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Genetic affinities of central China populations

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ABSTRACT. Hunan locates in the south-central part of China, to the south of the middle reaches of the Yangtze River and south of Lake Dongting. According to the historical records, the peopling of Hunan by modern human ancestors can ascend to 40 thousand years ago. Thus, to trace the ancient maternal components can offer further insight into the origin of south-central China. In this study, we investigated the mitochondrial DNA of 114 individuals from Hunan Province (including 34 Han, 40 Tujia and 40 Miao). Hypervariable regions I and II of the mtDNA control region were sequenced, and the relative diagnostic variations in coding region according to the updated worldwide phylogeny tree were selected and typed by restriction fragment length polymorphism analysis or direct sequencing. All individuals were classified into specific (sub)haplogroups. By comparison with the surrounding populations, southern China-prevalent haplogroups were detected with relative higher frequency in the Tujia and Miao ethnic

populations, such as haplogroup B, with more than 20%, lacking in the Han population, which illustrated its southern origin characters. In addition, we also detected northern of East Asia prevalent haplogroups with a relative higher frequency in Tujia populations than in the Miao and Yao ethnic groups, implying a gene flow from Han populations. However, the language-clustering tendency was supported by our principal component analysis and further genetic estimation results. Han and ethnic groups in central China exhibited specific ancestors related to their closer language affinity, although there was extensively genetic admixture between Han and ethnic groups.

Key words: mtDNA; Origin; Ethnic group; Hunan

INTRODUCTION

Mitochondrial DNA (mtDNA) harbors a series of molecular properties, lack of recombination, mode of inheritance, rapid evolutionary rate, and high population-specific polymorphisms, which make mtDNA a useful genetic marker for studies on the population genetics and molecular anthropology (Bandelt et al., 2001; Forster et al., 2002; Pakendorf and Stoneking, 2005). Especially, the updated phylogenetic tree of China with the entire mtDNA genomes (Kivisild et al., 2002; Zhao et al., 2009; Kong et al., 2003b, 2006, 2011), the quality strategy (Bandelt et al., 2001; Parson and Bandelt, 2007), and the coding region variations typing strategy for verifying the haplogroup status (Yao et al., 2004), mtDNA has offered the fundament for tracing the prehistory origin and migration scenarios at maternal aspect. Thus, the more mtDNA datasets from the crucial geographical region, Hunan, south-central China, will offer more data for tracing the migration and expansion of the anatomically modern human to this area.

Hunan is suited in south-central China, with ethnic enclaves including the Miao, Tujia, Dong, Yao, and Han populations belonging to different language groups. According to historical records, Miao, Tujia, Dong, and Yao peoples are the aboriginal residents of Hunan Province, and Han as well as other minorities settled this region during historic periods (Du and Ye, 1994). Hunan is a Province with many ethnic minority groups. By now, 50 ethnic minority groups are present, with a population of 5.2 million, accounting for 8% of the total population of Hunan. Except for the recent immigration of Han populations, there is still some debate regarding the origin of the major ethnic groups of Hunan Province, central of China, such as Tujia, Miao, and Yao. The prevalent opinion is that the Tujia was mainly the descendants of the ancient Ba People, settling in western Hunan and Hubei Province, and there were subsequent gene exchanges with other ethnic groups. The Miao ethnic group came from the Jiu-Li tribe in the Yellow River basin of central China, as early as 5000 years ago, and later the San-Miao tribe, who pushed southward to the Yangtze River basin. Thus, further analysis of the aboriginal settlers in this region and the Han populations will be helpful to explore the origin of the minorities and to trace the demographic history of the ethnic populations in Hunan Province.

In this article, we studied the mtDNA sequence polymorphisms of 2 ethnic populations and a Han population by amplifying the hypervariable regions I and II of mtDNA. To

further illustrate the genetic affinity between the populations investigated in this study, we compared the maternal component of the 3 populations with the surrounding groups retrieved from literature. Except for the genetic admixture results of the populations with the northern and southern China prevalent maternal components, it was significant that in the Hunan populations the consistent affinity between the maternal genetic datasets and their languages was detected. Tujia populations have closer genetic affinity with the Han populations for belonging to the Sino-Tibet language family, and the Yao population shows the greatest genetic distance with Han and Tujia populations, in line with the Hmong-Mien language of the Yao ethnic group.

MATERIAL AND METHODS

Subjects and data collection

One hundred and fourteen healthy individuals from 3 populations were collected in Hunan China: Han (N = 34), Miao (N = 40), and Tujia (N = 40). The individuals in the study were selected to avoid known maternal relatives, and genetic investigation ascertained that each sample belonged to the locality for at least 3 generations. Informed consent was obtained from every participant.

Comparative mtDNA data from southeast Asia (Figure 1 and Table 1) and southern China were taken from previously published literature (Wen et al., 2004a,b, 2005).

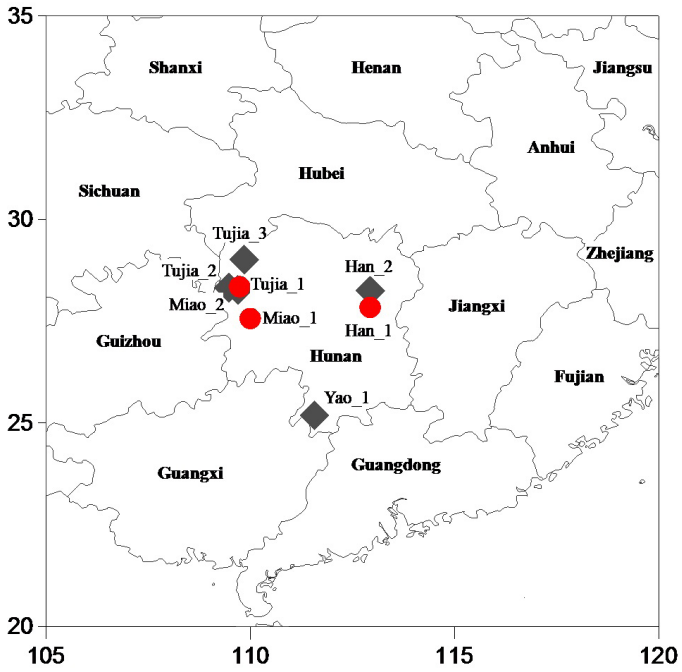

Figure 1. Location of the populations investigated in this study. The populations collected in this study are highlighted with solid circles, and the diamonds represent the populations retrieved from the literature.

Table 1. Populations studied.

	Nation	Abbreviation	Location	Country	Language	N	References
1	Han	Han_1	Xiangtan of Hunan	China	Sino-Tibetan	34	This study
2	Miao	Miao_1	Huaihua of Hunan	China	Hmong-Mien	40	This study
3	Tujia	Tujia_1	Xiangxi of Hunan	China	Tibeto-Burma	40	This study
4	Han	Han_2	Changsha of Hunan	China	Sino-Tibetan	16	Wen et al., 2004a
5	Tujia	Tujia_2	Western Hunan	China	Tibeto-Burma	66	Wen et al., 2004b
6	Tujia	Tujia_3	Yongshun of Hunan	China	Tibeto-Burma	31	Wen et al., 2004b
7	Miao	Miao_2	Jishou of Hunan	China	Hmong-Mien	103	Wen et al., 2005
8	Yao	Yao_1	Jianghua of Hunan	China	Hmong-Mien	24	Wen et al., 2005

Genomic DNA extraction, amplification, sequencing, and coding region information typing

Total DNA was isolated with the standard phenol/chloroform method and stored at 4°C. Hypervariable regions I and II were amplified with different primer pairs. Detailed information is provided in Table 2. PCR products were purified and sequenced by Shanghai Sangon Biological Engineering & Services Company. Both strands of the same mtDNA fragment were sequenced to reduce ambiguities in base calling. The strict strategy was followed as suggested (Parson and Bandelt, 2007). The sequences were edited and aligned by the DNASTAR software (DNASTAR) by comparison to the revised Cambridge reference sequence, and the variations were recorded (Andrews et al., 1999). The length of the polymorphisms of the A and C stretches from 16,180 to 16,189 (triggered by the 16,189 T to C substitution) was disregarded in the analysis (Yao et al., 2002; Kong et al., 2003a). All of the individuals were screened for the mtDNA 9-bp deletion in the COII/tRNA^{Lys} intergenic region. According to the HVSI and HVSII variations, all the mtDNA were roughly assigned into the specific haplogroup based on the updated East Asia the worldwide (mtDNA tree Build 15) phylogenetic trees (Kong et al., 2006; van Oven and Kayser, 2009). Subsequently, the specific coding variation(s) were selected and typed by RFLP and/or direct DNA sequencing. The detail information is shown in Table 2.

Table 2. Primers for amplification, sequencing, and RFLP analysis.

Primer pairs	Locations in rCRS	Annealing temperature (°C)	Polymorphisms at/in
L29/H408	8-29/429-408	54	HVSII
L15996/H16498	15975-15996/16517-16498	60	HVSI
L14575/H16048	14556-14575/16067-16048	53	15040, 15071, 15487T, 15784
L4210/ H5442	4189-4210/5461-5442	56	4491, 5178A
L4499/H5099	4480-4499/5118-5099	60	4831 *Hha*I (4833)
L9794/H10164	9774-9794/10181-10164	60	9824 *Hin*fI (9824)
L3179/H3674	3160-3179/3693-3674	59	3391 *Hae*III (3394)
L394/H902	375-394/922-902	60	663 *Hae*III (663)
L8215/H8297	8196-8215/8316-8297	57	9-bp deletion
L13209/H13458	13209-13229/13458-13439	59	13262 *Alu*I (13262)

rCRS = revised Cambridge reference sequence.

Data analysis

The mtDNAs of 5 Hunan populations (including 1 Han, 2 Tujia, 1 Miao, and 1 Yao group) were retrieved from the literature (Wen et al., 2004a,b, 2005), and the individuals were assigned to specific haplogroup by comparing the East Asian mtDNA data using a similar strategy (Yao et al., 2004). The haplogroup distribution frequencies for each of the 3 populations were calculated (Table 3). Principal component analysis was performed by taking the

Table 3. Haplogroup (HG) frequency of the populations investigated in this study.

HG	Xiangtan of Hunan Han_1 (N = 34)	Huaihua of Hunan Miao_1 (N = 40)	Xiangxi of Hunan Tujia_1 (N = 40)	Changsha of Hunan Han_2 (N = 16)	Western Huan Tujia_2 (N = 66)	Yongshun of Hunan Tujia_3 (N = 31)	Jizhou of Hunan Miao_2 (N = 103)	Jianghua of Hunan Yao_1 (N = 24)
A	0.147	0.075	0.175	0.063	0.136	0.000	0.087	0.042
B4	0.000	0.000	0.000	0.000	0.030	0.000	0.019	0.083
B4a	0.000	0.075	0.025	0.063	0.076	0.000	0.039	0.000
B4b1	0.000	0.050	0.025	0.063	0.030	0.000	0.029	0.000
B4c1	0.000	0.000	0.000	0.063	0.000	0.000	0.010	0.000
B4c2	0.000	0.000	0.000	0.000	0.000	0.032	0.010	0.000
B4g	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
B5a	0.000	0.150	0.000	0.000	0.030	0.065	0.058	0.292
B5b	0.000	0.025	0.025	0.000	0.045	0.000	0.010	0.000
B6	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000
C	0.029	0.075	0.050	0.000	0.106	0.032	0.087	0.000
C4	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
C5	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
CZ	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
D	0.118	0.050	0.150	0.000	0.076	0.129	0.039	0.000
D*	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000
D4	0.000	0.000	0.000	0.000	0.000	0.000	0.087	0.167
D4a	0.059	0.000	0.000	0.063	0.000	0.000	0.010	0.000
D4b	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000
D4b1	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D4i	0.000	0.025	0.000	0.000	0.000	0.000	0.010	0.000
D4o	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D5	0.000	0.000	0.025	0.000	0.015	0.065	0.029	0.042
D5a	0.000	0.000	0.000	0.000	0.061	0.000	0.019	0.000
F	0.029	0.000	0.000	0.000	0.000	0.000	0.029	0.000
F1	0.000	0.000	0.050	0.000	0.030	0.032	0.019	0.042
F1a	0.000	0.050	0.025	0.000	0.030	0.161	0.019	0.042
F1a1	0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.000
F1a1a	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000
F1c	0.000	0.000	0.025	0.000	0.015	0.065	0.000	0.000
F2	0.000	0.000	0.000	0.000	0.015	0.032	0.000	0.000
F2a	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
F2a2	0.000	0.000	0.000	0.000	0.000	0.032	0.000	0.000
F3a	0.029	0.000	0.000	0.063	0.030	0.000	0.010	0.042
F4a	0.000	0.000	0.000	0.000	0.000	0.097	0.000	0.000
G	0.000	0.050	0.000	0.000	0.000	0.000	0.010	0.000
G1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G1a1	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
G1a2	0.000	0.000	0.075	0.000	0.000	0.000	0.010	0.000
G2a	0.000	0.000	0.000	0.000	0.000	0.032	0.010	0.000
G2a1	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
G2a1	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G2b	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
G3	0.029	0.000	0.025	0.000	0.015	0.000	0.019	0.000

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Table 3. Continued.

HG	Xiangtan of Hunan Han_1 (N = 34)	Huaihua of Hunan Miao_1 (N = 40)	Xiangxi of Hunan Tujia_1 (N = 40)	Changsha of Hunan Han_2 (N = 16)	Western Huan Tujia_2 (N = 66)	Yongshun of Hunan Tujia_3 (N = 31)	Jizhou of Hunan Miao_2 (N = 103)	Jianghua of Hunan Yao_1 (N = 24)
G3a	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
M*	0.000	0.000	0.000	0.000	0.030	0.000	0.029	0.083
Uncertain	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042
M10	0.088	0.000	0.025	0.125	0.045	0.032	0.000	0.000
M11	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
M15	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.042
M33	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
M61	0.000	0.000	0.000	0.000	0.030	0.000	0.019	0.000
M7	0.000	0.100	0.025	0.000	0.000	0.000	0.010	0.000
M7b	0.059	0.025	0.050	0.063	0.015	0.000	0.039	0.000
M7b1	0.000	0.000	0.000	0.063	0.015	0.032	0.000	0.000
M7b1	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
M7b1'2	0.029	0.025	0.000	0.000	0.000	0.000	0.000	0.000
M7b3	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000
M7c	0.029	0.000	0.000	0.000	0.030	0.000	0.000	0.000
M8a	0.029	0.000	0.025	0.000	0.000	0.000	0.019	0.000
M8a2	0.029	0.025	0.000	0.000	0.000	0.000	0.000	0.000
M9	0.029	0.050	0.025	0.000	0.030	0.000	0.000	0.000
M9a	0.000	0.000	0.025	0.000	0.030	0.032	0.000	0.000
M9a1	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000
N*	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
N9a	0.000	0.000	0.000	0.188	0.000	0.000	0.019	0.083
M9b1	0.000	0.025	0.000	0.000	0.015	0.000	0.000	0.000
R*	0.000	0.000	0.000	0.000	0.015	0.032	0.000	0.000
R9b	0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.000
R9c	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
R11	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
R22	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Y1	0.029	0.000	0.000	0.000	0.000	0.000	0.010	0.000
Y2	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000
Z	0.029	0.025	0.050	0.000	0.000	0.000	0.010	0.000

haplogroup frequencies (Table 3) as input factors with the SPSS software (version 16). In addition, the genetic distance and haplotype diversity were evaluated with the Arlequin software package (version 3.1).

RESULTS AND DISCUSSION

With the updated East Asia and world mtDNA phylogeny tree, all the individuals were assigned to the specific East Asia and southeast Asia haplogroups (Table 2 and **Table S1**) according to the combined information from control-region and partial coding-region information. The northern East Asia prevalent haplogroup (54.39%, 62/114, including A, D, C, G, M8, Y, and Z), more than the southern Asia prevalent haplogroup (41.23%, 47/114, containing B, F, M7, M9, and R22), contained the major maternal components of the 3 investigated Hunan populations, which revealed the genetic admixture results of the Hunan populations. However, the 3 populations showed quite different maternal components. In detail, southern China prevalent haplogroups accounted for two thirds (~60%) of the maternal components of the Hunan Miao ethnic group, and the Hunan Han and Tujia populations accounted for the remaining third (~30%). The northern East Asia prevalent haplogroups were opposite, indicating that the Tujia populations had a higher frequency of northern-eastern Asia prevalent maternal components. The maternal components of the 3 investigated populations was in line with their language branch affinity; the Miao population in Hunan Province belonging to Hmong-Mien language had much higher maternal components popular in southern East Asia than the populations of Han, belonging to Tibeto-Burma language groups. In addition, our results supported that the Tujia of Tibeto-Burma languages group in the Hunan Province may have much closer affinity with the Han populations than the Miao and Yao ethnic groups with the Hmong-Mien language.

To obtain further insight into the affinity between the maternal components and their languages, we compared our datasets with the mtDNAs of the Han, Tujia, Miao, and Yao groups reported in the literature (Wen et al., 2004a,b, 2005). We found that the Yao ethnic groups showed a higher southern of East Asia contribution. This was similar with the Miao population in our dataset; however, the Miao ethnic in literature has a similar contribution from both northern and southern of East Asia maternal contributions. Rather, the 2 Tujia ethnic groups of Tibeto-Burma language branch had a relative higher southern East Asia maternal contribution by comparing with the Tujia population in this study (Figure 2). This implied that the Tujia population had a much more complex prehistory because of its mixture with surrounding populations. Figure 2 showed the principal component map for the first two principal components, which together accounted for 57.71%. In the principal component map, a quite clear clustering pattern between the populations and their language affinity was observed along the diagonal line. In detail, the populations of Hmong-Mien and Han language branch located at two different sides of the fitted line, with R at 0.063, and the Tujia populations of Tibeto-Burma family situated at middle between the populations of the Hmong-Mien and Han language families. This clustering pattern was significantly in line with their language affinity, although the populations of the same language family had great maternal genetic differences. The sharing southern of Eastern Asia components of haplogroups B, F, M7, and M9 of the Yao and Miao populations, as well as the A, C, D, G, and Z of the Han and Tujia populations, contributed to the separated clustering affinity.

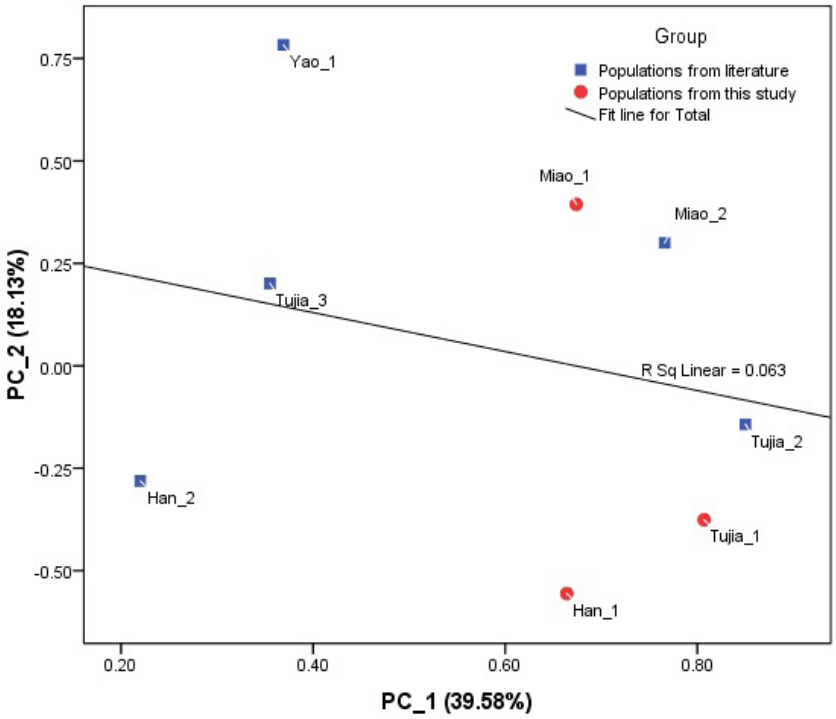

Figure 2. Principal component (PC) analysis of the populations under study.

To better understand the genetic affinities between the populations, the haplotype diversity and the F statistics were performed based on the haplotype frequencies at the significance level of 0.05. Except the lower haplotype diversity in the Yao population, we found similar haplotype diversities among the Han, Tujia, and Miao populations (Table 4). This result supported the plausible genetic admixture results with the immigration from the north of China and the aboriginal southern of China components, which was in line with the history record (Du and Ye, 1994). The relatively lower F_{ST} value indicated a closer genetic affinity between the Tujia and Han populations than between the Tunjia and Miao populations. However, the Yao ethnic group had the greatest F_{ST} value with its surrounding populations, matching well with the haplotype estimation results; however, this may be in line with the higher frequency of B and D4 haplogroups in Yao populations, which accounted more than half of the maternal components of the Yao population (Table 5).

Table 4. Haplotype diversity of the populations in this study.

Statistics	Han_1	Han_2	Miao_1	Miao_2	Tujia_1	Tujia_2	Tujia_3	Yao_1
Sample size	34	16	40	103	40	66	31	24
No. of alleles	33	14	34	82	31	56	28	14
Haplotype diversity	0.9982 ± 0.0077	0.9833 ± 0.0278	0.9923 ± 0.0073	0.9933 ± 0.0028	0.9808 ± 0.0125	0.9944 ± 0.0039	0.9935 ± 0.0100	0.9130 ± 0.0397

Significance level = 0.0500.

Table 5. F-statistic results from haplotype frequencies in the populations of this study.

	Han_1	Han_2	Miao_1	Miao_2	Tujia_1	Tujia_2	Tujia_3	Yao_1
Han_1	0	-	-	-	-	-	-	-
Han_2	0.0053	0	-	-	-	-	-	-
Miao_1	0.00034	0.00734	0	-	-	-	-	-
Miao_2	0.00257	0.00896	0.00281	0	-	-	-	-
Tujia_1	0.00543	0.01493	0.00413	0.00389	0	-	-	-
Tujia_2	-0.0003	0.00708	0.00248	-0.00005	0.0029	0	-	-
Tujia_3	0.00032	0.00538	0.00386	0.00093	0.00324	-0.00083	0	-
Yao_1	0.0365	0.04541	0.04027	0.03299	0.05209	0.04021	0.04481	0

Significance level = 0.0500.

In summary, our extensive analysis on the mtDNAs of the Han, Miao, Yao, and Tujia populations led to further insight into the maternal genetic structure of central China and a better understanding of the relationships between their maternal components and their languages. The formation and development of the central populations was a complex process, affecting each population by integrating genetic components from Han, Tibeto-Burma, and Hmong-Mien language families. Furthermore, the observed that genetic profiles were generally consistent with the language affinity for sharing population-specific maternal components.

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Supplementary material

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