DOI: 10.1002/ajpa.23823

# BRIEF COMMUNICATION



# The massive assimilation of indigenous East Asian populations in the origin of Muslim Hui people inferred from paternal Y chromosome

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### Funding information

National Natural Science Foundation of China, Grant/Award Numbers: 31071098, 31671297, 91731303, 31801040; Nanqiang Outstanding Young Talents Program of Xiamen University, Grant/Award Number: X2123302; the Fundamental Research Funds for the Central Universities, Grant/Award Number: ZK1144

## Abstract

**Objectives:** The Hui people are the adherents of Muslim faith and distributing throughout China. There are two contrasting hypotheses about the origin and diversification of the Hui people, namely, the demic diffusion involving the mass movement of people or simple cultural diffusion.

**Materials and methods:** We collected 621 unrelated male individuals from 23 Hui populations all over China. We comprehensively genotyped more than 100 informative Y-chromosomal single nucleotide polymorphisms and 17 Y-chromosomal short tandem repeats (STRs) on those samples. **Results:** Co-analyzed with published worldwide populations, our results suggest the origin of Hui people has involved massive assimilation of indigenous East Asians with about 70% in total of the paternal ancestry could be traced back to East Asia and the left 30% to various regions in West Eurasia.

**Discussion:** The genetic structure of the extant Hui populations was primarily shaped by the indigenous East Asian populations as they contribute the majority part of the paternal lineages of Hui people. The West Eurasian admixture was probably a sex-biased male-driven process since we have not found such a high proportion of West Eurasian gene flow on autosomal STRs and maternal mtDNA.

### KEYWORDS

gene flow, Hui people, population admixture, Y chromosome

1

## 1 | INTRODUCTION

The Hui people are one of 56 ethnic groups recognized by China (Q. Zheng, 2013) composing predominantly of adherents of the Muslim faith and distributing throughout China. The origin and diversification of Hui groups via demic diffusion involving the mass movement of people or simple cultural diffusion is a long-going debate. According to historical documents, Islam was first introduced in China during the Tang dynasty (~1,400 years ago). Since then a large number of soldiers, merchants, and political emissaries migrated from Central Asia, Arabia and Persia to China, probably mainly through the Silk Road (Gladney, 1998). The Chinese Muslim Hui people are believed to be the decedents of those immigrants. Nowadays the majority of Hui people speak Han Chinese languages, but their culture has distinct differences with Han due to their practice of Islam. For example, they follow Islamic dietary laws and reject the consumption of pork, and they wear white caps or headscarves.

The genetic evidence, however, suggest the origin of Hui people likely involving massive assimilation of indigenous East Asian populations. The paternal Y-chromosomal short tandem repeat (STR) clustering suggests there is an affinity between the Hui people of Liaoning in northeastern China and Ningxia in northwestern China with Sino-Tibetan speaking East Asian populations (Zhang, Li, Huang, Lu, & Hu, 2010). The study of autosomal STRs of Hui in Gansu province in northwest China also shows no evidence of substantial gene flow from the Middle East or Europe into the Hui people during their Islamization (H. B. Yao et al., 2016). The maternal mtDNA profile shows only about 6.7% of the lineages in Hui people of Xinjiang in northwest China having West Eurasian origin (Y. G. Yao, Kong, Wang, Zhu, & Zhang, 2004). Not only in northern China, previous studies also reveal the Hui people in southern China show a similar affinity with indigenous East Asians, for example, the Utsat Hui people in Hainan Island are thought to be descendants of the Champa Kingdom, but they are genetically much closer to the Hainan indigenous Hlai groups than to the Cham and other mainland Southeast Asian populations (Li et al., 2013). The evidence from physical measurements also indicate Hui people harbor lots of East Asian ancestry, for example, Hui people in Gansu and Ningxia have the common physical features of East Asians (L. B. Zheng, 1997).

The previous genetic studies with limited genetic markers and insufficient sampling have not been able to give a clear answer to the East-West admixture in the Muslim Hui people. Therefore, we analyzed 100 informative Y-chromosomal single nucleotide polymorphisms (SNPs) and 17 Y-chromosomal STRs in 621 unrelated individuals from 23 Hui populations all over China. Our results suggest the East Asian specific paternal lineages contribute the majority part of the gene pool of Hui people. The Hui people also have the signal of West Eurasian related admixture with diverse lineages associated with various regions of West Eurasia.

# 2 | MATERIALS AND METHODS

We collected blood or saliva samples of 621 unrelated individuals from 23 Muslim Hui populations all over China. The samples included

in this study were filtered for individuals for at least three generations belonging to Hui. Our study was approved by the Ethic Committee of School of Life Sciences, Fudan University. All individuals were adequately informed and signed their informed content before their participation. The sampling locations and sample sizes are shown in Figure 1 and Supporting Information Table S1. Genomic DNA was extracted using DP-318 Kit (Tiangen<sup>®</sup> Biotechnology, Beijing).

The samples were typed through seven panels of 100 SNPs as listed in the latest Y-chromosome phylogenetic tree as reported before (Wang et al., 2014) with additional SNPs in Supporting Information Table S2. Those binary markers were hierarchically genotyped by SNaPshot (ABI SNaPshot<sup>®</sup> Multiplex Kit) and fluorescent allele-specific PCR. PCR products were electrophoresed on a 3730xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Seventeen Y-chromosomal STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a, DYS385b, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635, and YGATAH4) were amplified using the AmpFISTR® YfilerTM PCR Amplification kit (Applied Biosystems, Carlsbad, CA). Amplified products were separated and detected using the ABI 3730xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA) according to the manufacturer's recommended protocol. The data were analyzed using GeneMapper ID v3.2 (Applied Biosystems, Carlsbad, CA). For use in the analyses, DYS389II was calculated by subtracting the DYS389I allele size.

The principal component analysis (PCA) was performed using SPSS 18.0 software (SPSS, Chicago, IL) using Y-chromosome haplogroup frequency data. We followed the ISOGG SNP tree to phylogenetically merge different haplogroup frequency datasets since different studies used a different set of markers to genotype Y-chromosome SNPs. The references for Y-chromosome frequency datasets used here can be found in Supporting Information Table S3. Molecular diversity, population structure estimates and Y-STR genetic distances between populations were calculated using Arlequin (v. 3.11; Excoffier, Laval, & Schneider, 2005) using Y-STR data. We prepared Supplementary Document 1 listing all the reference papers we obtained data used in the Y-STR analysis. Neighbor-joining unrooted trees based on R<sub>ST</sub> statistics calculated from Y-STR data were performed using MEGA (v. 5.1; Saitou & Nei, 1987; Tamura et al., 2011) to visualize relationships among Hui and worldwide populations. We also extracted Y-chromosomal STR data from Hui people in Ningxia published in Guo et al. (2008) and labeled as Hui-NX2 in genetic distance analysis.

## 3 | RESULTS

According to the nomenclature of Y Chromosome Consortium (ISOGG Y-DNA Version History—2014, Version: 9.129, Date: November 30, 2014), We identified 54 SNP lineages from the 621 male samples including haplogroup C-M130, D-M174, N-M231, O-M175, E-M96, F-M89, G-M201, H-M69, I-M258, J-M304, L-M20, Q-M242, R-M306, and T-M272, which suggest the diverse origins of Hui people. The Y-chromosome haplogroup frequency distributions of the 23 Hui populations are presented in Figure 2 and Supporting Information Tables S1 and S2. The haplogroup O-M175, C-M130, D-



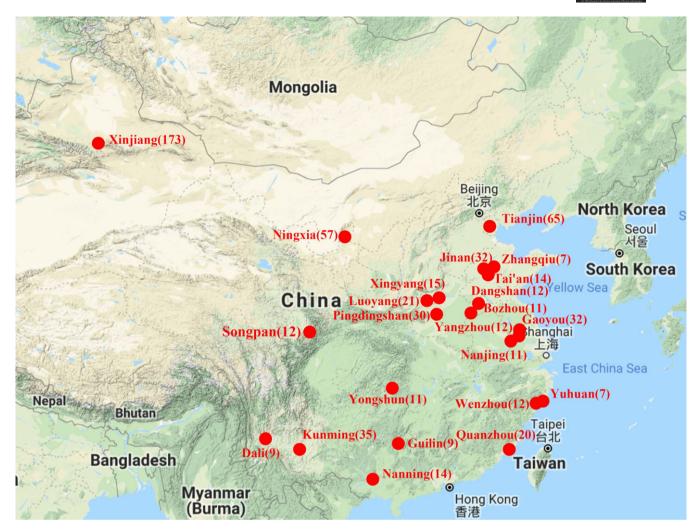


FIGURE 1 The sampling locations around China and the sample size collected from each region

M174, and N-M231 have a southern origin and show a south to north migration route in East Asia (Wang & Li, 2013). Those four haplogroups are regarded as specific lineages of East Asia and Southeast Asia as they comprise 92.87% of East Asian gene pool but are nearly absent in West Eurasia (except the high frequency of Haplogroup N in northern Europe; Zhong et al., 2010). Those four haplogroups are also prevalent in the studied Hui groups, contributing to 69.73% of the Hui people in total (when we use the words "in total" we mean we lumped all Hui samples into one group and then calculated frequencies). We note that if we sum the frequencies of those four haplogroups per population and then calculate the average frequency of East Eurasian lineages for all populations, it results in 74.25%. The proportion of East Eurasian lineages in Hui people ranges from about 50% in Tai'an to 100% in Guilin, Wenzhou and Yuhuan when we calculated frequencies in different Hui groups (Supporting Information Tables S1 and S2). Similar to the Han Chinese genetic profile (Wang et al., 2013; Yan et al., 2014; Yan, Wang, Li, Li, & Jin, 2011; X. Yao et al., 2017), the haplogroup C-M130, O1a1-P203, O3a1c-002611, O3a2c1\*-M134, and O3a2c1a-M117 also reach high frequencies in Hui population, comprising 8.21%, 5.64%, 12.2%, 11.3%, and 9.66% in total, respectively. The haplogroup O2b-M176, a lineage almost exclusively distributing in Japan and Korea (Wang & Li, 2013), comprises 3.54% of the Hui people in total. But we found O2b-M176 interestingly enriches in Gaoyou, Jiangsu, eastern coast China contributing to 37.5% of Gaoyou Hui group. We note that this O2b-M176 lineage is more frequent found in Hui (3.54% in total) than in Han Chinese of the same region as reported in Yan et al. (2011) since they have not detected this haplogroup in 167 eastern Han Chinese individuals and only found at an average frequency of 0.3% in all 361 sampled Han Chinese.

The West Eurasian specific haplogroup J-M304 and R-M306 reach high frequencies in Hui people, comprising 7.88% and 13.36% in total, respectively. Haplogroup J-M304 probably originated in Near East and then migrated to North Africa, Europe, Central Asia, Pakistan, and India (Cruciani et al., 2007; Di Giacomo et al., 2004; El-Sibai et al., 2009; Semino et al., 2004). The subclade J1-M267 is mainly distributed in the Levante region and North Africa (El-Sibai et al., 2009), reaching a frequency of 1.77% in total in Hui people. Another subclade J2-M172 probably originated in the Fertile Crescent and spread into the Mediterranean following the expansion of Neolithic farmers (Di Giacomo et al., 2004), which contributes 5.79% in total of the Hui people. There are also two main subclades within Haplogroup R-M306, namely R1-M173 and R2-M124. Haplogroup R1-M173 widely

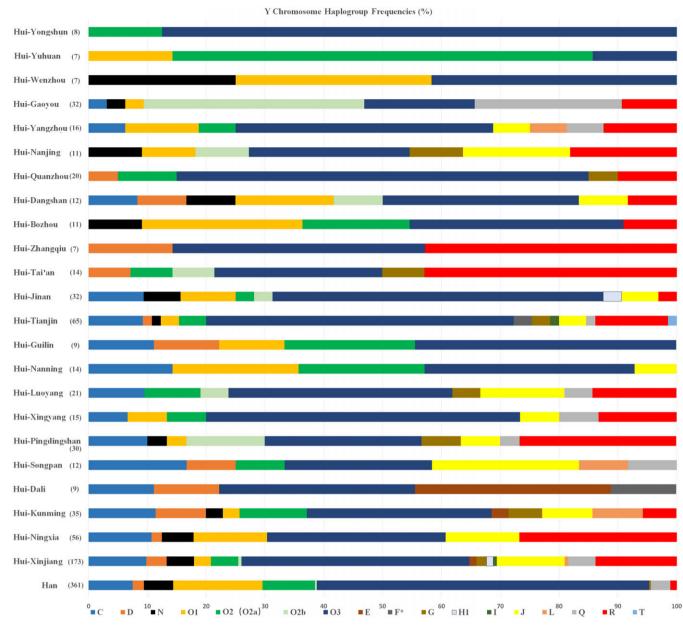
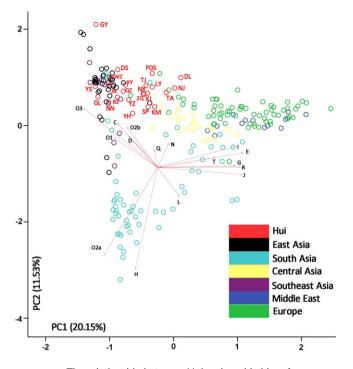


FIGURE 2 The Y-chromosomal haplogroup frequencies of different Hui groups compared with Han Chinese

distributed in West Eurasia, South Asia, and Central Asia (Underhill et al., 2015), which is observed at a frequency of 12.39% in total in Hui people. Haplogroup R2-M124 is mainly found in Asia, especially in the Indian subcontinent and Central Asia (Zhao, Khan, Borkar, Herrera, & Agrawal, 2009), comprising 0.97% in total of our studied Hui samples and only observed in Hui population of Jinan, Shandong. We also detected low frequencies of haplogroup E-M96, F\*-M89, G-M201, H1-M69, I-M258, L1-M76, M357, and T-M272 in Hui people, which are frequent in West Eurasia and the Middle East but rarely found in East Asia (Zhong et al., 2010). We also detected the Haplogroup Q-M242 reaching 3.54% in total of our Hui amples, which is however almost the same as observed in Han Chinese (3.3%; Yan et al., 2011). The West Eurasian related lineages comprise about 30% of Hui people in total when we consider haplogroup C-M130, D-M174, N-M231, and O-M175 as East Eurasian specific lineages and the other haplogroups as West Eurasian related lineages. The frequency of West Eurasian related lineages varies a lot in different Hui groups. We have not found any single individual with West Eurasian related haplogroups in Hui populations from Guilin, Wenzhou, Yuhuan, and Yongshun, but detected about 40% from Ningxia, Dali, Songpan, Pingdingshan, and Luoyang.

We carried out PCA to discern the genetic relationships of different Hui groups with West Eurasians and East Asians with the aid of additional published Y-chromosome frequency data from worldwide populations (Figure 3, Supporting Information Figure S1 and Supporting Information Table S3). The results of PCA are presented by the plots of the first two principal components (PCs), which together account for 31.68% of the Y-chromosome variation in these populations. The first PC revealed a clear north-south geographic division between West Eurasia and East Asia. The haplogroup I-M258, E-M96, R-M306, G-M201, J-M304, and L-M20 were found to contribute most to the pole of West Eurasia. Contrastingly, the haplogroup O-M175,



**FIGURE 3** The relationship between Hui and worldwide reference populations analyzed by PCA with the frequencies of haplogroup

C-M130, and D-M174 contributed most to the East Asian pole. The second PC distinguish the northern Asian populations with high frequencies of haplogroup O3-M122, C-M130, and D-M174 from South Asian populations with high frequencies of haplogroup H and O2a. We found that the Hui people in Dali (DL), Nanjing (NJ), and Tai'an (TA) are shifted toward European and Middle East populations. The Hui in Gaoyou (GY) look like an outlier due to the high frequency of Haplogroup O2b-M176. Our Hui populations are scattered in the middle of the plot right between the West Eurasian and East Asian poles together with the populations from Central Asia and South Asia, which is consistent with previous observation based on haplogroup classification that Hui people have both East Asian West Eurasian affinities.

To visualize the detailed relationships among Hui people with worldwide populations, we generated a neighbor joining (NJ) unrooted trees using Slatkin's R<sub>ST</sub> distance calculated from 15 Y-STR loci (the 17 Y-STRs excluding DYS385a/b) in 100 populations (Supporting Information Table S4). The Slatkin's R<sub>ST</sub> is a linearized  $F_{ST}$  suited for the stepwise STR mutation model. We observed an obvious East-West geographical division in the NJ tree (Figure 4). The Hui people in Yongshun, Wenzhou, Yuhuan, Quanzhou, Jinan, Yangzhou, Gaoyou, Nanjing, Tianjin, Xingyang, Luoyang, Pingdingshan, Dangshan, Bozhou, and Nanning cluster together into the East Asian and Southeast Asian branch, while the Hui people in Tai'an and Zhangqiu cluster with Bulgarian, Croatian, Hungarian, Pathan, and Sindhi probably due to the high frequency of haplogroup R in those two groups. The Hui people in Ningxia, Xinjiang, Kunming, Songpan, and Guilin cluster tightly together occupying a position right between the East and West, implying those groups harboring both East Asian and West Eurasian genetic profile.

## 4 | DISCUSSION

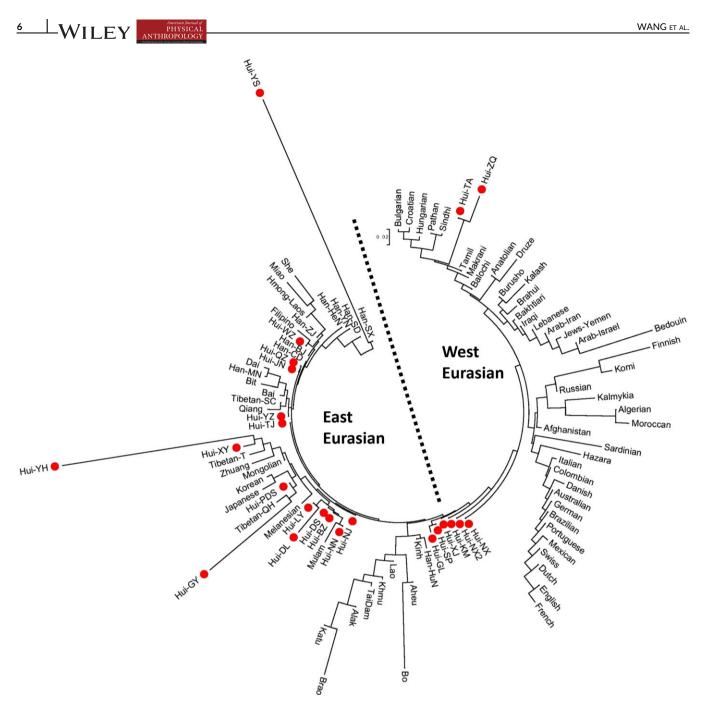
This is the most comprehensive study of the paternal history of Hui people in China so far by including 23 populations collected from all over China and genotyping informative Y-chromosomal SNP and STR markers. The haplogroup classification, frequency-based PCA, and genetic distance based cluster analysis consistently suggest the Hui people are admixed populations with paternal gene flow from West Eurasia and the Middle East. The West Eurasian related lineages comprise about 30% of the paternal gene pool of Hui people. On the other hand, we have shown that the genetic structure of the extant Hui populations was primarily shaped by the indigenous East Asian populations as they contribute the majority part of the paternal lineages of Hui people. For instance, the East and Southeast Asia specific and the frequent lineage of Han Chinese, Haplogroup O-M175 (Wang & Li, 2013), comprise 54.9% of the Hui population, implying the origin of Hui people involving massive assimilation of indigenous East Asian populations.

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The frequencies of West Eurasian related lineages E-M96, F-M89, G-M201, H-M69, I-M258, J-M304, L-M20, Q-M242, R-M306, and T-M272 vary a lot in different Hui groups. For example, the Haplogroup E enriches in Yunnan, southwest China, but Haplogroup T is only found in Tianjin. The scattered distribution of those various West Eurasian lineages indicates the diverse origins of Hui people, who probably came from the Middle East, Europe, Central Asia, and South Asia before randomly admixing into East Asians.

One interesting phenomenon is the inconsistency between paternal Y-chromosomal and maternal mtDNA ancestry estimated in Hui people. The West Eurasian related Y-chromosomal lineages in the Hui samples collected from Xinjiang reach a high frequency of 35.29%, but the West Eurasian related mtDNA lineages only comprise 6.7% of the Hui gene pool in Xinjiang as reported before (Y. G. Yao et al., 2004). We also have not found such a high proportion of West Eurasian gene flow on autosomal STRs compared with that from paternal Y chromosome (H. B. Yao et al., 2016). Therefore, we suspect the admixture in Hui people was probably a sex-biased male-driven process. The sex-biased pattern was also observed in southern Han Chinese and Tibeto-Burman-speaking populations that males played a larger role (about 1.5 times higher) than females in the admixtures (Wen, Li et al., 2004; Wen, Xie et al. 2004), which however is less drastic than that found in the Hui populations since the contribution of West Eurasian Y chromosomes is about 5 times higher than that of West Eurasian mtDNA. We consider two possible reasons for the observed huge sex-biased difference in Hui people. One reason is that the soldiers, merchants, and political emissaries migrated from Central Asia, Arabia, and Persia to China to initially form the Hui in history were mainly males. The second reason is the marriage mechanism they practice. The Hui practice endogamy that they mainly marry among themselves and the intermarriage is sex-biased that usually involves Han Chinese women converting to Islam when marrying the Hui males (Gladney, 1998). We note that the sex-biased pattern seen in Hui from Xinjiang might not be truth for all Hui

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**FIGURE 4** The neighbor joining (NJ) unrooted trees using Slatkin's  $R_{ST}$  distance calculated from 15 Y-STR loci in 100 populations. The populations were labeled as follows: Hui-KM: Kunming Yunnan; Hui-DL: Dali Yunnan; Hui-GL: Guilin Guangxi; Hui-NN: Nanning Guangxi; Hui-GY: Gaoyou Jiangsu; Hui-NJ: Nanjing Jiangsu; Hui-YZ: Yangzhou Jiangsu; Hui-LY: Luoyang Henan; Hui-PDS: Pingdingshan Henan; Hui-XY: Xingyang Henan; Hui-QZ: Quanzhou Fujian; Hui-SP: Songpan Sichuan; Hui-TA: Tai'an Shandong; Hui-ZQ: Zhangqiu Shandong; Hui-YJ: Yuhuan Zhejiang; Hui-YS: Yongshun Hunan; Hui-TJ: Tianjin; Hui-NX: Ningxia; Hui-XJ: Xinjiang; Hui-BZ: Bozhou Anhui; Hui-DS: Dangshan Anhui

populations since we currently do not have the mtDNA data for comparison.

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## ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (31071098 31671297 91731303, 31801040), Nanqiang Outstanding Young Talents Program of Xiamen University (X2123302), the Fundamental Research Funds for the Central Universities (ZK1144).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Wang C-C, Lu Y, Kang L, et al. The massive assimilation of indigenous East Asian populations in the origin of Muslim Hui people inferred from paternal Y chromosome. *Am J Phys Anthropol.* 2019;1–7. <u>https://doi.org/10.</u> 1002/ajpa.23823