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SHORT COMMUNICATION

A Novel α-Globin Chain Variant, Hb Nanchang [*HBA2*: c.46G>A, Codon 15 (*GGT*>*AGT*) (Gly→Ser)], Detected by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

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ABSTRACT

Here we report a new α chain variant accidentally discovered during Hb A_{1c} measurement by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) that revealed the presence of a variant α chain with a mass of 15155 Da. However, this hemoglobin (Hb) variant cannot be detected by the first-line methods such as cation exchange high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Sanger sequencing confirmed the presence of a heterozygous missense mutation [*HBA2*: c.46G>A, codon 15 (*GGT*>*AGT*), (Gly→Ser)]. The theoretical mass difference (30 Da) due to the substitution of amino acid glycine to serine matched the actual measured mass difference (29 Da). As this is the first report of the mutation, we named it Hb Nanchang after the place of residence of the proband.

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Hemoglobin (Hb) variants, characterized by the abnormal structure of one or more globin chains, are the most common inherited monogenic red blood cell disorders. More than 1800 hemoglobinopathies (including thalassemias and Hb variants) have been described [1,2]. Cation exchange high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are first-line screening methods to quantify various Hb variants. The development of matrix-assisted laser desorption ionization (MALDI) made it possible to use mass spectrometry (MS) to detect intact globin chains, and MALDI-TOF MS can discriminate the variant globin chains from the normal ones by the mass differences between them [3]. Here we report a new Hb variant accidentally discovered during the measurement of Hb A_{1c} by MALDI-TOF MS.

The proband is a 33-year-old Chinese woman from Nanchang of Jiangxi Province, People's Republic of China (PRC) who visited our hospital for an annual medical checkup. Her fasting glucose concentration was 5.0 mmol/L, and her Hb A_{1c}, initially measured by the CapillaryS3 TERA (Hb A_{1c} procedure; Sebia, Lisses, France), was 5.4% (36 mmol/mol, reference interval 4.0–6.0%) with a normal electropherogram. At that time, we were evaluating the performance of a MALDI-TOF MS system (QuanTOF; Intelligene Biosystems, Qingdao, Shandong, PRC) to assay Hb A_{1c}, and whole blood from the proband was included as one of the samples evaluated. However, we found a variant globin chain in the mass spectrogram of the sample. As shown in Figure 1(A), QuanTOF detected the intact α chain (15126 Da) and β chain (15868 Da), and the corresponding glycated α chain (15,289 Da) and β chain (16,031 Da). Remarkably, a variant chain (15156 Da) was present on the right side of the normal α chain [Figure 1(B)]. QuanTOF yielded Hb A_{1c} values of 5.1% (31.0 mmol/mol) through traditional β chain glycation and 6.8% (51 mmol/mol) through α chain glycation. The Ethics Committee of Peking University Shenzhen Hospital approved this study and we obtained informed consent from the proband.

Next, Hb analysis by the CapillaryS3 TERA (Sebia) [Figure 2(A)], and the β-Thalassemia Short program (VARIANT II^{TM}; Bio-Rad Laboratories, Hercules, CA, USA) [Figure 2(B)], showed no evidence of a Hb variant, along with normal Hb A_2 [2.4% for CapillaryS3 TERA (Sebia) and 2.5% for VARIANT II^{TM} (Bio-Rad Laboratories)] and Hb F. Sanger sequencing revealed a heterozygous mutation (*HBA2*: c.46G>A) at nucleotide 131 (forward primer) [Figure 3(A)] or nucleotide 367 (reverse primer) [Figure 3(B)] on the *HBA2* gene, resulting in a substitution of glycine (molecular weight: 75 Da) to serine (molecular weight: 105 Da) at codon 15. No missense mutations were seen on the *HBA2* and *HBB* genes when compared with the standard sequences. To the best of our knowledge, this mutation has not yet been reported, and we named it Hb Nanchang after the birthplace of the proband. Her hematological parameters were as follows: red blood cell (RBC) count 4.54×10^{12}/L (reference interval 3.8–5.1 $\times 10^{12}$/L), Hb 14.5 g/dL (reference interval 11.5–15.0 g/dL), mean corpuscular volume (MCV) 91.2 fL (reference interval 82.0–100.0 fL), and mean corpuscular Hb

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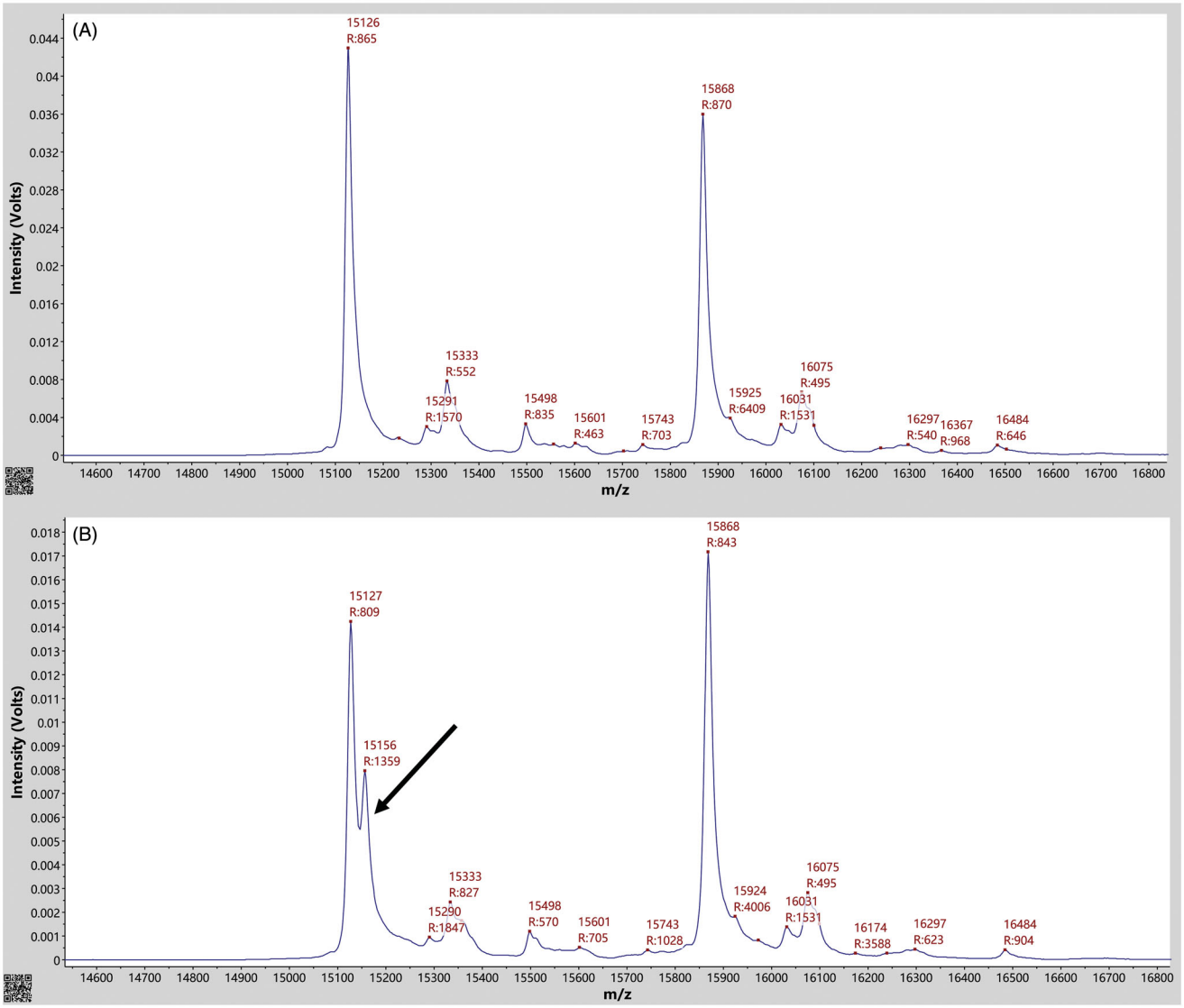
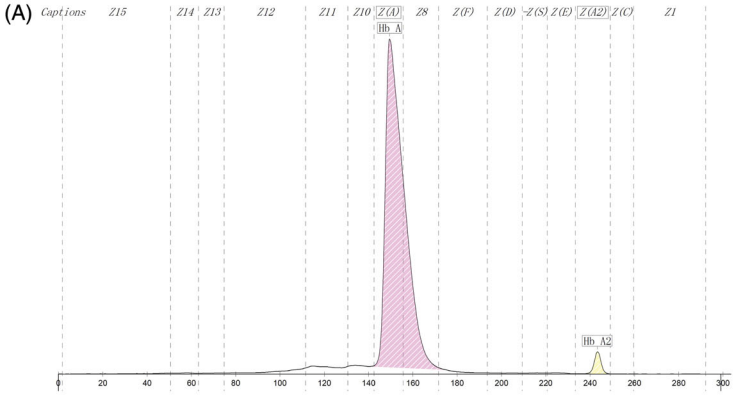


Figure 1. The MALDI-TOF MS of control and Hb Nanchang. (A) Mass spectrogram of control showed α chain (15,126 Da) and β chain (15,868 Da), and the corresponding glycated α chain (15,289 Da) and glycated β chain (16,031 Da). (B) The arrow indicates the variant α chain peak (15,156 Da).



Haemoglobin Electrophoresis

Name	%	Normal Values %
Hb A	**97.6**	96.8 - 97.8
Hb A2	**2.4**	2.2 - 3.2

(B)

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.99	1514
F	0.3	---	1.07	5315
Unknown	---	1.3	1.21	28079
P2	---	4.3	1.33	91025
P3	---	6.0	1.73	126019
Ao	---	85.8	2.40	1811467
A2	2.5	---	3.61	48594

Total Area: 2,112,014

F Concentration = 0.3 %
A2 Concentration = 2.5 %

Analysis comments:

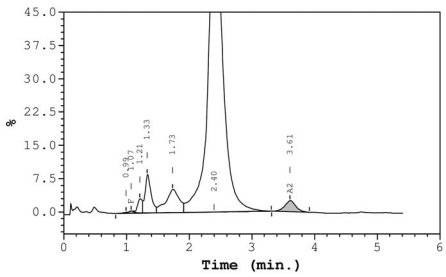


Figure 2. Hemoglobin analysis. (A) CapillaryS3 TERA (Hb program) (Sebia). (B) VARIANT II^{TM} (β-Thalassemia Short program) (Bio-Rad Laboratories).

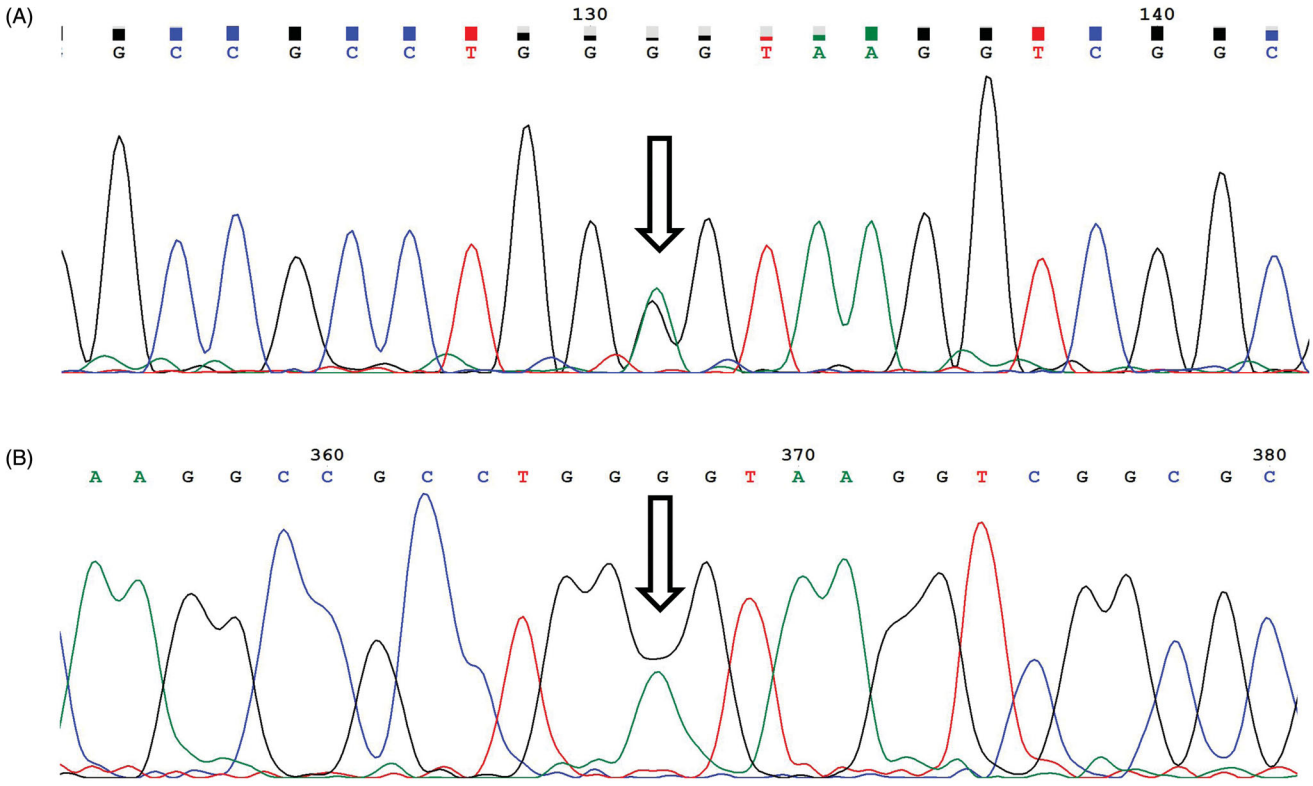


Figure 3. Sanger sequencing. (A) Forward primer. (B) Reverse primer. Arrows indicate the heterozygous mutation Hb Nanchang (*HBA2*: c.46G > A).

(MCH) 31.9 pg (reference interval 27.0–34.0 pg). All her hematological parameters were within the reference range.

Many Hb variants were previously discovered by measurement of Hb A_{1c} [4,5]. Theoretically, the readily detected variants are charge-difference substitutions; otherwise, they will not be detectable and result in the overlap of Hb A peaks and variant peaks. As seen in this study, cation exchange HPLC and CE methods faced challenges in discriminating Hb Nanchang from Hb A. However, MALDI-TOF MS easily detected this Hb variant *via* the sufficient mass difference between the wild and variant globin chains. The measured mass difference (29 Da), which matched the theoretical one (30 Da), can also validate the specific amino acid substitution. Although MALDI-TOF MS can only detect the variant chains with mass differences exceeding at least ± 6 Da from the wild-type chains [6], it is a useful supplementary method because it allows the recognition of some chromatographically or electrophoretically silent Hb variants.

The procedure for determining Hb A_{1c} by MALDI-TOF MS has been developed, and its performance has been evaluated [7–10]. Hb A_{1c} results obtained with α or β chain glycation were prone to the interference of the detected α or β chain variant [11]. As MALDI-TOF MS can quantify the intact globin chain and the corresponding glycated forms, allowing Hb A_{1c} estimation through α or β chain glycation, the interference of Hb variants on Hb A_{1c} quantification may be overcome by employing the glycation of the globin chain without a genetic variant to estimate Hb A_{1c} values [11]. In this case, MALDI-TOF MS gave a spuriously elevated Hb A_{1c} value based on α chain glycation and an accurate Hb A_{1c} result based on β chain glycation, confirming the previous findings.

To date, three Hb variants caused by the mutation at codon 114 in the *HBA* gene, Hb Ottawa (*HBA2*: c.46G>C), Hb St. Rose (*HBA1*: c.46G>T) and Hb I-Interlaken (*HBA1*: c.47G>A), have been documented in the database of Hb variants and thalassemias [1]. These Hb variant traits are not associated with abnormal oxygen affinity or hematological abnormalities. Unlike Hb Nanchang, these Hb variants were readily detectable by alkaline electrophoresis or cation exchange HPLC.

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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