Y Chromosomal STR Polymorphism in Northern Chinese Populations

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ABSTRACT

Y chromosomal STRs show sufficient variability among individuals in a population and a high degree of geographical differentiation, such that their polymorphic character makes them especially suited for population genetic studies. To investigate the polymorphism of a set of 17 Y-STR loci in northern China, we genotyped the 17 Y chromosomal STR loci in a population sample of 377 unrelated males from eight ethnic populations in northern China. We calculated the haplotype frequencies, *Rst* value and carried out the analysis of molecular variance (AMOVA). We then drew the multidimensional scaling analysis (MDS) plot and phylogenetic tree based on the *Rst* value. All populations showed a high level of haplotype diversity, with low inter-population variance as measured by an analysis of molecular variance. However, the genetic distances were significant when the eight populations were compared to other populations. By MDS and the phylogenetic tree, we found that the eight populations had a close relationship and Xibo had a northeast origination.

Key terms: China; population; STR; Y chromosome.

INTRODUCTION

The Y chromosome is inherited from father to son and the majority of its DNA lacks recombination. Therefore, all descendants of a male lineage share a common Y chromosomal haplotype in the non-recombining region, unless a mutation alters the haplotype. Y chromosomal STRs show sufficient variability among individuals in a population and a high degree of geographical differentiation (Jobling and Tyler-Smith 1995). Thus, their polymorphic character makes them especially suited for forensic, genealogical and population genetic studies (de Knijff et al. 1997; Gusmao et al. 2006; Jobling et al. 1997; Schultes et al. 1999).

The Y-STR Haplotype Reference database has been established for

populations around the world (Kayser et al. 2002; Lessig et al. 2003; Roewer et al. 2001; Willuweit and Roewer 2007). The minimal haplotype, which consists of the loci DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385, can distinguish approximately 76.1-95.5% of male individuals in various populations (Kayser et al. 2002; Roewer et al. 2001; Schoske et al. 2004). Despite the utility of the minimal haplotype, additional Y-STR loci are required to improve the ability to distinguish different paternal lineages. Also, empirical studies of the other Y-STR loci are needed to provide an efficient marker set with sufficient power of discrimination (Gusmao et al. 2006). Recently, a set of 17 Y-STR loci had been analyzed in some populations and exhibited

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a high degree of haplotype diversity (Huang et al. 2008; Kumagai et al. 2007; Pontes et al. 2007), while the polymorphism of these loci in the northern China population was unknown. Here, we present haplotype analysis and genetic tests of these 17 Y-STR loci in eight ethnic populations from northern China to explore the genetic relationships of these populations.

MATERIALS AND METHODS

DNA Samples:

A total of 377 unrelated male subjects were randomly selected from eight ethnic groups in northern China (Fig. 1). The male subjects were obtained from ethnic minority settlements. Prior to sampling, a questionnaire had been filled in by each participant regarding the ethnic nature of his parents and grandparents. Only the ones whose parents and grandparents were from

the same minority were included in this study. For the eight groups involved in the present study, Xibo was sampled from Xinjiang Municipality (northwest China); while the other ethnic groups came from Heilongjiang, Jinlin, Liaoning province and the Inner Mongolia Municipality (northeast China), respectively. Of the 377 individuals, 50 were Mongol, 71 were Man, 48 were Hezhen, 73 were Korean, 50 were Ewenk, 27 was Oroqen, 30 were Daur and 28 were Xibo. Details are listed in table 1. Blood samples were collected from randomly selected individuals in each ethnic group. All subjects were resident in their own hometown and everyone was the offspring of a non-sanguineous marriage of members of the same nationality for within at least three generations. Informed consent was obtained from all participants in this study. DNA was extracted from peripheral blood samples anticoagulated with ACD by the standard phenol-chloroform extraction method.

Figure 1: Geographic distribution of the study populations in China.

Y-STR Genotyping:

All samples were genotyped the 17 Y-STR loci (DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and GATA H4) by the AmpF/STR®Y-filer™ PCR Amplification kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The amplified products were analyzed with an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems). The genotyping designations were based on comparison with allelic ladders included in the Y-filer™ kit using GeneMapper 4.0 software (Applied Biosystems). We used the control DNA samples and allelic ladders provided by the manufacture of the kit, and followed the International Society for Forensic Genetics (ISFG) guidelines for STR analysis.

Analysis of data:

The number of haplotypes was counted for each population. Haplotype diversity (H) was calculated as $H = n(1 - \sum \pi^2) / (n - 1)$, where π is the frequency of the i th haplotype and n is the number of samples (Nei 1996). The discrimination capacity (D) was determined by dividing the number of haplotypes by the number of individuals in each population (Gene et al. 1999).

Genetic relationships between the eight populations, based on the 17 Y-STR loci haplotypes, were further explored by analysis of molecular variance (AMOVA), as implemented in Arlequin 3.01 (Excoffier et al. 2005) (<http://cmpg.unibe.ch/software/arlequin3/>). Subsequently, the haplotypes of these populations were compared to published data from eight populations: Tibetan, $n=167$ (Zhu et al. 2008); Taiwanese, $n=200$ (Huang et al. 2008); Portuguese, $n=175$ (Pontes et al. 2007); Serbian, $n=185$ (Veselinovic et al. 2008); Shandong Han, $n=131$ (Yan et al. 2007); Japanese, $n=1079$ (Mizuno et al. 2008); Western Russian, $n=545$ (Roewer et al. 2008) and Chinese Hui, $n=143$ (Guo et al. 2008), with R_{st} (the index of the population

pairwise genetic distance), which is analogous to F_{st} but is based on a stepwise mutation model. AMOVA and the R_{st} values were assessed by permutation tests with 10,000 permutations. Population relationships on the basis of the R_{st} values were determined by multidimensional scaling analysis (MDS) as implemented in Statistica (StatSoft Inc., Tulsa, USA). Based on the R_{st} genetic distances, phylogenetic tree was constructed based on the Neighbor-Joining method using the MEGA software ver.4.1 (Kumar et al. 2008).

RESULTS

A total of 377 unrelated males from eight northern China populations were investigated in this study and 361 different haplotypes were detected. Two haplotypes occurred three times, 12 occurred twice and 347 were unique (Supplementary Table 1). The discrimination capacity of these 17 loci in the total samples was 0.958, and the haplotype diversity value was 0.9991 (see Table 2).

AMOVA of the eight ethnic populations in northern China indicated that 96.36% of the variation was found within populations, whereas only 3.64% was among populations (Table 3).

We obtained data for the 17 loci haplotypes of another eight populations from the literature. We carried out the R_{st} analysis for the total 16 populations. The pairwise R_{st} results showed that there were significant differences between the northern China populations and the other populations ($P < 0.001$, Supplementary Table 2). On the basis of pairwise R_{st} values, we drew the MDS plot to learn the relationships of these populations. The results displayed a close relationship among the eight northern China populations, with an elevated distance to the other Asian and European populations (Fig. 2). We then constructed the phylogenetic tree by the MEGA. The results showed that the eight northern China populations and Shandong Han were in a clade, while the other populations were in another clade (Fig. 3).

TABLE 1

Details of the eight populations

Population	Number	Sampling place	Location (longitude, latitude)
Mongol	50	Hailar, Inner Mongolia Municipality	126.0, 51.0
Man	71	Xiuyan, Liaoning Province	123.2, 40.2
Hezhen	48	Tongjiang, Heilongjiang Province	132.5, 46.7
Korean	73	Yanji, Jilin Province	121.4, 40.2
Ewenk	50	Molidawa, Inner Mongolia Province	118.6, 48.0
Oroqen	27	Arlihe, Inner Mongolia Province	131.0, 53.5
Daur	30	Molidawa, Inner Mongolia Province	118.6, 40.8
Xibo	28	Yili, Xinjiang Municipality	80.5, 43.3

TABLE 2

Diversity parameters of 17 Y-STRs in eight different Chinese populations

Population	Number	diff Ht	<i>D</i>	<i>h</i>
Mongol	50	48	0.960	0.9992
Man	71	70	0.986	0.9998
Hezhen	48	39	0.813	0.9952
Korean	73	71	0.973	0.9996
Ewenk	50	50	1.000	1.0000
Oroqen	27	27	1.000	1.0000
Daur	30	29	0.967	0.9989
Xibo	28	28	1.000	1.0000
Total	377	361	0.958	0.9991

Diff Ht: number of different haplotypes; *D*: discrimination capacity; *h*: haplotype diversity

TABLE 3

Analysis of molecular variance in the eight populations

Source of variation	Sum of squares	Variance components	Percentage of variation
Among populations	103.257	0.203	3.64
Within populations	1982.544	5.373	96.36
Total	2085.801	5.576

Figure 2: Multidimensional scaling analysis (MDS) plot of the northern China populations and eight other populations, from pairwise *Rst* values. B is an enlargement of the square in A. Acronyms was as follows: Ma-Man; Mo-Mongol; He-Hezhen; Ko-Korean; Or-Oroquen; Da-Daur; Xi-Xibo; Ew-Ewenk and Sh-Shandong Han.

Figure 3: Phylogenetic tree of 16 populations based on the *Rst* values

DISCUSSION

Human Y-linked polymorphisms in the non-pseudoautosomal portion of the Y chromosome are haploid inherited with paternal lineage. This part of Y chromosome lacks recombination, which is the same as mitochondrial DNA, and is passed down from father to son unchanged except for mutations. The use of Y chromosome polymorphisms, especially Y-STR, has become commonplace in an application of human evolutionary study and population history research (Huang et al. 2008; Jobling et al. 1997; Kumagai et al. 2007; Mertens et al. 2007; Parkin et al. 2006), as well as in a variety of forensic situations for mixture analysis and paternity test (Diederichs et al. 2005; Hall and Ballantyne 2003; Hanson and Ballantyne 2004; Jobling et al. 1997).

In this study we genotyped 17 Y-STR loci in 377 male subjects from eight northern China populations. The results showed that there were 361 haplotypes in these 377 individuals, and most haplotypes appeared only once. There was only one shared haplotype among these populations, which appeared in two populations (Korean and Oroqen). These 17 loci had a high level of haplotype diversity and discrimination capacity in these eight populations (Table 2). These results indicated that this set of loci had a high level of diversity in northern China and that they were suitable for forensic discrimination.

The results of the AMOVA showed that 96.36% of the variation was found within populations, whereas only 3.64% was among populations. These results showed that the eight populations had a close relationship, which is in accordance with other studies carried on the basis of the classical genetic markers or the uniparental markers (Ma et al. 2005; Mao et al. 2008; Xue et al. 2005). To obtain a more comprehensive picture of the genetic structure of these populations, we obtained the Y-STR data of the other eight populations from Asia and Europe and carried out *Rst* analysis for all 16 populations. The results showed that the *Rst* value was low among the northern China

populations, while the difference between northern China and other populations was large (Supplementary Table 2). An MDS plot based on pairwise *Rst* values showed the northern China populations formed a close knit group. For the other populations, Shandong Han was close to the northern China population, while the other populations were far away from the core group. Shandong province is near northeast China so this could be the cause of their close relationship. As demonstrated in the MDS plot, Taiwanese and Japanese had a close genetic relationship. Hui was clustered with the Europe populations, Hui are descended from Arab and Persian Muslim traders who settled in China (Dillon 1999), so the patrilineal genetic structure is similar to that of Europeans. The result of the phylogenetic tree was in accordance with the MDS: Shandong Han was clustered with the eight northern China populations, Taiwanese and Japanese were in a sub-clade, Hui, Tibetan and three Europe populations were in a sub-clade.

From the MDS plot and the phylogenetic tree, we know that the distribution pattern was in good agreement with the geographic locations or the ethno-origins of these populations. The results showed that the haplotypes of these 17 loci had a good geographical characteristic and that the eight northern China populations had a close relationship.

In the eight populations, Xibo was sampled from Xinjiang province, which is located in the northwestern China, while the others are from the northeast. From the results of MSD plot, we found that Xibo was mixed with the northeast populations. In the phylogenetic tree, Xibo had the closest relationship to Mongols. The Xibo originated in northeastern China, where their ancestry can be traced for at least 3000 years. In 1764, about 1020 soldiers, together with 4000 dependants, were sent to defend the border of the northwestern province of Xinjiang and their descendants have remained there ever since (Ruofu 1994). They speak a language related to Man, reflecting their northeastern origins, quite distinct from the Uygur language spoken in the northwest. Our findings also

demonstrated the northeastern origin of the Xibo.

In this study, we examined 17 Y-STR loci in eight northern China populations and carried out the analysis of molecular variance, *Rst*, multidimensional scaling analysis and constructed the phylogenetic tree. By these analyses, we found that the eight populations had a close relationship and that the Xibo have a northeastern origin.

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