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SHORT COMMUNICATION

Characteristics of eight X-STR loci for forensic purposes in the Chinese population

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Abstract X-chromosomal short tandem repeats (ChrX STRs) loci are used for forensic practice in recent years. Considering the unique heredity characteristics of ChrX, recombination and linkage disequilibrium (LD) among ChrX STR loci vary between male and female and different populations as well. However, there is a lack of data for analysis of recombination and linkage disequilibrium on ChrX STR loci in the Chinese population. In this work, a total of 303 unrelated individuals (203 males and 100 females) in the Chinese Han population were analyzed with Mentype Argus X-8 PCR amplification kit (DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS10134-DXS7423). The recombination and linkage disequilibrium of the eight ChrX STR loci were investigated with HapMap LD plots and software ARLEQUIN 3.1. Allele frequencies of the eight loci and further population forensic genetic parameters were obtained. Our results revealed hotspots for recombination, and there was no obvious evidence for LD among the eight loci in the Chinese population. Our work implied that single locus frequencies rather than haplotype frequencies should be applied for forensic practice in the Chinese population.

Keywords ChrX · STR · LD · Haplotype · Chinese Han population

Introduction

X-chromosomal short tandem repeats (ChrX STRs) are potentially complementary to other genetic markers, such as autosomal STRs, Y-STRs, and mitochondrial DNA [1, 2]. It is very efficient for ChrX STRs in the mother–son kinship [2]. In the father–daughter parentage testing, ChrX STRs may be also valuable. Recently, typing of ChrX STRs has been established as a useful strategy in solving complex kinship cases [2–5].

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ChrX STRs are different from autosomal STRs in Hardy–Weinberg equilibrium. Recombination and linkage disequilibrium (LD) among ChrX STR loci vary between male and female and different populations as well. Males carry one X-chromosome, and the whole X-chromosome is transmitted to female offspring. The female offspring carry two X-chromosomes and the two X-chromosomes are prone to recombination during meiosis [6]. Thus, considering X-chromosomal heredity characteristics, recombination and linkage disequilibrium must be investigated before ChrX STRs can be applied for forensic practice in the appropriate population [4].

The Mentype Argus X-8 PCR amplification kit includes eight loci (DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS10134-DXS7423) located at Xp22, Xq12, Xq26, and Xq28, and they are assigned to four linkage groups 1–4 for practical reasons [7]. The kit is widely used in forensic practice [2, 6–8], but application of the kit in the Chinese Han population has not yet been reported. According to the studies in kinship testing by Pepinsk and Robino [9, 10], alleles of closely linked X-chromosomal loci can be analyzed as haplotype instead of single STR. But if they are not closely linked, taking them as haplotype will degrade efficacy of kinship testing. Thus, it is necessary to investigate the recombination and linkage disequilibrium of the eight loci and evaluate the efficacy of the eight loci with frequencies of single locus and haplotype for forensic practice in the Chinese Han population.

Materials and methods

Samples and DNA extraction

Blood samples were taken from 303 unrelated individuals (203 males and 100 females) of the Chinese Han population living in Sichuan province. Genomic DNA was extracted using the Chelex-100 protocol as described by Walsh et al. [11]. Written informed consent was obtained from all individuals.

PCR amplification

The multiplex test system Mentype Argus X-8 PCR amplification kit (Biotype, Dresden, Germany) was used to amplify the loci DXS8378, HPRTB, DXS7423, DXS7132, DXS10134, DXS10074, DXS10101, and DXS10135. Table 1 shows relevant information on the eight ChrX STRs.

PCR amplification was performed according to the manufacturer's instructions (25 μl PCR reaction volumes containing approximately 0.2–2 ng DNA). Samples were amplified in the MasterCycler epGradient S cycler (Eppendorf, Hamburg, Germany) under the following conditions, 94 C for 4 min (hot start), 94 C for 30 s, 58 C for 120 s, 72 C for 75 s for 30 cycles, and 68 C for 60 min.

Table 1 Information on the eight ChrX STRs in this study

Locus	Chromosomal mapping	Localization of locus
DXS8378	Xp22.31	9330122-9330512
HPRTB	Xq26.2	133443027-133443430
DXS7423	Xq28	149461462-149461884
DXS7132	Xq11.2	64572000-64572412
DXS10134	Xq28	149400574-149401094
DXS10074	Xq12	66893678-66894174
DXS10101	Xq26.2	133482013-133482455
DXS10135	Xp22.31	9266118-9266616

Details in localization came from http://www.genome.ucsc.edu

Typing and analysis of data

Amplified products were resolved and detected by capillary electrophoresis with the denaturing polymers POP4 (Applied Biosystems, Foster City, CA) in the ABI 310 genetic analyzer (Applied Biosystems) as recommended by the manual instructions. Amplicon sizing was supported with the DNA Size Standard 550 (Biotype). The 9947A, 9948, DNA XX74, and DNA XY1 control cell line samples (Biotype) were typed for calibrating allelic ladder. Results were typed automatically using GeneMapper ID ver. 3.2 with the binset provided by the manufacturer (www.biotype.de). New alleles were sequenced using ABI PRISMTM 3730XL DNA Analyzer (Applied Biosystems).

The allele frequency of male samples, female samples, and combined male and female samples at each locus were calculated by the gene counting method. With female samples, deviations from the Hardy–Weinberg equilibrium (HWE) were determined by an exact test with GENEPOP software [12]. The power of discrimination in females and males, mean exclusion chance, and the polymorphism information content were calculated by the online software (http://www.chrx-str.org) [13].

Linkage disequilibrium analysis

Due to the fact that the ChrX sequence has been recorded the physical localization of DXS8378, HPRTB, DXS7423, DXS7132, DXS10134, DXS10074, DXS10101, and DXS10135 could be requested using In-Silico PCR tool of the University of California Santa Cruz website (http://www.genome.ucsc.edu/). We used the physical localization of the eight loci (DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS7423-DXS10134) to down-

load HapMap LD Plot [data source: release 23a/phase II Mar08, on NCBI36 assembly, dbSNP b126, Han Chinese in Beijing (CHB)] from HapMap website (http://www.hapmap.org). The LD plot of DXS7132-DXS10074 using CHB was incomplete due to insufficient information for the SNP, so the LD plot could not represent linkage disequilibrium of the location. Instead, we downloaded the CEPH (Utah residents with ancestry from northern and western Europe) LD plot of DXS7132-DXS10074. The hotspots for recombination between all pairs of the eight loci were detected through the HapMap LD Plot. With male samples, pair-wise linkage disequilibrium between all pairs of the eight loci was tested by software ARLEQUIN 3.1 (http://cmpg.unibe.ch/software/arlequin3) [14].

Results

Tables S1 and S2 of Electronic supplementary material show the allele frequencies of the eight loci, further statistical information, and new alleles which were detected in the Chinese Han population. HWE was performed on female samples, and the genotype distributions did not deviate from HWE at the eight loci. Table S3 of Electronic supplementary material shows the sequencing data of new alleles.

Population genetic evaluation of the eight ChrX STR loci in the Chinese Han population revealed that when the forensic efficiency of the eight loci was calculated, the combined power of discrimination was at least 0.9999 in males (PD_M) and 0.9999 in females (PD_F), and the combined power of paternity exclusion was a minimum of 0.9999 in trio cases and 0.9997 in duo cases. The eight ChrX STR loci provided high polymorphism information for forensic identification and paternity testing.

The localization of the eight loci is listed in Table 1. The HapMap LD plots of DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS7423-DXS10134 are showed in Fig. S1 of Electronic supplementary material. Some hotspots for recombination were found on the LD plots of the four pairs. Significant linkage disequilibrium was not detected when the pair-wise linkage disequilibrium between all pairs of the eight loci was tested in male samples (Table 2).

Table 2 Results of pair-wise linkage disequilibrium testing (significance level=0.0500)

Pairs of loci	Exact p value
DXS8378, HPRTB	0.234676
DXS8378, DXS7423	0.443637
DXS8378, DXS7132	0.422234
DXS8378, DXS10134	0.741295
DXS8378, DXS10074	0.975183
DXS8378, DXS10101	0.880396
DXS8378, DXS10135	0.834275
HPRTB, DXS7423	0.663339
HPRTB, DXS7132	0.981103
HPRTB, DXS10134	0.919808
HPRTB, DXS10074	0.215729
HPRTB, DXS10101	0.801721
HPRTB, DXS10135	0.459170
DXS7423, DXS7132	0.663758
DXS7423, DXS10134	0.357435
DXS7423, DXS10074	0.690313
DXS7423, DXS10101	0.298806
DXS7423, DXS10135	0.969512
DXS7132, DXS10134	0.328395
DXS7132, DXS10074	0.257567
DXS7132, DXS10101	0.742114
DXS7132, DXS10135	0.697710
DXS10134, DXS10074	0.820269
DXS10134, DXS10101	0.926826
DXS10134, DXS10135	0.406561
DXS10074, DXS10101	0.480663
DXS10074, DXS10135	0.465959
DXS10101, DXS10135	0.480364

No evidence of linkage disequilibrium was detected

Discussion

In previous studies, the eight loci were evaluated with DNA samples from Germany, Japan, Ghana, Sweden, Hungary, and Korea [6–8, 15, 16], and some of them were described in the Chinese Han (Taiwan) population and Iberian and Latin American population studies [17, 18]. Data for the Mentype Argus X-8 PCR amplification kit in the Chinese Han population were not reported before, and this was the first time that the kit was applied in the Chinese Han population. Compared with the Korean, Japanese, and Chinese Han (Taiwan) population studies, allele frequencies for the loci obtained in this study were regarded as similar. However, nine alleles which were not included in the Argus X-8 kit ladder were obtained (Tables S1 and S2) in this study. For DXS7132, allele 18 was obtained; locus DXS10134 showed alleles 35.2, 36.2, 37.3, 39, and 40, and at the locus DXS10074, new allele 18.3 was observed. The allele 35 was detected at locus DXS10101, while a rare allele 31.1 at locus DXS10135 was also found. Among these alleles, 35.2, 18.3, and 31.1 were novel ones without any previous reports.

X-chromosomal haplotyping could be utilized in forensic practice, especially in complex kinship testing. The correct application of ChrX haplotypes takes genetic

linkage, linkage disequilibrium, and recombination of appropriate population in consideration [4, 18]. The eight ChrX STRs were assigned to four linkage groups for their close physical distance [7], but whether they are assigned linkage is determined by the hotspots for recombination between the loci. Even if the physical distance of the loci is very close, the recombination and crossing-over might occur in case of existence of hotspots for recombination between them. Recombination occurring at low physical distances of ChrX has been reported by Edelmann et al. [19]. Analysis of linkage and linkage disequilibrium for the eight ChrX STRs has also been reported in a previous study, and a mathematical model was created for the estimation of recombination frequencies [6]. In our study, HapMap LD plots were used to analyze recombination and linkage disequilibrium of the eight loci, and pair-wise linkage disequilibrium testing was applied to authenticate the results of HapMap LD plots study.

The human genome includes LD blocks within which single nucleotide polymorphisms show strong association with each other [20]. It is widely accepted that the linkage disequilibrium structure of the human genome is shaped by recombination, and the gaps between LD blocks are hotspots for recombination [21–24]. If two STR loci are in the same LD block, their alleles are transmitted to offspring together, and the STR loci show significant linkage disequilibrium. On the contrary, if two STR loci are in different LD blocks, hotspots for recombination may exist between them and the recombination and crossing-over may occur during meiosis. In this work, we downloaded HapMap LD Plots of four pairs (DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS10134-DXS7423) from the HapMap website to observe if two STR their loci are in the same LD block. The results showed DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS10134-DXS7423 were not in the same LD block, respectively, and the hotspots for recombination were detected. Consequently, the probability of recombination and crossing-over among the eight loci might be high and the eight loci might be in linkage equilibrium.

For the purpose of authentication, the pair-wise linkage disequilibrium of the eight loci was tested in male samples with software ARLEQUIN 3.1. Exact p values below 5% were not found, and no evidence of linkage disequilibrium was detected. The results support the study of the HapMap LD plots and verify the assumption of linkage equilibrium. It meant that the haplotypes which were made up of random two or more loci among the eight loci could not be used for forensic practice. For the time being, this result should be regarded as limited to our sample from the Chinese Han population.

In conclusion, in spite of the eight ChrX STRs being assigned to four linkage groups for their close physical distance, our study found the hotspots for recombination between the eight loci and no evidence of linkage disequilibrium was detected. The results indicated that the recombination and crossing-over among the eight loci in the Mentype Argus X-8 PCR amplification kit might occur in the Chinese Han population. In light of the high combined power of discrimination in males and females and the combined power of paternity exclusion in trio and duo cases, we suggest that single locus frequencies should be used rather than haplotype frequencies for forensic practice in the Chinese Han population.

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