Uncommon Combination of Hemoglobin Jax and Hemoglobin Constant Spring Leading to Microcytic Anemia

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Patient: Female, 59-year-old
Final Diagnosis: Compound heterozygosity of hemoglobin Jax and hemoglobin Constant Spring
Symptoms: Anemia
Clinical Procedure: Hematology
Objective: Rare disease
Background: Thalassemia and hemoglobin (Hb) variants are the most common hereditary red blood cell disorders worldwide. Alpha-thalassemia and alpha-globin variants are caused by mutations of the alpha-globin genes (HBA2 and HBA1), resulting in impaired alpha-globin production and structurally abnormal globin, respectively. Clinical severity of alpha-thalassemia correlates with the number of affected alpha-globin genes, yielding a spectrum of clinical manifestations from mild to severe anemia. Routine diagnosis involves Hb analysis and PCR-based methods, yet identifying rare variants necessitates comprehensive clinical and hematologic laboratory data. The knowledge of phenotype and genotype correlation is useful for genetic counseling and treatment planning.

Case Report: A 59-year-old Thai woman presented with chronic anemia. Her baseline Hb level ranged between 8.0 and 9.0 g/dL, with no history of transfusion. Physical examination showed mild pallor, without enlarged liver and spleen. Laboratory investigations showed microcytic, hypochromic anemia and abnormal Hb peak by Hb analysis (retention time 4.58 min by HPLC method). Common alpha-globin gene deletions, including the Southeast-Asian/Thai 3.7 kb and 4.2 kb deletions were tested using gap-PCR, with none of these deletions detected. Direct DNA sequencing revealed a compound heterozygosity of Hb Jax (HBA2: c.44G>C) and Hb Constant Spring (HBA2: c.427T>C).

Conclusions: Compound heterozygosity of Hb Jax and Hb Constant Spring results in microcytic anemia. Hb Jax can be identified by Hb analysis, and diagnosis can be confirmed by direct DNA sequencing method. Coinheritance of Hb Jax and alpha-globin variants should be considered in cases with microcytic anemia and a specific Hb peak seen in Hb chromatogram.

Keywords: Hemoglobin Constant Spring • alpha-Thalassemia • Hemoglobinopathies

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Introduction

Thalassemia and hemoglobin (Hb) variants represent the most prevalent hereditary red blood cell disorders worldwide, with a notable prevalence in regions previously endemic for malaria [1]. Thalassemia and Hb variants are caused by mutations of globin genes, resulting in impaired production or structurally abnormal Hb, respectively [2]. Alpha-thalassemia and alpha-globin variants are caused by mutations of the alpha-globin genes (HBA2 and HBA1) located on chromosome 16. Large deletions, involving one or both alpha-globin alleles on a single chromosome, constitute the most common mutation type. Less frequently observed are non-deletional mutations, typically giving rise to Hb variants characterized by abnormal structure or impaired production/stability of alpha-globin. The clinical severity of alpha-thalassemia correlates with the number of affected alpha-globin genes, yielding a spectrum of clinical manifestations from mild to severe anemia [3,4].

The standard diagnostic approach for alpha-thalassemia and alpha-globin variants involves Hb analysis and PCR-based methods. However, the identification of rare variants requires comprehensive clinical and hematologic laboratory data [5]. We describe a patient with microcytic anemia caused by compound heterozygosity of a rare alpha-globin variant, Hb Jax and Hb Constant Spring. This information contributes to the understanding of such cases and will be useful for genetic counseling and planning of treatment.

Case Report

A 59-year-old Thai woman was referred to our clinic for evaluation of unexplained microcytic anemia. Her baseline Hb level ranged between 8.0 and 9.0 g/dL. She denied any history of prior transfusions, abdominal heaviness, or pain, and her daily life activities remained unaffected by the anemia. Physical examinations revealed moderate pallor, without jaundice. No other significant clinical findings, such as organomegaly or thalassemic bone changes, were observed. Her son had previously received a diagnosis of microcytic anemia, although the definitive cause had not been confirmed. Other family members reported no health complaints and denied a history of blood transfusion.

The results of the laboratory investigations are presented in Table 1. Additionally, a peripheral blood smear demonstrated hypochromic microcytic red blood cells, anisocytosis, poikilocytosis, basophilic stippling, and increased polychromasia. Hb analysis was conducted using high-pressure liquid column chromatography (HPLC) with the Variant II HPLC system (Bio-Rad Laboratories, CA, USA), following the manufacturer’s recommendations. The results are illustrated in Figure 1, indicating an Hb pattern of AA with an abnormal Hb peak at a retention time of 4.58 min. Hb F was measured at 0.8%, Hb A2 at 1.7%, and abnormal Hb at 4.8%.

Common alpha-globin gene deletions, including the Southeast-Asian/Thai 3.7 kb and 4.2 kb deletions were tested using gap-polymerase chain reaction [6], with none of these deletions

| Table 1. Hematologic characteristics and genotype of the reported patient. |
|-----------------------------|-----------------------------|
| **Characteristics**         | **Results**                 |
| RBC (×10⁶ cells/µL)         | 3.63                        |
| Hb (g/dL)                   | 8.4                         |
| MCV (fl)                    | 78.8                        |
| MCH (pg)                    | 23.1                        |
| MCHC (g/dL)                 | 29.4                        |
| RDW (%)                     | 16.6                        |
| RBC (×10⁶ cells/µL)         | 212                         |
| Hemoglobin analysis         | AA, abnormal Hb at retention time 4.58 min |
|                            | Hb F 0.8, Hb A2 1.7, abnormal Hb 4.8%   |
| Alpha-globin gene mutations | HBA2: c.44G>C and HBA2: c.427T>C    |
| Genotype                    | α一位/α二位                            |

CS – Constant Spring; Hb – hemoglobin; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; MCV – mean corpuscular volume; N/A – not available; RBC – red blood cell; RDW – red cell distribution width.
detected. Given the presence of an abnormal Hb peak identified during Hb analysis, further Sanger DNA sequencing of alpha-globin genes (HBA2 and HBA1) was undertaken. The sequencing revealed compound heterozygosity for Hb Jax (HBA2: c.44G>C) and Hb Constant Spring (HBA2: c.427T>C), as depicted in Figure 2.

Discussion

This case report illustrates the clinical presentation of chronic microcytic anemia resulting from a rare alpha-globin variant, Hb Jax, co-inherited with a common unstable alpha-globin variant, Hb Constant Spring, in a Thai woman. The identification of an abnormal Hb peak through Hb analysis served as a crucial diagnostic clue, and the definitive diagnosis was subsequently confirmed through Sanger DNA sequencing.

More than 70 non-deletional alpha-globin gene mutations have been reported [7]. Changes include single nucleotide substitutions, small insertions, or deletions. They give rise to Hb variants with modified properties, leading to effects that span from clinically insignificant to severe anemia [4,8]. Additionally, the clinical severity can be influenced by the interaction between the alpha- and beta-globin proteins.

Hb Jax arises from a single nucleotide substitution in exon 1 of the HBA2 gene (HBA2: c.44G>C), resulting in an amino acid change (p.Trp15Ser). Initially described by Hoyer on the HbVar: A database of Human Hemoglobin Variants and Thalassemias website in 2022, Hb Jax was identified in an adult male with a history of deep vein thrombosis. Laboratory investigations revealed a normal Hb level of 13.5 g/dL, microcytosis with mean corpuscular volume of 76.6 fl, and an abnormal Hb peak with
a low, broad, elevated baseline at a retention time of 4.85 min. Isoelectric focusing and capillary electrophoresis showed no separation from Hb A. Hb Jax showed relative stability when tested by heat stability and isopropanol precipitation tests [9].

Hb Constant Spring is one of the most prevalent alpha-globin variants in the Southeast Asian population. This abnormal Hb results from a point mutation (TAAcCAA) at the termination codon of HBA2, leading to an elongated and unstable mRNA and subsequently unstable Hb [10,11]. The presence of Hb Constant Spring is typically challenging to detect, often registering at only 1% to 2% in heterozygous cases, owing to the inherent instability of the mRNA. Conventional Hb analysis can overlook the diagnosis of heterozygous Hb Constant Spring [12,13]. Therefore, using a higher sensitivity method, such as capillary electrophoresis, can provide potential benefits in areas with a high prevalence of Hb Constant Spring. Additionally, molecular analysis for the detection of Hb Constant Spring should be performed as a form of diagnostic confirmation [14,15]. In our case, the Hb Constant Spring peak was not identifiable and was potentially obscured by the presence of the Hb Jax peak, which shares the same retention time.

Generally, individuals with heterozygous Hb Constant Spring are asymptomatic and exhibit normal Hb levels, without laboratory indicators of hemolytic anemia [16]. The presence of heterozygous alpha-globin variants typically has a benign impact and does not significantly affect hematological parameters. However, when hemoglobinopathies are combined, this can lead to clinical significance [2,4]. For instance, individuals with homozygous Hb Constant Spring can exhibit some degree of microcytic anemia, with an average Hb level and mean corpuscular volume of 9.9±0.8 g/dL and 82.8±8.5 fl, respectively [12].

Coinheritance of Hb Constant Spring with other alpha-globin Hb variants, such as Hb Pakse, Hb Adana, Hb Q-Thailand, and Hb Quong Sze, have been reported in association with varying degrees of hemolytic anemia [17-20]. In light of this, it is plausible to attribute the microcytic anemia observed in our patient to a combination of Hb Jax and Hb Constant Spring. This report underscores the importance of not diagnosing unusual cases of microcytic anemia through conventional testing alone. Instead, it emphasizes the necessity of correlating findings with the patient’s clinical context and other laboratory results.

**Conclusions**

This is the first report of compound heterozygosity of Hb Jax and Hb Constant Spring in a Thai woman who presented with chronic microcytic anemia. Hb Jax can be identified by Hb analysis, and the diagnosis can be confirmed by direct DNA sequencing method. Coinheritance of Hb Jax and alpha-globin variant should be considered in cases with microcytic anemia and a specific Hb peak seen in Hb chromatogram.

**Ethics Statement**

The study protocol was approved by the Institutional Ethics Committee of Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (MED-2566-0597).

**Declaration of Figures’ Authenticity**

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**References:**

