

Genetic polymorphism analysis of 15 STR loci in Chinese Hui ethnic group residing in Qinghai province of China

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Abstract In the present study, we investigated the diversity distributions of allelic frequencies of 15 short tandem repeats (STRs) loci in a sample of Chinese Hui ethnic group in the Ningxia Hui Autonomous Region. The allelic frequencies of the 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) were obtained from 2975 unrelated healthy Hui individuals. The STR genotyping data of all the samples were generated by DNA extraction, multiple amplification, GeneScan and genotype analysis. The genetic distances among different populations were calculated by using Nei's method and a phylogenetic tree was constructed based on the allelic frequencies of the same 15 STR loci using the neighbor-

joining method. A total of 185 alleles were observed in the Hui population, with the corresponding allelic frequencies ranging from 0.0002 to 0.5322. Chi-Square tests showed that all STR loci were in Hardy–Weinberg equilibrium. The forensic statistical parameters of all the loci showed high values. The population data in this study were compared with the previously published population data from other ethnics or areas. The Hui population showed significant differences from the Minnan Han, Uigur, Ewenki, Yi, Tibetan, Maonan and Malay ethnic minority groups in some loci, and from the South Morocco population and the Moroccan population in all the loci. Our results are valuable for human individual identification and paternity testing in the Chinese Hui population and are expected to enrich the genetic information resources of Chinese populations.

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Introduction

The Hui ethnic group with a sizable population of over 9.81 million is the most widely distributed ethnic minority in China. The Hui ethnic group, typically distinguished by their practice of Islam, is one of the largest ethnic minorities in the country. Most of the Huis inhabit in the Ningxia Hui Autonomous Region, Gansu, Qinghai, Henan, Hebei, Shandong and Yunnan provinces and the Xinjiang Uygur Autonomous Region. The Huis speak Mandarin Chinese and write in Chinese characters although a number of Arabic and Persian words have remained in their daily interactions and religious activities. The Hui people living in the border areas of China often use the language of local ethnic minorities [1, 2].

The majority of the Hui people believe in Islamic religion, which has a deep influence on their daily life (<http://www.china.org.cn/english/null/139573.htm>). Usually a mosque is built and becomes the symbol of architecture in large Hui communities. The habitation of the Hui people is characterized by small scale concentration and wide distribution with mosques as the center of their social activities (<http://www.chinahighlights.com/travelguide/nationality/hui.htm>). Hui men usually wear Hui style hats, which are small black-colored and white-colored hats without brims. Most Hui men like wearing white hats while some do not wear hats and instead they wrap their heads with white towels or cloth. Women usually wear white round-edged hats and veils on their heads. Under the influence of Islamism, the Huis consume a large amount of beef and mutton in their diet and the meat of pig, dog, horse, donkey, mule and beasts of prey is forbidden [3, 4].

Qinghai province, where the samples for this study were collected, is located in the northeastern part of the Tibetan Plateau. The Yellow River originates in the middle of the province, while the Yangtze and Mekong have their sources in the southwestern part of the province. Qinghai is a multi-ethnic province inhabited by 44 ethnic groups, including the Han people (the China's majority) and the Tibetan, Hui, Tu, Salar, Bonan, Yugur, Dongxiang, and Mongol ethnic groups. Minority people account for 44% of the total population in the province (<http://www.china.org.cn/e-xibu/2JI/3JI/qinghai/qing-ban.htm>).

Short tandem repeats (STRs) are the most ideal genetic markers because of their numerous allelic genes, their power for human identification, and their stable heredity in the human genome [5, 6]. STR typing is also suitable for analyzing degraded or outmoded human DNA and minute amounts of human DNA [7, 8]. Recent advances in study on polymorphic STRs as genetic markers have led to their wide application to population genetics, establishment of forensic DNA database, human individual identification, paternity testing, genetic mapping, disease linkage analysis, archaeology and potential inference of the ethnic origin of an individual. Previously published population data have shown that the allelic distributions of STR loci differ from one population to another or between the members of the same population living in different geographic areas [9–12]. In the present study, we investigated the distributions of the allelic frequencies and forensic statistical parameters of 15 STR loci in the Chinese Hui ethnic group living in Qinghai province of China, established a forensic DNA database and compared the study population with other neighboring populations. In addition, a phylogenetic tree was constructed to illustrate the inter-population relationships based on allelic frequencies of 15 STR loci using neighbor-joining method.

Materials and methods

Sample collection and DNA extraction

After informed consent, bloodstain samples were obtained from 2,975 unrelated, healthy individuals from the Chinese Hui ethnic group living in Qinghai province of China. Donors were randomly chosen among the Hui individuals whose ancestors had lived in the region for at least three generations. Genomic DNA was extracted using the Chelex-100 protocol as described by Walsh et al. [13].

PCR amplification and DNA typing

PCR for 15 autosomal STRs was performed in a multiplex reaction using AmpF ℓ STR Identifier kit. 0.9 μ l (2 ng/ μ l) genomic DNA samples were amplified in a total reaction volume of 10 μ l consisting of 2.9 μ l deionized water, 4 μ l dNTP, 0.2 μ l AmpliTaqGold DNA polymerase and 2.0 μ l primer set. Thermal cycling was conducted under the following conditions: 95°C for 11 min; 28 cycles of 94°C for 60 s, 59°C for 60 s, 72°C for 60 s; a final extension of 60°C for 45 min. Detection and genotyping of all STR PCR products were carried out using ABI3130XL DNA Genetic Analyzer (Applied Biosystem, Foster City, CA, USA). Allele designations were determined by comparison of the sample PCR fragments with those of allelic ladders provided with the kit using Genemapper 3.2 (Applied Biosystem, Foster City, CA, USA). The alleles of all STR loci were named according to the number of repeat units present, as recommended by the DNA Commission of the International Society for Forensic Haemogenetics [14].

Quality control

All experimental steps were performed according to the laboratory internal control standards and kit controls.

Statistical and phylogenetic analysis

Allelic and genotypic frequencies were estimated by the direct counting method. The observed heterozygosity (HO), expected heterozygosity (HE), power of discrimination (DP), probability of paternity exclusion (EPP), polymorphism information content (PIC), probability values of exact tests of Hardy–Weinberg disequilibrium were calculated as previously reported [15, 16]. R \times C contingency test was employed for pairwise interpopulation comparisons [17]. Our present data were compared with previously published data for the same 15 STR loci in samples from the Chinese Yi ethnic minority group living in Yunnan province [9], from the Chinese Dongxiang [10], Salar [10], Tu [11] and Tibetan [12] ethnic minority groups

Table 1 Allelic frequencies and forensic statistical parameters regarding the 15 STR loci of the Chinese Hui ethnic group

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
6						0.1043		0.0002							
7			0.0032	0.0044		0.2692	0.0014	0.0006				0.0006		0.0164	
8	0.0037		0.1746	0.0008		0.0644	0.2392	0.0127				0.5322		0.0043	
8.3						0.0002									
9	0.0008		0.0788	0.0508		0.4809	0.1118	0.2523		0.0002		0.1290		0.0718	
9.2			0.0002							0.0002					
9.3						0.0613									
10	0.0985		0.1687	0.2319		0.0193	0.1410	0.1348				0.0220	0.0008	0.1686	
10.2															
11	0.0591		0.3011	0.2415		0.0004	0.2353	0.2703		0.0024		0.2863	0.0063	0.3516	
11.2										0.0010					
12	0.1398		0.2329	0.3850	0.0016		0.2016	0.2098		0.0396	0.0002	0.0287	0.0395	0.2517	
12.2										0.0090					
13	0.2422		0.0360	0.0752	0.0022		0.0539	0.1049		0.2652	0.0016	0.0012	0.1996	0.1240	
13.2										0.0508			0.0002		
14	0.1950		0.0043	0.0096	0.0417		0.0157	0.0131		0.2403	0.2013		0.2201	0.0110	
14.2										0.1148					
15	0.1740		0.0002	0.0008	0.3547		0.0002	0.0014	0.0008	0.0845	0.0355		0.1657	0.0006	
15.2										0.1369					
16	0.0703		0.3272		0.3272		0.0074	0.0208	0.0074	0.0208	0.2088		0.1178	0.0002	
16.2					0.0004					0.0290					
17	0.0147		0.1931		0.1931		0.0687	0.0020	0.0687	0.0020	0.2602		0.0768		0.0008
17.2										0.0024					
18	0.0018		0.0741		0.0741		0.1115	0.0006	0.1115	0.0006	0.1843		0.0428		0.0253
18.2										0.0004					
19	0.0002		0.0045		0.0045		0.1866	0.0004	0.1866	0.0004	0.0928		0.0501		0.0513
20					0.0006		0.1185	0.0006	0.1185	0.0006	0.0149		0.0344		0.0511
21							0.0280	0.0004	0.0280	0.0004	0.0004		0.0188		0.1046
21.2															0.0036
22							0.0453		0.0453				0.0163		0.1756
22.2															0.0065
23							0.2040		0.2040				0.0069		0.2257
23.2															0.0071
24							0.1450		0.1450				0.0026		0.1859
24.2															0.0075

Table 1 continued

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
25		0.0004							0.0649				0.0008		0.0927
25.2															0.0028
26		0.0004							0.0154						0.0416
26.2															0.0008
27		0.0046							0.0030				0.0002		0.0124
28		0.0493							0.0006						0.0041
28.2		0.0200													
29		0.2566													
29.2		0.0008							0.0002						0.0004
30		0.2667													
30.2		0.0210													
31		0.1013													
31.2		0.0795													
32		0.0275													
32.2		0.1231													0.0002
33		0.0034													
33.2		0.0406													
34		0.0008													
34.2		0.0038													
35		0.0002													
35.2		0.0002													
HO	0.8379	0.8176	0.7716	0.7421	0.7149	0.6617	0.8078	0.7816	0.8530	0.8199	0.8053	0.6119	0.8814	0.7571	0.8406
HE	0.8351	0.8256	0.7886	0.7313	0.7226	0.6771	0.8112	0.7898	0.8640	0.8273	0.8041	0.6168	0.8567	0.7637	0.8562
PIC	0.8143	0.8049	0.7569	0.6874	0.6744	0.6317	0.7842	0.7574	0.8492	0.8064	0.7751	0.5572	0.8410	0.7282	0.8404
DP	0.8351	0.8256	0.7886	0.7313	0.7226	0.6771	0.8113	0.7898	0.8640	0.8273	0.8041	0.6168	0.8567	0.7637	0.8562
EPP	0.6862	0.6765	0.6069	0.5219	0.5062	0.4646	0.6432	0.6068	0.7384	0.6776	0.6303	0.8010	0.7273	0.9842	0.7263
P	0.3061	0.4176	0.1538	0.0811	0.1353	0.0895	0.4058	0.1581	0.4432	0.1788	0.1581	0.3029	0.1034	0.3079	0.4487

HO observed heterozygosity; HE expected heterozygosity; DP power of discrimination; EPP probability of exclusion; PIC polymorphism information content; P probability values of exact tests for Hardy–Weinberg equilibrium

Table 2 Inter-population comparison results (*P* value) of the population data between the Chinese Hui ethnic group and the previously published ethnic groups

Comprised population	vWA	FGA	TH01	TPOX	CSF1PO	D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51	D19S433	D21S11
Hui vs. Dongxiang	0.537	0.872	0.967	0.893	0.996	0.986	0.698	0.998	0.989	0.702	0.970	0.970	0.674	0.783	0.739
Hui vs. Salar	0.629	0.896	0.878	0.757	0.684	0.998	0.563	0.940	0.891	0.837	0.993	0.802	0.835	0.802	0.912
Hui vs. Guangdong Han	0.736	0.752	0.642	0.628	0.927	0.941	0.645	0.727	0.719	0.743	0.957	0.883	0.486	0.535	0.824
Hui vs. Uigur	0.616	0.585	0.011	0.256	0.539	0.821	0.327	0.878	0.716	0.729	0.803	0.304	0.727	0.657	0.685
Hui vs. Ewenki	0.142	0.994	0.934	0.662	0.109	0.851	0.794	0.902	0.131	0.457	0.940	0.911	0.000	0.670	0.316
Hui vs. Tu	0.138	0.464	0.615	0.973	0.355	0.537	0.777	0.474	0.092	0.667	0.596	0.953	0.996	0.854	0.773
Hui vs. Maonan	0.000	0.000	0.048	0.011	0.000	0.221	0.099	0.171	0.012	0.000	0.000	0.000	0.000	0.133	0.393
Hui vs. Minnan Han	0.472	0.944	0.471	0.276	0.714	0.435	0.850	0.205	0.027	0.002	0.019	0.643	0.811	0.859	0.447
Hui vs. Malay	0.584	0.000	0.000	0.598	0.000	0.000	0.000	0.000	0.012	0.027	0.121	0.124	0.000	0.001	0.018
Hui vs. Morocco	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000
Hui vs. Moroccan	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Hui vs. Yi	0.000	0.000	0.020	0.673	0.541	0.000	0.021	0.194	0.513	0.392	0.442	0.575	0.587	0.001	0.104
Hui vs. Tibetan	0.858	0.001	0.001	0.208	0.912	0.002	0.108	0.005	0.952	0.084	0.013	0.072	0.000	0.503	0.094

living in Qinghai province of China, from the Chinese Ewenki ethnic group in the Inner Mongolia Autonomous Region [18], from the Han population in Guangdong province [19], from the Minnan Han population in Southeast China [20], from the Chinese Uigur ethnic group in Xinjiang province [21], from the Chinese Maonan ethnic group in Guangxi [22], from the South morocco population [23], from the Malay ethnic group [24], and from the Moroccan population [25]. Excel program and SPSS 13.0 for windows software was used for analysis of the data. A phylogenetic tree showing inter-population relationships was constructed using genetic distances with hierarchical cluster method by SPSS 13.0 software.

Results and discussion

Allelic frequencies and forensic statistical parameters regarding the 15 STR loci of the Chinese Hui population living in Qinghai province of China are shown Table 1. The comparison results (*P* value) of the population data between the Hui ethnic group and the previously published ethnic groups are shown in Table 2.

As shown in Table 1, a total of 185 alleles were observed in the Hui population, with the corresponding allelic frequencies ranging from 0.0002 to 0.5322. The combined probabilities of exclusion and power of discrimination for all 15 STR loci were 0.999999, and 0.9999999999, respectively. Chi-Square tests show that all STR loci were in the Hardy–Weinberg equilibrium ($P > 0.05$).

The Hui population data were compared with the previously published population data of other ethnic groups for the same 15 STR loci by the method of the $R \times C$ contingency test. No differences were found in the comparison results between our study population and the Chinese Dongxiang, Salar and Tu ethnic minority groups living in Qinghai Province [10, 11] and the Chinese Han population living in Gongdong province [19] at all 15 STR loci. We found that the study population showed significant difference from the Uigur ethnic population [21] at the TH01 locus ($P = 0.011$), the Ewenki group [18] at D18S51 ($P < 0.001$) locus, the Minnan Han population in Southeast China [20] at D7S820 ($P = 0.027$), D8S1179 ($P = 0.002$) and D13S317 ($P = 0.019$) loci, the Chinese Yi ethnic minority group [9] at vWA ($P < 0.001$), FGA ($P < 0.001$), TH01 ($P = 0.020$), D2S1338 ($P < 0.001$), D3S1358 ($P = 0.021$) and D19S433 ($P = 0.001$) loci, the Chinese Tibetan ethnic minority group [12] at FGA ($P = 0.001$), TH01 ($P = 0.001$), D2S1338 ($P = 0.002$), D5S818 ($P = 0.005$), D13S317 ($P = 0.013$) and D18S51 ($P < 0.001$) loci, the Maonan ethnic group [22] at CSF1PO ($P < 0.001$), TPOX ($P = 0.011$), D13S317 ($P < 0.001$), D16S539 ($P < 0.001$), D8S1179 ($P < 0.001$), vWA ($P < 0.001$), D18S51

Table 3 Genetic distances between the Hui population and the previously published ethnic groups

Populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0.000	0.246	0.119	0.148	0.298	0.119	0.185	0.050	0.622	0.394	0.535	0.176	0.827	0.324
2	0.246	0.000	0.280	0.317	0.496	0.214	0.399	0.188	0.358	0.572	0.542	0.356	0.544	0.615
3	0.119	0.280	0.000	0.119	0.334	0.118	0.229	0.073	0.636	0.427	0.550	0.179	0.890	0.414
4	0.148	0.317	0.119	0.000	0.292	0.173	0.251	0.081	0.736	0.460	0.599	0.186	0.965	0.387
5	0.298	0.496	0.334	0.292	0.000	0.323	0.377	0.253	0.894	0.537	0.525	0.255	1.070	0.421
6	0.119	0.214	0.118	0.173	0.323	0.000	0.259	0.079	0.464	0.436	0.506	0.221	0.665	0.432
7	0.185	0.399	0.229	0.251	0.377	0.259	0.000	0.150	0.732	0.472	0.506	0.229	0.913	0.335
8	0.050	0.188	0.073	0.081	0.253	0.079	0.150	0.000	0.543	0.356	0.494	0.115	0.746	0.329
9	0.622	0.358	0.636	0.736	0.894	0.464	0.732	0.543	0.000	0.933	0.732	0.748	0.183	0.852
10	0.394	0.572	0.427	0.460	0.537	0.436	0.472	0.356	0.933	0.000	0.624	0.472	1.128	0.697
11	0.535	0.542	0.550	0.599	0.525	0.506	0.506	0.494	0.732	0.624	0.000	0.614	0.964	0.649
12	0.176	0.356	0.179	0.186	0.255	0.221	0.229	0.115	0.748	0.472	0.614	0.000	0.954	0.360
13	0.827	0.544	0.890	0.965	1.070	0.665	0.913	0.746	0.183	1.128	0.964	0.954	0.000	1.044
14	0.324	0.615	0.414	0.387	0.421	0.432	0.335	0.329	0.852	0.697	0.649	0.360	1.044	0.000

Note: 1 Tu; 2 Uigur; 3 Dongxiang; 4 Ewenki; 5 Guangdong Han; 6 Salar; 7 Yi; 8 Hui; 9 South morocco; 10 Tibetan; 11 Malay; 12 Minnan Han; 13 Moroccan; 14 Maonan

($P < 0.001$) and FGA ($P < 0.001$) loci, the Malay ethnic group [24] at TH01 ($P < 0.001$), CSF1PO ($P < 0.001$), D3S1358 ($P < 0.001$), D2S1338 ($P < 0.001$), D7S820 ($P = 0.012$), D18S51 ($P < 0.001$), D5S818 ($P < 0.001$), D8S1179 ($P = 0.027$), D19S433 ($P = 0.001$), D21S11 ($P = 0.018$) and FGA ($P < 0.001$) loci, and the South morocco population [23] and the Moroccan population [25] at all loci.

The genetic distances between the Hui population and the previously published populations are shown in Table 3. Short genetic distances were found between the Hui population and the Tu ethnic minority (0.050), the Dongxiang ethnic minority (0.073), the Salar ethnic minority (0.079) and the Ewenki ethnic group (0.081), but further distances were found between the Hui population and the Minnan Han population (0.115), the Yi ethnic minority group (0.150), the Uigur ethnic group (0.188), the Han population in Guangdong (0.253), the Maonan ethnic group (0.329), the Tibetan ethnic minority group (0.356), the Malay ethnic group (0.535), the southern Morocco population (0.622) and the Moroccan population (0.827). The results show that the genetic distances between the Hui population and the other populations living in the same area (in Qinghai province of China) was the nearest, much smaller than its distances to the Chinese populations living in other provinces. More genetic diversity existed between the Hui population and the foreign populations in other countries. A neighbor-joining phylogenetic tree (Fig. 1) was constructed based on the allelic frequencies of the 15 STR loci in the Hui population and other 13 populations [9–12, 18–25]. Cluster analysis shows that two main clusters existed within the 14 populations: the first-level cluster consisted

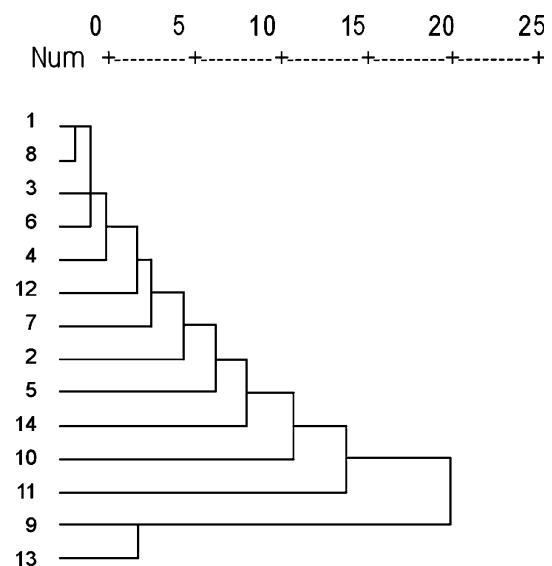


Fig. 1 A phylogenetic tree constructed by the neighbor-joining method based on the allelic frequencies of all 15 STR loci. The tree shows the relationships between the Hui population and other 13 populations (1 Tu; 2 Uigur; 3 Dongxiang; 4 Ewenki; 5 Guangdong Han; 6 Salar; 7 Yi; 8 Hui; 9 South morocco; 10 Tibetan; 11 Malay; 12 Minnan Han; 13 Moroccan; 14 Maonan)

of all the populations living in China, and the second-level cluster consisted of the foreign populations only (the Malay ethnic group, the southern Morocco population and Moroccan population). The Hui population clustered with the Tu, Dongxiang and Salar ethnic minority groups at the first level, followed in descending order by Ewenki, Minnan Han, Yi, Uigur, Guangdong Han, Maonan, and Tibetan. The results of the cluster analysis were consistent

Fig. 2 A map showing the locations of the Hui ethnic group and other populations in China (1 Tu; 2 Uigur; 3 Dongxiang; 4 Ewenki; 5 Guangdong Han; 6 Salar; 7 Yi; 8 Hui; 9 Tibetan; 10 Minnan Han; 11 Maonan)



with those of the genetic distance analysis. The best explanation for the first-level clustering and the short genetic distances of the Hui and other ethnic groups in Qinghai province may be that there have been strong gene flows among these populations since they lived in the same area. Living together for a long time may help promote the gene communion and amalgamation between the Hui population and the other populations in the same area. Locations of the Hui ethnic group and other populations in China are shown in Fig. 2.

In summary, our results are valuable for human identification and paternity testing of the Chinese Hui population in Qinghai province of China. The study is expected to enrich Chinese population genetic information resources and provide biological evidence for the origin of the Hui population.

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