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A novel case of Hb Bart's hydrops fetalis following prenatal diagnosis: Case report from Huizhou, China

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ABSTRACT

Objective: Presentation of a novel case of a patient with Hb Bart's hydrops fetalis, which was accurately identified by SMRT sequencing leading to expand the mutation spectrum of α-thalassemia.

Case report: A 26-year-old pregnant woman and her husband underwent molecular analysis of thalassemia due to abnormal hematological results. The molecular analysis showed that the pregnant woman carried -$\alpha^{3.7}$/–^{SEA}, while her husband exhibited a negative result. Accordingly, the pregnant woman continued the pregnancy until the 19-week gestational age. She was subsequently referred to our department for genetic counseling due to abnormal ultrasound findings in the fetus. A novel deletional α-thal mutation was detected for the husband by MLPA, and the precise location of the mutation was determined through SMRT sequencing, which revealed a 45.2 kb deletion. Later, an interventional umbilical cord blood puncture was offered for the pregnant woman. The cord blood was subjected to capillary electrophoresis, which revealed apparent Hb Bart's and Hb Portland peaks associated with Hb Bart's hydrops fetalis syndrome.

Conclusion: It is imperative that Hb Bart's hydrops fetalis syndrome be diagnosed with the utmost expediency. If results of molecular analysis are not consistent with the clinical hematological findings, the presence of a novel thalassemia could be suspected. To identify the novel genotype, the SMRT sequencing represents an effective method for achieving an accurate diagnosis.

1. Introduction

Alpha-thalassemia (α-thal) is one of the most frequent human autosomal-recessive disorders around the world. The pathogenesis of α-thals are due to a deficiency (α^+) or absence (α^0) of hemoglobin α functional globin chain synthesis (located on chromosome 16) and the clinical phenotype ranges from close to normal without complications to a severe lethal hemolytic anemia [1,2]. Of these, Hb Bart's hydrops fetalis syndrome is a clinically significant disease of lethal form in which no α-globin chain is synthesized (−/−) and characterized by prenatal onset of generalized edema and pleural and pericardial effusions as a result of congestive heart failure induced by severe anemia. Ineffective erythropoiesis, increased haemolysis, marked hepatosplenomegaly, and a massive placenta are common [3,4]. Death usually occurs *in utero* or shortly after birth unless sophisticated measures are implemented to save the fetus. In a case of

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continuing pregnancy, the affected pregnancy is usually associated with an increased risk of several serious complications, such as preeclampsia, psychic trauma of carrying a nonsurviving fetus to term and so on [5]. At conception, each sib of a proband with Hb Bart syndrome has a 25 % chance of having Hb Bart's hydrops fetalis syndrome. In order to avoid serious maternal morbidity, prenatal genetic testing should be carried out for couples who are at high risk of having a fetus with Hb Bart's hydrops fetalis syndrome. In this study, we report a case of Hb Bart's hydrops fetalis associated with a novel deletion α-thal, which is confirmed by single-molecule real-time (SMRT) sequencing, also known as third-generation sequencing.

2. Case report

We present a 26-year-old, gravida 1, para 0, pregnant woman with abnormal hematological results (Table 1), who was admitted to the outpatient clinic for genetic screening for thalassemia during prenatal examination. Her red blood cell (RBC) indices, low Hb A_2 and high Hb H values were indicative of a high probability of having Hb H disease. Iron deficiency was excluded. Therefore, gap polymerase chain reaction (gap-PCR) and reverse dot-blot hybridization assay were employed to identify 6 common Chinese α-thal mutations and 17 common Chinese β-thal mutations for her and her husband in our prenatal screening program. Finally, the pregnant woman was diagnosed with Hb H disease, with the genotype of $-\alpha^{3.7}/--^{SEA}$, while no common α-thal mutations were found in her husband. Further, novel deletional α-thal mutations in the Chinese population including $--^{THAI}$, $-\alpha^{27.6}$ and $-\alpha^{21.9}$, were detected for the husband, but also negative. Afterwards, she was referred to our department for further investigation due to abnormal ultrasound findings in the fetus.

The pregnant woman was not jaundiced and had no blood transfusion history, but thickened placenta (34mm), increased cardiothoracic ratio (CTR, 0.66) and bilateral choroid plexus cysts in fetus were present at 17 weeks' gestation. In the follow-up ultrasound examination at 19 weeks' gestation, it was observed that not only was the placenta thickened, but also that there were abnormalities, including fetal heart enlargement, thickening of the left and right ventricular walls, and an increase in the peak systolic velocity of the fetal middle cerebral artery (MCA-PSV) (Fig. 1). These ultrasound abnormalities indicated the possibility of the fetus suffering from hydrops fetalis syndrome.

Reviewing the test records of the pregnant woman and her husband, although the three novel deletional α-thal mutations were not detected in the husband, his hematological results suggested that he had a classical α-thal trait. Consequently, multiplex ligation-dependent probe amplification (MLPA) was used to detect copy-number variations (CNVs) in the husband's α-globin genes. The result showed that the husband had a heterozygous deletion ranging from upstream of *HBA2* to the exon 5 of *LUC7L* (Fig. 2A), but the exact location cannot be clarified. To validate the unknown deletion in the husband, SMRT sequencing of the α-globin genes was then performed. This method offers the advantages of high throughput and comprehensive site coverage, which enables the accurate identification of breakpoints of deletions. Finally, a novel 45.2 kb deletion located at the *HBA* gene was confirmed and the exact location (16p13.3, Chr16: 171252–216415, GRch38/hg38) was identified for the first time (Fig. 2B). As the couple was identified as being isotype α-thal, an interventional umbilical cord blood puncture was required for the pregnant woman. The umbilical cord blood obtained was determined by hemoglobin electrophoresis using a capillary electrophoresis (CE) system (Capillarys2 Flex Piercing; Sebia, Lisses, France). The CE demonstrated apparent Hb Bart's and Hb Portland peaks (Fig. 3) associated with Hb Bart's hydrops fetalis syndrome. So unfortunately, the pregnant woman could only choose to terminate the pregnancy.

3. Discussion

α-thal is a group of autosomal recessive genetic blood disorders that cause significant morbidity and mortality by mutations on the α-globin genes [6]. In China, it is especially prevalent in the Southern provinces and there are a large number of carriers for the same genotypes of α-thal that may give birth to fetuses with severe thalassemia [7]. Hb Bart's hydrops fetalis syndrome is the most severe form of α-thal and is usually lethal, resulting in hydrops or stillbirth. According to the mendelian inheritance, couples who have the same genotypes of α^0-thal will have a quarter chance of giving birth to a fetus with Hb Bart's hydrops fetalis syndrome, as in our case. Therefore, pregnant women are often offered prenatal diagnosis to avoid continued pregnancies with the Hb Bart's hydrops fetalis syndrome, which causes neonatal death, and indeed prenatal diagnosis of thalassemia has been approved to significantly reduce the births of severe thalassemia.

Analysis of hematologic parameters is an important screening tool in the prevention and treatment of α-thal, which has been widely used [8]. As shown in Table 1, deletions in the α-globin genes usually results in significant manifestations, with low mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Hb A_2 values, depending on the number of nonfunctional α-globin genes. Currently, hemoglobin electrophoresis is a powerful technique for screening hemoglobinopathies as well as for measuring the quantification of Hb Bart's in cord blood [4]. As illustrated in Fig. 3, Hb Bart's hydrops fetalis syndrome displays a special pattern of

Table 1
Phenotype and genotype data of the studied subjects.

Subject	Age (year)	Hb (g/L)	MCV (fL)	MCH (pg)	HbA (%)	HbF (%)	HbA_2 (%)	HbH (%)	Hb Bart's (%)	HBA genotype	HBB genotype
Husband	28	137	66.6	20.3	97.8	0.0	2.2	–	–	$--^{45.2kb}/\alpha\alpha$	N/N
Pregnant woman	26	93	76.7	22.3	85.9	0.7	0.9	11.8	0.7	$-\alpha^{3.7}/--^{SEA}$	N/N

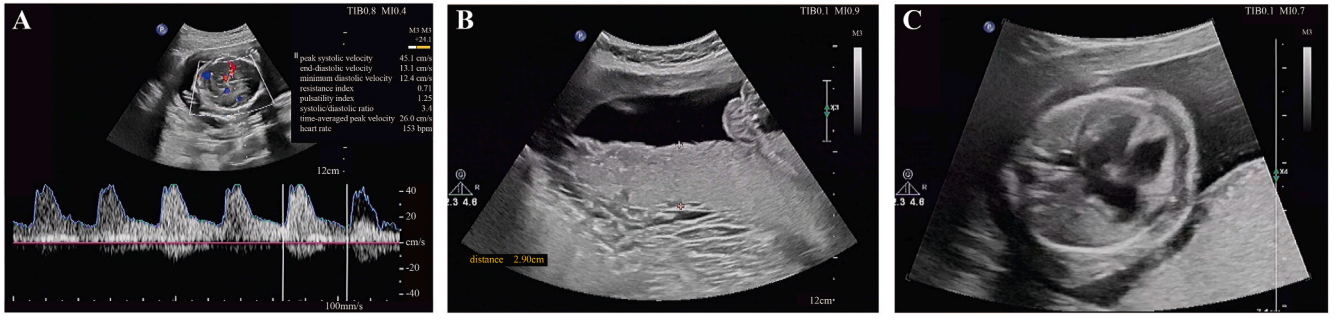

Fig. 1. Ultrasound images of the fetal at 19 weeks. A). Increased MCA-PSV. B) Increased placental thickness. C) Enlarged heart, thickening of the left and right ventricular walls.

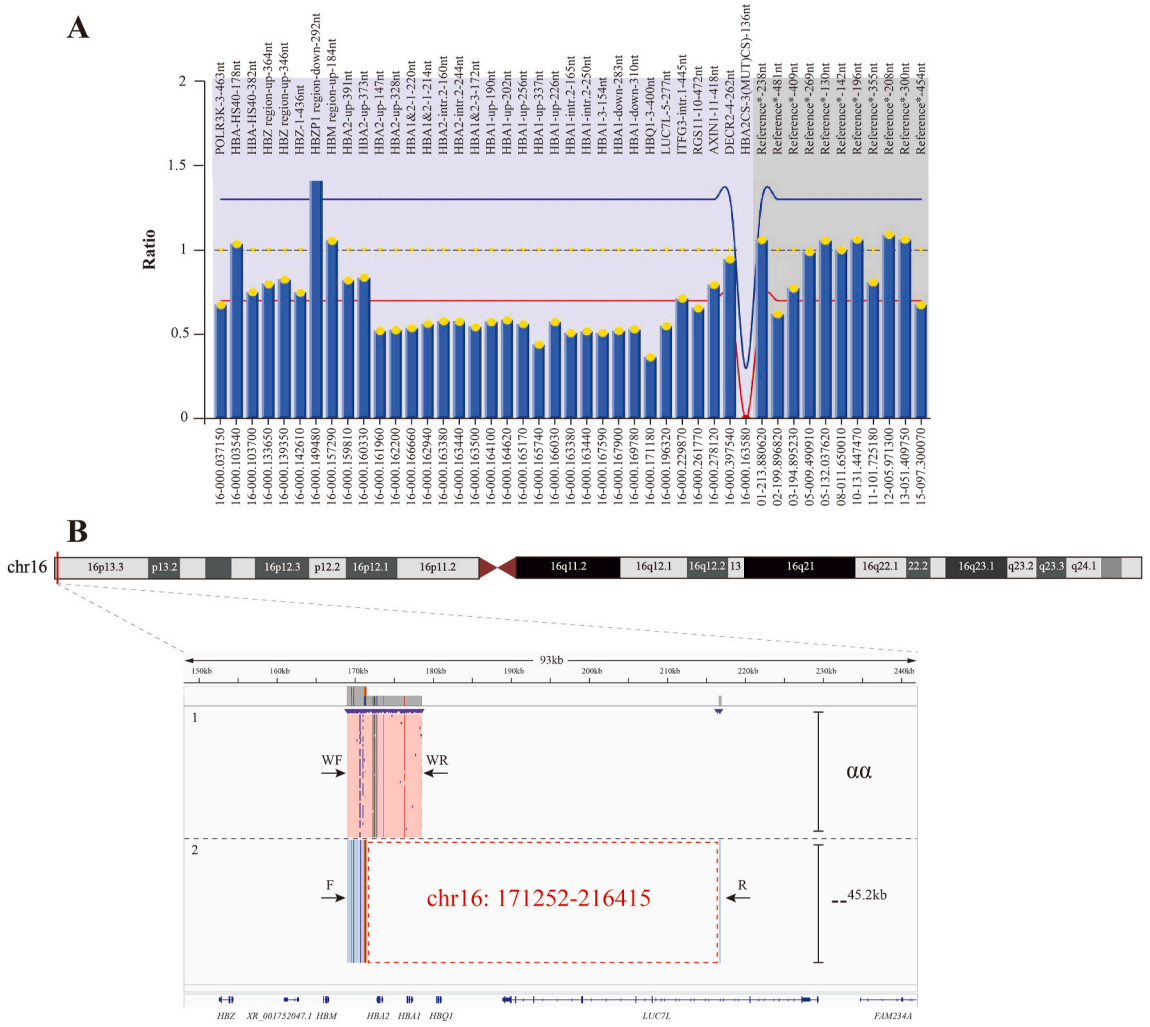

Fig. 2. The results of Multiplex ligation-dependent probe amplification (MLPA) and the SMRT sequencing for the subject. A) MLPA result showed the deletion ranging from upstream of *HBA2* to the exon 5 of LUC7L. B) SMRT sequencing result showed the exact length of the deletion is 45.2 kb, locating at *HBA* gene (Chr16: 171252–216415, GRch38/hg 38).

embryonic hemoglobin fractions that differs from those observed in other α-thal. Embryonic hemoglobin fractions (Epsilon4, Hb Gower1 and Hb Portland) can only be detected in the second or third trimester of pregnancy in cases of Hb Bart's hydrops fetalis syndrome. Hb Bart's hydrops fetalis syndrome is attributed to the deletion of all α-globin genes, which results in no production of α-globin chains. As a consequence, the γ-globin chain, which predominantly produced *in utero*, forms Hb Bart's (γ^4) [9,10]. Hb Bart's is non-functional hemoglobin that is incapable of facilitating oxygen transfer. The occurrence of Hb Bart's hydrops fetalis syndrome almost invariably results in fetal demise or neonatal mortality. Moreover, Hb Bart's disease is frequently associated with significant maternal morbidity and even mortality. Therefore, it is necessary to diagnose Hb Bart's hydrops fetalis syndrome as soon as possible. Several studies [5,11–13] have shown that ultrasound examination is reproducible and highly effective in detection of fetuses with Hb Bart's hydrops fetalis syndrome, even though in the late first trimester or early second trimester. In this study, thanks to the abnormal ultrasound results have been found in early detection and as shown in Fig. 1. Hence, placental thickening combined an increase in CTR and MCA-PSV that were predicting fetal Hb Bart's disease, even though in the late first trimester or early second trimester.

For parents with a pregnancy in which one partner was α^0-thal and the other presented a classical α-thal trait, it is crucial to identify novel large deletional mutations in the α-globin genes in prenatal diagnosis with the utmost expediency. To date, a number of molecular techniques for detecting globin gene variants have been developed, according to the genetic basis and various forms of α-thal, including gap-PCR, MLPA, droplet digital polymerase chain reaction (ddPCR), and so on [14–16]. Despite the extensive literature attesting to the efficacy of these techniques in accurately determining genotypes, their utility in identifying novel large deletional mutations in the α-globin genes remains constrained. Fortunately, with the rapid development of sequencing techniques, SMRT sequencing has been used to identify both common and novel variants in thalassemia. Therefore, using SMRT sequencing, we have identified a novel case of Hb Bart's hydrops fetalis with a typical α^0-thal ($-^{SEA}$ deletion) and a novel 45.2 kb deletion encompassing

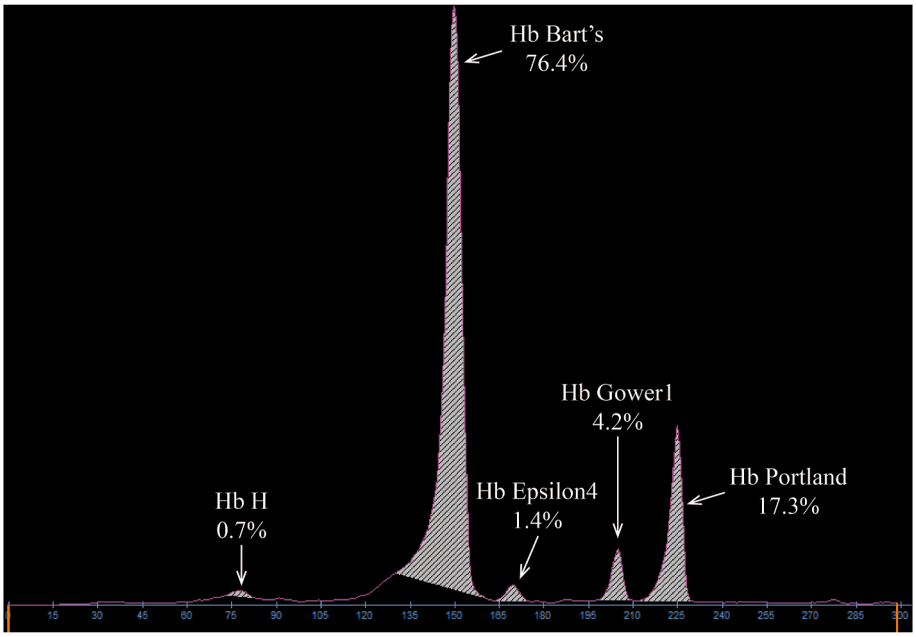

Fig. 3. Hb analysis of the fetal in cord blood.

three functional globin genes (*HBM*, *HBA2*, *HBA1*, Fig. 2), which was inherited from the husband. Compared with the conventional methods, SMRT sequencing, with the read length of product can be as long as dozens of kb without interrupting the DNA generated in a process termed Pacific Biosciences (PacBio) High-Fidelity (HiFi) sequencing via only a single experiment and it is more accurate in repeat regions and highly homologous regions [17,18].

In summary, given the high prevalence and broad mutation spectrum of α-thal across ethnic groups, it is necessary to select appropriate methods according to the type of variant associated with a specific population and clinical phenotype in a diagnostic laboratory. SMRT sequencing represents an effective method for achieving an accurate diagnosis.

CRediT authorship contribution statement

Zeyan Zhong: Conceptualization, Funding acquisition, Writing – original draft. **Dina Chen:** Data curation, Software. **Zhiyang Guan:** Formal analysis, Investigation. **Guoxing Zhong:** Methodology. **Zhiyong Wu:** Validation. **Jianmin Chen:** Visualization. **Jianhong Chen:** Resources, Supervision, Writing – review & editing.

Statement of ethics

The study was reviewed and approved by the Medical Ethics Committee of Huizhou First Maternal and Child Health Care Hospital (20231107-B3). All procedures were performed by The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Disclosure statement

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

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Zeyan Zhong reports financial support was provided by Medical Scientific Research Foundation of Guangdong Province. None reports a relationship with None that includes:. None has patent None pending to None. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in

this paper.

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Data availability

The data that has been used is confidential.

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