCTCAG (p.Ala87_Thr88delinsSerGln) in the man (proband) (Supplementary Figure 2) that was classified as a variant of uncertain significance (VOUS) based on the American College of Medical Genetics and Genomics (ACMG) classification guidelines. The two amino acid substitution does not seem to create a new splice site or change the structure of the Hb molecule according to commonly used variant effect prediction tools. The 10-bp deletion in the second exon of the β-globin gene was replaced with an additional 10 nucleotides, resulting in the substitution of two amino acids in the peptide chain with no change in the β-globin chain length. Multiplex ligation-dependent probe amplification (MLPA) with ProbeMix P102 HBB D1-0818 (MRC Holland, Amsterdam, The Netherlands) did not
detect dosage alteration in the β-globin gene clusters (Supplementary Figure 3). No abnormal peaks were demonstrated on the high performance liquid chromatography (HPLC) (Bio-Rad Laboratories, Hercules, CA, USA) using the proband’s hemolysate.

To explore the possible consequence of this mutation, the husband’s parents were tested (Table 1). Using Sanger sequencing, we found that the husband’s β-globin gene allele was inherited from his father. Like him, the father had normal hematological parameters and an Hb profile (Supplementary Figure 4). Using the NCBI BLAST sequence similarity search tool, we found that the insertion sequence of ‘TTTTTCTCAG’ came from the same position of the δ-globin (Figure 1). DNA sequencing of the δ-globin gene showed a wild-type sequence in both the husband and his father. Based on the hematological and molecular findings, this novel mutation seemed to produce a silent Hb variant that might move to the same position as Hb A and was not detectable on CE. This variant was named Hb Wanjiang after the place of residence of the proband. Although the wife was a β°-thal carrier, it could be assumed that the combination of these two alleles would not cause severe consequences. The pregnancy continued to term. The cord blood at delivery showed a normal Hb profile (Figure 2). A molecular study confirmed that the boy had inherited both mutant alleles from his parents. On a follow-up visit at the age of 12 months, the boy was healthy with a hematologic feature of the β-thal trait.

In this study, we present a fusion allele of unequal recombination events at the β-globin locus. The high sequence homology between δ- and β-globin genes favors these events. For example, Hb Lepore, a δβ variant, contains a hybrid globin chain with a δ amino terminus and a β-carboxyl terminus. Several Hb Lepore variants have been described with different crossover breakpoints (HbVar database: http://globin.cse.psu.edu); of these, Hb Lepore Boston–Washington is the most commonly reported Lepore...

### Table 1. Summary of the hematological profiles and molecular findings of the studied family.

| Parameters                  | Proband | Father | Mother |
|-----------------------------|---------|--------|--------|
| Sex-age (years)             | M-32    | M-64   | F-62   |
| Hb (g/dL)                   | 16.3    | 15.2   | 12.7   |
| RBC (10^12/L)               | 5.70    | 5.07   | 3.96   |
| MCV (fl)                    | 84.8    | 88.2   | 90.1   |
| MCH (pg)                    | 28.5    | 29.3   | 30.1   |
| MCHC (g/dL)                 | 33.6    | 33.0   | 33.3   |
| Hb A (%)                    | 96.3    | 97.1   | 97.2   |
| Hb A2 (%)                   | 3.0     | 2.9    | 2.8    |
| Heinz bodies                | [-]     | [-]    | [-]    |
| Stability test              | [-]     | [-]    | [-]    |
| α Genotype                  | αα/αα   | αα/αα  | αα/αα  |
| β Genotype                  | ββ/wanjiang/βA | ββ/wanjiang/βA | ββ°/βA |

Hb: hemoglobin; RBC: red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; MCHC: mean corpuscular Hb concentration.

Figure 1. The mechanism underlying the formation of Hb Wanjiang with two consecutive homologous recombinations. First crossover occurring at Block 1 between β and δ genes generates a βδ fusion allele and a δβ fusion allele. Second crossover occurring at Block 2 between the βδ fusion allele and β gene generates the βδβ fusion allele (Wanjiang variant).
variant. In all Lepore variants, carriers present with a β-thalassemic trait phenotype with microcytosis and hypochromia, suggesting the reduced synthesis of the δβ hybrid chain [3]. In the present case, no abnormalities were observed by hematological studies and by MLPA, suggesting that it is not a Lepore or anti-Lepore variant. Because of two homologous sequences adjoining the deletion of the β-globin gene, we suspect that two consecutive recombinations occur in gametogenesis and lead to the formation of the Hb Wanjiang allele (Figure 1). However, considering that the probability of a double cross-linking of the HBD and HBB genes in a region that covers a small stretch is extremely low, Hb Wanjiang most likely results from a cross-linking between a fusion gene (e.g., anti-Lepore type) and an HBB gene occurred in an ancestor. Similarly, a complex fusion variant, Hb Palencia, has been reported [4]. This fusion allele comprises two 3’ and 5’ untranslated region (UTR) ends formed via the HBB gene and a 196bp internal fragment of the HBD gene (βδβ). The carrier showed an Hb variant at the Hb D zone (44.6%) on CE with normal hematological parameters.

Although two amino acids at codons 86/87 are substituted, Hb Wanjiang was not detected by either CE or HPLC. However, other methods such as mass spectrometry and isoelectric focusing were not available for further attempts. Five variants with amino acid substitutions at codon 86 of the β-globin chain and four variants at codon 87 have been reported, and none of those exhibits thalassemic or unstable effects [5–12]. The prevalence of Hb Wanjiang is unknown because of its low red blood cell indices that will be missed by conventional thalassemia screening strategies. Recently, newer screening modalities such as genetic carrier screening based on NGS have become available for preconception or prenatal patients [13–15]. We can expect that rarer Hb variants will be disclosed, especially those with no notable hematological changes.

Figure 2. Capillary electrophoresis of cord blood.

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

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