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Correspondence

Genetic distribution of 39 STR loci in 1027 unrelated Han individuals from Northern China

Dear Editor,

The Han Ethnic Group is the largest among the 56 ethnic groups in China, accounting for 92% of the total population. The Han people are found in all parts of the country. One study showed that the Han Chinese population was actually substructured in a complex manner, with the main observed clusters roughly corresponding to the Northern-Han, Central-Han, and Southern-Han populations [1]. In the present study, population genetic data and forensic parameters of 39 autosomal short tandem repeats (STRs) were obtained from 1027 unrelated Chinese Han individuals residing in Northern China (Hebei, Henan, and Shanxi provinces) (Fig. S1).

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Bloodstain samples of 1027 (male 506, female 521) unrelated healthy individuals were collected after obtaining informed consent. DNA was extracted using the Chelex-100 protocol [2]. The quantity of recovered DNA was determined by Qubit® Quantitation System (Invitrogen, CA, USA), according to the manufacturer's specifications. DNA samples were amplified using two kits: Microreader™ 21 ID system (Microread Genetics Incorporation, China), which included Amelogenin and 20 autosomal loci (i.e., CSF1PO, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S82, D8S1179, FGA, Penta D, Penta E, THO1, TPOX, vWA), and Microreader™ 23sp ID system, which included Amelogenin and 22 autosomal loci (i.e., D6S477, D18S535, D19S253, D15S659, D11S2368, D20S470, D1S1656, D22-GATA198B05, D8S1132, D4S2366, D21S1270, D13S325, D9S925, D3S3045, D14S608, D10S1435, D12S391, D7S3048, D17S1290, D5S2500, D2S1338, D16S539). Both kits contain D12S391, D16S539, and D2S1338, which could be used for sample concordance. Polymerase chain reaction (PCR) was conducted with the GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Amplified products were separated by capillary electrophoresis on an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Data were analyzed using Genemapper ID 3.2 software (Applied Biosystems, Foster City, CA, USA).

Allelic frequencies and forensic parameters were evaluated using Powerstats version 1.2 (Promega, Madison, WI, USA). Hardy–Weinberg Equilibrium (HWE) of each locus and the linkage disequilibrium (LD) for all pair-wise STR loci were tested using the Genepop Version 4.2 software package (http://genepop.curtin.edu.au). To estimate the inter-population differentiation between the Chinese Northern Han population and 12 other reference populations with 13 CODIS STR loci overlapping with those used in our study were selected, including nine Chinese Han populations residing in different regions of China (ZhejiangHan, JiangsuHan, GuangdongHan, NeimengguHan, HunanHan, JilinHan, LiaoningHan, SichuanHan, and YunanHan) [3–11] and three Chinese ethnic minority groups (Miao, Uigur, and Tibet) [12–14]. A principal components analysis (PCA) was performed in MATLAB 2007a (MathWorks Inc.) based on allele frequencies. The locus-by-locus Fst and associated P-values were calculated using the analysis of molecular variance (AMOVA) with ARLEQUIN v3.1 software (http://cmpg.unibe.ch/software/arlequin3).

Allele frequencies and forensic statistics of each locus are shown in Table S1. All loci were found to be polymorphic. Among the 39 loci, Penta E showed the highest observed heterozygosity (Ho), discrimination power (DP), and probability of paternity exclusion in trios [PE (T)], with values of 0.9182, 0.9876, and 0.8327, respectively. TPOX showed the lowest Ho, DP and PE (T), which was 0.6212, 0.8017 and 0.3171, respectively. The cumulative match probability was approximately 4.7539×10^{-48}. Deviations from HWE were detected at D2S441 ($p = 0.0349$). However, after Bonferroni correction (i.e., p-value = 0.05/39 = 0.00128), no locus showed significant deviations from HWE.

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Thirty-six pairs of loci showed significant ($p < 0.05$) LD among 741 pairwise comparisons (Table S2). After Bonferroni correction (p-value = 0.05/741 = 0.0000675), only the pair of Penta E and D13S325 still showed significant LD (p-value = 0.000004). This result may be due to a sampling artifact, since these two loci are not on the same chromosome. Therefore, these 39 loci could be treated as independent loci at the population level. The PCA results are shown in Fig. S2. The first component explained 24.69% of the total variation and the second explained 12.05% of the overall variation. The results showed that the Northern Han, JilinHan, LiaoningHan, Neimengguhan, and Uigur populations clustered in the upper part, which was consistent with the fact that they all geographically belong to Northern China. The ZhejiangHan, JiangsuHan, HuannanHan, SichuanHan, GuangdongHan, YunnanHan, Miao, and Tibetan populations clustered in the lower part, which is consistent with their geographical location in Southern China. The present result is consistent with previous studies of other genetic markers [15].

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The result of single-locus Fst and p-values between the Northern Han and 12 reference populations are shown in Table S3. Relatively more differences were found between the Northern Han and three ethnic minority groups, after Bonferroni's correction (p-value = 0.05/13 = 0.0038), which had differences in 5

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loci with the Uigur, 3 loci with the Miao and 2 loci with the Tibetan populations, respectively. On the other hand, except for the Sichuanhan population at the D7S820 locus, there were no differences observed between the Northern Han and the other 9 Chinese Han populations at the 13 CODIS loci. This suggested that Han populations residing in different regions of China are more closely related to each other than they are to Chinese ethnic minority groups.

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We strictly followed the International Society for Forensic Genetics (ISFG) recommendations for the analysis of DNA polymorphisms and nomenclature [16] and the guidelines for publication of population data requested by the journal [17]. The DNA typing was performed at the laboratory, which was accredited according to the ISO 17,025 standard. The certification of approval by the China National Accreditation Service for Conformity Assessment (CNAS) was No. CNAS L0812.

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