Large-scale screening of thalassemia in Ji'an, P.R. China

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Abstract

Background: To evaluate the prevalence of alpha- and beta-thalassemia in Ji'an City, Jiangxi Province, 28,941 people in the region were genetically screened to identify various thalassemia genotypes. Methods: High-throughput amplicon sequencing and gap-PCR was used to screen 301 thalassemia alleles in 28,941 people in the region. Pregnant women were the focus of this screening, and if a pregnant woman harbored mutations in a thalassemia-inducing gene, her spouse was also genetically tested. Results: Of the participants, 2,380 people were carriers of thalassemia, with at least one thalassemia allele, including 1,742 alpha-thalassemia carriers, 686 beta-thalassemia carriers and 48 composite alpha- and beta-thalassemia carriers. The total carrying rate of thalassemia in Ji'an was 8.22%, and the carrying rates of alpha- and beta-thalassemia were 6% and 2.37%, respectively. In addition, the first measured carrier rate of composite alpha- and beta-thalassemia in Ji'an was 0.17%. According to the geographical distribution of the 1,742 alpha -thalassemia carriers, the city with the highest carrier rate was Suichuan, followed by Wan’an and Taihe. According to the geographical distribution of the 686 beta-thalassemia carriers, the top three cities with high carrier rates were Suichuan, Wan'an and Xiajiang, sequentially. Conclusions: This research emphasizes the importance of large-scale population screening and that comprehensive molecular epidemiology data are necessary for the proper prevention and treatment of thalassemia. The epidemiological data updated in this research may enable the local government to focus on the severity of this disease and determine a method for effective resource allocation under limited resource conditions.

Background

Hemoglobinopathy is any hereditary blood disease caused by an abnormal hemoglobin molecular structure or an abnormal rate of globin peptide synthesis (thalassemia) [1]. According to the affected globin gene, thalassemia can be divided into alpha-thalassemia or beta-thalassemia. Clinically, there are many symptoms of thalassemia, from asymptomatic to deadly. Patients with such symptoms can be divided into patients with thalassemia minor, intermedia and major according to the clinical severity. Patients with the latter two types are diagnosed with thalassemia. The phenotype severity of this disease is mainly related to the unbalanced degree of the α:non-α chain[2]. This monogenic disease is one of the most widespread and the most harmful, with the largest number of people suffering from it in the world[3, 4]. It is estimated that 1–5% of the global population are carriers of thalassemia mutations[5]. In many Asian countries, alpha-thalassemia and beta-thalassemia generate an increasingly serious health burden[6] The plans designed to avoid and better manage these diseases may significantly improve the health indexes in many developing countries[7]. Accurate population frequency data are the preconditions to formulate these plans.

In China, the high-incidence areas of thalassemia include Guangdong, Guangxi, Hainan, Yunnan, Guizhou, Sichuan, Chongqing, Hunan, Jiangxi and other southern regions[8, 9]. At present, in addition to hematopoietic stem cell transplantation, there is still a lack of an effective treatment for thalassemia major. Many patients need lifelong blood transfusion and chelation therapy, and the only effective

intervening measures to prevent the birth of babies with thalassemia major and thalassemia intermedia are carrier screening and prenatal diagnosis[10]. Comprehensive molecular epidemiology data are necessary for the correct prevention and treatment of thalassemia disease. So far, substantial amounts of genomic data have been generated through next-generation sequencing (NGS) to reveal people's genetic composition and evaluate potential health risks. Research indicates that the scope of variation in thalassemia is wider than previously reported[11], and NGS is an effective method to screen for variation related to thalassemia.

In recent years, a large-scale investigation of thalassemia was carried out in different regions of China[4, 8, 12], but the epidemiologic characteristics of thalassemia among people from Jiangxi Province, as one of the high-prevalence areas, especially those from Ji'an City, are not fully understood[13]. Ji'an is located in the central part of Jiangxi Province, with a total area of 25,300 square kilometers and a population of 4,956,600. It has jurisdiction over 2 districts, 1 city and 10 counties, namely, Jizhou District, Qingyuan District, Jinggangshan City, Ji'an County, Taihe County and Wan'an County, Suichuan County, Yongxin County, Yongfeng County and Jishui County, Xiajiang County, Anfu County, and Xingan County. The purpose of this study was to assess the presence of thalassemia mutations in Ji'an City, Jiangxi Province, China, and to explore the epidemiological characteristics of thalassemia.

Methods

Participants

In this study, our strategy was to screen pregnant women in the Ji'an region with high-throughput amplicon sequencing for the 301 thalassemia alleles (Table s1). This is a government-supported public health service program, and all pregnant women within the jurisdiction volunteered to attend the free testing. If a pregnant woman was identified as a thalassemia carrier, her spouse also agreed to accept a thalassemia genetic test. The detailed screening process is shown in Figure 1. This research was approved by the Ethics Committee of First People's Hospital of Ji'an, and all the participants signed written informed consent.

NGS library preparation

As previously reported, a series of primers were designed based on the characteristics of three genes related to alpha-thalassemia and beta-thalassemia, HBA1, HBA2 and HBB, to achieve the efficient amplification of genes[4]. The primers are related to the following patents: WO/2014/023076, WO/2014/023167, and CN102952877. The detection area of the nondeletion alpha-thalassemia is located on chr16:226667-227546 and chr16:222863-223733 in HBA1 and HBA2, respectively; the beta-thalassemia nondeletion variant is located on chr11:5247713-5248438 and chr11:5246655-5247209 in the HBB gene. Briefly, multiplex PCR techniques were used to amplify and enrich the HBA1, HBA2, and HBB genes in a sample and simultaneously introduce a tag sequence for sample identification by fragmentation and ligation. The PCR products of a plurality of samples (≤96 samples) were pooled into a library. After library preparation, the DNA sequences of the samples in each library were supplemented

with a linker sequence for sequencing and library identification. Libraries were pooled at equal molarity. The pool was sequenced with 2 × 100 paired end reads on a MGISEQ-2000 chip.

Bio-informatics analysis

We used an in-house developed pipeline that integrates multiple in-house programs and open-source softwares for raw reads data quality controls, reads alignment to human reference genome (UCSC build hg19), duplicates marking, SNVs and Indels calling and annotation. Firstly, we use an in-house program, named GaeaFastqQC, for quality control of sequencing data, where low quality reads (reads with more than 10% unidentified base calls or more than 50% low quality base calls) and reads with sequencing adaptor contamination were filtered out, and the clean data was exported. We then used an open-source BWA (BWA 0.7.10, http://bio-bwa.sourceforge.net/) to align the clean data obtained from above step to human genome reference sequences (hg19) using BWA-backtrack algorithm with parameters “-L -l 31 -i 10 -a 500 -e 21 -l -t 10”. This was followed by using two programs based on Picard tools (https://broadinstitute.github.io/picard/) and GATK-Lite-2.3-9 (ftp://anonymous:anon@ftp.broadinstitute.org/pub/gsa/GenomeAnalysisTK/GenomeAnalysisTKLite-2.3-9-gdcdccbb.tar.bz2) to mark duplicates, realign indel and recalibrate the base quality scores. Then program based on samtools was used to sort the bam result and generated the final bam files. Variants calling were performed by using GATK-Lite-2.3-9 with parameters “-genotype_likelihoods_model BOTH -stand_call_conf 30.0 -stand_emit_conf 10.0 -dbsnp file://${dbsnp} –noMultiSampleCall”, and SNVs and Indels were exported. The two sets of variants were merged, filtered and sorted using an in-house program named mergeVariant. Finally, the HbVar[12] and IthaGenes[14] Database was used to annotate the detected SNPs and Indels. Mutations were named according to the literature[15]. The detailed process is shown in Figure 2.

Gap-PCR tests

As for suspected α thalassaemia and β thalassaemia carriers, multiplex gap-PCR was used to detect deletion-type α thalassaemia : –SEA, α3.7, -α4.2, –FIL and –THAI and β thalassaemia deletions : Chinese Ggamma (Agammadeltabeta) 0, South-East Asia type hereditary persistence of fetal hemoglobin (SEA-HPFH) and Taiwanese.

Results

Samples and demographic data

From May 2018 to March 2019, 28,941 participants (1,370 men, 27,519 women and 52 persons unknown) in total participated in this research. The age distribution of the participants was as follows: ≥17 and <20, 438 persons; ≥20 and <30, 17,903 persons; ≥30 and <40, 9,434 persons; ≥40 and <50, 1,159 persons; and ≥50, 5 persons.

Thalassemia carriers identified by NGS

A total of 28,941 people were involved in thalassemia gene screening via NGS. Among these participants, 2,380 people were diagnosed as carriers of thalassemia, including 1,694 carriers of α-thalassemia, 638 carriers of beta-thalassemia and 48 carriers of composite alpha- and beta-thalassemia. The total carrier rate of thalassemia in Ji'an was 8.22%, and the carrier rates of alpha- and beta-thalassemia were 5.85% and 2.20%, respectively. In addition, the first measured incidence rate of composite alpha- and beta-thalassemia in Ji'an was 0.17%.

Among the 1,694 carriers of alpha-thalassemia, 15 different variations were identified in this study and accounted for 19 different genotypes (Table 1). αα/--^{SEA} was the most common alpha-thalassemia genotype, accounting for 41.97%, followed by αα/-α$^{3.7}$, αα/-α$^{4.2}$ and Hb Westmead/αα, accounting for 38.67%, 10.68% and 4.43%, respectively. Notably, compared with the conventional kit for alpha-thalassemia gene testing in China, the screening performed in this study revealed the rare mutations Hb Phnom Penh in 5 cases, and CD 61 AAG>TAG (Lys>Stop), Hb Evanston, initiation codon (A>G), initiation codon (-T), poly A (A>G) and -FIL in 1 case each. A novel alpha-thalassemia allele, HBA1: C.395insT, which can generate polypeptides completely different from the original alpha-globin peptide, was first identified in this study. In this cohort, we also found 20 beta-thalassemia mutations and 21 genotypes (Table 2) among 638 participants. IVS-II-654 (C>T)/β^{N}, codons 41/42 (-TTCT)/β^{N}, codon 17 (A>T) /β^{N}, -28 (A>G)/β^{N} and codons 27/28 (+C)/β^{N} were the most common genotypes in current research, accounting for 31.97%, 23.35%, 13.95%, 12.70% and 4.86%, respectively. In addition, alleles, such as -50 (G>A) beta+, Chinese Ggamma (Agammadeltabeta) 0, CAP +8 (C>T), SEA-HPFH, -72 (T>A) beta+ and codons 8/9 (+G) beta0, were also found with the screening method used in this study but could not be detected by conventional kits for beta-thalassemia genetic testing.

Forty-eight participants were carriers of both alpha- and beta-globin variations (Table 3). Among these carriers, 79.17% of the genotypes were composed of the common deletion of the alpha-globin gene (αα/-α$^{3.7}$, αα/--^{SEA}) and beta-globin gene point mutations, among which composite αα/--^{SEA} and IVS-II-654 (C>T) beta+ heterozygosis was the most common genotype.

Forty-six suspected couples were identified in this research. Among them, 22 couples, 16 couples and 8 couples had the risk of giving birth to Hb H disease patients, patients with Hb Bart's Hydrops fetalis syndrome and beta-thalassemia major patients, respectively.

Twenty-nine Hb variants were identified among 126 participants, and most of the variants corresponded to normal phenotypes, although Hb Groene Hart, Hb Port Phillip, Hb Shenyang and Hb Zurich-Albisrieden could manifest as cellule low pigment anemia (Table 4).

Geographical distribution of thalassemia carriers in Ji'an

The total carrier rate of thalassemia in Ji'an was 8.22%, and the carrier rate in the southern counties was the highest (Figure 3a). According to the geographical distribution of the 1,694 alpha-thalassemia carriers, the city with the highest carrier rate was Suichuan, followed by Wan'an and Taihe (Figure 3b).

According to the geographical distribution of the 638 beta-thalassemia carriers, the top three cities with high carrier rates were Suichuan, Wan'an and Xiajiang, sequentially (Figure 3c). Moreover, we also counted the carriers of αα/--^{SEA}, the common genotype of alpha-thalassemia. As shown in Figure 3, there was a significant difference within a small geographical distance (Figure 3d). IVS-II-654 (C>T)/β^N, the common genotype of beta-thalassemia, also presented the same trend (Figure 3e).

Discussion

Key results

This was the first study to conduct a molecular epidemiology investigation of thalassemia among the population in Ji'an. In our research, 28,941 people participated in the screening for alpha/beta-thalassemia. Most of the participants were women aged 20-30 years, accounting for 61.86%. Our data illustrate that the carrier rates of alpha- and beta-thalassemia in this area were 5.85% and 2.20%, respectively and that the incidence rate of composite alpha- and beta-thalassemia was 0.17%. The southern counties of Ji'an had a high rate of thalassemia carriers and differences in geographical distance.

Comparison with other cities inside and outside Jiangxi Province, China

It has been shown that the carrier rate of alpha-thalassemia is much higher than in Nanchang (1.49%) and Xinyu (2.2%) and slightly lower than in Ganzhou (7.19%) in Jiangxi[13]. The carrier rate of beta-thalassemia is higher than in Nanchang (1.14%), Xinyu (1.7%) and also slightly lower than Ganzhou (2.3%)[13]. In addition, the composite carrier rates of alpha-thalassemia and beta-thalassemia in Jiangxi are similar to those in Ganzhou (0.18%)[13]. Consistent with previous reports[16, 17], αα/--^{SEA} was the most common alpha-thalassemia mutation. Among the beta-thalassemia alleles, codons 41/42 (-TTCT) and IVS-II-654 (C> T) were the two most common. The ranking of the two main alleles was different from the previous research results in Jiangxi Province[13]. We think this may be caused by the new genetic screening method. Our research indicated that the carrier rates of alpha- and beta-thalassemia in this area were much lower than those in Guangdong Province (11.07%)[16] and Guangxi Zhuang Autonomous Region (24.51%)[18], two high-incidence areas of alpha/beta-thalassemia. The frequency spectrum of alpha- and beta-thalassemia mutations in this study is similar to that previously described in South China, e.g., Chenzhou[12]. For the composite genotypes, αα/--^{SEA} and IVS-II-654 (C>T) /β^N were more than other genotypes. Similar to the general geographical distribution of people with thalassemia in China, the thalassemia carrier rate in South China was higher than that in North China. We also found a significant difference between the common mutation of alpha-thalassemia, αα/--^{SEA}, and the common mutation of beta-thalassemia, IVS-II-654 (C>T) beta+, within a small geographical distance. The alpha/beta-thalassemia carrier rates in Suichuan were higher than those in other cities in this province. Suichuan, located in southwest Ji'an, is adjacent to Ganzhou where borders Meizhou in Guangdong Province. The alpha/beta-thalassemia carrier rate in Meizhou is higher than the average carrier rate in

Guangdong Province, and population migration may be one of the main reasons for the higher alpha/beta-thalassemia carrier rate in Suichuan.

Technological advancement and limitations

High-throughput amplicon sequencing were adopted to screen the 301 thalassemia alleles in this research, and the molecular epidemiology data of Ji'an was first comprehensively reported. In recent years, with the constant progress and development of sequencing techniques, high-throughput sequencing can test almost all mutations in thalassemia gene sequences; moreover, it features a high throughput and a low cost and therefore is suitable for the screening of thalassemia genes and the identification of rare alleles of thalassemia. At present, the three-step thalassemia screening program of routine blood samples (indexes: MCV≤80 fL, MCH≤27 and MCHC<320), Hb electrophoresis (indexes: HbA2 ≥2.5%, HbA2≥3.5% and HbF increases with abnormal Hb strips) and routine genetic testing is mainly adopted clinically in China. Although the three-step screening program plays an important role in the prevention and control of thalassemia, with the advancement of screening, the shortcomings of this method have become increasingly apparent, which are mainly reflected in the following aspects. (1) Routine blood examination can only suggest the possibility of thalassemia, but it cannot be used for definitive diagnosis, and failed detection may occur, as people with normal MCV and MCH may be thalassemia carriers. In 2017, Zhu Baosheng from the First People's Hospital of Yunnan Province led his team to analyze the thalassemia-carrying condition of 951 individuals of the Dai Ethnic Minority Group in Yunnan by use of the routine blood and high-throughput sequencing strategies, and they found through this research that the failed detection in the routine blood screening reached 17.1-23.4%[4]. (2) Hb electrophoresis testing is suitable for the definitive diagnosis of most carriers of β-thalassemia minor and thalassaemia patients (α and β), but it is not sensitive enough for the diagnosis of α-thalassemia carriers. According to research by Zhu et al., due to HbA2 >2.5, more than 90% of $-\alpha^{3.7}/\alpha\alpha$-type carriers failed to be detected using hemoglobin electrophoresis[4]. Hemoglobin electrophoresis has a high failed diagnosis ratio for thalassemia detection. (3) Routine genetic testing is mainly directed to the testing of 23 common gene types (3 deletion types and 3 mutation types of α-thalassemia and 17 mutation types of β-thalassemia); therefore, it cannot be used to test for rare types, thus leading to the approximately 2% rate of failed diagnosis. However, the NGS technique still has some limitations. For instance, Zebisch et al. identified the new mutation of ε-γ-δ-β thalassemia with MLPA and CGH, while NGS cannot test this mutation[19]. In light of the different variations in thalassemia, based on cost, different methods should be used in combination to comprehensively test for all variations of thalassemia.

Summary

In summary, we investigated the carrier rates of variations related to thalassemia in Ji'an using NGS and demonstrated the diversity of relevant variations. A novel mutation was identified in this research, which fully illustrates that the application of NGS to routine thalassemia gene screening can effectively reduce the failed detection ratio of unique or rare genotypes and can contribute to the improvement of the prevention and control of thalassemia. Domestic and foreign practices have illustrated that the birth rate

of babies with thalassemia major can be effectively reduced through premarital and pregnancy physical examination, prenatal screening, prenatal diagnosis and other prevention and control measures. The per capita cost for this program was controlled within RMB 200 during the implementation process. The screening mode of examining the pregnant women first and then determining whether to examine their spouses based on the result was adopted, and this method may be a better method to perform thalassemia gene screening.

Conclusions

Our research results are of great significance for the prevention and treatment of thalassemia in this area and other high-prevalence areas. The epidemiological data updated in this research may enable the local government to focus on the severity of this disease and determine methods for effective resource allocation under limited resource conditions.

Abbreviations

PCR: Polymerase Chain Reaction

NGS: Next-generation Sequencing

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

Hb: Hemoglobin

MLPA: Multiplex Ligation-dependent Probe Amplification

CGH: Comparative Genome Hybridization

Declarations

- Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of First People's Hospital of Ji'an. All the participants signed written informed consent.

- Consent to publish

Not applicable

- Availability of data and materials

Not applicable

- Competing interests

The authors declare that they have no competing interests.

- Funding

No funding was obtained for this study.

- Authors' Contributions

LWM performed the data generation, processing, and analysis and prepared the paper and figures; YQ,SPC,HL and HRW contributed to data analysis and interpretation; LPG, JH, XW, YL contributed to result interpretation; JX, YF designed the study and carried out sample collection, storage and transport; All authors have read and approved the manuscript.

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Tables

Due to technical limitations, Tables 1-4 are only available as a download in the supplemental files section.

Table Legends

Table 1 Distribution of α-thalassemia genotypes in Ji'an Region

Table 2 Distribution of β-thalassemia genotypes in Ji'an Region

Table 3 Genotypes of composite α and β-thalassemia in Ji'an Region

Table 4 Hemoglobin variants in this cohort

Additional Files

Table S1 301 kinds of thalassemia genotype

Figures

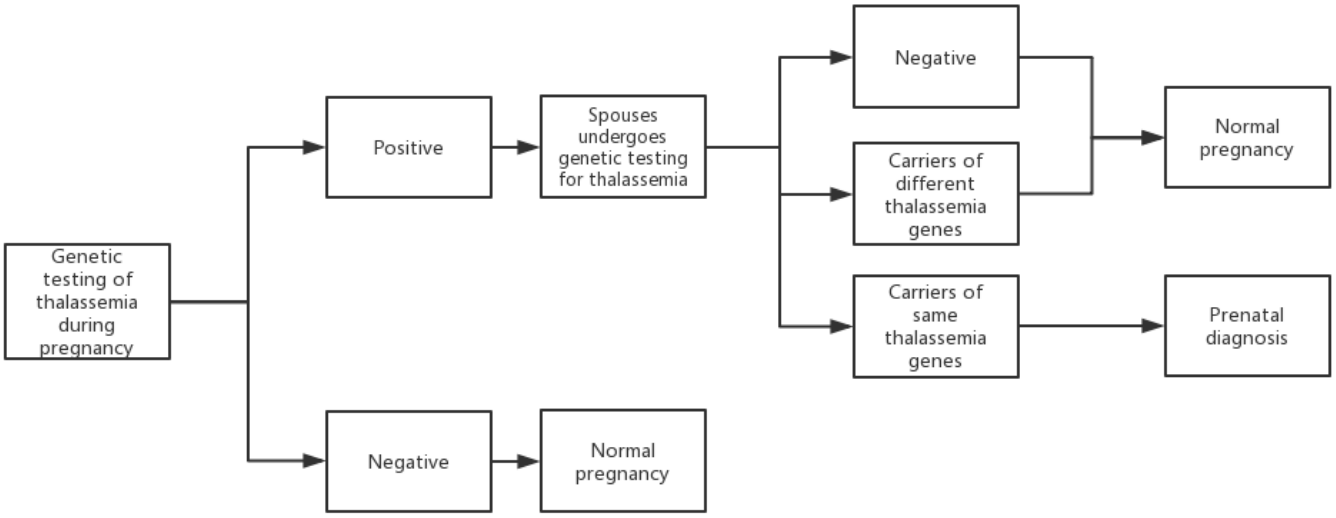

Figure 1

Flow chart for genetic screening of thalassemia.

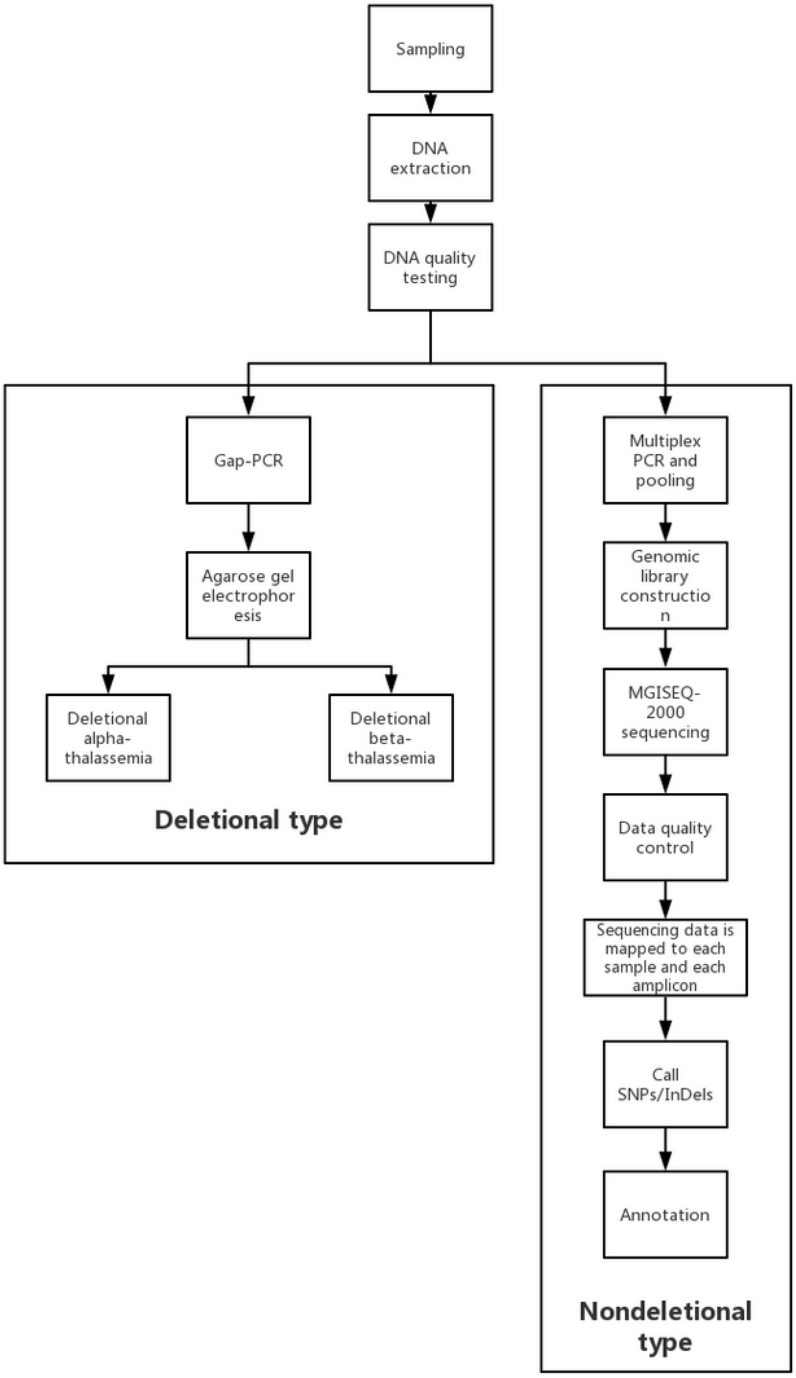

Figure 2

Diagram for the screening of hemoglobin variants and deletional and nondeletional alpha/beta-thalassemia.

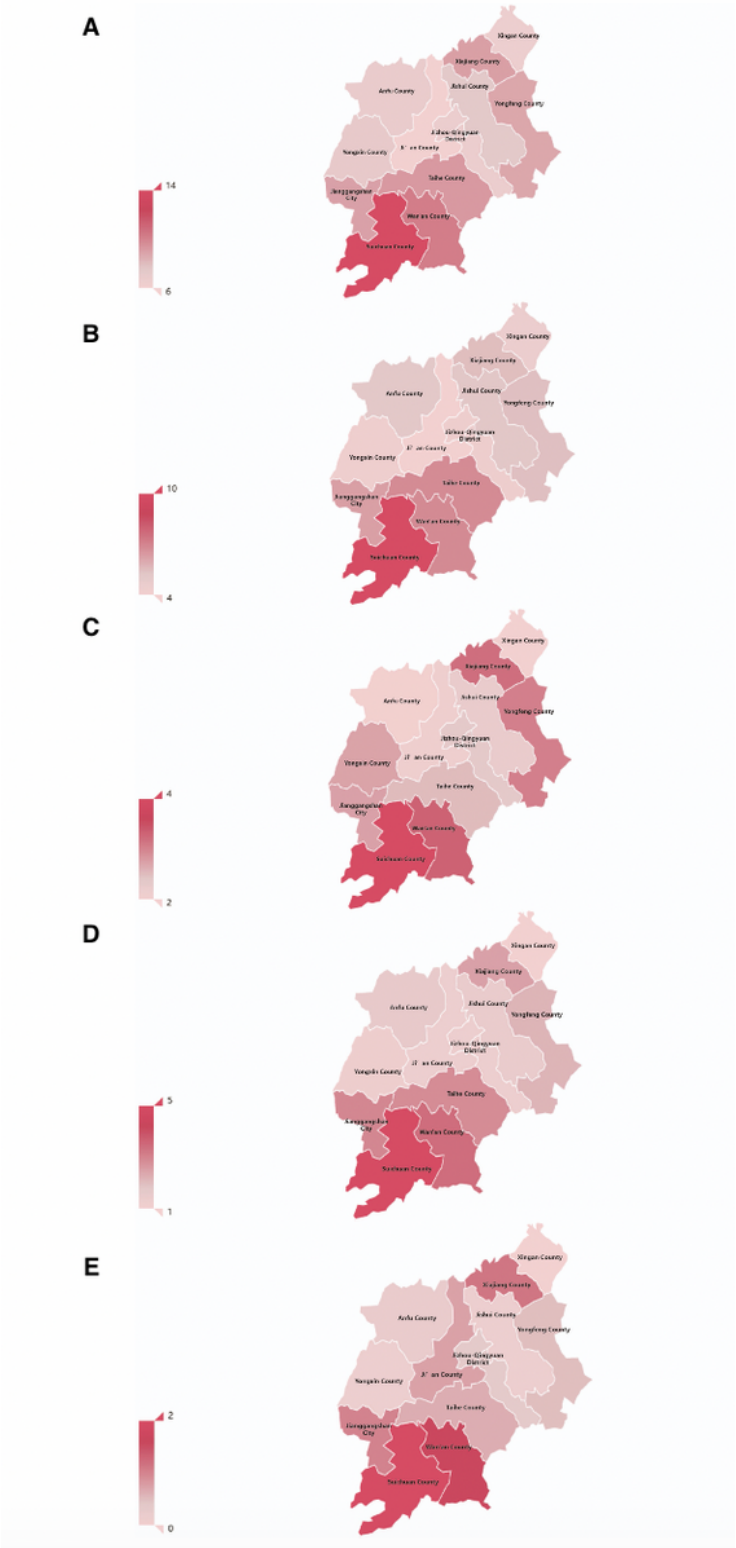

Figure 3

Geographical distribution of thalassemia carriers in this study. (a) thalassemia carrier rate; (b) α-thalassemia carrier rate ; (c) αα/–SEA genotype carrier rate; (d) β-thalassemia carrier rate; (e) IVS-II-654 (C>T)/βN carrier rate. The map depicted in figure taken from https://www.tubiaoxiu.com. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country,

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