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# Genetic polymorphisms of 27 Yfiler® Plus loci in the Daur and Mongolian ethnic minorities from Hulunbuir of Inner Mongolia Autonomous Region, China

*Dear Editor,*

Y-chromosomal specific short tandem repeat (Y-STR) markers are inherited paternally, and their haplotype distribution may be distinct from other populations of different geographic regions or different ethnic backgrounds. These properties make Y-STRs useful tools in population and forensics genetics [1–3]. The Yfiler® Plus Kit (Thermo Fisher Scientific, Waltham, MA, USA) with an expanded set of Y-STR markers was produced in light of the continuous demand for developing more efficient and discriminative Y-STR haplotyping systems [4]. To take full advantage of the increased resolution of Y chromosome haplotype analysis offered by Yfiler® Plus, it is necessary to collect haplotype frequency data for as many population samples as possible.

Hulunbuir is located on the northeastern border of China and the eastern part of the Eurasian steppe, which has a long history and is one of the birthplaces of northern hunting or nomadic peoples. According to the latest census (2010), the aboriginal population of the Daur ethnic minority was 131,992, which is considerably smaller than other minorities. They are thought to be descendants of the Khitan, an ancient nomad tribe who lived in the lower reaches of the Heilong River and founded the Liao Dynasty (916–1125). The Daur language belongs to the Mongolian Austronesian of Altai Phylum.

The Mongolian minority is the most populous ethnic minority in Hulunbuir, with a population of 230,008 (in 2010), accounting for 9.02% of the total population. This ethnic minority is brave and unconstrained with a profound history. Having their own spoken and written language, which belongs to the Mongolian group of the Altaic language family. So far, Y haplotype diversity data in the Chinese Daur and Mongolian minorities are lack, and the genetic relationships between them and neighboring populations keep unclear [5–7]. The aim of this study was to provide Yfiler® Plus haplotypes data of Daur and Mongolian minorities residing in Hulunbuir of Inner Mongolia Autonomous Region, China, and compare their genetic relationships with other neighboring and/or linguistically close populations.

All samples were collected after receiving informed consent, and individuals were considered autochthonous if their ancestors had lived in Hulunbuir, Inner Mongolia Autonomous Region of China for at least three generations. The Ethical Committee of Fudan University, Shanghai, People's Republic of China approved the study. Genomic DNA was extracted from blood spots collected on FTA cards (Wuhan Jiteng Biotech Co., Ltd., China) using the Chelex-100 protocol as described by Walsh [8]. Y-STR amplification was performed on a GeneAmp® PCR System 9700 Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) using the Yfiler® Plus PCR Amplification kit, according to the manufacturer's protocol, with modifications in the final reaction volume (10.0 μl), which was composed of 4.0 μl of master mix, 2.0 μl of primers set, 3.0 μl of deionized ddH2O and 0.5–1.0 ng of genomic DNA. Electrophoresis and STR genotyping were performed in an upgraded ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Allele designations were made using the allelic ladders provided with the Yfiler Plus kit and following the recommendations of the DNA Commission of the ISFG on Y-STR analysis [9].

Experiments were performed in the Key Laboratory of Evidence Science (China University of Political Science and Law), which is accredited according to ISO 17025 standards and passed the YHRD Quality Control Exercise in 2009. Data were submitted to the YHRD (Y-chromosomal haplotype reference database, https://yhrd.org [10]) under accession number YA004277 for the Daur minority and YA004555 for the Hulunbuir Mongolian minority.

Haplotype and allelic frequencies were estimated by direct gene counting. Gene and haplotype diversities were calculated according to the formula by Nei [11]. The discrimination capacity was calculated as the proportion of different haplotypes in the sample. Pairwise genetic distances of Rst and corresponding *P*-values between different populations were evaluated by analysis of molecular variance (AMOVA) and visualized using multidimensional scaling (MDS) plots using statistical online tool available on the YHRD website. The *P*-values were then calculated at a significance level of 0.05 using 10,000 permutations. Haplotypes that presented as null, intermediate, duplicate (except for DYS385 and DYF387S1) or triplicate alleles were removed. A Bonferroni correction was also applied to adjust for potential type I errors [12].

The haplotype distributions of the two minorities are listed in Table S1. A total of 196 different haplotypes were observed in the sample of 203 Daur individuals. Deletions were detected at the DYS448 locus in 5 individuals, and the null type display was confirmed using the PowerPlex® Y23 STR Kit (Promega Corporation, Madison, WI, USA). The overall haplotype diversity was calculated as 0.9997 with a discrimination capacity of 0.9655. A total of 279 different haplotypes were identified from 282 unrelated Mongolian samples, of which 276 (98.92%) were distinct. There were deletions at the DYS448 locus in 6 samples. The overall observed haplotype diversity was calculated as 0.9999 with a discrimination capacity of 0.9894.

To evaluate the utility of the new markers for forensic casework, haplotype-based analyses were repeated for another subset of Y-STRs, namely, Yfiler® panel analysis (17 loci). An increase in the number of analyzed Y-STR markers decreased the number of shared haplotypes and increased the number of unique haplotypes. This is reflected in the discriminatory capacity increasing from 75.86% with the Yfiler® loci haplotype to 96.55% with the Yfiler® Plus kit for the Daur samples and from 96.45% with the Yfiler® loci haplotype to 98.94% with the Yfiler® Plus kit for the Mongolian samples (Table S2).The results indicated that the Yfiler® Plus kit offers a high power of discrimination and can be useful for forensic investigation and paternal lineage identification in the Daur and Mongolian minorities.

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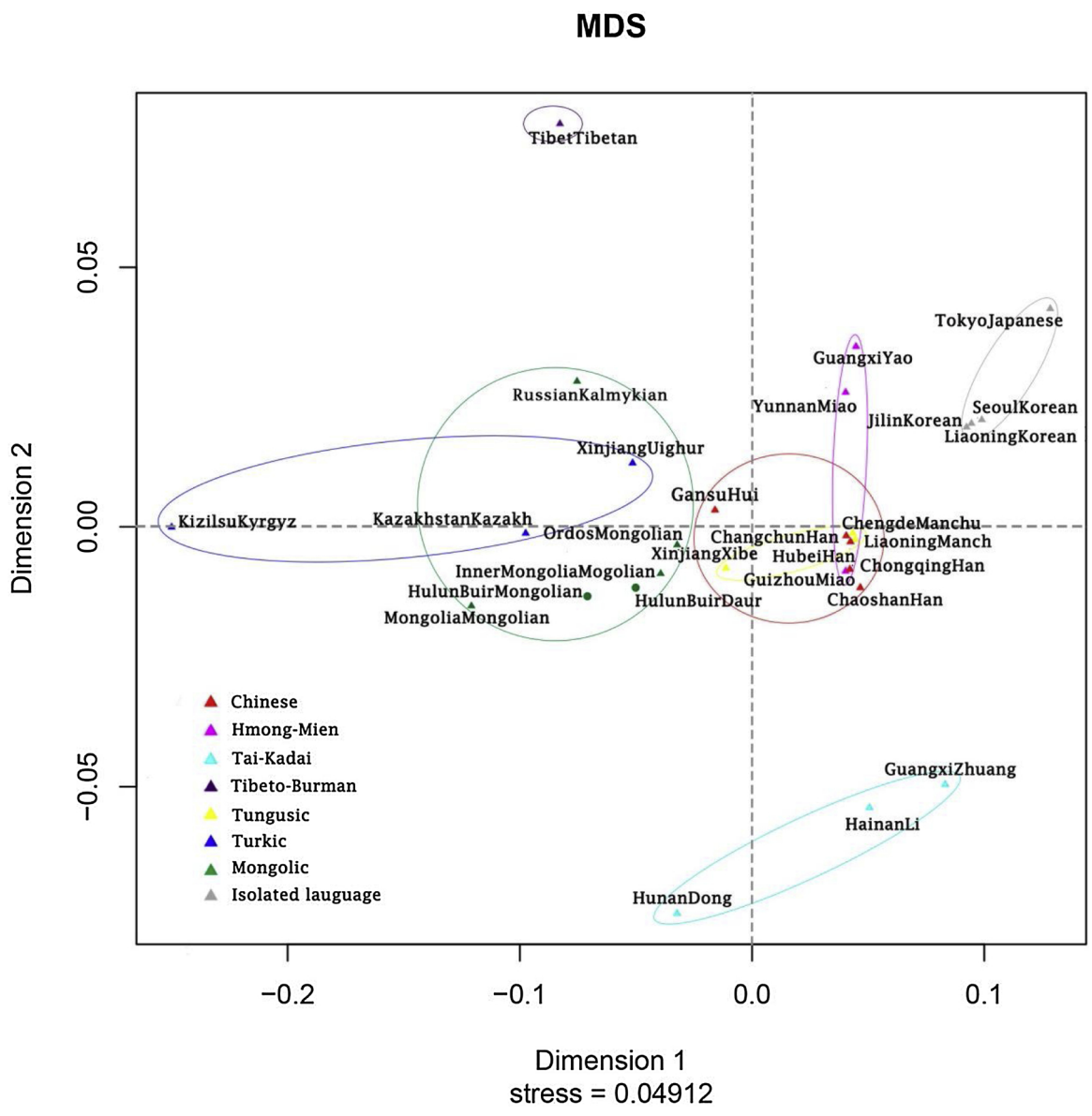


**Fig. 1.** Multidimensional scaling (MDS) analysis for the Hulunbuir-Daur, Hulunbuir-Mongolian and 26 other reference populations based on Rst values. Triangles indicate the reference populations and circles indicate the populations in this study. All populations are marked with different colours according to the language classification, as shown in the lower left corner of the Figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To explore the genetic relationship with relative populations, we compared the 17 Yfiler haplotypes data of the two minorities with 26 neighboring populations according to the language classification (Table S3): Dong, Hui [13–15], Korean [16,17], Kyrgyz, Li [18], Manchu [19,20], Miao [21], Mongolian [22,23], Tibetan, Uighur [24,25], Xibe [26], Yao, Zhuang [27,28], Chinese Han [29,30], as well as 5 neighboring nations: Tokyo-Japanese [31], Seoul-Korean [32], Kazakh from Kazakhstan [33], Ulaanbaatar Mongolian, and Kalmykian from Russion Federatiion [34]. Table S4 shows that no significant differences ($P > 0.0018$, after Bonferroni's correction) between the Hulunbuir Daur and the Hulunbuir Mongolian samples (Rst = 0.0079), and the Mongolians of Inner Mongolia (Rst = 0.0097), indicating little genetic difference. Although significant, low Rst values between the Daurs and the other three Mongolic-speaking populations (Ordos-Mongolian, Mongolia-Mongolian, Russian-Kalmykian) (0.0166–0.0488) and other four groups in Central Asia and Western China (Kazakh, Xibe,Uighur, Hui) (0.0329–0.0575) were obtained. In comparison to the remaining Chinese minority ethnic groups, China Han and two Asian nations (Seoul-Korean and Tokyo-Japanese), highly significant distances were observed (0.0975–0.2473, $P < 0.0018$). Similarly, the Hulunbuir Mongolian minority was not significantly different ($P > 0.0018$) from the Mongolians residing in Inner Mongolia (Rst = 0.0045), Ordos-Western Inner Mongolia (Rst = 0.0110), and Xinjiang Xibe (Rst = 0.0163). Although significant, low Rst values between Hulunbuir Mongolians and Mongolians from Mogolia, Kalmykians from Russian, Hui, Xibe, Kazakh and Uighur were also obtained (0.0163–0.0558). By comparing the Hulunbuir Mongoliansin with the remaining populations, highly significant distances were observed (0.0743–0.2013, $P < 0.0018$).

Multidimensional scaling plot constructed to portray the patterns of population genetic relationships based on linearized Rst values of all 28 population data (Fig. 1) showed that, except for Russian-Kalmykian, the other five Mongolic-speaking groups, Hulunbuir-Daur, Hulun buir-Mongolian, Inner Mongolia-Mongolian, Ordos–Mongolian and Mongolia-Mongolian, are gathered in the lower left quadrant, which confirms their historical ancestry. Although they all belong to Mongolic-

speaking groups, Kalmyks have their own different origins. Kalmyks originated from the famous four Oirats and experienced a long-term migration from east to west [35]. They finally settled in the Kalmyk Republic, which is far from the other eastern Mongolia populations. In addition to the Mongolic-speaking groups, the two studied Hulunbuir minorities are also close to Kazakhstan-Kazakh, Xinjiang-Uighur, Xinjiang-Xibe and Gansu-Hui. It is widely accepted that many ancient Mongolian tribes participated in the formation of the Kazakhs during different historical periods [36]. Xibes in Xinjiang have always lived among Kazakhs, so we speculate that Xibes indirectly obtained some Mongolian genetic components from Kazakhs. It is not surprising that the two studied Hulunbuir minorities are close to Xinjiang-Uighur and Gansu-Hui, which are generally considered to be two typical admixtures of eastern and western genetic components [37,38], and were ruled by the Mongols for many years. Further, populations with the same language background tend to be together, such as Tai-kadai speaking groups (Guangxi-Zhuang, Hainan-Li and Hunan-Dong), and Isolated language speaking groups (Jilin Korean, Liaonin Korean, Seoul Korean, and Tokyo-Japanese). Additionally, the Tibetan minority demonstrated significant population heterogeneities different from all of the other populations included, in this case attributable to the differentiation of China, presumably because of differences in ancestral components.

In conclusion, these data in Chinese Hulunbuir Daur and Mongolian minorities could be potentially useful for the regional specific and prerequisite reference to the forensic, genealogical, and evolutionary purposes. The work presented here follows the recommendations of the ISFG on the use of Y-STR in forensic analysis and the guidelines for publication of population data proposed by the journal [39].

## Conflict of interest

The authors state that they have no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2019.02.003.

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