Haplotype and mutation analysis for newly suggested Y-STRs in Korean father-son pairs

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Introduction

Spontaneous germline mutations, which lead to differentiation of Y-STR haplotypes between the father and his sons, can result in an erroneous exclusion of biological paternity. However, frequent germline mutations at specific loci have been proposed to facilitate the identification of male individual in forensic investigations. Because high correlation exists between the degree of polymorphism and the mutation rate of a given locus, highly polymorphic STR loci may have high mutation rates. For forensic purposes highly polymorphic loci are more attractive, but markers with a high mutation rate should be carefully used in paternity testing. Therefore, reliable estimates of mutation rates are required to be calculated for the proper use of Y-STRs and non-erroneous interpretation of the genetic profiles. Recently developed Y-STR analysis kits, the PowerPlex® Y23 system and the YFiler® Plus system, include several new Y-STR loci with high gene diversity. Some of these newly added loci were reported to have mutation rates of > 1%, and thereby considered rapidly mutating Y-STR (RM Y-STR). Therefore, we constructed an in-house multiplex PCR system for 14 Y-STRs (DYS385a/b, DYF387S1, DYS391, DYS449, DYS460, DYS481, DYS518, DYS533, DYS549, DYS570, DYS576, DYS627 and DYS643) which include 11 newly added loci to the PowerPlex Y23 system or the Yflier Plus system, and analyzed haplotype transfers between 363 Korean father-son pairs.

Materials and Methods

DNA samples

DNA from 726 male samples already typed for 22 Y-STRs in a previous report were selected and analyzed (according to the protocol approved by Institutional Review Board of Severance Hospital at Yonsei University). These samples consisted of 351 unrelated individuals who had one or two respective sons. Genomic DNA was extracted from buccal swab samples using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Multiplex PCR amplification of 14 Y-STRs

An in-house multiplex PCR system was constructed for the amplification of 14 Y-STRs. The multiplex PCR system included 11 Y-STR which were newly added to the PowerPlex® Y23 system (Promega, Madison, WI, USA) or the YFiler® Plus system (Applied Biosystems, Foster City, CA, USA), and commonly used DYS385 a/b and DYS391. PCR amplifications were conducted in a final volume of 10 µl containing 1.0 ng template DNA, 1.0 µl of Gold ST*R 10X buffer (Promega), 2.5 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems) and the proper concentration of primers. Thermal cycling was performed under the following conditions : 95°C for 11 m; 28 cycles of 94°C for 20 s, 60°C for 90 s, 72°C for 60 s; and a final extension of 60°C for 45 min.

Electrophoresis and genotyping

The PCR products were mixed with GeneScan™ 500 LIZ® size standard (Applied Biosystems) and separated by capillary electrophoresis using a 3130 Genetic Analyzer (Applied Biosystems). The genotyping of PCR products at each STR locus was performed by comparing to an allelic ladder using GeneMapper® ID Software v3.2 (Applied Biosystems). Allele nomenclature at each locus followed the recommendations of the International Society of Forensic Genetics (ISFG) Commission.

Identification of mutations

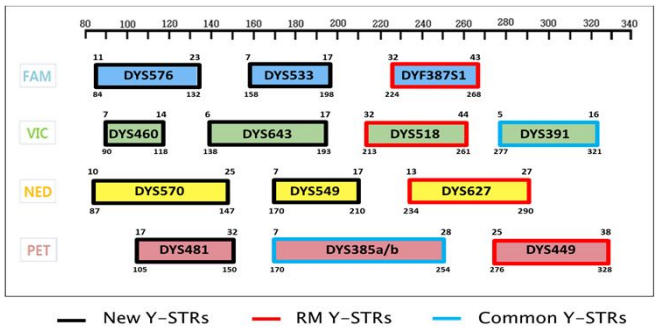
Mutations were analyzed by electrophoresis as allele length differences between father and son. To confirm mutations, father-son pairs who showed mutations were reanalyzed using the same method as described above. In addition, all pairs with a mutation were analyzed using the PowerPlex®16 HS system (Promega) to confirm biological paternity.

Statistical analysis

Haplotype frequencies and haplotype diversities were estimated according to the Nei's formula and mutation rates were calculated as the number of mutations divided by the number of allele transmissions.

Results

Allelic size range for 14 loci of an in-house multiplex PCR system, Euplex Y14



Haplotype diversities of each different multiplex system in 351 unrelated Koreans

	AmpFℓSTR® Yfiler™ (17)[a]	PowerPlex®Y23 (23)	AmpFℓSTR®Yfiler™ Plus (NGY) (27)	Euplex Y14 (11)[b]	Total (28)
No. of samples	351	351	351	351	351
Different haplotypes	338	347	351	350	351
Unique haplotypes	330	343	351	349	351
Discrimination capacity (%)	96.30	98.86	100.00	99.72	100.00
Haplotype diversity	0.99963	0.99994	1.00000	0.99998	1.00000

[a] The number in parenthesis is the number of Y-STRs

[b] Number of loci except for DYS385a/b and DYS391

Gene diversities and mutation estimates for 28 Y-STRs

Locus	Gene Diversity	Mutation rates: Korean No. of mutations	Korean No. of allele transmissions	Korean Mutation rate (x10^{-3})[b]	Previous literature summary[a] No. of mutations	Previous literature summary No. of allele transmissions	Previous literature summary Mutation rate (x10^{-3})	Total No. of mutations	Total No. of allele transmissions	Total Mutation rate (x10^{-3})
385	0.96117	2	738	2.70	63	28683	2.20	33	14711	2.45
387S[c]	0.94566	2	726	2.80	28	1804	15.90	15	1265	9.35
518	0.86134	2	363	5.50	28	1556	18.40	15	960	11.95
449	0.85635	7	363	19.30	19	1617	12.20	13	990	15.75
627	0.83279	4	363	11.00	21	1766	12.30	13	1065	11.65
570	0.79689	1	363	2.80	24	1981	12.12	13	1172	7.46
481	0.79407	3	363	8.30	11	2147	5.12	7	1255	6.71
458	0.79300	3	369	8.10	55	8119	6.77	29	4244	7.44
643	0.78997	0	363	0.00	2	2328	0.86	1	1346	0.43
576	0.77436	6	363	16.50	33	2282	14.46	20	1323	15.48
448	0.76855	0	369	0.00	10	8111	1.23	5	4240	0.62
389-II	0.72558	2	369	5.40	55	15188	3.62	29	7779	4.51
19	0.70232	2	369	5.40	42	16981	2.47	22	8675	3.94
392	0.68090	0	369	0.00	7	16281	0.43	4	8325	0.22
390	0.67704	1	369	2.70	33	16505	2.00	17	8437	2.35
635	0.67196	3	369	8.10	31	8943	3.47	17	4656	5.79
389-I	0.66919	2	369	5.40	43	15225	2.82	23	7797	4.11
439	0.65008	2	369	5.40	57	11518	4.95	30	5944	5.18
460	0.64943	1	363	2.80	10	1717	6.22	6	1040	4.51
393	0.63028	1	369	2.70	17	15149	1.12	9	7759	1.91
549	0.60645	1	363	2.80	8	2239	3.57	5	1301	3.19
H4	0.60200	1	369	2.70	23	9150	2.51	12	4760	2.61
438	0.59038	0	369	0.00	5	11559	0.43	3	5964	0.22
456	0.53843	2	369	5.40	35	8121	4.31	19	4245	4.86
533	0.50740	2	363	5.50	8	1730	5.01	5	1047	5.26
437	0.44182	1	369	5.40	14	11547	1.21	8	5958	3.31
391	0.24991	0	369	0.00	43	16380	2.63	22	8375	1.32

[a] Mutation rate estimate from Ballantyne *et al.* Am J Human Genet: 2010, Burgarella *et al.* Eur J Human Genet: 2011 and YHRD

[b] Mutation rate estimates for Yfiler loci from Lee *et al.* Int J Leg Med: 2006 and mutation raters for the rest 11 Y-STRs from the present study

[c] 11 Y-STRs from the Euplex Y14 underlined and indicated in bold

Conclusions

This study demonstrates the haplotype diversities and mutation rates for 11 Y-STRs obtained from 363 father-son pairs of 351 Korean families using the Euplex Y14 system. In haplotype analysis, 11 Y-STRs showed high discrimination capacity in Koreans with 350 unique haplotypes from 351 unrelated individuals. In addition, these 11 Y-STRs had high locus-specific mutation rates up to 1.93×10^{-2}. Especially, DYS449, DYS576 and DYS627 loci showed mutation rate estimates over 1%. However, DYF387S1, DYS518 and DYS570 loci which showed mutation rate estimates over 1% in other populations had considerably lower mutation rate in Koreans. The results of this study will be useful for the interpretation of Y-STR profiles in forensic cases as well as in paternity analysis.

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