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Population genetic study of six closely linked groups of X-STRs in a Japanese population

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Abstract

X chromosome STR (X-STR) polymorphisms are a useful tool in the fields of human population genetics and personal identification and are quite informative in the investigation of complex kinship or deficiency cases, especially where it is necessary to determine relationships with second-generation offspring in which the same X chromosome may have been inherited. We investigated eight X-STR systems using the Mentype Argus X-8 kit and further developed decaplex PCR for the DXS10148, DXS10161, DXS10160, DXS10159, DXS10079, DXS10075, DXS6799, DXS10102, DXS10106, and DXS10146 loci with the aim of constructing closely linked groups on the X chromosome. The studied population comprised 569 Japanese individuals (390 males and 179 females). Heterozygosity among the present 18 X-STRs showed a distribution of from 54.2% to 90.5%. We constructed six closely linked groups, each comprising three to five X-STRs: DXS10148- DXS10135-DXS8378, DXS10161-DXS10160-DXS10159, DXS7132-DXS10079-DXS10074-DXS10075-DXS981, DXS6809-DXS6789-DXS6799, DXS10102-HPRTB-DXS10101-DXS10106, and DXS8377-DXS10146-DXS10134-DXS7423. The forensic utility of these groups as haplotypes was then evaluated. Haplotype diversity values showed a distribution of from 0.9699 to 0.9959. Analysis of the present closely linked haplotypes will contribute to solving complex kinship cases involving X chromosome inheritance.

Keywords: X chromosome ・ STR ・ Japanese ・ Multiplex PCR ・ Closely linked group

Introduction

The human X chromosome has been the focus of much research in the fields of population genetics and forensics in recent years, and closely linked groups of markers are becoming more attractive [1–9]. X-STRs can be used to complement autosomal STRs in paternity testing of female children. Closely

linked groups of markers on the X chromosome, in particular, are highly effective in determining relationships with second-generation offspring and can thus serve as a complement to autosomal STRs and mitochondrial DNA polymorphisms. For the forensic application of X-STR polymorphisms, however, it is important to collect population data and construct reference databases documenting genetic variation in specific STRs and possible haplotype frequencies within a given population. Furthermore, it is desirable to be able to obtain haplotype data with only a small number of multiplex PCR amplifications. The aim of this study was to increase the X-STR database, with the particular aims of identifying closely linked groups of more than three loci distributed throughout the entire X chromosome and obtaining the haplotype frequencies of those loci in the Japanese population. We have already reported a population study of 16X-STR polymorphisms in the Japanese population [10]. Three of those loci are included in the Mentype Argus X-8 kit. In this study, we investigated eight X-STR markers using the Mentype Argus X-8 kit and further developed decaplex PCR to complement construction of six closely linked groups of data in the Japanese population.

Materials and methods

Samples

Genomic DNA was extracted from blood samples obtained from 569 unrelated Japanese individuals (390 male and 179 female). Informed consent was obtained from all donors. This study was approved by the ethics committee of Tokyo Dental College (approval no. 202 and 204). DNA extraction was performed as described previously [10].

PCR amplification and typing of X-STRs

The DXS10135, DXS8378, DXS7132, DXS10074, HPRTB, DXS10101, DXS10134, and DXS7423 loci

(Fig. 1) were typed using the Mentype Argus X-8 kit (Biotype AG, Dresden, Germany) according to the manufacturer's instructions, apart from with a reduction in the amount of reaction mix used (10 µl). Electrophoresis was performed using the ABI PRIZM 310 Genetic Analyzer (Applied Biosystems) under the condition described in the manufacturer's recommendations. Decaplex PCR for DXS10106, DXS10159, DXS0148, DXS6799, DXS10146, DXS10075, DXS10079, DXS10161, DXS10102, and DXS10160 was carried out (Fig. 1). The primer sequences, concentrations used in the multiplex, type of labeling dye, and range of amplified fragment sizes are listed in Table 1. Sequences of primers were obtained from X-STR org (http://www.chrx-str.org) and other studies [4, 6, 11] but were partly changed except for one sequence depending on the profile of the multiplex electrophoretogram. Multiplex PCR was performed in a volume of 20 µl reaction mix containing: 3 ng genomic DNA, 1×10 mM Tris–HCl at pH 8.3, 50 mM KCl, 2.5 mM MgCl2, 0.001% gelatin, 200 µM dNTP, 1.75 U AmpliTaq Gold (Applied Biosystems), and an appropriate volume of each primer (Table 1). The PCR temperature profile was as follows: 11 min at 95°C followed by 50 s at 95°C and 105 s at 60°C for 28 cycles, with a final extension at 72°C for 60 min. Samples for electrophoresis were prepared as described in the direction for use of GeneScan-500 LIZ internal size standard (Applied Biosystems). Electrophoresis was performed using the ABI PRIZM 310 Genetic Analyzer (Applied Biosystems) with POP4 polymer. Fragment sizes were automatically determined using the GeneScan Analysis software 3.1 (Applied Biosystems) and results analyzed using the Genotyper ver. 2.5 (Applied Biosystems). Genotyping was performed through comparison with the sequenced samples and the DNA control reference sample 9947A (Applied Biosystems) to validate the typing protocol for multiplex X-chromosomal STRs [12].

Sequencing of the common and new alleles was performed on ABI 3130 Genetic Analyzer using BigDye Terminator v 1.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions”

Statistical analysis

The chromosomal location of the 22 markers was determined by querying the NCBI map viewer. Observed heterozygosity (Hobs) was calculated using female data with the PowerStatsV12 software (http://www.promega.com). Polymorphism information content, power of discrimination in females, power of discrimination in males, and power of exclusion were also calculated with the PowerStatsV12 software. Linkage disequilibrium and Hardy–Weinberg equilibrium were tested with the exact test using GENEPOP software (ver. 3.4) (http://genepop.curtin.edu.au).

Results and discussion

Population studies by Argus X-8 kit

A total of 390 unrelated male and 179 unrelated female individuals were analyzed using the Mentype Argus X-8 kit. The total number of male samples for DXS10134 was slightly less (388) because two samples were degraded and had been consumed (Table S1). Many of the individuals (339 male and 173 female) were the same as those previously described for 16-X-STR systems [10]. Therefore, these samples had already been typed for loci DXS7132, HPRTB, and DXS7423 by different multiplex systems [10] and were therefore repeatedly typed in this study. In the present study, no significant differences were observed in allele frequencies between male and female ($P>0.042$). The combined allele frequencies for both male and female are shown in Table S1 together with the statistical parameters obtained. The distribution of allelic frequencies in females was not significantly different from the Hardy–Weinberg equilibrium ($P>0.057$). Although the degree of diversity differed depending on the parameters (Table S1), most of the loci in the Argus X-8 kit were fairly informative in the Japanese population. Four earlier studies using the Argus X-8 kit to examine the same loci targeted in this study in East Asian populations were found, three on Japanese populations [13–15] and one on a Korean population [16]. In addition, four (DXS8378, DXS7132, HPRTB, and DXS7423) of the eight loci had been examined in the Japanese population by in-house multiplex PCR [17, 18]. Only

DXS10135 showed a significant difference in allelic frequency between that observed in these earlier reports in Japanese populations of similar size (P=0.0007 and P=0.0100 for Tamura et al. [14] and Tie et al. [15], respectively), while only DXS10135 and DXS7132 showed a significant difference in a Korean population [16]. However, significant differences were observed in all eight loci between the present data and those of Germans [13]. A comparison of allelic distribution of DXS10074 in our Japanese population with that in German, Hungarian, Finnish, Swedish, Italian, Ghanaian, and Somali populations [7, 13, 19, 20] revealed a characteristic difference: the distribution in the European populations was bimodal. The highest frequencies occurred at alleles 8 and 16, 17, and 18. A peak at allele 8 was also found in African populations [13, 20]. An absence of a peak at allele 8 in the Korean [16], Chinese [21], and Japanese populations suggests that this is characteristic of East Asian populations as a whole. DXS7132, HPRTB, and DXS7423 by different multiplex systems [10] and were therefore repeatedly typed in this study. In the present study, no significant differences were observed in allele frequencies between male and female (P>0.042). The combined allele frequencies for both male and female are shown in Table S1 together with the statistical parameters obtained. The distribution of allelic frequencies in females was not significantly different from the Hardy–Weinberg equilibrium (P>0.057). Although the degree of diversity differed depending on the parameters (Table S1), most of the loci in the Argus X-8 kit were fairly informative in the Japanese population.

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Construction of decaplex PCR system and allele designation

The ten X-STR loci in this study were selected to construct closely linked STR clusters as will be described below (Fig. 2). We determined the sequences of the common alleles of the ten loci. The repeat structure and allele designation were then further compared with those described at X-STR org (http://www.chrx-str.org) and in other reports [2–6, 11, 22] to ascertain whether they matched the established allele nomenclature. DXS10148 showed a highly complex repeat structure [4, 8]. The repeat structures of microvariant alleles not shown in a previous report [8] are shown in Table S2. There were essentially three types of allele in DXS10148: one designated by an integer, one including a decimal point and 1 (.1), and one including a decimal point and 2 (.2). Each type of allele showed a characteristic repeat structure. However, the second type including a decimal point and 1 in its structure could be classified into at least two types depending on the difference in the II and III repeat region, as shown in Table S2. Therefore, the same number of alleles (25.1) possessed a different structure. Because we constructed the 3' end of the DXS10148 forward primer at 10 bp upstream to that described by Hundertmark et al. [4], a new allele with an additional G following the primer sequence was found. This allele was designated as 28.3. This allele would not be found by the primers described previously [4]. We also typed the present 10-X-STRs for control reference sample 9947A to compare our results with those of panel cells. All of the allele types were identical to those described at X-STR org or in other reports [4, 6] except that for DXS6799.

Population studies of loci in decaplex PCR

A total of 390 unrelated male and 170 unrelated female individuals in a Japanese population were analyzed by our new decaplex PCR system, although the total number differed slightly between each locus due to consumption or degradation of samples (Table S3). No significant differences were observed in allele frequencies between male and female ($P>0.128$). The combined allele frequencies for both male and female are shown in Table S3.The distribution of allelic frequencies in females was not significantly different from the Hardy–Weinberg equilibrium ($P>0.093$). The degree of diversity was highest at the DXS10148 loci, and the other loci were also fairly informative in the Japanese population, except for DXS10106.

Because the loci included in the present decaplex PCR have not been examined in other Japanese populations, we compared the allele frequencies of the three loci DXS10148, DXS10079, and DXS10146 with those in a Korean population [8]. No significant differences were observed between the data obtained here and those of this earlier study ($P>0.064$) [8].

Linkage equilibrium analysis

Based on the results of typing for the 10 X-STR loci, tentative haplotypes composed of six physically closely linked locus groups (group 1: DXS10148-DXS10135-DXS8378, group 2: DXS10161-DXS10160-DXS10159, group 3: DXS7132-DXS10079-DXS10074-DXS10075-DXS981, group 4: DXS6809-DXS6789-DXS6799, group 5: DXS10102-HPRTB-DXS10101-DXS10106, and group 6: DXS8377-DXS10146-DXS10134-DXS7423) were constructed (Fig. 1, Table 2), each spanning <3.54 cM. Among these 22 loci, the data of four loci (DXS981, DXS6809, DXS6789, and DXS8377) were quoted from our previous population data [10]. A test for linkage disequilibrium was performed for all pairs of loci (Table S4). A significant deviation was observed between all pairs of loci within groups 1 ($P<0.0004$) and 2 ($P<0.0000$) and was observed between at least some pairs of loci within groups 3, 5,

and 6. Only in group 4 was no significant difference observed between DXS6809 and each of the other loci. Tests between each locus within the six groups and each locus in the other groups also showed a significant association at some loci, although each pair of loci was not closely linked. It is difficult to evaluate the results of linkage disequilibrium based on the present data alone.

In summary, significant linkage disequilibrium was observed for all pairs of loci in the combinations of DXS10148-DXS10135-DXS8378, DXS10161-DXS10160-DXS10159, and DXS10102-HPRTB-DXS10101 in the present Japanese population. These STR combinations span distances of 130, 770, and 110 kb, respectively (Table 2, Fig. 1). Although the STR combination in group 6 spans distances of as much as 140 kb, no significant linkage disequilibrium was observed in the present Japanese samples. The trio STR clusters of DXS6801-DXS6809-DXS6789, DXS10079-DXS10074-1DXS10075, DXS10146-DXS10134-DXS10147, DXS10148-DXS10135-DXS8378, and DXS10103-HPRTB-DXS10101 have been proposed as haplotypes [1–4, 6]. These STRs were studied further in other populations [7–9]. Although no linkage disequilibrium has been shown in recent studies [7, 21], linkage disequilibrium among these loci should also be investigated in even less-differentiated populations than those addressed hitherto. To allow for future comparison and possible usage in forensic cases in the Japanese population, we have presented data on haplotypes of the above-mentioned six groups (Table S5). All haplotypes showed a high diversity of more than 0.9699, suggesting their usefulness as tools in determining relationships in complex kinship cases (Table S5).

In conclusion, we investigated eight X-STRs using the Argus X-8 kit and ten X-STRs using our newly developed decaplex PCR system in a Japanese population. Six closely linked haplotypes composed of three to five loci were analyzed using the current and previously obtained data. The haplotype diversity values of these six closely linked haplotypes composed of at least three loci exceeded 0.9699 with a highest value of 0.9959, and the frequencies of the most common type showed less than 8.0% (Table S5). Although the stability of these haplotypes must be further studied, analysis of these possible linkage groups may provide indispensable information in solving complex kinship cases, such as

deficiency cases and second-generation family member analysis.

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Figure legend

Fig. 1 Location and combination of closely linked 22 X-STR loci. Physical localization is given in mega-base pairs. Five loci (DXS10135, DXS8378, DXS10074, DXS10101, and DXS10134) were analyzed by Argus X-8 kit and ten loci by our new decaplex kit (DXS10148, DXS10161, DXS10160, DXS10159, DXS10079, DXS10075, DXS6799, DXS10102, DXS10106, and DXS10146); seven loci (DXS7132, DXS981, DXS6809, DXS6789, HPRTB, DXS8377, and DXS7423) had been analyzed previously [10]

Fig. 2 Electrophoretic profiles of female and male subjects by decaplex PCR. All peaks showed single, balanced pattern

Table.1 Primer sequences used in this study.

locus	Size(bp)	Primer sequence		Dye labeled	Final primer concentration(pmol)
DXS 10106	94-122	5' 6-FAM-ATATCTGAATCTTGCACCCTTTTTTTC 3'	5' CAACAGAGCAAGACTCTGTC 3'	6-FAM	4
DXS 10159	131-175	5' 6-FAM-GACATAAGCGAAACTCTATCTCA 3'	5' TGACATGTTGCTGGATTTGG 3'*	6-FAM	2
DXS 10148	179-237	5' 6-FAM-GATTCTGTCTCAACAAAAAAAAAAAAAAGG 3'	5' GCTTCCTTTAATCTTTTGCTGGT 3'	6-FAM	2
DXS 6799	113-149	5' VIC-CTAGCAAACTGAATTTAGTAATGT 3'	5' TTTCTGAGATAACTACTTGCACATG 3'	VIC	2
DXS 10146	176-270	5' VIC-TGCCTTGCCCTTCCTACCT 3'	5' GAAAAAGAAAGAAAGACAGAGAAAGAAAGA 3'	VIC	1.6
DXS 10075	105-144	5' NED-CCAAGTTATTGCAGAGAAGAATCAT 3'	5' AGACTACCTCTGCTCCCTTTAAAAG 3'	NED	1.4
DXS 10079	162-206	5' NED-GCTGAGATTGTGCCAATGCT 3'	5' GCATCAATAATCATCAGGTATATTG 3'	NED	1
DXS 10161	222-238	5' NED-TCTGTATAAGCATCATGGGACTTCT 3'	5' CAAAATGTCTTTGAATTAGTGAAGGTTATC 3'	NED	1.4
DXS 10102	108-140	5' PET-GTGATGCTTGCCTCAATCTTAA 3'	5' ATAAATGTTTGTCAGAGTAAAAAAAAAAAG 3'	PET	4
DXS 10160	146-203	5' PET-TTATTTTCTGGGTTGAACACTTCTC 3'	5' GACTTTCCCTAGGTTAGGGGTATCA 3'	PET	6

* Edelmann et al. 2009

Table 2 Compiled data of haplotypes.

Group 1	No. of samples	No. of haplotypes	The most common haplotype (%)	No. of unique haplotypes	Haplotype diversity	Whole distance(Mb)
DXS10148 DXS10135 DXS8378	373	232	11 (2.9%)	152	0.9927	0.13
Group 2						
DXS10161 DXS10160 DXS10159	385	81	31 (8.1%)	24	0.9699	0.77
Group 3						
DXS7132 DXS10079 DXS10074 DXS10075 DXS981	356	308	5 (1.4%)	272	0.9962	3.54
Group 4						
DXS6809 DXS6789 DXS6799	364	133	23 (6.3%)	60	0.9837	2.44
Group 5						
DXS10102 HPRTB DXS10101 DXS10106	369	186	10 (2.7%)	114	0.9904	0.18
Group 6						
DXS8377 DXS10146 DXS10134 DXS7423	351	286	4 (1.1%)	239	0.9957	0.14