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TECHNICAL NOTE

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Identification and Characterization of Variant Alleles at CODIS STR Loci*

ABSTRACT: Short tandem repeat (STR) profiles from 32,671 individuals generated by the ABI Profiler Plus and Cofiler systems were screened for variant alleles not represented within manufacturer-provided allelic ladders. A total of 85 distinct variants were identified at 12 of the 13 CODIS loci, most of which involve a truncated tetranucleotide repeat unit. Twelve novel alleles, identified at D3S1358, FGA, D18S51, D5S818, D7S820 and TPOX, were confirmed by nucleotide sequence analysis and include both insertions and deletions involving the repeat units themselves as well as DNA flanking the repeat regions. Population genetic data were collected for all variants and frequencies range from 0.0003 (many single observations) to 0.0042 (D7S820 '10.3' in North American Hispanics). In total, the variant alleles identified in this study are carried by 1.6% of the estimated 1 million individuals tested annually in the U.S. for the purposes of parentage resolution. A paternity case involving a recombination event of paternal origin is presented and demonstrates how variant alleles can significantly strengthen the genetic evidence in troublesome cases. In such instances, increased costs and turnaround time associated with additional testing may be eliminated.

KEYWORDS: forensic science, DNA typing, STR, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, allele, variant, paternity analysis

Length polymorphism in STRs is exploited by gel electrophoretic resolution of PCR amplicons to distinguish between alleles (1,2). For most forensic and parentage testing applications, amplified STR alleles of tested individuals are compared to alleles comprising commercially-produced allelic ladders in order to accurately assign allele designations (3,4). Most DNA profiles are comprised of STR alleles represented within allelic ladders but a small number of individuals harbor poorly characterized 'variant' alleles absent from allelic ladders (5). Unless such variants are confirmed to be non-artifactual and population genetic information is available, the loci involved are typically omitted from genetic analyses. This practice can diminish the discriminatory power of STR test batteries, particularly in cases involving other confounding factors such as degraded DNA, single genetic inconsistencies or when the racial compositions of the tested parties are mixed or unknown.

The most widely accepted method of confirming the existence of an uncharacterized allele is re-amplification of the sample containing the suspected variant (1). If the variant again fails to align with a ladder allele and no artifactual factors are identified, the variant is typically sequenced in order to fully characterize its structure and assign an allele designation (5). The investigation described herein was undertaken in order to both identify novel STR variants as well as to generate population genetic data for previously described variant alleles. To demonstrate the value of characterizing variant alleles found in commonly used STR systems, a paternity case is presented that involves a variant examined in this study.

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Methods

Using standard methodologies, genomic DNA from 32,671 unrelated North American adults was isolated from buccal swabs submitted for parentage testing (6). Individuals included in this study were chosen randomly from the normal influx of casework entering our laboratories. Since our casework involves individuals from a diverse mixture of North American geographies, population substructure within this large sample set is highly unlikely.

Genetic profiles comprised of Combined DNA Index System (CODIS) loci were generated with either both the Applied Biosystems (ABI) AmpFlSTR Profiler Plus and COfiler multiplex systems (maximum 13 distinct loci) or with the Profiler Plus system only (maximum 9 distinct loci) per recommendations of the manufacturer (7,8). Previously described alleles not recognized by the allele-calling software were confirmed as non-artifactual variants by analyzing 1–2 additional alleles of the same size from other samples. FGA allelic variants were additionally confirmed with the AmpFlSTR SGM Plus multiplex system (9). Novel variants were isolated from their sister alleles by amplifying samples with single-locus primer pairs (Integrated DNA Technologies), staining electrophoresed bands with ethidium bromide and excising gel slices containing the variant alleles. Gel slices were then placed in H_2O, vortexed and centrifuged. Serial dilutions were prepared from the supernatants and used as templates for specific re-amplification of the variant alleles using the single-locus primer pairs. Amplifications yielding single products were purified with QiaQuick columns (Qiagen) and subjected to cycle sequencing with dye terminators.

Designations for variants lying between two ladder alleles were approximated by comparing allele sizes with the mean sizes of the flanking ladder alleles given by the manufacturer (7,8). Variant allele designations were also calculated using the following equations

TABLE 1—*Confirmed variant alleles*.

Locus	Variant Allele	Size Range (bp)*	Locus	Variant Allele	Size Range (bp)*
D3S1358	9	102.36–102.60	D5S818	**12.3**	158.72–158.76
	15.1†	127.25–127.30		**18**	179.32–179.39
	16.2‡	132.58–132.74			
	17.1	135.69–135.85			
	20.1	147.86–148.09			
vWA	18.3§	188.02	D13S317	5	194.99–195.09
				6	199.10–199.16
				7.1	204.27–204.34
FGA	15¶	207.83–207.87	D7S820	**5.2**	257.77–257.78
	16¶	211.87–211.92		6.3	262.44–262.56
	16.1	212.91–213.10		8.1	268.32–268.36
	16.2	213.96–214.20		8.3	270.28–270.34
	19.3	227.47–227.53		9.1 (2)	272.14–272.46
	20.1	229.56–229.62		9.3 (2)	274.10–270.20
	20.3 (2)	231.59–231.71		10.1	275.93–276.20
	21.1	233.70–233.82		10.3	277.94–278.15
	22.3 (2)	239.77–240.12		11.1	279.85–280.08
	23.3	243.89–244.27		11.3	281.89–281.98
	24.1	246.02–246.21		12.1 (2)	283.74–283.85
	24.3	248.09–248.35		13.1	287.66–287.78
	25.1	250.24–250.34			
	25.3	252.11–252.57			
	31.2 (2)	274.35–274.71			
	32.2	278.29–278.59			
	33.1	281.66–281.79			
	34.1	285.64–285.70			
	34.2	286.32–286.42			
	41.2	314.31–314.41			
	42.2	318.28–318.60			
	43.2 (2)	322.32–322.65			
	44.2 (2)	326.40–326.79			
	45.2 (3)	330.53–330.94			
	46.2 (2)	334.64–335.09			
	47.2 (3)	338.85–339.49			
	48.2	343.11–343.45			
	49.2	347.53–347.54			
D8S1179	7	122.98–123.15	TH01	4	165.61–165.66
				8.3	184.59–184.72
				13.3	204.89–204.93
D21S11	24.3	189.93–190.07	TPOX	**7.3**	225.65–225.67
	25.3	194.05–194.11		14	250.84–251.00
	27.1	200.09–200.19			
	29.1	208.37–208.44			
	29.3 (2)	210.43–210.62			
	30.3	214.60–214.68			
	31.1 (2)	216.59–216.61			
	32.1	220.68–220.70			
	33.1 (3)	224.69–224.88			
	34.1	228.84–229.01			
	35.1 (2)	232.89–233.05			
	36.1	237.01–237.06			
D18S51	7	266.45–266.53	CSF1PO	10.2	298.86
	11.2	284.09–284.24		16	320.56–320.64
	12.2	287.94–288.28			
	13.3	293.03–293.18			
	15.2	299.75–299.92			
	16.1	303.01			
	16.2	303.85–303.94			
	17.2	307.86–308.06			
	17.3	308.97–309.05			
	18.1	311.01–311.10			
	20.2	320.05–320.30			
	21.2 (2)	324.03–324.44			
	27	347.33–347.35			
	28.1**	351.48–351.52			

* Observed nucleotide lengths vary depending on equipment calibration, electrophoretic inconsistencies, etc.; allelic size ranges were determined from all alleles identified whether or not they were re-analyzed (allele sizes shown as a single number indicate that only a single allele was identified); † X.1 variants are 1bp larger than the X allele; ‡ X.2 variants are 2bp larger than the X allele; § X.3 variants are 3bp larger than the X allele; novel alleles are noted in **bold-face**; ¶ originally designated as novel FGA '14.3' and '15.3' variants but DNA sequencing revealed their structures as previously described '15' and '16' alleles, respectively; ** a novel confirmed variant for which nucleotide sequencing failed to reveal the genetic event underlying its anomalous electrophoretic migration; unless indicated by a number in parentheses, all alleles were confirmed by re-analysis of a single sample; alleles falling within ladder ranges are shown between dashed horizontal lines (note: D8S1179 '7' is below the ladder, D5S818 '18' is above the ladder, all variants at D13S317 are below the ladder, TPOX '14' is above the ladder and CSF1PO '16' is above the ladder).

TABLE 2—*Novel variant alleles*.

Locus	Allele Designation	Novel Nucleotide Sequence
D3S1358	15.1*	ACCCTGTCTCAT**T**A[GATA]$_{15}$
FGA	21.1*	[TTTC]$_3$TTTTTTCT[CTTT]$_{10}$**T**[CTTT]$_3$CTCC[TTCC]$_2$
	33.1	[TTTC]$_3$TTTTTTCT[CTTT]$_{13}$TTTCT[CTTT]$_{11}$CTCC[TTCC]$_2$
	34.1	[TTTC]$_3$TTTTTTCT[CTTT]$_{13}$TTTCT[CTTT]$_{12}$CTCC[TTCC]$_2$
	41.2	[TTTC]$_4$TTTTTT [CTTT]$_{11}$[CTGT]$_3$[CTTT]$_{11}$[CTTC]$_3$[CTTT]$_3$CTCC[TTCC]$_4$
D18S51	13.3†	[AGAA]$_{14}$ . . . 76bp‡ . . . **Δ**A**Δ**. . .
	16.1*	[AGAA]$_3$A[**A**GAA]$_{13}$
	28.1*	?
D5S818	12.3†	[AGAT]$_3$**Δ**A**Δ**GAT[AGAT]$_9$
	18*	[AGAT]$_{14}$**ACAT**[AGAT]$_3$
D7S820	5.2†	[GATA]$_9$ . . . 41bp‡ . . . **Δ**AGTAAACATTTAAT**Δ**. . .
TPOX	7.3†	[AATG]$_8$ . . . 48bp‡ . . . **Δ**A**Δ**. . .

* Denotes alleles involving insertions (inserted nucleotides are indicated in **bold type**); the mutational event underlying the generation of D18S51 '28.1' was not identified but likely involves insertion of a single nucleotide into a '28' allele. † Denotes alleles involving deletions (deleted nucleotides are flanked by **Δ**); ‡ deletions involving D18S51 '13.3', D7S820 '5.2' and TPOX '7.3' occurred 76bp, 41bp and 48bp downstream of the simple repeat sequences, respectively; all sequences shown correspond to (+)-strand DNA in the 5′-3′ direction.

derived elsewhere (10):

$$\delta_1 = S_Y - L_Y \quad \delta_2 = S_{OL} - L_X \quad c = |\delta_1 - \delta_2|$$

δ_1 represents the size difference (in base pairs) between sister allele Y (S_Y) and ladder allele Y (L_Y) and δ_2 represents the size difference between the variant (S_{OL}) and ladder allele X (L_X), the smaller allele adjacent to the variant. The c value is the absolute size difference between the variant and the ladder allele and indicates how many additional base pairs are present in the variant.

Allele designations for variants lying outside of the ladder ranges were extrapolated by comparing variant allele sizes to the sizes of the smallest or largest ladder alleles. It was determined empirically by DNA sequence analysis that designations of the novel variants identified in this study lying outside allelic ladder ranges were accurately extrapolated.

Likelihood ratios in the form of Paternity Indices (PIs), as well as Random Man Not Excluded statistics, were calculated using allele frequencies from an in-house database and formulae derived elsewhere (11). Since discernible data were not generated at all loci tested in each individual, variant allele frequencies were calculated by dividing the number of observations by the product of 2N (where N = the number of individuals studied from each population) and our success rate at each locus, generally ranging from 80 to >90%. Occurrences of the same variant in parent-child pairs involved in parentage testing and in individuals submitting for kinship analyses were regarded as only single observations of that variant since such alleles are identical-by-descent. STR loci described in this study have been demonstrated to obey Mendel's law of independent assortment and the multiplication rule was used to calculate the Combined Paternity Index (7,8,9,11).

Results and Discussion

Eighty-five distinct variant alleles were identified in 757 of the 32,671 individuals examined. However, 242 of the 757 individuals expressing variants were children submitting for parentage testing with one or both parents. These children were discarded from the analysis since their alleles are identical-by-descent with those of their parents. The remaining 515 variant alleles (85 are distinct) identified in 32,429 individuals (1.6%) are presented in Table 1. These variants occurred at 12 of the 13 CODIS loci and all were observed as heterozygotes, paired in each case with well-characterized sister alleles represented in allelic ladders. The single CODIS locus at which no variants were identified is D16S539. Table 2 provides DNA sequence information for 12 previously unreported variants identified in this study. Five of the 12 novel alleles involve insertions of nucleotides either directly into the STR repeat regions or into DNA closely flanking the repeats. Deletions involving simple repeat loci generated 4 of the 12 variants and the remaining 3 novel alleles involved rearrangements of complex FGA repeats. Fifty-two of the 85 confirmed variants were flanked by ladder alleles while the remaining 33 were outside of ladder ranges.

The number of variant allele observations and corresponding allele frequencies in each population group examined in this study are shown in Table 3. The most common variant overall, D21S11 '33.1', was identified in 57 samples, represents 11.1% of all variants confirmed in this study and comprises over 0.16% and 0.07% of the allelic diversity at D21S11 in the North American Black and Hispanic populations, respectively. The variant comprising the largest fraction of alleles at any single locus, is the '10.3' at D7S820, occurring in 0.23% of samples from Hispanics. Other more common variants include D3S1358 '20.1' in Caucasians and Hispanics, D18S51 '15.2' in Blacks and Hispanics, D3S1358 '9', FGA '16.1', '31.2', '43.2' and '44.2', D21S11 '24.3' and D18S51 '21.2' in Blacks and FGA '45.2', D21S11 '33.1', D13S317 '7.1' and TH01 '4' in Hispanics, all exceeding frequencies of 0.05% in the indicated populations. However, most alleles were quite rare with many occurring in only a single profile reviewed in this study.

A representative paternity case involving the D21S11 '33.1' allele identified in this study is presented in Table 4 to demonstrate how the inclusion of variant alleles in parentage analyses can resolve troublesome casework. This particular case involves a single genetic inconsistency (possible mutation) at D8S1179 producing a very low locus-specific PI. In the absence of frequency information for the '33.1' allele, data generated at D21S11 in this case would ordinarily be discarded. In this typical scenario, the Combined Paternity Index (CPI) would be less than 3 to 1 in favor of paternity. Since it is generally regarded in the United States that only CPIs in excess of 100 to 1 comprise sufficient genetic evidence to conclusively assign paternity, this particular case would require additional testing (12). Alternatively, inclusion of D21S11 in the analysis using frequency data generated in this study yields a locus-specific PI of 298 to 1, raises the CPI to 777 to 1 and no additional testing is needed. It is also worth noting that the Random Man Not Excluded value (the likelihood that a particular AF would

TABLE 3—*Variant allele observations and frequencies.*

Locus	Variant Allele	# of Observations	Caucasian $N^* = 16,185$	African American $N = 14,015$	Hispanic $N = 1,872$	Other $N = 357$
D3S1358	9	35	1 (0.003235)	32 (0.120172)	...	**2**
	15.1	1	...	1 (0.003755)	...	...
	16.2	4	...	4 (0.015021)	...	...
	17.1	2	2 (0.006470)	...	...	...
	20.1	27	19 (0.061462)	2 (0.007511)	3 (0.116128)	**3**
vWA	18.3	1	1 (0.003152)	...	...	...
FGA	15	2	2 (0.006442)	...	...	...
	16	3	3 (0.009621)	...	...	...
	16.1	13	...	13 (0.050031)	...	...
	16.2	5	...	5 (0.019243)	...	...
	19.3	1	1 (0.003221)	...	...	...
	20.1	1	1 (0.003221)	...	...	...
	20.3	2	...	1 (0.003849)	1 (0.028445)	...
	21.1	1	1 (0.003221)	...	...	...
	22.3	4	...	3 (0.011546)	1 (0.028445)	...
	23.3	9	...	8 (0.030788)	1 (0.028445)	...
	24.1	3	...	3 (0.011546)	...	...
	24.3	4	1 (0.003221)	3 (0.011546)	...	...
	25.1	1	...	...	1 (0.028445)	...
	25.3	4	...	4 (0.015394)	...	...
	31.2	28	1 (0.003221)	25 (0.096215)	...	**2**
	32.2	9	1 (0.003221)	8 (0.030788)	...	...
	33.1	1	...	1 (0.003849)	...	...
	34.1	2	...	2 (0.007697)	...	...
	34.2	1	...	1 (0.003849)	...	...
	41.2	4	...	2 (0.007697)	1 (0.028445)	**1**
	42.2	12	...	11 (0.042334)	1 (0.028445)	...
	43.2	18	1 (0.003221)	17 (0.065425)	...	...
	44.2	16	1 (0.003221)	15 (0.057729)	...	...
	45.2	14	...	12 (0.046183)	2 (0.056889)	...
	46.2	13	...	11 (0.042334)	1 (0.028445)	**1**
	47.2	5	1 (0.003221)	4 (0.015394)	...	...
	48.2	1	...	1 (0.003849)	...	...
	49.2	1	...	1 (0.003849)	...	...
D8S1179	7	1	...	1 (0.003659)	...	...
D21S11	24.3	21	...	16 (0.059646)	1 (0.039106)	**4**
	25.3	1	...	1 (0.003728)	...	...
	27.1	4	...	4 (0.014912)	...	...
	29.1	1	1 (0.003198)	...	...	...
	29.3	11	7 (0.022386)	4 (0.014912)	...	...
	30.3	2	...	1 (0.003728)	1 (0.039106)	...
	31.1	1	...	...	1 (0.039106)	...
	32.1	2	1 (0.003198)	1 (0.003728)	...	...
	33.1	57	4 (0.012792)	45 (0.167756)	2 (0.078212)	**6**
	34.1	13	2 (0.006396)	10 (0.037279)	1 (0.039106)	...
	35.1	9	...	9 (0.033551)	...	...
	36.1	1	...	1 (0.003728)	...	...
D18S51	7	2	...	...	1 (0.041282)	**1**
	11.2	4	...	4 (0.015295)	...	...
	12.2	6	...	6 (0.022943)	...	...
	13.3	3	...	3 (0.011471)	...	...
	15.2	27	1 (0.003238)	24 (0.091771)	2 (0.082564)	...
	16.1	1	...	...	...	**1**
	16.2	1	...	1 (0.003824)	...	...
	17.2	1	...	...	...	**2**
	17.3	1	...	...	1 (0.041282)	...
	18.1	2	2 (0.006476)	...	...	...
	20.2	7	...	6 (0.022943)	...	**1**
	21.2	16	1 (0.003238)	14 (0.053533)	...	**1**
	27	2	1 (0.003238)	...	1 (0.041282)	...
	28.1	1	...	...	1 (0.041282)	...
D5S818	12.3	2	...	2 (0.007273)	...	...
	18	1	...	1 (0.003637)	...	...
D13S317	5	2	1 (0.003162)	1 (0.003648)	...	...
	6	2	2 (0.006324)	...	...	...
	7.1	2	...	...	2 (0.075987)	...
D7S820	5.2	1	...	1 (0.003747)	...	...
	6.3	3	3 (0.009644)	...	...	...

TABLE 3—*Continued.*

Locus	Variant Allele	# of Observations	Caucasian $N^* = 16,185$	African American $N = 14,015$	Hispanic $N = 1,872$	Other $N = 357$
	8.1	1	···	···	1 (0.028445)	···
	8.3	1	···	1 (0.0033747)	···	···
	9.1	9	4 (0.012859)	2 (0.007495)	···	3
	9.3	4	···	2 (0.007495)	1 (0.028445)	1
	10.1	12	2 (0.006429)	9 (0.033727)	1 (0.028445)	···
	10.3	9	1 (0.003215)	···	8 (0.227556)	···
	11.1	5	4 (0.012859)	1 (0.0033747)	···	···
	11.3	2	···	2 (0.007495)	···	···
	12.1	1	1 (0.003215)	···	···	···
	13.1	3	1 (0.003215)	1 (0.003747)	1 (0.028445)	···
TH01	4	2	···	1 (0.004460)	1 (0.094380)	···
	8.3	1	1 (0.005040)	···	···	···
	13.3	1	1 (0.005040)	···	···	···
TPOX	7.3	1	···	···	1 (0.044814)	···
	14	1	···	1 (0.004372)	···	···
CSF1PO	10.2	1	···	···	···	1
	16	1	···	···	···	1
TOTALS	···	**515**	**78**	**366**	**40**	**31**

* N = total number of samples tested from each population; the number of observations of each allele is presented with the corresponding allele frequency in parentheses (shown as a percentage of successfully typed alleles at that locus); allele frequencies are not given for variants identified in the 'Other' population group since this does not refer to a single population.

TABLE 4—*Variant alleles in paternity analyses.*

STR Locus	Tested Party Phenotype			Paternity Index	
	Mother	Child	Alleged Father	Variant Allele Omitted	Variant Allele Included
D13S317	11, 13	11, **12**	12*	2.33	2.33
D18S51	18	16, **18**	16, 17	2.78	2.78
D21S11	31.2, 33.2	**33.1**, 33.2	29, 33.1	···	298.00
D3S1358	15, 17	**15, 17**	15, 17	2.01	2.01
D5S818	11, 12	**11, 12**	9, 11	0.85	0.85
D8S1179†	15, 16	13, 15	14, 16	0.0025	0.0025
FGA	20, 21	20, **27**	21, 27	15.63	15.63
vWA	15, 16	16, **17**	15, 17	2.46	2.46
D7S820	8, 13	**8**	8, 10	2.45	2.45
		Combined Paternity Index		<3 to 1	777 to 1
		Random Man Not Excluded		7.35×10^{-4}	4.75×10^{-6}

* Single allele designations indicate homozygosity; † D8S1179 involves a single genetic inconsistency, likely representing a recombination event involving the paternally-derived gamete; **bold type** STR alleles in the child represent the paternal obligate alleles (or possible paternal obligate alleles).

not be excluded if he were falsely accused) is decreased by a factor of roughly 100 when D21S11 is included in the analysis of this case.

In conclusion, we have identified and generated population genetic data for 85 distinct variant alleles at 12 of the 13 CODIS STR loci that are not represented in commercially available allelic ladders. Twelve of the 85 variants have not been previously reported. We estimate that 1.6% of the disputed parentage caseload performed annually by accredited U.S. laboratories involves one of the variants described in this study. This is very likely a conservative estimate because additional variants surely remain unidentified. Use of these variant alleles in statistical calculations of the odds of paternity can reduce costs and turnaround time associated with additional testing in problematic cases such as those involving single genetic inconsistencies. Finally, in addition to their benefit to parentage analyses, the variant alleles described herein comprise valuable tools for forensic and other human identification laboratories that use CODIS STRs.

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