Original Article

Molecular epidemiological survey of haemoglobinopathies in the Guangxi Zhuang Autonomous Region of southern China


Accurate and up-to-date data on the frequency of haemoglobinopathies among the populations of Guangxi Zhuang Autonomous Region, where haemoglobinopathies are most endemic in China, are required. In our study, a total of 5789 samples obtained from members of the Han, Zhuang, and Yao ethnic groups in six geographical areas of Guangxi Province were analysed systematically in terms of both haematological and molecular parameters. The results presented that the total heterozygote frequency of thalassaemias and other haemoglobinopathies was 24.51%, of which 17.55% was due to α-thalassaemia, 6.43% to β-thalassaemia, 0.38% to structural haemoglobin variants, and 0.16% to δ-thalassaemia. The mutational spectrum among the local population for each type of disorder was described, including the first report on the true prevalence of three silent α thalassaemia defects, −α3.7/4.78%, −α4.2/1.61% and Hb Westmead (αWSαf) (1.57%) and of δ-thalassaemia resulting from five novel and two rare mutations never before identified in Chinese individuals. Comparison of the frequencies of α-globin mutations among the ethnic groups showed that there was a statistically significant difference between the Han (15.71%) and Zhuang (20.12%), and between the Han (15.71%) and Yao (20.84%) ethnic groups. In addition, we have performed the first extensive study of haematological parameters of the Hb Westmead mutation using a group of Chinese subjects with compound heterozygosity for this variant and an α-thalassaemia deletion. The knowledge gained in this study will enable us to estimate the health burden in this high-risk population and to elucidate the various genetic alterations that underlie haemoglobinopathies.

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Key words: Guangxi Zhuang Autonomous Region – haemoglobinopathies – molecular epidemiological survey – thalassaemia

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The haemoglobinopathies are a diverse group of autosomal recessive disorders. They include the thalassaemias, which are characterized by the reduced synthesis of one or more normal globin chains, and disorders caused by haemoglobin (Hb) variants, where there is a structural alteration in one of the globin chains (1, 2). A few haemoglobinopathies can involve both phenotypes, for example haemoglobin E (Hb E) was caused by a mutation in the β-globin gene to produce abnormal Hb E or to reduce transcription of β-globin gene by the mechanism of aberrant splicing (3). Another heterogeneous group of related Hb disorders, hereditary persistence of fetal Hb (HPFH), is involved in general in ‘the thalassemia syndromes’ because frequently they are inherited together with the different forms of thalassaemia and modify the clinical phenotype of the latter (1, 4). The highest frequencies of various haemoglobinopathies, which are the most common single-gene diseases worldwide, occur in developing countries, particularly those of Sub-Saharan Africa and Southeast Asia including southern China (1, 5, 6). Homozygous Hb defects cause the most severe pathology with respect to inherited haemolytic anaemia and remain a major public health problem in these high-risk regions owing to their high prevalence and incidence.

Guangxi Zhuang Autonomous Region is a coastal province that is located in the southwest of mainland China and borders on the northern part of Vietnam. It represents the first point of settlement on the ‘Out of Africa’ migration route of modern humans from Southeast Asia into mainland China (7). Guangxi Province is an autonomous region which is known to have the largest minority group (the Zhuang ethnic minority) in China. Over 32% (15.38 million) of the population belong to the Zhuang ethnic minority, which corresponds to 95% of the total Zhuang population in China (8). It is interesting that, among all the high-risk regions of southern China, the highest frequency of haemoglobinopathies is found in Guangxi. However, further analysis of the incidence and molecular nature of these disorders to understand better the health burden posed by genetic disorders of Hb according to geographic region and ethnic distribution is required (9, 10–14).

In the study described herein, we performed a complete molecular characterization of the α, β, and δ-globin genes in 5789 consecutive samples from Guangxi using haematological and molecular analyses. We also assessed the geographic and ethnic distributions of the thalassaemias and other haemoglobinopathies among three ethnic groups (Han, Zhang, and Yao) in the local population. In addition, we report the finding of six novel α- and δ-thalassaemia mutations and two other δ-thalassaemia mutations that were identified firstly in Chinese patients. Our main aim was to clarify the true prevalence of various haemoglobinopathies and their patterns of co-inheritance in the area of southern China where these disorders are most endemic.

**Materials and methods**

**Population samples**

A total of 5789 students (2841 males and 2948 females) from 12 local primary and middle schools in six regions of the Guangxi Zhuang Autonomous Region was enrolled for this study between October 2007 and May 2008 (Fig. 1). All of these students were of Guangxi descent. Overall, 58.1% (3361/5789) and 28.8% (1670/5789) of the students were from the two major local ethnic groups, Han and Zhang, respectively. In addition, 619 subjects from the Yao ethnic minority (10.7%) and 139 subjects from 9 other local minorities (2.4%) also asked to be tested. All the students were educated about the thalassaemias beforehand by a haematologist or a geneticist and a consent form was signed by the parent of each participant before peripheral blood was collected.

**Screening strategy and experimental analysis**

The diagnostic flowchart used in this study is illustrated in Fig. 2. We designed a strategy that combined phenotypic/molecular screening and genotyping. All 5789 participants were screened for the presence of defects by two procedures. We used a hematological screening protocol to detect all of the suspected subjects who had hematological phenotypes by using full blood counts (FBCs) and haemoglobin test as described before (15) and we found 924 positive samples, then all such positive samples were further characterized by molecular diagnosis as described previously (15–17), guided by blood analysis, for identifying four different types of haemoglobin disorders. We identified 523 α-thalassaemia cases, 370 β-thalassaemia cases, 22 structural Hb variants, and 9 δ-thalassaemia cases, respectively, by this confirmative test. For those subjects who had no positive hematological phenotypes, we directly used a DNA-based molecular screening protocol to detect α-thalassemia silent carriers. A total of 363 such cases were detected by this protocol. The criteria that indicated the possibility of heterozygosity for various types of thalassaemias were referenced by published manuscript (15, 18, 19). It was
worth noting that all samples were directly tested for ‘silent’ α globin variants by using molecular screening; thus, our results were not affected by iron levels. Considering its expenditure a serum iron was not detected in our study.

Fig. 1. The six regional centres in Guangxi Zhuang Autonomous Region that formed the basis of our study.

Statistical analysis

Current and predicted vital statistics were obtained from the ‘Population Bulletin of the Family Planning Commission of Guangxi Autonomous Region’ (8). Statistical analyses were conducted

Fig. 2. Diagnostic flowchart for the detection of Hb disorders in this study. The positive sample numbers detected at each step are indicated in the leftmost position. In the molecular screening step, Gap-PCR and ARMS-PCR were used for genotyping of the \(-\alpha^{3.7}/\) and \(-\alpha^{4.2}/\) deletions and the \(\alpha^{WS}/\) mutation, respectively. In the confirmative test step, Gap-PCR was used to type the common known α-thalassaemia deletions or β-thalassemia/HPFH deletions; MLPA method was used to detect unknown gross deletions in the α- or β-globin gene cluster; RDB was used to detect known point mutations causing α- or β-thalassaemia; DNA sequencing was used to identify novel or rare point mutations in the entire α1- and α2- or β-globin gene. The DHPLC method followed by DNA sequence analysis was applied for rapid genotyping of the underlying point mutations in the δ-globin gene. The detailed information on the primer design and PCR/DHPLC conditions is available. In addition, DNA sequencing was used to characterize point mutations in the α-, β- or δ-globin gene, in all samples that contained a Hb band that migrated abnormally. All individuals with β-thalassaemia were analysed to determine whether they had co-inherited one of the six α-thalassaemia defects (\(-\alpha^{SEA}/\), \(-\alpha^{3.7}/\), \(-\alpha^{4.2}/\), \(\alpha^{WS}/\), \(\alpha^{8S}/\) and \(\alpha^{+}/\)) that are common among the Chinese. In samples with HbF values >5%, the promoters of the Gγ- and Aγ-globin genes were sequenced or the MLPA method was used to detect gross deletions in the β-globin gene cluster. ARMS: amplification refractory mutation system; MLPA: multiplex ligation-dependent probe amplification; RDB: reverse dot blot; DHPLC: denaturing high performance liquid chromatography; HPFH: hereditary persistence of fetal haemoglobin.
with the SPSS 17.0 statistical software. The prevalence of different thalassaemia alleles was calculated from the modified Hardy–Weinberg formula.

**Results**

Population prevalence of haemoglobinopathies

As listed in Table 1, we identified a total of 1419 mutant chromosomes, which corresponded to 1016 α-thalassaemia, 372 β-thalassaemia, and 9 δ-thalassaemia alleles and 22 structural Hb variants; this gave a heterozygote frequency of 24.51% for haemoglobinopathies in the Guangxi populations. A total of 79 out of the 370 β-thalassaemia carriers was also found to carry α-thalassaemia, which gave a frequency of 1.36% for coincidence of these two common disorders in the local population. As expected, the 79 patients presented the very variable α-globin alterations in combination with β-globin mutations (data not shown), showing 31 genotype combinations of the coincidence of both Hb disorders. The δ-thalassaemia and structural Hb variants were found to occur at relatively low frequency in this region (Table 1), but the cases identified did show considerable genetic heterogeneity. We identified nine previously reported variants of Hb (data not shown) and seven novel or rare δ-thalassaemia mutations that had not been reported previously in Chinese patients (see below). To more clearly delineate the incidence of various haemoglobinopathies in our study, a group of common structural Hb variants was classified into the category of α-thalassaemia considered as a non-deletional type of α-thalassaemia (Hb Westmead, Hb CS, and Hb QS) or the category of β-thalassaemia (Hb E), thus obtaining a relatively low frequency of structural Hb variants in the local population.

The spectrum of α-, β-, and δ-thalassaemia mutations

The number and percentage of chromosomes that carried the three different types of thalassaemia and that were identified among the 5789 samples from three ethnic groups (Han, Zhuang, and Yao) are listed in Table 2. A total of 1016 chromosomes that carried known α-thalassaemia mutations and 372 chromosomes that carried known

<table>
<thead>
<tr>
<th>Haemoglobinopathy</th>
<th>Subgroup</th>
<th>Case number</th>
<th>Percentage</th>
<th>Total</th>
<th>Allele number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Thalassaemia</td>
<td></td>
<td>886</td>
<td>15.32</td>
<td></td>
<td>1016⁺</td>
<td>17.55</td>
</tr>
<tr>
<td>α-Thalassaemia silent</td>
<td></td>
<td>363</td>
<td>6.27</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>α-thalassaemia trait</td>
<td></td>
<td>498</td>
<td>8.6</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Hb H disease</td>
<td></td>
<td>25</td>
<td>0.43</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>β-thalassaemia</td>
<td></td>
<td>370</td>
<td>6.39</td>
<td></td>
<td>372⁺</td>
<td>6.43</td>
</tr>
<tr>
<td>β-thalassaemia trait</td>
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<td>287</td>
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<td>β/α-thalassaemia trait</td>
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<td>1.36</td>
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<td>0.00</td>
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<td>0.07</td>
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<td>0.00</td>
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<td>δ-thalassaemia</td>
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<td>0.16</td>
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<td>0.16</td>
</tr>
<tr>
<td>Carrier</td>
<td></td>
<td>6</td>
<td>0.1</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>δ/α-thalassaemia trait</td>
<td></td>
<td>3</td>
<td>0.05</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Structural haemoglobin variants</td>
<td></td>
<td>22</td>
<td>0.38</td>
<td>22</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Abnormal haemoglobin carrier</td>
<td></td>
<td>17</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb variant/α-thalassaemia trait</td>
<td></td>
<td>4</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb variant/β-thalassaemia trait</td>
<td></td>
<td>1</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1287</td>
<td>22.25</td>
<td></td>
<td>1419</td>
<td>24.51</td>
</tr>
</tbody>
</table>

⁺⁺⁺All the cases are caused by α-thalassaemia co-inherited with different types of β-thalassaemia or δ-thalassaemia or Hb variant. These numbers are involved in the total number of α-thalassaemia alleles, including five cases with homozygous or compound heterozygous silent carrier α-thalassaemia.

⁺⁺⁺⁺⁺Including three types of silent heterozygous mutation, –α^3.7/αα, –α^4.2/αα, and α^WS/αα.

⁺⁺⁺⁺⁺⁺⁺Including 14 cases with homozygous or compound heterozygous silent carrier α-thalassaemia. Ninety-one individuals with two common abnormal Hbs including seventy cases with Hb CS and twenty-one cases with Hb QS are considered as a non-deletion type of α-thalassaemia mutations.

⁺⁺⁺⁺⁺⁺⁺⁺This case is caused by a heterozygous Hb variant co-inherited with a heterozygous β-thalassaemia. This number is involved in the total number of β-thalassaemia alleles; 12 cases with Hb E variant are considered as ones having β-thalassaemia trait.

⁺⁺⁺⁺⁺⁺⁺⁺⁺Three of them were caused by heterozygous β-thalassaemia and α-globin triplication; one was caused by a compound heterozygosity for two β-thalassaemia mutations.
β-thalassaemia mutations were characterized in our study. The six common mutations that cause α-thalassaemia and eight common mutations responsible for β-thalassaemia accounted for approximately 98.9% and 98.1% of these mutations, respectively (Table 2). Comparison of the frequency of α-globin mutations among the three ethnic groups, Han 15.71% (528/3361), Zhuang 20.12% (336/1670), and Yao 20.84% (129/619), respectively, showed that there was a statistically significant difference between the Han and Zhuang ethnic groups (p < 0.0001), and between the Han and Yao ethnic groups (p = 0.002). However, no statistically significant difference was found between the Zhuang and Yao ethnic groups (p = 0.704). In addition, there were no statistically significant differences among the Han, Zhuang, and Yao populations with respect to the frequency of β-globin mutations (6.43%, 261/3361; 7.01%, 117/1670; and 5.17%, 32/619, respectively; p = 0.217). Comparison of the distribution of the α- and β-thalassaemia mutations among the six representative geographical areas showed no significant differences among the six regions, except that there was a higher prevalence of α-thalassaemia in Baise and Hezhou than in the other regions (data not shown). Only nine δ-thalassaemia alleles were detected by our
large-scale survey; hence the mutant alleles could not be compared among different ethnic groups. The results indicated that two variants, c.−77T>C and c.−130A>G seemed to be the relatively dominant forms in this region.

Six novel mutations and two rare mutations

We identified one novel mutation in the α-globin gene and five novel δ-globin mutations in the course of our study (Fig. 3). The novel α-globin mutation was HBA2: c.40G>T (Genbank ID. GU145033), which involved a GCC→TCC change at codon 13 within exon 1 of the α2-globin gene; it was found in two unrelated individuals. The HbA2 level was 1.97% in one carrier state, and 4.47% in another individual who was heterozygous for both this α-globin variant and a β-thalassaemia mutation (HBB: c.52A>T).

With respect to the five novel mutations in the δ-globin gene, the first was HBD:c−180 A>G (Genbank ID. GU145029), which involved an A→G substitution at nucleotide (nt) −130 of the promoter of the δ-globin gene and was detected in two unrelated individuals. The level of HbA2 was 1.7% in one carrier, and 2.65% in another individual who co-inherited with the −SEA/mutation. The second mutation was HBD:

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**Fig. 3.** DHPLC analysis and partial nucleotide sequence of the six novel α- and δ-globin mutations, as well as two δ-globin mutations that were identified in Chinese individuals for the first time. The DHPLC patterns (from A to H) and the corresponding portion of the chromatographic DNA sequencing profile (from a to h) are presented for the mutations HBA2: c.40G>T (A–a), HBD: c−180 A>G (B–b), HBD: c−94G>A (C–c), HBD: c−58−52del (D–d), HBD: c.94T>C (E–e), HBD: c.*+36T>G (F–f), HBD: c.130G>A, HBD: c−127T>C (G–g), and HBD: c.130G>A (H–h). The black arrow indicates the location of the mutation or deletion. The possibility that these mutations corresponded to single nucleotide polymorphisms (SNPs) was excluded by analysing these sequences in at least 150 samples from random Chinese individuals.
c.−94G>A (Genbank ID. GU145030), which involved a G→A substitution at position −44 of the δ-globin promoter; this variant was identified in an α+ -thalassaemia carrier (−α^3.7/αα) with a low level of HbA2 (1.12%). The third was HBD: c.−58−52del (Genbank ID. GU145032), which involved a deletion from nt −8 to nt +102 and was also found in an α0-thalassaemia carrier (−α^0/αα) with a low level of HbA2 (1.69%). The fourth was HBD: c.94T>C (Genbank ID. GU145031), which involved a T→C substitution at codon 31 of the δ-globin gene, and it was found in an individual with a low level of HbA2 (1.83%). The fifth mutation was HBD: c. +136T>G (Genbank ID. GU145034), which involved a T→G change at position 136 of the 3’ UTR; the level of HbA2 in the carrier state was 1.98%.

In addition, two other rare mutations were characterized in the Chinese population for the first time. One is HBD: c.−127T>C, which corresponded to a T→C substitution at nt −77 within the promoter region. This mutation had been observed previously in the Japanese population and we identified it in three independent individuals. The other mutation is a GAG→AAG change at codon 43 (HBD: c.130G>A), which resulted in the abnormal Hb A2-Melbourne that was first reported in an Italian family.

Haematological features of Hb Westmead mutations

We identified a total of 89 individuals (1.55%) with the Hb Westmead mutation and found that this variant was the fourth most prevalent α-thalassaemia allele in the local population. Among the 89 individuals with this mutation, 11 subjects (12.36%) were diagnosed as co-inheriting β-thalassaemia and 5 individuals (5.61%) showed compound heterozygosity for the Hb Westmead mutation with α-thalassaemia deletion. To assess the haematological features of the Hb Westmead mutations, we compared the haematological phenotype of this variant in the presence or absence of thalassaemia mutations among 76 individuals from our study and 62 from the clinical laboratory. The differences for most FBC indices between the paired groups were shown in Table 3. Our conclusion is that an additional Hb Westmead mutation could aggravate the mild phenotype of the α-thalassaemia carrier (αα/−αSEA) to the level of an intermediate (Hb H disease caused by the αWSα/−αSEA); moreover, which could make more contribution to the phenotypic effects of mutations occurred in females than males, and what causes this appearance to form needs further study.

Discussion

We have performed a survey of the incidence and molecular characteristics of thalassaemias and other haemoglobinopathies in the Guangxi Zhuang Autonomous Region of southern China. The results of the survey indicated that there was an extremely high prevalence of inherited Hb disorders in this region: the heterozygote frequency was 24.51%. α-Thalassaemia showed the highest heterozygote frequency (17.55%), whereas the heterozygote frequency of δ-thalassaemia was very low (0.16%), reported herein for the first time. We also determined the prevalence with four different types of co-inheritance of two Hb disorders and thalassaemia intermediate (TI) (Table 1). We determined for the first time the true prevalence of silent α-thalassaemia by screening all the participants for the three common silent α-thalassaemia defects (−α^3.7/-, −α^3.2/-, and αWSα/). By using such a strategy, which combined phenotypic/molecular screening and genotyping, we were able to perform a population-based investigation of various haemoglobinopathies in Guangxi, rather than relying on a hospital-based or patient-based strategy to survey a partial region of Guangxi as previous studies did (12, 13). Furthermore, comparison of our data with that previously reported for this region (10, 12, 13) revealed the higher carrier frequency of various haemoglobinopathies, especially in α-thalassemias. The main reason we think that various defects including some silent mutations, such as the αWSα/ alleles contributed to overall incidence of inherited Hb disorders could be well detected based on our analysis strategy both by use of a large number of representative samples and by screening all the participants.

In the study reported herein, the prevalence of thalassaemias and other haemoglobinopathies and the carrier frequency of mutations were characterized for three major ethnic groups, Han, Zhuang and Yao (Table 2). The results showed that the frequency of both α- and β-thalassaemia and the distribution of mutations were correlated roughly among these three groups, except for statistically significant differences in the frequency of α-thalassaemia between the Han (15.71%) and Zhuang (20.12%, p = 0.0001) ethnic groups, and between the Han (15.71%) and Yao (20.84%, p = 0.002) ethnic groups. The results further confirm the hypothesis that these three Chinese populations originated from a common evolutionary group (20, 21). The current difference in carrier frequency between the Han and the other two ethnic groups was probably caused by a founder effect event initiated by genetic drift and followed by
Table 3. Comparison of haematological parameters between the Hb Westmead mutation and compound heterozygosity for the Hb Westmead mutation and \( \alpha \)-thalassaemia deletion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \alpha^{WS}_a/a_a )</th>
<th>( \alpha^{WS}_a/a_M^b )</th>
<th>( \alpha^{WS}_a/--)SEA )</th>
<th>( \alpha/--SEA )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>135.83 ( \pm ) 13.04</td>
<td>125.18 ( \pm ) 15.76</td>
<td>107.6 ( \pm ) 13.61</td>
<td>125.00 ( \pm ) 15.31</td>
</tr>
<tr>
<td>(n = 42)</td>
<td>(n = 49)</td>
<td>(n = 49)</td>
<td>(n = 5)</td>
<td>(n = 40)</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>84.98 ( \pm ) 6.48</td>
<td>84.51 ( \pm ) 8.02</td>
<td>72.78 ( \pm ) 6.95</td>
<td>76.54 ( \pm ) 6.44</td>
</tr>
<tr>
<td>(n = 42)</td>
<td>(n = 49)</td>
<td>(n = 8)</td>
<td>(n = 5)</td>
<td>(n = 40)</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.03 ( \pm ) 2.17</td>
<td>27.41 ( \pm ) 2.75</td>
<td>23.44 ( \pm ) 4.26</td>
<td>24.52 ( \pm ) 3.23</td>
</tr>
<tr>
<td>(n = 42)</td>
<td>(n = 49)</td>
<td>(n = 8)</td>
<td>(n = 5)</td>
<td>(n = 40)</td>
</tr>
<tr>
<td>HbA2 (%)</td>
<td>2.88 ( \pm ) 0.53</td>
<td>2.64 ( \pm ) 0.61</td>
<td>2.89 ( \pm ) 1.03</td>
<td>3.11 ( \pm ) 0.88</td>
</tr>
<tr>
<td>(n = 42)</td>
<td>(n = 49)</td>
<td>(n = 8)</td>
<td>(n = 5)</td>
<td>(n = 40)</td>
</tr>
</tbody>
</table>

Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean cell Hb; \( \alpha^{WS}_a \), Hb Westmead [2, 122 (H5) His\( \rightarrow \)Gln].

\( ^a \)There existed significant differences for most FBC indices between the paired groups (\( \alpha^{WS}_a/a_a \) vs \( \alpha^{WS}_a/--SEA \), \( p < 0.01 \); \( \alpha^{WS}_a/--SEA \) vs \( \alpha^{WS}_a/a_M^b \), \( p < 0.05 \); and \( \alpha^{WS}_a/a_a \) vs \( \alpha^{WS}_a/a_M^b \), \( p < 0.05 \)), except that there was no significant difference in Hb and MCH in the males and in Hb in females between the \( \alpha^{WS}_a/--SEA \) and \( \alpha^{WS}_a/a_M^b \) groups (\( p > 0.05 \)), and in Hb between males in the \( \alpha^{WS}_a/a_M^b \) and \( \alpha^{WS}_a/a_a \) groups (\( p > 0.05 \)). Simultaneously, there was statistically significant differences for Hb, MCV and MCH in the females between the \( \alpha^{WS}_a/--SEA \) and \( \alpha/--SEA \) groups (\( p < 0.01 \)); however, there was statistically significant difference only in MCV in the males between the \( \alpha^{WS}_a/--SEA \) and \( \alpha/--SEA \) groups (\( p < 0.01 \)). There was no statistically significant different in the level of HbA2 between all the paired groups (\( p > 0.05 \)).

\( ^b \)The genotype \( \alpha^{WS}_a/a_M^b \) involved three genotypes, which corresponded to \( \alpha^{WS}_a/a^{4.2} \), \( \alpha^{WS}_a/a^{3.7} \) and \( \alpha^{WS}_a/a^{WS}_a \). Values are given as the mean \( \pm \) SD of (n) determinations. Same sex groups for different genotypes of compound heterozygosity for Hb Westmead and \( \alpha \)-thalassaemia were compared by t-tests. Reference range values: Hb, 120–170 g/l (male), 110–160 g/l (female); MCV, 82–94 fl; MCH, 26–32 pg; and HbA2, 2.5–3.5%.
isolated living conditions and religious customs such as endogamous marriage. Furthermore, our results indicated that the prevalence of thalassemias and other haemoglobinopathies throughout southern China is highest in Guangxi, with the exception of the carrier frequency for silent α-thalassaemia in Hainan Island, which is greater than 50% (16, 22, 23). The observed geographical distribution and prevalence of mutant alleles for various Hb disorders in Guangxi is reminiscent of the situation found for glucose-6-phosphate dehydrogenase (G6PD) deficiency in this region (24).

This confirms further the hypothesis that alleles of the human α- and β-globin genes and the G6PD gene are involved in a protective effect against malaria (25–28).

On the basis of our epidemiological results, with a current annual approximately 700,000 births in Guangxi, the expected number of affected births each year therefore would be 724 (95% CI, 603–897) for β thalassaemia major or TI, 1095 (95% CI, 935–1337) for Bart’s hydrops fetalis, and 1327 (95% CI, 1148–1610) for Hb H disease, respectively, which are arising from the estimated rates per 1000 pregnancies with 4.14, 6.26, and 7.58 for these three genetic disorders, respectively, assuming a 1/4 reproductive risk. No deviation from Hardy–Weinberg or genotypic equilibrium was observed for the allele and genotype distributions of the different thalassaemia loci.

Our study shows that the inherited Hb disorders, in particular α- and β-thalassaemia, are a very severe public health issue among the local populations of Guangxi. Characterization of the mutational spectrum will enable us to design a rational strategy to control α- and β-thalassaemias in this region. The findings of this study suggest that it will be necessary to consider the following aspects when implementing a large-scale prevention program (1). Both α- and β-thalassaemia have a narrow mutational spectrum in this region, six and eight mutations account for 99% of α-thalassaemia and 98% of β-thalassaemia defects, respectively. This should allow us to design cost-effective molecular tests (2). As many as 1.36% of the local population co-inherited α- and β-thalassaemia mutations. We suggest that all couples where both partners show variation in any Hb gene in the Guangxi region should be screened for α-thalassaemia mutations (3). The occurrence of both δ-thalassaemia and structural Hb variants was lower than expected except for those common Hb variants causing α-thalassemia (Hb Westmead, Hb CS, and Hb QS) or β-thalasssemia (Hb E); the incidence of δ-thalassaemia in Guangxi of 0.16% was far less than that in Italy (2.5%) or Cyprus (1.47%) (17, 29). Thus, co-inheritance of β- and δ-thalassaemia, which might lead to misdiagnosis, is rare. Among the nine structural Hb variants identified in this study, only Hb Q-Thailand displays a slight thalassaemic phenotype, and it needs to be considered during screening for α-thalassaemia in order to give comprehensive genetic counselling to those couples in whom α-thalassaemia co-exists with Hb Q-Thailand (4). Hb H disease and TI are important issues for prenatal diagnosis in this region (30–32). Close attention should be paid to two forms of these disorders that occurred at a relatively high rate. The first is Hb H disease caused by compound heterozygosity for the Hb Westmead mutation and a deletion mutation for α-thalassaemia (αWSa/–). The second is a type of TI that results from the coexistence of a γ-globin gene triplication (aaαα) with heterozygosity for a β-thalassaemia mutation. These two disorders appear to be milder forms of TI according to our observation of several such cases. We need to help at-risk couples to recognize this type of disease and then to make their decision as to whether to choose to have prenatal diagnosis and to terminate a pregnancy if necessary.

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Conflict of interest
Authors report no conflicts of interest.

References


