HEMOGLOBIN
INTERNATIONAL JOURNAL FOR HEMOGLOBIN RESEARCH

Taylor & Francis
Taylor & Francis Group

Hemoglobin

international journal for hemoglobin research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ihem20

A New Hemoglobin Variant: Hb Tangshan [*HBA1*: c.239C > T, CD79(GCG > GTG)(Ala > Val)] Detected by MALDI-TOF MS

Anping Xu, Song Ge, Yueying Huang, Weijie Xie, Yinghui Ye, Cheng Lin & Ling Ji

To cite this article: Anping Xu, Song Ge, Yueying Huang, Weijie Xie, Yinghui Ye, Cheng Lin & Ling Ji (01 Nov 2023): A New Hemoglobin Variant: Hb Tangshan [*HBA1*: c.239C > T, CD79(GCG > GTG)(Ala > Val)] Detected by MALDI-TOF MS, Hemoglobin, DOI: 10.1080/03630269.2023.2277445

To link to this article: https://doi.org/10.1080/03630269.2023.2277445

Published online: 01 Nov 2023.

HEMOGLOBIN
https://doi.org/10.1080/03630269.2023.2277445

Taylor & Francis
Taylor & Francis Group

RAPID COMMUNICATION

A New Hemoglobin Variant: Hb Tangshan [*HBA1*: c.239C > T, CD79(GCG > GTG) (Ala > Val)] Detected by MALDI-TOF MS

Anping Xu[a*], Song Ge[a*], Yueying Huang[a], Weijie Xie[a], Yinghui Ye[a], Cheng Lin[b] and Ling Ji[a]

[a]Department of Laboratory Medicine, Peking University Shenzhen Hospital, Shenzhen, Guangdong Province, People's Republic of China; [b]Department of Laboratory Medicine, Dongguan Shipai Hospital, Dongguan, Guangdong Province, People's Republic of China

ABSTRACT

In this report we decribed a new α-chain variant found during the measurement of hemoglobin A_{1c} (Hb A_{1c}) using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS). MALDI-TOF MS analysis detected an α-chain variant with a mass of 15,155 Da. However, this Hb variant was not detected during Hb A_{1c} measurement by cation-exchange high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) methods. Sanger sequencing validated the presence of a heterozygous missense mutation [*HBA1*: c.239C > T, CD79(GCG > GTG)(Ala > Val)]. The observed 28 Da mass difference exactly matches the theoretical mass difference (28 Da) resulting from the substitution of alanine (89.079) with valine (117.133). As this represents the initial documentation of the mutation, we named it Hb Tangshan after the proband's residence.

ARTICLE HISTORY
Received 20 July 2023
Revised 22 September 2023
Accepted 23 October 2023

KEYWORDS
Hb Tangshan (*HBA1*: c.239C > T); MALDI-TOF MS; Hb A_{1c}

Hemoglobinopathies are the most prevalent monogenic genetic disorders worldwide. Over 2400 thalassemias and hemoglobin (Hb) variants, have been documented in the database of human hemoglobin variants and thalassemia mutations [1,2]. Cation-exchange high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are commonly used to screen and quantify different Hb variants. Cation-exchange HPLC is a method that uses an ion-exchange column and a mobile phase to separate substances with different charges. CE, on the other hand, is a technique that separates substances with different charges in the liquid phase through electric field migration. MALDI-TOF MS offers accurate and rapid analysis of Hb constituents based on mass differences [3]. HbA_{1c} is a modified hemoglobin with a stable adduct of glucose covalently linked to the N-terminal valine of the N-chain. Hb A_{1c} is a widely used biomarker for diabetes mellitus management. In this report we present a new α-chain variant found during the measurement of Hb A_{1c} using MALDI-TOF MS.

This study was approved by the Ethics Committee of Peking University Shenzhen Hospital, and informed consent was obtained from the participant. The practice of informed consent plays a critical role in medicine because patients have the right to dispose of their samples. The proband, a 46-year-old Chinese woman from Tangshan city in Hebei Province, People's Republic of China (PRC), came to our hospital for her annual checkup. Her HbA_{1c} was initially measured using a MALDI-TOF MS system (QuanTOF; Intelligene Biosystems, Qingdao, Shandong, PRC). A variant globin chain was observed in the mass spectrogram of the sample. As shown in Figure 1A, QuanTOF detected the intact α-chain (15127 Da) and β-chain (15868 Da). QuanTOF yielded Hb A_{1c} values of 5.3% (34.0 mmol/mol) through traditional β-chain glycation. Given that Hb variants may have an impact on Hb A_{1c} results [4], we conducted a further analysis of her Hb A_{1c} using two different methods: capillary electrophoresis (CE) with the CapillaryS3 TERA system (Sebia, Lisses, France) and cation-exchange high-performance liquid chromatography (HPLC) with the D-100 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The results obtained from the CapillaryS3 TERA (Figure 1B) and the D-100 (Figure 1C) were 5.54% and 5.49% (reference interval 4.0–6.0%), respectively, which were similar to those obtained from QuanTOF. However, unlike the mass spectrogram, neither the electropherograms nor the chromatograms showed any abnormal peaks.

Sanger sequencing was performed to determine the presence of point mutations in the *HBA1*, *HBA2* and *HBB* genes using an ABI PRISMTM 3730 XL sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing analysis identified a heterozygous mutation [HBA1: c.239C > T] in the *HBA2* gene, which caused a substitution of alanine (89 Da) with valine (117 Da) at codon 79 (Figure 1D). The theoretical mass difference due to this amino acid substitution is 28 Da, which is in exact agreement with the actual observed mass difference. Additionally, no missense mutations were detected in the *HBA2* and *HBB* genes when compared to the reference sequence.To the best of our knowledge, this mutation has not yet been reported, and we named it Hb Tangshan based on her birthplace. Her hematological parameters were

CONTACT Dr. Cheng Lin 156617245@qq.com Department of Laboratory Medicine, Dongguan Shipai Hospital, Guangdong Province, People's Republic of China; Dr. Ling Ji 1120303921@qq.com Department of Laboratory Medicine, Peking University Shenzhen Hospital, Guangdong Province, People's Republic of China.
*Anping Xu and Song Ge contributed equally to this study.

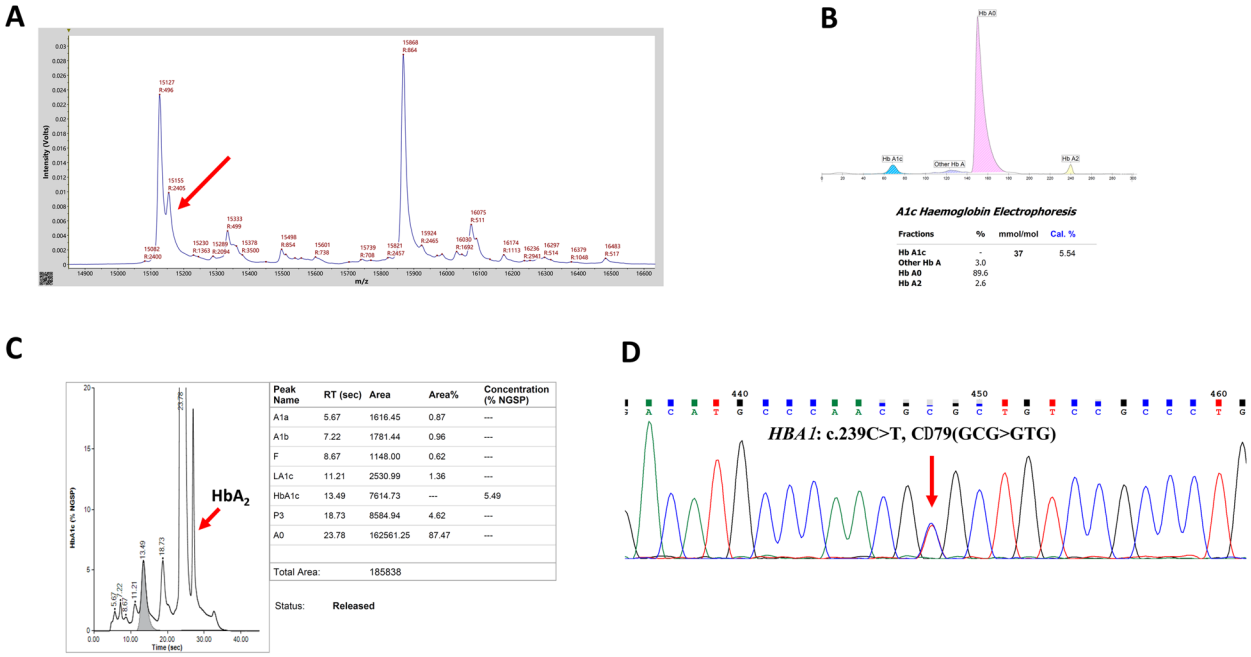

Figure 1. Identification of hemoglobin (Hb) Tangshan. (A) Mass spectrometry showed α-globin chain (15,127 Da) and β-globin chain (15,868 Da). the arrow indicates the variant α-chain peak (15,155 Da). (B) Hb A_{1c} measurement by the capillary electrophoresis method (CapillaryS3 TERA). (C) Hb A_{1c} measurement by the cation-exchange high-performance liquid chromatography method (Bio-Rad D-100). the arrow indicates the HbA_2 fraction. (D) Sanger sequencing revealed a heterozygous mutation of *HBA1*: c.239C > T.

within the normal range: red blood cell (RBC) count 4.67×10^{12}/L, Hb 13.3 g/dL, mean corpuscular volume (MCV) 89.3 fL, and mean corpuscular Hb (MCH) 28.5 pg.

To date, two Hb variants caused by mutation at codon 79 in the *HBA* gene have been documented in the database of Hb variants and thalassemias: Hb J-Singapore [HBA2:c.239C > G] and Hb Mantes-La-Jolie [HBA1 or HBA2:c.238G > A]. Hb J-Singapore [HBA2:c.239C > G] was first identified in 1972 using the cation exchange HPLC VARIANT™ β-Thalassemia Short Program (Bio-Rad Laboratories, Hercules, CA, USA). Electrospray ionization mass spectrometry (ESI/MS) revealed that the variant has a mass of 15113.1 Da, which is 13 Da lower than the normal α-chain (15126.4 Da) [5]. Wajcman *et al.* first reported the presence of Hb Mantes-La-Jolie in a 6-month-old baby and his mother from the Republic of Chad, and electrospray mass spectrometry detected a 30 mass unit increase in the abnormal globin chain, indicating a substitution of alanine with threonine [6].

Many new Hb variants have been identified during the measurement of Hb A_{1c} by cation exchange HPLC or CE methods [7,8]. For cation-exchange HPLC and CE methods, the detectable variants are typically charge-difference substitutions. If there are no charge differences, the Hb A peak and the variant peak may overlap, making it difficult to detect the variant in the analysis. As observed in this study, cation-exchange HPLC and CE methods faced challenges in distinguishing Hb Tangshan from Hb A. However, MALDI-TOF MS easily detected this Hb variant due to the significant mass difference between the wild and variant intact globin chains.

We have found dozens of globin chain mutations in clinical practice for the measurement of Hb A_{1c} using MALDI-TOF MS. The most common was Hb New York, followed by Hb J-Bangkok, Hb Q-Thailand, Hb G-Coushatta, Hb G-Taipei, Hb G-Honolulu and others. This demonstrates the usefulness of MALDI-TOF MS as a supplementary method, as it can identify Hb variants that may not be detectable through HPLC or CE techniques [9]. The discovery of new variants provides a diagnostic basis for hemoglobinopathies. Additionally, detecting variants during the Hb A_{1c} assay alerts to potential interference and prevents reporting of inaccurate Hb A_{1c} results.

Disclosure statement

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

Funding

This study was supported by Guangdong Basic and Applied Basic Research Foundation (2023A1515010618) and Dongguan Social Development Science and Technology Program (20231800938022).

ORCID

Anping Xu http://orcid.org/0000-0002-9827-7272

References

[1] Patrinos GP, Giardine B, Riemer C, *et al.* Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res. 2004;32(Database issue):D537–D541. doi: 10.1093/nar/gkh006.

[2] Kountouris P, Lederer CW, Fanis P, *et al.* IthaGenes: an interactive database for haemoglobin variations and epidemiology. PLoS One. 2014;9(7):e103020. doi: 10.1371/journal.pone.0103020.

[3] Kleinert P, Schmid M, Zurbriggen K, *et al.* Mass spectrometry: a tool for enhanced detection of hemoglobin variants. Clin Chem. 2008;54(1):69–76. doi: 10.1373/clinchem.2007.089961.

[4] Little RR, Rohlfing CR, Hanson S, *et al.* Effects of hemoglobin E and D traits on glycated hemoglobin (HbA1c) measurements by twenty-three methods. Clin Chem. 2008;54(8):1277–1282. doi: 10.1373/clinchem.2008.103580.

[5] Blackwell RQ, Boon WH, Liu CS, *et al.* Hemoglobin J Singapore: alpha 78 Asn–Asp; alpha 79 Ala–Gly. Biochim Biophys Acta. 1972;278(3):482–490. doi: 10.1016/0005-2795(72)90008-6.

[6] Wajcman H, Blouquit Y, Lahary A, *et al.* Three new neutral alpha chain variants: Hb Bois Guillaume [alpha 65(E14(Ala–>Val], Hb Mantes-la-Jolie [alpha 79(EF8)Ala-Thr], and Hb Mosella [alpha 111(G18)Ala–>Thr]. Hemoglobin. 1995;19(5):281–286. doi: 10.3109/03630269509005815.

[7] Cai W-J, Xie X-M, Zhang Y-L, *et al.* A new hemoglobin variant: Hb Henan [β90(F6)Glu→Gln; HBB: c.271G<C]. Hemoglobin. 2014;38(2):127–129. doi: 10.3109/03630269.2014.880062.

[8] Ju AP, Zheng LH, Fu XT, *et al.* Hb Huadu [α124(H7)Ser→Thr (TCC>ACC), HBA2: c.373T>A]: a novel variant of the α-globin gene. Hemoglobin. 2022;46(6):335–337. doi: 10.1080/03630269.2022.2151464.

[9] Xu M, Wang Y, Xu A. A Comparative evaluation of capillary electrophoresis, cation-exchange high-performance liquid chromatography, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the screening of hemoglobin variants. Am J Clin Pathol. 2021;156(3):445–454. doi: 10.1093/ajcp/aqaa260.