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Full Length Research Paper

Population genetic data for six non-combined DNA index system (non-CODIS) miniSTR loci from the Xhosa and Cape Muslim populations of South Africa

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Allele frequency and forensic parameters for six non-combined DNA Index System (non-CODIS) miniSTR loci: D1S1627, D3S4529, D5S2500, D6S1017, D8S1115, and D9S2157 were calculated from a sample of 226 unrelated individuals from two populations (that is, Xhosa and Cape Muslim) of South Africa. No significant deviation from Hardy-Weinberg equilibrium was found. All loci showed a moderate degree of polymorphism with heterozygosity values >0.6 for both populations across all loci. The combined power of discrimination was 0.999999675 and 0.999999037 for Xhosa and Cape Muslim populations, respectively. The respective values for the combined power of exclusion in these populations were 0.94 and 0.99. The allele frequency data generated can be used for estimating DNA profile frequencies for the studied populations residing in South Africa.

Key words: Allele frequencies, MiniSTR, DNA typing, population data, South Africa.

INTRODUCTION

Genotyping systems have been developed and used world-wide to help with forensic casework involving DNA. It is the most frequently used with the identification of individuals in mass fatality disasters, complex paternity cases, and missing person's cases (Budimlija et al., 2003; Goodwin et al., 2004; Kleyn, 2009). The most thoroughly researched and used genotyping system is known as the COmbined DNA Index System (CODIS) which comprised 13 core short tandem repeat (STR) loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, and D16S539). Recently, it has come to light that the CODIS loci could not produce complete genotype profiles when analyzing degraded DNA samples. Therefore, miniSTR loci were developed specifically to aid the genotyping of degraded or compromised DNA samples (Butler et al., 2003; Coble and Butler, 2005). Majority of loci selected were not occupied by CODIS loci and were thus referred to as non-CODIS (NC) loci (Hill et al., 2008). When forensic data is presented in court, it has to be substantiated with background information on local population structures.

In developing countries like South Africa, the use and development of non-commercial genotyping systems is extremely important to cut the cost of forensic casework. Population data from countries like South Africa where great ethnic and cultural diversity is displayed is important world-wide. Previously, Abrahams et al. (2010) published allelic frequency and forensic parameter data for three South African populations using six NC loci (that is, D1S1627, D3S4529, D5S2500, D6S1017, D8S1115, and D9S2157). The aim of this study was to investigate allelic frequencies and forensic parameters of the same six NC loci in an additional two South African populations, that is, Xhosa and Cape Muslim populations.

Majority of inhabitants of South Africa are referred to as 'Black Africans' and these can be more accurately defined by their cultures and languages. The Xhosa population is a ' Black African' population of South Africa

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indigenous to the Eastern Cape and comprises approximately 17.6% of the total population (Leat et al., 2004; Ikediobi et al., 2011). The Cape Muslim population is a more complex population since it can trace its roots back to slave trade, colonialism, trade routes, and political prisoners from Africa and Asia (Da Costa, 1994). The Cape Muslim population is therefore characterized by complex population structures with an admixture of European, African, South Asian, and Indonesian ancestries (Ikediobi et al., 2011).

The availability of population data from both indigenous and admixed populations is valuable for a number of human genetic areas of research, such as, forensic genetics, genetic association studies, and anthropological studies (Cloete et al., 2010).

MATERIALS AND METHODS

DNA extraction

Samples were collected from 120 Xhosa individuals (Leat et al., 2004; Ikediobi et al., 2011) and 106 Cape Muslim individuals (Cloete et al., 2010; Ikediobi et al., 2011). All donors were healthy unrelated individuals from respective communities living in South Africa. Samples were collected as whole blood or buccal swab samples. DNA was extracted as previously described by Lahiri and Nurnberger (1991). DNA samples were quantified using a NanoDrop ND-1000 UV-Vis spectrophotometer.

PCR amplification, typing and data analysis

DNA samples were analysed using NC miniSTR primer sets previously published by Hill et al. (2008). Primers were synthesized by Applied Biosystems and all forward primers were labeled with either 6FAM™, VIC™ and NED™ dyes which enabled colour separation on an ABI 377 genetic analyzer. All reverse primers were unlabelled and had an additional 5' guanine base added to produce fully adenylated polymerase chain reaction (PCR) products (Hill et al., 2008). Complete primer sequences and final concentrations of each of the loci used in this study are presented in Table 1.

Amplification of samples were performed in reaction volumes of 10 µl using a master mix containing 1X Supertherm PCR buffer (containing 1.5 mM $MgCl_2$); 250 µM of each deoxynucleotide triphosphate (Roche dNTP's: dATP, dCTP, dGTP, dTTP); 1 µl of template DNA at a concentration of 200 pg/µl, 160 µg/ml BSA and 1 U Supertherm Gold Taq DNA polymerase. Primers were added to produce final concentrations as indicated in Table 1. Amplification reactions were performed using a 96-well Gene-Amp® PCR system 9700 thermal cycler (Applied Biosystems). Thermal cycling conditions for miniplex 1 were: 95°C for 10 min; 35 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, followed by a final incubation at 60°C for 45 min and 4°C forever. Thermal cycling conditions for miniplex 2 were: 95°C for 10 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a final incubation at 60°C for 45 min and 4°C forever.

Amplified samples were electrophoretically separated using an ABI Prism® 377 Genetic Analyzer (Applied Biosystems) and GeneScan™ 500 LIZ™ size standard (Applied Biosystems, Warrington, UK). All data were collected with the ABI 377 collection software (Applied Biosystems) and analyzed using the GeneScan 3.1 (Applied Biosystems) software. Size fragments were converted to allele numbers by making use of the Genotyper 2.5 (Applied Biosystems) software. Allele numbers were assigned to samples according to the known allele numbers in the allelic ladders.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was determined by an exact test using GENEPOP (Version 4.0.7) software package designed by Raymond and Rousset (1995). The Bonferroni correction was applied to test for deviations from the HWE (Butler, 2005). Forensic parameters: match probability, power of discrimination, polymorphism information content, power of exclusion, and typical paternity index were calculated using PowerStats v1.2 software package (Promega). (Tereba, 1999).

RESULTS AND DISCUSSION

The observed allele frequencies and statistical parameters for forensic testing based on the six NC loci in the selected two South African populations are summarized in Tables 2 to 4. The six NC miniSTR loci displayed HWE P-values ranging between 0.00462 and 0.7810 for the studied populations (Table 4). Amongst these, D8S1115 and D9S2157 showed P-values below the 0.05 limit set for HWE in the Cape Muslim population. This suggests possible deviation from HWE. After the application of the Bonferroni correction (Butler, 2005), only P-values below 0.004166666 (0.05/12) would be considered significant. Thus, in this study, no loci displayed deviation from HWE.

The observed heterozygosity values ranged from 0.733 to 0.908 and 0.679 to 0.726 for the Xhosa and Cape Muslim populations, respectively (Table 4). These values hereby imply that there is a minimum of 73.3 and 67.9% chance of random samples matching in the respective populations. Furthermore, these values are indicative of good allele diversity for each locus.

Combined power of discrimination values of 0.999999675 and 0.999999037 for Xhosa and Cape Muslim populations demonstrates the high degree of discrimination which will be obtained when using these loci combined in the respective populations. Individual power of exclusion values (which ranged between 0.440 and 0.812 for both populations) displayed a low degree of exclusionary power (Table 4). However, when combining the power of exclusion values for the six loci per population, they displayed exclusionary values of 0.94 in the Xhosa population and 0.99 in the Cape Muslim population.

As expected, combined paternity index values display greater significance in each population than the individual values (Table 4). The Xhosa population shows the greatest combined paternity index value of 495.3 with the Cape Muslim population only showing a combined paternity index value of 17.5. The Xhosa population also displayed highly informative polymorphism information content values for all loci (that is, values >0.7). The Cape Muslim population on the other hand only displayed values >0.7 for the loci D3S4529, D5S2500, and

Table 1. Primer sequences, dye labels and final concentration of primers used in miniplex reactions 1 and 2.

STR locus	Miniplex set	Primer sequence (5'-3')	Primer concentration (µM)
D1S1627	1	F-[VIC]-CATGAGGTTTGCAAATACTATCTTAAC R-GTTTTAATTTTCTCCAAATCTCCA	0.3
D3S4529	2	F-[VIC]-CCCAAAATTACTTGAGCCAAT R-GAGACAAAATGAAGAAACAGACAG	0.1
D5S2500	1	F-[NED]-CTGTTGGTACATAATAGGTAGGTAGGT R-GTCGTGGGCCCCATAAAATC	0.1
D6S1017	2	F-[NED]-CCACCCGTCCATTTAGGC R-GTGAAAAAGTAGATATAATGGTTGGTG	0.1
D8S1115	1	F-[6FAM]-TCCACATCCTCACCAACAC R-GCCTAGGAAGGCTACTGTCAA	0.1
D9S2157	2	F-[6FAM]-CAAAGCGAGACTCTGTCTCAA R-GAAAATGCTATCCTCTTTGGTATAAAT	0.5

Table 2. Allele frequency distribution of three miniSTR loci (D1S1627, D5S2500, and D8S1115) amongst the 226 individuals from two South African population groups.

Allele	Miniplex 1							
	D1S1627		D5S2500			D8S1115		
	Xhosa	Cape Muslim	Allele	Xhosa	Cape Muslim	Allele	Xhosa	Cape Muslim
10	0.0625	0.094	13		0.005	7	0.013	0.009
11	0.142	0.075	14	0.379	0.302	9	0.438	0.325
12	0.154	0.071	15	0.042	-	10	0.008	0.009
13	0.304	0.429	16	0.004	-	11	0.054	0.005
14	0.246	0.316	17	0.179	0.283	12	0.004	-
15	0.0875	0.014	18	0.058	0.259	13	0.033	-
17	0.004	-	19	0.079	0.009	14	0.021	0.024
-	-	-	20	0.217	0.075	15	0.042	0.047
-	-	-	21	0.004	-	16	0.092	0.387
-	-	-	22	0.004	-	17	0.133	0.156
-	-	-	23	0.033	0.061	18	0.117	0.024
-	-	-	24	-	0.005	19	0.0375	0.009
-	-	-	-	-	-	20	0.004	-
-	-	-	-	-	-	21	0.004	0.005

D9S2157.

The data generated from this study showed high discriminatory and exclusionary power for both studied populations. This thereby indicates that these loci can be used to compliment any existing genotyping systems.

Table 5 presents the results obtained for the population differentiation test when the two selected populations to other populations were compared as studied by Abrahams et al. (2010). The locus D9S2157 is the only locus that shows significant differentiation for all the five populations. Furthermore, the Afrikaner : Xhosa, Asian-Indian: Xhosa, the Mixed Ancestry : Xhosa, and the Xhosa : Cape Muslim population pairs showed significant differentiation across all loci. The Mixed Ancestry : Cape Muslim pair showed no significant differentiation for all loci except D6S1017 and D9S2157. The Afrikaner : Asian-Indian population pair displayed no significant differentiation for loci D5S2500, D6S1017, and D8S1115.

Table 3. Allele frequency distribution of three miniSTR loci (D3S4529, D6S1017, and D9S2157) amongst the 226 individuals from two South African population groups.

Allele	Miniplex 2							
	D3S4529		D6S1017			D9S2157		
	Xhosa	Cape Muslim	Allele	Xhosa	Cape Muslim	Allele	Xhosa	Cape Muslim
12	0.067	0.009	7	0.333	0.057	7	0.096	0.094
13	0.108	0.250	8	0.200	0.165	8	0.004	0.014
14	0.342	0.311	9	0.125	0.024	9	0.208	0.075
15	0.250	0.175	10	0.225	0.429	10	0.108	0.033
16	0.175	0.198	11	0.008	0.038	11	0.283	0.208
17	0.058	0.057	12	0.083	0.236	12	0.079	0.047
-	-	-	13	0.021	0.038	13	0.029	0.241
-	-	-	14	-	0.014	14	0.046	0.094
-	-	-	18	0.004	-	15	0.092	0.113
-	-	-	-	-	-	16	0.042	0.075
-	-	-	-	-	-	17	0.013	0.005

Table 4. Population statistics of six miniSTR loci among the 226 individuals from two South African populations.

Population parameter	Miniplex 01					
	D1S1627		D5S2500		D8S1115	
	Xhosa	Cape Muslim	Xhosa	Cape Muslim	Xhosa	Cape Muslim
Ho	0.908	0.726	0.733	0.708	0.817	0.708
He	0.795	0.699	0.768	0.755	0.764	0.720
MP	0.092	0.146	0.087	0.105	0.083	0.144
PD	0.908	0.854	0.913	0.895	0.917	0.856
PE	0.812	0.470	0.482	0.440	0.630	0.440
PIC	0.76	0.65	0.73	0.71	0.74	0.67
TPI	5.45	1.83	1.88	1.17	2.73	1.71
HWE P-value	0.3045	0.7810	0.4758	0.3092	0.3324	0.0073

Population parameter	Miniplex 02					
	D3S4529		D6S1017		D9S2157	
	Xhosa	Cape Muslim	Xhosa	Cape Muslim	Xhosa	Cape Muslim
Ho	0.775	0.726	0.792	0.679	0.850	0.717
He	0.774	0.771	0.778	0.730	0.839	0.858
MP	0.096	0.095	0.096	0.112	0.053	0.041
PD	0.904	0.905	0.904	0.888	0.947	0.959
PE	0.553	0.470	0.584	0.397	0.695	0.455
PIC	0.74	0.73	0.74	0.69	0.82	0.84
TPI	2.22	1.83	2.40	1.56	3.33	1.77
HWE P-value	0.3926	0.5515	0.0591	0.1839	0.6767	0.00462

Ho, observed heterozygosity; He, expected heterozygosity; MP, matching probability; PD, power of discrimination; PE, paternity power of exclusion; PIC, polymorphism information content; TPI, typical paternity index; P, probability values of exact tests for Hardy-Weinberg disequilibrium.

The Afrikaner : Mixed Ancestry and the Asian-Indian : Mixed Ancestry population pairs displayed significant differentiation for all loci except for D3S4529 and D8S1115, respectively. The Afrikaner : Cape Muslim and the Asian-Indian : Cape Muslim population pairs showed no significant differentiation for loci D3S4529 and D8S1115, with the latter population pair also displaying no significant differentiation for the locus D5S2500.

The study of the genetic diversity of the selected two populations can be used in conjunction with other

Table 5. Exact test of population differentiation based on allele frequencies for five population groups in South Africa.

Population pair	D1S1627	D3S4529	D5S2500	D6S1017	D8S1115	D9S2157
Afrikaner:Asian-Indian	*0.00000*	*0.03308*	0.07772	0.10292	0.23804	*0.00000*
Afrikaner:Mixed Ancestry	*0.00014*	0.24798	*0.00002*	*0.00000*	0.00445	*0.00131*
Afrikaner:Xhosa	*0.0000*	*0.00018*	*0.00000*	*0.00000*	0.00000	*0.00000*
Afrikaner:Cape Muslim	*0.00905*	0.05161	*0.01743*	*0.00061*	0.16055	*0.00102*
Asian Indian:Mixed Ancestry	*0.00029*	*0.00830*	*0.01900*	*0.00000*	0.05606	*0.00000*
Asian-Indian:Xhosa	*0.00000*	*0.00000*	*0.00000*	*0.00000*	0.00000	*0.00000*
Asian-Indian:Cape Muslim	*0.00417*	0.68281	0.54335	*0.00155*	0.17337	*0.03492*
Mixed Ancestry:Xhosa	*0.00711*	*0.02457*	*0.00000*	*0.00000*	0.00113	*0.00000*
Mixed Ancestry:Cape Muslim	0.30907	0.09026	0.39613	*0.00290*	0.05451	*0.00582*
Xhosa:Cape Muslim	*0.00015*	*0.00470*	*0.00000*	*0.00000*	0.00000	*0.00000*

Values in italic represent significant differentiation with P-value < 0.05.

investigated populations of South Africa (Abrahams et al., 2010; Benjeddou et al., 2006; Cleote et al., 2010) to help fill any missing gaps of both indigenous and admixed populations of the country. This kind of population data will be very important to help with the identification of individuals in an array of different circumstances.

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REFERENCES

Abrahams Z, D'Amato ME, Davison S, Benjeddou M (2010). Allele frequencies of six non_CODIS miniSTR loci (D1S1627, D3S4529, D5S2500, D6S1017, D8S1115 and D9S2157) in three South African populations. Forensic Sci. Int. Genet. 5(4):354-355.

Benjeddou M, Leat N, Davison S (2006). Use of molecular genetics and historical records to reconstruct the history of local communities. Afr. J. Biotechnol. 5:2516-2519.

Budimlija Z, Prinz MK, Zelson-Mundorff A, Wiersema J, Bartelink E, MacKinnon G, Nazzaruolo BL, Estacio SM, Hennessey MJ, Shaler RC (2003). World Trade Center human identification project: Experiences with individual body identification cases. Croat. Med. J., 44(3):259-263.

Butler JM, Shen Y, McCord B (2003). The development of reduced size STR amplicons as tools for analysis of degraded DNA. J. Forensic Sci. 48(5):1054-1064.

Butler JM (2005). Forensic DNA Typing: biology, technology, and genetics of STR markers. Elsevier Academic Press, London.

Cloete K, Ehrenreich L, D'Amato ME, Leat N, Davison S, Benjeddou M (2010). Analysis of seventeen Y-chromosome STR loci in the Cape Muslim population of South Africa. Legal Med. (Tokyo) 12:42-45.

Coble MD, Butler JM (2005). Characterization of new miniSTR loci to aid analysis of degraded DNA. J. Forensic Sci. 50:43-53.

Da Costa Y (1994) In: Da Costa Y, Davids A, editors Cape Muslim history, Cape Town. Shuter and Shooter.

Goodwin W, Ballard D, Simpson K, Thacker C, Syndercombe-Court D, Gow J (2004). Case study: paternity testing-when 21 loci are not enough. Int. Congress Series 1261:460-462.

Hill CR, Kline MC, Coble MD, Butler JM (2008). Characterization of 26 MiniSTR loci for improved analysis of degraded DNA samples. J. Forensic Sci. 53:73-80.

Ikediobi O, Aouizerat B, Xiao Y, Gandhi M, Gebhardt S, Warnich L (2011). Analysis of pharmacogenetic traits in two distinct South African populations. Human Genomics 5(4):265–282.

Kleyn EL (2009). The Internal Validation and Casework Application of MiniSTR Systems. Unpublished–Thesis.

Lahiri DK, Nurnberger JI (1991). A rapid non-enzyme method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 19:5444.

Leat N, Benjeddou M, Davison S (2004). Nine-locus Y-chromosome STR profiling of Caucasian and Xhosa populations from Cape Town South Africa. Forensic Sci. Int. 144:73–75.

Raymond M, Rousset F (1995). GENEPOP (version 4.0.7): population genetics software for exact tests and ecumenicism. J. Heredity 86:248-249. Available online at: http://wbiomed.curtin.edu.au/genepop.

Tereba A (1999). Tools for analysis of population statistics. Profiles in DNA 2:14–16. Available online at: http://www.promega.com/geneticidtools/powerstats/.