RS12574989 and haplotype associated with α/β-chain imbalance and population HbA2 reduction
Qiyin Lin1,2†, Yingjun Xie1,2†, Xuan Zhong3, Xiaofang Sun1,2 and Ding Wang1,2*

Abstract
Determining the associated relationship of genotype and phenomenon would benefit the understanding of disease and renew disease intervention means. 14,518 patients who underwent haemoglobin electrophoresis from June 2020 to December 2020 were enrolled in our study, and additional data including sex, age and routine blood examination results were collected. We focused on individuals with normal red blood cell indices and no common thalassemia pathogenic mutation and selected three groups for the following study: the control group (2.5% ≤ HbA2 ≤ 3.5%), the HbA2 under 2.5 group (HbA2 < 2.5%) and the HbA2 under 2.4 group (HbA2 < 2.4%). Four regions of β-globin regulation were sequenced. Statistical analysis was conducted to compare the collected information of the three groups and the genotype distributions in the control group and sequenced group. The HbA2 under 2.5 group was characterized by a majority of females and lower red blood cell counts and haemoglobin compared with the control group. There were genotypes associated with the grouping as the T of rs12574989 and TTT AGC of the haplotype were significantly increased in the HbA2 under 2.4 group and CTT AGC was significantly decreased in the HbA2 under 2.4 group. This study demonstrated that the genotypes of the population associated with HbA2 were reduced in southern China.

Keywords: α/β-chain imbalance, Thalassemia, Haemoglobin distribution, Single nucleotide polymorphisms, Haplotypes

Introduction
Thalassemia, as the natural selection against Plasmodium falciparum malaria [1], originates from certain warm areas, including the Mediterranean, Middle East, central Asia, India, and southern China; and has spread worldwide to be one of the most common genetic disorders [2, 3]. Guangdong located in the south of China with a high incidence of thalassemia [4], and over 10% of the population in this area are thalassaemia carriers [5]. According to the clinical spectrum, some thalassemia carriers have mild anaemia and are asymptomatic without needing regular transfusions, but they require occasional transfusions during some physiopathological state [6]. In addition, the offsprings of some carriers are at high risk of severe thalassemia [7]. Thalassemia screening to identify carriers will provide useful advice for people with special medical conditions and genetic counselling for healthy fertile individuals. In the clinical, the haematological examination results show that MCH (mean red blood cell hemoglobin) and MCV (mean red blood cell volume) are lower than the critical value, indicating that the risk of thalassemia is higher, and further gene diagnosis would be used for more accurate and efficient diagnosis for identification of the positive population.

†Qiyin Lin and Yingjun Xie contributed equally to the manuscript.
*Correspondence: largestone_1984@163.com

1 Present Address: Department of Obstetrics and Gynecology, Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, No. 63 Duobao Road, Guangzhou 510150, Guangdong, China
Full list of author information is available at the end of the article
However, there were contradictory results as there is no clear genetic understanding of some odd haematological phenotypes during thalassemia screening. Genetic studies targeting specific populations with specific haematological phenotypes will enhance our understanding of the disease, even when determining a cure for the disease.

Thalassemia is a typical genetic disease with defects in haemoglobin [8]. The management of thalassemia depends on its clinical characteristics [9], which is a response to the genetic basis of Hb gene variation. There were two main human Hbs in normal adults, which were HbA and HbA2: HbA accounts for 96.5%-97.5% of Hbs and is the major protein. HbA consists of two α-and two β-globin chains and the rest of HbA2 consists of two α-and two δ-globin chains [10]. The balance of α- and β-globin chains comprises functional HbA, which is the molecular basis for Hb to perform its function [11].

Patients with thalassemia have genetic variants that cause abnormal α- or β-globin chains, which can be classified as α-thalassemia and β-thalassemia, as fail to produce these chains [12]. For those results of haemoglobin electrophoresis in α-thalassemia positive patients showed that HbA increased to more than 97.5%, but decreased to 96.5%, indicating a higher risk of β-thalassemia. The decreased in globin output of the β-globin caused by β-globin gene mutations was the genetic basis of β-thalassemia, which is one of the most important haemoglobinopathy in clinic [13].

The β-like globin genes are located in 11p15.5 [14], the expression of the human β-globin gene is switched after birth and replaces the foetal period expression gene γ-globin [15]. The β-globin gene is regulated by the 3’ enhancer [16] and 5’ locus control region (LCR), which is several kilobases upstream of named DNase I hypersensitive sites (HS) 2, HS3 and HS4 [17, 18]. Single nucleotide polymorphisms (SNPs) were identified in the 3’ enhancer, and we found that RS12574989 together with two haplotypes were associated with the clinical grouping. Collectively, we identified that genetic polymorphisms of the β-globin 3’ enhancer was associated with increased HbA and decreased HbA2. These results benefit the genetic understanding of β-globin regulation and β-thalassemia gene therapy.

Materials and methods

Study population

Our study was approved by the Academic Committee of the Third Affiliated Hospital of Guangzhou Medical University. From June 2020 to December 2020, 14,518 patients who underwent haemoglobin electrophoresis assays were enrolled in this study, and all of them signed informed consent before the study started. In order to eliminate the confounding factors, such as blood disease, age (especially new-borns) and blood transplantation, we selected patients from the reproductive department as the control and HbA2 under 2.5 group for further statistical analysis. The study items included sex, age, clinical examination and genotype.

Haemoglobin electrophoresis

The peripheral blood of each subject was anticoagulated with EDTA for examination in this study. Whole blood was lysed using haemolysing solution. Haemoglobin electrophoresis was performed on a capillary electrophoresis instrument (Capillarys 2 Flex Piercing, Sebia, Evry, France) according to the manufacturer’s instructions.

Bloods routine examination

The haematological parameters of the peripheral blood were determined on an automatic haematology analyser (Sysmex XN-9000, Shanghai, China) according to the manufacturer’s instructions.

Genetic diagnosis of thalassemia

The genomic DNA of each individual was extracted from peripheral blood using a QIAamp DNA Blood Mini Kit (Cat# 51,104, Qiagen, Dusseldorf, Germany). The genetic diagnosis of thalassemia was performed using commercialized kits purchased from Yaneng Biosciences (Shenzhen, Guangdong, China). The deletions of α-globin were detected using gap polymerase chain
and HBB:c.-11_-8delAAACA. HBB:c.92
HBB:c.85dupC, HBB:c.-82C > A, HBB:c.-80 T > C,
HBB:c.-79A > G, HBB:c.2 T > G, HBB:c.45dupG,
HBB:c.79G > A, HBB:c.217dupA, HBB:c.130G > T,
HBB:c.316-197C > T, HBB:c.52A > T, HBB:c.-78A > C,
and for β-globin, they were HBB:c.126_129delCTTT,
(HBA2:c.377 T > C) and ααWS (HBA2:c.369C > G);
provide the range (minimum–maximum) while qualita-
tive manufacturer’s instructions. The mutations were deter-
mined by alignment with Genome Reference Consortium
Human GRCh38.

DNA segments sequencing

The DNA segments were amplified by PCR using AceTaq
Master Mix (Cat# P412, Vazyme, Nanjing, China) with
primers for HS2, HS3, HS4 and 3’ enhancer (Table 1).
The sizes of the segments were confirmed by agarose
electrophoresis, and Sanger sequence was using BigDye
Terminator v3.1 Cycle Sequencing Kit (Cat #4,337,455,
Thermo Fisher Scientific, CA, USA) according to the
manufacturer’s instructions. The mutations were deter-
mined by alignment with Genome Reference Consortium
Human GRCh38.

Statistical analysis

Quantitative results are expressed as the means ± SD and
provide the range (minimum–maximum) while qualita-
tive results are expressed as numbers of cases and ratios
in tables. For quantitative data, ANOVA was used for
multiple group comparisons, and the two-tailed Student’s
t-test was applied to compare the differences between
the two groups. For qualitative data, the Pearson’s chi
squared test was used for two-group distribution differ-
ence comparisons and odds ratios (ORs) with respec-
tive confidence interval (95% CI) calculations. Statistical
analysis was performed using the SPSS 20.0 software
(SPSS Inc., Chicago, IL, USA). P < 0.05 was regarded as a
statistically significant difference. The linkage imbalance
among the six alleles was analysed using the online SHE-
SIS software (http://analysis.bio-x.cn/myAnalysis.php).

Results

Characteristics of high HbA, low HbA2 and normal MCV
and MCH populations

In total, 14,518 individuals who underwent thalassemia
screening by haemoglobin electrophoresis were included
in this study (Fig. 1). The control group included 183 ran-
domly selected individuals in the negative part of haemo-
globin electrophoresis who possessed an HbA2 from 2.5%
to 3.5% (Fig. 2. A and B), MCV ≥ 82 fl, MCH ≥ 27 pg
and no thalassemia diagnose by genetic testing (Fig. 1). A
total of 25.14% of individuals who underwent haemoglo-
bin electrophoresis screening were positive for an abnor-
mal HbA2 ratio or haemoglobin variants, and 82.99% of
people were suspected to possess α-thalassemia with a
low HbA2 (Fig. 1). In the HbA2 < 2.5% population, 55.91%of
the people had normal MCV and MCH, 6.95% of the
people were confirmed to possess α-thalassemia via
genetic diagnosis and the rest were normal individu-
als (Fig. 1). The HbA2 under 2.5 group (Fig. 2. A and B)
included 931 individuals excluding newborns, blood
transplantation patients and patients with haematologi-
malignancies, who shared similar MCV, MCH and
thalassemia genetic diagnosis results with the control
group but obtained an HbA2 below 2.5% (Table 2).

Significant differences in sex and routine blood indices
were found between the HbA2 under 2.5 group
and the control group.

165 individuals in the HbA2 under 2.5 group and HbA
higher than 97.6% were randomly selected and included
in the HbA2 under 2.4 group (Fig. 2. A). The distribu-
tion of HbA and HbA2 in the HbA2 under 2.4 group
was similar to that in the HbA2 under 2.5 group (Fig. 2.
A and B). The basic information and clinical haemato-
logical information between the HbA2 under 2.5 group
(n=931), HbA2 under 2.4 group (n=165) and control
(n=183) were statistically analysed. There were no
significant differences between the HbA2 under 2.5 group
and HbA2 under 2.4 group (Table 2). The HbA2 under
2.5 group and HbA2 under 2.4 group had a high propor-
tion of females compared with the control group, and
the sex ratio of the control group was near 1:1 (Table 2).
There was no significant difference of MCV and MCH
in groups (Table 2). There were consistent trends as a
reduce of MCHC and RDW in HbA2 reduce groups com-
pare with control group without statistical difference
(Table 2). There was no significant difference of MCV and MCH
in groups (Table 2). There were consistent trends as a
reduce of MCHC and RDW in HbA2 reduce groups com-
pare with control group without statistical difference
(Table 2). There were consistent results in RBC and Hb
as significant decreases in RBC (P value < 0.05) and Hb

| Primer name | Base sequence (5’-3’) | Size (bp) |
|-------------|-----------------------|----------|
| HS4-F       | CTGACTTGTAAAGCTTCTC   | 1099     |
| HS4-R       | CCTGGTGAAAGTGAATT     |          |
| HS3-F       | AGAAGAGTCAAGCTTGGCT   | 845      |
| HS3-R       | CTGGTACAGGGTCTTACGG   |          |
| HS2-F       | GTGCAGCTGAGCTGAGTC    | 618      |
| HS2-R       | CACATTGTCTCAGGCATC    |          |
| 3’ enhancer-F| GAATGGGGAGGCATGGT    | 806      |
| 3’ enhancer-R| GTGGTTGATGGTACACTAG   |          |
(P value < 0.01) were observed in the HbA2 reduce group compared to the control group (Table 2).

**Genotype associated with clinical grouping**
To determine the gene variations of β-globin regulation, four segments were sequenced for genotyping in a total of 20 individuals (10 for the HbA2 reduce group and 10 for the control group). Because there was no variation found in HS2, HS3 and HS4, further study was focused on the 3’ enhancer. There were no deletions or insertions, but six single nucleotide polymorphisms were found: rs12574989, rs7110263, rs10837631, rs78928216, rs10837630 and rs11036351. In the comparison of the HbA2 reduce group and control group, a significant increase in the rs12574989 mutation type (heterozygous type-CT and homozygous type-TT, Fig. 2. C) was found in the HbA2 under 2.4 group, but no difference was found in the other loci (Table 3). Statistical analysis of six SNPs formed a haplotype prompt that CTT AGC significantly decreased and TTT AGC significantly decreased in the HbA2 reduce group compared with the control group (Table 3).

**Discussion**
According to clinical diagnosis and management differences, thalassemia can be divided into three levels: transfusion-dependent thalassemia (TDT), nontransfusion-dependent thalassemia (NTDT) and minor thalassemia [12]. Thalassemia has affected individuals since birth with clinical criteria including anaemia, reduced Hb, significant hepatosplenomegaly, retarded growth and height and frequent intercurrent infections [12, 20]. The effective management of thalassemia could improve clinical symptoms but not provide fundamental solutions, such as blood transfusions, a splenectomy, iron chelation, and even stem cell transplantation [12, 21]. In clinical, the commercial kit could provide over ninety-persents conformed genetic thalassemia diagnosis report, but there was not certain genetic explanation for some clinical features. To completely avoid severe thalassemia, it is necessary to screen thalassemia carriers.
that could affect offspring [7] and study the pathogenesis-related genetics to produce available treatments.

Thalassemia screening aimed to identify individuals who were potential pathogenic mutation carriers using red blood cell indices and haemoglobin analysis. The widely used indices for screening red blood cell indices were Hb, MCV and MCH as values lower than the cut-offs (11 g/dL for Hb, 27 pg for MCH and 82 fl for MCV) prompt high risk [21, 22]. However, for clinical usage, the cut-offs were slightly adjusted for local people as we defined high risk as Hb < 11.5 g/dL and MCV < 82 fl. Hb analysis could be conducted by capillary electrophoresis, which is an approved platform of Hb analysers [23], and used to identify if there are some Hb variants and determine the ratio of HbA and HbA2. In our study, the majority of the HbA2 < 2.5% population (82.99%) was Hb positive, and more than half (55.91%) of these patients had a normal MCV and MCH. In this population, a small percentage (6.95%) of patients were diagnosed as thalassemia if they were diagnosed as α-thalassemia without any β-thalassemia, the majority (93.05%) of these patients were not diagnosed with any pathogenic mutations. It is worth noting that the individuals in our HbA2 reduce group are those with normal heredity and erythrocyte index, and their HbA2 proportion has decreased. Statistical analysis showed that the majority of this population

![Fig. 2](image_url)  
Fig. 2 The SNP was identified associated with HbA2 reduction. A indicated distribution of HbA and HbA2 in different grouping. B indicated the haemoglobin electrophoresis of patients in the HbA2 reduce group and control (normal) group. C indicated genotype of rs12574989 C/T which was associated with HbA2 reduce grouping.
was female and had a low RBC and Hb compared with the control group.

The theory of α/β-chain imbalance refers to that the pathogenic mutation leads to the restriction of one globin expression and induces the excess of another globin, which directly leads to ineffective erythropoiesis, and further leads to iron overload, anemia and organ damage [19]. Like β-thalassemia, α-thalassemia is caused by a disease-causing genotype that makes defective production of α globin. The typical haematology characteristics of α-thalassemia patients were up-regulation of HbA and down-regulation of HbA2. Multi genetic reasons leading this clinical phenomenon, beside the α-globin abnormal, multi genetic reasons leading this clinical phenomenon. We focus on genetic variation analysis of β-globin regulation regions.

Genetic variants in functional regions are associated with gene expression and disease pathological progress [24]. The β-globin regulation regions, including HS regions [25, 26] and their 3′ enhancers [16], were found decades ago. As their function was proven in cell cultures and in transgenic mice [17], an engineered lentiviral product was created for β-thalassemia treatment, which could spatially and temporally mimic functional β-globin expression [27]. Furthermore, the clinical symptoms of β-thalassemia were effectively alleviated by transplantation of autologous haematopoietic stem cells (HSCs), in which lentiviruses transferred normal and functional β-globin [28]. The functional regions of β-globin regulation in the lentiviral product were detected in our study. HS was a conserved region without any variants, and six SNPs were found in the 3′ enhancer. Only one SNP (rs12574989) was associated with the grouping. As C turned to T, the CpG island of the wild type was destroyed by this mutation. CpG islands are classical methylation sites. This is consistent with our hypothesis that the loss of wild type results in increased gene expression. Furthermore, the two haplotypes, which were generated because of SNP variation, were also associated with the grouping. CTT AGC was significantly decreased and TTT AGC was increased in HbA2 under 2.4 group. The variation of RS12574989 was different in regions base on the database of dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and the ALFA project and 1000Genomes reported similar results, the alt allele ‘T’ was under 5% in African (0.15%), Europe (2.68%) and American (1.3%). The top occurrence was East Asian (10.91%) and South Asian (8.7%), according the study of 1000Genomes (https://www.ncbi.nlm.nih.gov/snp/rs12574989#frequency_tab). However, there were seldom published literature covered the individual pathophysiological phenomenon associated with variant RS12574989 and haplotypes. In the current study, it is identified that significantly increased SNP ‘T’ of rs12574989 was only 20.9% of individuals with the HbA2 under 2.4 group, that means the relationship of the clinical phenomenon and genetic was associated but not causal, and there were other potential reasons to lead the HbA abnormal. However, the HbA

| Table 2 Characteristics of the HbA2 reduce group and control group |
|---------------------------------------------------------------|
| HbA2 under 2.5 group (n = 931) | HbA2 under 2.4 group (n = 165) | Control group (n = 183) | P value |
|--------------------------------|--------------------------------|--------------------------|---------|
| HbA2 under 2.5 group (n = 934) VS. HbA2 under 2.4 (n = 165) | HbA2 under 2.5 group (n = 934) VS. Control (n = 183) | HbA2 under 2.4 group (n = 165) VS. Control (n = 183) |
| Age (Mean ± SD) | 32.87 ± 5.81 | 33.21 ± 5.67 | 32.46 ± 5.08 | 0.487 | 0.377 | 0.1953 |
| Gender [n(%)] Male | 347(37.15) | 50(30.3) | 85(46.45) | 0.065 | 0.025 | <0.001 |
| Female | 587(62.85) | 115(69.7) | 98(53.55) |
| MCV [fL] Mean ± SD | 88.10 ± 3.30 | 88.48 ± 3.39 | 88.34 ± 3.55 | 0.17 | 0.362 | 0.7118 |
| MCH [pg] Mean ± SD | 30.12 ± 1.28 | 30.08 ± 1.24 | 30.24 ± 1.14 | 0.735 | 0.213 | 0.2007 |
| MCHC [g/dL] Mean ± SD | 34.19 ± 0.99 | 34.04 ± 0.84 | 34.27 ± 0.83 | 0.068 | 0.325 | 0.0112 |
| RBC [10^12/L] Mean ± SD | 4.64 ± 0.5 | 4.62 ± 0.46 | 4.74 ± 0.47 | 0.517 | 0.111 | 0.0037 |
| Hb [g/dL] Mean ± SD | 13.97 ± 1.51 | 13.9 ± 1.38 | 14.34 ± 1.4 | 0.073 | 0.003 | 0.0646 |
| RDW [%] Mean ± SD | 12.6 ± 0.74 | 12.58 ± 0.83 | 12.44 ± 0.6 | 0.831 | 0.007 | 0.0646 |
| Range | 27–34.4 | 27±33.7 | 27.7–33.9 | 0.735 | 0.213 | 0.2007 |
| Range | 27–33.7 | 27.7–33.9 | 0.735 | 0.213 | 0.2007 |
| Range | 31.2–38.1 | 31.7–36.3 | 32.1–36.5 | 0.068 | 0.325 | 0.0112 |
| Range | 2.94–6.17 | 3.24–6.03 | 3.8–5.84 | 0.517 | 0.111 | 0.0037 |
| Range | 8.7–18 | 9.9–18 | 11.5–17.5 | 0.073 | 0.003 | 0.0646 |
| Range | 10.9–18.4 | 10.9–18.4 | 11.2–15.5 | 0.831 | 0.007 | 0.0646 |
level of the individuals who got ‘T’ variation were 97.5% to 98%, this prompt the genetic variation might related to the clinical phenomenon. Although the HbA2 under 2.4 group is 165, they were sufficient to conclude with clinical and genetic features in southern China. Firstly, the HbA2 under 2.4 group was the representative of the HbA2 under 2.5 (n = 931), at which share consistent clinical information and distribution of HbA and HbA2. Secondly, the HbA2 reduce group was 6.4% (931/14518) of the total, as there were 14,518 thalassemia screening people were included in this study. Further large scale genetic testing to find out more CTT AGC haplotype and comparison study for the interplay between the genetic and haematological phenotype was needed.

In the present study, we demonstrated specific genotype enrichment in a HbA2 reduced population, but the influence on disease pathological progress was unknown. Further studies are needed to define if the single base difference leading the gene expression difference and the involved mechanism, such as epigenetic.

**Conclusion**

In summary, our study focused on a population with normal red blood cell indices and no common pathogenic mutation of the α- and β-globin genes but a reduced HbA2 ratio. We demonstrated that the population was primarily female and had low RBC and Hb compared with the control group, and a genetic variation in the functional region of the β-globin gene was associated with this population. Further functional studies are needed to define the biological function of haplotypes if they cause β-globin expression changes, which would benefit gene therapy strategy designs.

### Table 3

| SNP            | HbA2 under 2.4 group (n = 165) | Control group (n = 183) | OR[95%CI]      | P value |
|----------------|-------------------------------|-------------------------|----------------|---------|
| rs 12574989 C/T|                               |                         |                |         |
| CC             | 102(0.618)                    | 159(0.869)              | 1              |         |
| CT             | 57(0.345)                     | 23(0.126)               | 3.863[2.242–6.658] | <0.001  |
| TT             | 6(0.036)                      | 1(0.005)                | 9.353[1.110–78.826] | <0.001  |
| CT + TT        | 63(0.382)                     | 24(0.131)               | 9.353[1.847–2.268] | <0.001  |
| C              | 261(0.791)                    | 341(0.932)              | 17.613[1.007–1.259] | <0.001  |
| T              | 69(0.209)                     | 25(0.068)               |                |         |
| rs 7110263 T/G |                               |                         |                |         |
| TT             | 44(0.267)                     | 50(0.273)               | 1              |         |
| CT             | 84(0.509)                     | 98(0.536)               | 0.469[0.313–0.702] | 0.746   |
| GG             | 37(0.224)                     | 35(0.191)               | 0.365[0.212–0.630] | 0.746   |
| G              | 158(0.479)                    | 168(0.459)              | 1.083[0.804–1.459] | 0.602   |
| T              | 172(0.521)                    | 198(0.541)              |                |         |
| rs 10837631 T/A|                               |                         |                |         |
| TT             | 82(0.497)                     | 82(0.448)               | 1              |         |
| AT             | 66(0.400)                     | 84(0.459)               | 0.786[0.504–1.226] | 0.54    |
| AA             | 17(0.103)                     | 17(0.093)               | 1[0.478–2.093] | 0.54    |
| A              | 100(0.303)                    | 118(0.322)              |                |         |
| T              | 230(0.697)                    | 248(0.678)              | 0.914[0.663–1.260] | 0.582   |
| rs 78928216 A/C|                               |                         |                |         |
| AA             | 109(0.661)                    | 135(0.738)              | 1              |         |
| AC             | 51(0.309)                     | 46(0.251)               | 0.188[1.373–0.857] | 0.183   |
| CC             | 5(0.030)                      | 2(0.011)                | 0.182[3.096–0.589] | 0.183   |
| A              | 269(0.815)                    | 316(0.863)              |                |         |
| C              | 61(0.185)                     | 50(0.137)               | 0.698[0.464–1.049] | 0.083   |
| rs 10837630 G/C|                               |                         |                |         |
| GG             | 45(0.273)                     | 51(0.279)               | 1              |         |
| CG             | 82(0.497)                     | 96(0.525)               | 0.898[0.968–0.589] | 0.741   |
| CC             | 38(0.230)                     | 36(0.197)               | 0.563[1.196–0.652] | 0.602   |
| C              | 158(0.479)                    | 168(0.459)              | 1.083[0.803–1.459] | 0.083   |
| G              | 172(0.521)                    | 198(0.541)              |                |         |
| rs 11036351 C/T|                               |                         |                |         |
| CC             | 110(0.667)                    | 136(0.743)              | 1              |         |
| CT             | 51(0.309)                     | 46(0.251)               | 0.19[1.371–0.856] | 0.083   |

### Table 3 (continued)

| SNP            | HbA2 under 2.4 group (n = 165) | Control group (n = 183) | OR[95%CI]      | P value |
|----------------|-------------------------------|-------------------------|----------------|---------|
| TT             | 4(0.024)                      | 1(0.005)                | 0.155[4.945–0.545] | 0.143   |
| C              | 271(0.821)                    | 318(0.869)              |                |         |
| T              | 59(0.179)                     | 48(0.131)               | 0.693[0.458–1.049] | 0.082   |
| Haplotype      |                               |                         |                |         |
| C G A A C C     | 96(0.291)                     | 112(0.306)              | 0.906[0.653–1.257] | 0.555   |
| C G T C C T     | 57(0.173)                     | 46(0.126)               | 1.425[0.935–2.173] | 0.098   |
| C T T A G C     | 101(0.306)                    | 170(0.464)              | 0.487[0.356–0.668] | <0.001  |
| T T T A G C     | 69(0.209)                     | 24(0.066)               | 3.710[2.267–6.072] | <0.001  |

(continued)
Acknowledgements
We sincerely thank all of the participated patients and normal volunteers.

Author contributions
QL, XS and DW conceived and designed the study. QL collected the clinical data. XZ and YX analyzed the data. DW wrote the manuscript. All authors read and approved the final manuscript.

Funding
This study was supported by Guangzhou Education Bureau-funded project (202032888), Guangzhou Science and technology project (202102010105) and Guangdong Education Department project.

Availability of data and materials
The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: ClinVar SCV001809802.

Declarations

Ethics approval and consent to participate
All the methods of this study were established and have been performed in accordance with the Declaration of Helsinki, and approved by the Academic Committee of the Third Affiliated Hospital of Guangzhou Medical University (No. YLKS2022-005). All the participated individuals signs the written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest.

Author details
1Present Address: Department of Obstetrics and Gynecology, Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510000, Guangdong, China. 2Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, Guangdong, China. 3Medical Intensive Care Unit, Guangdong Women and Children Hospital, Guangzhou 510000, Guangdong, China. 4Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, Guangdong, China. 5Medical Intensive Care Unit, Guangdong Women and Children Hospital, Guangzhou 510000, Guangdong, China.

Received: 8 April 2022 Accepted: 8 August 2022
Published online: 15 August 2022

References
1. Weatherall DJ. Genetic variation and susceptibility to infection: the red cell and malaria. Br J Haematol. 2008;141(3):276–86.
2. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86(6):480–7.
3. Weatherall DJ. The inherited diseases of haemoglobin are an emerging global health burden. Blood. 2010;115(22):4331–6.
4. Zeng YT, Huang SZ. Disorders of haemoglobin in China. J Med Genet. 1987;24(10):578–83.
5. Xu XM, Zhou YQ, Luo GX, Liao C, Zhou M, Chen PY, Lu JP, Jia SQ, Xiao GF, Shen X, et al. The prevalence and spectrum of alpha and beta thalassemia in Guangdong Province: implications for the future health burden and population screening. J Clin Pathol. 2004;57(5):S17–22.
6. Taher AT, Radwan A, Viprakasit V. When to consider transfusion therapy for patients with non-transfusion-dependent thalassemia. Vox Sang. 2015;108(1):1–10.
7. Viprakasit V, Limwongse C, Sukpanichnant S, Ruangvutilert P, Kanjanakorn C, Giomglas W, Sinkong M, Utto W, Tanphaichitr V. Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand. Clin Chem Lab Med. 2013;51(8):1605–14.
8. Mettananda S, Fisher CA, Hay D, Badat M, Quek L, Clark K, Hublitz P, Dowdes D, Kerry J, Gosden M, et al. Editing an alpha-globin enhancer in primary human hematopoietic stem cells as a treatment for beta-thalassemia. Nat Commun. 2017;8(1):424.
9. In: Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT). Edited by rd, Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. Nice, 2013.
10. Stamatoyannopoulos G. Control of globin gene expression during development and erythroid differentiation. Exp Hematol. 2005;33(3):259–71.
11. Mettananda S, Higgins DR. Molecular basis and genetic modifiers of thalassemia. Hematol Oncol Clin North Am. 2018;32(2):193–211.
12. Viprakasit V, Ekawattanakit S. Clinical classification, screening and diagnosis for thalassemia. Hematol Oncol Clin North Am. 2018;32(2):193–211.
13. Thein SL. Molecular basis of beta thalassemia and potential therapeutic targets. Blood Cells Mol Dis. 2018;70:54–65.
14. Smith EC, Orkin SH. Hemoglobin genetics: recent contributions of GWAS and gene editing. Hum Mol Genet. 2016;25(R2):R99–105.
15. Higgins DR, Engel JD, Stamatoyannopoulos G. Thalassemia. Lancet. 2012;379(9813):373–83.
16. Trudel M, Costantini F. A 3’ enhancer contributes to the stage-specific expression of the human beta-globin gene. Genes Dev. 1987;1(1):954–61.
17. Fraser P, Pruzina S, Antoniou M, Grosfeld V. Each hypersensitive site of the human beta-globin locus control region confers a different developmental pattern of expression on the globin genes. Genes Dev. 1993;7(1):106–13.
18. Amid A, Cheong M, Eng B, Hanna M, Hohenadel BA, Nakamura LM, Walker L, Odame I, Kirby-Allen M, Wave JS. Hb S/beta+4-thalassemia due to Hb sickle and a novel deletion of DNase I hypersensitive sites HS3 and HS4 of the beta locus control region. Haematologica. 2015;100(5):e166–168.
19. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391(10116):155–67.
20. Weatherall DJ. The definition and epidemiology of non-transfusion-dependent thalassemia. Blood Rev. 2012;26(Suppl 1):53–6.
21. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V. In: Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT). Edited by Weatherall D. Nice, 2013.
22. Sanguanvanich T, Phumy N, Chomchuensan S, Steger HF. Screening for alpha-thalassemia-1 heterozygotes in expecting couples by the combination of a simple erythrocyte osmotic fragility test and a PCR-based method. Commun Genet. 1999;2(1):26–9.
23. Sae-ung N, Srivorakun H, Fucharoen G, Yamsri S, Sanchaisuriya K, Fucharoen S. Phenotypic expression of hemoglobins A(2), E and F in various hemoglobin E related disorders. Blood Cells Mol Dis. 2012;48(1):11–6.
24. Wang D, Zhong X, Huang D, Chen R, Bai G, Li Q, Yu B, Fan Y, Sun X. Functional polymorphisms of interferon-gamma affect pneumonia-induced sepsis. PLoS ONE. 2014;9(1):e87049.
25. Forrester WC, Thompson C, Elder JT, Grousdale M. A developmentally stable chromatin structure in the human beta-globin gene cluster. Proc Natl Acad Sci USA. 1986;83(5):1539–63.
26. Tuan D, Solomon W, Lü Q, London IM. The “beta-like-globin” gene domain in human erythroid cells. Proc Natl Acad Sci USA. 1985;82(19):6384–8.
27. Negre O, Eggimann AV, Beuzard Y, Ribeil JA, Bourget P, Borwornpinyo S, Hongeng S, Hacein-Bey S, Cavazzana M, Leboulch P, et al. Gene Therapy of the beta-Hemoglobinopathies by Lentiviral Transfer of the beta(A(T87Q))-Globin Gene. Hum Gene Ther. 2016;27(2):148–65.
28. Thompson AA, Walters MC, Kwiatkowski J, Rasko JEJ, Ribeil JA, Hongeng S, Magrin E, Schiller GI, Payen E, Semeraro M, et al. Gene therapy in patients with transfusion-dependent beta-thalassemia. N Engl J Med. 2018;378(16):1479–93.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.