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# Analysis of Glucose-6-Phosphate Dehydrogenase Genetic Polymorphism in the Hakka Population in Southern China

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Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
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**Background:** In southern China, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a significant health problem. The aim of this study was to investigate the molecular epidemiological characteristic of the *G6PD* gene among Chinese Hakka in southern Guangdong province.

**Material/Methods:** We screened 611 unrelated subjects for G6PD genetic polymorphism analyzed by a gene chip analysis for common Chinese G6PD mutations. G-6-PD enzyme activity was determined by use of the G-6-PD quantitative detection kit.

**Results:** Seven mutation sites were detected from subjects in our study. G6PD Canton (c.1376 G→T)(33.06%), G6PD Kaiping (c.1388 G→A)(30.67%), and polymorphism (c.1311 C→T)(25.89%) account for 89.62% of mutations, followed by G6PD Gaohe (c.95 A→G)(5.97%), G6PD Chinese-5 (c.1024 C→T)(3.58%), G6PD Maewo (c.1360 C→T)(0.39%), and G6PD Viangchan (c.871G→A)(0.39%).

**Conclusions:** We studied the genetic polymorphisms and frequencies of G6PD gene in the Hakka population of Meizhou. Our results coincide with the results among the Chinese Jiangxi Hakka population. It was consistent with previous research reports on Chinese people. There were differences in the results of reports from some other Asian populations. Our results could be useful for future prevention and control of G6PD deficiency aimed at the Chinese Hakka population.

**MeSH Keywords:** **Asian Continental Ancestry Group • Glucosephosphate Dehydrogenase Deficiency • Polymorphism, Genetic**

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## Background

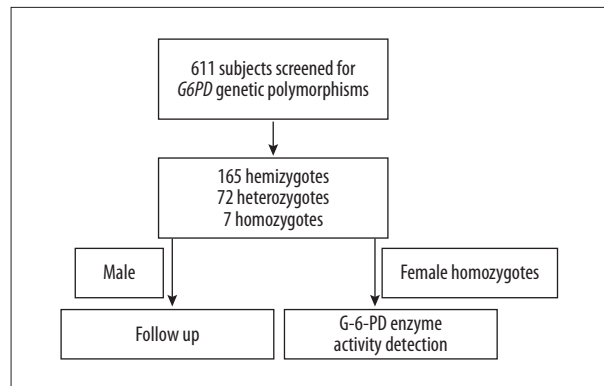
Glucose-6-phosphate dehydrogenase (G6PD) plays a key role in the production of ribose 5-phosphate and the generation of NADPH in the hexose monophosphate pathway. Because this pathway is the only NADPH-generation access in mature red cells, which lack the citric acid cycle, a genetic deficiency of G6PD (OMIM 305900) is often associated with adverse physiologic effects [1–3].

There are 3 types of G6PD deficiency. We were prompted to undertake this studies to help patients with symptoms such as myalgia, cramps, and muscle weakness under conditions of stress, particularly physical exertion. All 3 variants – Mediterranean (305900.0006), Seattle-like (305900.0010), and G6PD A (305900.0002) – showed the enzyme was defect in muscle [4,5]. A growing number of studies have found the relationship between G6PD deficiency and some physiological functions.

Hereditary glucose 6 phosphate dehydrogenase deficiency is the most common genetic enzyme deficiency in the world. G6PD deficiency is an incomplete dominant inheritance of X. The *G6PD* gene is located on chromosome Xq28. It consists of 13 exons and 12 introns, encoding 515 amino acids, and is a typical housekeeping gene. The *G6PD* gene has many variants due to its molecular structure [6–8]. The deficiency is widely distributed and occurs in about 400 million people worldwide [9]. G6PD deficiency has an obvious geographical distribution in the mainland is China; it is higher in the provinces south of the Yangtze River, including Guangdong, Hainan, Guangxi, Yunnan, Guizhou, and Sichuan provinces [10–12].

Meizhou is a city in northeast Guangdong province, with an area of 15 876 km<sup>2</sup> and a population 5.44 million. It located at the junction of Fujian, Guangdong, and Jiangxi provinces [13]. Meizhou is one of the most representative Hakka settlements in the world. The vast majority of people living in Meizhou are Hakka. It is the last foothold of Hakka migration to the south and one of places where the Hakka ethnic group first developed. Hakka is an intriguing Han Chinese population that mainly inhabits southern of China, originally migrating to the south from the Yellow River area. Some studies preferred the northern origins of Hakka [14,15].

We performed population screening of *G6PD* genetic polymorphisms in 611 Chinese Hakka subjects living in Meizhou city in Guangdong province, documenting the polymorphism in this region and providing references for the clinical genetic counseling of G6PD deficiency in Hakka ethnic areas. This topic is rarely reported in the Meizhou Hakka area.



**Figure 1.** Flow chart of screening for *G6PD* genetic polymorphisms and enzyme activity detection.

## Material and Methods

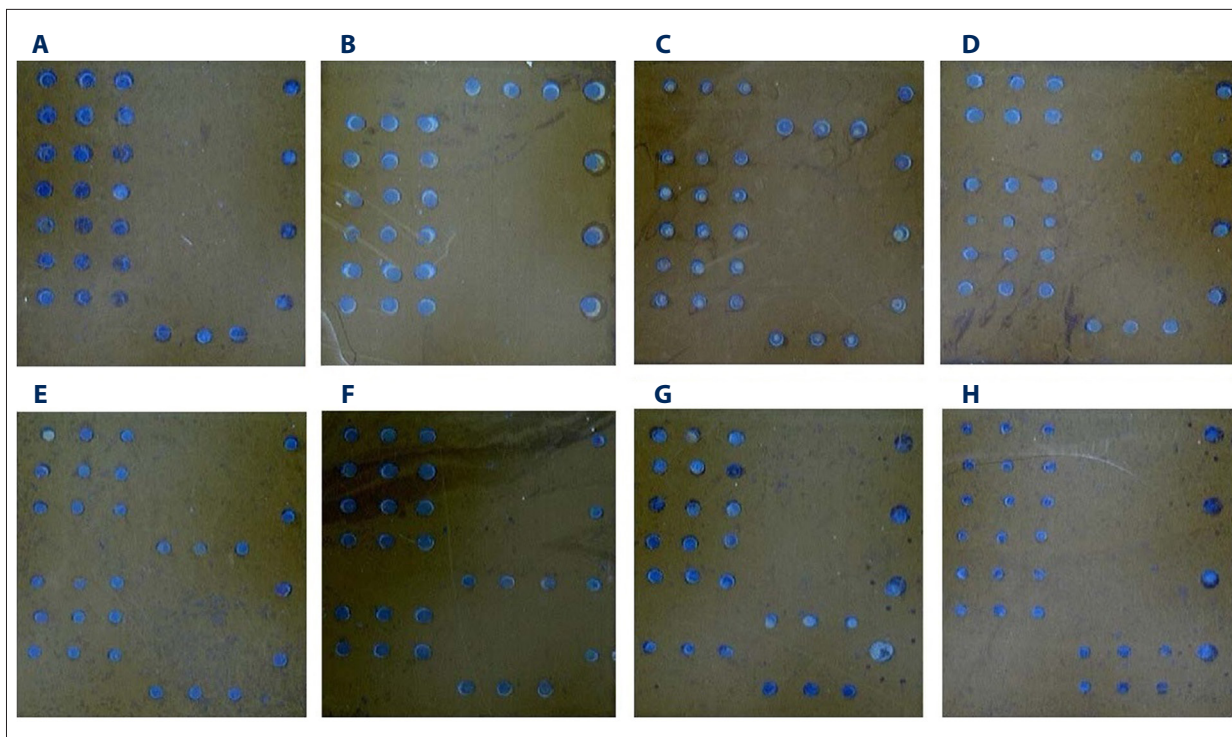
### Subjects

From February 2016 to October 2017, we enrolled 611 subjects ages 2 days to 94, including 403 males and 208 females (1.937: 1). Subjects visited Meizhou People's Hospital located in Guangdong province, China. These patients came to the hospital for various reasons, including threatened abortion, neonatal jaundice, anemia, pneumonia, allergy, and fever. The flow chart of screening for *G6PD* genetic polymorphisms and enzyme activity detection is shown in Figure 1.

### G6PD quantitative detection

G-6-PD was determined by the rate method, which is used to determine the content of human serum G-6-PD *in vitro* (Korfang Biotechnology Co., Guangzhou, Guangdong, China). This kit is used to screen for neonatal jaundice and G6PD deficiency caused by acute hemolytic anemia, as well as for routine checkups. Blood samples were stored in 2-ml vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 4000 rpm for 5 min. The hematocrit was accurately absorbed in 20  $\mu$ l with a sampler and dissolved in 1 ml of dissolved solution. The red blood cells were completely dissolved. Then, a 10- $\mu$ l sample were added into the 220- $\mu$ l buffer R1 (containing NADP) at 37°C for 3–5 min. Then, we added buffer R2 (containing G6P) at 37°C for 2 min. At the 340-nm wavelength, the absorbance was measured for 2 min, and the rate of change of the absorbance per minute ( $\Delta A/\text{min}$ ) was calculated using the formula  $G-6-PD(KU/L) = \Delta A/\text{min} \times 4099.7(K \text{ value}) \times 51(\text{dilution times}) = \Delta A/\text{min} \times 209.0847$ .

The reference range of G6PD activity is 1.70–4.00KU/L in children and 1.30–3.60 KU/L in adults. The reference range of G6PD activity in neonatal venous blood or umbilical cord blood is 2.50–5.80 KU/L.



**Figure 2.** The hybridization results of *G6PD* gene chip. (A) Wild-type; (B) c.95 A→G; (C) c.871G→A; (D) c.1024 C→T; (E) c.1311 C→T; (F) c.1360 C→T; (G) c.1376 G→T; (H) c.1388 G→A.

**DNA extraction and genotyping**

Blood samples were stored in 2-ml vacuum tubes containing EDTA. Genomic DNA was extracted from the blood of patients and controls using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) following the manufacturer’s instructions, and DNA concentration was quantified using a Nanodrop 2000™ Spectrophotometer (Thermo-Fisher Scientific, Waltham, MA). Polymerase chain reaction was performed according to the following protocol: 50°C for 2 min, pre-denaturation at 95°C for 15 min, followed by 45 cycles at 94°C for 30 s and 65 °C for 45 s. Amplification was performed using the *G6PD* Gene Typing Detection kit (gene chip assay) (Sinochips Bioscience Co., Zhuhai, Guangdong, China).

Using a commercial detection kit, we analyzed the 7 known *G6PD* gene mutations most commonly seen in the Chinese population: *G6PD* Gaohe (c.95 A→G, 32 His→Arg), *G6PD* Viangchan (c.871G→A, 291 Val→Met), *G6PD* Chinese-5 (c.1024 C→T, 342 Leu→Phe), *G6PD* Maewo (c.1360 C→T, 454 Arg→Cys), *G6PD* Canton (c.1376 G→T, 459 Arg→Leu), *G6PD* Kaiping (c.1388 G→A, 463 Arg→His), and one polymorphism (c.1311 C→T, rs2230037).

**Statistical analysis**

SPSS statistical software version 19.0 was used for data analysis. Data are reported as the means±SD. The prevalence of *G6PD* gene mutations was evaluated using descriptive statistics.

**Table 1.** Allele distribution of *G6PD* variants.

Alleles	n	Frequency, %
c.1376 G→T	83	33.06
c.1388 G→A	77	30.67
c.1311 C→T	65	25.89
c.95 A→G	15	5.97
c.1024 C→T	9	3.58
c.1360 C→T	1	0.39
c.871G→A	1	0.39
<b>Total</b>	<b>251</b>	

**Results**

A total of 611 subjects, including 403 (65.96%) men and 208 (34.04%) women, were recruited in the study. The mean age was 23.83±27.52 years, which was 22.99±27.42 in men and 25.45±27.69 in women. All of them lived in 7 areas of Meizhou city, Guangdong province, and all of them were ethnic Hakka.

Seven mutation sites were detected from subjects in our study. Mutations of *G6PD* gene were shown by hybridization testing on chip (Figure 2). The allelic frequencies of *G6PD* variants

**Table 2.** The prevalence of G6PD variants in all subjects.

Nucleotide change	Male (n=403)			Female (n=208)			Total (n=611)		
	Hemizy-gote	n	Frequency, %	Heterozy-gote	Homozy-gote	n	Frequency, %	n	Frequency, %
Wild type		238	59.06			129	62.02	367	60.07
c.1376 G→T	61		15.14	20	0	20	9.62	81	13.26
c.1388 G→A	52		12.90	22	1	23	11.06	75	12.27
c.1311 C→T	37		9.18	20	2	22	10.58	59	9.66
c.95 A→G	10		2.48	4	0	4	1.92	14	2.29
c.1024 C→T	4		0.99	5	0	5	2.40	9	1.47
c.1360 C→T	0		0.00	1	0	1	0.48	1	0.16
c.871G→A, c.1311 C→T	1		0.25		1	1	0.48	2	0.33
c.95 A→G, c.1311 C→T/c.1311 C→T					1	1	0.48	1	0.16
c.1311 C→T, c.1376 G→T					1	1	0.48	1	0.16
c.1376 G→T, c.1388 G→A					1	1	0.48	1	0.16

**Table 3.** Seven female patients with homozygous mutations in the G6PD gene.

Case No.	Gender	Age	Homozygote	G-6-PD enzyme activity (U/L)
1	Female	63 years	c.871G→A, c.1311 C→T	0.99
2	Female	24 years	c.1311 C→T, c.1376 G→T	1.05
3	Female	32 years	c.95 A→G, c.1311 C→T/c.1311 C→T	0.97
4	Female	6 months	c.1311 C→T/c.1311 C→T	–
5	Female	40 years	c.1376 G→T, c.1388 G→A	0.07
6	Female	46 years	c.1311 C→T/c.1311 C→T	–
7	Female	9 days	c.1388 G→A/c.1388 G→A	0.06

are shown in Table 1. We identified 18 genotypes, including 6 kinds of heterozygotes, 6 kinds of hemizygotes, and 6 kinds of homozygotes. The results are listed in Table 2.

We detected 7 female patients with homozygous mutations in the *G6PD* gene and with G6PD deficiency. Their clinical data are shown in Table 3.

## Discussion

The human *G6PD* gene has many variants because of its molecular structure. At present, more than 400 kinds of biochemical variants have been reported worldwide [16]. There are at least 25 *G6PD* mutations in China, of which 3 mutations – 1376G>T, 1388G>A, and 95A>G – account for more than 60% [17–19]. Guangdong province is a high-incidence area of

*G6PD* deficiency in China. We analyzed *G6PD* genetic polymorphisms and distribution among the Hakka population, so as to analyze the difference of *G6PD* gene mutation distribution and enrich the *G6PD* gene mutation data. This is also one of the purposes of our research.

Seven mutation sites were detected from subjects in our study. G6PD Canton (c.1376 G→T)(33.06%), G6PD Kaiping (c.1388 G→A)(30.67%) and polymorphism (c.1311 C→T) (25.89%) account for 89.62% mutations, followed by G6PD Gaohe (c.95 A→G)(5.97%), G6PD Chinese-5 (c.1024 C→T) (3.58%), G6PD Maewo (c.1360 C→T)(0.39%), G6PD Viangchan (c.871G→A)(0.39%). The finding was similar to Guangxi [20], Yunnan [21–23], and Taiwan [24–26].

In Malaysia, the 3 most common variants are G6PD Viangchan c.871G→A (39.5%), G6PD Mediterranean c.563 C→T (26.7%),

and G6PD Mahidol c.487 G→T (15.1%) [27]. G6PD Viangchan c.871G→A has been reported to be a common variant in Laos and showed a frequency of 54% in a group of G6PD-deficient Thais [28]. This indicates that the polymorphism of *G6PD* gene is differs among various populations. It may also be due to our small sample size.

In conclusion, this study is the first to provide information about the genetic polymorphisms of *G6PD* gene in Meizhou city of Guangdong province, China. The most common mutations are G6PD Canton (1376 G→T) and G6PD Kaiping (1388 G→A), and the following mutations were 1311 polymorphism (1311 C→T), G6PD Gaohe (95 A→G), G6PD Chinese-5 (1024 C→T), G6PD Maewo (1360 C→T), and G6PD Viangchan (871 G→A). Our results coincide with the results among the Chinese Jiangxi Hakka population reported by Hu et al. This study complements the *G6PD* polymorphisms data in the Hakka population. It could be useful for future prevention and control of G6PD deficiency aimed at the Chinese Hakka population.

This study was a retrospective analysis of *G6PD* gene results in patients treated in our hospital, and some of these patients were not tested for G-6-PD enzyme activity. Therefore, we did not have the corresponding data to analyze the correlation between *G6PD* gene polymorphisms and G-6-PD enzyme activity in the Hakka population. This is the subject that we will focus on next. On the other hand, in order to obtain more accurate and detailed data on the *G6PD* polymorphisms in the Meizhou Hakka area, the focus of our work is to expand the small sample size of the study and to set up the disease group and the control group for research. We also intend to perform research on abnormal hemoglobin disease, thalassemia, and G6PD deficiency [29].

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To date, approximately nearly 200 *G6PD* gene mutations have been documented. The relationship between many sites and G-6-PD enzyme activity has not been clearly defined, and we plan to study of these relationships and their pathogenesis in the future.

## Conclusions

We studied the genetic polymorphisms and frequencies of G6PD gene in the Hakka population of Meizhou. Our results coincide with the results among Chinese Jiangxi Hakka population and are consistent with previous research reports on Chinese people. There were differences in the results of reports from some other Asian populations. Our findings could be useful for future prevention and control of G6PD deficiency aimed at the Chinese Hakka population. There were some shortcomings in our research, which we intend to address in future research. We also plan to study the relationship between *G6PD* gene mutations and G-6-PD enzyme activity and their pathogenesis.

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## Conflict of interests.

None.

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