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## Mutation Screening for Thalassaemia in the Jino Ethnic Minority Population of Yunnan Province, Southwest China

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6 2 Mutation Screening for Thalassaemia in the Jino Ethnic  
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9 3 Minority Population of Yunnan Province, Southwest China

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## 22 ABSTRACT

### 23 Objectives

24 This study aimed to detect  $\alpha$ - and  $\beta$ -thalassaemia mutations in the Jino ethnic minority  
25 population of Yunnan Province, southwest China.

### 26 Design

27 A total of 1,613 Jino adults were continuously recruited from February 2012 to April  
28 2012. Fasting venous blood samples were obtained to determine haematological  
29 parameters. Haemoglobin analysis was conducted via high-performance liquid  
30 chromatography. Participants with hypochromic microcytic anaemia or positive  
31 haemoglobin analysis profiles were confirmed via  $\alpha$ - and  $\beta$ -globin genetic testing,  
32 including DNA microarray analysis, direct sequencing methods and multiplex  
33 gap-polymerase chain reaction assays.

### 34 Setting

35 Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai  
36 Jiao Tong University Affiliated Sixth People's Hospital

### 37 Results

38 We found 363 suspected cases via primary screening of haematological parameters  
39 and haemoglobin analysis. After further genetic testing, six types of  $\alpha$ - and  
40  $\beta$ -thalassaemia mutations were detected in 203 out of 363 individuals. Both of  $\alpha^0$ - and  
41  $\alpha^+$ -thalassaemia mutations, -<sup>-SEA</sup> and - $\alpha^{3.7}$  were identified. Additionally, 13 Hb E  
42 carriers had coexisting  $\alpha^0$  or  $\alpha^+$ -thalassaemia deletions. Clinical haematological  
43 parameters indicated that in this study, carriers of all thalassaemic genotypes had

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4 44 more severe hypochromic microcytic anaemia compared with non-thalassaemic  
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6 45 individuals.  
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## 9 46 **Conclusion**

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11 47 Our results provide information on the Jino ethnic minority that may be useful for  
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13 48 further genetic counselling, prenatal screening and clinical diagnosis of thalassaemia  
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15 49 in this region.  
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## 21 51 **Strengths and limitations of this study:**

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24 52 1. As Jino, the last ethnic minority confirmed in China, was reported to have a high  
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26 53 prevalence of thalassaemia according to a previous research of children under 10  
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28 54 years old, this study aimed to detect the mutations of  $\alpha$ - and  $\beta$ -thalassaemia in Jino  
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30 55 adults.  
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33 56 2.  $\alpha$ - and  $\beta$ -thalassaemia mutation spectrum shown in this research may help to  
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35 57 explain further genotype – phenotype correlations and to establish a thalassaemia  
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37 58 prevention program in this area.  
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39 59 3. The sample size of controls we used in the genetic testing was relatively small and  
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41 60 may not have the validity to detect the other genotypes of silent  $\alpha$ -thalassaemia  
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43 61 from this group.  
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## INTRODUCTION

As a group of monogenic disorders, thalassaemia is a serious health problem worldwide, especially in Mediterranean areas, Southeast Asia and southern China [1-3]. Yunnan Province, which is located along the border areas of China-Myanmar-Laos, is notable for its ethnic diversity. According to a survey of children under 10 years old, several ethnic minorities in this region have a high prevalence of thalassaemia, with the prevalence of  $\alpha$ -thalassaemia ( $\alpha$ -thal) being highest (22.1%) in Dai from Xishuangbanna and the prevalence of  $\beta$ -thalassaemia ( $\beta$ -thal) being highest in Achang (40.6%) [4].

Jino is the last ethnic minority confirmed in China, and the prevalences of  $\alpha$ -thal and  $\beta$ -thal among Jino children are 3.1% and 29.3%, respectively. Thalassaemic children can exhibit various clinical symptoms; some are asymptomatic carriers, whereas others have severe haemolytic anaemia [5]. Blood transfusion therapy, which is needed for severe carriers, imposes a heavy burden on families and public health management [6]. Although genetic screening is essential to prevent and control this inherited disease, systematic investigations of thalassaemia mutations in Jino adults are rare.

The Jino population comprises nearly 20,000 individuals, and most (approximately 90%) live around Jino Mountain, which is located in east-central Yunnan Province [7].

A large number of thalassaemic mutations have been found in the general population worldwide [8-10]; however, little is known regarding this isolated population. Indeed, the molecular mechanism and genetic variations of thalassaemia in Jino individuals

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4 86 may be different from those in other ethnicities. Our study aimed to detect  $\alpha$ -thal and  
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6 87  $\beta$ -thal gene mutations in Jino adults to provide basic information for further prenatal  
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9 88 consulting and thalassaemia diagnosis.  
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## 14 90 **MATERIALS AND METHODS**

### 15 16 91 **Participants and clinical screening**

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19 92 This cross-sectional study was conducted in eight villages of Jino Mountain Township  
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21 93 in southern Yunnan Province, China (Fig. 1).  
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24 94 A total of 1,613 Jino adults, including 762 males and 851 females, were randomly  
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26 95 selected from February 2012 to April 2012 (haematological and demographic  
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28 96 characteristics of the total population included in the study are described in Table 1).  
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30 97 Haematological parameters were measured, including haemoglobin (Hb), mean  
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32 98 corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell  
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34 99 (RBC), and red cell distribution width (RDW). Haemoglobin was analysed by HPLC  
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36 100 (high-performance liquid chromatography) using the VARIANT II<sup>TM</sup> haemoglobin  
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38 101 analysing system (Bio-Rad Laboratories, Hercules, CA, USA).  $\alpha$ - and  $\beta$ -globin  
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40 102 genetic testing was performed in participants (n=363) with hypochromic microcytic  
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42 103 anaemia (MCV<80 fl and/or MCH<27 pg) and/or positive HPLC profiles. The  
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44 104 remaining participants (n=1250) were considered normal, and controls (n=50) were  
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46 105 randomly selected from this group for further genetic testing.  
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54 106 **Table 1. Haematological and demographic characteristics of 1,613 Jino ethnic**  
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56 107 **minority adults included in the study.**  
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Parameter	Total	Males	Females
Samples(n)	1613	762	851
Age(years)	40.43 ± 14.78	40.11 ± 15.21	40.71 ± 14.38
BMI(kg/m <sup>2</sup> )	21.79 ± 3.20	22.12 ± 3.17	21.50 ± 3.21
RBC(10 <sup>12</sup> /L)	4.85 ± 0.56	5.08 ± 0.57	4.65 ± 0.47
RDW(%)	12.76 ± 1.29	12.60 ± 1.10	12.89 ± 1.42
MCV(fL)	83.42 ± 8.08	84.97 ± 7.54	82.03 ± 8.30
MCH(pg)	29.21 ± 3.26	29.91 ± 3.07	28.59 ± 3.30
HCT(%)	40.25 ± 4.36	42.88 ± 3.74	37.90 ± 3.44
Hb(g/dL)	14.09 ± 1.70	15.09 ± 1.49	13.20 ± 1.34

108 The data are shown as the means ± SD; BMI: body mass index; RBC: red blood cell; RDW:  
 109 red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular  
 110 haemoglobin; HCT: haematocrit; Hb: haemoglobin

### 111 Genetic testing

112 Genomic DNA was extracted from venous blood leukocytes. Three methods were  
 113 utilized to detect thalassaemic mutations.

114 A CapitalBio Thalassaemia Gene Mutation Detection Kit (CapitalBio, Beijing, China)  
 115 was used to determine 25 common mutations in globin genes in the Chinese  
 116 population via DNA microarray. Six  $\alpha$ -thal gene mutations and nineteen  $\beta$ -thal gene  
 117 mutations were included. A BioMixer<sup>TM</sup> II Microarray Hybridization Station  
 118 (CapitalBio, Beijing, China) was used for hybridization after multiplex polymerase  
 119 chain reaction amplification. Then, chips were scanned using a LuxScan<sup>TM</sup> 10K-B

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4 120 Microarray Scanner (CapitalBio, Beijing, China).

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6 121 To validate  $\beta$ -thal mutations, three fragments of the  $\beta$ -globin gene were amplified.

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9 122 The first fragment was amplified with 5'-CCT AAG CCA GTG CCA GAA GAG C-3'

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11 123 as the forward primer and 5'-TGC CCA GTT TCT ATT GGT CTC C-3' as the reverse

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14 124 primer, the second fragment was amplified with 5'-TAG AAA CTG GGC ATG TGG

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16 125 AG-3' as the forward primer and 5'-TGT ACC CTG TTA CTT ATC CC-3' as the

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19 126 reverse primer, and the third fragment was amplified with 5'-TCA GGG CAA TAA

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21 127 TGA TAC AA-3' as the forward primer and 5'-TTA GTA GTT GGA CTT AGG GA-3'

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24 128 as the reverse primer. The fragments were sequenced using a 3500 Genetic Analyser

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26 129 (Applied Biosystems, Foster City, CA, USA).

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29 130 Moreover, we also confirmed three  $\alpha$ -thal deletions [i.e., the Southeast Asian deletion

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31 131 (-<sup>SEA</sup>), rightward deletion (- $\alpha^{3.7}$ ), and leftward deletion (- $\alpha^{4.2}$ )] via multiplex

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34 132 gap-polymerase chain reaction assays [11, 12].

### 35 36 133 **Statistical analysis**

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39 134 A statistical analysis was carried out using SAS for Windows (version 9.2; SAS

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41 135 Institute, Cary, NC, USA). All quantitative traits were tested for normality, and

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44 136 skewed quantitative traits were logarithmically transformed to approximate univariate

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46 137 normality. The data are shown as the means  $\pm$  standard deviation (SD). The

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49 138 quantitative traits (RBC, Hb, MCV, MCH, RDW) were compared between two groups

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51 139 by using Wilcoxon test, and ANOVA tests were performed for comparing the

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54 140 differences in three subgroups of thalassaemia carriers ( $\alpha$ -Thal,  $\beta$ -Thal and  $\alpha\beta$ -Thal).

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56 141 Two-tailed statistical significance was considered at  $p < 0.05$ .



## 142 RESULTS

### 143 Mutations identified in Jino

144 Due to mutations in different globin genes, we observed three groups of thalassaemic  
 145 carriers, including individuals with only  $\alpha$ -thal gene mutations or  $\beta$ -thal gene  
 146 mutations and individuals with combined  $\alpha\beta$ -thalassaemia ( $\alpha\beta$ -thal) gene mutations.  
 147 Six different thalassaemia mutations were detected in 203 individuals among 363  
 148 suspected cases. No mutations were observed in the 50 controls. Table 2 shows the  
 149 allele frequency of  $\alpha$ - and  $\beta$ -thalassaemia mutations found in our study.

150 **Table 2. Allele frequency of  $\alpha$ - and  $\beta$ -Thalassaemia mutations found in our study.**

Mutation	Phenotype	n	Number of Alleles	Allele Frequency (ratio)
$\alpha$ -thalassaemia				
-- <sup>SEA</sup>	$\alpha^0/\alpha$	42	42	70.00% (42/60)
- $\alpha^{3.7}$	$\alpha^+/\alpha$	16	16	30.00% (18/60)
- $\alpha^{3.7}/-$ $\alpha^{3.7}$	$\alpha^+/\alpha^+$	1	2	—
$\beta$ -thalassaemia				
codon 17	$\beta^0/\beta^A$	20	20	12.35% (20/162)
Hb E	$\beta^E/\beta^A$	132	132	87.65% (142/162)
Hb E/ Hb E	$\beta^E/\beta^E$	5	10	—

151  $\alpha$ : the normal  $\alpha$ -globin chain;  $\alpha^0$ : the  $\alpha$ -globin chain is totally deletion;  $\alpha^+$ : the  $\alpha$ -globin chain is  
 152 partly deletion;  $\beta^A$ : the normal  $\beta$ -globin chain;  $\beta^E$ : the abnormal  $\beta$ -globin chain of Hb E mutation;  
 153  $\beta^0$ : the  $\beta$ -globin chain is totally deletion.

### 154 Mutations in the $\alpha$ -thal gene

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4 155 None of the three common non-deletion  $\alpha^+$ -thal genes were found in the 203  
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6 156 participants with thalassaemia mutations. Forty-six of the 203 participants carried  
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8 157  $\alpha$ -thal gene deletions only; -<sup>SEA</sup> and - $\alpha^{3.7}$  were observed, accounting for 16.7%  
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11 158 (34/203) and 5.9% (12/203) of the mutations, respectively. Among these individuals,  
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13 159 we identified both  $\alpha^+/\alpha$  and  $\alpha^+/\alpha^+$  for - $\alpha^{3.7}$  and  $\alpha^0/\alpha$  for -<sup>SEA</sup> (gel electrophoresis  
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15 160 results are shown in Fig. 2). However, no - $\alpha^{4.2}$  mutations were observed.

### 161 **Mutations in the $\beta$ -thal gene**

162 We observed mutations in codon 17 (A>T) (Fig. 3) and Hb E [codon 26 (G>A)] (Fig.  
163 4). Codon 17 (A>T), which accounted for 9.9% (20/203) of mutations, was found to  
164 be  $\beta^0/\beta^A$  in this population. Participants with only the Hb E [codon 26 (G>A)] variant,  
165 either  $\beta^E/\beta^A$  or  $\beta^E/\beta^E$ , accounted for 61.1% (124/203) of mutations. Furthermore, 13 Hb  
166 E carriers harboured -<sup>SEA</sup> (n=8) or - $\alpha^{3.7}$  (n=5) at a combined frequency of 6.4%  
167 (13/203).

### 168 **Haematological features of different thalassaemia genotypes**

169 The haematological data of different thalassaemia genotypes are summarized in Table  
170 3. Compared with normal individuals, thalassaemic carriers had significantly lower  
171 Hb, MCV and MCH levels ( $p<0.001$ , respectively) and higher RBC and RDW levels  
172 ( $p<0.001$ , respectively). Furthermore, we compared the differences in those five  
173 indexes among three groups of carriers ( $\alpha$ -thal,  $\beta$ -thal and  $\alpha\beta$ -thal). Significant

174 **Table 3. Haematological data of 1,613 Jino ethnic minority individuals with different thalassaemia subtypes.**

Thalassaemia types	n	RBC( $10^{12}/L$ )	Hb(g/dL)	MCV(fL)	MCH(pg)	RDW(%)
$\alpha$ -Thalassaemia	46	5.63 $\pm$ 0.78	13.03 $\pm$ 1.61	68.99 $\pm$ 6.50	23.33 $\pm$ 2.45	13.64 $\pm$ 1.38
$\alpha^0/\alpha$	34	5.88 $\pm$ 0.68	12.98 $\pm$ 1.62	65.69 $\pm$ 2.65	22.08 $\pm$ 0.81	14.01 $\pm$ 1.37
$\alpha^+/\alpha$	11	4.86 $\pm$ 0.58	13.19 $\pm$ 1.70	78.96 $\pm$ 4.43	27.14 $\pm$ 1.87	12.52 $\pm$ 0.74
$\alpha^+/\alpha^+$	1	5.34	12.7	71.5	23.8	13.1
$\beta$ -Thalassaemia	144	5.27 $\pm$ 0.48	13.31 $\pm$ 1.70	72.46 $\pm$ 7.09	25.29 $\pm$ 2.75	13.21 $\pm$ 0.96
$\beta^0/\beta^A$	20	5.41 $\pm$ 0.38	10.68 $\pm$ 0.53	58.70 $\pm$ 2.31	19.77 $\pm$ 0.83	14.23 $\pm$ 0.40
$\beta^E/\beta^A$	120	5.23 $\pm$ 0.48	13.77 $\pm$ 1.41	75.17 $\pm$ 3.88	26.34 $\pm$ 1.49	12.98 $\pm$ 0.85
$\beta^E/\beta^E$	4	5.91 $\pm$ 0.51	12.55 $\pm$ 1.22	60.23 $\pm$ 3.61	21.23 $\pm$ 1.15	15.00 $\pm$ 0.57
$\alpha\beta$ -Thalassaemia	13	5.67 $\pm$ 0.70	13.63 $\pm$ 1.67	69.40 $\pm$ 6.85	24.17 $\pm$ 2.59	13.12 $\pm$ 0.92
$\beta^E/\beta^A$ with $\alpha^0/\alpha$	7	6.02 $\pm$ 0.69	13.57 $\pm$ 1.58	65.94 $\pm$ 2.82	22.57 $\pm$ 1.07	13.66 $\pm$ 0.71
$\beta^E/\beta^A$ with $\alpha^+/\alpha$	5	5.21 $\pm$ 0.53	14.08 $\pm$ 1.88	76.46 $\pm$ 3.86	27.00 $\pm$ 1.33	12.22 $\pm$ 0.36
$\beta^E/\beta^E$ with $\beta^0/\beta^A$	1	5.57	13.8	58.3	21.2	11.8
Total thalassaemia	203	5.38 $\pm$ 0.60	13.27 $\pm$ 1.68	71.48 $\pm$ 7.08	24.77 $\pm$ 2.79	13.30 $\pm$ 1.08
Non-thalassaemia	1410	4.78 $\pm$ 0.51	14.21 $\pm$ 1.67	85.14 $\pm$ 6.64	29.85 $\pm$ 2.79	12.68 $\pm$ 1.30
$P^a$ value		<0.001	<0.001	<0.001	<0.001	<0.001
$P^b$ value		0.0012	>0.05	0.0111	0.0002	0.0573

175 The data are shown as the n, means  $\pm$  SD, medians (interquartile range) or raw data when necessary; RBC: red blood cell; Hb: haemoglobin; MCV: mean

176 corpuscular volume; MCH: mean corpuscular haemoglobin; RDW: red cell distribution width.

177 <sup>a</sup>Non-thalassaemic individuals compared with thalassaemia group.

178 <sup>b</sup> Compare among three subgroups of thalassaemia ( $\alpha$ -Thal,  $\beta$ -Thal and  $\alpha\beta$ -Thal).

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181 differences in MCV ( $p=0.0111$ ), MCH ( $p=0.0002$ ) and RBC ( $p=0.0012$ ) were  
182 observed between the groups. MCV and MCH levels in the  $\alpha$ -thal group were  
183 significantly lower than those in the  $\beta$ -thal group ( $p<0.05$ ), whereas RBC levels were  
184 higher ( $p<0.05$ ). In contrast, no difference was observed between the  $\alpha\beta$ -thal and  
185  $\alpha$ -thal groups/ $\beta$ -thal groups. Moreover, there was a tendency towards increased RDW  
186 levels in the  $\alpha$ -thal group compared with the  $\beta$ -thal group ( $p=0.0573$ ).

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## 188 DISCUSSION

189 Thalassaemia is a common monogenic disease with a relatively high prevalence in  
190 Southeast Asia. In China, this disease is mainly prevalent in areas near the southern  
191 bank of the Yangtze River, such as Guangdong, Guangxi, Fujian and Yunnan  
192 Provinces [13-15]. Prenatal screening and related molecular diagnoses are crucial for  
193 preventing and treating thalassaemia. Many thalassaemia studies have been conducted  
194 in Yunnan Province [16, 17]. However, data on the Jino population are limited  
195 because this population is the last ethnic minority confirmed in China.

196 We randomly selected 1,613 Jino adults from eight villages of Jino Mountain  
197 Township in Jinghong, Southern Yunnan. Among the gene mutations identified, the  
198 most prevalent  $\alpha$ -thal and  $\beta$ -thal genotypes in this region were -<sup>SEA</sup> and Hb E, in  
199 agreement with previous data from Yunnan Province [18,19]. According to our results,  
200 the overall prevalence of thalassaemia in Jino was nearly 12.6%, which is similar to  
201 the prevalence observed in Kunming [20]. The prevalence of  $\alpha\beta$ -thal (8%) in our

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3 202 population was equal to that in the Li population in Hainan Province (7.99%), where  
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5 203 thalassaemia prevalence is high [21]. Although Yunnan Province has a high  
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7 204 prevalence of thalassaemia with diverse genotypes, globin gene mutations among the  
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9 205 Jino population are relatively limited.  
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11 206 Hb E, a type of haemoglobinopathy, can be observed in most regions of southeast  
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13 207 China [22]. Due to a point mutation in the  $\beta$ -globin gene, the balance of various  
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15 208 globin products is disrupted, leading to a structural haemoglobin variant. Although Hb  
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17 209 E carriers may only have slight anaemia, their offspring will exhibit severe clinical  
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19 210 symptoms in the presence of other  $\beta$ -thal types [23]. Therefore, potential Hb E carriers  
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21 211 should undergo genetic testing and prenatal counselling. HPLC is often used as an  
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23 212 efficient primary screening method to detect abnormal Hb, as was done in this study  
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25 213 and previous studies [24,25]. In our study, 95.6% (131/137) of Hb E carriers were  
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27 214 identified by HPLC, and 13 of these individuals had concomitant  $\alpha$ -thal deletions.  
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29 215 Different genotypes lead to different clinical phenotypes [26]. We found that  
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31 216 thalassaemic carriers had significantly lower MCV and MCH levels. Regarding those  
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33 217 with  $\beta$ -thal mutations, MCV and MCH levels were significantly decreased in codon  
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35 218 17 carriers compared with Hb E carriers, suggesting that a nonsense mutation in the  
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37 219  $\beta$ -globin gene causes greater erythrocyte impairment. Hypochromic microcytic  
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39 220 anaemia was moderate in individuals with  $\beta^E/\beta^A$  and  $\alpha^+/\alpha$  compared with  $\beta^E/\beta^A$  carriers.  
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41 221 This paradox may be explained by the fact that changes in the  $\alpha$ - and  $\beta$ -globin chains  
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43 222 could balance each other out when both mutations coexist in an individual.  
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45 223 Accordingly, rapidly estimating the genetic state of an illness based on haematological  
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47 224 parameters is difficult. Therefore, genetic screening of both  $\alpha$ - and  $\beta$ -globin gene  
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49 225 mutations in potential parents is of utmost importance to prevent births with severe  
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51 226 defects [27].  
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3 227 In conclusion, this study revealed  $\alpha$ - and  $\beta$ -thalassaemia mutations in the Jino ethnic  
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5 228 minority population in Yunnan Province. Of these mutations, -<sup>SEA</sup> and Hb E were the  
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7 229 most prevalent  $\alpha$ -thal and  $\beta$ -thal gene mutation types. In addition, data based on  
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9 230 clinical haematological parameter analysis indicated that the severity of hypochromic  
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11 231 microcytic anaemia is associated with the genotype of thalassaemia. Our results  
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13 232 provide evidence that may be useful for further genetic counselling, prenatal  
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15 233 screening and clinical diagnosis of thalassaemia in this region.  
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## 19 234 **Contributors**

20  
21 235 WJ and CH conceived and designed the experiments. SW and RZ performed the  
22  
23 236 experiments and analyzed the data. GX and YL contributed materials and analysis  
24  
25 237 tools. SW prepared the article. CH and WJ revised the manuscript. All the authors  
26  
27 238 have read and approved the final version of this manuscript.  
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## 49 246 **Competing interests**

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52 247 None declared.  
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## 54 248 **Ethics approval**

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57 249 According to the Helsinki Declaration II, ethical approval for the study was granted  
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3 250 by the Institutional Review Board of Shanghai Jiao Tong University affiliated with the  
4  
5 251 Sixth People's Hospital, Shanghai, China. Written informed consent was obtained  
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7 252 from all participants.  
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## 10 253 **Data sharing statement**

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12 254 No additional data are available.  
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## 18 256 **REFERENCES**

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## 334 **Figure legends**

335 **Fig. 1: Geographical location of the Jino ethnic minority populations in Yunnan**  
336 **Province, Southwest China.** The solid black triangle represents Jino Mountain. The  
337 solid black circles represent the eight villages of Jino Mountain Township where the  
338 1,613 subjects were randomly selected.

339 **Fig. 2: Three  $\alpha$ -thal deletions.** Lane 1 represents homozygous -  $\alpha^{3.7}$ ; lanes 2 and 3  
340 represent heterozygous -  $\alpha^{3.7}$ ; and lanes 4 and 5 represent heterozygous -  $\alpha^{-SEA}$ . The 2.0  
341 kb, 1.7 kb, 1.4 kb, and 1.2 kb marked bands represent the -  $\alpha^{3.7}$ ,  $\alpha\alpha$ , -  $\alpha^{4.2}$ , and -  $\alpha^{-SEA}$   
342 genotypes, respectively.

343 **Fig. 3: Heterozygous codon 17 (A>T) mutation (a) and the corresponding normal**  
344 **sequence of  $\beta$ -globin.** Red arrows indicate the position of this point mutation.

345 **Fig. 4: Heterozygous codon 26 (G>A) mutation (a) and the corresponding normal**  
346 **sequence of  $\beta$ -globin.** Red arrows indicate the position of this point mutation.

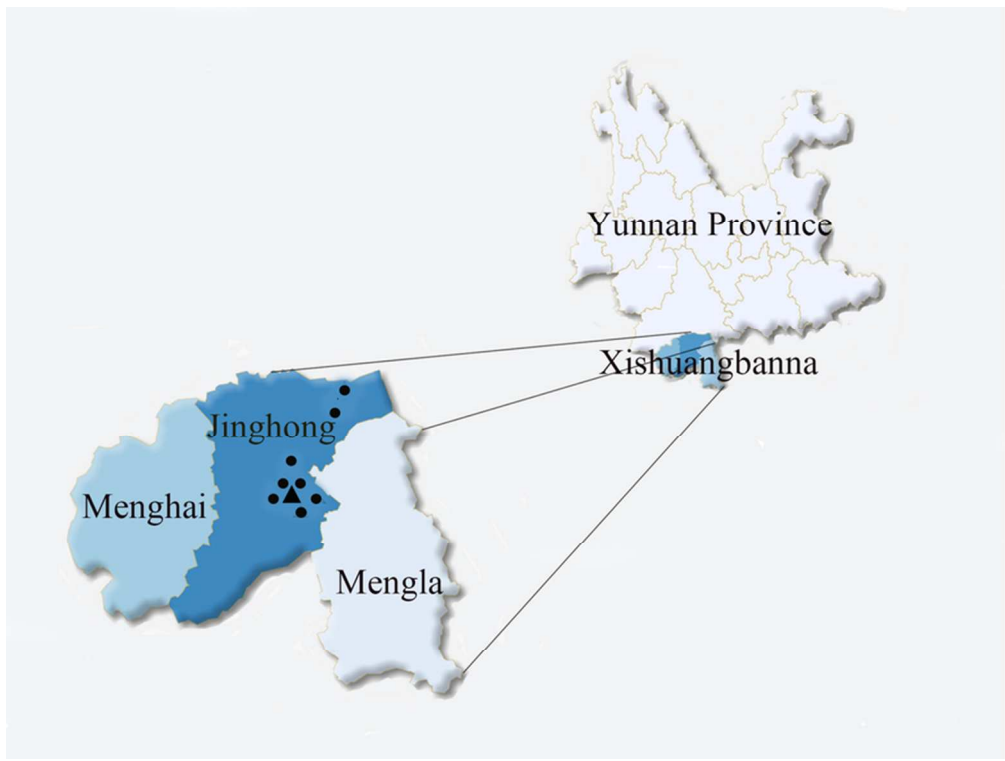
## 348 **Table legends**

349 **Table 1.** Haematological and demographic characteristics of 1,613 Jino ethnic  
350 minority adults included in the study.

351 **Table 2.** Allele frequency of  $\alpha$ - and  $\beta$ -Thalassaemia mutations found in our study.

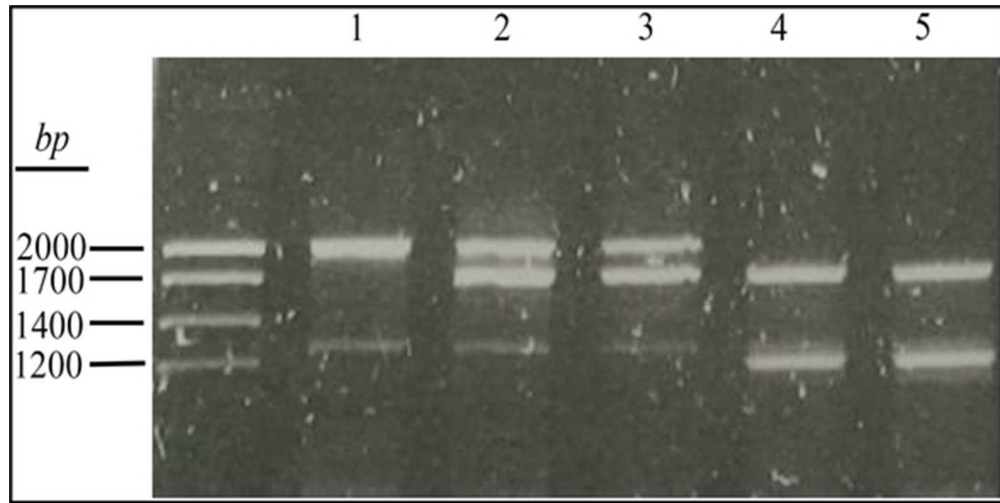
352 **Table 3.** Haematological data of 1,613 Jino ethnic minority individuals with different  
353 thalassaemia subtypes.

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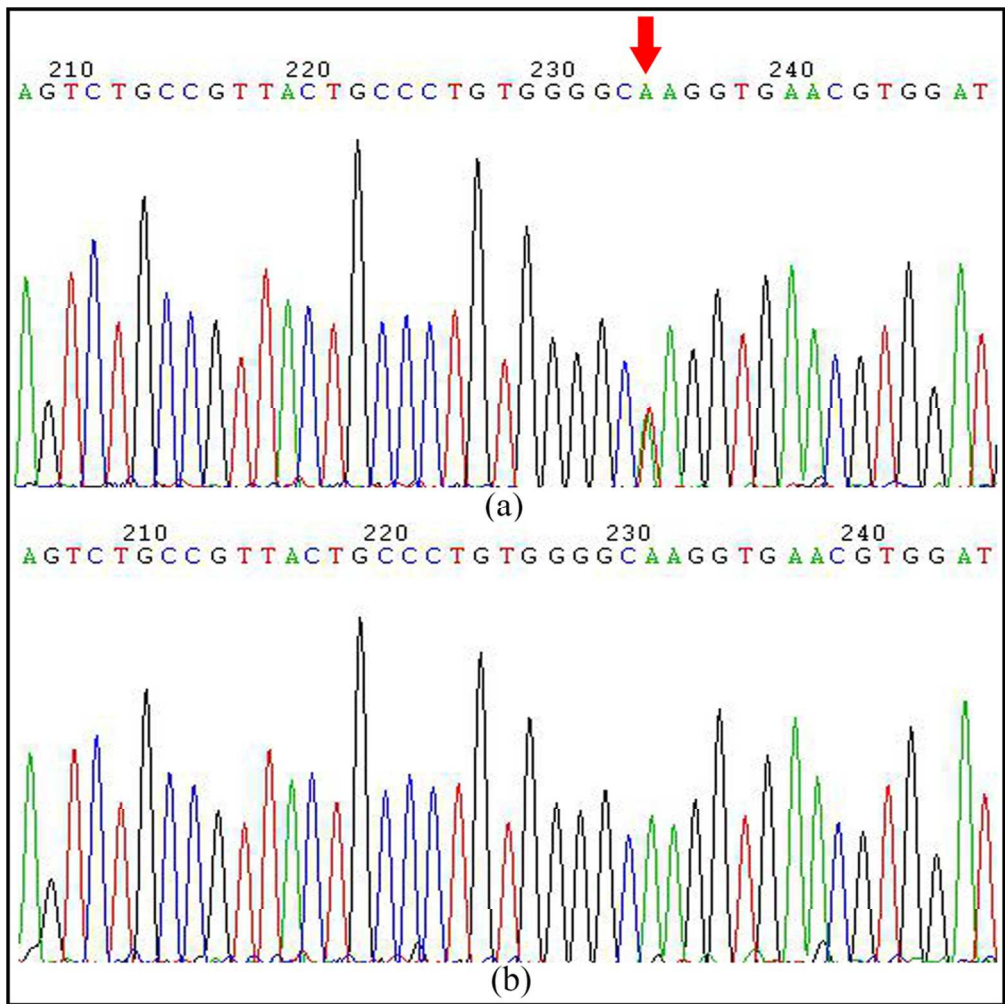


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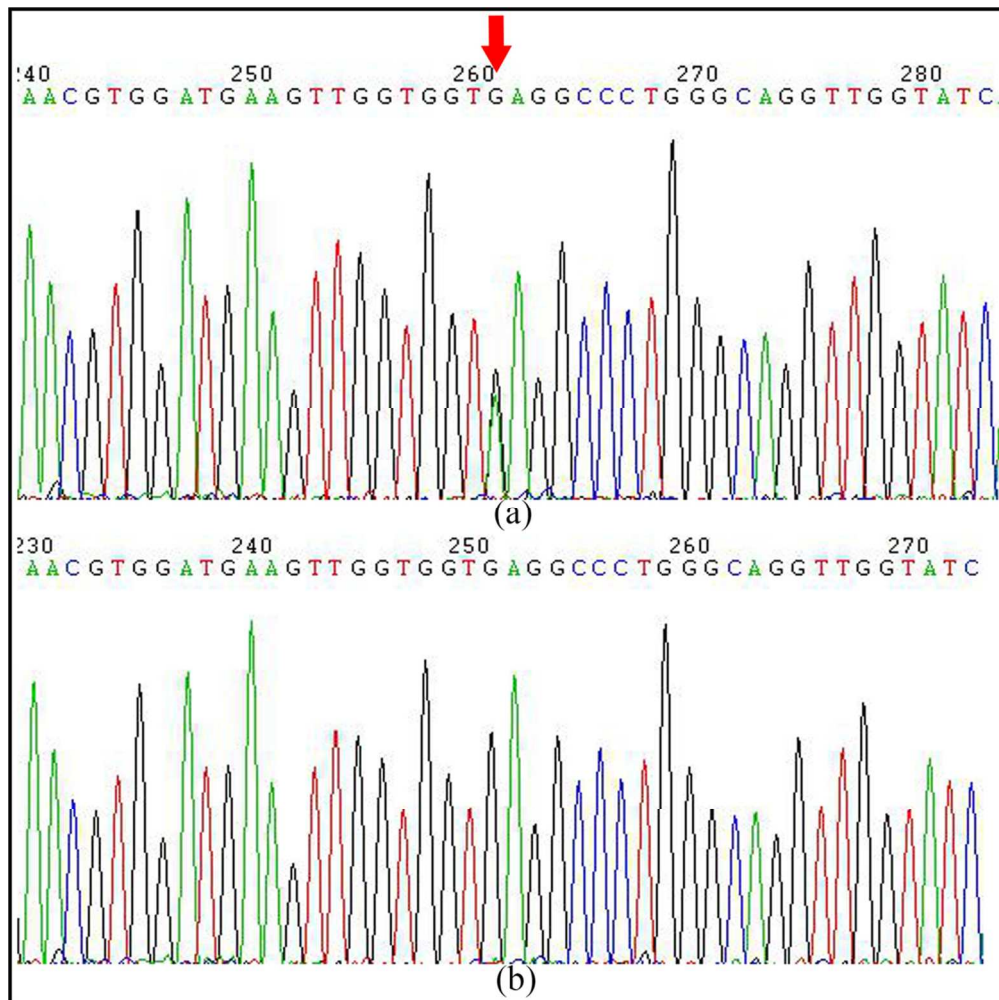
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# BMJ Open

## Mutation Screening for Thalassaemia in the Jino Ethnic Minority Population of Yunnan Province, Southwest China

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Secondary Subject Heading:	Epidemiology, Genetics and genomics, Haematology (incl blood transfusion)
Keywords:	EPIDEMIOLOGY, Antenatal < GENETICS, Anaemia < HAEMATOLOGY

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Manuscripts



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6 2 Mutation Screening for Thalassaemia in the Jino Ethnic  
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9 3 Minority Population of Yunnan Province, Southwest China

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49 19 **Keywords:**

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51 20 thalassaemia, gene mutation, haemoglobin, anaemia, Jino, Yunnan Province

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54 21 **Word count: 2,129**

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## 23 ABSTRACT

### 24 Objectives

25 This study aimed to detect  $\alpha$ - and  $\beta$ -thalassaemia mutations in the Jino ethnic minority  
26 population of Yunnan Province, southwest China.

### 27 Design

28 A total of 1,613 Jino adults were continuously recruited from February 2012 to April  
29 2012. Fasting venous blood samples were obtained to determine haematological  
30 parameters. Haemoglobin analysis was conducted via high-performance liquid  
31 chromatography. Participants with hypochromic microcytic anaemia or positive  
32 haemoglobin analysis profiles were confirmed via  $\alpha$ - and  $\beta$ -globin genetic testing,  
33 including DNA microarray analysis, direct sequencing methods and multiplex  
34 gap-polymerase chain reaction assays.

### 35 Setting

36 Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai  
37 Jiao Tong University Affiliated Sixth People's Hospital

### 38 Results

39 We found 363 suspected cases via primary screening of haematological parameters  
40 and haemoglobin analysis. After further genetic testing, four types of  $\alpha$ - and  
41  $\beta$ -thalassaemia mutations were detected in 203 out of 363 individuals. Both of  $\alpha^0$ - and  
42  $\alpha^+$ -thalassaemia mutations,  $--^{SEA}$  and  $-\alpha^{3.7}$  were identified.  $\beta$ -thalassaemia mutations  
43 included CD17 (HBB:c.52A > T) and CD26 (HbE or HBB:c.79G>A). Additionally,  
44 13 HbE carriers had coexisting  $\alpha^0$  or  $\alpha^+$ -thalassaemia deletions. Clinical

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4 45 haematological parameters indicated that in this study, carriers of all thalassaemic  
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6 46 genotypes had more severe hypochromic microcytic anaemia compared with  
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9 47 non-thalassaemic individuals.

## 11 **Conclusion**

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14 49 Our results provide information on the Jino ethnic minority that may be useful for  
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16 50 further genetic counselling, prenatal screening and clinical diagnosis of thalassaemia  
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19 51 in this region.

## 23 **Strengths and limitations of this study:**

- 20  
21 52
- 24 53 1. As Jino, the last ethnic minority confirmed in China, was reported to have a high  
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26 54 prevalence of thalassaemia according to the previous research of children under  
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28 55 10 years old, this study aimed to detect the mutations of  $\alpha$ - and  $\beta$ -thalassaemia in  
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30 56 10 years old, this study aimed to detect the mutations of  $\alpha$ - and  $\beta$ -thalassaemia in  
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32 57 Jino adults.
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34 58 2.  $\alpha$ - and  $\beta$ -thalassaemia mutation spectrum shown in this research may help to  
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36 59 explain further genotype – phenotype correlations and to establish a thalassaemia  
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38 60 prevention program in this area.
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40 61 3. The sample size we used in the genetic testing was relatively small and may not  
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42 62 have the validity to identify the rare thalassaemia from this ethnic group.  
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## INTRODUCTION

As a group of monogenic disorders, thalassaemia is a serious health problem worldwide, especially in Mediterranean areas, Southeast Asia and southern China [1-3]. Yunnan Province, which is located along the border areas of China-Myanmar-Laos, is notable for its ethnic diversity. According to a previous study of children under 10 years old, several ethnic minorities in this region have a high prevalence of thalassaemia, with the prevalence of  $\alpha$ -thalassaemia ( $\alpha$ -thal) being highest (22.1%) in Dai from Xishuangbanna and the prevalence of  $\beta$ -thalassaemia ( $\beta$ -thal) being highest in Achang (40.6%) [4].

Jino is the last ethnic minority confirmed in China, and the prevalence of  $\alpha$ -thal and  $\beta$ -thal among Jino children are 3.1% and 29.3%, respectively. Thalassaemic children may exhibit various clinical symptoms; some are asymptomatic carriers, whereas others have severe haemolytic anaemia [5]. Blood transfusion therapy, which is needed for severe carriers, imposes a heavy burden on families and public health management [6]. Although genetic screening is essential to prevent and control this inherited disease, systematic investigations of thalassaemia mutations in Jino adults are rare.

The Jino population comprises nearly 20,000 individuals, and most (approximately 90%) live around Jino Mountain, which is located in east-central Yunnan Province [7].

A large number of thalassaemic mutations have been found in the general population worldwide [8-10]; however, little is known regarding this isolated population. Indeed, the molecular mechanism and genetic variations of thalassaemia in Jino individuals

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4 86 may be different from those in other ethnicities. Our study aimed to detect  $\alpha$ -thal and  
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6 87  $\beta$ -thal gene mutations in Jino adults to provide basic information for further prenatal  
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9 88 consulting and thalassaemia diagnosis.  
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## 14 90 **MATERIALS AND METHODS**

### 15 16 91 **Participants and clinical screening**

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19 92 According to the Helsinki Declaration II, ethical approval for the study was granted  
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21 93 by the Institutional Review Board of Shanghai Jiao Tong University affiliated with the  
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23 94 Sixth People's Hospital, Shanghai, China. This cross-sectional study was conducted  
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25 95 between February 2012 to April 2012 in eight villages (Luote, Jiama, Balai, Situ, New  
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27 96 Situ, Baka, Baya, Dapingzhang) around Jino Mountain in Jinghong, southern Yunnan  
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29 97 Province, China (Fig. 1). List of Jino adults from those eight villages was obtained  
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31 98 from local Villager Committee Offices. Participants were sampled by a simple  
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33 99 computer program of randomization from those villages. Staffs of local health center  
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35 100 who understand both Chinese and Jino language contacted the subjects and introduced  
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37 101 the purpose of this study. Oral and written informed consent was obtained from all the  
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39 102 individuals. Basic demographic information and fasting venous blood samples were  
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41 103 collected by researchers.  
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49 104 A total of 1,613 Jino adults, including 762 males and 851 females, were attending this  
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51 105 survey (haematological and demographic characteristics of the total population  
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53 106 included in the study are described in Table 1). Haematological parameters were  
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55 107 measured, including haemoglobin (Hb), mean corpuscular volume (MCV), mean  
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4 108 corpuscular haemoglobin (MCH), red blood cell (RBC), and red cell distribution  
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6 109 width (RDW). Haemoglobin was analysed by HPLC (high-performance liquid  
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9 110 chromatography) using the VARIANT II™ haemoglobin analysing system (Bio-Rad  
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11 111 Laboratories, Hercules, CA, USA).  $\alpha$ - and  $\beta$ -globin genetic testing was performed in  
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13 112 participants (n=363) with hypochromic microcytic anaemia (MCV<80 fl and/or  
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15 113 MCH<27 pg) and/or positive HPLC profiles. In order to evaluate the validity of  
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17 114 primary screening approaches for detection of thalassaemia carriers, part of the  
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19 115 individuals with negative screening results (n=50) were randomly selected from  
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21 116 remaining participants (n=1250) for further genetic testing.  
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31 **Table 1. Haematological and demographic characteristics of 1,613 Jino ethnic**  
32 **minority adults included in the study.**  
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Parameter	Total	Males	Females
Samples(n)	1613	762	851
Age(years)	40.43 ± 14.78	40.11 ± 15.21	40.71 ± 14.38
BMI(kg/m <sup>2</sup> )	21.79 ± 3.20	22.12 ± 3.17	21.50 ± 3.21
RBC(10 <sup>12</sup> /L)	4.85 ± 0.56	5.08 ± 0.57	4.65 ± 0.47
RDW(%)	12.76 ± 1.29	12.60 ± 1.10	12.89 ± 1.42
MCV(fL)	83.42 ± 8.08	84.97 ± 7.54	82.03 ± 8.30
MCH(pg)	29.21 ± 3.26	29.91 ± 3.07	28.59 ± 3.30
HCT(%)	40.25 ± 4.36	42.88 ± 3.74	37.90 ± 3.44
Hb(g/dL)	14.09 ± 1.70	15.09 ± 1.49	13.20 ± 1.34

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57 119 The data are shown as the means ± SD; BMI: body mass index; RBC: red blood cell; RDW:

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4 120 red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular  
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6 121 haemoglobin; HCT: haematocrit; Hb: haemoglobin  
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## 9 122 **Genetic testing**

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11 123 Genomic DNA was extracted from venous blood leukocytes. Three methods were  
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13 124 utilized to detect thalassaemic mutations.  
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16 125 A CapitalBio Thalassaemia Gene Mutation Detection Kit (CapitalBio, Beijing, China)  
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18 126 was used to determine 25 common mutations in globin genes in the Chinese  
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20 127 population via DNA microarray. Six  $\alpha$ -thal gene mutations and nineteen  $\beta$ -thal gene  
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22 128 mutations were included. Among them, there were three  $\alpha$ -thal deletions [i.e., the  
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24 129 Southeast Asian deletion ( $--^{SEA}$ ), rightward deletion ( $-\alpha^{3,7}$ ), and leftward deletion ( $-$   
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26 130  $\alpha^{4,2}$ )] and three nondeletional  $\alpha$ -thal mutations [i.e., Hb Constant Spring  
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28 131 (HBA2:c.427T>C), Hb Quong Sze (HBA2:c.377T>C or HBA1) and Hb Westmead  
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30 132 (HBA2:c.369C>G)]. Nineteen  $\beta$ -thal gene mutations were CD14/15  
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32 133 (HBB:c.45\_46insG), CD27/28 (HBB:c.84\_85insC), CD41/42  
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34 134 (HBB:c.126\_129delTCTT), CD71/72 (HBB:c.216\_217insA), -32 (HBB:c.-82T>C),  
35  
36 135 -30 (HBB:c.-80T>C), -29 (HBB:c.-79A>G), -28(HBB:c.-78A>G), CD17  
37  
38 136 (HBB:c.52A>T), CD26 (HBB:c.79G>A), CD30 (HBB:c.91A>G), CD37  
39  
40 137 (HBB:c.113G>A), CD43 (HBB:c.130G>T), IVS1-1 (HBB:c.92+1G>T), IVS1-5  
41  
42 138 (HBB:c.92+5G>T), IVS2-5 (HBB:c.315+5G>C), IVS2-654 (HBB:c.316-197C>T),  
43  
44 139 Int (HBB:c.2T>G), CAP (HBB:c.-11\_-8delAAAC). A BioMixer™ II Microarray  
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46 140 Hybridization Station (CapitalBio, Beijing, China) was used for hybridization after  
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48 141 multiplex polymerase chain reaction amplification. Then, chips were scanned using a  
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4 142 LuxScan™ 10K-B Microarray Scanner (CapitalBio, Beijing, China).  
5  
6 143 To validate  $\beta$ -thal mutations, three fragments of the  $\beta$ -globin gene were amplified.  
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8  
9 144 The first fragment was amplified with 5'-CCT AAG CCA GTG CCA GAA GAG C-3'  
10  
11 145 as the forward primer and 5'-TGC CCA GTT TCT ATT GGT CTC C-3' as the reverse  
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13 146 primer, the second fragment was amplified with 5'-TAG AAA CTG GGC ATG TGG  
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15 147 AG-3' as the forward primer and 5'-TGT ACC CTG TTA CTT ATC CC-3' as the  
16  
17 148 reverse primer, and the third fragment was amplified with 5'-TCA GGG CAA TAA  
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19 149 TGA TAC AA-3' as the forward primer and 5'-TTA GTA GTT GGA CTT AGG GA-3'  
20  
21 150 as the reverse primer. The fragments were sequenced using a 3500 Genetic Analyser  
22  
23 151 (Applied Biosystems, Foster City, CA, USA).

24  
25  
26 152 Moreover, we also confirmed three  $\alpha$ -thal deletions [i.e., the Southeast Asian deletion  
27  
28 153 ( $--^{SEA}$ ), rightward deletion ( $-\alpha^{3.7}$ ), and leftward deletion ( $-\alpha^{4.2}$ )] via multiplex  
29  
30 154 gap-polymerase chain reaction assays. Primers and PCR conditions were designed as  
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32 155 described in classical literatures [11,12].  
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### 39 **Statistical analysis**

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41 157 A statistical analysis was carried out using SAS for Windows (version 9.2; SAS  
42  
43 158 Institute, Cary, NC, USA). All quantitative traits were tested for normality, and  
44  
45 159 skewed quantitative traits were logarithmically transformed to approximate univariate  
46  
47 160 normality. The data are shown as the means  $\pm$  standard deviation (SD). The  
48  
49 161 quantitative traits (RBC, Hb, MCV, MCH, RDW) were compared between two groups  
50  
51 162 by using Wilcoxon test, and ANOVA tests were performed for comparing the  
52  
53 163 differences in three subgroups of thalassaemia carriers ( $\alpha$ -Thal,  $\beta$ -Thal and  $\alpha\beta$ -Thal).  
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164 Two-tailed statistical significance was considered at  $p < 0.05$ .

## 165 RESULTS

### 166 Mutations identified in Jino

167 Due to mutations in different globin genes, we observed three groups of thalassaemic  
 168 carriers, including individuals with only  $\alpha$ -thal gene deletions or  $\beta$ -thal gene  
 169 mutations and individuals with combined  $\alpha\beta$ -thalassaemia ( $\alpha\beta$ -thal) gene mutations.  
 170 Four different thalassaemia mutations were detected in 203 individuals among 363  
 171 suspected cases. No mutations were observed in 50 individuals with negative primary  
 172 screening results. Table 2 shows the allele frequency of  $\alpha$ - and  $\beta$ -thalassaemia  
 173 mutations found in our study.

174 **Table 2. Allele frequency of  $\alpha$ - and  $\beta$ -Thalassaemia mutations found in our study.**

Mutation	Phenotype	n	Number of Alleles	Allele Frequency (ratio)
$\alpha$ -thalassaemia				
-- <sup>SEA</sup>	$\alpha^0/\alpha$	42	42	70.00% (42/60)
- $\alpha^{3.7}$	$\alpha^+/\alpha$	16	16	30.00% (18/60)
- $\alpha^{3.7}/-\alpha^{3.7}$	$\alpha^+/\alpha^+$	1	2	—
$\beta$ -thalassaemia				
CD17	$\beta^0/\beta^A$	20	20	12.35% (20/162)
HbE	$\beta^E/\beta^A$	132	132	87.65% (142/162)
HbE/HbE	$\beta^E/\beta^E$	5	10	—

175  $\alpha$ : the normal  $\alpha$ -globin chain;  $\alpha^0$ : the  $\alpha$ -globin chain is totally deletion;  $\alpha^+$ : the  $\alpha$ -globin chain is  
 176 partly deletion;  $\beta^A$ : the normal  $\beta$ -globin chain;  $\beta^E$ : the abnormal  $\beta$ -globin chain of HbE mutation;  
 177  $\beta^0$ : the  $\beta$ -globin chain is totally deletion.

## 178 **Mutations in the $\alpha$ -thal gene**

179 None of the three common nondeletional  $\alpha^+$ -thal mutations, Hb Constant Spring  
180 (HBA2:c.427T>C), Hb Quong Sze [HBA2:c.377T>C (or HBA1)] and Hb  
181 Westmead (HBA2:c.369C>G), were found in 203 participants with thalassaemia  
182 mutations. Forty-six of 203 participants carried  $\alpha$ -thal deletions only; --<sup>SEA</sup> and  $-\alpha^{3.7}$   
183 were observed, accounting for 16.7% (34/203) and 5.9% (12/203) of the mutations,  
184 respectively. Among these individuals, we identified both  $\alpha^+/\alpha$  and  $\alpha^+/\alpha^+$  for  $-\alpha^{3.7}$  and  
185  $\alpha^0/\alpha$  for --<sup>SEA</sup> (gel electrophoresis of PCR amplifying results are shown in Fig. 2).  
186 However, no  $-\alpha^{4.2}$  deletion was observed.

## 187 **Mutations in the $\beta$ -thal gene**

188 We observed mutations in CD17 (HBB:c.52A>T) (Fig. 3) and CD26 (HbE or  
189 HBB:c.79G>A) (Fig. 4). CD17, which accounted for 9.9% (20/203) of mutations, was  
190 found to be  $\beta^0/\beta^A$  in this population. Participants with HbE variant only, either  $\beta^E/\beta^A$  or  
191  $\beta^E/\beta^E$ , accounted for 61.1% (124/203) of mutations. Furthermore, 13 HbE carriers  
192 harboured --<sup>SEA</sup> (n=8) or  $-\alpha^{3.7}$  (n=5) at a combined frequency of 6.4% (13/203).

## 193 **Haematological features of different thalassaemia genotypes**

194 The haematological data of different thalassaemia genotypes are summarized in Table  
195 3. Compared with normal individuals, thalassaemic carriers had significantly lower  
196 Hb, MCV and MCH levels ( $p<0.001$ , respectively) and higher RBC and RDW levels  
197 ( $p<0.001$ , respectively). Furthermore, we compared the differences in those five  
198 indexes among three groups of carriers ( $\alpha$ -thal,  $\beta$ -thal and  $\alpha\beta$ -thal). Significant

199 **Table 3. Haematological data of 1,613 Jino ethnic minority individuals with different thalassaemia subtypes.**

Thalassaemia types	n	RBC( $10^{12}/L$ )	Hb(g/dL)	MCV(fL)	MCH(pg)	RDW(%)
$\alpha$ -Thalassaemia	46	5.63 $\pm$ 0.78	13.03 $\pm$ 1.61	68.99 $\pm$ 6.50	23.33 $\pm$ 2.45	13.64 $\pm$ 1.38
$\alpha^0/\alpha$	34	5.88 $\pm$ 0.68	12.98 $\pm$ 1.62	65.69 $\pm$ 2.65	22.08 $\pm$ 0.81	14.01 $\pm$ 1.37
$\alpha^+/\alpha$	11	4.86 $\pm$ 0.58	13.19 $\pm$ 1.70	78.96 $\pm$ 4.43	27.14 $\pm$ 1.87	12.52 $\pm$ 0.74
$\alpha^+/\alpha^+$	1	5.34	12.7	71.5	23.8	13.1
$\beta$ -Thalassaemia	144	5.27 $\pm$ 0.48	13.31 $\pm$ 1.70	72.46 $\pm$ 7.09	25.29 $\pm$ 2.75	13.21 $\pm$ 0.96
$\beta^0/\beta^A$	20	5.41 $\pm$ 0.38	10.68 $\pm$ 0.53	58.70 $\pm$ 2.31	19.77 $\pm$ 0.83	14.23 $\pm$ 0.40
$\beta^E/\beta^A$	120	5.23 $\pm$ 0.48	13.77 $\pm$ 1.41	75.17 $\pm$ 3.88	26.34 $\pm$ 1.49	12.98 $\pm$ 0.85
$\beta^E/\beta^E$	4	5.91 $\pm$ 0.51	12.55 $\pm$ 1.22	60.23 $\pm$ 3.61	21.23 $\pm$ 1.15	15.00 $\pm$ 0.57
$\alpha\beta$ -Thalassaemia	13	5.67 $\pm$ 0.70	13.63 $\pm$ 1.67	69.40 $\pm$ 6.85	24.17 $\pm$ 2.59	13.12 $\pm$ 0.92
$\beta^E/\beta^A$ with $\alpha^0/\alpha$	7	6.02 $\pm$ 0.69	13.57 $\pm$ 1.58	65.94 $\pm$ 2.82	22.57 $\pm$ 1.07	13.66 $\pm$ 0.71
$\beta^E/\beta^A$ with $\alpha^+/\alpha$	5	5.21 $\pm$ 0.53	14.08 $\pm$ 1.88	76.46 $\pm$ 3.86	27.00 $\pm$ 1.33	12.22 $\pm$ 0.36
$\beta^E/\beta^E$ with $\beta^0/\beta^A$	1	5.57	13.8	58.3	21.2	11.8
Total thalassaemia	203	5.38 $\pm$ 0.60	13.27 $\pm$ 1.68	71.48 $\pm$ 7.08	24.77 $\pm$ 2.79	13.30 $\pm$ 1.08
Non-thalassaemia	1410	4.78 $\pm$ 0.51	14.21 $\pm$ 1.67	85.14 $\pm$ 6.64	29.85 $\pm$ 2.79	12.68 $\pm$ 1.30
$P^a$ value		<0.001	<0.001	<0.001	<0.001	<0.001
$P^b$ value		0.0012	>0.05	0.0111	0.0002	0.0573

200 The data are shown as the n, means  $\pm$  SD, medians (interquartile range) or raw data when necessary; RBC: red blood cell; Hb: haemoglobin; MCV: mean

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3 201 corpuscular volume; MCH: mean corpuscular haemoglobin; RDW: red cell distribution width.

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5 202 <sup>a</sup>Non-thalassaemic individuals compared with thalassaemia group.

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7 203 <sup>b</sup>Compare among three subgroups of thalassaemia ( $\alpha$ -Thal,  $\beta$ -Thal and  $\alpha\beta$ -Thal).

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11 206 differences in MCV ( $p=0.0111$ ), MCH ( $p=0.0002$ ) and RBC ( $p=0.0012$ ) were  
12  
13 207 observed between those groups. MCV and MCH levels in the  $\alpha$ -thal group were  
14  
15 208 significantly lower than those in the  $\beta$ -thal group ( $p<0.05$ ), whereas RBC levels were  
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17 209 higher ( $p<0.05$ ). In contrast, no difference was observed between the  $\alpha\beta$ -thal and  
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19 210  $\alpha$ -thal groups/ $\beta$ -thal groups. Moreover, there was a tendency towards increased RDW  
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21 211 levels in the  $\alpha$ -thal group compared with the  $\beta$ -thal group ( $p=0.0573$ ).  
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## 27 213 **DISCUSSION**

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30 214 Thalassaemia is a common monogenic disease with a relatively high prevalence in  
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32 215 Southeast Asia. In China, this disease is mainly prevalent in areas near the southern  
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34 216 bank of the Yangtze River, such as Guangdong, Guangxi, Fujian and Yunnan  
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36 217 Provinces [13-15]. Prenatal screening and related molecular diagnoses are crucial for  
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38 218 preventing and treating thalassaemia. Many thalassaemia studies have been conducted  
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40 219 in Yunnan Province [16, 17]. However, data on the Jino population are limited  
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42 220 because this population is the last ethnic minority confirmed in China.

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45 221 We randomly selected 1,613 Jino adults from eight villages around Jino Mountain in  
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47 222 Jinghong, Southern Yunnan. Among the gene mutations identified, the most prevalent  
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49 223  $\alpha$ -thal and  $\beta$ -thal genotypes in this region were --<sup>SEA</sup> and HbE, in agreement with  
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51 224 previous data from Yunnan Province [18,19]. According to our results, the overall  
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53 225 prevalence of thalassaemia in Jino was nearly 12.6%, which is similar to the  
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55 226 prevalence observed in Kunming [20]. Prevalence of  $\alpha\beta$ -thal (8%) in our population  
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3 227 was equal to that in the Li population in Hainan Province (7.99%), where  
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5 228 thalassaemia prevalence is high [21]. Although Yunnan Province has a high  
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7 229 prevalence of thalassaemia with diverse genotypes, globin gene mutations spectrum  
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9 230 among the Jino population are relatively limited.  
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11 231 HbE, a type of haemoglobinopathy, can be observed in most regions of southeast  
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13 232 China [22]. Due to a point mutation in  $\beta$ -globin gene, the balance of various globin  
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15 233 products is disrupted, leading to a structural haemoglobin variant. Although HbE  
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17 234 carriers may only have slight anaemia, their offspring will exhibit severe clinical  
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19 235 symptoms in the presence of other  $\beta$ -thal types [23]. Therefore, potential HbE carriers  
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21 236 should undergo genetic testing and prenatal counselling. HPLC is often used as an  
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23 237 efficient primary screening method to detect abnormal Hb, as was done in this study  
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25 238 and previous studies [24,25]. In our study, 95.6% (131/137) of HbE carriers were  
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27 239 identified by HPLC, and 13 of these individuals had concomitant  $\alpha$ -thal deletions.  
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29 240 Different genotypes lead to different clinical phenotypes [26]. We found that  
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31 241 thalassaemic carriers had significantly lower MCV and MCH levels. Regarding those  
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33 242 with  $\beta$ -thal mutations, MCV and MCH levels were significantly decreased in CD17  
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35 243 carriers compared with HbE carriers, suggesting that a nonsense mutation in the  
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37 244  $\beta$ -globin gene causes greater erythrocyte impairment. Hypochromic microcytic  
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39 245 anaemia was moderate in individuals with  $\beta^E/\beta^A$  and  $\alpha^+/\alpha$  compared with  $\beta^E/\beta^A$  carriers.  
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41 246 This paradox may be explained by the fact that changes in the  $\alpha$ - and  $\beta$ -globin chains  
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43 247 could balance each other out when both mutations coexist in an individual.  
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45 248 Accordingly, rapidly estimating the genetic state of an illness based on haematological  
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47 249 parameters is difficult. Therefore, genetic screening of both  $\alpha$ - and  $\beta$ -globin gene  
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49 250 mutations in potential parents is of utmost importance to prevent births with severe  
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51 251 defects [27].  
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3 252 However, there are some limitations in this study. First, the sample size we used in the  
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5 253 genetic testing was relatively small and may not have the validity to identify the rare  
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7 254 thalassaemia variants from this ethnic group. Second, investigations of population and  
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9 255 family structure were not performed in this study, though  $\alpha$ -thal and  $\beta$ -thal gene  
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11 256 mutations were common among Jino ethnic minority. As a result, further studies about  
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13 257 the inbreeding levels and consanguinity structure are warranted to reveal the  
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15 258 underlying mechanism of gene flow and then assess the occurrence and persistence of  
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17 259  $\alpha$ -thal and  $\beta$ -thal gene mutations, especially co-existing  $\alpha$ -thal and  $\beta$ -thal gene  
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19 260 mutations within Jino individuals.

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23 261 In conclusion, this study revealed  $\alpha$ - and  $\beta$ -thalassaemia mutations in the Jino ethnic  
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25 262 minority population in Yunnan Province. Of these mutations, --<sup>SEA</sup> and HbE were the  
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27 263 most prevalent  $\alpha$ -thal and  $\beta$ -thal gene mutation types. In addition, data based on  
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29 264 clinical haematological parameter analysis indicated that the severity of hypochromic  
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31 265 microcytic anaemia is associated with the genotype of thalassaemia. Our results  
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33 266 provide evidence that may be useful for further genetic counselling, prenatal  
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35 267 screening and clinical diagnosis of thalassaemia in this region.

## 38 268 **Contributors**

39  
40  
41 269 WJ and CH conceived and designed the experiments. SW and RZ performed the  
42  
43 270 experiments and analyzed the data. GX, YL, XH, Fusong Jiang and Feng Jiang  
44  
45 271 contributed materials and analysis tools. SW prepared the article. CH and WJ revised  
46  
47 272 the manuscript. All the authors have read and approved the final version of this  
48  
49 273 manuscript.

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2  
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9

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16

## 17 281 **Competing interests**

18  
19  
20  
21 282 No, there are no competing interests.  
22

## 23 283 **Ethics approval**

24  
25  
26 284 According to the Helsinki Declaration II, ethical approval for the study was granted  
27  
28 285 by the Institutional Review Board of Shanghai Jiao Tong University affiliated with the  
29  
30 286 Sixth People's Hospital, Shanghai, China. Written and Oral informed consent was  
31  
32 287 obtained from all participants.  
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## 35 288 **Data sharing statement**

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38 289 No additional data are available.  
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## 369 **Figure legends**

370 **Fig. 1: Geographical location of the Jino ethnic minority populations in Yunnan**  
371 **Province, Southwest China.** The solid black triangle represents Jino Mountain. The  
372 solid black circles represent the eight villages in Jinghong where the 1,613 subjects  
373 were randomly selected.

374 **Fig. 2: Gel electrophoresis of PCR amplify in  $\alpha$ -thal deletions.** M: marker, 200 bp  
375 DNA Ladder; Lane 1: rightward deletion (genotype of  $-\alpha^{3.7}/-\alpha^{3.7}$ ); Lane 2 and Lane 3:  
376 rightward deletion (genotype of  $-\alpha^{3.7}/\alpha\alpha$ ); Lane 4 and Lane 5: Southeast Asia deletion  
377 (genotype of  $--^{SEA}/\alpha\alpha$ ).

378 **Fig. 3: Heterozygous CD17 (A>T) mutation (a) and the corresponding normal**  
379 **sequence of  $\beta$ -globin.** Red arrows indicate the position of this point mutation.

380 **Fig. 4: Heterozygous CD26 (G>A) mutation (a) and the corresponding normal**  
381 **sequence of  $\beta$ -globin.** Red arrows indicate the position of this point mutation.

## 382 **Table legends**

383 **Table 1.** Haematological and demographic characteristics of 1,613 Jino ethnic  
384 minority adults included in the study.

385 **Table 2.** Allele frequency of  $\alpha$ - and  $\beta$ -Thalassaemia mutations found in our study.

386 **Table 3.** Haematological data of 1,613 Jino ethnic minority individuals with different  
387 thalassaemia subtypes.



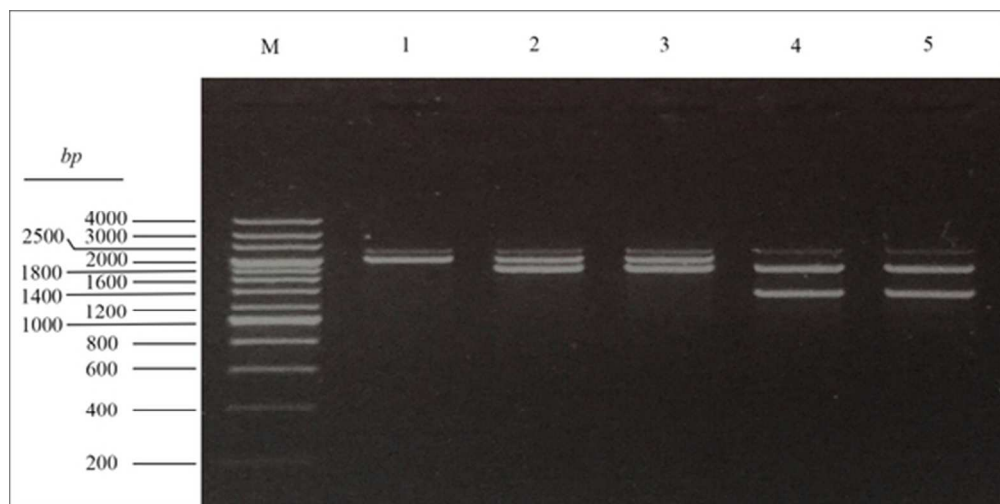


Fig. 2: Gel electrophoresis of PCR amplifying  $\alpha$ -thal deletions. M: marker, 200 bp DNA Ladder; Lane 1: rightward deletion (genotype of  $-\alpha^{3.7}/-\alpha^{3.7}$ ); Lane 2 and Lane 3: rightward deletion (genotype of  $-\alpha^{3.7}/\alpha\alpha$ ); Lane 4 and Lane 5: Southeast Asia deletion (genotype of  $--^{SEA}/\alpha\alpha$ ).  
49x24mm (300 x 300 DPI)

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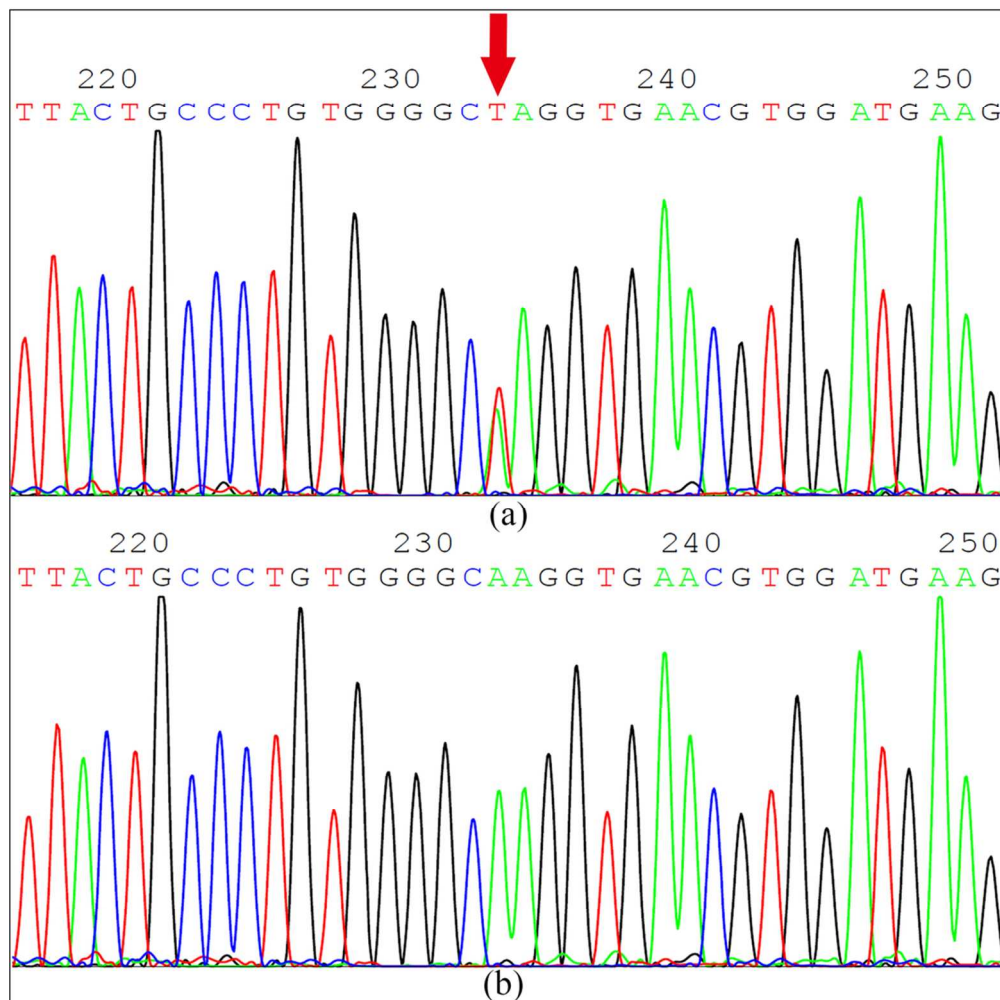


Fig. 3: Heterozygous CD17 (A>T) mutation (a) and the corresponding normal sequence of  $\beta$ -globin. Red arrows indicate the position of this point mutation.  
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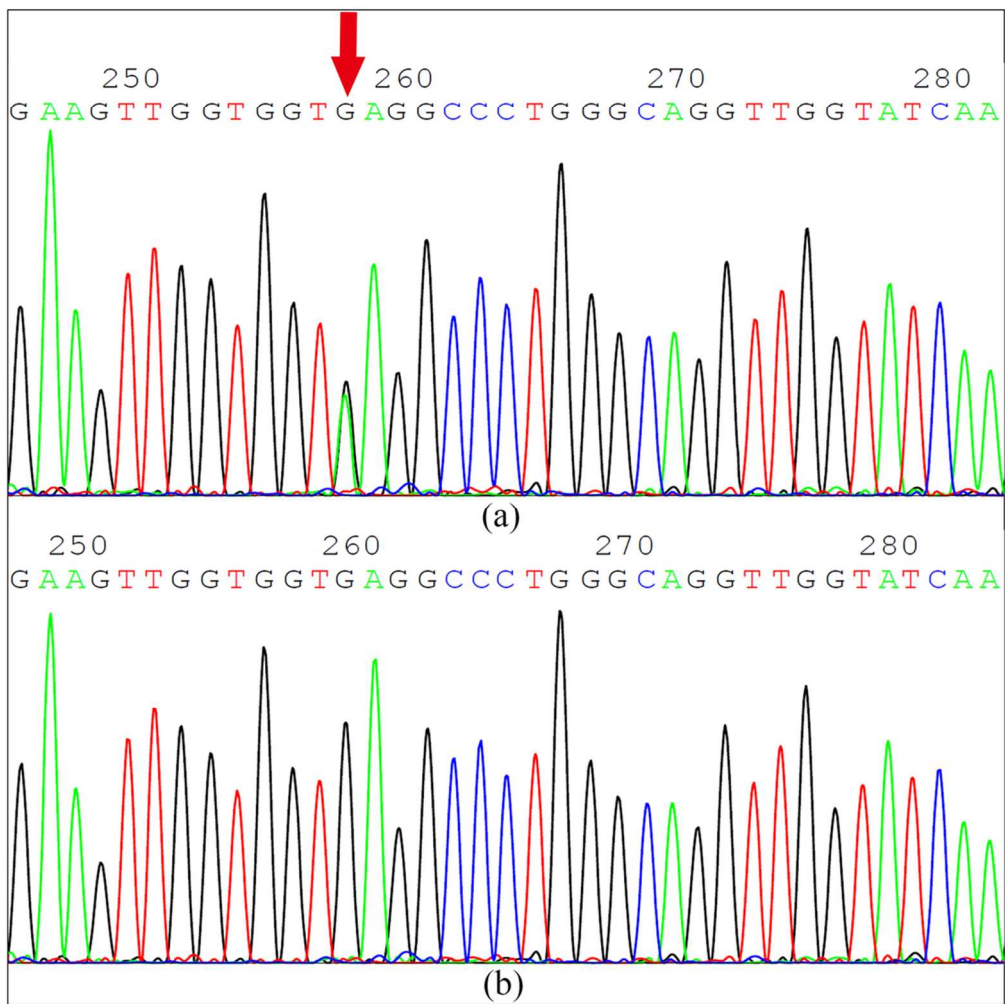


Fig. 4: Heterozygous CD26 (G>A) mutation (a) and the corresponding normal sequence of  $\beta$ -globin. Red arrows indicate the position of this point mutation.  
99x99mm (300 x 300 DPI)