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SHORT COMMUNICATION

Genetic study of 12 X-STRs in Malay population living in and around Kuala Lumpur using Investigator Argus X-12 kit

Michinaga Samejima · Yasutaka Nakamura · Phrabhakaran Nambiar · Kiyoshi Minaguchi

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Abstract We investigated 12 X-chromosomal short tandem repeat (STR) polymorphisms in 283 unrelated Malay individuals (160 males and 123 females) living in and around Kuala Lumpur using the Investigator Argus X-12 kit. Heterozygosity among the present 12 X-STRs showed a distribution of from 55.3 to 93.5 %. The diversity values of the haplotypes constructed using four closely linked groups were all higher than 0.9865. A comparison of allelic frequency in each system and haplotype variation indicated that the nature of these X-STRs in the Malay population differed from that in East Asian, European, or African populations. Several microvariant alleles found in the Malay population were characterized and compared with known sequence data. The present data may be helpful in forensic casework such as personal identification and kinship testing in the Malay population in Malaysia.

Keywords X chromosome · STR · Malay · Argus X-12 · Microvariant allele · Closely linked group

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M. Samejima · Y. Nakamura · K. Minaguchi (✉)
Department of Forensic Odontology, Tokyo Dental College,
1-2-2 Masago, Mihama-ku,
Chiba City 261-0011, Japan
e-mail: minaguci@tdc.ac.jp

P. Nambiar
Department of General Dental Practice and Oral & Maxillofacial Imaging, Faculty of Dentistry, University of Malaya,
50603 Kuala Lumpur, Malaysia

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 [1–15]. X-chromosomal short tandem repeats (X-STRs) can be used to complement autosomal STRs in paternity testing in 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. Recently, the Argus X-12 kit, which can analyze four closely linked groups, each composed of three X-STR markers, became commercially available. To our knowledge, no studies have been published on X-STR polymorphisms in the Malay population. Therefore, we conducted a study of 12 X-STRs using the Argus X-12 kit and compared allelic distribution of each locus with that in other populations.

Materials and methods

Samples

Genomic DNA was extracted from tooth samples obtained from 283 unrelated Malay individuals (160 male and 123 female) living in and around Kuala Lumpur. Appropriate consent was obtained from the patients. A family history was also taken to ensure that their parents were of Malay origin. Tribal population samples were not included in the study. This study was approved by the ethics committee of Tokyo Dental College (approval no. 202 and 204) and met the conditions for cooperative study at the University of

Table 1 Allele frequencies of 12 X-STR loci in Malay population determined by Argus X-12 kit

Allele	DXS10103	DXS8378	DXS7132	DXS10134	DXS10074	DXS10101	DXS10135	DXS7423	DXS10146	DXS10079	HPRTB	DXS10148
6	–	0.002	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	0.002	–	–	–	–	–	–	–
8	–	–	–	–	0.025	–	–	–	–	–	–	–
9	–	0.032	–	–	0.002	–	–	–	–	–	–	–
10	–	0.495	–	–	–	–	–	–	–	–	0.002	–
11	–	0.305	0.002	–	–	–	–	–	–	–	0.138	–
12	–	0.140	0.049	–	–	–	–	–	–	–	0.259	–
13	–	0.025	0.244	–	–	–	–	0.002	–	–	0.392	–
14	0.005	–	0.276	–	0.022	–	–	0.534	–	–	0.145	–
15	0.007	–	0.345	–	0.052	–	–	0.374	–	–	0.062	–
16	0.261	–	0.071	–	0.128	–	0.002	0.086	–	0.012	0.002	–
17	0.081	–	0.012	–	0.281	–	0.034	0.002	–	0.140	–	0.002
18	0.207	–	–	–	0.328	–	0.027	–	–	0.128	–	0.138
18.3	–	–	–	–	–	–	–	–	–	0.002	–	–
19	0.387	–	–	–	0.140	–	0.084	–	–	0.219	–	0.052
19.1	–	–	–	–	–	–	0.002	–	–	0.002	–	0.005
20	0.049	–	–	–	0.020	–	0.081	–	–	0.212	–	0.047
21	0.002	–	–	–	–	–	0.099	–	–	0.180	–	0.047
21.1	–	–	–	–	–	–	–	–	–	–	–	0.005
22	–	–	–	–	–	–	0.101	–	0.005	0.076	–	0.017
22.1	–	–	–	–	–	–	0.002	–	–	–	–	0.022
23	–	–	–	–	–	–	0.099	–	0.012	0.020	–	–
23.1	–	–	–	–	–	–	–	–	–	–	–	0.106
24	–	–	–	–	–	–	0.116	–	0.032	0.002	–	–
24.1	–	–	–	–	–	–	–	–	–	–	–	0.111
25	–	–	–	–	–	–	0.079	–	0.062	0.005	–	–
25.1	–	–	–	–	–	–	–	–	–	–	–	0.121
26	–	–	–	–	–	0.002	0.059	–	0.177	–	–	–
26.1	–	–	–	–	–	–	–	–	–	–	–	0.131
27	–	–	–	–	–	0.015	0.039	–	0.209	–	–	–
27.1	–	–	–	–	–	–	–	–	–	–	–	0.074
27.2	–	–	–	–	–	0.010	–	–	–	–	–	–
27.3	–	–	–	–	–	0.007	–	–	–	–	–	–
28	–	–	–	–	–	0.015	0.052	–	0.163	–	–	–

Table 1 (continued)

Allele	DXS10103	DXS8378	DXS7132	DXS10134	DXS10074	DXS10101	DXS10135	DXS7423	DXS10146	DXS10079	HPRTB	DXS10148
28.1	–	–	–	–	–	–	–	–	–	–	–	0.054
28.2	–	–	–	–	–	0.057	–	–	–	–	–	–
28.3	–	–	–	–	–	0.002	–	–	–	–	–	–
29	–	–	–	0.002	–	0.027	0.030	–	0.143	–	–	–
29.1	–	–	–	–	–	–	–	–	–	–	–	0.032
29.2	–	–	–	0.002	–	0.071	–	–	–	–	–	–
30	–	–	–	0.002	–	0.089	0.027	–	0.089	–	–	–
30.1	–	–	–	–	–	–	–	–	–	–	–	0.025
30.2	–	–	–	–	–	0.099	–	–	–	–	–	–
31	–	–	–	0.017	–	0.163	0.020	–	0.044	–	–	0.002
31.1	–	–	–	–	–	–	–	–	–	–	–	0.005
31.2	–	–	–	–	–	0.067	–	–	–	–	–	–
32	–	–	–	0.012	–	0.167	0.007	–	0.012	–	–	–
32.1	–	–	–	–	–	–	–	–	–	–	–	0.002
32.2	–	–	–	–	–	0.049	–	–	–	–	–	–
33	–	–	–	0.059	–	0.108	0.015	–	0.017	–	–	–
33.2	–	–	–	–	–	0.010	–	–	–	–	–	–
34	–	–	–	0.067	–	0.030	0.010	–	0.012	–	–	–
34.2	–	–	–	0.002	–	0.002	–	–	–	–	–	–
35	–	–	–	0.145	–	0.007	0.010	–	0.002	–	–	–
36	–	–	–	0.202	–	0.002	–	–	–	–	–	–
36.3	–	–	–	0.005	–	–	–	–	–	–	–	–
37	–	–	–	0.251	–	–	0.002	–	–	–	–	–
37.3	–	–	–	0.010	–	–	–	–	–	–	–	–
38	–	–	–	0.145	–	–	–	–	–	–	–	–
38.3	–	–	–	0.010	–	–	–	–	–	–	–	–
39	–	–	–	0.042	–	–	0.002	–	–	–	–	–
39.3	–	–	–	0.005	–	–	–	–	–	–	–	–
40.1	–	–	–	–	–	–	–	–	–	–	–	0.002
40.2	–	–	–	–	–	–	–	–	0.005	–	–	–
40.3	–	–	–	0.002	–	–	–	–	–	–	–	–
41.2	–	–	–	–	–	–	–	–	0.005	–	–	–
41.3	–	–	–	0.010	–	–	–	–	–	–	–	–
42.2	–	–	–	–	–	–	–	–	0.005	–	–	–
42.3	–	–	–	0.005	–	–	–	–	–	–	–	–

Table 1 (continued)

Allele	DXS10103	DXS8378	DXS7132	DXS10134	DXS10074	DXS10101	DXS10135	DXS7423	DXS10146	DXS10079	HPRTB	DXS10148
43.2	–	–	–	–	–	–	–	–	0.005	–	–	–
43.3	–	–	–	0.002	–	–	–	–	–	–	–	–
No. of males	160	160	160	160	160	160	160	160	160	160	160	160
No. of females	123	123	123	123	123	123	123	123	123	123	123	123
P	0.277	0.312	0.239	0.926	0.352	0.199	0.224	0.287	0.759	0.531	0.782	0.234
H_{obs}	0.715	0.585	0.813	0.805	0.805	0.862	0.935	0.553	0.854	0.813	0.740	0.886
PDf	0.876	0.826	0.855	0.951	0.908	0.975	0.983	0.746	0.964	0.948	0.890	0.977
PDm	0.731	0.627	0.737	0.856	0.765	0.893	0.915	0.550	0.856	0.811	0.723	0.906
PE	0.453	0.274	0.623	0.608	0.608	0.718	0.867	0.238	0.702	0.623	0.493	0.767
PIC	0.68	0.58	0.69	0.81	0.75	0.89	0.93	0.49	0.85	0.82	0.70	0.90

P values of exact test for Hardy–Weinberg equilibrium

H_{obs} observed heterozygosity, *PDf* power of discrimination in women, *PDm* power of discrimination in men, *PE* power of exclusion, *PIC* polymorphism information content

Malaya. Isolation of genomic DNA from tooth samples was performed as described previously [16], except that tooth samples were decalcified with EDTA for 2 days.

PCR amplification and typing of X-STRs

The DXS10148, DXS10135, DXS8378, DXS7132, DXS10079, DXS10074, DXS10103, HPRTB, DXS10101, DXS10146, DXS10134, and DXS7423 loci were typed using the Investigator Argus X-12 kit (Qiagen, Hilden) according to the manufacturer's instructions, apart from 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 conditions described in the manufacturer's recommendations. 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). Moreover, microvariant alleles of DXS10148, DXS10134, and DXS10079 were amplified using primers as described previously [11]. Sequencing of new alleles was performed on the ABI 3130 Genetic Analyzer using the BigDye Terminator v 1.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions.

Statistical analysis

Observed heterozygosity (H_{obs}), polymorphism information content, power of discrimination in females, power of discrimination in males, and power of exclusion were also calculated with the PowerStatsV12 software (http://www.promega.com). Comparison of allele frequencies between populations were compared by the *G* test using R software (version 2.14.1, http://www.r-project.org/). Linkage disequilibrium and Hardy–Weinberg equilibrium were tested with the exact test using GENEPOP software (version 4.0.10, http://genepop.curtin.edu.au).

Results and discussion

Population study using Argus X-12 kit

A total of 160 unrelated male and 123 unrelated female individuals from the Malay population living in and around Kuala Lumpur were analyzed using the Investigator Argus X-12 kit. No significant differences were observed in allele frequencies between male and female in any of the loci ($P > 0.02284$). The combined allele frequencies for both male and female are shown in Table 1 together with the statistical parameters. The distribution of allelic frequencies in females was not significantly different from the Hardy–Weinberg equilibrium ($P > 0.1988$). Although degree of diversity

differed depending on the parameters (Table 1), most of the loci in the Argus X-12 kit were fairly informative in the Malay population, with observed heterozygosities of over 0.553 (DXS7423). Calculation of the combined power of discrimination of the 12 loci showed 0.999999997 and 0.999999999999993 for males and females, respectively.

Because no data have been reported on X-STR polymorphisms in the Malay population, we compared allelic frequencies of the 12 loci with those of East Asian [6, 11, 12], European [Germans from X-STRorg (http://www.chrx-str.org)], and African populations [13] to determine the general features of the X-STRs in the Malay population (Table S1). Among 11 of these 12 loci (excluding DXS10103), significant differences were observed in allele frequencies in 9 loci ($P<0.0023$, excluding DXS10135 and DXS8378) between the present data and the data of a Japanese population [11]. The present data showed significant differences in 9 of the 12 loci in comparison with the Korean population ($P<0.0021$; excluding DXS7132, DXS10103, and DXS10146) [6], and in 7 loci in comparison with the South Chinese population ($P<0.0000001$; excluding DXS10135, DYS8378, HPRTB, DXS10146, and DXS10134) [12]. The data also revealed significant differences in 10 of the loci in comparison with the German population ($P<0.0012$; excluding DXS10135 and DXS10103; X-STRorg) and in 11 loci in comparison with the Somali population (excluding DXS7132) [13]. These features suggest that allelic distribution of many X-STRs in the Malay population differs from that in East Asian, European, and African populations. Allelic distribution of DXS10074 in the European population was bimodal, with the highest frequencies at alleles 8, 16, and 17 or 18 [8, 17–19]. A peak at allele 8 was also found in African populations [17, 19], but has not been reported in East Asian populations [6, 11, 12, 20]. This group of alleles was observed in the Malay population at a frequency of 2.65 % (11 individuals), suggesting that this is yet another group not derived from East Asia that is contained in the Malay X chromosome.

Microvariant alleles

Several rare alleles were found in the Malay population (Table 2). In DXS10148, large alleles 40.1 and 31 were observed. Although allele 40.1 is the largest one found so far in an East Asian population [6, 11, 12], large alleles are fairly frequent in Algerians and Ivorians [14, 21]. The largest allele so far found in East Asians was 33.1 in Korean [6, 11, 12]. The frequencies of larger allele than 35.1 found in the African populations were 5.1 % in Algerians and 12.1 % in Ivorians. Because no repeat structure for allele 40.1 has yet been demonstrated in Ivorians, we compared it with allele 38.1 in the Danish population [13], alleles 38.1 and 41.1 in the Algerian population [21] (Table 2), and other alleles reported so far [4, 6, 11, 13] (Table S2). As shown in Table 2, a few mutational events appear to have occurred in the common allelic structure. However, structure appears to be partly similar to the allele in Danes but dissimilar among the known alleles. Allele 31 was also found in Swedish population [15], and similar-sized alleles in Somalian populations [13], but was the largest one found in East Asians among alleles designated by an integer (Table 2). The repeat structural pattern was the same as that of the common allele designated by an integer. The second largest repeat number of an allele designated by an integer in the Malay population

Table 2 Comparison of the repeat structure of microvariant alleles

	Allele	Repeat structure		Reference
DXS10148	17–21	[GGAA]4 -[AAGA]n -[AAAG]4-N8-[AAGG]2	n=7~11	[11]
	31[a]	[GGAA]4 -[AAGA]21 -[AAAG]4-N8-[AAGG]2		This study
	38.1	[GGAA]4 -[AAGA]13 -A-[AAGA]15 -[AAAG]4-N8-[AAGG]2		[21]
	38.1	[GGAA]4 -[AAGA]12-[AAGG]3 -A-[AAGA]13 -[AAAG]4-N8-[AAGG]2		[21]
	41.1	[GGAA]4 -[AAGA]17 -A-[AAGA] 9-TAGA-[AAGA]4 -[AAAG]4-N8-[AAGG]2		[21]
	38.1	[GGAA]2 -AGAA-GGAA -[AAGA]15 -A-[AAGA] 8-TAGA-[AAGA]4 -[AAAG]4-N8-[AAGG]2		[13]
	40.1[a]	[GGAA]2 -AGAA-GGAA -[AAGA]18 -A-[AAGA]12 -[AAAG]4-N8-[AAGG]2		This study
DXS10134	29.2[a]	(GACAGA)2-[GAAA]-GTAA-[GAAA]3-AAA-[GAAA]3-AAA -[GAAA]12		This study
	34.2[a]	(GACAGA)3-[GAAA]-GTAA-[GAAA]3-AAA-[GAAA]4-AAA-[GAAA]7 -AA -[GAAA]7		This study
	37.2–39.2	(GACAGA)3-[GAAA]-GTAA-[GAAA]3-AAA-[GAAA]4-AAA-[GAAA]4 -AA -[GAAA]n	n=13~15	[18]
DXS10079	14–25	[AGAA]n -AGAG-[AGAA]3	n=10~21	[2]
	19.1[a]	[AGAA]3 -A -[AGAA]12 -AGAG-[AGAA]3		This study
	18.3[a]	[AGAA]2 -AGA -[AGAA]12 -AGAG-[AGAA]3		This study
	19.3	[AGAA]5 -AGA -[AGAA]10 -AGAG-[AGAA]3		[2]

[a] Microvariant alleles found in this study

was 22, suggesting the uniqueness of the allele 31 in the Malay population.

Allele 29.2 was the smallest allele including 0.2 at DXS10134 as reported so far. It had a deletion of a 6-bp repeat motif, GACAGA, in its complex repeat structure, unlike in common alleles designated by an integer (Table 2 and S2). A Japanese microvariant, 35.2, however, had the same motif (Table 2 and S2). Another microvariant allele 34.2 including 0.2 was also found in the present study. Its repeat structure was partly similar to that in alleles 37.2 38.2 and 39.2 in Hungarians [18], although a 2-bp insertion with the partial sequence $[GAAA]_7AA[GAAA]_7$ occurred at a different position (Table 2 and S2). Allele 33.2 has been reported in the Somali population [13]. Although its repeat structure remains to be determined, various patterns appeared at 10134 due to the complexity of its repeat motif.

Allele sizes other than those indicated by an integer are not frequent in many populations at DXS10079 [6, 11, 12, 13, 14, 15, 21, X-STRorg]. Alleles 18.3 and 19.1 in DXS10079 were found in the Malay population. The repeat structure of the 18.3 allele shared the same pattern as the 19.3 allele found in Germans [2], which may have resulted from A deletion in the AGAA repeat structure. The structure of allele 19.1 showed a new pattern with A insertion in the AGAA repeat structure. Because no other information on repeat structures of 20.3 in Chinese [12], 18.3 and 19.3 in Algerians [21], or 16.1 in Somalis [13] is available, we could not compare their differences any further.

Linkage equilibrium analysis and haplotype

Based on the results of typing for the 12 X-STR loci, a test for linkage disequilibrium was performed for all pairs of loci in the Malay population (Table S3). A significant deviation was observed between pairs DXS10103–HPRTB and DXS10103–DXS10101 in linkage group 3, and between pair DXS10146–DXS10134 in linkage group 4. However, no significant difference was observed between each of the other loci. The tendencies of deviation for linkage disequilibrium were different between the Malay and other populations, such as Japanese [11], Chinese [12], Swedish [15], and Ivorian [14]. To allow further comparisons and possible usage in forensic cases in the Malay population, the data have been presented as haplotypes (Table S4).The compiled data on haplotypes of the four linkage groups in the present study are shown in Table S5 together with those of Japanese [11] Korean [6], Greenlander [13], German [X-STRorg], Swedish [15], Danish, Somali [13], and Ivorian populations [14]. Haplotype diversity in the present study in each group showed distribution within 0.9865–0.9973. In the Malay population, the order of values was highest in linkage group 1, followed by groups 4, 2, and 3. The value of linkage group 1 was the highest among all reference populations. However, the order of values among linkage groups 2, 3, and 4 differed depending on the reference population.

In conclusion, we characterized 12 X-STR polymorphisms in the modern Malay population using the Investigator Argus X-12 kit. Most of the loci examined were fairly or highly polymorphic, and the four closely linked groups, each composed of 3 X-STR markers, also showed a highly polymorphic nature in the Malay population. The present results will afford useful information for forensic casework in Malaysia.

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