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Molecular characterization of similar Hb Lepore Boston-Washington in four Chinese families using third generation sequencing

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Hemoglobin (Hb) Lepore is a rare deletional $\delta\beta$ -thalassemia caused by the fusion between delta-beta genes, and cannot be identified by traditional thalassemia gene testing technology. The aim of this study was to conduct molecular diagnosis and clinical analysis of Hb Lepore in four unrelated Chinese families using third generation sequencing. Decreased levels of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and an abnormal Hb band were observed in the probands of the four families. However, no common α and β -thalassemia variants were detected in the enrolled families using polymerase chain reaction-reverse dot blot hybridization based traditional thalassemia gene testing. Further third-generation sequencing revealed similar Hb Lepore-Boston-Washington variants in all the patients, which were resulted from partial coverage of the *HBB* and *HBD* globin genes, leading to the formation of a delta-beta fusion gene. Specific gap-PCR and Sanger sequencing confirmed that all the patients carried a similar Hb Lepore-Boston-Washington heterozygote. In addition, decreased levels of MCH and Hb A2 were observed in the proband's wife of family 2, an extremely rare variant of Hb Nanchang (GGT > AGT) (HBA2:c.46G > A) was identified by third-generation sequencing and further confirmed by Sanger sequencing. This present study was the first to report the similar Hb Lepore-Boston-Washington in Chinese population. By combining the utilization of Hb capillary electrophoresis and third-generation sequencing, the screening and diagnosis of Hb Lepore can be effectively enhanced.

Keywords Thalassemia, Third-generation sequencing, Gap-PCR, Sanger sequencing, Hb Lepore

Thalassemia is an inherited hemolytic anemia caused by the reduced or absent synthesis of one or more globin chains in hemoglobin due to globin gene deletion or mutation, it is commonly categorized into two groups: α -thalassemia and β -thalassemia¹⁻³. Among them, β -thalassemia is mainly caused by point mutations in the β -globin gene, while deletional variants are rare^{4,5}. Chinese $G\gamma^+(\Lambda\gamma\delta\beta)^0$ -thalassemia and SEA-HPFH are the most common $\delta\beta$ -thalassemia in Chinese populations caused by partial *HBB* and *HBD* deletion⁶. The Hb Lepore are a group of structural defects, which are composed of two normal α chains and two $\delta\beta$ fusion chains, causing by recombination events between the δ - and β -globin genes, resulting in δ and β fusion gene and $\delta\beta$ -thalassemia. Hb Lepore occurs worldwide, the Boston-Washington, Baltimore, Hollandia and Leidan have been described in the database and literature⁷⁻¹¹.

Common α - and β -thalassemia gene variants in China are typically diagnosed by PCR-reverse dot blot hybridization (PCR-RDB) and gap-PCR¹². However, traditional diagnostic tools may lead to misdiagnosis of

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rare thalassemia genotypes. At present, the third-generation sequencing (TGS) technology has been gradually used for thalassemia molecular diagnosis, with an obvious advantage in revealing globin genes single-nucleotide variants, short insertions/deletions and structural variants^{13,14}. Increasing rare and novel globin gene variants have been identified using long-read sequencing^{13–16}.

In this study, four families with abnormal hematological screening results indicating of rare thalassemia carriers underwent further investigation using third-generation sequencing. All of the probands in these families harbored Hb Lepore variants, which were resulted from partial coverage of the *HBB* and *HBD* globin genes, leading to the formation of a delta-beta fusion gene. Moreover, our findings could provide valuable reference in conducting TGS for rare and novel thalassemia diagnosis.

Materials and methods

Subjects

Four unrelated families from the Quanzhou region in Southeast China, with abnormal routine blood analysis and Hb capillary electrophoresis screening results, were enrolled in this study. All the probands had similar abnormal Hb Lepore bands located in Hb zone 6, manifested decreased levels of MCV and MCH, and suspected as thalassemia carriers. All the enrolled members in the four families denied recent blood transfusions. After written informed consents were signed, peripheral blood samples were collected for further molecular diagnosis in the enrolled subjects. This study was approved by the ethics committee of The Women's and Children's Hospital of Quanzhou (2021No.61).

Hematological screening

Routine blood analysis for mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels detection was conducted using an automated cell counter (Sysmex XS-1000i; Sysmex Co., Ltd., Kobe, Japan). Hb capillary electrophoresis (Sebia, Evry Cedex, France) was performed to detect the levels of Hb A, Hb A2 and Hb F and other abnormal hemoglobin bands. Positive hematological screening results included at least one of the following criteria: MCV < 82 fL, MCH < 27 pg, Hb A2 > 3.4%, Hb A2 < 2.6%, Hb F > 2.0% and abnormal Hb bands.

Traditional thalassemia gene testing

All of the members in the four families did further thalassemia gene testing. Genomic DNA of the members in the enrolled families were extracted using an automatic nucleic acid extractor (Ruibao Biological Co., Ltd). Twenty-three types of common α -thalassemia and β -thalassemia variants in the Chinese populations were detected through PCR-RDB according to the manufacturer's protocol (Yaneng Biological technology Co., Ltd., Shenzhen)¹⁷.

Third-generation sequencing

The third-generation sequencing based on PacBio Sequel II platform for globin genes variants detection was conducted as the description of our previous study¹⁶. Genomics DNA were extracted and then sent to Berry Genomics laboratory for third-generation sequencing. Briefly, the purified DNA samples were quantified using the Qubit dsDNA BR assay kit (ThermoFisher Scientific). Then, optimized primers were used to generate specific amplicons that encapsulated known structural variation regions, as well as single nucleotide variations in the *HBA1/2* and *HBB* globin genes, based on databases such as HbVar, Ithanel, LOVD, and LOVD-China. After purification and end repair, double barcode adaptors were ligated to the 5' and 3' ends and Sequel Binding and Internal Ctrl Kit 3.0 (PacBio) was used to prepare SMRT bell libraries. Finally, third-generation sequencing was performed on the PacBio Sequel II System after primed DNA-polymerase complexes were loaded onto the SMRT cells¹⁶.

Following alignment of the subreads, the consensus circular sequence was mapped to the GRCh38 reference and variants were called (FreeBayes software, version 1.2.0). Cis- and trans-configuration between two variants in the long reads was analyzed using WhatsHap (version 0.18) software. The alignments of variant and wild-type molecules were visualized using the Integrative Genomics Viewer¹⁶.

Specific gap-PCR amplification and Sanger sequencing

Specific gap-PCR or Sanger sequencing were used to confirm the rare or novel globin gene variants detected by third-generation sequencing. Gap-PCR was used to identify the deletion breakpoints. We designed specific primers according to the known DNA sequences around the breakpoints or the globin gene sequence variants. The primers sequences were as followed, P1: AGAGATGCGGTGGGGAGATA and P2: AACGATCCTGAGACTTCCACA. All primers were synthesized at Sangon Biotech (Shanghai). Gap-PCR reaction system: 5 × buffer 5 μ L, 25 mmol dNTPs 0.2 μ L, 25 mmol MgCl₂ 1.5 μ L, Taq enzyme 2.5U, 10 μ mol primers 1 μ L each, template 2 μ L, and plus ultra-pure water to 25 μ L. The amplification conditions were 95 °C for 10 min, then 35 cycles of 94 °C for 1 min, 62 °C for 30 s, 72 °C for 1 min, and finally 72 °C for 5 min. Electrophoresis analysis was performed and then the purified electrophoresis products were sent for Sanger sequencing. In addition, Sanger sequencing was also performed for globin gene variants. The sequenced data were analyzed with GenBank NG_000007.3 as their reference sequences.

Ethics approval and consent to participate

This study was approved by the ethics committee of The Women's and Children's Hospital of Quanzhou (2021No.61). We received informed consent from the study participants and their parents, and they agreed to the publication of a report on the study. All procedures performed involving human participants were in

accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

Hematological screening results

The hematological screening results of the enrolled four families were described in Table 1. All of the members in four families had decreased levels of MCV and MCH detected by routine blood analysis. As elicited in Table 1 and Fig. 1, subsequent Hb capillary electrophoresis results demonstrated similar four Hb bands in the four probands of the families as well as the proband's daughter in family 1, including Hb A, Hb A2, Hb F, and Hb zone 6 bands. Among these five subjects, the abnormal Hb zone 6 (Hb Lepore) levels was obvious range from 7.3 to 10.5%, and Hb F levels range from 0.7 to 14.1%. In addition, both of the proband's wife in family 2 and family 3 only had a decreased level of Hb A2, who were suspected as α -thalassemia carriers.

| | Family 1 | | Family 2 | | Family 3 | | Family 4 |
|-----------------------|-----------------------------|-----------------------------|-----------------------------|--|-----------------------------|-----------------------------|-----------------------------|
| | Proband | Daughter | Proband | Wife | Proband | Wife | Proband |
| Sex-age | F-37 | F-2 | M-29 | F-28 | M-31 | F-28 | M-27 |
| RBC($10^{12}/L$) | 4.73 | 5.10 | 7.31 | 5.15 | 5.04 | 4.53 | 6.19 |
| Hb(g/L) | 108 | 112 | 146 | 138 | 97 | 124 | 150 |
| MCV(fl) | 70 | 66.7 | 63.7 | 84.3 | 69.1 | 80.4 | 65 |
| MCH(pg) | 22.8 | 21.7 | 20 | 26.8 | 22.8 | 28 | 20.9 |
| Hb A(%) | 85.4 | 76.1 | 84.4 | 97.8 | 83.2 | 97.5 | 86.6 |
| Hb A2(%) | 2.5 | 2.5 | 2.9 | 2.2 | 2.3 | 2.5 | 2.3 |
| Hb F(%) | 1.6 | 14.1 | 2.5 | 0 | 5.1 | 0 | 0.7 |
| Hb zone 6(%) | 10.5 | 7.3 | 10.2 | 0 | 9.4 | 0 | 10.4 |
| α -thalassemia | $\alpha\alpha/\alpha\alpha$ | $\alpha\alpha/\alpha\alpha$ | $\alpha\alpha/\alpha\alpha$ | $\alpha\alpha^{HBA2:c.46G>A}/\alpha\alpha$ | $\alpha\alpha/\alpha\alpha$ | $\alpha\alpha/\alpha\alpha$ | $\alpha\alpha/\alpha\alpha$ |
| β -thalassemia | Hb Lepore-BW | Hb Lepore-BW | Hb Lepore-BW | β^N/β^N | Hb Lepore-BW | β^N/β^N | Hb Lepore-BW |

Table 1. The hematological and molecular analysis of the enrolled families. M, Male; F, Female; N, Normal; Hb, Hemoglobin; BW, Boston-Washington.

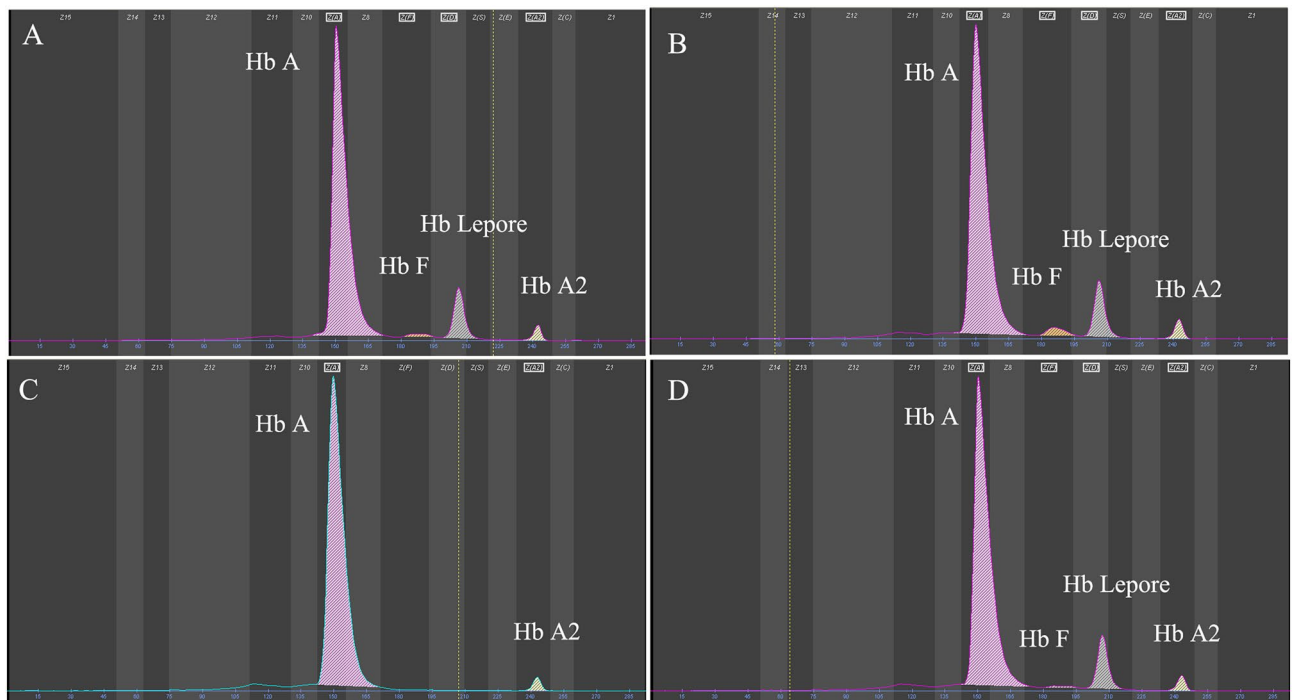


Figure 1. Hemoglobin capillary electrophoresis results of the enrolled families. (A,B,D) All of the probands in family 1, 2, and 4, elicited abnormal Hb bands in zone 6, which indicated existence of Hb variants. (C) Hemoglobin capillary electrophoresis results revealed a decreased level of Hb A2 in proband's wife of family 2.

Traditional thalassemia gene testing results

Firstly, 23 common α -thalassemia and β -thalassemia variants in Chinese populations were investigated using PCR-RDB technique in the four families. As delineated in Table 1, no common α -thalassemia and β -thalassemia variants was detected. Given that the positive hematological screening results in the enrolled subjects, rare or novel globin genes variants were suspected and all of them were subject to further genetic diagnosis.

Third-generation sequencing results

In order to further investigate the thalassemia genotype of the suspected families, TGS based on single-molecule real time sequencing was performed for globin genes detection including single nucleotide variation and structure variants. As depicted in Table 2 and Fig. 2, TGS detection revealed large deletions that partially encompassed the *HBB* and *HBD* globin genes in four probands from the families, which located in the region of Hb Lepore-Boston-Washington. In family 1, the proband's daughter who exhibit similar hematological screening results also had the same deletion. In addition, a rare variant of Hb Nanchang (GGT > AGT) (*HBA2*:c.46G > A) at codon 15 in $\alpha 2$ (Fig. 2) was identified in the proband's wife of family 2 with decreased level of Hb A2 and slightly low level of MCH. However, no relative globin genes variants was observed in the proband's wife of family 3 who also had decreased level of Hb A2.

Specific gap-PCR amplification verification results

In order to confirm the deletions in the enrolled families, the specific gap-PCR amplification was further conducted. Subsequently, two specific primers were designed to amplify the breakpoints using gap-PCR technique. The gap-PCR detection results demonstrated a large deletion covering the *HBB* and *HBD* globin gene cluster in all the proband's of the four families. Subsequently, the electrophoretic results delineated a 800 bp PCR product in the four families, which amplified by the primers P1 and P2 (Fig. 3).

Sanger sequencing results

To further verify the specific location of the breakpoints in the four enrolled families, Sanger sequencing was conducted using the specific products that amplified by gap-PCR. As shown in Table 2 and Fig. 3, after alignment between sequencing results and NG_000007.3 sequence through BLAST analysis, all the patients in the four families had a partial *HBB* and *HBD* deletion fragment range from 63,633–71,046 bp, with a fragment length of 7.414-kb (NG_000007.3:g.63633_71046 del), which was almost the same with Hb Lepore-Boston-Washington (NG_000007.3:g.63632_71046 del). In addition, the Hb Nanchang (GGT > AGT) (*HBA2*:c.46G > A) identified in proband's wife in family 2 was further confirmed by Sanger sequencing (Fig. 3).

Discussion

Thalassemia is a hereditary blood disorder caused by globin gene synthesis disorders⁴, which included α , β , γ , $\delta\beta$ -thalassemias. Thalassemia is highly prevalent in South China, especially in Guangdong, Guangxi, and Hainan provinces^{18–20}. Quanzhou region of Fujian province, located in Southeast China, displays a high prevalence of thalassemia, with a diversity and complexity of thalassemia^{21–23}. Common α - and β -thalassemia gene variants in China are typically diagnosed by PCR-RDB and gap-PCR¹², but they have limitations in the diagnosis of rare thalassemia variants. Third-generation sequencing technology has significant advantages in the diagnosis of thalassemia, and can detect almost all known globin gene variants^{13,14}. In our study, third generation sequencing was performed to investigate globin gene variants in four Chinese families with abnormal Hb variants, all of the suspected members harbored a large 7.414-kb deletion that partially affected the *HBB* and *HBD* globin genes (NG_000007.3:g.63633_71046 del), which closely resembled the Hb Lepore-Boston-Washington variant (NG_000007.3:g.63632_71046 del).

$\delta\beta$ -thalassemia is a rare condition, which is caused by partial deletions in *HBB* and *HBD* globin genes. In China, the $\text{G}\gamma^+(\text{A}\gamma\delta\beta)^0$ and SEA-HPFH genotypes were the two most common $\delta\beta$ -thalassemia variants⁶. In addition, our previous study indicated a higher prevalence of SEA-HPFH of $\delta\beta$ -thalassemia in Quanzhou region, while, Hb Lepore had not been identified^{22,23}. The Lepore hemoglobins are a group of $\delta\beta$ -thalassemia, which is more frequently identified in Southern Europeans¹¹, Caucasians in Central Portugal and in the Spanish Alta Extremadura²⁴. The Hb Lepore Boston-Washington, Baltimore, Hollandia and Leidan are commonly described in the database and literature, with different recombination crossover breakpoints^{7–10}. Moreover, several novel Hb Lepore variants have been identified, including Hb Lepore-Hongkong and Hb Lepore-ARUP^{25,26}.

Hb Lepore has been rarely reported in Chinese populations, while, the Hb Lepore-Boston-Washington seem more prevalent in China²⁷. Hb Lepore cannot be diagnosed through routine thalassemia gene testing based on

| Families | TGS (NG_000007.3) | Sanger sequencing (NG_000007.3) |
|----------|-------------------|---------------------------------|
| Family 1 | 63633_71046del | 63633_71046del |
| Family 2 | 63632_71046del | 63633_71046del |
| Family 3 | 63633_71046del | 63633_71046del |
| Family 4 | 63626_71040del | 63633_71046del |

Table 2. *HBB* and *HBD* deletions detected by third-generation sequencing (TGS) and verified by Sanger sequencing.

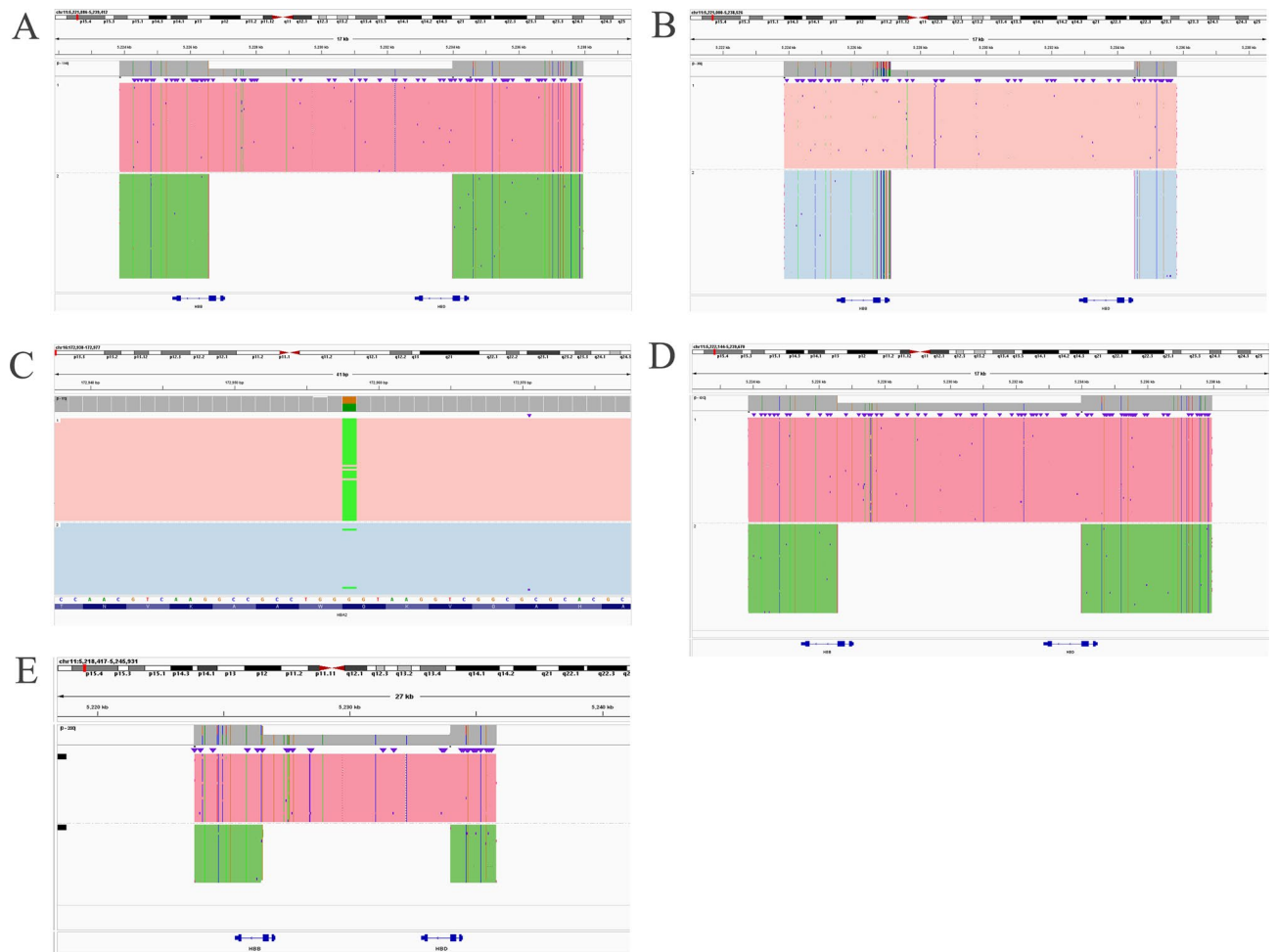


Figure 2. Third-generation sequencing (TGS) results of the enrolled families. (A) In the probands of family 1, a 7.414 kb deletion (NG_000007.3:g.63633_71046del) that partially covering *HBB* and *HBD* globin genes was identified. (B) In the proband of family 2, a 7.415 kb deletion (NG_000007.3:g.63632_71046del) in *HBB* and *HBD* globin genes was detected using TGS. (C) A variant of Hb Nanchang (GGT > AGT) (*HBA2*:c.46G > A) was identified in the proband's wife of family 2. (D) A 7.414 kb deletion (NG_000007.3:g.63633_71046del) that partially covering *HBB* and *HBD* globin genes was identified in family 3. (E) A 7.415 kb deletion (NG_000007.3:g.63626_71040del) was identified in the proband of family 4.

PCR-RDB technology, but it can be well indicated through hemoglobin electrophoresis or high performance liquid chromatography (HPLC) technology. Heterozygotes of Hb Lepore commonly exhibit a mild hypochromic microcytic anaemia with 10–15% Hb Lepore and a slightly increased level of Hb F⁷. However, a novel Hb Lepore-Hong Kong was identified with increased level of Hb A2 and Hb F, but without a Hb Lepore band, which may due to the delta-beta fusion gene of Hb Lepore-Hong Kong that sharing the same coding sequences as *HBB*²⁵. In the present study, five members in four Chinese families had decreased levels of MCV and MCH, with an abnormal Hb band in zone 6 (7.3–10.5%), and various Hb F levels (0–5.1%), which was consistent with the previous reports. Finally, all of the suspected members were identified carrying a same 7.414-kb deletion (NG_000007.3:g.63633_71046 del) in *HBB* and *HBD* globin genes, which manifest one bp difference with Hb Lepore-Boston-Washington, and we proposed to classify them as Hb Lepore-Boston-Washington heterozygosity carriers.

Hb Lepore is associated with a β -thalassemia phenotype, thus, individuals with homozygous or compound heterozygosity of Hb Lepore(s) would lead to β -thalassemia intermedia or major²⁸. In this study of family 2 and family 3, the probands' wife were also subject to TGS detection, while, no mutations in β -globin gene was observed. Interestingly, a rare Hb Nanchang (GGT > AGT) (*HBA2*:c.46G > A) variant at codon 15 in *HBA2* globin gene was identified in the proband's wife of family 2. The *HBA2*:c.46G > A variant, which involved a GGT \rightarrow AGT change and resulting in a Gly \rightarrow Ser replacement at CD15 was first identified in a girl with normal hematological parameters and classified as silent α -thalassemia²⁹. Subsequently, the variant of Hb Nanchang (*HBA2*:c.46G > A) was reported by Xu et al.³⁰ in a Chinese population with normal hematological parameters. Notably, in our present study, the patient who harbored Hb Nanchang (GGT > AGT) (*HBA2*:c.46G > A) variant exhibited a slightly low level of MCH (26.8 pg), which may causing α^+ -thalassemia (silent α -thalassemia). In addition, as elicited in the IthaGenes database (<https://www.ithanet.eu/>), *HBA2*:c.46G > A was indicated to result in α^+ -thalassemia.

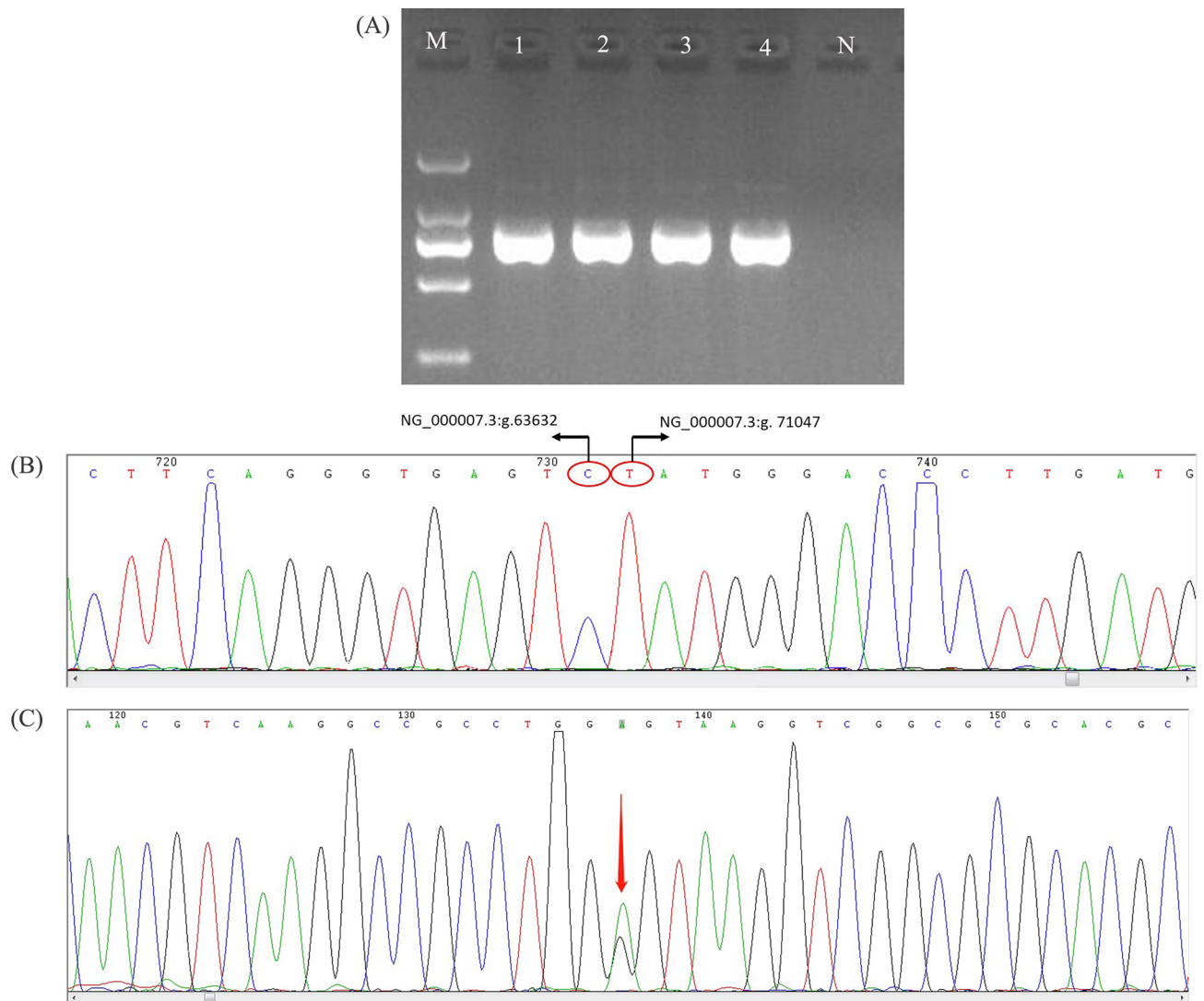


Figure 3. Specific gap-PCR amplification and Sanger sequencing results in the four enrolled families. (A) 1: family 1. 2: family 2. 3: family 3. 4: family 4. N: Normal control. (B) Sanger sequencing results showed that the deletion fragments of fracture in the four families was range from 63,633–71,046 bp, which was similar to Hb Lepore-Boston-Washington. (C) The Hb Nanchang (GGT > AGT) (HBA2:c.46G > A) variant in the proband's wife of family 2 was verified by Sanger sequencing.

To summarize, our study first described the similar Hb Lepore (NG_000007.3:g.63633_71046del) in Chinese population. In addition, Hb Nanchang variants was identified in Fujian province for the first time. Our findings suggest that combining the use of Hb capillary electrophoresis and third-generation sequencing would effectively screen and diagnose Hb Lepore.

Data availability

The raw sequence data reported in this study have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: <https://ngdc.cncb.ac.cn/gsa-human>, accession number: HRA006741).

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References

1. Zaino, E. C. & Tien, Y. Y. Hemoglobinopathy and thalassemia in China. *N. Engl. J. Med.* **305**(13), 766 (1981).
2. Muncie, H. L. Jr. & Campbell, J. Alpha and beta thalassemia. *Am. Fam. Physician.* **80**(4), 339–344 (2009).
3. Taher, A. T., Otrrock, Z. K., Uthman, I. & Cappellini, M. D. Thalassemia and hypercoagulability. *Blood Rev.* **22**(5), 283–292 (2008).
4. Weatherall, D. J. Phenotype-genotype relationships in monogenic disease: Lessons from the thalassaemias. *Nat. Rev. Genet.* **2**(4), 245–255 (2001).

5. Zhuang, J. *et al.* A first clinical and molecular study of rare IVS-II-806 (G > C) (HBB:c.316–45G > C) variant in the β -globin gene: A possibly benign variant. *Indian J. Hematol. Blood Transfus.* **39**(1), 102–106 (2023).
6. Ju, A. P. *et al.* Molecular epidemiological characteristics and differential diagnosis of common $\delta\beta$ -Thalassemia/HPFH. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* **30**(4), 1182–1187 (2022).
7. Ribeiro, M. L. *et al.* Hb Lepore-Baltimore (delta 68Leu-beta 84Thr) and Hb Lepore-Washington-Boston (delta 87Gln-beta IVS-II-8) in central Portugal and Spanish Alta Extremadura. *Hum. Genet.* **99**(5), 669–673 (1997).
8. Lad, H. *et al.* First observation of Hb Lepore Hollandia in the Baiga Tribal Family. *Indian J. Hematol. Blood Transfus.* **34**(3), 581–584 (2018).
9. Harteveld, C. L. *et al.* Hb Lepore-Leiden: A new delta/beta rearrangement associated with a beta-thalassemia minor phenotype. *Hemoglobin* **32**(5), 446–453 (2008).
10. McKeown, S. M. *et al.* Rare occurrence of Hb Lepore-Baltimore in African Americans: Molecular characteristics and variations of Hb Lepores. *Ann. Hematol.* **88**(6), 545–548 (2009).
11. Roper, P. *et al.* Identification of the Hb Lepore phenotype by HPLC. *Haematologica* **84**(12), 1081–1084 (1999).
12. Luo, H. *et al.* Molecular prevalence of HBB-associated hemoglobinopathy among reproductive-age adults and the prenatal diagnosis in Jiangxi Province, southern central China. *Front. Genet.* **13**, 992073 (2022).
13. Xu, L. *et al.* Long-molecule sequencing: A new approach for identification of clinically significant DNA variants in α -Thalassemia and β -Thalassemia carriers. *J. Mol. Diagn.* **22**(8), 1087–1095 (2020).
14. Liang, Q. *et al.* A more universal approach to comprehensive analysis of Thalassemia Alleles (CATSA). *J. Mol. Diagn.* **23**(9), 1195–1204 (2021).
15. Liu, Q. *et al.* Identification of rare thalassemia variants using third-generation sequencing. *Front. Genet.* **13**, 1076035 (2023).
16. Zhuang, J. *et al.* Third-generation sequencing as a new comprehensive technology for identifying rare α - and β -globin gene variants in thalassemia alleles in the Chinese population. *Arch. Pathol. Lab. Med.* **147**(2), 208–214 (2023).
17. Liu, Q. *et al.* Analysis on the Genotype of 5018 Cases of Thalassemia in Hunan Area. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* **27**(6), 1938–1942 (2019).
18. Lu, H. *et al.* Genetic diagnosis of thalassemia in Baise, Guangxi Zhuang Autonomous Region. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* **29**(3), 865–868 (2021).
19. Zhang, C. M. *et al.* Molecular epidemiology investigation of beta-thalassemia in Zhongshan City, Guangdong Province People's Republic of China. *Hemoglobin.* **34**(1), 55–60 (2010).
20. Yao, H. *et al.* The spectrum of α - and β -thalassemia mutations of the Li people in Hainan Province of China. *Blood Cells Mol. Dis.* **53**(1–2), 16–20. <https://doi.org/10.1016/j.bcmd.2014.01.003> (2014).
21. Huang, H. *et al.* Molecular characterization of thalassemia and hemoglobinopathy in Southeastern China. *Sci. Rep.* **9**(1), 3493 (2019).
22. Zhuang, J. *et al.* Molecular analysis of α -thalassemia and β -thalassemia in Quanzhou region Southeast China. *J. Clin. Pathol.* **73**(5), 278–282 (2020).
23. Zhuang, J. *et al.* Molecular characterization analysis of thalassemia and hemoglobinopathy in Quanzhou, Southeast China: A large-scale retrospective study. *Front. Genet.* **12**, 727233 (2021).
24. Gilsanz, F., Vela, J. G. & Núñez, G. M. Age and sex matched analysis of Hb Lepore trait in a new population in Spain. *Nouv. Rev. Fr. Hematol.* (1978) **34**(2), 163–166 (1992).
25. Jiang, F. *et al.* Hb Lepore-Hong Kong: First report of a novel δ/β -globin gene fusion in a Chinese family. *Hemoglobin* **45**(4), 220–224 (2021).
26. Nussenzveig, R. H., Vanhille, D. L., Hussey, D., Reading, N. S. & Agarwal, A. M. Development of a rapid multiplex PCR assay for identification of the three common Hemoglobin-Lepore variants (Boston-Washington, Baltimore, and Hollandia) and identification of a new Lepore variant [published correction appears in *Am J Hematol.* 2012 Dec;87(12):E136. Reading, N Scott [added]]. *Am. J. Hematol.* **87**(10), E74–E75 (2012).
27. Jiang, F. *et al.* Molecular epidemiology and hematologic characterization of $\delta\beta$ -thalassemia and hereditary persistence of fetal hemoglobin in 125,661 families of greater Guangzhou area, the metropolis of southern China. *BMC Med. Genet.* **21**(1), 43 (2020).
28. Guo, L., Kausar, A., Old, J. M., Henderson, S. J. & Gallienne, A. E. Characterization of Hb Lepore variants in the UK population. *Hemoglobin* **39**(1), 58–61 (2015).
29. Yao, X. Y. *et al.* Prevalence and genetic analysis of α -thalassemia and β -thalassemia in Chongqing area of China. *Gene* **532**(1), 120–124 (2013).
30. Anping, X., Chen, W., Xie, W., Huiqi Zheng, Y. & Zhou, L. J. 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. *Hemoglobin* **45**(4), 250–253. <https://doi.org/10.1080/03630269.2021.1956946> (2021).

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Author contributions

J.Z. and N.Z. designed the study and wrote the article; Y.Z., A.M. and J.Z. performed traditional thalassemia gene testing, Sanger sequencing and third-generation sequencing; Y.J. and Y.C. contributed in patients recruitment and clinical consultation; J.Z. and C.C. revised and polished the paper. All authors approved the final article. We confirmed that all subjects participated in this study signed written informed consent for publication their own and their children's genetic data and relevant information.

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Competing interests

The authors declare no competing interests.

Additional information

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