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Announcement of population data

Analysis of mutations in father–son pairs with 17 Y-STR loci

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Abstract

We have examined 389 father/son sample pairs from U.S. Caucasians, African Americans, Hispanics and Asians using the 17 Y-STR loci in the Yfiler™ kit and observed a total of 24 differences between father and son. Thirteen mutations resulted in the gain of a repeat in the son and 11 resulted in a loss of a repeat. All samples resulted in single repeat mutations except one sample which contained a two repeat loss at Y-GATA-H4. Furthermore, two different sample pairs were found to have two mutations. An African American sample pair had a mutation at DYS458 and a second at DYS635 and an Asian sample pair had mutations at DYS439 and Y-GATA-H4.

Keywords: Short tandem repeat; DNA; Y-STR; Mutation rate; DYS19; DYS385a/b; DYS389I; DYS389II; DYS390; DYS391; DYS392; DYS393; DYS437; DYS438; DYS439; DYS448; DYS456; DYS458

1. Introduction

Y-chromosome short tandem repeats (Y-STRs) have proven beneficial in a number of fields and applications including paternity, anthropology and genealogy studies [1–3]. An increasing number of forensic DNA laboratories are adopting Y-STR analyses into their routine casework, especially since the release of commercially available kits. Reliable estimations of mutation rates for these loci are a valuable asset to assist in the interpretation of Y-STR test results [4]. While there are a large number of articles reporting mutation rates for the minimal haplotype loci, only a few articles [5–8] have reported results with the 17 Y-STR loci in the AmpFℓSTR® Yfiler™ PCR amplification kit (Applied Biosystems, Foster City, CA). These studies primarily involved samples from populations outside of the U.S. Therefore, this is the first study with father/son sample pairs from major U.S. populations using the Yfiler loci.

This study reports the mutation rates of the 17 Yfiler loci in 389 father/son sample pairs representing U.S. Caucasians, African Americans, Hispanics and Asians. A compilation of mutation rate data for these loci both from this study and from previous literature is presented. Furthermore, duplications and deletions are described which are known to occur on the Y chromosome [9] that may complicate data interpretation.

2. Materials and methods[1]

2.1. DNA samples

Buccal swab samples from 399 father/son pairs (798 total samples) were collected by DNA Diagnostics Center (Fairfield, OH) representing U.S. Caucasian, African American, Hispanic and Asian populations. These sample pairs were from previously analyzed parentage tests each with a male child and an alleged father that was not excluded as a biological parent. In total, four buccal swabs were collected from each individual; two swabs were tested at DNA Diagnostics Center and two were distributed anonymously to NIST for testing. Buccal swabs were extracted at NIST using the DNA IQ™ System (Promega Corporation, Madison, Wisconsin). One half

[1] Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

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of each buccal swab was cut and placed into a 1.5 mL centrifuge tube. One hundred and fifty microliter of prepared lysis buffer was added to each tube. The tubes were then vortexed and centrifuged for 3 min at 10,000 × *g* and placed in a heat block for 30 min at 95 °C. After heating, the tubes were removed from the heat block and the swabs were transferred into the DNA IQ™ spin basket. The liquid in the bottom of the 1.5 mL tube was re-pipetted over the swab after the basket was placed in a fresh 1.5 mL tube. The tube and basket were then centrifuged for 3 min at 10,000 × g. At this step, the protocol was followed according to the manufacturer's instructions except that the DNA samples were eluted in 200 μL of elution buffer instead of the recommended 100 μL.

2.2. DNA quantification

The samples were quantified on the 7500 Real-Time PCR Instrument (Applied Biosystems) with an Alu qPCR assay [10]. For 20 μL reactions, 10 μL of either the Platinum® SYBR® Green qPCR SuperMix UDG (Invitrogen, Carlsbad, CA) or the 2× Power SYBR® Green PCR Master Mix (Applied Biosystems) was used with 0.2 μL of 20× 1 mg/mL ultrapure, non-acetylated BSA (Invitrogen) and 0.4 μL of 20 μM each primer. The Invitrogen mix required the addition of 0.3 μL of ROX reference dye. The reaction mix was brought to a total volume of 18 μL with DI H_2O. Each sample well contained 18 μL of reaction mix and a 2 μL aliquot of the father/son samples. PCR conditions using the Invitrogen mix included 50 °C for 2 min and 95 °C for 2 min followed by 30 cycles of 95 °C for 15 s and 68 °C for 1 min. PCR conditions for the Applied Biosystem's mix consisted of 95 °C for 10 min (''hotstart'') followed by 30 cycles of 95 °C for 15 s and 68 °C for 1 min. The sample concentration values ranged from 0.02 ng/μL to 49 ng/μL and were then adjusted to 0.5 ng/μL and placed in 96 well plates.

2.3. PCR amplification and detection

For quality assurance purposes, all samples were run with the AmpFℓSTR® Identifiler kit (Applied Biosystems) to confirm that the samples were male and to check for autosomal allele sharing that would suggest the father/son sample pairs were related. Samples were then examined with the 17 Y-STRs in the AmpFℓSTR® Yfiler™ kit using the following conditions: PCR amplification of the Yfiler loci was performed using 0.5 ng to 1 ng of DNA template and half volume reactions for a total of 12.5 μL instead of the 25 μL suggested by the manufacturer. The thermal cycling conditions followed the

Table 1
Twenty-four mutations and nine duplications or deletions observed in 389 father/son sample pairs with 17 Yfiler loci

Ethnicity	Sample	Locus	Allele (father)	Allele (child)	Comments
African American	16B	DYS458	18	19	Gain of 1 repeat
African American	16B	DYS635	23	22	Loss of 1 repeat
African American	18B	DYS390	24	23	Loss of 1 repeat
African American	39B	DYS458	18	19	Gain of 1 repeat
African American	46B	DYS389I and DYS389II	14, 30	13, 29	Loss of 1 repeat
African American	47B	DYS635	22	23	Gain of 1 repeat
African American	58B	DYS389I and DYS389II	14, 32	15, 33	Gain of 1 repeat
African American	65B	Y GATA H4	11	9	Loss of 2 repeats
African American	72B	DYS635	22	23	Gain of 1 repeat
African American	90B	DYS456	15	16	Gain of 1 repeat
African American	22B	DYS448	19, 20	19, 20	**Duplication**
African American	56B	DYS19	14, 15	14, 15	**Duplication**
African American	33B	DYS389I and DYS389II			**Deletion**
African American	33B	DYS439			**Deletion**
African American	72B	DYS448	19, 20	19, 20	**Duplication**
African American	97 B	DYS448	17.2, 19, 20	17.2, 19, 20	**Triplication**
Caucasians	11C	DYS439	14	15	Gain of 1 repeat
Caucasians	87C	DYS389I and DYS389II	13, 30	14, 31	Gain of 1 repeat
Caucasians	94C	Y-GATA-H4	12	13	Gain of 1 repeat
Hispanic	48H	DYS439	13	12	Loss of 1 repeat
Hispanic	53H	DYS437	15, 16	15, 16	**Duplication**
Asian	29A	DYS389I and DYS389II	14, 30	13, 29	Loss of 1 repeat
Asian	45A	DYS389I and DYS389II	15, 30	14, 29	Loss of 1 repeat
Asian	24A	DYS458	19	18	Loss of 1 repeat
Asian	26A	DYS389II only	30	31	Gain of 1 repeat
Asian	31A	DYS439	12	13	Gain of 1 repeat
Asian	101A	DYS439	13	12	Loss of 1 repeat
Asian	101A	Y-GATA-H4	12	11	Loss of 1 repeat
Asian	52A	DYS19	17	18	Gain of 1 repeat
Asian	57A	DYS439	13	12	Loss of 1 repeat
Asian	58A	DYS458	18	19	Gain of 1 repeat
Asian	77A	DYS456			**Deletion**
Asian	83A	Y-GATA-H4			**Deletion**

Table 2
Summary of autosomal mutations in 389 father/son sample pairs using Identifiler

Ethnicity	Sample	Locus	Allele (father)	Allele (child)
African American	96B	D3S1358	17, 17	15, 16
African American	99B	FGA	19, 23	22.2, 24
Hispanic	42H	D21S11	29, 31	30, 32
Asian	45A	D19S433	14, 14	13, 13
Asian	52A	D8S1179	11, 13	12, 15

manufacturer's instructions using the GeneAmp® 9700 (Applied Biosystems). Separation and detection of the 17 Y-STR loci were performed using the ABI Prism 3100 Genetic Analyzer 16-capillary array system and filter set G5. Each sample was prepared by adding 1 μL PCR product to 14 μL of Hi-Di™ formamide and 0.4 μL GS500-LIZ internal size standard (Applied Biosystems). Samples were injected for 10 s at 3 kV and separated in approximately 36 min using a 36 cm array and POP™-6 polymer (Applied Biosystems). Samples were analyzed using GeneMapper ID v3.2 (Applied Biosystems). Comparison information of the father and son sample data was generated using an in-house software program involving Microsoft Excel (Richmond, WA) macros designed to check for allele sharing across all loci. Paternity indexes were calculated using DNA-View software, version 28.18.

3. Results and discussion

A total of 798 samples from alleged fathers and sons were analyzed in this study which produced 788 samples with full profiles. In the 389 father/son pairs with full profiles, 24 mutations were observed with the 17 Y-STR loci in the Yfiler™ kit. Eleven of the mutations between father and son resulted in the loss of a repeat in the son while 13 mutations resulted in the gain of a repeat (Table 1). In five samples, a gain or loss of one repeat was observed in both DYS389I and DYS389II for each sample. Since DYS389I is a subsection of DYS389II, these events were counted as a single mutation. All samples resulted in single repeat mutations except one sample which displayed a two repeat loss at Y-GATA-H4. Furthermore, two different sample pairs were found to have double mutations. An African American sample pair (16B) had one mutation at DYS458 and a second at DYS635. This sample exhibited a gain of a single repeat in the son at DYS458 and a loss of a repeat at DYS635. An Asian father/son pair (101A) was also found to have two mutations with the Yfiler loci at DYS439 and Y-GATA-H4 (Table 1). In order to confirm the paternity of these two alleged father/son pairs, the Identifiler results were reviewed. Neither of these sample pairs were found to have observable mutations with the 15 autosomal STRs. In other words, all loci showed allele sharing between the father and the son with these two sample pairs. The paternity index (PI) value for sample pair 16B is 3.8×10^7 in the African American population and for sample pair 101A is 5.5×10^4 in the Asian population using the 15 autosomal Identifiler STR loci genotyping results. When the mothers' profile is included for sample pair 16B, the PI is 1.8×10^9 and for 101A is 4.3×10^6.

The autosomal mutations observed in 389 father and child sample pairs with the Identifier loci are summarized in Table 2. A total of five mutations were observed, two in the African Americans, one in Hispanics and two in the Asians. No autosomal mutations were seen in the Caucasian samples and no mutations occurred more than once for the same father/son pair. A comparison of results from Tables 1 and 2 show a higher number of mutations in the African American and Asian samples than in the Caucasian and Hispanic populations. Also, there are less mutations between father and son in the autosomal STRs than the Y-STRs. Interestingly, two Asian samples (45A and 52A) were found to have mutations in both autosomal and Y-STR loci. Paternity was confirmed by the paternity index value of 5.2×10^3 for 45A and 2.6×10^3 for 52A in the Asian population when the mothers' alleles are included.

Table 3
Summary of mutation rates from literature and NIST results for 17 Yfiler STR loci

Yfiler™ kit loci	Literature summary			NIST results			
Locus	Mutations	# Meioses	Mutation rate (%)	Mutations	# Meioses	Mutation rate (%)	Total (%)
DYS19	22	9241	0.238	1	389	0.257	0.239
DYS389I	14	7445	0.188	5	389	1.285	0.243
DYS389II	22	7432	0.296	6	389	1.542	0.358
DYS390	21	8723	0.241	1	389	0.257	0.241
DYS391	25	8672	0.288	0	389	<0.003	0.276
DYS392	5	8636	0.058	0	389	<0.003	0.055
DYS393	6	7425	0.081	0	389	0.003	0.077
DYS385a/b	30	13765	0.218	0	389	<0.003	0.212
DYS438	2	4075	0.049	0	389	0.003	0.045
DYS439	22	4052	0.543	5	389	1.285	0.608
DYS437	6	3971	0.151	0	389	0.003	0.138
DYS448	1	557	0.180	0	389	0.003	0.106
DYS456	4	557	0.718	1	389	0.257	0.529
DYS458	6	557	1.077	4	389	1.028	1.057
DYS635	6	1430	0.420	3	389	0.771	0.495
GATA-H4	4	1593	0.251	3	389	0.771	0.353

Thus far, in the four other published studies describing mutation rates with the 17 Yfiler loci [5–8] there have been no reports of a double mutation or a two repeat loss within a single father/son sample pair. However, previous studies involving fewer Y-STR loci have identified more than one mutation in a father/son pair. For example, Kayser and Sajantila [11] tested nine Y-STR loci and found on two separate occasions mutations at two Y-STRs in a single father/son sample pair. Our study supports these earlier findings, which is important because this data suggests that two differences in a father/son pair with 17 Y-STR loci would still be an inclusion when considered along with autosomal markers.

As mentioned earlier, duplications and deletions are known to occur on the Y chromosome and can be seen in both the father and the son [9]. In fact, a triplication was observed at DYS448 in one father/son sample pair. Additional deletions and duplications observed in the data set are provided in Table 1 and were observed in both the father and son in all cases. For example, African American sample 22B displayed a duplication (alleles 19 and 20) at DYS448 in both the father and son which demonstrates that these allele patterns can be inherited. In African American sample pair 33B, a deletion was observed at DYS389I/II and a second deletion was observed at DYS439. In both the father and son, the other 15 loci in the multiplex had sufficiently strong signal (around 4000 rfu's) which discounts allele dropout as a reason for these deletions. We have only run these samples with the primer pairs contained in the Yfiler™ kit in this study, but future studies could be attempted using other primer pairs to confirm the noted deletions. However, both DYS389I/II and DYS439 are in close proximity on the q-arm of the Y chromosome and it is likely that this entire region has been deleted [12].

A large amount of mutation rate information is available in the literature on various Y-STR loci for multi-generational work. In Table 3, we report the most current mutation rate information available on the 17 Y-STR loci in the Yfiler™ kit, as this will be important for laboratories using this technology. The final mutation rates for the Y-STR loci were determined by pooling the data in this study with mutation rate data provided by the Y Chromosome Haplotype Reference Database (YHRD) www.YHRD.org, published articles and papers in press [5–8,13–17]. Haplotype information for the 778 samples considered in this study across the 17 Yfiler loci is available in Supplementary material in Table S1.

This paper follows the guidelines for publication of population data requested by the journal [18]. The Y-STR haplotypes and mutation rates reported here for the father/son pairs were submitted to YHRD and are also available in electronic format at http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm. Haplotype information was submitted only for the 389 son samples to prevent overestimation of these haplotype frequencies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2007.08.016.

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