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X-STR Allele and Linkage Haplogroup Frequencies in the Lebanese Population and the Potential of X-STR Polymorphism in Forensic Casework

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Abstract

When autosomal STR profiling fails to supply satisfactory outcome in human identification and/or in forensic casework, scientists may use Y-STR and/or X-STR profiling. However, representative X-STR allele and haplogroup frequencies should be established in the studied population for proper use of X-STR profiles. In this study, 507 non-related males and females representative of the Lebanese population as to the religious and geographic distributions were tested to establish the X-STR allele frequencies. 500 non-related males were tested to establish the X-STR haplogroup frequencies. DNA was amplified using Investigator™ Argus X-12 multiplex PCR system (Qiagen). Results were analyzed using in-house software (FIMS), Arlequin v3.5 software, MEGASTAT software and an online software: http://www.chrx-str.org. System DXS10135 showed to be the most informative while system DXS8378 was the least informative. The combined discrimination power (CDP) in both males and females was equal to one. The combined mean exclusion chance (CMEC) was 0.999999548 in duo paternity cases and 0.999999998 in trio paternity cases. The haplotype assessment showed that all 500 tested haplotypes were unique. The haplogroups assessment using the Investigator™ Argus X-12 kit, which determines four haplogroups named haplogroup one, two, three and four, showed high number of haplogroups 329, 194, 185 and 257 respectively, which reflects a relatively high level of X-STR polymorphism in the Lebanese population compared to Y-STR. The most frequent linkage group showed a relatively low frequency of 0.048. However, when compared to the most frequent linkage group in the German population (0.037), it showed a higher frequency, Results indicate high potentials in the field of forensic science and mandate the use of X-STR analysis for complex cases based on their ability to complement autosomal and Y-STR analysis.

Keywords: X-STR, Allele Frequency; Linkage Group; Lebanese Population

Abbreviations: CMEC: Combined Mean Exclusion Chance, CDP: Combined Discrimination Power, STR: Short Tandem Repeat.

Introduction

Many polymorphic DNA loci have been utilized for PCR-based typing, including variable number of tandem repeat (VNTR) markers and short tandem repeat (STR) loci [1,2]. The latter are tandem repeats of 2-6 bp which have become a wide-spread routine in human identification as well as forensic case work and kinship testing owing to their high individualization power and practicality [3-5].

However, sometimes autosomal STRs may show microsatellite mutations (gain or loss of repeats in the alleles) at one or more loci. Knowledge about mutation rates and the mutational process of STRs used in forensic analysis is crucial for the correct interpretation of resulting genetic profiles. Moreover, when there are one or two alleles mismatch, data from other DNA markers become essential to resolve the relatedness between the parent and offspring especially in paternity deficient cases. The most widely used markers in combination with the autosomal STR in such aberrant situations are mtDNA and Y-STR to resolve maternity and paternity disputes, respectively [6].

Although mitochondrial DNA is highly polymorphic, yet the mitochondria DNA-based assay is time consuming. That is why, Typing of X chromosomal STRs (X-STRs) could be highly useful for establishing paternity when the child is a female because fathers transmit their X-STRs to all their daughters.

X-chromosomal short tandem repeats (STRs) have a wide range of applications such as solving complex kinship cases and deficiency paternity cases especially when the disputed child is a female [7-9]. The utility of such loci comes from the fact that males are hemizygous for all X chromosomal markers reveals their haplotypes directly from STR typing. In other words, men transmit to his daughters one X-chromosome they received from their mothers. Therefore, in the absence of the father, paternal grandmother and uncles can be used to infer the father's profile [10]. Moreover, in pedigrees with proved kinship relations ChrX haplotypes can also be determined for females in many instances. True daughters carry always the whole paternal X-chromosome (ChrX) and therefore both of the daughters ChrX haplotypes can also be completely recognized when a father and his daughter are submitted to a jointed typing [11,12]. Other cases include when the alleged father is related to the true biological father, and the exclusion power of the genetic systems is greatly diminished, may be of special interest [13]. In population and forensic genetics, many studies have also focused on the analysis of X-chromosome short tandem repeats (X-STRs) based on the comparison with first- or second-degree relatives and on the collection of frequency and forensic efficiency information [14-19].

So far, there are a reduced number of population genetic studies regarding X-STR markers. Lebanon for example, has just recently developed an autosomal and Y-STR allele frequency database [20,21]. Lebanon is a small country of around 4.5 million. Moreover, practices such as endogamy and consanguinity have reached high rates of 36% and 88% respectively [22,23]. Results of these databases have shown so far that there might be an effect of consanguinity and endogamy on these population and subpopulation genetics. Such data are not yet available regarding X-STR analysis for the Lebanese population, and it would be highly informative to assess these frequencies where consanguinity and endogamy are widely spread practices. Moreover, in order to increase the knowledge and applicability of X-STRs in the forensic genetics field and before a new locus can be introduced in the forensic current practices, further population genetic database for the relevant population must be established in order to evaluate its effectiveness.

Materials and Methods

Population

EDTA blood tubes or buccal swabs (from right cheek, left cheek and tongue) from 507 unrelated healthy volunteers were collected with informed consent for X-STR allele frequency assessment in the Lebanese population. In addition, 99 unrelated male individuals were sampled to reach 502 samples for the haplogroup studies. The donors were selected based on religious and geographic distribution as described by Ansar, et al. [20].

DNA Extraction and Quantitation

DNA was extracted from whole blood leukocytes using the salting out method and from buccal swabs using a modified phenol-chloroform-isoamyl alcohol method. DNA quantification was performed using Nanodrop 2000C spectrophotometer equipment (Thermo Fisher

Scientific Inc.) and diluted accordingly to approximately 0.5 - 1 ng/µl.

PCR Amplification

Investigator Argus X-12 PCR amplification kit (Qiagen) was used to amplify 12 X-Chromosomal STR systems (DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148, HPRTB and Amelogenin) according to the manufacturer's recommendations [24,25].

DNA Typing

The amplified PCR products were electrophoresed on an ABI Prism 3130 Genetic Analyzer with POP-7 polymer using BTO 500 as a size standard. The data was analyzed with GeneMapper _ ID Software v4.0 (Applied Biosystems, Foster City, CA, USA).

Data Analysis

All genotypes were transferred from the Genemapper to in-house software Forensic Information and Management System (FIMS) in Addition to Arlequin v3.5 to determine the allele frequencies. ANOVA test was performed using MEGASTAT software to determine if the difference in allele frequency between males and females is statistically significant.

In order to evaluate the usefulness and potential applications of these twelve X-chromosome markers in forensic practice we performed HWE using chi-square test in female samples, and calculated GD and LD values. For the evaluation of forensic efficiency, various statistical parameters (CMECD, CMECT, PDM, PDF and CPD) were calculated using Chromosome X website software (http://www.chrx-str.org).

Results and Discussion

A Lebanese population sample of genetically unrelated individuals was studied for twelve X chromosome markers DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148, HPRTB and Amelogenin. Tables 1a, 1b, 1c and 1d describe the allele frequencies for each X-STR marker.

Allele	DXS10074	DXS10079	DXS10101	DXS10103	DXS7132
7	0.03105				
8	0.1879				
9	0.01471				
10	0.00163				
11	0.00163				0.01307
12	0.00163				0.12582
13	0.00654				0.28595
14	0.01471				0.33824
15	0.0768	0.01471		0.0049	0.20425
16	0.17974	0.01797		0.05556	0.03105
16.2	0.01471				
17	0.23366	0.0719		0.10131	0.00163
18	0.15686	0.17484		0.21732	
19	0.06536	0.27614		0.48529	
20	0.01144	0.2549		0.12418	
21	0.00163	0.12908		0.01144	
22		0.04739			
23		0.01307			
24.2			0.00327		
26.2			0.01144		
27			0.00327		
27.2			0.04085		
28			0.02451		
28.2			0.12092		
29			0.01634		

29.2			0.15686		
30			0.07516		
30.2			0.17647		
31			0.08333		
31.2			0.10294		
32			0.0866		
32.2			0.04248		
33			0.03431		
33.2			0.01307		
34			0.00817		

Table 1: Allele frequency for DXS10074, DXS10079, DXS10101, DXS10103 and DXS7132 Systems.

Allele	DXS10134	DXS10135	DXS10146	DXS10148
13.3				0.01471
16		0.00327		
17		0.0098		0.00654
17.1		0.00327		
17.3				0.00163
18		0.04902		0.11601
18.1		0.0098		
19		0.06373		0.02778
19.1		0.02451		
20		0.06373		0.00817
20.1		0.00817		0.00163
21		0.0915		0.0049
21.1		0.01471		
22		0.07353		0.01307
22.1		0.0098		0.02451
23		0.05556	0.00163	0.01797
23.1		0.00817		0.06863
23.3		0.00163		
24		0.0866	0.01307	0.01471
24.1				0.12418
25		0.07516	0.04575	
25.1				0.1781
26	0.00164	0.0866	0.09804	
26.1				0.14869
27		0.07843	0.09641	
27.1				0.10131
28	0.00327	0.05882	0.1781	
28.1				0.07843
29	0.00655	0.03922	0.13725	0.00163
29.1				0.03595
30	0.00327	0.03595	0.09967	0.00163
30.1				0.00654
31	0.00491	0.02124	0.03268	

Allele	DXS10134	DXS10135	DXS10146	DXS10148
31.1			0.00163	
32	0.01473	0.01307	0.0098	
32.1				0.00327
32.2	0.00164			
33	0.08183	0.00817	0.00654	
33.2	0.00164			
34	0.14403	0.00163	0.00163	
35	0.22095			
35.2			0.00327	
36	0.15385	0.00327		
37	0.13748			
37.3	0.00164			
38	0.07365			
38.2	0.00327		0.00163	
38.3	0.03764			
39	0.00982			
39.2		0.00163	0.02288	
39.3	0.03601			
40	0.00164			
40.2			0.01961	
40.3	0.02619			
41.2			0.02124	
41.3	0.01964			
42.2			0.04248	
42.3	0.01146			
43.2			0.06373	
43.3	0.00327			
44.2			0.0719	
45.2			0.01961	
46.2			0.00654	
47.1			0.0049	

Table 2,3: Allele frequency for DXS100103, DXS10135, DXS10146 and DXS10148 Systems.

Allele	DXS7423	DXS8378	HPRTB
8			0.00163
9		0.0098	
10		0.39379	0.01307
11		0.38399	0.12745
12		0.19935	0.37255
13	0.03595	0.0098	0.30065
14	0.40686	0.00327	0.13399
15	0.32516		0.04575
16	0.19444		0.0049
17	0.03758		

Table 4: Allele frequency for DXS7423, DXS8378 and HPRTB Systems.

After determining the allele frequencies, the gene diversity values of the tested X-STR systems in Lebanese population were calculated (Table 3). The most polymorphic X-STR marker was DXS10135 while the least polymorphic marker was DXS8378.

In order to evaluate the usefulness and potential application of these twelve X-STR systems in a forensic practice in the Lebanese population, we performed HWE (Hardy-Weinberg Equilibrium) using Chi-square test on female samples, and no significant deviations ($P < 0.05$) were observed at the twelve studied loci except for the DXS10148 locus Table 5 [26,27].

X-STR Locus	*HWE P value*
DXS10103	0.81649
DXS8378	0.78279
DXS7132	0.23935
DXS10134	0.11926
DXS10074	0.12904
DXS10101	0.59922
DXS10135	0.45176
DXS7423	0.77072
DXS10146	0.15791
DXS10079	0.60388
HPRTB	0.37241
DXS10148	0.0097

Table 5: HWE results 12 X-STR in the Lebanese females population.

Moreover, in paternity testing the usefulness of a genetic marker is measured through the probability of finding, in randomly chosen individuals, inconsistencies with parent to child Mendelian rules of transmission. This parameter is called power of exclusion (PE), paternal exclusion chance or probability, can be defined for duos (mother not typed) or trios (random false fathers are matched against mother/child pairs) and performed both for autosomal and X-chromosomal markers (restricted to paternity testing involving daughters). PE is an a priori statistic, in the sense of not depending on the individual's genetic data of a case, being dependent however on the estimates of genetic markers allele (or haplotype) frequencies [28-30].

Mean exclusion chance in trios involving daughters (MECT) and in father/daughter duos (MECD) as well as power of discrimination in females (PDF) and in males (PDM) were calculated using formulae according to Desmarais, et al. [31].

The polymorphism information content was calculated according to the formula stated in Botstein, et al. [32]. Unbiased estimate of expected heterozygosity was computed as described by Edwards, et al. Table 6 summarizes the above mentioned parameters [33].

Forensic Parameter	DXS8378	DXS7423	DXS7132	DXS10148	DXS10146	DXS10135	DXS10074	DXS10079	DXS10101	DXS10103	DXS10134	HPRTB
MEC_DTrio	0.64925	0.58763	0.70199	0.85868	0.82287	0.88364	0.93399	0.63103	0.89877	0.77605	0.69177	0.88288
MEC_DDuo	0.50436	0.44068	0.56295	0.76391	0.71354	0.80014	0.87962	0.48563	0.82359	0.65244	0.55212	0.79911
PD_F	0.86378	0.81281	0.89188	0.97074	0.95591	0.97916	0.99255	0.84561	0.98396	0.93399	0.88686	0.97892
PD_M	0.68834	0.65754	0.74517	0.87141	0.84199	0.89303	0.93755	0.68822	0.90597	0.80342	0.73433	0.89238
PIC	0.64925	0.58763	0.70199	0.85868	0.82287	0.88364	0.93399	0.36103	0.89877	0.77605	0.69177	0.88288
HET	0.68834	0.65754	0.74517	0.87141	0.84199	0.89303	0.93755	0.68822	0.90597	0.80342	0.73433	0.89238
PE	0.41045	0.36567	0.50153	0.73746	0.67914	0.78121	0.87257	0.41028	0.80764	0.60539	0.48334	0.77988

PIC, polymorphic information content; HET, observed heterozygozity; PE, power of exclusion; PD, power of discrimination in females and males; MEC, mean exclusion chance calculated according to Desmarais in trios and duos.
Table 6: Forensic parameters for 12 X-STRs assessed on the Lebanese population.

Lebanon	CPD females	CPD males	Combined MEC Desmerais trio	Combined MEC Desmerais duo
Lebanese Population	1	1	0.999999998	0.999999548

Table 7: X-STR Kit Assessment.

A high power of discrimination in females and males as well as high CMEC in trios and duos was encountered which highlights the efficiency of all the 12 X-STR systems combined in our Lebanese population. Haplotypes assessment revealed 500 unique male haplotype using Argus X-12 kit.

Haplogroups assessment using the Investigator™ Argus X-12 kit showed high number of haplogroups 329, 194, 185 and 257 respectively. The most frequent haplogroup showed a relatively low frequency of 0.048.

Conclusion

High level of CPD (1) and CMEC (1) for Argus X-12 kit (Qiagen) in the lebanese population, shed the light on the importance it adds to forensic case work especially when an incomplete autosomal profile is recorded. Despite males being hemizygous, high levels of CPDmales and

CMEC for duos and trios were observed which is directly related to the high level of polymorphism encountered due to the presence of new alleles and micro variants.

Haplotypes and haplogroups result come to back-up our previous results and stress on the power Argus X-12 adds to forensic cases.

These facts are of high significance in the field of forensic science and mandate the use of X-STR analysis for complex cases based on their ability to conclude where autosomal and Y-STR fail to prevail.

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