



Genetic Polymorphism and Haplotype Analysis of Mentype® Argus X-8 STR Loci in Koreans

Eun Jin Lim¹ · Jeong Eun Sim¹ · Hwan Young Lee¹ · Myung Jin Park¹ · Na Young Kim¹ · Hyung-Joon Ahn² · Woo Ick Yang¹ · Kyoung-Jin Shin¹

¹Department of Forensic Medicine and Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea.

²Department of Oral Medicine, Yonsei University College of Dentistry, Seoul 120-752, Korea

Introduction

Gonosomal markers have been efficiently used for solving deficiency cases. Due to their hemizyosity and high mean exclusion chances, X-chromosomal STRs (X-STRs) are particularly helpful in paternity testing and kinship analyses, such as father-daughter, mother-son, and grandmother-granddaughter kinship testing, or the kinship testing of putative sisters. However, since the degree of polymorphism is limited in STRs and highly polymorphic STRs like DXS10011 are prone to mutations, stable haplotypes of closely linked loci were suggested for use in kinship testing instead of a single STR.

Recently, for practical aspects, four X-chromosomal STR duos in the linkage groups 1-4 (DXS10135-DXS8378, DXS7132-DXS10074, HPRTB-DXS10101, and DXS10134-DXS7423) were chosen and used to construct the commercially available forensic Chromosome X typing kit, Mentype® Argus X-8 (Biotype AG, Dresden, Germany). Since each of the four STR clusters spans less than 0.5 cM and the genetic distance between linkage groups is more than 50 cM, the clusters represent stable haplotypes that can be treated as unlinked, thereby providing highly informative tools for kinship testing.

In the present study, we investigated the polymorphisms and haplotypes of the Argus X-8 loci in Koreans and evaluated their efficiency in forensic practice. In addition, we provided relevant information about population differences with respect to allele distribution patterns and haplotype diversities.

Materials and Methods

DNA Samples

Blood or buccal samples from 450 unrelated Koreans (i.e. 300 males and 150 females) were analyzed. Genomic DNA was extracted using a QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

PCR Amplification and Genotyping

The genomic DNA was amplified using the Mentype® Argus X-8 PCR amplification Kit (Biotype AG) following the manufacturer's protocols. The PCR products were analyzed by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The fragment sizes were determined using the GeneScan 3.7 software (Applied Biosystems) and the allele designations were carried out using Genotyper 3.7 software (Applied Biosystems) with the Argus X-8_v1.gta macro (Biotype AG).

Sequence Analysis of Off-ladder Alleles

Sequence analyses were performed on samples which showed alleles that could not be designated using allelic ladder of the Mentype® Argus X-8 PCR amplification kit. Primers for PCR amplification of off-ladder alleles were designed using the Primer 3 software (<http://frodo.wi.mit.edu/primer3/input.htm>). Each PCR products was cloned using TOPO TA Cloning® Kit (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation. Then, at least two clones were chosen and sequenced on an ABI 3130xl Genetic Analyzer using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems).

Statistical Analysis

Polymorphism information content (PIC), expected heterozygosity (Het), mean exclusion chances (MEC) for trio and duo cases, and power of discrimination (PD) for males and females were calculated at the ChrX research homepage (<http://www.chrx-str.org>). Exact tests of Hardy-Weinberg equilibrium (HWE) and population differentiation were carried out using the Arlequin 2.0 software (<http://lgb.unige.ch/arlequin>). Data from Germans (Becker D *et al.*, *FSI Genetics* 2008;2:69-74), Ghanaian (Thiele K *et al.*, *FSI Genetics Suppl* 2008;1:167-9) and Japanese (Hashiyada M *et al.*, *FSI Genetics Suppl* 2008;1:150-2) were employed for the exact test of population differentiation. Haplotypes of four closely linked STR pairs were obtained from male genotypes and haplotype diversity was calculated using Arlequin 2.0 software.

Results

Table 1. Allele frequencies of the Mentype® Argus X-8 STRs in a Korean population

DXS7132		DXS7423		DXS8378		HPRTB		DXS10074		DXS10101		DXS10134		DXS10135	
Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.
10	0.0017	13	0.0017	7	0.0017	10	0.0033	12	0.0033	26.2	0.0017	30	0.0017	16	0.0100
11	0.0067	14	0.2833	9	0.0050	11	0.0400	13	0.0017	27	0.0117	31	0.0017	17	0.0100
12	0.0833	15	0.6583	10	0.5767	12	0.3183	14	0.0117	28	0.0167	32	0.0250	18	0.0666
13	0.2017	16	0.0533	11	0.3000	13	0.4067	15	0.0617	28.2	0.0167	33	0.0317	19	0.0882
14	0.3133	17	0.0033	12	0.1117	14	0.1917	16	0.1833	29	0.0317	34	0.0717	20	0.1032
15	0.2983			13	0.0050	15	0.0300	17	0.3067	29.2	0.0450	34.2	0.0017	21	0.1314
16	0.0717					16	0.0100	17.3	0.0033	30	0.1017	35	0.1567	21.3	0.0033
17	0.0183							18	0.2750	30.2	0.0917	35.3	0.0033	22	0.1265
18	0.0050							19	0.1267	31	0.1767	36	0.2217	22.2	0.0017
								20	0.0250	31.1	0.0017	37	0.2183	23	0.0799
								21	0.0017	31.2	0.1467	37.1	0.0017	24	0.0765
								22		32	0.1633	37.3	0.0117	25	0.0599
								23		32.2	0.0783	38	0.1250	26	0.0333
								24		33	0.0683	38.3	0.0267	27	0.0499
								25		33.2	0.0300	39	0.0650	28	0.0516
								26		34	0.0150	39.3	0.0083	29	0.0349
								27		34.2	0.0033	40	0.0150	29.2	0.0017
								28		41	0.0017	30	0.0150	30	0.0150
								29		41.3	0.0050	31	0.0166	31	0.0166
								30		42.3	0.0017	32	0.0183	32	0.0183
								31		43.3	0.0050	33	0.0100	33	0.0100
								32				34	0.0033	34	0.0033
								33				35	0.0033	35	0.0033
								34				36	0.0033	36	0.0033
								35				37	0.0017	37	0.0017
								36				38	0.0033	38	0.0033
								37				39	0.0033	39	0.0033
								38				40	0.0033	40	0.0033
								39				41	0.0033	41	0.0033
								40				42	0.0033	42	0.0033
								41				43	0.0033	43	0.0033
								42				44	0.0033	44	0.0033
								43				45	0.0033	45	0.0033
								44				46	0.0033	46	0.0033
								45				47	0.0033	47	0.0033
								46				48	0.0033	48	0.0033
								47				49	0.0033	49	0.0033
								48				50	0.0033	50	0.0033
								49				51	0.0033	51	0.0033
								50				52	0.0033	52	0.0033
								51				53	0.0033	53	0.0033
								52				54	0.0033	54	0.0033
								53				55	0.0033	55	0.0033
								54				56	0.0033	56	0.0033
								55				57	0.0033	57	0.0033
								56				58	0.0033	58	0.0033
								57				59	0.0033	59	0.0033
								58				60	0.0033	60	0.0033
								59				61	0.0033	61	0.0033
								60				62	0.0033	62	0.0033
								61				63	0.0033	63	0.0033
								62				64	0.0033	64	0.0033
								63				65	0.0033	65	0.0033
								64				66	0.0033	66	0.0033
								65				67	0.0033	67	0.0033
								66				68	0.0033	68	0.0033
								67				69	0.0033	69	0.0033
								68				70	0.0033	70	0.0033
								69				71	0.0033	71	0.0033
								70				72	0.0033	72	0.0033
								71				73	0.0033	73	0.0033
								72				74	0.0033	74	0.0033
								73				75	0.0033	75	0.0033
								74				76	0.0033	76	0.0033
								75				77	0.0033	77	0.0033
								76				78	0.0033	78	0.0033
								77				79	0.0033	79	0.0033
								78				80	0.0033	80	0.0033
								79				81	0.0033	81	0.0033
								80				82	0.0033	82	0.0033
								81				83	0.0033	83	0.0033
								82				84	0.0033	84	0.0033
								83				85	0.0033	85	0.0033
								84				86	0.0033	86	0.0033
								85				87	0.0033	87	0.0033
								86				88	0.0033	88	0.0033
								87				89	0.0033	89	0.0033
								88				90	0.0033	90	0.0033
								89				91	0.0033	91	0.0033
								90				92	0.0033	92	0.0033
								91				93	0.0033	93	0.0033
								92				94	0.0033	94	0.0033
								93				95	0.0033	95	0.0033
								94				96	0.0033	96	0.0033
								95				97	0.0033	97	0.0033
								96				98	0.0033	98	0.0033
								97				99	0.0033	99	0.0033
								98				100	0.0033	100	0.0033

Table 2. Forensic statistical parameters of the Mentype® Argus X-8 STRs calculated in a Korean population

Parameter	DXS7132	DXS7423	DXS8378	HPRTB	DXS10074	DXS10101	DXS10134	DXS10135
PIC	0.722	0.412	0.494	0.639	0.742	0.875	0.834	0.914
Het	0.761	0.485	0.565	0.695	0.777	0.888	0.852	0.921
MEC _{trio}	0.722	0.412	0.494	0.639	0.742	0.875	0.834	0.914
MEC _{duo}	0.586	0.276	0.350	0.494	0.610	0.788	0.729	0.847
PD _{female}	0.904	0.662	0.740	0.852	0.916	0.976	0.961	0.988
PD _{male}	0.760	0.484	0.565	0.694	0.776	0.886	0.851	0.920

* No significant deviation from Hardy-Weinberg equilibrium was observed in all eight loci. ($p > 0.01$)

Table 3. Off-ladder alleles of the Mentype® Argus X-8 kit in a Korean population

Locus	Allele ^a	Repeat structure ^b	N
DXS7132	10	(TCTA) ₁₀	1
	18	(TCTA) ₁₈	3
DXS8378	7	(CTAT) ₇	1
DXS10074	17.3	(AAGA) ₄ AGA(AAGA) ₁₀ AAGG(AAGA) ₂	1
	17.3	(AAGA) ₁₂ AAA(AAGA) ₂ AAGG(AAGA) ₂	1
DXS10134	30	(GAAA) ₃ gaga(GAAA) ₂ aa(GAAA) ₃ gaga(GAAA) ₃ gaga(gacaga) ₃ (GAAA) ₃ gtaa(GAAA) ₃ aaa(GAAA) ₄ aaa(GAAA) ₁₀	1
	34.2 (36)	(GAAA) ₃ gaga(GAAA) ₄ aa(GAAA) ₃ gaga(GAAA) ₄ gaga(gacaga) ₂ gagaga(GAAA) ₃ gtaa(GAAA) ₃ aaa(GAAA) ₄ aaa(GAAA) ₁₆	1
	37.1 (37)	Δ(GAAA) ₃ gaga(GAAA) ₄ aa(GAAA) ₃ gaga(GAAA) ₄ gaga(gacaga) ₃ (GAAA) ₃ gtaa(GAAA) ₃ aaa(GAAA) ₄ aaa(GAAA) ₁₇	1