Gene 535 (2014) 353–358

Contents lists available at ScienceDirect

Gene

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

Tri-allelic pattern at the TPOX locus: A familial study

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ARTICLE INFO

Article history:
Accepted 10 October 2013
Available online 18 October 2013

Keywords:
Family pedigree
STR
Tri-allelic pattern
TPOX

ABSTRACT

Alleles at the TPOX STR locus have 6–14 different numbers of a four-nucleotide (AATG) repeat motif arranged in tandem. Although tri-allelic genotypes are generally rare, the TPOX tri-allelic pattern has a higher frequency, varying widely among populations. Despite this, there are few accurate reports to disclose the nature of the TPOX third allele. In this work we present data obtained from 45 individuals belonging to the same pedigree, in which there are cases of tri-allelic TPOX genotypes. The subjects were apparently healthy with a normal biological development. We noticed six tri-allelic cases in this family, and all of them were women. Karyotype analysis showed no occurrence of partial 2p trisomy. All the tri-allelic cases had the genotype 8–10–11, probably due to three copies of the TPOX STR sequence in all cells (Type 2 tri-allelic pattern). Based on previous data we assumed the allele 10 as the TPOX third allele. The pedigree analyses show evidences that the TPOX extra-allele was the allele10, it is placed far from the main TPOX locus, and that there is a potential linkage of the TPOX extra-allele-10 with Xq. This was the first study that included a large pedigree analysis in order to understand the nature TPOX tri-allelic pattern.

1. Introduction

A forensic paternity test follows simple Mendelian inheritance where the child inherits one allele from the mother and another from the father at each locus. Rare events, such as point mutations in primer binding regions, slippage mutations, and other events such as gene conversion and copy number variations (CNVs) (Freeman et al., 2006) can occasionally cause an aberrant result, including an abnormal number of alleles, which seemingly break the rules of Mendelian inheritance (Lukka et al., 2006). Clayton et al. (2004) have distinguished two types of tri-allelic pattern. Type 1: when after PCR amplification two alleles have different intensity of the third allele and Type 2: when the three peaks have similar intensity. 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 short tandem repeats (STR) tri-allelic genotypes can be unusually frequent in the TPOX human STR locus. Alleles at the TPOX locus have different numbers of a four-nucleotide repeat motif arranged in tandem (Anker et al., 1992). Therefore, TPOX polymorphism is widely used for paternity testing and personal identification, and is one of the FBI's CODIS STR loci (http://www.cstl.nist.gov/strbase/fbicore.htm). The TPOX tri-allelic profiles are due to some duplication of the STR segment and flanking regions recognized by the TPOX primers.

For more than a decade, TPOX tri-allelic genotypes have been reported with a widely varied frequency among human populations. Crouse et al., 1999 reported 18 tri-allelic genotypes in a sample of over 10,000 individuals drawn from Alabama, USA, which is equivalent to a frequency of 0.18% (18/10,000). Later, Huel et al. (2007) typed 32,800 individuals from Bosnia, Kosovo, and Serbia, but found only one subject with a TPOX tri-allelic genotype (1/32,800; 0.003%), and Fridman et al. (2008) analyzing 561 unrelated individuals (410 females and 151 males) from Brazil also observed one single occurrence of a tri-allelic pattern at the TPOX locus (1/561; 0.2%). In contrast, more than 2% of indigenous South Africans exhibit tri-allelic TPOX genotypes: 165 tri-allelic genotypes (116 females and 49 males) were found among a total of 6827 black South Africans (3399 females and 3428 males) (Lane, 2008). And in a larger study from Brazil, Poiares et al. (2010) typing 12,886 unrelated individuals were unable to find any TPOX tri-allelic genotype.

Abbreviations: CNVs, copy number variations; STR, short tandem repeat; TPOX, thyroid peroxidase; FBI, Federal Bureau Investigation; CODIS, Combined DNA Index System; DNA, deoxyribonucleic acid; GTG, G-banded chromosome; pb, base pair; PCR, polymerase chain reaction; X-STR, short tandem repeat of X-chromosome; IBGE, Instituto Brasileiro de Geografia e Estatística.

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http://dx.doi.org/10.1016/j.gene.2013.10.019

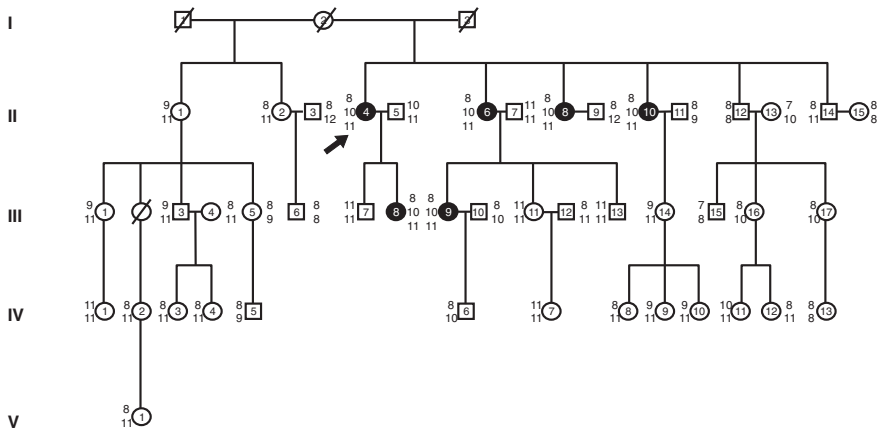

Fig. 1. Family pedigree with the DNA profiles of the TPOX locus. Roman numerals show the five generations of this family. Females are represented by circles. Males are represented by squares. Shaded circles indicate women with tri-allelic TPOX genotype. Numbers within the symbols (circles and squares) indicate each subject. Numbers outside the symbols indicate the individual's genotype for the TPOX STR locus.

In the African population (Lane, 2008), Dr. Lane was able to suggest that the TPOX extra allele were the allele 10. Indeed, according to STRBase website (with 102 TPOX tri-allelic reported subjects) the allele 10 is present in 90% of individuals reported with TPOX tri-allelic pattern, and all other alleles were present in lower percentage. In this database, comparing TPOX allele frequencies among bi and tri-allelic subjects, the frequency of the allele 10 in tri-allelic subjects is around five times higher than in bi-allelic subjects. Thus, it was possible to strengthen the hypothesis that the third-extra allele would be the allele 10. Only ten tri-allelic subjects from the STRBase without the allele 10 were reported. All of them had the allele 9 or 11 (genotypes were: 8–9–11; 8–11–12; 8–11–14.3; 9–11–12), which could denote slippage mutation from an ancestral third-extra allele 10.

As pointed out by Clayton et al. (2004), a Type 2 tri-allelic pattern may result from a chromosomal duplication and, in this case, it may be associated with severe clinical syndromes (Lukka et al., 2006). However, the partial maternal isodisomy for chromosome 2p (2pter–2p12) that also produces a tri-allelic pattern might be compatible with a minimal influence on normal development (Bakker et al., 2001). In this context, Muna Al-Saffar et al. (2000), have shown a 7-month-old well-developed girl with the karyotype 46, XX, der(13) t(2;13)(p23; p11.2).ish der(13)(wcp2+) de novo. A chromosome painting strategy confirmed that the additional segment of chromosome 2 was on 13p, resulting in trisomy of 2p23-2pter. Megarbane et al. (1997) also demonstrated that the partial trisomy of 2p was compatible with adulthood. Another possibility is that the third TPOX allele may not be linked to chromosome 2. Lane showed that two thirds of the TPOX tri-allelic adults were females, and TPOX tri-allelic fathers only transmitted the TPOX tri-allelic genotype to their daughters (Lane, 2008). With this evidence, he suggested that the inserted allele was on an X chromosome. This report was enhanced by Díaz et al. (2009).

Despite the substantial frequency of the TPOX tri-allelic pattern, the nature of the third allele is still poorly understood. In this work we present data obtained from 45 individuals of the same family where there were cases of TPOX tri-allelic pattern, and employ these data to investigate the underlying inheritance of the third allele detected at this locus.

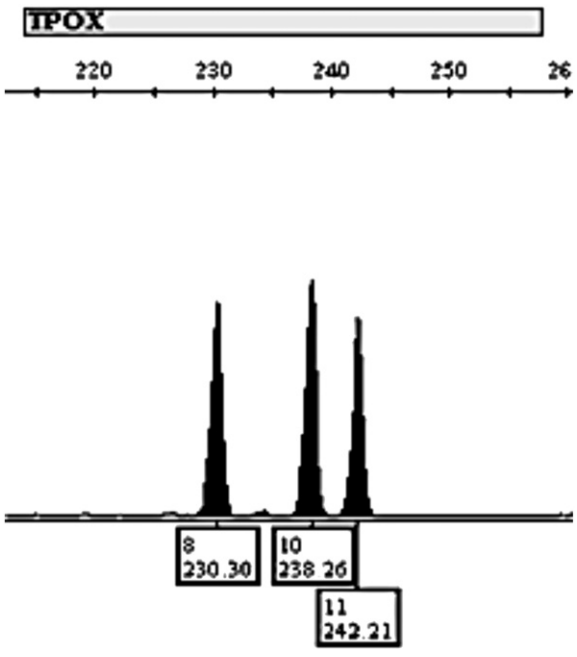

Fig. 2. Tri-allelic TPOX genotype as depicted by the ABI GeneMapper3.2 software. The three TPOX STR peaks represent alleles 8, 10, and 11.

2. Material and methods

The 45 studied subjects, belonging to the same family pedigree, were born and are living in four different cities in the Rio Grande do Sul State, southern Brazil. The proband (or propositus) was a female called LTMV, she was the first tri-allelic family member who received attention for her genetic characteristic. The DNA profiles of her and her daughter were analyzed in a kinship lab routine. Then, a large number of her relatives were invited to participate in this study. This project was approved by the Research Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) (Protocol #09/04688-OfCEP943/29); and the informed written consent and assent to participate were obtained from all subjects or their surrogates.

All subjects participated in an interview on their own residence when they response a questioner about health and development conditions. Data about important medical disorders, syndromic conditions, typical diseases, pathological sickness, abnormal features, atypical appearances, distinctive symptoms, intellectual capacities, and other characteristics were enquired. No medical exams (biochemical or images) were evaluated.

Blood samples were collected on FTA cards and DNA was extracted from blood spots using the manufacturer's protocols. In all 45 subjects, we confirmed kinship between them, by using autosomal STRs (AmpF/STR® Identifiler™ PCR Amplification Kit; Applied Biosystems; Life Technologies, USA), with paternity index values above 10,000. A total of 0.5–1.0 ng of DNA was used to amplify 15 autosomal STR loci (D8S1179,