RESEARCH ARTICLE

Molecular prenatal diagnosis of alpha and beta thalassemia in pregnant Hakka women in southern China

Pingsen Zhao^{1,2,3} | Heming Wu^{1,2,3} | Zhixiong Zhong² | Liubing Lan^{3,4} | Mei Zeng^{3,4} | Hualan Lin^{3,4} | Huaxian Wang^{1,2,3} | Zhiyuan Zheng^{1,2,3} | Luxian Su^{1,2,3} | Wei Guo^{1,2,3}

¹Clinical Core Laboratory, Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, Meizhou, China

²Center for Precision Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, Meizhou, China

³Prenatal Diagnosis Center, Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, Meizhou, China

⁴Department of Obstetrics, Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, Meizhou, China

Correspondence

Pingsen Zhao, Clinical Core Laboratory, Center for Precision Medicine, Meizhou People's Hospital, Meizhou Hospital Affiliated to Sun Yat-sen University, Meizhou, China.
Email: zhaopingsen01@163.com, zhaopingsen@hotmail.com.

Funding information

Medical Scientific Research Foundation of Guangdong Province, China, Grant/Award Number: A2016306; National Major Scientific and Technological Special Project for "Significant New Drugs Development", Grant/Award Number: 2015ZX09102025; The National Key Research and Development Program of China, Grant/Award Number: 2016YFD0050400 and 2017YFD0501705; Natural Science Foundation of Guangdong Province, China, Grant/Award Number: 2014A030307042 and 2016A030307031; Key Scientific and Technological Project of Meizhou People's Hospital (Huangtang Hospital); Meizhou Hospital Affiliated to Sun Yat-sen University, Guangdong Province, China, Grant/Award Number: MPHKSTP-20170102

Background: To date, there has been no systematic study of DNA-based prenatal diagnosis of thalassemia in pregnant Hakka women in southern China.

Methods: A total of 279 pregnant Hakka women with confirmed cases of thalassemia who had been treated at the Meizhou People's Hospital in China's Guangdong Province from January 2014 to December 2016 were here enrolled. Genomic DNA was extracted from peripheral blood of couples and villus, amniotic fluid, or fetal cord blood. DNA-based diagnosis was performed on the tissues of fetuses whose parents had tested positive for α - and β -globin gene mutations were found using polymerase chain reaction (PCR) and flow-through hybridization technique. Follow-up visits were performed 6 months after the fetuses were born. Prenatal diagnosis was performed on 279 fetuses in at-risk pregnancies.

Results: Here, 211 α -thalassemia fetuses were confirmed, including 41 (19.43%) that tested positive for Bart's hydrops syndrome and 15 (7.11%) for Hb H disease. There were 103 (48.81%) heterozygotes. β -thalassemia was confirmed in 68 fetuses, including 23 (33.82%) with severe thalassemia and 27 (39.71%) heterozygotes. Another 12 cases were confirmed with α + β -thalassemia, including three cases of severe β -thalassemia. DNA-based testing prenatal diagnosis of thalassemia was found to be highly reliable.

Conclusions: Our findings provide key information for clinical genetic counseling of prenatal diagnosis for major thalassemia in pregnant Hakka women in southern China.

KEYWORDS

fetuses, genetic mutations, Hakka women, pregnant women, prenatal diagnosis, southern China, thalassemia

1 | INTRODUCTION

Thalassemia is an inherited autosomal recessive disorder characterized by microcytic hypochromic anemia resulting from reduced or absent synthesis of one or more of the globin chains of hemoglobin.^{1,2} There are two main types of thalassemia, α and β .^{3,4} The incidence of thalassemia is high among people living in parts of Mediterranean countries, the Middle East, Africa, and Southeast Asia, including southern China.⁵

The major cause of α -thalassemia includes deletions that remove one or both α -globin genes from the affected chromosome 16.⁵ The symptoms of α -thalassemia are caused by deletion or non-deletional mutation of one ($-\alpha/\alpha\alpha$, $\alpha^T\alpha/\alpha\alpha$, or $\alpha\alpha^T/\alpha\alpha$) or both ($-\alpha/\alpha\alpha$ or $-\alpha/-\alpha$) α -globin genes. Hb H (β_4) disease is caused by loss or inactivation of three α -globin genes ($-\alpha/\alpha$ or $-\alpha^T\alpha$ or $-\alpha\alpha^T$). Severe anemia associated with Hb Bart's (γ_4) disease ($-\alpha/-\alpha$) can cause fetal death at the time of birth.⁶ β -thalassemia is a group of diseases of hemoglobin, most of which result from point mutations or deletions within the β -globin gene on the chromosome 11.^{7,8} β -thalassemia is classified into two types, mutations affecting the β -globin gene resulting in β^+ -thalassemia with a reduction in β -globin and β^0 -thalassemia with total absence of β . More than 30 different mutations of β -thalassemia have been identified in Chinese populations.⁹ Parents who are carriers for the thalassemia gene may give birth to fetuses with major thalassemia. Prenatal diagnosis can prevent the births of children with major thalassemia. Prenatal diagnosis of thalassemia is widely practiced using molecular biological technology.

The Hakka people are an intriguing Han Chinese populations that mainly inhabit southern China, which is characteristic of their unique culture and is distinct from the traditional culture of southern Hans, but show lots of similarities to that of Northern Hans, including some features in dialects, life styles, customs, and habits.¹⁰ The prevalence of thalassemia in the Meizhou Hakka population in Guangdong Province of China is high.^{11,12} However, there has been no systematic study on DNA-based prenatal diagnosis of thalassemia in this area. Our study provides a retrospective analysis of fetuses from 279 pregnant Hakka women confirmed to have thalassemia using molecular prenatal diagnosis in Meizhou, Guangdong Province of China.

2 | MATERIALS AND METHODS

This retrospective study was conducted from January 2014 to December 2016. Here, 279 pregnant Hakka women with thalassemia who sought treatment at the Meizhou People's Hospital (Huangtang Hospital) in Guangdong Province. Most of them come from seven areas within Meizhou City, Guangdong Province. There are 21 women from Ganzhou City in Jiangxi Province. All of them were Hakka. Before the prenatal diagnosis, the genotypes of both father and mother were tested. This study was approved by the Ethics Committee of Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated with Sun Yat-sen University. All participants signed informed consent forms.

Fetal sampling was performed in three ways: (1) Chorionic villi sampling (CVS) was conducted at 10-12 weeks of gestation; (2) amniotic fluid (10 mL) was collected at 15-22 weeks; and (3) cord blood (1.0-2.0 mL) was sampled at 18-28 weeks.^{13,14} All procedures were carried out under ultrasonography guidance. At the same time, 2 mL maternal peripheral blood was sampled for short tandem repeats (STR) analysis using Sanger sequencing.^{15,16}

Villi were washed twice with normal saline. Villus tissue was removed from 15 mL centrifuge tubes with hook tweezers, placed in Petri dishes with saline. Parts of villi were removed from stripped maternal decidua with hooks at low magnification. Some of the tissues were used for the extraction of fetal DNA. The remaining villi were soaked in saline and frozen at -80°C .^{17,18}

Amniocentesis cells were isolated from 10 mL amniocentesis fluid using a centrifuge at 1000 g for 10 minutes. The supernatant was discarded and 800 μL sterile saline was used to resuspend cell pellets. Then 400 μL of pellet was removed for DNA extraction.^{17,19} As for samples of umbilical cord blood, 200 μL of umbilical cord blood was taken to extract DNA.²⁰

Maternal contamination was tested by STR analysis using agents from Beijing Microread Genetics. Because fetal samples are always contaminated by maternal tissue, STR analysis was conducted before testing of the fetal samples.^{21,22} The STR markers (D19S433, D5S818, D21S11, D18S51, D6S1043, AMEL, D3S1358, D13S317, D7S820, D16S539, CSF1PO, Penta D, D2S441, vWA, D8S1179, TPOX, Penta E, TH01, D12S391, D2S1338, and FGA) were sequenced using an ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). When all the polymorphic alleles of the mother were absent from fetal DNA, the fetal sample was considered free of maternal contamination.

DNA was extracted from the cells of chorionic villus, amniotic fluid or cord blood using a Qiagen DNA extraction kit (Qiagen, Valencia, CA, USA). Gap-polymerase chain reaction (gap-PCR) and flow-through hybridization technology (HybriBio Limited, Chaozhou, China) were used to detect the α -thalassemia mutations, including deletion of $-\text{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$ and non-deletion of Hb Constant Spring ($\alpha^{\text{CS}}\alpha$) (CD142, TAA $\rightarrow$ CAA), Hb Quong Sze ($\alpha^{\text{QS}}\alpha$) (CD125, CTG $\rightarrow$ CCG), and Hb Westmead ($\alpha^{\text{WS}}\alpha$) (CD122, CAC $\rightarrow$ CAG). Mutations in the β -globin gene were detected using polymerase chain reaction (PCR) and flow-through hybridization technology (HybriBio Limited, China), specifically detection of the following 16 common non-deletion β -globin gene mutations: CD41-42(-TCTT), CD43(G $\rightarrow$ T), IVS-II-654(C $\rightarrow$ T), CD17(A $\rightarrow$ T), CD14-15(+G), -28(A $\rightarrow$ G), -29(A $\rightarrow$ G), CD71-72(+A), βE (G $\rightarrow$ A), IVS-I-1(G $\rightarrow$ T), IVS-I-1(G $\rightarrow$ A), CD27-28(+C), IVS-I-5(G $\rightarrow$ C), Cap+40-43(-AAAC), initiation codon (T $\rightarrow$ G) and CD31(-C). Two pairs of primers were used to span most sequences of the β -globin gene.

Statistical analysis was conducted using SPSS 17.0 software (IBM, Armonk, NY, USA). The prevalence of thalassemia was evaluated using descriptive statistics.

3 | RESULTS

From January 2014 to December 2016, prenatal diagnosis was performed in 279 fetuses in 278 mothers. The age group for father and

TABLE 1 Summary of prenatal diagnosis of thalassemia as indicated by DNA analysis

Samples	α -thalassemia			β -thalassemia		$\alpha\beta$ -thalassemia			Normal	Total
	Major	HbH	Carrier	Major	Carrier	Major	Intermedia	Carrier		
CVS	6		6		2			1	5	20
AF	32	14	97	20	25	2	2	7	56	255
CB	3								1	4
Total	41	14	103	20	27	2	2	8	62	279

CVS, chorionic villi sample, AF, amniotic fluid, CB, cord blood.

TABLE 2 Molecular prenatal diagnosis of α -thalassemia in pregnant Hakka women in southern China

Genotype	No. of fetuses	Constituent ratio (%)	Genotype	No. of fetuses	Constituent ratio (%)
$-\alpha^{3.7}/\alpha\alpha$	8	5.06	$-\text{SEA}/-\alpha^{3.7}$	6	3.80
$-\alpha^{4.2}/\alpha\alpha$	2	1.27	$-\text{SEA}/-\alpha^{4.2}$	1	0.63
$\alpha^{\text{CS}}\alpha/\alpha\alpha$	4	2.53	$-\text{SEA}/\alpha^{\text{CS}}\alpha$	4	2.53
$\alpha^{\text{WS}}\alpha/\alpha\alpha$	2	1.27	$-\text{SEA}/\alpha^{\text{WS}}\alpha$	3	1.90
$-\text{SEA}/\alpha\alpha$	87	55.06	$-\text{SEA}/-\text{SEA}$	41	25.95

TABLE 3 Molecular prenatal diagnosis of β -thalassemia in pregnant Hakka women in southern China

Genotype	No. of fetuses	Constituent ratio (%)	Genotype	No. of fetuses	Constituent ratio (%)
-28/N	3	6.25	CD17/CD27-28	1	2.08
-29/N	1	2.08	CD17/CD41-42	1	2.08
CD17/N	2	4.17	CD17/IVS-II-654	1	2.08
CD27-28/N	2	4.17	CD27-28/CD41-42	1	2.08
CD41-42/N	8	16.67	CD41-42/IVS-II-654	7	14.58
IVS-II-654/N	10	20.83	-28/CD41-42	1	2.08
Cap+40-43(-AAAC)/N	1	2.08	-28/IVS-II-654	2	4.17
Cap+40-43(-AAAC)/Cap+40-43(-AAAC)	1	2.08	CD41-42/CD41-42	2	4.17
-28/-28	1	2.08	IVS-II-654/IVS-II-654	1	2.08
CD17/CD17	1	2.08	IVS-II-654/CD71-72	1	2.08

mother ranged from 19 to 48 years old and 16 to 42 years old, respectively. The mean gestational age at which this invasive procedure was carried out was 19.7 ± 3.34 weeks. The parents with α -thalassemia showed Hb A₂ <3.5% or normal. The parents with β -thalassemia showed Hb A₂ over 3.5% and mean corpuscular volumes (MCV) of <80 fL.

Here, 279 fetuses from at-risk pregnancies were subjected to prenatal diagnosis. We tested 20 CVS samples, 255 amniocentesis fluid samples, and four cord blood samples (Table 1). The 211 α -thalassemia families consisted of 41 (19.43%) Bart's hydrops syndrome, 15 (7.11%) Hb H disease, 103 (48.81%) heterozygote (Tables 2 and 4), and the 68 β -thalassemia families included 23 (33.82%) cases of severe thalassemia and 27 (39.71%) heterozygotes (Table 3). There were 12 cases with $\alpha\beta$ -thalassemia, including three cases of severe β -thalassemia (Table 4).

There were no normal controls for individual loci in the kit we used. In this study, we detected a Cap+40-43 (-AAAC) homozygous

mutation of β -thalassemia by sequencing (Fig. S1). The fetus inherited Cap+40-43 (-AAAC) mutations from his/her mother and father. Prenatal diagnoses indicated 41 cases of Bart's hydrops syndrome and 23 cases of severe β -thalassemia. Patients provided informed consent for the testing.

We detected 15 cases of HbH disease, eight cases of deletion HbH disease, three cases of $-\text{SEA}/\alpha^{\text{WS}}\alpha$, and four cases of $-\text{SEA}/\alpha^{\text{CS}}\alpha$. The mothers of three cases of $-\text{SEA}/\alpha^{\text{CS}}\alpha$ and one case of fetal malformation ($-\text{SEA}/-\alpha^{3.7}$ at the same time) decided to terminate the pregnancy. The remaining parents chose to continue the pregnancy, and the infants showed no severe anemia during the first 6 months of follow-up after birth.

All the mothers who decided to terminate the pregnancy were followed up until termination, at which time tissues were examined and the diagnoses were confirmed. Cases of non-severe thalassemia showed no severe anemia phenotype within the first half year after

TABLE 4 Molecular prenatal diagnosis of α + β -thalassemia in pregnant Hakka women in southern China

Genotype	No. of fetuses	Constituent ratio (%)	Genotype	No. of fetuses	Constituent ratio (%)
$-\alpha^{4.2}/\alpha\alpha$, CD41-42/N	1	8.33	$-\text{SEA}/\alpha\alpha$, CD41-42/N	1	8.33
$-\text{SEA}/\alpha\alpha$, IVS-II-654/N	1	8.33	IVS-II-654/N, $-\text{SEA}/-\alpha^{3.7}$	1	8.33
$-\alpha^{3.7}/-\alpha^{3.7}$, CapM/N	1	8.33	$-\alpha^{3.7}/\alpha\alpha$, IVS-II-654/-28	1	8.33
$-\alpha^{3.7}/\alpha\alpha$, IVS-II-654/N	2	16.67	$-\alpha^{3.7}/\alpha\alpha$, CD41-42/IVS-II-654	1	8.33
$-\alpha^{4.2}/\alpha\alpha$, IVS-II-654/N	1	8.33	$-\text{SEA}/\alpha\alpha$, IVS-II-654/IVS-II-654	1	8.33
$-\text{SEA}/\alpha\alpha$, -28/N	1	8.33			

birth, which was fully consistent with the results of prenatal diagnosis. Our prenatal diagnosis proved to be highly accurate and reliable.

4 | DISCUSSION

Thalassemia is widely distributed in southern China, particularly in the Guangdong, Guangxi, and Hainan Provinces.^{23,24} In Guangdong, 8.53% of the general population carries the α -thalassemia gene and 2.54% carries the β -thalassemia gene.¹¹ Meizhou is located in eastern Guangdong Province with a resident population of 5.28 million and an annual birth rate of 12.45‰ (official web site of the Bureau of Health and Family Planning of Meizhou, China), such that about 1808 babies each year are expected to be born with a severe form of thalassemia, which poses a public health problem. However, there have been no systematic studies of prenatal diagnosis of thalassemia in this region. In this study, all participants were Hakka from southern China. They were tested for mutations in the α - or β -globin genes using DNA-based molecular diagnosis. Molecular prenatal diagnosis is one of the most effective and direct methods of preventing major thalassemia. These are several useful procedures available to couples at risk to provide them with the information they need to decide whether to continue their pregnancies. This study is the first systematic prenatal diagnosis of thalassemia in the Hakka region in southern China.

This study covered 159 couples in which both partners were heterozygous for the Southeast Asian type deletion ($-\text{SEA}$), accounting for 56.99% of the total study population. The $-\text{SEA}$ deletion is the most common type of thalassemia in this area. Such couples have a 1/4 chance of producing a fetus with Hb Bart's.²⁵ We identified 41 fetuses with Bart's hydrops syndrome, accounting for 25.79%. We also identified 41 fetuses with Hb Bart's hydrops syndrome and 23 cases with severe β -thalassemia. All these mothers decided to terminate their pregnancies. Altogether, the use of this test prevented the birth of 23 newborns with major β -thalassemia syndrome.

HbH disease can be divided into two types according to genotype, deletion HbH disease and non-deletion HbH disease. Because the vast majority of non-deletion mutations affect the function of the $\alpha 2$ gene, the clinical manifestation of non-deletion HbH disease is more serious than deletion HbH disease, excepting $-\text{SEA}/\alpha$

$\text{WS}\alpha$.²⁶ The clinical phenotype of deletion HbH disease and genotype $-\text{SEA}/\alpha$ $\text{WS}\alpha$ varies from mild anemia to transfusion-dependent severe anemia. Prenatal diagnosis allows the parents to decide whether to continue the pregnancy. Here, we detected 15 cases of HbH disease, eight cases of deletion HbH disease, three cases of $-\text{SEA}/\alpha$ $\text{WS}\alpha$, and four cases of $-\text{SEA}/\alpha$ $\text{CS}\alpha$. The three cases of $-\text{SEA}/\alpha$ $\text{CS}\alpha$ and one case of fetal malformation ($-\text{SEA}/-\alpha^{3.7}$ at the same time) were terminated. All other parents made the informed choice to continue the pregnancy. They showed no severe anemia phenotype within the first 6 months after birth and showed good growth upon follow-up.

We identified two fetuses with Hb Bart's disease and one fetus with deletion Hb H disease. In all three cases, one parent was an α -thalassemia carrier and the other was an α + β -thalassemia carrier. These results indicate fetuses at risk for being homozygous for α -thalassemia. We suggest that it should be considered standard to test for α -thalassemia mutations in the β -thalassemia gene when the other partner in the couple is also an α -thalassemia carrier because there is a one-in-four chance that they will produce a fetus with Hb Bart's or Hb H disease.

At first, to eliminate the risk of maternal cell contamination (MCC), we performed two rounds of DNA analysis. The first analysis was performed after amniocentesis or CVS, and the second analysis was carried out after fetal DNA was re-extracted from amniocyte or CVS after culture. When we used STR detection technology to rule out maternal cell contamination, we performed two rounds of DNA analysis after confirming that there was no maternal cell contamination. Using these methods, we successfully minimized the risk of maternal cell contamination to guarantee the accuracy and reliability of the results.²¹

The term invasive prenatal diagnosis refers to obtaining fetal material by CVS, amniocentesis, or cordocentesis. Mutations in the α - and β -globin genes can be detected by molecular prenatal diagnosis and further definitive diagnosis of the fetus can be performed. The most critical issue in any type of invasive prenatal molecular testing is maternal cell contamination, especially when a fetus is found to have inherited a particular mutation from the mother. The best practice is to test all prenatal samples for maternal cell contamination. The recent successful studies of fetal DNA in maternal plasma may foster the development of a new form of prenatal testing that is non-invasive for the fetus.^{27,28}

In conclusion, we here conducted a systematic study of DNA-based prenatal diagnoses of 279 fetuses in at-risk pregnancies to in parents thalassemia in the Meizhou, a very high-incidence area in China. Diagnoses of α -thalassemia were confirmed, 19.43% of these cases were Bart's hydrops syndrome, 7.11% Hb H disease, and 48.81% of study population. Cases of β -thalassemia were 33.82% severe and 39.71% heterozygous. There were 12 confirmed cases of $\alpha\beta$ -thalassemia. This showed DNA-based testing prenatal diagnosis of thalassemia to be highly reliable. Our findings provide key information for clinical genetic counseling of prenatal diagnosis of major thalassemia in pregnant Hakka women in Meizhou, China.

ACKNOWLEDGMENTS

This study was supported by Natural Science Foundation of Guangdong Province, China (Grant No.: 2014A030307042 to Pingsen Zhao), Natural Science Foundation of Guangdong Province, China (Grant No.: 2016A030307031 to Pingsen Zhao), the National Key Research and Development Program of China (Grant No.: 2016YFD0050400 to Pingsen Zhao), Medical Scientific Research Foundation of Guangdong Province, China (Grant No.: A2016306 to Pingsen Zhao), and Key Scientific and Technological Project of Meizhou People's Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, Guangdong Province, China (Grant No.: MPHKTSP-20170102 to Pingsen Zhao). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of Meizhou People's Hospital (Huangtang Hospital), and Meizhou Hospital Affiliated with Sun Yat-sen University and all patients signed informed consent forms.

AUTHOR CONTRIBUTIONS

Pingsen Zhao conceived and designed the experiments; Heming Wu, Zhixiong Zhong, Liubing Lan, Mei Zeng, Hualan Lin, Huaxian Wang, Zhiyuan Zheng, Luxian Su, and Wei Guo performed the experiments; Pingsen Zhao and Heming Wu analyzed the data; Pingsen Zhao and Heming Wu wrote the article. Pingsen Zhao, Heming Wu, and Zhixiong Zhong reviewed the article. All authors have read and approved the final article.

EMPLOYMENT OR LEADERSHIP

None declared.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

REFERENCES

- Muncie HL Jr, Campbell J. Alpha and beta thalassemia. *Am Fam Physician*. 2009;80:339-344.
- Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bull World Health Organ*. 2001;79:704-712.
- Cohen AR, Galanello R, Pennell DJ, Cunningham MJ, Vichinsky E. Thalassemia. *Hematology Am Soc Hematol Educ Program* 2004;1:14-34.
- Weatherall DJ. Thalassemia: the long road from bedside to genome. *Nat Rev Genet*. 2004;5:625-631.
- Zeng YT, Huang SZ. Alpha-globin gene organisation and prenatal diagnosis of alpha-thalassaemia in Chinese. *Lancet*. 1985;1:304-307.
- Hartevelde CL, Higgs DR. Alpha-thalassaemia. *Orphanet J Rare Dis*. 2010;5:13.
- Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis*. 2010;5:11.
- Rund D, Rachmilewitz E. Beta-thalassemia. *N Engl J Med*. 2005;353:1135-1146.
- Xie J, Zhou Y, Xiao Q. [Screening and molecular diagnosis for a rare genotype of beta-thalassemia intermedia]. *Zhonghua Er Ke Za Zhi*. 2016;54:223-224.
- Wang WZ, Wang CY, Cheng YT, et al. Tracing the origins of Hakka and Chaoshanese by mitochondrial DNA analysis. *Am J Phys Anthropol*. 2010;141:124-130.
- Xu XM, Zhou YQ, Luo GX, et al. The prevalence and spectrum of alpha and beta thalassaemia in Guangdong Province: implications for the future health burden and population screening. *J Clin Pathol*. 2004;57:517-522.
- Al-Owain M, Imtiaz F, Shuaib T, et al. Smith-Lemli-Opitz syndrome among Arabs. *Clin Genet*. 2012;82:165-172.
- Kaviani A, Perry TE, Dzakovic A, Jennings RW, Ziegler MM, Fauza DO. The amniotic fluid as a source of cells for fetal tissue engineering. *J Pediatr Surg*. 2001;36:1662-1665.
- Old J, Petrou M, Varnavides L, Layton M, Modell B. Accuracy of prenatal diagnosis for haemoglobin disorders in the UK: 25 years' experience. *Prenat Diagn*. 2000;20:986-991.
- Press MO, Carlson KD, Queitsch C. The overdue promise of short tandem repeat variation for heritability. *Trends Genet*. 2014;30:504-512.
- Darby BJ, Erickson SF, Hervey SD, Ellis-Felege SN. Digital fragment analysis of short tandem repeats by high-throughput amplicon sequencing. *Ecol Evol*. 2016;6:4502-4512.
- Chan AY, So CC, Ma ES, Chan LC. A laboratory strategy for genotyping haemoglobin H disease in the Chinese. *J Clin Pathol*. 2007;60:931-934.
- Yong PJ, McFadden DE, Robinson WP. Developmental origin of chorionic villus cultures from spontaneous abortion and chorionic villus sampling. *J Obstet Gynaecol Can*. 2011;33:449-452.
- Savickiene J, Treigyte G, Baronaite S, et al. Human amniotic fluid mesenchymal stem cells from second- and third-trimester amniocentesis: differentiation potential, molecular signature, and proteome analysis. *Stem Cells Int*. 2015;2015:319238.
- Kile ML, Baccarelli A, Hoffman E, et al. Prenatal arsenic exposure and DNA methylation in maternal and umbilical cord blood leukocytes. *Environ Health Perspect*. 2012;120:1061-1066.
- Weida J, Patil AS, Schubert FP, et al. Prevalence of maternal cell contamination in amniotic fluid samples. *J Matern Fetal Neonatal Med*. 2016;212:1-5.
- Attwell M, Pontikinas E, Nicholls C. Three step maternal mutation detected by STR analysis. *Forensic Sci Int Genet*. 2015;16:138.
- Lin M, Wang Q, Zheng L, et al. Prevalence and molecular characterization of abnormal hemoglobin in eastern Guangdong of southern China. *Clin Genet*. 2012;81:165-171.
- Zheng CG, Liu M, Du J, Chen K, Yang Y, Yang Z. Molecular spectrum of alpha- and beta-globin gene mutations detected in the population

- of Guangxi Zhuang Autonomous Region, People's Republic of China. *Hemoglobin*. 2011;35:28-39.
25. Chui DH. Alpha-thalassaemia and population health in Southeast Asia. *Ann Hum Biol*. 2005;32:123-130.
26. Fucharoen S, Viprakasit V. Hb H disease: clinical course and disease modifiers. *Hematology Am Soc Hematol Educ Program*. 2009;1: 26-34.
27. Drury S, Hill M, Chitty LS. Cell-free fetal DNA testing for prenatal diagnosis. *Adv Clin Chem*. 2016;76:1-35.
28. Capoluongo E, Plebani M. Circulating fetal cell-free DNA and prenatal molecular diagnostics: are we ready for consensus? *Clin Chem Lab Med*. 2014;52:609-611.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Zhao P, Wu H, Zhong Z, et al.

Molecular prenatal diagnosis of alpha and beta thalassemia in pregnant Hakka women in southern China. *J Clin Lab Anal*. 2018;32:e22306. <https://doi.org/10.1002/jcla.22306>