A Genome-Based Study of the Muslim Hui Community and the Han Population of Liaoning Province, PR China

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Abstract To investigate the proposed historical origins of the Hui, a Chinese Muslim minority resident in Liaoning Province, PR China, DNA samples obtained from 53 individuals were analyzed at ten autosomal and six Y-chromosome microsatellite loci. As reference sources, equivalent samples were investigated from the coresident Han majority population. Both the Hui and the Han exhibited appreciable genetic heterogeneity in terms of the size, number, and size range of alleles, suggestive of population substructure resulting from their particular cultural and historical backgrounds. The contrast in the patterns of autosomal and Y-chromosome diversity of the two communities was obvious. Analysis of molecular variance showed that only 4.6% of total autosomal molecular variance was due to differences between the Hui and Han. The comparable value for Y-chromosome haplotype distributions of 14.0% indicated that the Hui and Han of Liaoning have separate paternal genetic histories.

The term *Hui* developed from the Mandarin word *Huihui* used by the Yuan Dynasty to describe Central Asians, Persians, and Arabs resident in China (Lipman 1997). Within the Peoples Republic of China (PR China), the Hui nationality encompasses all Muslims who do not have their own specific language or culture and are not registered as members of the other nine officially recognized Muslim minorities: the Bo'an, Dongxiang, Kazakh, Kirghiz, Salar, Tatar, Tajik, Uygur, and Uzbek. Chinese historical records indicate that the Hui community originated from the marriage of non-Chinese males with Han females (Du and Yip 1993; Gladney 1996, 1998; Leslie 1986; Lipman 1997; Wong and Dajani 1988). Today the community comprises some 8.6 million individuals resident in 19 provinces of PR China. A sizeable proportion of Hui male forebears are believed to have been Arab merchants from the Middle East and traders from Iran and Central Asia who traveled the Silk Road between Xi'an in China and Constantinople/Istanbul on the Bosphorous from approximately 120 B.C. to A.D. 1600 (Gladney 1996; Wong and Dajani 1988). Because of their occupation and life style, these persons were mainly unaccompanied males who settled in China, married Han women,

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and procreated, a practice that was specifically encouraged by a formal edict issued by the Emperor Hongwu in A.D. 1372 (Du and Yip 1993).

The different historical backgrounds of the Hui and Han populations thus offer the possibility of investigating the effects of male-mediated gene flow. For this reason, a comparison of autosomal and Y-chromosome microsatellite marker diversity was undertaken in coresident Hui and Han communities in Liaoning Province, PR China.

Materials and Methods

Subjects. All individuals sampled were from coresident Hui and Han communities in and adjacent to the city of Liaoyang, located in central Liaoning province, PR China. Historically the region formed the power base of the Manchu (Qing) Dynasty, which ruled China from 1662 to 1908 (Roberts 1999). Liaoning has a current population of approximately 40 million people, including 33 million Han and the Hui minority of 263,000 (Chinese Family Planning Commission 1997).

Blood samples were obtained from Hui communities with the initial permission of local religious leaders, the *Ahong*. *Ahong* who were interested in the proposed study arranged for finger-prick blood samples to be collected from 53 volunteer community members (26 male/27 female), either in their residences or in village centers. At the same time, 102 samples were collected from randomly selected volunteer individuals in the Han community, all of whom were male.

Markers. A set of 10-dinucleotide short tandem repeat (STR) markers on chromosomes 13 and 15 (*D13S126, D13S133, D13S192, D13S270, D15S11, D15S97, D15S98, D15S101, D15S108,* and *GABRB3*) were selected from the Stanford Human Genetic Diversity Panel on the basis of their reported high levels of heterozygosity (Bowcock et al. 1994). For the Y-chromosome analysis, six tri- and tetranucleotide STR markers (*DYS19, DYS388, DYS389I, DYS390, DYS392, DYS393*) were chosen from a panel of markers recommended by the Forensic Laboratory for DNA Research in the Department of Human Genetics, Leiden University, (http://ruly70.medfac.leidenuniv.nl/~fldo).

Genotyping. The primer sequences and polymerase chain reaction (PCR) conditions used were as described in the Genome Data Base (GDB) or Centre d'Etudes du Polymorphisme Humain (CEPH) protocols for autosomal markers, with incorporation of the following modifications. PCR was performed in 5 μL volumes containing 10–20 ng of genomic DNA, 50 m*M* KCl, 10 m*M* Tris-HCl (pH 8.30), 1.5m*M* $MgCl_2$, 0.2m*M* of each dNTP (Biotech), and 0.32μ*M* of each primer, with the forward primer marked by fluorescent label (FAM, TET, or HEX), and 0.05 U of Amplitaq polymerase (Perkin Elmer). Y-chromosome STR amplification was conducted according to Seielstad et al. (1994). Separation of

the subsequent PCR products was undertaken on 6% denaturing polyacrylamide gels (ABI Prism 373A automatic sequencer, Perkin Elmer). The Genotyper program was used to detect and analyze PCR products by reference to an internal size standard Genescan-500 TAMRA (Perkin Elmer).

Statistical Analysis. Basic statistical computations including allele frequency, observed heterozygosity, gene diversity, and Hardy-Weinberg equilibrium (HWE) tests were performed using the GENEPOP program (Rousset 1995). An exact probability test was used to assess the significance of deviation from HWE (Guo and Thompson 1992). If deviation from HWE was confirmed, a U test was used to further indicate whether it was due to heterozygote deficiency (Raymond and Rousset 1995). The correlation of genes within individuals within populations (F_{IS}) was calculated for each population (Weir and Cockerham 1984). Differences between the Hui and Han allele distributions were assessed by performing Wilcoxon signed ranks tests, a nonparametric equivalent of the paired t test. The degree of population differentiation was ascertained by analysis of molecular variance (AMOVA), based on the squared difference in the number of repeats. The analysis was completed by using the ARLEQUIN software program (Excoffier et al. 1992). ARLEQUIN was also used to calculate the average variance of the number of repeats per locus averaged over each population. The difference in the effective population size of approximately 1:4 between Y-chromosome and autosome markers was taken into account in the relevant calculation (Pérez-Lezuan et al. 1997).

Genetic distances were calculated with the MICROSAT software package, a program specifically designed for the processing of microsatellite data (Minch 1998). Three distances were chosen, the standard distance D_S (Nei 1987), $(\delta\mu)^2$ or D_{DM} (Goldstein et al. 1995), and R_{ST} (Slatkin 1995). From each of these distances, unrooted neighbor-joining trees (Saitou and Nei 1987) were generated by the PHYLIP software package, version 3.5c (Felsenstein 1989). The statistical robustness of the trees was based on a comparison of 1000 bootstrap iterations (Felsenstein 1985).

Results

As shown in Table 1, the allele distributions at each autosomal locus exhibited a high level of polymorphism, ranging from 6 alleles (locus *D13S126* in the Hui) to 16 alleles (locus *D13S133* in the Han). An average of 10.1 alleles per locus was recorded for the Hui and 11.3 for the Han (Table 1). The pattern of allele distribution varied slightly between the two populations, with an average repeat variance of 16.23 recorded for the Hui and 15.52 for the Han (Table 1). No significant difference was observed in repeat variance ($p = 0.456$) or number of alleles ($p = 0.062$) between the two communities. The Hui had a higher mean level

Table 1. Distribution of Autosomal Microsatellite Alleles in the Hui and Han Populations of Liaoning Province

		Allele Distribution Data						
Marker	*Population*	*Allele range (bp)*	*No. of alleles*	*MFA*[a]	*RV*[b]	*Ho*[c]	*He*[d]	F_{IS}[e]
D13S126	Hui	100–110	6	102	3.50	0.396	0.735	0.470
	Han	102–106	7	106	5.81	0.667	0.702	0.057
D13S133	Hui	126–183	12	132	20.26	0.490	0.697	0.305
	Han	128–189	16	132	31.12	0.621	0.838	0.265
D13S192	Hui	93–119	14	103	17.50	0.520	0.883	0.419
	Han	87–121	14	97	21.14	0.790	0.823	0.045
D13S270	Hui	77–97	8	79	4.67	0.509	0.820	0.221
	Han	75–97	10	81	6.00	0.443	0.535	0.177
D15S11	Hui	240–272	10	244	30.49	0.396	0.478	0.181
	Han	242–266	13	244	15.17	0.618	0.623	0.013
D15S97	Hui	172–188	8	180	13.07	0.373	0.829	0.557
	Han	172–196	10	182	7.12	0.652	0.801	0.191
D15S98	Hui	131–175	14	153	36.68	0.528	0.808	0.354
	Han	145–171	9	157	15.23	0.817	0.779	–0.041
D15S101	Hui	95–123	10	105	11.57	0.415	0.845	0.516
	Han	99–117	12	109	14.08	0.781	0.811	0.044
D15S108	Hui	139–165	10	145	12.09	0.490	0.652	0.257
	Han	131–161	12	145	29.24	0.556	0.536	–0.032
GABRB3	Hui	181–201	9	183	12.50	0.320	0.737	0.573
	Han	181–201	10	185	10.27	0.430	0.663	0.357
Mean	Hui	—	10.1	—	16.23	0.457	0.746	0.385
	Han	—	11.3	—	15.52	0.637	0.711	0.108

a. MFA, most frequent allele.
b. RV, repeat variance (variance of allele repeat size).
c. H_o, observed heterozygosity.
d. H_e, expected heterozygosity.
e. F_{IS}, correlation of genes within individuals within populations (Weir and Cockerham 1984).

of expected heterozygosity (0.746) than the Han (0.711), but again the difference was not statistically significant ($p = 0.155$). In contrast, observed heterozygosity was significantly lower ($p = 0.03$) in the Hui (0.45) than the Han (0.657).

Calculation of the correlation of genes within individuals within populations, F_{IS} (Weir and Cockerham 1984) also showed a deficiency of heterozygosity in both communities (Table1). For the Hui, F_{IS} estimates varied from 0.181 to 0.557, with a mean of 0.385. In contrast, F_{IS} values calculated for the Han ranged from –0.041 to 0.357, with a mean value of 0.108.

Exact probability tests were next performed and the results indicated that, averaged across all loci, both the Hui and Han populations deviated significantly from HWE (Tables 2A and 2B). Further investigation of noncompliance with HWE by calculation of *U* tests indicated a deficiency of heterozygosity at 9 of the

Table 2A. Evaluation of Hardy-Weinberg Equilibrium in the Hui

	Exact Probability Test		*U Test*	
Locus	*p Value*	*S.E.*	*p Value*	*S.E.*
D13S126	<0.0001	<0.0001	<0.0001	<0.0001
D13S133	0.0013	0.0013	<0.0001	<0.0001
D13S192	<0.0001	<0.0001	<0.0001	<0.0001
D13S270	<0.0001	<0.0001	<0.0001	<0.0001
D15S11	0.0733	0.0223	0.0889	0.0269
D15S97	<0.0001	<0.0001	<0.0001	<0.0001
D15S98	<0.0001	<0.0001	<0.0001	<0.0001
D15S101	<0.0001	<0.0001	<0.0001	<0.0001
D15S108	<0.0001	<0.0001	<0.0001	<0.0001
GABRB3	<0.0001	<0.0001	<0.0001	<0.0001

Table 2B. Evaluation of Hardy-Weinberg Equilibrium in the Han

	Exact Probability Test		*U Test*	
Locus	*p Value*	*S.E.*	*p Value*	*S.E.*
D13S126	0.4881	0.0252	0.0445	0.0120
D13S133	<0.0001	<0.0001	<0.0001	<0.0001
D13S192	0.0302	0.0165	0.3295	0.0466
D13S270	0.0033	0.0029	0.0100	0.0047
D15S11	0.4847	0.0557	0.1925	0.0469
D15S97	0.0008	0.0008	0.0022	0.0014
D15S98	0.1860	0.0220	0.2948	0.0341
D15S101	0.0667	0.0277	0.3067	0.0316
D15S108	0.1707	0.0351	0.4742	0.0452
GABRB3	<0.0001	<0.0001	0.0032	0.0027

10 loci surveyed in the Hui, *D15S11* being the exception (Tables 2A and 2B). The *U* test results were less pronounced in the Han, with 5 of 10 loci exhibiting significant heterozygote deficiency.

Y-chromosome allele distributions indicated that the Hui had both a lower average number of alleles per locus than the Han (4.3 versus 5.2) and a lower mean allele size variance (2.3 versus 3.1). However, neither difference attained statistical significance. By comparison, the variance of allele size difference between the two communities was significant ($p = 0.03$). The mean gene diversity values for Y-chromosome markers in the Hui and Han were 0.659 and 0.644, respectively (Table 3). This difference was not statistically significant, but it was noticeable that the values calculated for both communities were much higher than

Table 3. Summary of Allele Distributions of Y-Chromosome Markers in the Hui and Han

		Allele Distribution Data				
Marker	*Population*	*Allele Range (bp)*	*No. of Alleles*	*MFA*[a]	*RV*[2]	*Gene Diversity*
DYS19	Hui	190–202	4	194	1.7	0.676
	Han	186–202	5	194	3.5	0.663
DYS388	Hui	129–144	6	129	3.5	0.649
	Han	126–144	6	129	4.7	0.649
DYS3891	Hui	249–257	3	253	1.0	0.612
	Han	245–261	5	249	2.5	0.590
DYS390	Hui	203–223	6	215	3.5	0.716
	Han	203–223	6	215	3.5	0.768
DYS392	Hui	251–260	3	251	2.3	0.547
	Han	251–269	5	257	2.5	0.694
DYS393	Hui	120–132	4	124	1.7	0.665
	Han	120–132	4	120	1.7	0.592
Mean	Hui	—	4.3	—	2.3	0.644
	Han	—	5.2	—	3.1	0.659

a. MFA, most frequent allele.
b. RV, repeat variance (variance of allele repeat size).

those reported for European populations (Péréz Lezuan et al. 1997; Roewer et al. 1996). This could be the result of the Han and Hui having ancestries that are more diverse, with greater historical admixture than European populations. It also has been claimed that the peoples of Eastern Asia originated from modern African populations who migrated directly to the region approximately 60,000 years ago (Su et al. 1999), which would have allowed greater time for the development of genetic diversity than in Europe.

Y-chromosome diversity was further studied via the construction of haplotypes (*DYS19-DYS388-DYS389I-DYS390-DYS392-DYS393*). In a number of cases ($n = 32$), specific markers could not be amplified from all samples because of partial sample degradation. As a result, 18 complete haplotypes were defined from the 26 male Hui samples, and 76 complete haplotypes were resolved from the 102 Han samples.

No haplotypes were shared between the two populations. Only one haplotype was shared by two individuals within the Hui sample (haplotypic diversity of 0.944), and no haplotypes were shared between any individuals in the Han sample (haplotypic diversity of 1.00). The mean number of pairwise differences between haplotypes within both populations were 3.98 repeats in the Hui and 3.72 repeats in the Han, indicating the presence of diverse male ancestries in both populations. AMOVA of the Y-chromosome haplotypes demonstrated a significant

between-population molecular variance of $\Phi_{ST} = 0.1399$ ($p < 0.001$). This variance compares with the Φ_{ST} value of 0.0463 calculated for autosomal allele distributions.

To explore the possible genetic ancestries of the Hui and Han populations, genetic distances between the Hui and Han and other geographically adjacent populations (Chinese, Mongolian, Kazakh, Kirghiz, Uygur) were calculated, incorporating the results of previously reported Y-chromosome analyses (de Knijff et al. 1997; Péréz-Lezuan et al. 1999). The three consensus trees generated were very similar (Figures 1**A–C**). In all three trees the Hui and Han clustered together and were separated from the other populations. The first two trees, D_s and D_{DM}, had the same physical structure, with the D_{DM} tree being more statistically robust. The third tree, based on R_{ST}, more clearly separated the populations into three distinct clusters, European, Central Asian, and East Asian.

Discussion

China is usually portrayed as a culturally homogenous, monoethnic state. In fact, PR China is a multicultural and ethnically diverse nation with great cultural, geographic, and linguistic diversity (Gladney 1998). To fully understand the patterns of genetic diversity of the Hui and Han in Liaoning, it is appropriate to consider briefly the nature of the two populations. Following the establishment of the Peoples Republic in 1949, anthropologists and demographers were recruited to survey the entire population. As a result, 56 separate ethnic groups or nationalities (*minzu*) were recognized. Currently the majority Han *minzu* forms 92% of the population, or approximately 1100 million individuals (Chinese Family Planning Commission 1997). The intention of the State in categorizing and recognizing the different *minzu* was to create some semblance of order out of the complex ethnography of PR China, and thus to encourage political stability (Gladney 1996, 1998). To this end, autonomous regions, prefectures, and counties were created, where the various officially recognized ethnic groups were in the majority. In practice, these communities enjoyed little power until the reforms of 1978, when the minority nationalities gained greater political influence (Gladney 1996, 1998).

The results of the present study suggest that the Han of Liaoning may be more of a political construct than a homogenous population. Conflicting results were obtained where, for example, the Han exhibited a wide range of autosomal and Y-chromosome alleles, and yet also showed heterozygote deficiency with deviation from HWE. On these grounds, it appears that there is some form of population substructure within the Han of Liaoning, possibly arising from earlier admixture with the Manchu.

There also was a deficiency of heterozygosity in the Hui, which probably reflects their complex ethnography. As previously indicated, the modern Hui *minzu* comprises Chinese Muslims who do not have a language of their own but

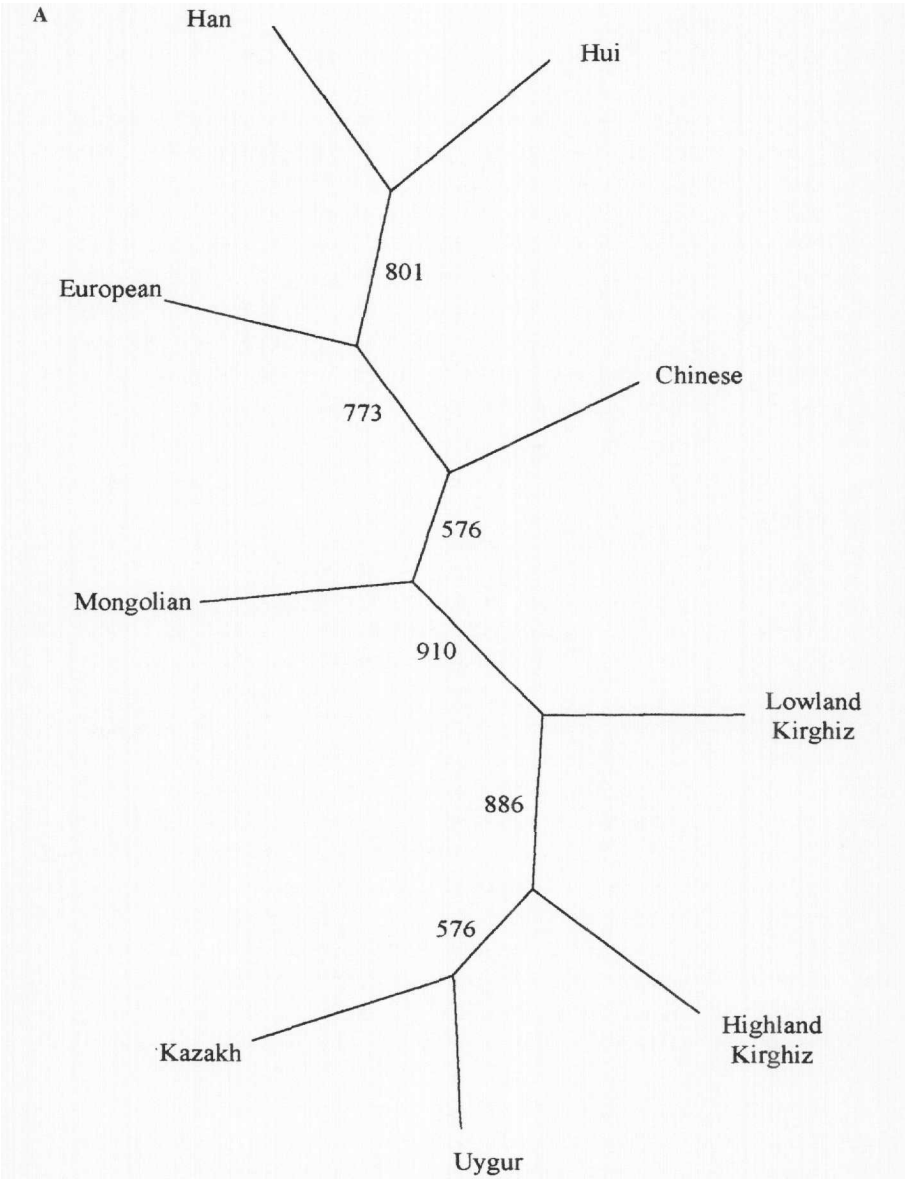

Figure 1. Unrooted neighbor-joining trees showing Y-chromosome phylogenetic affinities between nine populations using three different genetic distances. The numbers at each node indicate the number of trees out of 1000 bootstrap replicates with such a node. **A.** Nei's distance (D_S). **B.** Delta mu squared (D_{DM}). **C.** Slatkin's distance (R_{ST}).

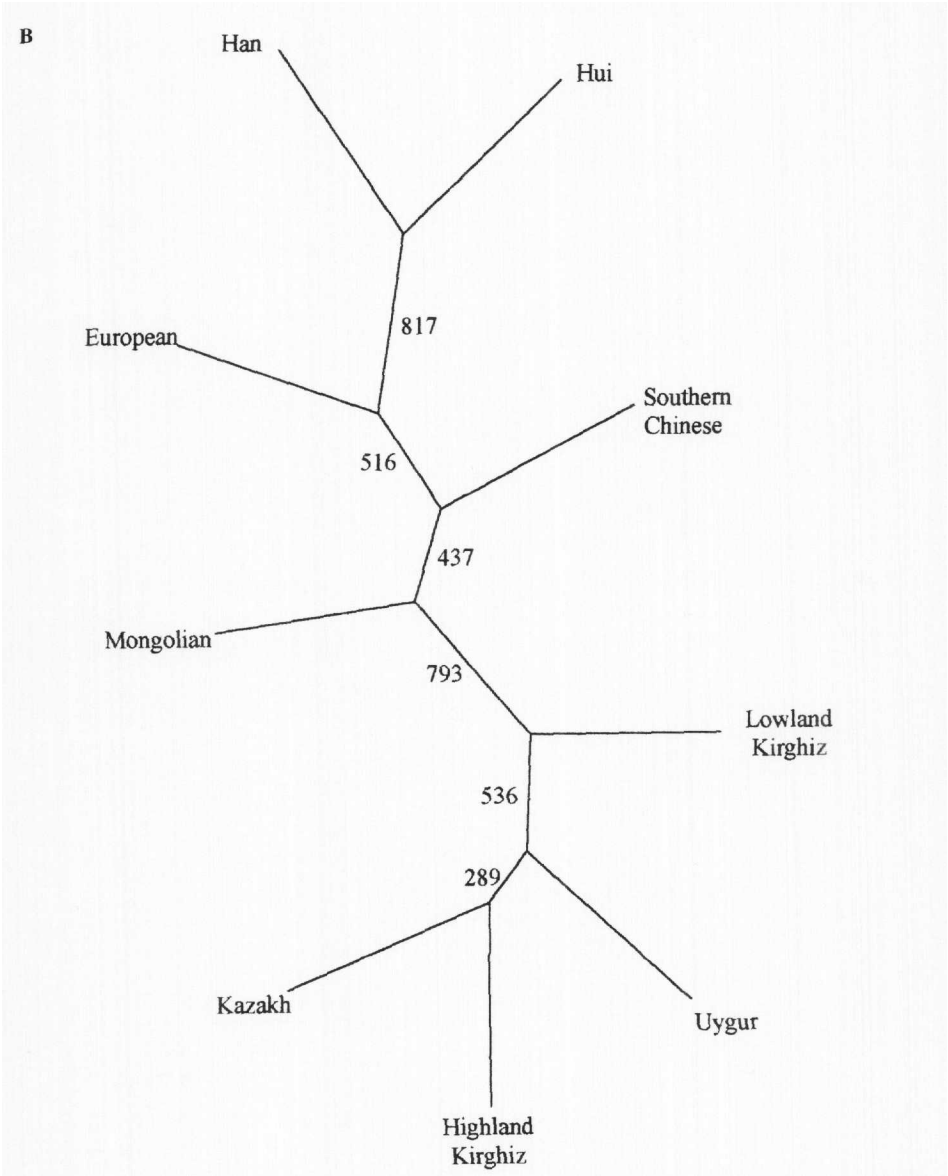

Figure 1B.

who have adopted the dialects spoken in the region where they live (Gladney 1996, 1998). This results in an ethnic group that is culturally diverse and spread virtually nationwide, linked only in that they could not be categorized into any other specific Muslim *minzu*. It is therefore probable that the modern Hui *minzu* is composed of numerous subpopulations with diverse origins.

C

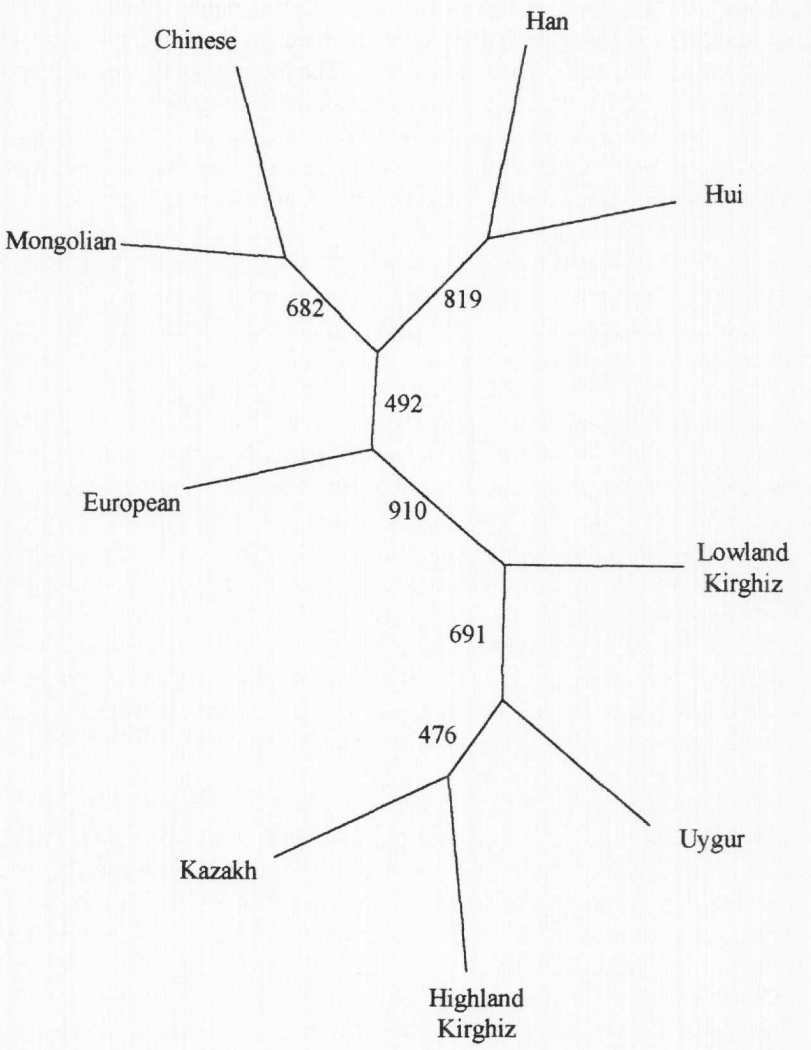

Figure 1C.

However, the long-term practice of consanguineous marriage in the Hui may also be a contributory factor in their low level of observed heterozygosity. Evidence of consanguineous marriages in Hui communities was reported in Gansu, Guandong, and Guizhou provinces, with mean inbreeding coefficients of $\alpha = 0.0012$ to 0.0065 (Wu 1987). A preliminary investigation on two extended Liaoning Hui pedigrees (data not shown) indicated similar high levels of consanguinity. Thus, both population substructure and preferential inbreeding may contribute

to the low levels of heterozygosity observed. Demographic studies of consanguinity in different Han populations have recorded α values of 0.0003 to 0.0045 (Du et al. 1981; Wu 1987; Zhan et al. 1992). The present study provided no evidence of significant levels of consanguinity in the Liaoning Han (Table 1).

The most striking aspect of the study was the apparent contrast in autosomal and Y-chromosome diversities between the Hui and Han, highlighted by AMOVA analysis. According to available historical evidence, Hui male ancestry is predominantly Western and Central Asian in origin (Gladney 1996; Lipman 1997). The AMOVA results suggest a firm genetic basis for the separate historical origins of the Hui and Han paternal ancestries. However, in attempting to further define the possible male ancestries of the Hui, a conflict between historical and genetic evidence was apparent. As shown in Figures 1**A–C**, Hui males clustered more closely with Eastern Asian groups than with the Central Asian group, casting in doubt the assumed Central Asian origins of the Hui. Therefore, either a significant proportion of the male Hui in Liaoning are of Eastern Asian descent, or the clustering of populations mainly reflects the complexity of their genetic structure rather than any implied shared genetic ancestry.

The latter explanation seems to be more persuasive. For example, reported mean gene diversity for the combined Central Asian populations analyzed by Péréz-Lezuan et al. (1999) was 0.432, and for the European population 0.451, much smaller than the gene diversities of 0.672 and 0.656 calculated for the Hui and Han. As Nei's distance, D_S, is based on differences in gene diversity, the tree constructed from this distance would primarily represent the clustering of populations with similar levels of genetic diversity and not necessarily reflect their direct genetic ancestry.

The distances D_{DM} and R_{ST} are based on allele size difference and, in theory, would more accurately define coalescent ancestry (Goldstein et al. 1995). In order to define ancestry, it should be possible to assume that one population was indeed the origin of the founders of a second population. The populations sampled in the present study appear to be too diverse to permit any such assumption, especially since both have Y-chromosome pools indicating the possible existence of multiple ancestries.

To better resolve the question of genetic ancestry, it may therefore be prudent to further subdivide the Hui and Han on demographic and anthropological grounds. Alternatively, future studies could investigate a possible association of lineages by means of deeper and population-independent genealogies, rather than by focusing on a population-based approach (Bosch et al. 1999). Hence the question could most likely be resolved by expanding the current Y-chromosome haplotype analysis to include biallelic markers.

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