Clin. Lab. 2025;71:XXX-XXX

CASE REPORT

Rare Hemoglobin Variant Hb Handsworth (HBA1:c.55 G>C): Leads to False Positive Diagnosis of Hb S

Chengxin Wang, Qingfu Li, Weidong Huang, Bingxin Shao

Medical Laboratory Center, Xinhui Maternity and Children's Hospital, Jiangmen, Guangdong, China

SUMMARY

Hemoglobin Handsworth (*HBA1*: c.55 G>C) is a structural hemoglobin variant. This study examined the molecular and genetic characteristics of a proband and four family members using complete blood count (CBC), capillary electrophoresis (CE), PCR, and direct sequencing. In the capillary electrophoresis, the proband, father, and son all displayed an abnormal band for HbS. Direct sequencing revealed a heterozygous mutation at CD18 (GGC>CGC) in the *HBA1* gene, confirming the presence of hemoglobin Hb Handsworth. It is important to note that individuals carrying only Hb Handsworth did not exhibit any abnormalities in the CBC, suggesting that Hb Handsworth is a non-pathological variation. However, the CE system cannot differentiate it from HbS, which can lead to misdiagnosis; thus, DNA sequencing is necessary for an accurate diagnosis.
(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.241220)

Correspondence:
Chengxin Wang
Medical Laboratory Center
Xinhui Maternity and Children's Hospital
Jiangmen, Guangdong
China
E-mail: wangccdoctor@163.com

KEYWORDS

Hb Handsworth, rare disease, hemoglobin variant, family analysis, false positives

Case Report accepted December 24, 2024

INTRODUCTION

Abnormal hemoglobins are among the most globally prevalent monogenic disorders [1], with routine blood tests and hemoglobin analysis being the primary screening methods. In the southern regions of China, the prevalence of abnormal hemoglobin carriers is about 0.337% [2], with frequently reported abnormal hemoglobins including Hb E, Hb Q-Thailand, Hb New York, and others. [3]. Although most carriers of abnormal hemoglobins do not show significant clinical symptoms, the coexistence of Hb variants with alpha or beta thalassemia may lead to changes in red blood cell parameters [4], resulting in moderate to severe anemia [5]. Thus, it is essential to accurately identify and differentiate clinically relevant Hb variants from non-pathological variations. Conventional Hb electrophoresis or HPLC analysis often cannot diagnose many Hb variants, which typically require confirmation through DNA sequencing. The case described here, Hb Handsworth, is a rare alpha-chain variant hemoglobin caused by a point

mutation c.55 G>C on the *HBA1* gene, leading to the substitution of Gly>Arg (glycine to arginine), and resulting in the formation of Hb Handsworth. There have been few studies on Hb Handsworth, mostly published as individual case reports. For the first time, we have investigated three generations of a Chinese family, presenting hematological and hemoglobin variant data, including neonatal and adult periods, to enhance our understanding of this rare hemoglobin variant.

CASE PRESENTATION

Materials and Methods

The research subject is a 37-year-old Chinese Zhuang woman who, at 32 weeks of gestation, was transferred from another hospital to our facility for prenatal checkups. During capillary electrophoresis, an abnormal hemoglobin was detected. To conduct a more detailed study of this abnormal hemoglobin, with the informed consent of all participants, we enrolled a three-generation family consisting of five members. We performed routine blood tests, hemoglobin electrophoresis, thalassemia gene testing, and DNA sequencing analysis. This study was approved by the Medical Ethics Committee of Xinhui Maternal and Child Health Hospital.

Detection Methods

Blood routine detection: A Mindray BC-7500 fully automatic blood analyzer was used, and the reference values for red blood cell parameters were: MCV: 82 - 100 fL, MCH: 27 - 34 pg, Hb: 115 - 150 g/L.

Hemoglobin analysis: A Capillarys 2 capillary electrophoresis instrument (Sebia Company, France) was used, with a normal reference value of Hb A2 2.5% - 3.5%.

Gene detection: Gap-PCR was used to detect common α-thalassemia deletion mutation genes, and the PCR-RDB method was used to detect common β-thalassemia and α-thalassemia gene point mutations. DNA sequencing was performed by Sanger sequencing of the *HBA1* gene.

RESULTS

Capillary electrophoresis (CE) performed for hemoglobin analysis identified the presence of HbA, HbA2, and an abnormal hemoglobin in the Z (S) region of the electropherogram (Figure 1), which was identified as a variant of HbS. This variant was present in the proband, her father, and her son (Figure 2), accounting for 12.6%, 13.1%, and 11.6% of their total hemoglobin, respectively, but was absent in the mother. The hematological data of this family pedigree are presented in (Table 1). Despite positive hemoglobin analysis, their thalassemia genotype results were normal using conventional kits. DNA sequencing of the proband revealed a heterozygous mutation CD18 (GGC>CGC) in the *HBA1* gene region, with the genomic position NC_000016.10: g.176771G>C, affecting the translation of the *HBA1* gene and causing a Gly>Arg (glycine to arginine) substitution, and causing Gly>Arg (glycine to arginine), leading to the abnormality of hemoglobin Handsworth. Genetic analysis showed that the proband, her father, and son carry the same genetic variant (Figure 3). Her mother and daughter did not carry this gene, and her husband's hematological phenotype and Hb typing analysis also showed no abnormalities.

DISCUSSION

This study utilized capillary electrophoresis (CE) technology, which, compared to traditional cellulose acetate electrophoresis and high-performance liquid chromatography (HPLC), provides a more precise method for protein separation. The CE technique, analyzed through electrophoretic software, can divide the electropherogram into 15 distinct regions (z1 to z15), effectively identifying and detecting various rare hemoglobin variants [6]. In this instance, although routine blood analysis and thalassemia gene testing did not uncover any abnormalities, CE technology revealed an anomaly in the Z (S) zone, where the abnormal hemoglobin is typically associated with HbS. However, considering that HbS is more prevalent in populations of African descent [7], we also excluded the possibility of this variant being HbS through a sickle cell test.

Through DNA sequencing, we identified a heterozygous mutation c.55 G>C in the *HBA1* gene region, which led to the identification of Hb Handsworth. Since the molecular structure of Hb Handsworth involves the conversion of glycine to arginine at position α-chain a18 (A16), this mutation, which introduces a larger basic amino acid side chain, might affect the stability of the molecule. However, since the mutation occurs on the molecular surface, its impact on the overall structure of the hemoglobin molecule is limited [8], and the protein structure, including adjacent residues, remains largely intact. Therefore, heterozygous carriers of this mutation do not exhibit any clinical symptoms or hematological changes associated with this mutation.

The mutation, inherited from the proband's father, is also present in the proband's son. The serum ferritin levels of the proband and her daughter were found to be slightly below the normal reference range, which may be related to their reduced HbA2 and MCV levels. Considering that the proband's son is still in the neonatal stage, his MCV and HbA2 levels have not yet fully stabilized and are not assessed. In this family, the Handsworth content in the three carriers ranges from 11.6% to 13.1%, a level that does not affect the Hb levels, and no decrease in MCV values was observed.

Hemoglobin Handsworth, a rare alpha-chain hemoglobin variant, was first reported in a case from India in 1977 [9], marking the global recognition of this particular hemoglobin variant. Subsequently, in 1981, Chinese scholars Liang Zhiquan and colleagues conducted

Table 1. Hematological data and genotypes of the family members.

Parameters	Father	Mother	Proband	Daughter	Son
Gender-age	M - 56	F - 57	F - 37	F - 6	M - 0
Hb (g/dL)	150	140	123	144	221
RBC (10^{12}/L)	4.98	4.50	4.32	5.20	4.70
MCV (fl)	88.8	92.1	86	80.0	99
MCH (pg)	30.20	31.20	28	27.7	34
Hb A2 (%)	2.6	2.8	2.3	2.6	0.2
Hb Handsworth (%)	13.1	-	12.6	-	11.6
HBA **Genotype**	$\alpha^{Handsworth}\alpha/\alpha\alpha$	αα/αα	$\alpha^{Handsworth}\alpha/\alpha\alpha$	αα/αα	$\alpha^{Handsworth}\alpha/\alpha\alpha$
HBB **Genotype**	βN/βN	βN/βN	βN/βN	βN/βN	βN/βN

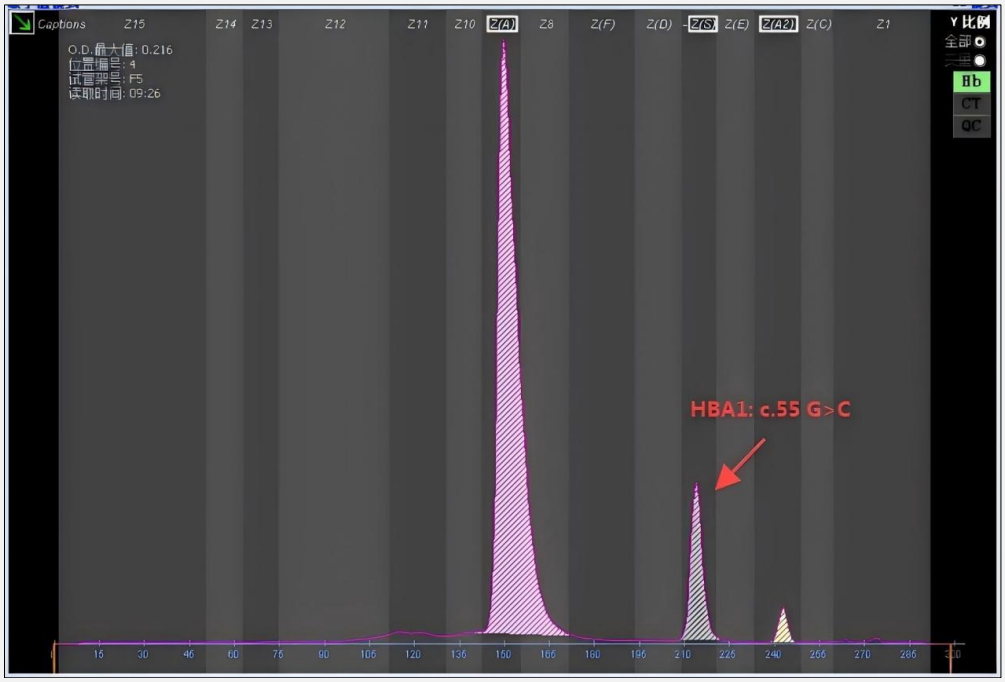

Figure 1. Electrophoresis diagram of hemoglobin Hb Handsworth, with peaks from left to right being Hb A, Hb Handsworth, Hb A2.

an in-depth structural analysis of this variant, revealing a point mutation from GGC to CGC at the CD18 site of the *HBA1* gene, leading to the amino acid change from glycine to arginine [10]. This key discovery laid the foundation for understanding the molecular mechanism of Hb Handsworth. In Liang Zhiquan's study, the patient's Hb Handsworth content was 31.9%, and the patient only exhibited mild anemia [10]. Additionally, Kyrri and colleagues found in their study in Cyprus that there was no consistent correlation between the content of Hb Handsworth and clinical symptoms, with patients ranging from mild anemia to asymptomatic carriers [11]. In 1997, Zhu Xiaoming and colleagues conducted a detailed structural analysis of a Hb Handsworth case in a mixed Uyghur and Han Chinese individual, further confirming the genetic characteristics of this variant. In

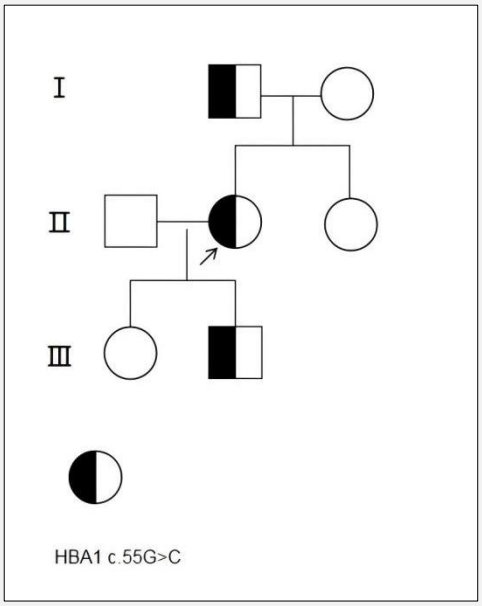

Figure 2. Family lineage: The proband is marked with an arrow.

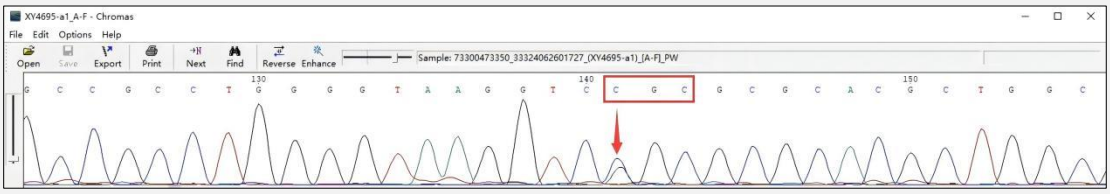

Figure 3. DNA sequencing analysis showing a heterozygous mutation of c.55G>C in the HBA1 gene. (The arrow indicates the HBA c.55 G>C mutation).

newborns reported in Saudi Arabia, the proportion of Hb Handsworth was 21%, and in two individuals reported in Iran, it was 12% and 13%, with mild anemia [12]. These studies indicate that there is significant heterogeneity between the expression levels of Hb Handsworth and clinical symptoms, but they are all mild anemia or even asymptomatic. In our study, the patient's Hb Handsworth content was 12.8%, lower than in other previously reported cases, and the routine blood phenotype was normal, further proving that Hb Handsworth is a non-pathological variant. Given that the number of global case reports on Hb Handsworth is still limited, we recommend expanding the screening for abnormal hemoglobins in future research to gain a more comprehensive understanding of its genetic patterns and clinical significance.

CONCLUSION

Hemoglobin Handsworth is a non-pathological hemoglobinopathy that does not significantly affect red blood cell parameters or clinical manifestations. Even neonatal and elderly carriers can have a normal hematological phenotype, but it cannot be distinguished from HbS in CE testing, which may lead to misdiagnoses of hemo-

globinopathies. This study provides, for the first time, a molecular diagnosis and clinical characteristic analysis across various age groups within a family, offering foundational data for future research on Hb Handsworth.

Acknowledgment:
The authors would like to thank the family for their participation in this study and the laboratory staff for their technical assistance.

Declaration of Interest:
No potential conflict of interest was reported by the author(s).

References:

1. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010 Jun 3;115(22):4331-6. (PMID: 20233970)

2. Ye L. New progress in laboratory detection of abnormal hemoglobinopathy. J Clin Hematol 2023;36(2):140-4. https://lcxy.whuhzzs.com/article/doi/10.13201/j.issn.1004-2806.2023.02.015

3. Xu A, Chen W, Xu M, Xie W, Ji L. Identification of Hemoglobin Variants Prevalent in China and Their Effects on Hemoglobin A1c Measurements. Am J Clin Pathol 2022 Jun 7;157(6):852-7. (PMID: 34871348)

4. Satthakarn S, Srisuwan W, Kunyanone N, Panyasai S. Novel Insights into Hb Shaare Zedek Associated with β^0-Thalassemia: Molecular Characteristics, Genetic Origin and Diagnostic Approaches. Int J Mol Sci 2024 Aug 6;25(16):8578. (PMID: 39201263)

5. Chen L, Tang N, Huang J, et al. Distribution characteristics and clinical phenotype analyses of hemoglobin variants in the Z region of Central Guangxi, Southern China. Hematology 2023 Dec; 28(1):2188651. (PMID: 36995301)

6. Tang B, Wang J, Qin D, et al. Hb Chapel Hill or Alpha2 74(EF3) Asp>Gly, a mildly unstable variant found in a Chinese family. Hematology 2023 Dec;28(1):2187154. (PMID: 36939273)

7. Al Zadjali S, Al-Riyami AZ, Gravell D, et al. Potential pitfalls in the diagnosis of Hb Handsworth in areas with high prevalence of HbS. Int J Lab Hematol 2014 Aug;36(4):488-92. (PMID: 24165563)

8. Al-Awamy BH, Niazi GA, Naeem MA, Wilson JB, Huisman TH. Hemoglobin Handsworth or alpha2 18(A16)Gly-Arg beta2 in a Saudi newborn. Hemoglobin 1985;9(2):183-6. (PMID: 4030381)

9. Griffiths KD, Lang A, Lehmann H, Mann JR, Plowman D, Raine DN. Haemoglobin Handsworth alpha 18 (A16) glycine leads to arginne. FEBS Lett 1977 Mar 15;75(1):93-5. (PMID: 852596)

10. Chih-chuan L, Hai-nan T, Kuo-feng C. Hemoglobin Handsworth (gamma 18 (A16) Gly leads to Arg) in a Chinese. Hemoglobin 1981;5(2):191-3. (PMID: 7216818)

11. Kyrri AR, Felekis X, Kalogerou E, et al. Hemoglobin variants in Cyprus. Hemoglobin 2009;33(2):81-94. (PMID: 19373583)

12. Akbari MT, Hamid M. Identification of α-globin chain variants: a report from Iran. Arch Iran Med 2012 Sep;15(9):564-7. (PMID: 22924376)