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CASE REPORT

Hb Guigang [α90 (FG2)Lys→Asn; *HBA1*:c.273G>T]: a Novel α-Globin Chain Variant

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SUMMARY

Background: **New hemoglobin (Hb) variants are constantly being updated as assays are developed and the testing population expands. Here, we report a novel Hb variant, named Hb Guigang.**
Methods: **Hemoglobin (Hb) analysis was analyzed by capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Glycated hemoglobin was performed by CE and HPLC. Routine genetic analysis was done with Gap-PCR and PCR-reverse dot-blot hybridization. The hemoglobin variant was identified by Sanger sequencing.**
Results: **CE of three cases showed the presence of Hb variants in Zone 5 and Zone 12, respectively. HPLC indicated an elevated P3 peak, suggesting the possible presence of the Hb variant. Hb A_{1c} was measured by CE and HPLC, and the results were 6.7% and 4.76%, respectively. Sanger sequencing confirmed an AAG>AAT mutation at codon 90 of the *HBA1* gene. This mutation was reported for the first time, and we named it Hb Guigang based on the proband's place of residence.**
Conclusions: **Hb Guigang with normal hematological parameters was separated and quantified by CE, whereas HPLC suggested that Hb Guigang co-eluted with the P3 peaks and could not be quantified.**
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KEYWORDS

Hb Guigang, variant, capillary electrophoresis (CE), high-performance liquid chromatography (HPLC)

INTRODUCTION

Hemoglobinopathies are the most common monogenic disorders worldwide, including thalassemia and hemoglobin (Hb) variants [1,2]. Hb variant is an amino acid mutation of hemoglobin, and more than 1,300 types have been described in the HbVar and IthaGenes databases. About 20% of Hb variants have hemolytic anemia, polycythemia, or methemoglobinemia symptoms; the other 80% of cases are asymptomatic [3]. With the wide spread use of CE and HPLC, more and more new Hb variants are being found in glycated hemoglobin testing and hemoglobinopathy screening [4,5]. Here, we described a novel Hb variant in three Chinese discover-

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ed through thalassemia screening using CE.

CASE REPORT

The proband of this investigation, a 30-year-old Chinese female living in Guigang of Guangxi Zhuang Autonomous Region, was referred to our hospital for a thalassemia screen. Her complete blood count (CBC) included: hemoglobin 11.60 g/dL (reference: 11.50 - 16.00 g/dL), mean corpuscular volume (MCV) 96.5 fL (reference: 82.0 - 100.0 fL), and mean corpuscular Hb (MCH) 31.4 pg (reference: 27.0 - 31.0 pg) (Sysmex XN 2800; Sysmex Corporation, Kobe, Japan). The hematological parameters of the other patients are shown in Table 1. Her fasting blood glucose was 5.32 mmol/L (reference: 3.90 - 6.11 mmol/L).

Hb analysis for the proband by CE showed the presence of Hb variants in Zone 5 and Zone 12 (Figure 1A) (CapillaryS2 Flex Piercing; Sebia, Lisses, Paris, France). Chromatographic analysis was done by HPLC with the β-thalassemia short program (VARIANT II™; Bio-Rad, Hercules, CA, USA). HPLC indicated an elevated P3 peak, suggesting the possible presence of the Hb variant (Figure 2B). Glycated Hb was determined in the proband by CE and HPLC (D-100; Bio-Rad, Hercules, CA, USA). CE showed an Hb A_{1c} value of 6.7% and a Hb variant, but the blood sample was stored in a refrigerated fridge for over ten days (Figure 1B). Hb A_{1c} was measured at 4.76% with an unknown peak in the P3 window, which often indicates the presence of a suspected Hb variant when the value is elevated (Figure 2A). The results of Hb analysis in the other patients are shown in Table 1.

Due to the reduced Hb A_2 value, α-thalassemia was detected by Gap-PCR and PCR-reverse dot-blot hybridization (Yaneng Biosciences, Shenzhen, Guangdong, China). No mutations were observed in these three patients. The Hb variant was identified by Sanger sequencing on the ABI 3500 XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The Sanger sequencing confirmed an AAG＞AAT mutation at codon 90 of the *HBA1* gene, resulting in a missense variant with asparagine instead of lysine.

DISCUSSION

In this present study, we detected the same mutation in three patients. After consulting the literature and databases (HbVar and IthaGenes), it is the first Hb variant at codon 90 of the *HBA1* gene and changes the protein residue lysine to asparagine at position α90 (FG2) Lys>Asn. We named this variant Hb Guigang [α90 (FG2)Lys→Asn; *HBA1*:c.273G>T] after the city where the proband lives. Subsequently, this variant data was uploaded to the IthaGenes database, and a registration ID 3378 was obtained.

Hb Guigang was separated from Hb A and electrophoresed in Zone 12, accompanied by Hb Guigang-A2 in Zone 5. However, the HPLC chromatogram suggested that the Hb Guigang overlapped with the P3 peak and could not be separated and quantified. Although HPLC also indicated the presence of variants, the detection of variants like this one was more advantageous with CE than HPLC. To date, eight variants have been reported in codon 90 of the α-globin gene: Hb Luocheng [α90 (FG2)Lys>Gln; *HBA1*:c.271A>C], Hb Clinico Madrid II [α90(FG2)Lys>Arg; *HBA1*:c.272A>G], Hb J-Rajappen [α90(FG2)Lys>Thr; *HBA1*:c.272A>C], Hb J-Broussais [α90(FG2)Lys>Asn; *HBA1*:c.273G>C], Hb Sudbury [α90(FG2)Lys>Glu; *HBA1*:c.271A>G or (*HBA2)*], Hb Handa [α90(FG2)Lys>Met; *HBA1*:c.272A>Tor (*HBA2)*] (Hb Munakata), Hb Clinico Madrid [α90(FG2) Lys>Arg; *HBA2*:c.272A>G], and Hb Bergerac [α90 (FG2)Lys>Gln; *HBA2*:c.271A>C]. All these variants were discovered by CE or HPLC methods, as the mobility of Hb is changed when a positively charged polar amino acid, like lysine, is replaced with a neutral or negatively charged one.

It is well recognized that erroneous Hb A_{1c} readings can be found in several hematologic disorders, and the Hb variant is one of these disorders [6]. The primary cause of inaccurate Hb A_{1c} levels in individuals with the Hb variant is a variation in mobility as detected by HPLC [7]. In this study, as shown in Figure 1B, the glycated Hb mode isolated Hb Guigang on CE, while it continued to co-elute with Hb Guigang and the P3 peak on HPLC (Figure 2A). In addition, the HPLC determined that the values of Hb A_{1c} were lower than FBG in the proband, while CE was higher than FBG. This was because the variant interferes with the results of the HPLC assay, reducing the Hb A_{1c} values, whereas the blood sample for the CE analysis is stored blood with degradation Hb A_0. Either HPLC or CE methods are unreliable when the patient is a carrier of the Hb variant, and the assay needs to be changed to an immunoassay or other indicators. Laboratory technicians should also be careful to observe the chromatograms or electrophoretic profiles and provide notes and recommendations on the report.

The hematological findings of three individuals were normal, demonstrating that the Hb Guigang was a benign mutation. Therefore, no prenatal diagnosis is required for couples with this mutation. However, the Hb Guigang interferes with Hb A_{1c} measurements, and clinicians should be concerned about the diagnosis of diabetes and monitoring of therapy in patients with this variant.

CONCLUSION

Hb Guigang is a novel α-globin chain variant with no clinical symptoms. The variant detection and quantification were done by CE but not by HPLC.

Table 1. Hematological and molecular results of Hb Guigang.

Parameters	Proband	Case 2	Case 3
Gender/age (years)	F/30	M/3	M/41
Hb (g/dL)	11.6	11.9	15.9
MCV (fL)	96.5	84.6	85.3
MCH (pg) CE	31.4	27.0	28.9
Hb A (%)	75.1	74.5	75.5
Hb Guigang (%)	22.5	22.3	22.1
Hb A2 (%)	2.1	2.1	2.0
Hb Guigang-A2 (%)	0.3	0.4	0.4
HPLC			
Hb A0 (%)	71.4	68.2	N
Hb Guigang + P3 (%)	20.4	23.8	N
Hb A2 (%)	2.3	1.7	N
α-globin Genotype	$\alpha\alpha^{Guigang}/\alpha\alpha$	$\alpha\alpha^{Guigang}/\alpha\alpha$	$\alpha\alpha^{Guigang}/\alpha\alpha$

N - No detection.

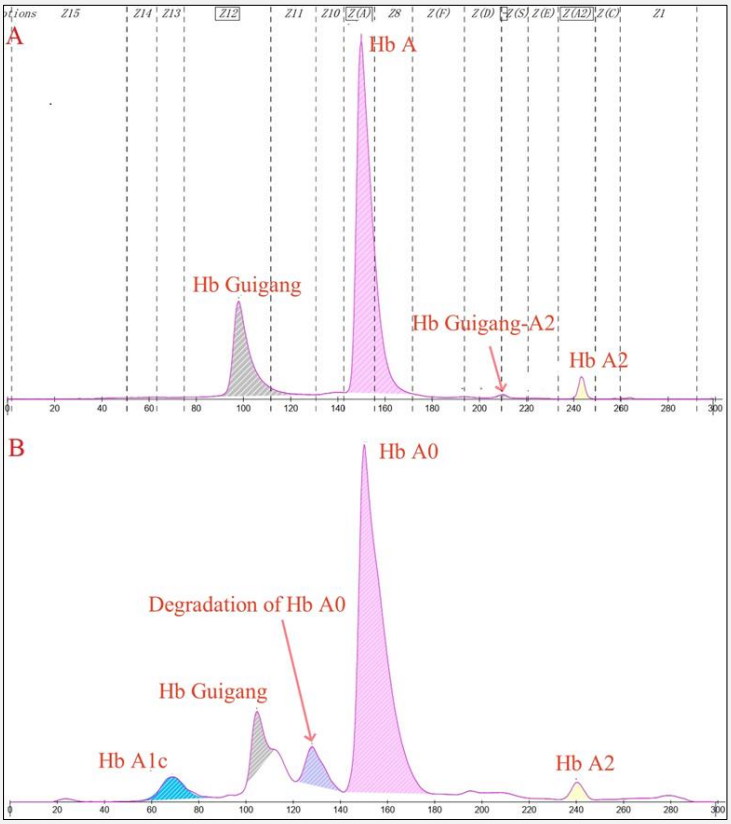

Figure 1. Analysis of the proband with Hb Guigang using CE different modes.

A - Hb analysis, B - Glycated Hb measurement.

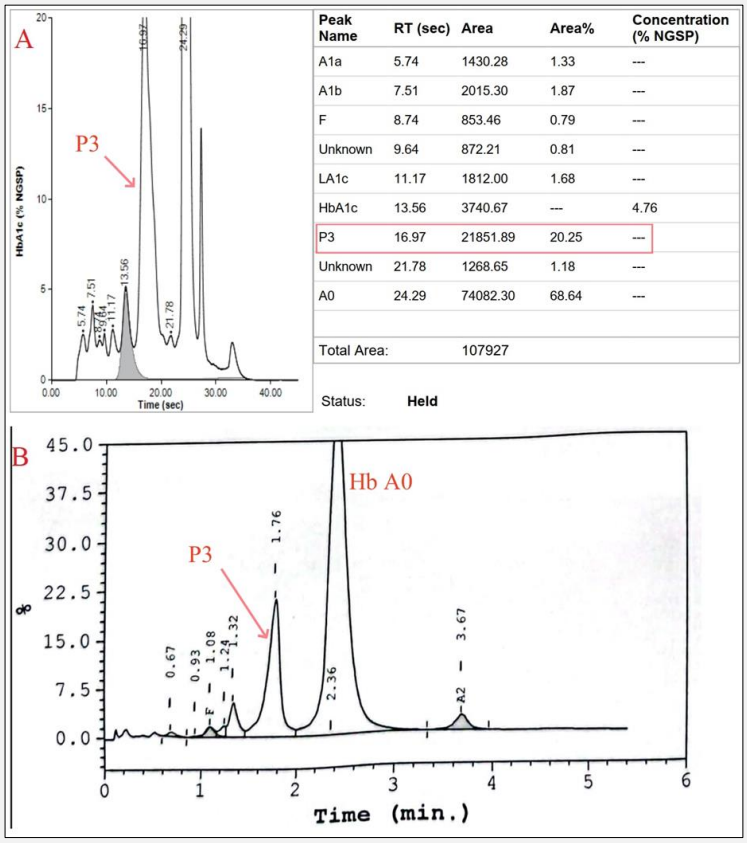

Figure 2. Analysis of the proband with Hb Guigang using HPLC different modes.

A - Glycated Hb measurement, B - Hb analysis.

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Declaration of Interest:
The authors report no conflicts of interest relevant to this article.

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