Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics 2 (2008) 372–375
FSI GENETICS
www.elsevier.com/locate/fsig

Case report

A case of tri-allelic pattern at locus D3S1358 on chromosome 3p21 inherited from paternal grandmother

Christopher Vidal *, Marisa Cassar

MLS BioDNA Ltd, KBIC 2150–2160, Industrial Estate, Kordin PLA08, Malta

Received 19 March 2008; received in revised form 13 May 2008; accepted 6 June 2008

Abstract

We are reporting a case of tri-allelic inheritance at locus D3S1358 commonly used for genetic identification in forensic DNA testing. This case was encountered during routine paternity testing using commercial DNA profiling kits. The tri-allelic inheritance identified was probably a result of duplication at this locus, supported by the equal peak intensities and inheritance pattern from grandparent to child.

Keywords: STRs; Tri-allelic inheritance; DNA profiling; Forensics; D3S1358

1. Introduction

Genotyping of highly polymorphic short tandem repeats (STRs) is widely used for the genetic identification of individuals in paternity disputes, familial relationship testing and forensic DNA analysis. Genotypes of a set of STRs accepted by the International community are rapidly determined using commercial kits and a simple multiplex polymerase chain reaction (PCR) followed by fragment analysis using fluorescent capillary electrophoresis [1]. Statistical analysis is performed to determine the combined paternity index and probability of relationship using population specific allele frequencies, now having been determined in different populations [2–4]. A paternity test follows simple Mendelian inheritance where a child inherits one allele from the mother and another from the father at every locus. In rare cases, abnormal patterns of inheritance can be encountered and forensic scientists have to be aware of these rare events in order to tackle them appropriately with minimal effects on the outcome of the result. Abnormal DNA profiles can result from both technical and biological processes such as allele drop-out due to rare mutations in the primer binding sites, mutations due to slippage and other events such as gene conversion and copy number variations (CNVs) [5]. In this article we report a rare case of tri-allelic inheritance of marker D3S1358 encountered at our facility.

2. Material and methods

2.1. DNA extraction

Genomic DNA was extracted from buccal swabs collected from mother, child and grandmother (mother of alleged father), using the AccuPrep™ Genomic DNA extraction kit (Bioneer Corporation, Daejong, Korea) (Fig. 1).

2.2. STR genotyping

The AmpFl STR® Identifiler kit (Applied Biosystems, Foster City, CA, USA) was used to amplify 15 STR loci and Amelogenin according to manufacturer's instructions, in a 12.5 μl total reaction volume. The amplified products were analysed using an ABI 3130 genetic analyser (Applied Biosystems, Foster City, CA, USA) followed by data analysis using GeneMapper® v3.5 software. Marker D19S433 was not successfully amplified and so was excluded from the analysis. Re-analysis was performed using the PowerPlex 16 identification kit (Promega Corporation, Madison, USA).

Eleven additional STRs across chromosome 3 (Table 1) were individually analysed by PCR in a 10 μl reaction, using 15 pmol of each primer (forward primer labelled with FAM or HEX), 2.5 mM $MgCl_2$, 0.2 mM dNTPs and 1 unit of Hot FIREPol® polymerase (Solis BioDyne, Tartu, Estonia), using the following thermal profile: 15 min at 95 °C followed by 30 cycles of 45 s at 95 °C, 45 s at 55 °C, 1 min at 72 °C and a final extension at 72 °C for 10 min, in a GeneAmp® 9700 PCR

* Corresponding author. Tel.: +356 23980148; fax: +356 23980161.
E-mail address: chrisv@mlsbiodna.com (C. Vidal).

1872-4973/$ – see front matter
doi:10.1016/j.fsigen.2008.06.002

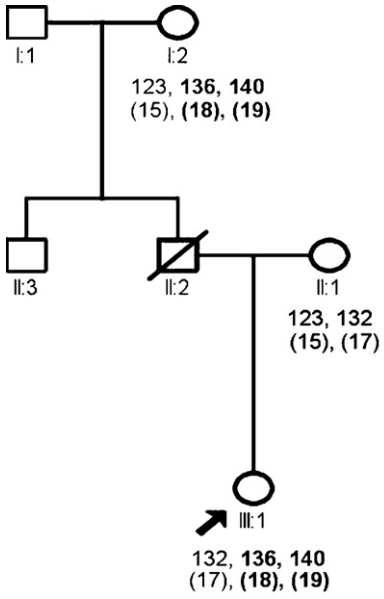

Fig. 1. Pedigree of studied members. Tested child is indicated with an arrow. Alleles of marker D3S1358 are shown in tested individuals in base pairs and standard STR nomenclature (in parenthesis).

thermalcycler (Applied Biosystems, Foster City, CA, USA). The amplified products were analysed as described above.

3. Results

Fig. 2 shows partial profiles of child and grandmother where three peaks at locus D3S1358 were observed, instead of two (heterozygous) or one (homozygous) as expected. When comparing the three DNA profiles and excluding those alleles inherited from the mother, the child's and alleged grandmother's profiles matched at all loci with the exception of CSF1PO, D2S1338 and D5S818. At locus D3S1358, the child inherited allele 17 from the mother while alleles 18 and 19 compared with those of the grandmother, showing that these were inherited from her father. Allele 19 was rarely found in the Maltese population (0.3%) [4]. A combined avuncular index (CAI) of 588 and a probability of paternity of 99.8302% were obtained when performing statistical analysis using the avuncular index [6]. Re-analysis using PowerPlex 16 (Promega Corporation, Madison, USA) (Fig. 2c and d) confirmed tri-allelic inheritance at locus D3S1358.

Table 1
Genotypes of STRs tested on chromosome 3

Locus	Position (cM)[a]	Mother	Child	Alleged grandmother
D3S4545	26	206, 218	206, 229	225, 229
D3S3038	45	194, 198	190, 198	190, 202
D3S2432	58	144, 148	148, 148	135, 148
D3S1768	62	*189*, 197	*189*, 197	192, 197
D3S1358	**69**	**123**, **132**	**132**, **136**, **140**	**123**, **136**, **140**
D3S2409	71	117, 120	117, 117	110, 117
D3S3644	91	172, 172	165, 172	165, 172
D3S4529	112	154, 158	149, 158	149, 161
D3S3045	124	183, *190*	183, *190*	183, 194
D3S1744	161	ND	156, 160	160, 164
D3S2398	209	271, 291	271, 283	283, 283
D3S1311	225	130, 145	143, 145	140, 143

Genotypes (alleles) are given in base pairs.
Underlined alleles are those matching with mother. Alleles in bold are those showing tri-allelic inheritance.
ND: not determined.
[a] Position on chromosome 3 according to Marshfield's genetic map.

To test for the possibility of partial duplication of chromosome 3, 11 STRs were analysed across the whole chromosome. As shown in Table 1, no triple alleles were observed to be inherited for any of the STRs tested, except for those at locus D3S1358. It is also evident that no obvious meiotic recombinational events occurred in the alleged father on chromosome 3 inherited from grandmother, since all alleles were in concordance with those of the child after excluding the ones inherited from mother.

4. Discussion

In this article we are reporting a case of aberrant DNA profiling, where locus D3S1358 yielded a tri-allelic pattern, inherited from grandmother to granddaughter through the paternal line. To our knowledge, until now, a case of tri-allelic pattern at this locus was never reported in the literature although six tri-allelic patterns (including pattern of alleles 17, 18 and 19) were reported on the Short Tandem Repeat DNA Internet Database (STRBase) (http://www.cstl.nist.gov/biotech/strbase/var_D3S1358.htm#Tri). This is the first time we encountered aberrant inheritance of STRs at our facility from around 1000 individuals tested so far.

Tri-allelic inheritance was reported for other markers used for DNA profiling with the most common locus being D18S51 [7] (STRBase reported 21 tri-allelic patterns) and in other loci including TPOX, vWA, D21S11, CSF1PO, Penta D, D8S1179 and FGA [7,8]. In a recent study, it was reported that an extra TPOX allele 10 was observed in 2.4% of South Africans with a higher incidence in females when compared to males [9]. This suggests that the extra TPOX allele was a result of a duplication most probably on the X chromosome rather than on chromosome 2p where TPOX is normally found. The extra allele observed in our case was a type 2 allele since it had an equal intensity to those of the other two alleles, suggesting that it was a result of a duplication event. The inheritance of the allele through two generations was also evidence that the allele was caused by a duplication rather than by a somatic mutation at a heterozygous region (type 1, three peaks of unequal intensities) [10]. Aberrant DNA profiles can result from a number of biological processes including somatic mutations (which might vary between different tissues tested), aneuploidy (trisomy 21), mosaicism, deletions and duplications [11]. Other causes of abnormal DNA profiles include allele drop-out due to mutations in primer binding sites and mutations due to replication slippage, which is the most common mechanism for STRs. Mutations due to replication slippage can be affected by the length and number of homogenous repeats with an increased mutation rate observed for the longer alleles with higher number of repeats [12]. Brinkmann et al. [12] also