β-globin gene cluster haplotypes in ethnic minority populations of southwest China

Hao Sun*, Hongxian Liu*, Kai Huang, Keqin Lin, Xiaoqin Huang, Jiayou Chu, Shaohui Ma & Zhaoqing Yang

The genetic diversity and relationships among ethnic minority populations of southwest China were investigated using seven polymorphic restriction enzyme sites in the β-globin gene cluster. The haplotypes of 1392 chromosomes from ten ethnic populations living in southwest China were determined. Linkage equilibrium and recombination hotspot were found between the 5′ sites and 3′ sites of the β-globin gene cluster. 5′ haplotypes 2 (+−−−), 6 (−+++−), 9 (−++++) and 3′ haplotype FW3 (−++) were the predominant haplotypes. Notably, haplotype 9 frequency was significantly high in the southwest populations, indicating their difference with other Chinese. The interpopulation differentiation of southwest Chinese minority populations is less than those in populations of northern China and other continents. Phylogenetic analysis shows that populations sharing same ethnic origin or language clustered to each other, indicating current β-globin cluster diversity in the Chinese populations reflects their ethnic origin and linguistic affiliations to a great extent. This study characterizes β-globin gene cluster haplotypes in southwest Chinese minorities for the first time, and reveals the genetic variability and affinity of these populations using β-globin cluster haplotype frequencies. The results suggest that ethnic origin plays an important role in shaping variations of the β-globin gene cluster in the southwestern ethnic populations of China.

According to the 2010 population census, excluding the Han, who forms the majority of the Chinese population, there are 55 other officially recognized ethnic minority populations accounting for 8.49% of the Chinese national population. More than 30 Chinese ethnic minorities inhabit in southwest China, most of them inhabit only in the region, such as Thai, Achang, Deang, and Tibetan. Due to the plateau and mountainous geographic features of the region, these populations have adapted the distinctive natural environment including climate, high altitude and epidemic infectious diseases, also experienced founder effect resulted from regional geographic isolation. Therefore, ethnic minority populations of southwest China present abundant diversity and distinction on their historic origins, languages, cultures, and genetic characteristics. Many studies have focused on understanding the genetic structures and relationships in these ethnic populations using microsatellite, mitochondrial DNA (mtDNA) and Y chromosome markers1–3. The evidence from these separate analyses concludes that the ancestors of East Asians originated in Africa and entered Asia from the southeast, but the migration of the ancient populations to the north of China and the strong south/north distinction in genetic patternation have been disputed2,4. In spite of the controversy, southwest China played an important role either as a passage of migration from Southeast Asia toward China or as an interface of ethnic mixture, and therefore impacted on the formation and diversity of Chinese ethnic populations. Furthermore, correlation between linguistic affinity and genetic diversity has been observed in southwest Chinese minorities5,6. However, the contributing genetic factors responsible for the diversities and affinities of these Chinese minorities remain unclear.

The value and power of β-globin gene cluster markers in resolving origins, migration and evolutionary relationships within human populations worldwide have been well demonstrated7–12. Seven neutral polymorphic restriction enzyme sites, especially the five sites within the 5′ region of the β-globin gene cluster, have been commonly employed to construct haplotypes for assessing genetic variation and the relationship between human populations. This method has revealed the origins of the β-globin gene mutations and the clinical implications of

The Department of Medical Genetics, Institute of Medical Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, 935 Jiaoling Road, Kunming 650118, China. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to S.M. (email: shaohuima@imbcams.com.cn) or Z.Y. (email: zyang@imbcams.com.cn)
variability and relationship among the ethnic Chinese populations. Results with those data previously published for Chinese and other world populations, and evaluated the genetic gene cluster in 10 ethnic minority populations, mainly from southwestern China. This study also integrated these understanding of Chinese ethnic diversity, differentiation and genetic relationships.

southwestern Chinese minority populations have not been investigated for β-globin gene cluster in 10 ethnic groups. These lack of comparable data from southwestern minorities significantly restricts our understanding of Chinese ethnic diversity, differentiation and genetic relationships.

We used a likelihood-ratio test of Arlequin software to evaluate linkage disequilibrium between a pair of loci according to the positions relative to the recombination hotspot. These suggest a recombination hotspot between the HincII 3′Ψβ and AvaII β loci (Fig. 1), the haplotypes derived from these seven loci were divided into 5′ sub-haplotypes and 3′ sub-haplotypes according to the positions relative to the recombination hotspot.

### Results

#### β-globin gene cluster polymorphism in southwest Chinese ethnic minority groups.

Table 1 presents the allelic frequencies detected for the seven polymorphic restriction sites of the β-globin gene cluster, in the 10 minority populations from southwest China. We found all restriction sites were polymorphic in the Hardy-Weinberg equilibrium with a few exception. We also found HincII 5′ε and Hinf I 3′β had the lowest frequency, while HincII 5′Ψβ had the lowest frequency among the minority groups. This indicates that the distribution patterns of allelic frequencies were homogenous among the populations.

We used a likelihood-ratio test of Arlequin software to evaluate linkage disequilibrium between a pair of loci in the studied populations from southwest China. Pairwise linkage disequilibrium (LD) were observed among the loci HincII 5′ε, HindIII Gγ, HindIII Aγ, HincII 5′Ψβ and HincII 3′Ψβ at the 5′ end of the β-globin gene cluster, as well as between AvaII β and Hinf I 3′β loci at the 3′ end of the cluster. But LD was not observed between loci of the 3′ and 5′ ends of the cluster (Table 2). These suggest a recombination hotspot between the HincII 3′Ψβ and AvaII β loci (Fig. 1), the haplotypes derived from these seven loci were divided into 5′ and 3′ sub-haplotypes according to the positions relative to the recombination hotspot.

#### 5′ Haplotypes of the β-globin gene cluster in southwest Chinese ethnic groups.

The haplotypes derived from the five polymorphic sites of the 5′β-globin cluster from the 10 southwestern minority groups and other populations are reported in Table 3. Twenty-six of the 32 (25) possible 5′ haplotypes were observed in the southwestern minority populations, but only haplotype 2, 5, 6 and 9 reached frequencies greater than 0.02. Haplotypes 25 (++++++) and 26 (++++−) have never been described before this study. Seven haplotypes, 3, 7, 8, 13, 16, 20, and 25, are novel haplotypes that have never been described before in any populations.

### Table 1. Allele frequencies for the presence (+) of the seven restriction loci of the β-globin cluster in the ethnic minorities of southwest China. *Hardy-Weinberg disequilibrium (p < 0.05).

| Locus   | HincII 5′ε | HindIII Gγ | HindIII Aγ | HincII 5′Ψβ | HincII 3′Ψβ | AvaII β | Hinf I 3′β |
|---------|------------|------------|------------|-------------|-------------|---------|------------|
| HincII 5′ε | 0.001      | 0.070      | 0.048      | 0.082       | 0.561       | 0.049   | 0          |
| HindIII Aγ | 0.017      | 0.042      | 0.028      | 0.049       | 0           | 0       | 0          |
| HincII 5′Ψβ | 0.004      | 0.070      | 0.048      | 0.082       | 0.561       | 0.049   | 0          |
| HincII 3′Ψβ | 0.001      | 0.070      | 0.048      | 0.082       | 0.561       | 0.049   | 0          |
| AvaII β  | 0.001      | 0.070      | 0.048      | 0.082       | 0.561       | 0.049   | 0          |
| Hinf I 3′β | 0.001      | 0.070      | 0.048      | 0.082       | 0.561       | 0.049   | 0          |
and distribution patterns of the haplotypes among the study populations. Our results demonstrate that current characterization of the variability, origin, migration and evolutionary relationships between populations worldwide. Our present study that the populations with longer history have higher levels of genetic variability.

17, 19, 20, 27, 28 and 29, were identified for the first time in Chinese populations with low frequencies as in other populations elsewhere. Haplotype 2 (+−−−−) was the most and 6 (++++−+) was the second most prevalent in the minorities of southwestern China with frequencies range of 0.570–0.779 and 0.035–0.174, respectively. The distribution patterns of common haplotypes 2, 6 and 5 in the southwestern minorities are generally consistent with that in other world populations. However, haplotype 2 was somewhat less frequent in Achang and Deang, and haplotype 5 was absent in Khmus. Achang participants had a significantly higher frequency of haplotype 4 (0.125), which is only slightly less frequent than that in African populations (0.152).

The distribution of the β-globin gene cluster haplotype showed geographic variation. The Chinese populations from the southwest and from north are distinguished by the distribution of haplotype 9 (−+++−−). Haplotype 9 is the third most prevalent haplotype with frequency of 2.2–8.5% in the southwest Chinese populations, but it is absent or very rare (<1%) in all other populations elsewhere (Table 3). On the contrary, common haplotype 5 (−+++−+) in southwest China is less frequent than that in northern China. In addition, haplotypes 12 is observed in the ethnic minorities of Yunnan, but it is absent in other regions of China.

We also measured genetic variability of Chinese populations using heterozygosity and Gini-Simpson index (GSI). We found that heterozygosity and GSI of Achang, Deang and Khmus are much higher than that of all other Chinese populations except Oroqens from northern China, and are comparable with African. This suggests that the populations with longer history have higher levels of genetic variability.

3′ Haplotype analysis. We identified 3′ haplotypes based on the presence (+) and absence (−) of AvaII and HinfI restriction sites (Fig. 1). We found gene framework (FW) 1–4 were polymorphic in all Chinese ethnic groups (Table 4). FW3 (−−−+) was the most common and FW4 (−−−−) was rare 3′ haplotypes in all ethnic Chinese populations examined. In general, the distribution patterns of FW1–4 among different Chinese ethnic populations were homogenous. In addition, we found different Thai subpopulations had the most frequent FW2, while Tibetan and northern Chinese Han subpopulations living in different regions had the highest FW3 haplotype frequencies. This suggests 3′ haplotype distribution pattern of these Chinese populations accords with their ethnic origin.

Genetic diversity for the β-globin cluster in Chinese ethnic populations. The measures of genetic diversity in Chinese ethnic populations and other world populations are presented in Table 5. Overall, total heterozygosity (Ht) of the southwestern Chinese population is 52.5%, of which 88% may be ascribed to genetic variation within populations. The level of heterozygosity observed in the southwestern Chinese is higher than that in northern Chinese, but the correction for gene differentiation coefficient (Gst) is lower in southwest Chinese (2.5%) than in northern Chinese (3.7%), indicating less interpopulation differentiation among the southwest populations. The lowest Gst were observed in northern Chinese Han (0.5%) and Thai (0.7%) subpopulations, while high degree of interpopulation differentiation was observed in Tibetan (Gst = 1.2%), indicating genetic heterogeneity among different Tibetan subpopulations from different inhabitation regions. Likewise, when the northern Han were analyzed together with southern Han (Han), the Gst was significantly increased, suggesting high differentiation between south and north Chinese Han populations.

Genetic relationship among Chinese populations. Using pairwise Fst (F-statistics) and exact test of non-differentiation based on 5′ haplotype frequencies, the most significant differences and greatest genetic heterogeneity were observed in inter-ethnic comparisons of the Chinese populations (Supplementary Tables 1 and 2). Khmus and Deang were significantly different from other southwestern minority populations even though they live within a rather small geographic region. The phylogenetic relationships among the minorities of southwestern China and other populations are shown in the dendrogram Neighbor-joining (NJ) tree (Fig. 2), which is based on the matrix of genetic distances (DA) between the populations (Supplementary Table 3). The genetic affinities showed clear ethnic and linguistic patterns among Chinese populations (Fig. 2a). A southwest/northeast geographic pattern was observed as well, but the north/south division was not distinct. On a global scale, the ethnic minorities from southwestern China are closely clustered to other Chinese and Asian populations, but are far away from African and European populations. Amerindian from the American continent is clustered close to Chinese populations (Fig. 2b).

Discussion
The β-globin gene cluster haplotypes have been largely shown as important and useful for investigating genetic variability, origin, migration and evolutionary relationships between populations worldwide. Our present study is the first report characterizing the β-globin gene cluster haplotypes in southwestern Chinese minorities, and reveals genetic variation and relationships in these ethnic populations. We found the allelic and haplotypic characteristics of the β-globin gene cluster, and the significant differences in genetic variability, characteristics and distribution patterns of the haplotypes among the study populations. Our results demonstrate that current
### Table 3. 5′ haplotype frequencies of the β-globin cluster in the minorities from Southwest China, other Chinese populations and world populations.

| Population | Thai (DH) | Thai (BN) | Thai (YY) | Jingpo | Achang | Deang | Khmus | Tibetan (YN) | Tibetan (TB) | Tibetan (QH) | Han<sup>α</sup> (KM) | Han<sup>α</sup> (XA) | Han<sup>α</sup> (BJ) | Han<sup>α</sup> (HK) | Evenki<sup>β</sup> | Oroqen<sup>α</sup> | Euro-Peans<sup>γ</sup> | Amer-Indians<sup>δ</sup> | Africans<sup>γ</sup> |
|------------|-----------|-----------|-----------|--------|--------|-------|-------|-------------|-------------|-------------|-----------------|---------------|----------------|--------------|----------------|----------------|------------------|------------------|
| 1          | 0.015     | 0.007     | 0.0790    | 0.011  | 0.012  | 0.005 | 0.020 | 0.026       |             |             |                 |               |                |               |                |                |                |                |
| 2          | 0.779     | 0.707     | 0.717     | 0.700  | 0.566  | 0.570 | 0.607 | 0.739       | 0.729       | 0.659       | 0.763          | 0.737         | 0.686          | 0.809        | 0.669          | 0.372          | 0.609          | 0.728          | 0.063           |
| 3          | 0.036     |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 4          | 0.012     | 0.008     | 0.008     | 0.125  | 0.048  | 0.021 | 0.008 | 0.008       | 0.013       | 0.019       | 0.004          | 0.022         | 0.032          | 0.532        | 0.008          | 0.022          | 0.015          |                |
| 5          | 0.021     | 0.041     | 0.046     | 0.032  | 0.096  | 0.072 | 0.065 | 0.042       | 0.012       | 0.141       | 0.149          | 0.134         | 0.064          | 0.079        | 0.129          | 0.233          | 0.031          | 0.139          |
| 6          | 0.078     | 0.103     | 0.065     | 0.084  | 0.095  | 0.078 | 0.036 | 0.174       | 0.115       | 0.183       | 0.067          | 0.087         | 0.118          | 0.1         | 0.099          | 0.178          | 0.112          | 0.025          |
| 7          | 0.004     | 0.014     |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 8          |           |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 9          | 0.004     | 0.049     | 0.049     | 0.079  | 0.038  | 0.057 | 0.036 | 0.022       | 0.083       | 0.085       | 0.005          | 0.004         | 0.004          | 0.003        | 0.004          | 0.002          | 0.013          |                |
| 10         | 0.040     | 0.012     | 0.006     |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 11         |           |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 12         |           |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 13         | 0.011     | 0.014     | 0.024     | 0.006  | 0.029  |        | 0.004 | 0.014       | 0.005       | 0.027       | 0.038          |                |                |               |                |                |                |                |
| 14         |           |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 15         | 0.010     |           |           |        |        |       |       |             |             | 0.012       | 0.042          | 0.025         |                |               |                |                |                |                |
| 16         | 0.004     | 0.007     | 0.006     | 0.018  |        |       |       |             |             | 0.005       | 0.003          | 0.004          | 0.012         | 0.013        |                |                |                |                |
| 17         | 0.008     |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 18         | 0.007     | 0.006     |           |        |        |       |       |             |             | 0.012       | 0.007          | 0.006         |                |               |                |                |                |                |
| 19         |           |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 20         | 0.004     | 0.014     | 0.020     | 0.021  | 0.071  |        |        |             |             |             |                 |               |                |               |                |                |                |                |                |
| 23         |           |           |           |        |        |       |       |             |             |             |                 |               |                |               |                |                |                |                |                |
| 24         | 0.008     | 0.016     |           |        | 0.017  |        |        |             |             |             |                 |               |                |               |                |                |                |                |                |
| 25         | 0.011     | 0.007     |           |        |        |       | 0.036  | 0.0211      | 0.012       |             |                 |               |                |               |                |                |                |                |                |
| 26         |           |           |           |        |        |       | 0.017  |           |             |             |                 |               |                |               |                |                |                |                |                |
| 27         |           |           |           |        |        |       | 0.007  |           |             |             |                 |               |                |               |                |                |                |                |                |
| 28         |           |           |           |        |        |       | 0.016  |           |             |             |                 |               |                |               |                |                |                |                |                |
| 29         |           |           |           |        |        |       | 0.007  |           |             |             |                 |               |                |               |                |                |                |                |                |

**Number of haplotype:** 29

**Heterozygosity:** 0.384

**GSI:** 0.382

**Ne:** 1.618

---

haplotypic variation of the β-globin gene cluster in Chinese ethnic populations mirrors their ethnic origin and linguistic classifications.

Our finding reveals the distribution characteristics of β-globin gene cluster haplotype in ethnic minority populations of southwest China. In the southwestern Chinese minorities, 5′ haplotype 2, 6, 9 and 5 are more prevalent overall, while haplotypes 12 and 4 are less common but not rare (Table 3). Haplotype 2 is the most common haplotype, and its distribution in southwestern China is consistent with that of the global pattern. Haplotype 6 was found to be the second most common haplotype in our study groups, which agree with its distribution pattern in Asian populations except in northern Chinese Han. Remarkably, we also found haplotype 9 is the third most frequent haplotype with frequencies of 2.2–8.5% in southwestern Chinese minorities (Table 3), which is much higher than that in northern Chinese populations (0–0.04) and other world populations<sup>8,14</sup>. Haplotype 9 is a characteristic haplotype across the southwestern Chinese minorities, suggesting gene flow and admixture among the adjoining populations within this geographic region. Moreover, in the Asian continent, haplotype 9 has only been reported in ethnic populations of China, to date. Comparable frequencies of haplotype 9 have been most clearly observed in native Americans that presumably migrated from Asia<sup>10,16,18</sup>. Our findings provide further evidence for Asian, probably Chinese, gene flow toward Native Americans.

The origin of β-globin gene cluster haplotypes in the populations of southwest China has not been explored. The haplotypes 2, 5 and 6 are considered primitive and first-order haplotypes as they are separated from each other by at least two genetic events, mutation or recombination. The origin of the rest of the haplotypes is derived from the first-order types by recombination<sup>8,17</sup>. Since at least two steps of genetic variations are required for...
conversion among haplotypes 2, 5 and 6, these three common haplotypes were likely present in the original populations settled in southwestern China. However, the prevalent haplotype 9 ($−++++$) in the populations of southwest China could be derived from all three common first-order haplotypes (2, 5, 6), most likely from haplotype 6 ($−++−+$) and 5 ($−+−++$), as only one conversion or mutation is needed at the HindIII $G_γ$ or HincII $5′Ψβ$ sites, respectively. Nevertheless, there is not a one-to-one correspondence between pairs of ancestor haplotypes and their products of genetic conversion events. Most second-order haplotypes are rare in the southwestern minorities; their origin could be attributed to genetic recombination.

Our results show linkage disequilibrium between the five sites within the $5′$ region of the $β$-globin gene cluster and between the other two sites within the $3′$ terminal, whereas linkage equilibrium was observed between $5′$ and $3′$ haplotypes as a result of the recombination hotspot between the two regions (Table 5, Fig. 1). These results confirm the presence of the recombination hotspot among the Chinese minority populations, consistent with findings in other populations. As the polymorphic sites within $5′$ haplotypes are in linkage disequilibrium and significantly associated to each other (Table 2), and the most common first-order haplotypes—presumably formed by a single round of recombination—were present in the study groups, our findings support the previous hypothesis that the rate of recombination within the $5′$ haplotypes is not particularly rapid. Therefore, the most common haplotypes 2, 6, 5 in southwestern Chinese minority populations would not be the result of recent recombination events, while it remains unclear if the same can be inferred for the common second-order haplotype 9.

$5′$ haplotypes provide information on microevolutionary processes, while $3′$ haplotypes reveal the ancestral origins of populations and can be traced through the origin of $β$-globin gene mutations. Southwestern China is a region with endemic malaria and thalassemia, with the highest frequencies of hemoglobin E ($βE$, HBB codon 26 $G > A$) reported in the Achang, Deang and Jingpo minority populations living in the region. The $βE$ gene was

| Population | No. of sub-populations | $H_T$ | $H_s$ | $D_{st}$ | $D_{mt}$ | $G_{ST}$ | $G_{ST′}$ |
|------------|-------------------------|-------|-------|---------|---------|---------|---------|
| Thai       | 3                       | 0.449 | 0.447 | 0.002   | 0.003   | 0.005   | 0.007   |
| Tibetan    | 3                       | 0.467 | 0.463 | 0.004   | 0.006   | 0.008   | 0.012   |
| Northern Han | 3                     | 0.441 | 0.439 | 0.002   | 0.002   | 0.004   | 0.005   |
| Han       | 4                       | 0.416 | 0.412 | 0.004   | 0.005   | 0.009   | 0.013   |
| Southwestern Chinese | 10     | 0.525 | 0.513 | 0.012   | 0.013   | 0.022   | 0.025   |
| Northern Chinese | 5      | 0.476 | 0.462 | 0.014   | 0.018   | 0.030   | 0.037   |
| Chinese   | 16                      | 0.509 | 0.496 | 0.013   | 0.014   | 0.025   | 0.027   |
| Asian     | 24                      | 0.532 | 0.508 | 0.024   | 0.025   | 0.045   | 0.047   |
| Americans | 31                      | 0.413 | 0.383 | 0.030   | 0.031   | 0.073   | 0.075   |
| European  | 5                       | 0.622 | 0.588 | 0.034   | 0.043   | 0.055   | 0.067   |
| African   | 6                       | 0.715 | 0.651 | 0.064   | 0.077   | 0.090   | 0.106   |

Table 4. Frequencies of the $β$-globin gene cluster frameworks (FW, AvaII $β$-Hinf 3$′$) in Chinese ethnic populations. Details of populations are described in Table 6.

Table 5. Genetic diversity for the $β$-globin gene cluster in world populations. The present study. Shimizu et al.14, Shimizu et al.14, Cheng et al.15, and Chan et al.20. The present study and Shimizu et al.13,14. Reference (number of populations): The present study (10), Shimizu et al. (9)13,14, Long et al. (2)8, Chen et al. (1)17, Aggarwal et al. (1)15, and Chan and Chen et al. (1)20,31. Callegari-Jacques SM et al.10.
found exclusively linked to haplotype 9 and FW2 in southwestern Chinese minorities in our previous study. But wide type HBB gene βA (HbAA) was linked to 5′ haplotypes 2, 6, 9, 5 (Table 3) and all gene frameworks, mostly to FW3 (−+) in the populations (Table 4). The distribution pattern of the 3′ haplotype frameworks is homogeneous in southwestern minorities and similar to that in southeastern Asian populations, which indicates a common origin in these Asian populations. More importantly, our results found that haplotype 9 is a characteristic type across all of the southwestern minority groups, meaning it must have been present in very early colonies of these populations. The findings on βA linked haplotypes in this study provide additional information for inferring evolution of the βE mutation. We speculate that the predominant βE genes in southwestern minorities occurred on a common haplotype 9 bearing chromosomal background and spread into different populations through the adjoining effect. The haplotype 9 linked βE is unlikely to form from haplotype 2 linked βE through recombination events in the respective populations.

It is well known that high heterozygosity is attributed to long population histories or interpopulation gene flow. In the present study, haplotypic heterozygosity, GSI, considering both frequency and number of haplotypes, and number of effective haplotypes (Ne) in the Achang, Deang and Khus from southwestern China was found to be much greater than that of other Chinese populations with the exception of Oroqens (Table 3). Deang is one of the oldest original populations, having lived in the region for more than 2000 years; Khus is another older aborigine population in the same region as well—the higher heterozygosities reflect their longer ethnic history. As Achang originated from an ancient Di-qiang population living in the Qinghai-Tibet Plateau of China, migration and admixture are expected. The higher heterozygosity of Achang could mirror the evolutionary action of both ethnic history and gene flow. Our findings provide more genetic evidence for interpreting the history and migration of southwestern Chinese minorities.

Our study explores how ethnic minority populations of southwest China are related to each other and other populations. Our findings demonstrate that genetic affinities among ethnic Chinese populations show ethnic and linguistic patterns. Some studies using microsatellite and mitochondrial DNA (mtDNA) markers have found distinct genetic divergence between southern and northern Chinese populations, and have argued that northern populations are derived from southern Chinese populations. Whereas, other studies found that DNA markers did not support the south/north division but rather suggest simple distance isolation. In addition, the correlation between genetic diversity and linguistic affinity in Chinese ethnic groups was demonstrated by autosomal microsatellite markers. By using the β-globin cluster markers, our study does not support the distinct south/north geographic division found in other studies using microsatellite, Y Chromosomal STR and mtDNA markers, but tends to support the hypothesis that DNA marker patterns suggest simple isolation by geographic distance. Alternatively, it is possible that the β-globin cluster markers and the other genetic markers may have evolved differently in these populations.

When genetic relationships between world populations were analyzed using haplotypic frequencies, the majority of Chinese ethnic groups were clustered together and close to other Asia and Amerindian populations as expected (Fig. 2b). As a result of the limitations in the number of examined populations and sample sizes, the unrooted polygenic tree may only represent the genetic affinities but not evolutionary relationships among the populations. This study reveals genetic relationships of southwestern minorities of China using the β-globin gene
cluster markers, for the first time, and provides new evidence supporting the consistency of genetic and linguistic evolution in Chinese populations. Moreover, our findings on the characteristic haplotype 9 distribution and phylogenetic relationship among populations strongly support the notion that Asian, and most likely Chinese, gene flow migrated toward native American populations.

In conclusion, the $\beta$-globin haplotype is useful for elucidating genetic variation, affinity and ethnic origin of human populations. Here we have shown that the distribution of $\beta$-globin haplotypes is significantly heterogeneous in minority populations of southwest China, the distribution pattern is significantly different with that of populations in other regions of China. Moreover, we have demonstrated that the genetic affinity of Chinese populations show ethnic and linguistic patterns. The genetic heterogeneity and differentiation presented in the southwest Chinese ethnic populations deepen our understanding of their ethnic history and gene flow. The diversity of the $\beta$-globin gene cluster in Chinese populations is mainly attributed to ethnic origin. Meanwhile, admixture, geographic isolation and genetic recombination are important factors accounting for the genetic variations observed. Overall, our findings provide new comparable data for revealing genetic diversity and the relationships of Chinese populations, and once again, and show that the $\beta$-globin gene cluster can provide a large amount of substantial information on elucidating human history and evolution.

Materials and Methods

**Studied populations.** Ten ethnic minority groups belonging to six Chinese nationalities: Achang, Deang, Khmus (officially recognized as a subpopulation of Bulang nationality), Jingpo, Thai (named Dai in Chinese) and Tibetan from south-to-west China regions including Yunnan, Tibet and Qinghai provinces, were studied. The chosen groups well represent ethnic populations of southwest China as they inhabit only in the region, with different historic origins and cultures. The sample size was chosen to satisfy the needs of genetic statistics. It varied with size and sample availability of the different ethnic groups. The sampling, geographic location and linguistic affiliation of...
frequencies, and phylogenetic trees were constructed using DA distances through the DISPAN program. G ST gene cluster: HincII 5′ these sites were genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) protocols as previously described with a few modification27,28. The genotypes were recorded as “−” or “+” according to the presence or absence of the respective restriction enzyme sites.

Table 6. Sample information of Chinese ethnic populations examined in the present study and previous reports.

| Populations       | No. of Chromosomes | Linguistic family, subfamily | Location (City, Province) | Geographic classification | reference |
|-------------------|--------------------|-------------------------------|---------------------------|---------------------------|-----------|
| 1. Achang         | 104                | Sino-Tibetan, Tibeto-Burman   | Lianghe, Yunnan           | southwest                 | Present study |
| 2. Deang          | 166                | Austro-Asiatic, Mon-Khmer     | Mangshi, Yunnan           | southwest                 |           |
| 3. Jingpo         | 164                | Sino-Tibetan, Tibeto-Burman   | Mangshi, Yunnan           | southwest                 |           |
| 4. Thai (BN)      | 144                | Dai, Tai-Kadai                | Jinghong, Yunnan          | southwest                 |           |
| 5. Thai (DH)      | 268                | Dai, Tai-Kadai                | Mangshi, Yunnan           | southwest                 |           |
| 6. Thai (YX)      | 136                | Dai, Tai-Kadai                | Yuci, Yunnan              | southwest                 |           |
| 7. Khmus          | 140                | Austro-Asiatic, Mon-Khmer     | Menhai, Yunnan            | southwest                 |           |
| 8. Tibetan (YN)   | 92                 | Sino-Tibetan, Tibeto-Burman   | Zhongdian, Yunnan         | southwest                 |           |
| 9. Tibetan (TB)   | 96                 | Sino-Tibetan, Tibeto-Burman   | Lhasa, Tibet              | southwest                 |           |
| 10. Tibetan (QH)  | 82                 | Sino-Tibetan, Tibeto-Burman   | Guinan, Qinghai           | southwest                 |           |
| 11. Han (KM)      | 240                | Sino-Tibetan, Chinese         | Kunming, Yunnan           | south origin              | [14]      |
| 12. Han (HK)      | 110                | Sino-Tibetan, Chinese         | HongKong                  | south                     | [20, 31]  |
| 13. Han (BJ)      | 226                | Sino-Tibetan, Chinese         | Beijing                   | north                     | [14]      |
| 14. Han (XA)      | 254                | Sino-Tibetan, Chinese         | Xiang, Shangxi            | north                     | [14]      |
| 15. Evenki        | 228                | Altai, Tungus                 | Hailar, Inner Mongolia    | north                     | [13]      |
| 16. Oroqen        | 162                | Altai, Tungus                 | Heihe, Heilongjiang       | north                     | [13]      |

the populations are presented in Fig. 3 and Table 6. All experiments and methods were approved and in accordance with the Ethics Committee of the Institute of Medical Biology, Chinese Academy of Medical Sciences. Unrelated healthy individuals from different minority populations were randomly selected. Informed consent was obtained from all subjects. Individual information on ethnic identification, ancestry and migration history were recorded to ensure the representativeness of their own minority communities. Genomic DNA was extracted from peripheral blood samples collected using anticoagulant sodium citrate. Carriers of the β-globin gene (HBB) mutations were excluded based on hematologic and molecular genetics analyses for thalassemia. A total of 1392 chromosomes with normal β-globin genotype HbAA (β0) from 696 individuals were examined in the present study.

Genotyping of the β-globin gene cluster. There are seven polymorphic restriction sites in the β-globin gene cluster: HincII 5′, HindIII G−γ, HindIII A−γ, HincII 5′Ψβ, HincII 3′Ψβ, AvaiI β and Hinf I 3′β (Fig. 1); these sites were genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) protocols as previously described with a few modification27,28. The genotypes were recorded as “+” or “−” according to the presence or absence of the respective restriction enzyme sites.

Haplotype analysis and statistical analysis. Allele frequencies for different restriction sites were calculated using a direct counting method. The Hardy-Weinberg equilibrium, estimation of haplotype frequencies of the β-globin gene cluster, linkage disequilibrium between pairs of loci, pairwise Fst (F-statistics) and the exact test of population differentiation, were evaluated using the population genetics analysis software Arlequin V3.5.29,30. The genetic distance and phylogenetic analysis program DISPAN (http://www.personal.psu.edu/nxm2/dispan2.htm, copyright 1993 by Tatsuya Ota and the Pennsylvania State University) was used to measure genetic diversity parameters HT (the average heterozygosity for the entire population), Hs (the average heterozygosity within populations) and G ST (gene differentiation coefficient). The haplotype frequencies in the present study were integrated with those from other populations in China and around the world using data from previous reports8,10,11,13–15,17,20,31. The matrix of DA genetic distances between populations was calculated using the haplotype frequencies, and phylogenetic trees were constructed using DA distances through the DISPAN program. G ST was used as a correction of G ST affected by the number of examined populations16. Genetic diversity was also measured by the Gini-Simpson index (GSI) and the effective number of haplotypes (Ne) as previously described8.

References

1. Chu, J. Y. et al. Genetic relationship of populations in China. Proc Natl Acad Sci USA 95, 11763–11768 (1998).
2. Su, B. et al. Y-Chromosome evidence for a northward migration of modern humans into Eastern Asia during the last Ice Age. Am J Hum Genet 65, 1718–1724, doi: 10.1086/302680 (1999).
3. Yao, Y. C. et al. Genetic relationship of Chinese ethnic populations revealed by mtDNA sequence diversity. Am J Phys Anthropol 118, 63–76, doi: 10.1002/aja.10052 (2002).
4. Ding, Y. C. et al. Population structure and history in East Asia. Proc Natl Acad Sci USA 97, 14003–14006, doi: 10.1073/pnas.240441297 (2000).
5. Lin, H. et al. Genetic relationships of ethnic minorities in Southwest China revealed by microsatellite markers. PLoS One 5, e9895, doi: 10.1371/journal.pone.0099895 (2010).
6. Sun, H. et al. Correlation between the linguistic affinity and genetic diversity of Chinese ethnic groups. J Hum Genet 58, 686–693, doi: 10.1038/jhg.2013.79 (2013).
7. Wainscoat, J. S. et al. Evolutionary relationships of human populations from an analysis of nuclear DNA polymorphisms. Nature 319, 491–493, doi: 10.1038/319491a0 (1986).
8. Long, J. C., Chakravarti, A., Boehm, C. D., Antonarakis, S. & Kazazian, H. H. Phylogeny of human beta-globin haplotypes and its implications for recent human evolution. Am J Phys Anthropol 81, 113–130, doi: 10.1002/aja.1330810112 (1990).
Competing financial interests: The authors declare no competing financial interests.

Acknowledgements
This project was supported by the Applied Basic Research Programs of Yunnan Province, China (No. 2013FA023), and the National High Technology Research and Development Program of China (No. 2012AA021802). In addition, we would like to acknowledge all the ethnic individuals who participated in this study.

Author Contributions
Z.Y., S.M. designed experiment and wrote the manuscript. H.S., H.L., K.H., X.H., K.L. performed the experiments. H.S. and J.C. analyzed the data, helped design the experiments, and wrote the paper.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Sun, H. et al. β-globin gene cluster haplotypes in ethnic minority populations of southwest China. Sci. Rep. 7, 42909; doi: 10.1038/srep42909 (2017).

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017