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Research paper

Molecular newborn screening of four genetic diseases in Guizhou Province of South China

Shengwen Huang [a,*], Yin Xu [b], Xingmei Liu [a], Man Zhou [b], Xian Wu [a], Yankai Jia [c,*]

[a] Department of Laboratory, Guizhou Provincial People's Hospital, Guiyang 550002, P.R. China
[b] Department of Gynaecology and Obstetrics, Guizhou Provincial People's Hospital, Guizhou, Guiyang 550002, P.R. China
[c] 3L Bioservices LLC, Midlothian, Virginia 23113, USA

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ABSTRACT

Genetic disorders have been a major concern for public health in China, especially in the rural regions. However, there is little information available about prevalence of many common single-gene disorders in Guizhou Province in the south western part of China. In the present study, we performed a molecular newborn screening for four genetic disorders, including beta-thalassemia (β-thal), glucose-6-phosphate dehydrogenase (G6PD) deficiency, phenylketonuria (PKU), and non-syndromic hearing loss and deafness (NSHL) in this region. A total of 515 newborns were genotyped using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) developed for screening the mutations causing these four disorders, and then confirmed by Sanger sequencing. The results showed that 48 out of 515 newborns were carriers of mutations related to these four diseases, with a frequency of 1 in 11 (9.32%). The carrier frequencies for each disease are: β-thal 2.72%; G6PD deficiency 1.94%; PKU 0.78% and NSHL 4.47%. The genotyping results by MALDI-TOF MS were concordant with Sanger sequencing results within 30 randomly selected samples. This is the first study that reveals carrier frequencies of these four diseases in Guizhou Province. These data provide valuable information for the genetic counseling and disease prevention in Guizhou and southwest China.

1. Introduction

Mendelian disorders or single-gene disorders are caused by mutations in a single gene and can be classified on the basis of their hereditary patterns as autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive. In general, most Mendelian disorders are relatively uncommon but will be serious enough to require medical treatment or hospital care. In some genetic disorders, the affected neonates may have no obvious symptoms during the newborn period or even beyond. These patients often cannot be diagnosed and treated at early age. Newborn screening with different testing methods can identify individuals who have an increased risk of a certain genetic disease. This allows the patients with a serious condition to be identified and treated before symptoms occur (Therrell et al., 2015).

In the past few decades, many new methods and technologies were developed and successfully used for newborn screening, such as various biochemical, immunological and electrophoretic tests. Especially, since the early 1990s, when the tandem mass spectrometry was proposed for newborn screening, an increasing number of potentially detectable congenital metabolic diseases can be identified (Chace et al., 2003). Most of these methods are effective for detecting both symptomatic patients and those patients undergoing newborn screening who are asymptomatic. With the rapid development and application of molecular biology techniques, many molecular techniques have been employed for diagnosis of genetic disorders and newborn screening (Baker et al., 2009; Baker et al., 2015). Molecular screening may allow predictive and pre-symptomatic testing for adult-onset disorders, and additionally, carriers of genetic mutations can be identified. Such diagnoses raise many ethical issues, and are not at present favoured in most newborn screening jurisdictions.

The frequency of each Mendelian disorder often varies with ethnic background, with each ethnic group having one or more Mendelian disorders in high frequency when compared to the other ethnic groups. Beta-thalassemia (β-thal), glucose-6-phosphate dehydrogenase (G6PD) deficiency, phenylketonuria (PKU), non-syndromic hearing loss and deafness (NSHL) are relatively common Mendelian disorders in Chinese population (Liu et al., 2014; Yang et al., 2015; Mei et al., 2013; Xu et al., 2014). Except the frequency of β-thal was well studied in Guizhou province, there is little information available on prevalence of the other three disorders in the region of Guizhou Province located in the south western part of China. In the present study, we aimed to investigate the carrier frequencies of these four genetic disorders in Guizhou Province, and to evaluate the technical efficacy of Matrix-Assisted Laser

* Corresponding author.
E-mail addresses: hsw713@sina.com (S. Huang), yankaijia@gmail.com (Y. Jia).

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Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) for molecular newborn screening of these four Mendelian disorders.

2. Materials and methods

2.1. Subjects

Between February 2013 to November 2013, 515 newborns delivered at the People's Hospital of Guizhou Province were enrolled, 264 males and 251 females. Umbilical cord blood samples were collected and avoided to be contaminated by maternal blood. Blood samples (500 μl) were collected into tubes containing EDTA then stored at 4 °C for genomic DNA extraction. This study was approved by the ethics review committee of the People's Hospital of Guizhou Province. Informed consents were obtained from parents of the newborns before the blood samples were collected.

2.2. Target mutations

Among the four genetic disorders investigated in this study, beta-thal, G6PD deficiency and PKU are each caused by pathogenic gene named *HBB*, *G6PD* and *PAH* respectively. NSHL is a heterogeneous class of disorders showing different pattern of inheritance, involving a multitude of different genes (Xiao and Xie, 2002). As the main inheritance pattern of NSHL is autosomal recessive (AR), three major AR genes (*GJB2*, *GJB3* and *SLC26A4*) were selected in this study. *MT-RNR1* gene was also selected because it is the most common pathogenic mitochondrial gene resulting in NSHL. Totally 44 pathogenic variants in the seven genes which are common in the Chinese population and one silent polymorphism of 1311C > T in *G6PD* were selected arbitrarily for this study (Table 1).

2.3. DNA preparation and primers design

Genomic DNA was extracted from 500 μl of EDTA-anticoagulated blood with TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's recommendations. DNA Samples were diluted to a standard concentration of 10–20 ng/μl. Primers for the polymerase chain reaction (PCR) and single-base primer extension reaction were designed by the MassArray Assay Design 3.1 software (Sequenom, San Diego, CA, USA).

2.4. PCR and iPLEX reaction

Each PCR had a total volume of 5 μl, which contained 0.5 μl of PCR buffer, 0.4 μl of 25 mM $MgCl_2$, 0.1 μl of 25 mM dNTP Mix, 1 μl of 0.5 μM Primer Mix, 0.2 μl of 5 U/μl HotStar Taq, 1 μl of standardized DNA sample. PCR master mix was prepared for 384-well PCR plate and dispensed to each well. PCR amplification was performed with the following condition: initial denaturation at 94 °C for 15 min, followed by 45 cycles of denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, and at last extension at 72 °C for 3 min. In order to remove remaining, nonincorporated dNTPs from amplification products, 2 μl of shrimp alkaline phosphatase enzyme (SAP) cocktail was added to each well of the 384-well plate after amplification, and incubated at 37 °C for 40 min, followed by incubation at 80 °C for 5 min. After cleanup, 2 μl of primer extension reaction cocktail was added to each well. Locus-specific primer extension reaction (iPLEX assay) was performed with the following cycling program: denaturation at 94 °C for 30s, followed by 40 cycles of denaturation at 94 °C for 5 s, annealing at 52 °C for 5 s, and extension at 80 °C for 5 s, then last extension step at 72 °C for 3 min.

Table 1
Diseases and target mutations selected in this study.

Disease	Gene	Mutation
β-thalassemia	*HBB*	−29 A > G, −28 A > G, CD17 A > T, CD26 G > A, CD41–42 delCTTT, CD43 G > T, CD71–72 insA, IVS-II-654 C > T, IVS-I-1G > T, CD27–28 insC
G6PD deficiency	*G6PD*	1388 G > A, 1387 C > T, 1381 G > A, 1376 G > T, 1360 C > T, 1024 C > T, 1004 C > A, 871 G > A, 835 A > G, 592 C > T, 519 C > T, 517 C > T, 487 G > A, 392 G > T, 95 A > G, 1311 C > T
NSHL	*GJB2*	35 delG, 176–191 del16, 235 delC, 299–300 delAT
	GJB3	538 C > T
	MTRNR1	1555 A > G, 1494 C > T
	SLC26A4	2168 A > G, IVS 7–2 A > G
PKU	*PAH*	R111X, R243Q, W326X, Y356X, R413P, R252Q, R241C, Y204C, V399V, IVS4-1G > A

2.5. MALDI-TOF MS analysis

The final products were desalted by resin treatment and transferred to a 384-element SpectroCHIP array (Sequenom, CA). The chip was placed into the MassARRAY Compact Mass Spectrometer (Sequenom, CA) and MALDI-TOF MS analysis was performed according to the manufacturer's protocol. The resultant mass spectrogram data were analyzed using MassArray Typer software that automatically translated the mass of the observed primers into a genotype for each reaction.

2.6. Validation of MALDI-TOF MS method

Thirty DNA samples were randomly selected from each genotype groups of 1311C > T polymorphism determined by MALDI-TOF MS method to re-genotyping by Sanger sequencing. Bidirectional sequencing was done conventionally on an ABI Prism DNA Analyzer Model 377 (Applied Biosystems, USA). The concordance between MALDI-TOF MS method and Sanger sequencing were compared.

3. Results

A total of 48 newborns were identified as carriers of these four diseases, with a total carrier frequency of 1 in 11 (9.32%). Of the 48 carriers, 45 carry only one mutation in one of these genes mentioned above, three carry two mutations in different genes. That is one carry *HBB* CD17 A > T with *G6PD* 1388G > A, one carry *HBB* CD17 A > T with *G6PD* 1024C > T, the third one carry *HBB* CD41–42 delCTTT with *GJB2* 235 delC. Except four male newborns were hemizygous for a mutation on *G6PD* gene, other 44 were heterozygous carriers for one of these mutations.

Among the ten mutations in the *HBB* gene selected in this study, four were detected in 14 newborns. As shown in Table 2, all of the 14 individuals were heterozygous for one of the four mutations, and thus the carrier frequency of β-thal was 2.72% (14/515). CD41–42 was the most frequently encountered mutation with a constituent ratio of 42.9%, followed by CD17 (35.7%), IVS-II-654 (14.4%) and CD71–72 (7.1%).

For G6PD deficiency, ten newborns were identified to have *G6PD* mutations with a detection rate of 1.94%. The numbers of males and females carrying *G6PD* mutation were four and six respectively, with the detection rates of 1.52% (4/264) and 2.39% (6/251) respectively, and all of the six females were heterozygous carriers. Five kinds of *G6PD*

Table 2
Carrier frequency of β-thalassemia.

Mutation	Number of carriers (n)	Frequency (%)	Ratio (%)
CD41–42	6	1.17	42.9
CD17	5	0.97	35.7
IVS-II-654	2	0.39	14.3
CD71–72	1	0.19	7.1
Total	14	2.72	100.0