Forensic Science International: Genetics 16 (2015) 88–93

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Identification of the third/extra allele for forensic application in cases with TPOX tri-allelic pattern

Juliane Bentes Picanço [a], Paulo Eduardo Raimann [a], Carlos Henrique Ares Silveira da Motta [b], Rodrigo Rodenbusch [a], Leonor Gusmão [c], Clarice Sampaio Alho [a,*]

[a] PUCRS, Faculdade de Biociências, Laboratório de Genética Humana e Molecular, Av. Ipiranga, 6681, P12C, sala 233, Porto Alegre, RS 90619-900, Brazil
[b] Instituto de Medicina Social e de Criminologia de São Paulo – IMESC, Rua Barra Funda, 824, São Paulo, SP 01152-000, Brazil
[c] IPATIMUP – Institute of Molecular Pathology and Immunology, Universidade do Porto, Porto, Portugal

ARTICLE INFO

Article history:
Received 24 September 2013
Received in revised form 24 October 2014
Accepted 19 November 2014

Keywords:
Tri-allelic pattern
Extra allele
TPOX STR profile

ABSTRACT

Genotyping of polymorphic short tandem repeats (STRs) loci is widely used in forensic DNA analysis. STR loci eventually present tri-allelic pattern as a genotyping irregularity and, in that situation, the doubt about the tri-allele locus frequency calculation can reduce the analysis strength. In the TPOX human STR locus, tri-allelic genotypes have been reported with a widely varied frequency among human populations. We investigate whether there is a single extra allele (the third allele) in the TPOX tri-allelic pattern, what it is, and where it is, aiming to understand its genomic anatomy and to propose the knowledge of this TPOX extra allele from genetic profile, thus preserving the two standard TPOX alleles in forensic analyses. We looked for TPOX tri-allelic subjects in 75,113 Brazilian families. Considering only the parental generation (mother + father) we had 150,226 unrelated subjects evaluated. From this total, we found 88 unrelated subjects with tri-allelic pattern in the TPOX locus (0.06%; 88/150,226). Seventy three of these 88 subjects (73/88; 83%) had the Clayton's original Type 2 tri-allelic pattern (three peaks of even intensity). The remaining 17% (15/88) show a new Type 2 derived category with heterozygote peak imbalance (one double dose peak plus one regular sized peak). In this paper we present detailed data from 66 trios (mother + father + child) with true biological relationships. In 39 of these families (39/66; 59%) the extra TPOX allele was transmitted either from the mother or from the father to the child. Evidences indicated the allele 10 as the extra TPOX allele, and it is on the X chromosome. The present data, which support the previous Lane hypothesis, improve the knowledge about tri-allelic pattern of TPOX CODIS' locus allowing the use of TPOX profile in forensic analyses even when with tri-allelic pattern. This evaluation is now available for different forensic applications.

1. Introduction

Genotyping of polymorphic short tandem repeats (STRs) loci is widely used in forensic DNA analysis, in familial relationship testing, and for genetic identification of individuals in paternity disputes. Tri-allelic patterns are categorized as a genotyping irregularity that can be found in STR profiling [1,2]. Clayton and co-workers [3] have distinguished two types of tri-allelic patterns. Type 1 has two alleles with a different intensity (after PCR amplification) of a third allele and Type 2 can be manifested as three peaks with the same height. Type 1 is believed to be the result of a mutation in an early somatic cell, while Type 2 is thought to represent a constitutional chromosomal rearrangement. Although tri-allelic genotypes are generally rare, data presented on the STRBase website (http://www.cstl.nist.gov/biotech/strbase) indicate that tri-allelic STR genotypes can be unusually frequent.

Alleles of the TPOX STR locus have different numbers of a four nucleotide repeat motif arranged in tandem [4] and, for more than a decade, TPOX tri-allelic genotypes have been reported with a widely varied frequency among human populations. The frequencies to the tri-allelic pattern varies; 0.18% in 10,000 subjects for Alabama, USA [5], 0.003% in 32,800 individuals from Bosnia, Kosovo and Serbia [6], 2% in 6827 South Africans [7], 0.2% in 561 subjects from Brazil [8]. On the other hand, Poiares et al. [9] typed 12,886 unrelated subjects from Brazil and were unable to find any TPOX tri-allelic genotype. In the African population [7],

* Corresponding author. Tel.: +55 51 3320 3545.
E-mail address: csalho@pucrs.br (C.S. Alho).

http://dx.doi.org/10.1016/j.fsigen.2014.11.016
1872-4973/

Dr. Lane was able to demonstrate that two thirds of the TPOX tri-allelic adults were females, and TPOX tri-allelic fathers only had transmitted the TPOX tri-allelic genotype to their daughters. With this evidence, he suggested that the extra allele (usually allele 10) would be inserted in the X chromosome. This report was enhanced by Díaz et al. [10].

Despite the substantial frequency of the TPOX tri-allelic pattern, the nature of the extra allele is still poorly understood. In this work, we present data obtained from 66 families with cases of TPOX tri-allelic pattern (105 tri-allelic subjects). We used these data to investigate which would be the extra allele and in which chromosome it would be.

2. Materials and methods

Data were obtained from routine paternity tests performed in the states of Rio Grande do Sul, Brazil (~4000 cases/year from Fundação Estadual de Produção e Pesquisa em Saúde, FEPPS) and São Paulo (~12,000 cases/year from Instituto de Medicina Social e de Criminologia de São Paulo, IMESC), from 2008 to 2012. In this routine, the genomic DNA was purified from dried blood samples preserved in FTA cards following the manufacturer's protocols. A total of 0.5–1.0 ng of DNA was used to amplify the STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) with the AmpFℓSTR® Identifiler™ Amplification kit (Applied Biosystems, Foster City, USA), using the manufacturer's instructions, followed by analysis on a capillary ABI 3130*xl* Genetic Analyzer. Familias Software v.1.81 [11] was used to evaluate the statistical parameters and to calculate the combined paternity/maternity index in all cases considering the Combined Paternity Index (IPC) of at least 10,000. All families that presented cases of TPOX tri-allelic pattern were also typed using a different system for human identification (PowerPlex®2.1 System, Promega, Madison, WI).

In order to prevent the possibility of a false tri-allelic genotype caused by micro-variants (shorter alleles) of the adjacent bins (e.g.: D18S51 - AmpFlSTR® Identifiler® or FGA - PowerPlex®2.1 System) occupying the bins of the TPOX locus, 10% of our tri-allelic subjects had their DNA amplified by the singleplex PCR-based analysis of the TPOX-locus. In all cases, the presence of three alleles was confirmed based on fragment analysis by capillary electrophoresis.

To avoid that the true tri-allelic pattern was confused with a tri-allelic pattern-like (caused either by mixtures of DNA samples or by some primer binding issues) we included in this study subjects whose three alleles were observed only in TPOX-locus. Also, the Peak Height Ratio (PHR) from the other locus was confirmed to be bi-allelic pattern.

This project was approved by the Research Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS (Protocol #10/05317).

3. Results and discussion

Analyzing the electropherograms from all heterozygote locus profiles and their PHR, we observed that heterozygosis peak imbalance appeared in some subjects, showing that one peak was the highest (double dose) and the second one was regular sized. This would be a tri-allelic profile hidden by the heterozygote peak imbalance. With this observation, we used Clayton's Type 2 tri-allelic pattern to create derivate categories (Fig. 1). In these derived categories the electropherogram would show the following patterns: Type 2-A: three peaks of three different alleles each one with the same intensity (e.g. 8-10-11); Type 2-B: one peak double height, referring to two identical alleles, and one peak with regular height of another different allele (e.g. 8-10-10); Type 2-C: one single peak with triplicate height, referring to three identical alleles (e.g. 10-10-10). This last pattern (Type 2-C) has not yet been verified in a real subject, but it can occur.

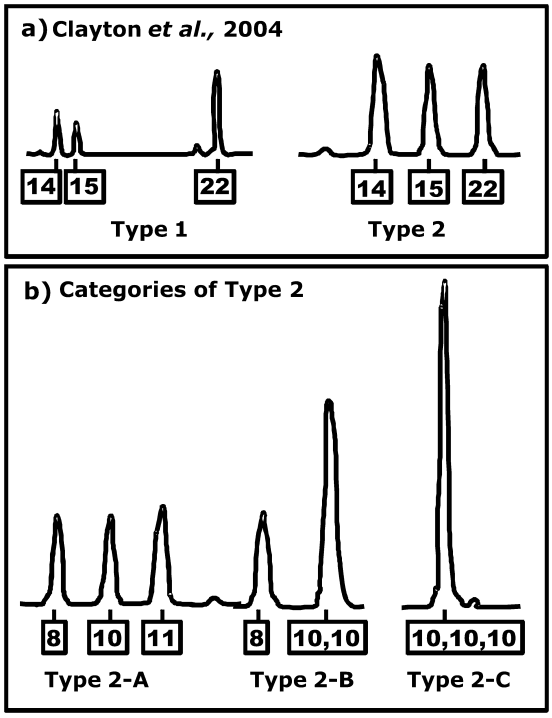

Fig. 1. (a) Clayton et al. (2004) tri-allelic pattern categories (Type 1 and Type 2); (b) Our suggestion for three categories derived from Clayton's Type 2 tri-allelic pattern. In these derived categories the electropherogram would show the following patterns: Type 2-A (8-10-11): three regular peaks of three different alleles; Type 2-B (8-10-10): one peak double dose and one regular peak; Type 2-C: one single peak triple dose.

We looked for tri-allelic subjects in 75,113 families (54,863 from IMESC, and 20,250 from FEPPS). Considering only the parental generation (mother + father) we had 150,226 unrelated subjects evaluated. From this total, we found 88 unrelated subjects with tri-allelic pattern in TPOX locus, which is equivalent to the frequency of 0.06% (88/150,226). Seventy three of these 88 subjects (73/88; 83%) were into the Type 2-A category. Fifteen of these 88 subjects (15/88; 17%) show the Type 2-B category (Fig. 2); this result indicates that 17% of TPOX tri-allelic genotypes would have been hidden if the heterozygosis peak imbalance were disregarded.

Sixty-six trios (66/88; 75%) belonged to families with true biological relationships (mother + father + child). For further analyses we worked only with these 66 trios, where the mother or the father had tri-allelic pattern in TPOX locus. The 66 families are presented in Fig. 3. In 39 families (39/66; 59%) the third allele was transmitted from the mother or from the father to the child. Thus, a total of 105 (39 + 66) tri-allelic subjects were studied here. The alleles present in all 105 tri-allelic subjects were: 6, 7, 8, 9, 10, 11, 12, and 13. To identify which one could be the extra allele, we performed two analyses: In the first one, we evaluated the possible transmission of the extra allele; evaluating the 39 families where the extra allele was transmitted, we observed that the alleles 6, 8, 9, 11, 12, and 13 could be excluded as being the third allele, because the parents had the allele, but it was absent on the child. Thus, only the alleles 7 and 10 could not be excluded as being the extra allele (Table 1). Considering the alleles 7 and 10 as possible third allele, we analyzed the 66 families and observed that the allele 7 appeared as possible third allele in only one family (FAM002), but allele 10 was present as a possible third allele in 100% of the families (66/66). Besides, in 51.5% (34/66) of families, the allele 10 was mandatorily transmitted by the tri-allelic parent.

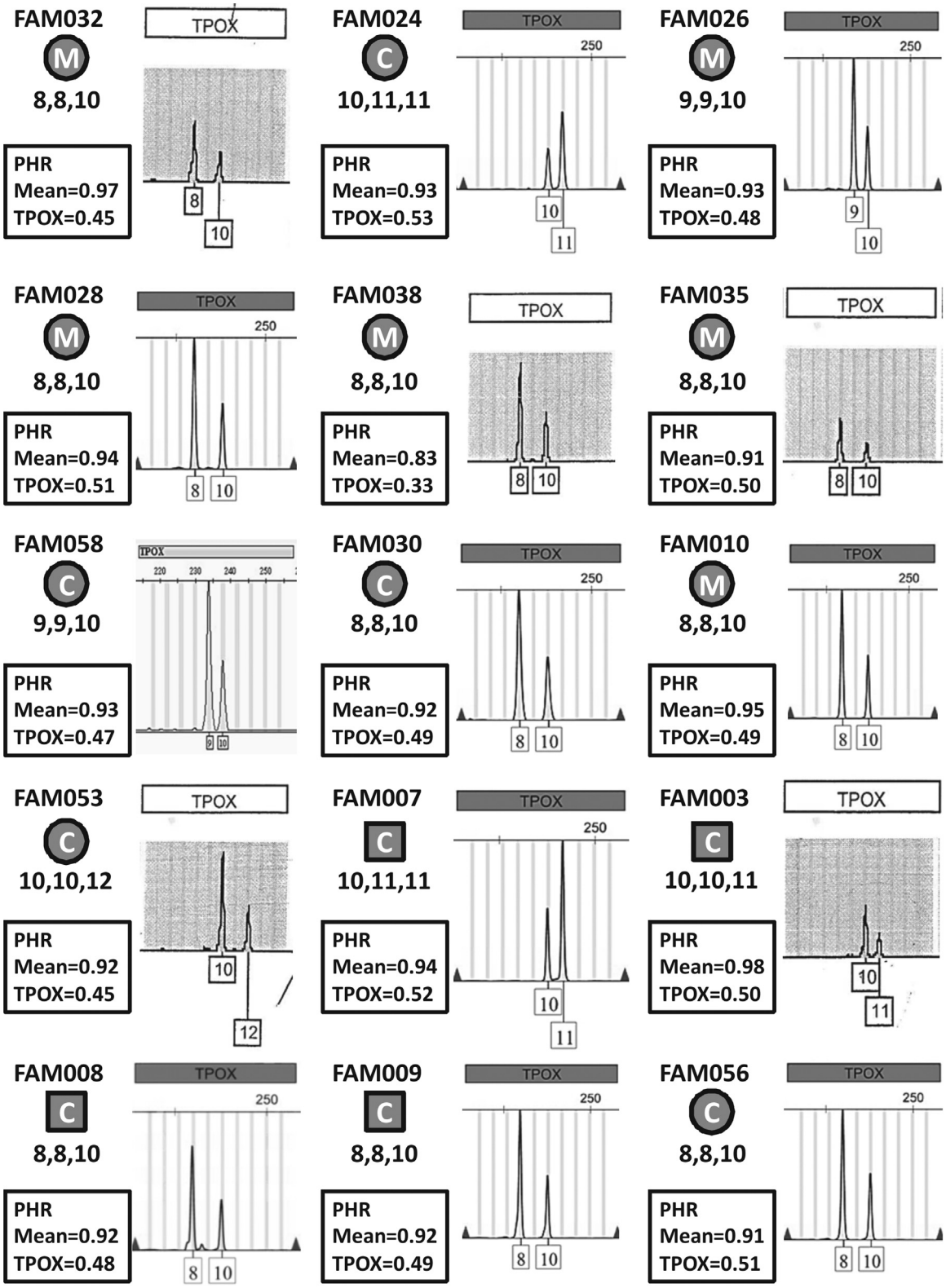

Fig. 2. Electropherograms are showing the heterozygote peak imbalance in TPOX locus. Circle: female; Square: male; M: mother; C: child. The numbers below the subjects' symbols represent the alleles of TPOX locus. The 15 subjects presented a higher peak (double dose homozygous) and a regular sized peak. The Peak Height Ratio (PHR) is shown: PHR Mean is equivalent to PHR average of all regular heterozygous locus in the subject; PHR TPOX is the PHR value to TPOX locus.

Based on the premise described by Dr. Lane [7], that the third/extra allele would be the allele 10, our result led us to accept that the extra allele should be the allele 10. This hypothesis was confirmed by the second analysis made by the investigation of the absolute and relative frequencies of each allele in the tri-allelic subjects. We analyzed our 66 families plus the tri-allelic individuals already reported in STRBase. Table 2 shows how many times each allele appears in each tri-allelic subject.

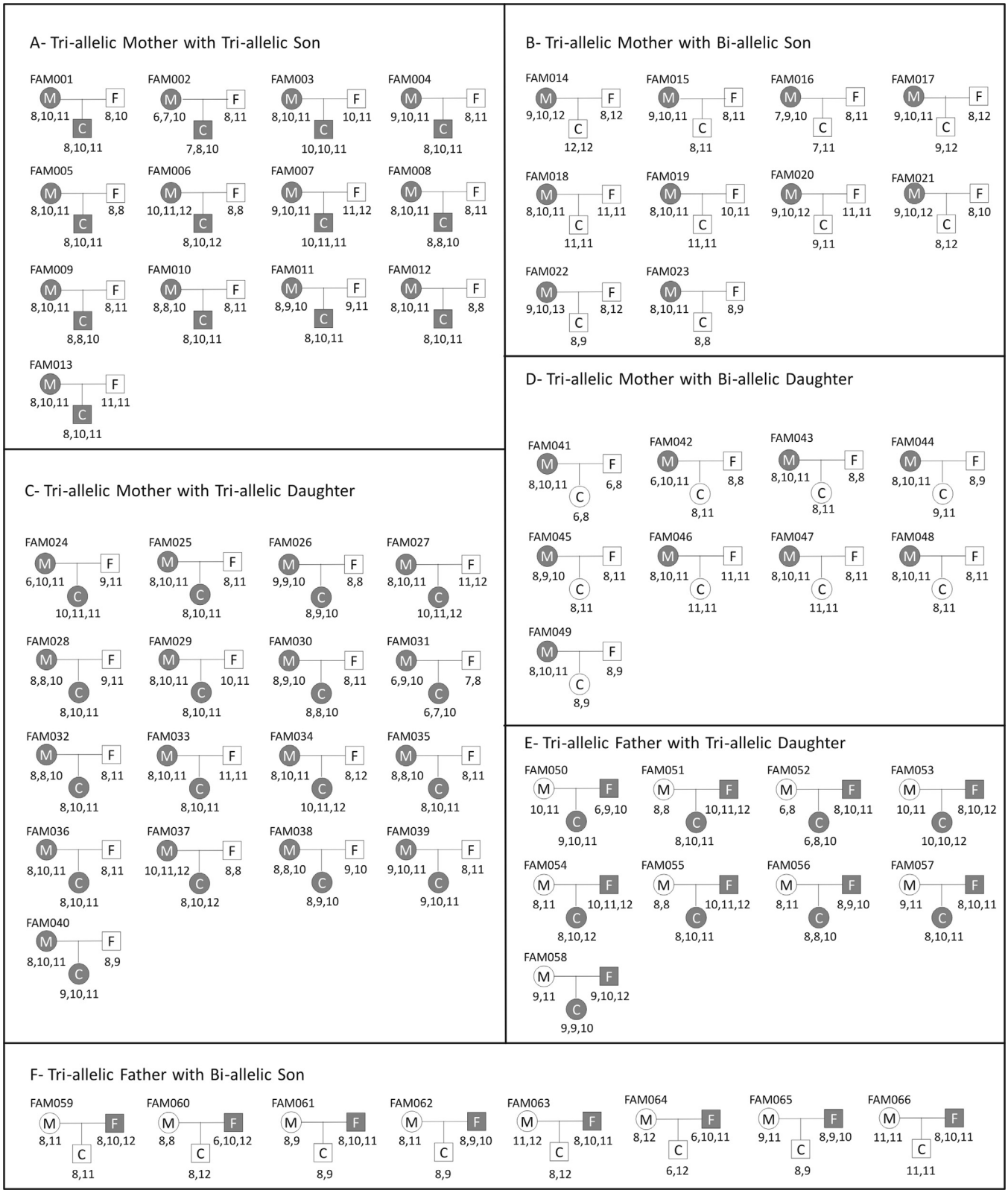

Fig. 3. Our 66 families with TPOX tri-allelic pattern. Circle: female; Square: male; M: mother; F: father; C: child. The numbers below the subjects' symbols represent the alleles of TPOX locus. The families are organized by tri-allelic parent (grey symbol); in 49 families the mother was tri-allelic (A, B, C, D), and in 17 families the father was tri-allelic (E, F). The mother transmitted the extra allele to her child (son or daughter equally) in 61% (30/49) of families. The father transmitted the third allele only when the child was a daughter.

According to Table 2, allele 10 was present in 100% of our tri-allelic subjects and in 90% of individuals reported on the tri-allelic STRBase. All other alleles were present in lower percentage. Ten tri-allelic subjects from the STRBase without the allele 10 had always the alleles 9 or 11 (genotypes were: 8-9-11; 8-11-12; 8-11-14.3; 9-11-12), what could denote slippage mutation from an ancestral extra allele 10. The comparison between each allele frequency in bi- and

Table 1
Transmission analysis of the third allele in 39 families to identify which allele was not transmitted from tri-allelic parent to tri-allelic child.

Allele	Families that exclude the transmission of this allele as third/extra allele[a]
6	FAM002, FAM024, FAM050
7	–
8	FAM026, FAM003, FAM027, FAM034, FAM053
9	FAM004, FAM030, FAM031
10	–
11	FAM008, FAM052
12	FAM051

[a] The number indicates the family that is shown in Fig. 3.

Table 2
Absolute frequency of each allele in tri-allelic subjects in our 66 families (with 105 tri-allelic subjects) and in STRBase (with 102 tri-allelic reported subjects).

Allele	Our 66 families ($N = 105$)	STRBase ($N = 102$)[a]
6	0.086 [09/105]	0.156 [16/102]
7	0.038 [04/105]	0.058 [06/102]
8	0.667 [70/105]	0.637 [65/102]
9	0.248 [26/105]	0.450 [46/102]
10	1.00 [105/105]	0.901 [92/102]
11	0.638 [67/105]	0.598 [61/102]
12	0.171 [18/105]	0.176 [18/102]
13	0.009 [01/105	–
14.3	–	0.009 [01/102]

[a] From: http://www.cstl.nist.gov/strbase/ [accessed on December 2012].

tri-allelic subjects and the ratio of these frequencies is showed in Table 3.

Comparing the allele frequencies among bi- and tri-allelic subjects, we observed that the frequency of the allele 10 in tri-allelic subjects was around five times higher than in bi-allelic subjects in both Brazilian and Global populations. With these results, it was possible to strengthen the hypothesis that the extra allele would be the allele 10. Additionally, we noted that in 100% of cases from 27 families where there was no transmission of the extra allele from the parent to the child, the allele 10 was absent in the bi-allelic offspring.

After this, we tested where the extra allele 10 would be. We noticed that in 100% of cases the tri-allelic father transmitted the extra allele 10 to his daughter, but never to his son (Fig. 3E–F). Moreover, the tri-allelic mothers transmitted the third allele 10 in around 50% of cases, both to her daughter and to her son (Fig. 3A–D). This last result allowed us to believe that the extra allele 10 would be on the X chromosome as previously suggested by Dr. Lane [7].

In our study with 75,113 Brazilian families any tri-allelic case was detected only in the offspring, i.e. here we do not detect any "de novo" TPOX tri-allelic mutation.

When STR loci present tri-allelic pattern it causes doubt on the tri-allele locus calculation, possibly reducing the total profile analysis strength. Knowing the extra TPOX allele may allow excluding it from genetic profile, thus preserving the two true TPOX alleles in forensic analyses (calculations). On the other hand, an extra allele transmission from a father to a daughter could improve paternity analysis especially in some special cases with partial profile, for example. The tri-allelic pattern study has important consequences for forensic applications, including also the criteria for locus interpretation in cases of admixtures. So, we assume the possibility to identify the extra allele is worthy to be considered for improve the frequency calculations and the quality of reports.

4. Conclusions

In our study of with Brazilian families the frequency of tri-allelic subjects in TPOX locus was 0.06% (88/150,226), and 17% (15/88) of TPOX tri-allelic genotypes presented heterozygosis peak imbalance. Our data support the previous hypothesis that the extra TPOX allele was the allele 10, and that it is localized on X chromosome. These data improve the knowledge about tri-allelic pattern of TPOX CODIS' locus allowing the use of TPOX profile in forensic analysis even when with tri-allelic pattern.

Table 3
Frequency of each allele in bi- and tri-allelic subjects in our 66 families and in STRBase, and tri-allelic/bi-allelic frequency ratios in Brazil and Global populations.

Population	Allele	Allelic frequency in tri-allelic subjects	Allelic frequency in bi-allelic subjects	Relative tri-allelic/bi-allelic Ratio
Our cases		Subjects $N = 105$ TPOX alleles $N = 315$	Subjects $N = 123{,}102$[a] TPOX alleles $N = 246{,}202$	
	6	0.028 [09/315]	0.018	1.555
	7	0.012 [04/315]	0.008	1.500
	8	0.248 [79/315]	0.456	0.544
	9	0.088 [28/315]	0.124	0.710
	10	0.339 [108/315]	0.067	5.060
	11	0.216 [69/315]	0.275	0.785
	12	0.059 [19/315]	0.048	1.230
	13	0.003 [01/315]	0.002	1.500
Population	Allele	Allelic frequency in tri-allelic subjects	Allelic frequency in bi-allelic subjects	Relative tri-allelic/bi-allelic Ratio
Global		Subjects $N = 102$ TPOX alleles $N = 306$	Subjects $N = 700$[b] TPOX alleles $N = 1400$	
	6	0.052 [16/306]	0.039	1.333
	7	0.019 [06/306]	0.008	2.375
	8	0.212 [65/306]	0.463	0.458
	9	0.150 [46/306]	0.138	1.087
	10	0.303 [93/306]	0.064	4.734
	11	0.199 [61/306]	0.241	0.826
	12	0.058 [18/306]	0.046	1.261
	14.3	0.003 [01/306]	–	–

[a] Aguiar et al., 2012: 123,102 individuals; $N = 246{,}202$ alleles [12].
[b] Butler et al., 2003: 700 individuals; $N = 1400$ alleles (U.S. Caucasian, African American, and Hispanic populations) [13].

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

This investigation was approved by Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul, Brazil. (CEP Resolution n°. 10/05317).

Acknowledgements

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS, Brasil.

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