

ALLELES' FREQUENCY DISTRIBUTION OF TWO STR LOCI IN EGYPTIAN POPULATION

BY

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ABSTRACT

Deoxyribonucleic acid (DNA) typing is widely used for personal identification. The allele frequency distribution of two STR loci (CSF1PO and TPOX) was studied in Egyptian population. A sample of 100 unrelated individuals inhabiting Dakahlia governorate (in Delta region) in Egypt was chosen randomly. Blood samples were collected after obtaining an informed consent. The DNA was extracted by using the standard phenol-chloroform extraction method and then amplified using the HYBAID Thermal Cycler. Four alleles were identified at each locus with frequencies 0.34 to 0.18 for CSF1PO locus and 0.37 to 0.085 for TPOX locus. By studying MP, PE, PD, PIC and TPI parameters; CSF1PO locus has the highest MP, PE and TPI values (0.227, 0.033 and 0.63 respectively). Meanwhile PD and PIC have the highest values in TPOX locus (0.807 and 0.69 respectively). It is concluded that the presence of certain alleles and absence of others only in the studied population compared to other presented studies on Egyptians, Arabs and Israeli populations can be specific. TPOX locus was found to be highly polymorphic and could be used in forensic investigations and issue of identity. Meanwhile both CSF1PO and TPOX loci are less informative in paternity testing.

Keywords: *DNA, STRs, Allele frequency, CSF1PO, TPOX.*

INTRODUCTION

Deoxyribonucleic acid (DNA) typing is widely used for personal identification, parentage cases and tracing the source of biological samples found in the crime scene (Atmadja, 1989). In view of their high level of variability, autosomal short tandem repeats (STRs) are very useful as

markers in both disciplines of forensic and population genetics studies (El Ossmani et al., 2009).

Potentially suitable STRs loci have repeat units of 3 -5 bp and allele sizes of about 100 - 300 bp. Some loci have alleles differing by a number of complete repeat units, whereas others display complex

polymorphism with some alleles differing in size by only 1 bp (Delghandi et al., 2001).

Since 1990, Federal Bureau of Investigation has recommended the forensic labs to use 13 STR loci, known as CODIS 13, as the loci of choice for forensic use. This recommendation has been accepted by forensic laboratories all over the world, thus the DNA typing can be compared to each other (Untoro et al., 2009).

This study was executed to get the allele frequencies and the measure of foren-

sic parameters for two STR loci (CSF1PO and TPOX) in a representative sample of Egyptian population.

SUBJECTS & METHODS

Population: A sample of 100 unrelated volunteers (50 males and 50 females; aged from 30 - 55 y) from Dakahlia governorate (in Delta region) in Egypt (Fig. 1) was chosen randomly for this study. After obtaining consent, a peripheral blood sample (5 ml) was collected from each subject on the anticoagulant ethylene - diamine - tetracetic acid (EDTA).



Fig. (1) : Map of Egypt. (Coudray et al., 2007)

DNA extraction: The blood was either used freshly or stored in deep freezer at -20°C for determining DNA fingerprinting of two loci (CSF1PO and TPOX) by using polymerase chain reaction (PCR) amplification. The test was done in the Genetics Unit of Mansoura University Children Hospital. DNA was extracted from blood using the standard phenol-chloroform extraction method (Albariño and Romanowski, 1994), followed by ethanol precipitation.

PCR amplification of DNA: PCR amplification was carried out in 0.2 ml thin-walled PCR tubes with an oil overlay. The volume of the reaction mixture was 50 µl containing 5 µl of template DNA, 5 µl of 10 X reaction buffer, 5 µl of 25 mmol MgCl₂ solution, 3 µl of 2 mmol of dNTPs solution, 3 µl of 5 picomol of each primer pair solution (= 6 µl), 26 µl of distilled water. Taq polymerase enzyme (0.3 µl of 5 unit/ µl) was added to the reaction mixture. Amplification of DNA was performed by using HYBAID Thermal Cycler at these parameters: 95 °C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 2 min and extension at 72°C for 2 min then a final extension at 72°C for 5 min (Frieze and Halldorsson, 2001).

Sequences of STRs loci primers (Frieze and Halldorsson, 2001): -
CSF1PO locus on short arm (q) of

chromosome (5q) (281-331 bp):

Primer Dy 1-1 sequence is (sense): 5'AACCTGAGTCTGCCAAGGACTAGC3.

Primer Dy 1-2 sequence is (antisense): 5'TTCCACACACCACTGGCCATCTTC3'.

TPOX locus on long arm (p) of chromosome (2p) (220 - 232 bp):

Primer Dy 2-1 sequence is (sense): 5'ACTGGCACAGAACAGGCACTTAGG'.

Primer Dy 2-2 sequence is (antisense): 5'GGAGGAAGTGGGAACACACAGGT 3'.

Statistical Analysis: Allele frequencies, power of discrimination (PD), power of exclusion (PE), polymorphism information content (PIC), match probability (MP) and typical paternity index (TPI) were calculated using the Excel Power Stats spreadsheet (Promega Corporation, Madison, WI, USA) (Tereba, 1995) for each locus of the studied population.

RESULTS

Allele frequency data of the two STR loci are presented in table 1. Four alleles (7, 8.3, 10.3 and 15) out of the 23 alleles of STR CSF1PO locus are detected in the studied group. Alleles are in the following order of frequency; allele 8.3 (0.340), followed by allele 7 (0.240), allele 10.3 (0.235) and lastly allele 15 (0.180). As regards STR TPOX locus, four alleles (5, 7.3, 10 and 14) out of the 17 alleles are detected. Allele frequency is allele 10 (0.370), followed by

alleles 5 and 14 (0.275 and 0.270 respectively) and lastly allele 7.3 (0.085).

Table (2) shows the statistical parameters (MP, PE, PD, PIC and TPI) for both CSF1PO and TPOX loci in the studied population. Also, combined match probability (CMP), combined power of discrimination (CPD), combined polymorphism information (CPI) and combined total paternity index (CTPI) are shown.

DISCUSSION

Short tandem repeat polymorphism has proven to be extremely useful in forensic medicine (Mastana and Singh, 2002). Over the past decade, the human identity testing community has settled on a set of STR loci that are widely used for DNA typing applications. These STR loci, which form the basis for DNA database worldwide, will continue to play an important role in forensic science for many years (Butler, 2006).

Proper DNA database should be available and employed for forensic genetics calculation, especially in populations where inter-population genetic exchange is extremely reduced both due to ethno-religious and geographical reasons (Barni et al., 2007). The aim of the present study is to establish database for two STR loci (CSF1PO and TPOX) in a sample of Egyptian population inhabiting

Dakahlia governorate in Delta region.

The present study reported that four alleles (7, 8.3, 10.3 and 15) from the 23 alleles of STR CSF1PO locus were detected in the studied group. Allele 8.3 was the predominant, followed by alleles 7 and 10.3 and lastly allele 15.

In comparison with other studies, Omran et al. (2009) revealed that in Upper Egyptian population; 8 alleles (7, 8, 9, 10, 11, 12, 13 and 14) were detected at CSF1PO locus and the most frequent allele was allele 12 (0.338) while the least were alleles 7 and 14 (0.008 for each).

Also Coudray et al. (2007), studied alleles' frequency of 15 STRs in three Egyptian populations of different ethnic groups (Berbers, Muslims and Copts). They stated that six alleles (8, 9, 10, 11, 12 and 13) were detected at CSF1PO locus in Egyptian Berbers (from Siwah, fig. 1). The most frequent allele was allele 10 (0.291) while the least frequent was allele 9 (0.005). In Egyptian Muslims from Adaima (an Upper Egyptian village near Esna, fig. 1), 8 alleles (8, 9, 10, 10.2, 11, 12, 13 and 14) were detected at CSF1PO locus. Allele 10 also was the most frequent allele (0.31) while the least were alleles 8 and 10.2 (0.005 for each). In the same village, alleles' frequency of Egyptian Copts showed that 7 alleles (8, 9, 10, 11, 12, 13 and 15) were detected at CSF1PO locus. The most frequent allele

was allele 12 (0.385) then allele 10 (0.365) while the least was allele 15 (0.005).

It can be noted that allele 8.3 (the most frequent allele in the present study) and allele 10.3 were not detected at locus CSF1PO in the other Egyptian studies. This may be due to small studied sample size and different ethnic origin. Allele 15 was detected only in the studied population from Dakahlia and in Egyptian Copts.

In spite of historical admixture with other ethnic groups who ruled Egypt in its past, e.g. Ottomans, Arabs (who constituted the major migration waves), English, French and Greeks (Omran et al., 2009), Berbers from the Siwah Oasis always tried to preserve their culture and their identity. Muslims and Copts are neighboring cultural groups where inter-population genetic exchange is extremely reduced. That is why alleles' frequency differed between Egyptians.

Furthermore, for neighboring countries, Alenizi et al. (2008), in Kuwait found that 8 alleles (7, 8, 9, 10, 11, 12, 13 and 14) at CSF1PO locus were detected. Allele 12 was the most frequent allele (0.347) then allele 10 (0.322) while the least was allele 7 (0.002). In Iraqi population, Barni et al. (2007) stated that 7 alleles (8, 9, 10, 11, 12, 13 and 14) were detected at the same locus. The most frequent allele was allele 12 (0.328) and the least was allele 14 (0.009).

Moreover, Cherni et al. (2005) study in Tunisian Berber population revealed that 6 alleles (8, 10, 11, 12, 13 and 14) were detected. Similarly, allele 12 was the most frequent allele (0.342) and the least frequent alleles were 8 and 14 (0.023 for each).

Alleles 8, 10, 11, 12 and 13 were detected in all available presented Arab population studies and in Egyptians apart from the present study. This indicates that these alleles are not specific for certain population. Allele 15 that was detected only in Dakahlia population and in Egyptian Copts was not detected in the presented Arab populations. Allele 14 was detected in Tunisian Berber population but not in Egyptian Berber population.

In the Jewish Israeli population Motro et al. (2002), detected 10 alleles (6, 7, 8, 9, 10, 11, 12, 13, 14 and 15) at CSF1PO locus. The most frequent was allele 11 (0.326) and the least frequent were alleles 7 and 15 (0.0009 for each). Also, in the Arab Israeli population 10 alleles (6, 7, 8, 9, 10, 11, 12, 13, 14 and 15) were detected at the same locus. But the most frequent was allele 10 (0.307) while the least frequent were alleles 6 and 15 (0.000 for each).

It can be noted that Israeli population share Arab and some Egyptian populations in the following alleles; 8, 9, 10, 11, 12, 13 and 14 indicating that they are not

specific. However, allele 6 was detected only in Israeli population indicating that it is specific for them. Allele 15 that was detected only in Dakahlia population and in Egyptian Copts was also detected in Israeli population but not in Arabs and other Egyptians (Berbers and Muslims). The most frequent alleles were 10 and 12 in presented studies except allele 8.3 in the present Dakahlia population and allele 11 in Jewish Israeli population. Meanwhile the least frequent alleles differed in all.

As regards the allele frequency of STR TPOX, the present study reported that four alleles (5, 7.3, 10 and 14) from the 17 alleles of this locus were detected in the studied subjects. Allele 10 was the predominant, followed by alleles 5 and 14 and lastly allele 7.3.

In their study on TPOX locus, Coudray et al. (2007) found that 7 alleles (6, 7, 8, 9, 10, 11 and 12) were detected in Egyptian Berbers from Siwah. The most frequent allele was allele 8 (0.480) while the least frequent was allele 12 (0.015). While in Egyptian Muslims from Adaima, 6 alleles (6, 8, 9, 10, 11 and 12) were detected. The most frequent was also allele 8 (0.424) and the least frequent was allele 6 (0.02). In the same village, allele frequencies of Egyptian Copts showed that 5 alleles (6, 8, 9, 10 and 11) were detected. Similarly, the most frequent allele was allele 8 (0.52) while the least frequent was allele 6 (0.005).

On Contrary to the present study, Omran et al. (2009) detected 7 alleles (6, 7, 8, 9, 10, 11 and 12) at TPOX locus in Upper Egyptians. The most frequent allele was allele 8 (0.483) while the least frequent was allele 7 (0.006).

It is concluded that alleles 5, 7.3 and 14 were detected in the studied group meanwhile allele 6 was not detected compared to other Egyptian studies. Allele 12 was not detected in Dakahlia population and in Egyptian Copts.

As regards Kuwaiti population, Alenizi et al. (2008) detected 5 alleles (8, 9, 10, 11 and 12) at TPOX locus. The most frequent allele was allele 8 (0.527) while the least frequent was allele 12 (0.0267). Meanwhile, Barni et al. (2007) study in Iraqi population revealed that 6 alleles (6, 8, 9, 10, 11 and 12) were detected. Similarly, the most frequent allele was allele 8 (0.544) and the least frequent was allele 12 (0.0194). Furthermore, in Tunisian Berber population, Cherni et al. (2005) detected 6 alleles (7, 8, 9, 10, 11 and 12). Also, allele 8 was the most frequent allele (0.477) and the least frequent was allele 12 (0.011).

Motro et al. (2002) study in Jewish Israeli population revealed that 7 alleles (6, 7, 8, 9, 10, 11 and 12) were detected at TPOX locus. The most frequent allele was allele 8 (0.526) while the least frequent was allele 6 (0.0009). Also, in the Arab Israeli

population 7 alleles (6, 7, 8, 9, 10, 11 and 12) were detected. Similarly the most frequent allele was allele 8 (0.513) and the least frequent was allele 7 (0.004).

Alleles 8, 9, 10, 11 and 12 were detected in all presented Arab populations, Israeli population and in some Egyptians indicating non specificity (apart from allele 12). Alleles 6 and 7 were inconstant. Allele 6 was not detected in Dakahleyian, Kuwaiti and Tunisian populations meanwhile allele 7 was not detected in Kuwaiti, Iraqi and some Egyptian populations. Again, the racial differences, the immigration and the different samples size may contribute to these frequency differences between populations.

By studying the forensically important parameters (MP, PE, PD, PIC and TPI) the present study revealed that match probability (MP), power of exclusion (PE) and typical paternity index (TPI) have the highest values in CSF1PO locus (0.227, 0.033 and 0.63 respectively). While power of discrimination (PD) and polymorphism information content (PIC) have the highest values in TPOX locus (0.807 and 0.69 respectively).

According to Shriver et al. (1995), when the power of discrimination value of one STR locus is over 0.80 and/or its power of exclusion value is over 0.50, STR locus could be considered as highly polymor-

phic. The results of the present study showed that TPOX locus is highly polymorphic (PD = 0.8). Both loci (CSF1PO and TPOX) have low power of exclusion, so they can not be used alone to exclude paternity. By using both loci, indicated by the combined parameters, their values are still low. The combined power of discrimination of two loci reaches 0.616, the combined match probability reaches 0.0438, the combined power of exclusion reaches 0.0006, the combined polymorphism information content reaches 0.4485 and the combined total paternity index reaches 0.378.

So, it can be concluded that in the studied Egyptian population, at CSF1PO locus, allele 8.3 was the most predominant followed by alleles 7 and 10.3. To our knowledge alleles 8.3 and 10.3 was not recorded in any of the studies we could obtain and review. Meanwhile allele 15 was recorded only in Egyptian population examined in the present study, Egyptian Copts and Israeli population. It could also be observed that alleles 5, 7.3 and 14 were detected only in the Egyptian population under study. Allele 10 of TPOX locus exhibited the higher frequency in the present study was not demonstrated as the most frequent allele in any of the populations examined in the previously mentioned studies. Allele 12 was absent in the studied Dakahlian population and Egyptian Copts.

Also, it could be concluded that TPOX locus is highly polymorphic and can be used in forensic investigations and issue of identity. While both loci are less informative in paternity testing and they can

not be used alone in exclusion or inclusion of paternity. Overall, these data indicated the possible utility of these STR loci for forensic personal identification in the Egyptian population.

Table (1): Alleles' frequency of the CSF1PO and TPOX loci in the studied group.

Alleles	CSF1PO	Alleles	TPOX
5	-	4	-
6	-	5	0.275
6.3	-	6	-
7	0.240	7	-
7.3	-	7.3	0.085
8	-	8	-
8.3	0.340*	9	-
9	-	10	0.370*
9.1	-	10.1	-
9.3	-	10.3	-
10	-	11	-
10.1	-	12	-
10.2	-	13	-
10.3	0.235	13.1	-
11	-	14	0.270
11.1	-	15	-
11.3	-	16	-
12	-		
12.1	-		
13	-		
14	-		
15	0.180		
16	-		

* is the highest frequency.

Table (2): Forensic important parameters for CSF1PO and TPOX loci in the studied group.

Parameters	CSF1PO	TPOX
MP	0.227	0.193
PE	0.033	0.020
PD	0.773	0.807
PIC	0.65	0.69
TPI	0.63	0.60
CMP	0.044	
CPD	0.616	
CPIC	0.449	
CTPI	0.378	

MP: match probability, **PE:** power of exclusion, **PD:** power of discrimination,
PIC: polymorphism information content, **TPI :** typical paternity index,
CMP: combined match probability, **CPD:** combined power of discrimination,
CPIC: combined polymorphism information content,
CTPI: combined total paternity index.

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معدل تكرار الصور الجينية لموضوعين من الجينات فى المصريين

المشتركون فى البحث

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أستهدفت الدراسة الحالية بحث معدل تكرار الصور الجينية لموضوعين من الجينات وهما CSF1PO و TPOX فى عينة من المصريين ودراسة بعض القياسات ذات الأهمية الطبية الشرعية (MP, PE, PD, PIC and TPI).

وقد تم إجراء هذه الدراسة على ١٠٠ شخص (٥٠ ذكر و ٥٠ أنثى تتراوح أعمارهم من ٣٠ إلى ٥٥ عام) تم إختيارهم عشوائياً ولاتوجد بينهم صلة قرابة من قاطنى محافظة الدقهلية - مصر، وقد تم أخذ عينة من الدم (٥سم) من كل شخص بعد أخذ موافقتهم لتعيين معدل تكرار الصور الجينية (alleles) لموضوعين (loci) للحامض النووى باستخدام تفاعل البلمرة المتسلسل.

أظهرت نتائج البحث تعيين أربعة صور جينية لكل من الموضوعين يتراوح تكرارها بين ٠.١٨ - ٠.٣٤ بالنسبة للموضع CSF1PO وبين ٠.٠٨٥ - ٠.٣٧ بالنسبة للموضع TPOX.

وبعد إجراء بعض القياسات ذات الأهمية الطبية الشرعية تبين أن الموضع الجينى CSF1PO ذو قيم أعلى لكل من (MP; PE and TPI) (٠.٢٢٧، ٠.٣٣، و ٠.٦٣ على التوالي) بينما أظهر الموضع TPOX أعلى قيم لكل من (PD and PIC) (٠.٨٠٧ و ٠.٦٩ على التوالي).

وعلى هذا يمكن أن نستنتج أن الموضع TPOX هو الأكثر تعدداً للأنماط الظاهرية ومن ثم يمكن إستخدامه فى القضايا الطبية الشرعية المنوط بها حل مشكلة الاستعراف، فى حين أنه لايمكن الاعتماد على الموضوعين فى قضايا إثبات أو نفي النسب لعدم امتلاكهما نسبة عالية فى نفي أو إثبات النسب.