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Observation of a previously unseen tri-allelic pattern in hydroxylase tyrosine (TH01) locus

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Abstract

The occurrence of two alleles in a human individual comes from the fact that one of them was inherited from the mother and the other from the father. Through molecular PCR technology, both alleles inherited by an individual can be amplified and identified. When three alleles of a genetic marker are detected this is named tri-allelic pattern. There are two types of tri-allelic pattern: type1, which are more common, where there is an imbalance in the size of the peaks and the type 2, where the peaks are of similar magnitude. The locus TH01 seems particularly stable in any type of rearrangement considered; however, during the routine forensic cases in our laboratory, we detected a type 1 tri-allelic pattern, in a genetic profile of a fragment of a femur received for the identification of an individual. The occurrence of the specific TH01 tri-allelic pattern had not been reported in the literature of forensic science prior to the time that this article was written.

Introduction

The occurrence of two alleles in a human individual comes from the fact that one of them was inherited from the mother and the other from the father. Through molecular technology of polymerase chain reaction (PCR), both alleles inherited by an individual can be amplified and identified. After PCR, by means of the capillary electrophoresis, is held the nomenclature derived from automated reading of fragments of DNA, so that the alleles with changes in size are displayed as two heterozygous and alleles of the same size, as a single peak homozygous. In this reading, removal of artifacts as stutter, pull up, any extra peaks (i.e. more than two) would as a tri-allelic pattern [1].

There are two types of tri-allelic pattern: type 1, which is more common, where there is an imbalance in the size of the peaks and, the sum of the maximum intensity of the affected allele variants being equivalent to the intensity of the non-mutant allele; and type 2, where the peaks are of similar magnitude. Tri-allelic patterns of type 1 indicate a somatic mutation of one allele at one *locus* heterozygous during development of the individual, resulting in chimerism. Usually the mutational event is the addition or loss of a repeating unit. Tri-allelic patterns of type 2 indicate a duplication event located on the same chromosome or translocated or aneuploidy chromosomal (trisomy). When it comes to duplication, it is likely that the two alleles are inherited together because they will be strongly linked [2].

The occurrence of a tri-allelic pattern is usually due to genetic duplications in tandem or dispersion of a small region of chromosome (partial trisomies); or incorrect segregation caused by a chromosomal meiotic or mitotic nondisjunction which leads to a full trisomy. For these reasons, the occurrence of tri-allelic pattern can give in only a few cell-groups and not in every cell of the organism – due to mosaicism and/or chimerism [3].

TH01, also called TC11 or HUMTH01, is a tetrameric short tandem repeat *locus*, located in intron 01of the tyrosine hydroxylase gene, which regulates gene expression and production of catecholamines with the 9.3 allele exercising a particularly effect strong on the production of noradrenaline. Tyrosine hydroxylase catalyzes the hydroxylation of L-tyrosine to L-dopa and is the speed limiting enzyme in the synthesis of catecholamines such as noradrenaline or adrenaline, which are crucial in the regulation of arterial pressure [4].

Stutter peaks are found in almost every electropherogram. Stutter peaks are small peaks that occur immediately before or after a real peak. During the PCR amplification process, the polymerase can lose its place when copying a strand of DNA, usually slipping forwards or backwards four base pairs. The result is a small number of DNA fragment copies that are either one repeat larger or smaller than the true fragment being amplified.

Pull-up (sometimes called bleed-through) is a failure of the analysis software to discriminate between the different dye colors used during the generation of test results. A peak observed in one dye (such as Blue) is recorded by sensor for another dye (such as Green or Yellow) and generates a second peak that is a technical artifact. The artifact peak can of substantial height to be considered a true allele. There is a danger that pull-up will go unrecognized, particularly when the result it produces is consistent with what the analyst expected or wanted to find.

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The TH01 marker is part of the 20 loci STR panel of the Combined DNA Index System (CODIS), whose autosomal *loci* are: TPOX, D3S1358, D5S818, FGA, D7S820, D8S1179, CSF1PO, TH01, D13S317, D16S539, vWA, D18S51, D21S11, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433 and D22S1045, plus amelogenin gene homolog of (X and Y), that determines the sex [5]. To be included in CODIS, the locus TH01 is widely used for analysis of human identification.

A tri-allelic pattern is a rare phenomenon and according to Clayton et al. [6] the locus TH01 seems particularly stable in any type of rearrangement. In a study on allelic variation, tri-allelic patterns and punctual mutations observed in nuclear STR typing of the populations of Bosnia and Serbia, in other study was observed a total of 15 tri-allelic patterns involving the following nine loci: TH01, D21S11, D18S51, PentaE, CSF1PO, PentaD, D8S1179, vWA, FGA and TPOX. For the locus TH01, was observed only a tri-allelic pattern with the alleles (7/8/9) [7].

Although the locus TH01 is stable, the NIST STRBase [8] database contains 7 cases of tri-allelic patterns for the TH01 locus. The tri-allelic pattern most frequent to the locus TH01 is (7, 8 and 9), having been found in a total of 3 cases. This data is available in the database of STRs: <https: strbase.nist.gov/tri_tab.htm> and are updated often by the National Institute of Justice of the United States of America.

Table 1 describes the different TH01 tri-allelic patterns identified, registering the tri-allelic pattern and the number of times that the genotype was found by analysis of STR genotypes.

A previously unseen pattern (6, 7 and 9.3) has been detected during routine forensic testing for identification of an individual.

During the routine forensic cases, fragments of a femur and dental elements of two individuals were received to determine whether there was a genetic link to fatherhood among them being the individual 1 (femur), dead and buried 17 years ago, supposed father of the individual 2 (teeth), dead and buried 3 years ago [9].

Materials and methods

The DNA of the samples of these biological materials were extracted through the organic method, with phenol/chloroform and prior total demineralization. The extracted DNA was submitted to amplification, using the commercial kit PowerPlexFusion (Promega Corporation), which is used in the work routine of our laboratory. The polymerase chain reaction (PCR) of all samples were conducted in thermal cycler Proflex brand, of the company Life Technologies for amplification cycles 29, with two phases, the first being with two stages and the second, with only one stage, according to manufacturer's instructions.

The amplification products samples were genotyped for the following loci STR: CSF1PO, TH01, TPOX, D5S818, D7S820, vWA, D13S317, D16S539, D21S11, FGA, D8S1179, D21S11, D3S1358, D18S51, D10S1248, D22S1045, D2S441, D1S1656, D12S391, Penta D, Penta E and the male-specific DYS391, In addition to the amelogenin gender identification.

Approximately 1 ng/μL of DNA was subjected to capillary electrophoresis in ABI 3500 Genetic Analyzer with POP-4 polymer and 36 cm capillary (Applied Biosystems). The data were analyzed in GeneMapper ® ID-X program version 1.5.

Results and conclusion

The profile obtained from the individual 1 was compared with the profile of the individual 2 and there was a paternity between them. Table 2 and figures 2,3 presents the results of STR analysis of the individual 1 and the individual 2. A type 1 tri-allelic pattern was observed in the locus TH01, with 6, 7 and 9.3 alleles.

Point out that none of the other loci exhibited additional peaks suggesting that the TH01 result is a tri-allelic pattern, rather than contamination or a sample mixture.

Table 1. Representation tri-allelic genotypes of locus TH01 and their respective frequencies. Retrieved from https://strbase.nist.gov/tri_tab.htm accessed September 2018.

TH01Tri-allelic Genotypes	Genotypes number[1]
6, 8, 9	1
6, 9, 10	1
7, 8, 9	3
7, 9, 9.3	1
8, 9,10	1

[1]Represents the number of times that the genotype was detected by analysis STRs genotypes.

Table 2. Comparison between the genetic profile between two individuals for determination of paternity

Locus	Individual 1	Individual 2
Amelogenin	X/Y	X/Y
D3S1358	15/17	15/17
D1S1656	13/17	12/17
D2S441	11.3/14	11/14
D10S1248	15/16	13/15
D13S317	11/12	12/14
Penta E	8/10	7/8
D16S539	12/13	12/13
D18S51	12/19	12/19
D2S1338	17/20	17/20
CSFIPO	10/12	12
Penta D	9/13	9
TH01	6/7/9.3	9.3
VWA	16	16/17
D21S11	27/30	30/31.2
D7S820	10	10/11
D5S818	11/12	11/12
TPOX	8	8/12
DYS391	10	10
D8S1179	10/12	10/13
D12S391	18/20	20
D19S433	13/14	13
FGA	24	24/26
D22S1045	16	16

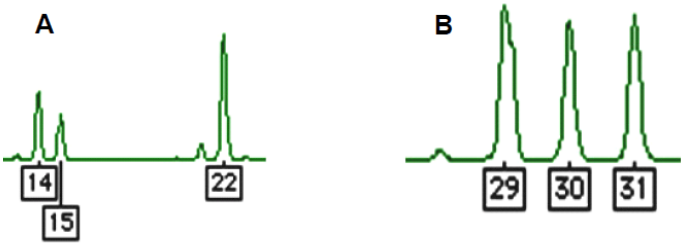

Figure 1. Schematic representation of the profile of the two types of tri-allelic pattern when they appear in capillary electrophoresis analysis. A) Type 1 standard: three alleles showing imbalanced intensities; B) type 2 pattern: three alleles with similar signal strengths. Retrieved from https://strbase.nist.gov/tri_tab.htm accessed April 2018.

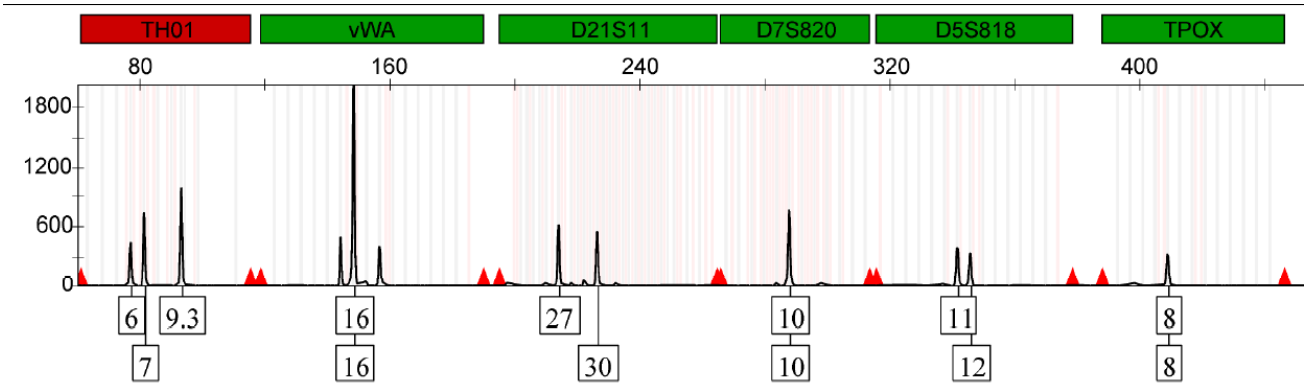

Figure 2. Electropherogram of individual 1, showing the three peaks at the TH01 locus.

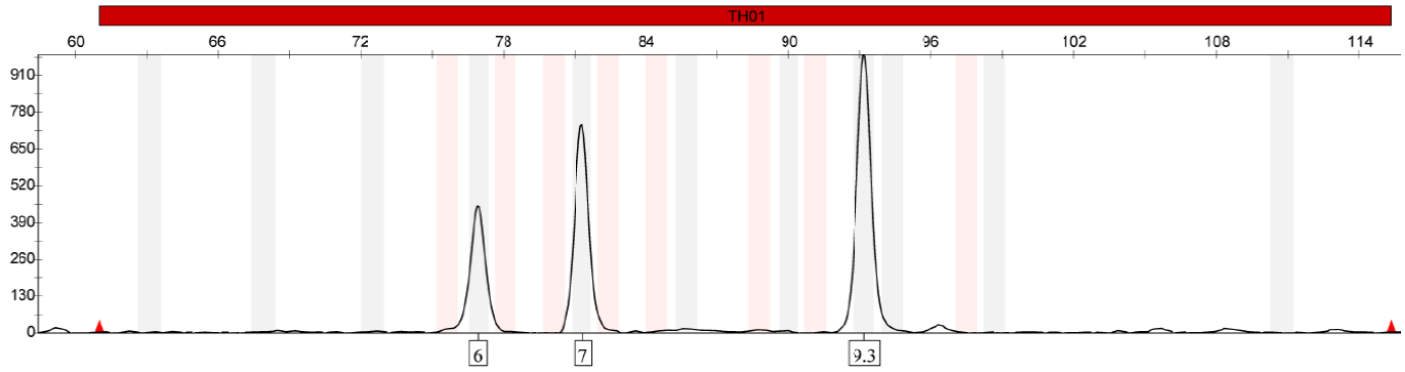

Figure 3. Tri-allelic pattern of type 1 found at locus TH01 of individual one.

The two peaks observed at "-4" and "+8" positions in vWA marker in figure 2 are sttuters, most likely this can be caused stochastic effects, which is the observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples.

The analysis was performed again in forensic laboratory of the Forensic Institute of Paraíba, using a fresh extraction of bone & teeth material, having been confirmed the same tri-allelic pattern (Figure 4). The small peak at the 8 position is a stutter, which is an artifact of PCR amplification that is typically one repeat unit less than the corresponding main allele peak resulting from strand slippage during amplification.

The peak observed at "+8" position in TH01 marker in figure 4 is sttuter.

It is considered a rare phenomenon, of a tri-allelic pattern STRs in a single locus. The occurrence of the 6/7/9.3 tri-allelic pattern to the locus TH01 had not been reported in the literature of forensic science prior to the time that this article was written. Only on the site of the NIST STRBase (strbase http://www.cstl.nist.gov/biotech/) seven records of tri-allelic profile standards for locus TH01, being the tri-allelic pattern more often: 7, 8 and 9, which was found in 3 cases, not having yet any description for the pattern 6, 7 and 9.3.

To calculate the statistical probability of this tri-allelic event within the profile, often uses the default 2pq for heterozygous and pick the two most common allele frequencies of the three for a value for p and q [10]. A second approach avoids any statistical calculations in the report about the frequency of these three alleles at this locus due to the rarity of occurrence. Due to the fact we've never encountered previously

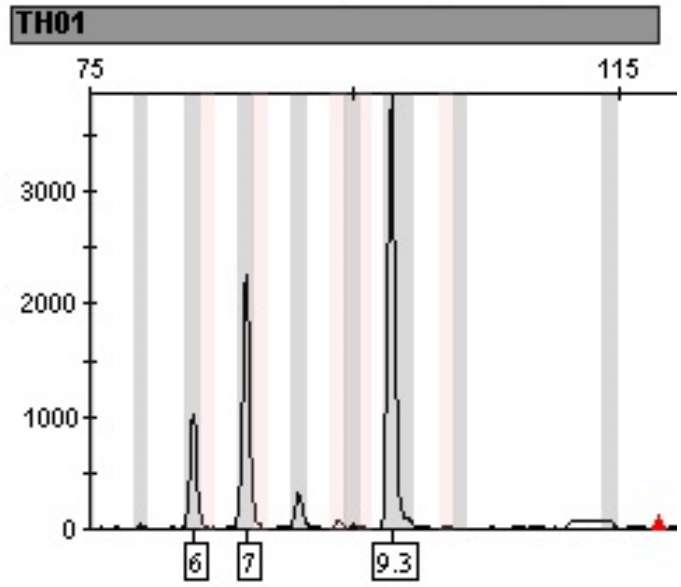

Figure 4. Electropherogram of individual 1 realized in Forensic Science Institute of Paraíba, Brazil confirming the three peaks at the TH01 locus.

such phenomenon in locus TH01 or any other loci in our laboratory, we take a more conservative approach and include the locus TH01 in the results, but do not include statistical calculations. We calculate the statistical probability of paternity link using only twenty-two loci, of the 23 analyzed, resulting a frequency of approximately 1 in 30 million, according to the Brazilian population using a theta correction of 2%.

Due to the fact that our report is a routine forensic case, a deeper analysis of family could not be performed. We believe that the lack of scientific information and data on the tri-allelic patterns in the locus TH01 presents a challenge to the analysis of the reasons that make this pattern occur. This lack of information is mainly due to the rarity of occurrence of rearrangement of alleles at this locus.

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