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Null allele can bring to interpretative problems in a deficitary paternity case

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ABSTRACT

An STR null allele is an allele at a microsatellite locus that fails to amplify. A possible cause is poor primer annealing due to nucleotide sequence divergence in the flanking primers. In this study, a woman (ZAM) wanted to know whether a man (PGAF) was the father of her child (ZGC). During the court settlement, PGAF died. PGAF's parents refused to undergo DNA investigation and denied the access to biological fragments from their dead son. Although, DNA specimens were obtained from buccal swabs of ZAM, ZGC and PGAF's paternal sister (PTFS). Initially, only autosomal profiles were studied, and kinship assignment was inconclusive. Following our requests, PGAF's parents (PRGF and LLGM) led us to obtain their DNA specimens. Only with the PTFS genetic profile, we were not able to demonstrate a kinship assignment. PTFS showed a homozygosis at D8S1179 locus. Then, merely comparing PTFS, LLGM and ZGC autosomal genetic profiles it was possible to underline that they were three different homozygous at D8S1179 locus. Hence, comparing the peak heights in different loci and according to literature, they had to carry a null allele at this locus. Parental studies were completed by Y haplotype analysis.

1. Introduction

In human identity testing, it is known DNA sequence variation to exist in and around the repeat region of short tandem repeat (STR) loci used. The STR alleles detected in forensic DNA laboratories worldwide – typed as "normal" – are the vast majority, while several variant alleles have been revealed. Furthermore, an allele drop-out can be brought about by a sequence difference at a polymerase chain reaction (PCR) primer binding site in the DNA template, with one set of primers and not with another. By definition, a microsatellite null allele is any allele at a microsatellite locus that consistently fails to amplify to detected levels via the polymerase chain reaction (PCR). Thus, poor primer annealing is one potential cause of microsatellite null alleles, and it is due to nucleotide sequence divergence in one or both flanking primers. Key mutations in the 3′ end of the priming site, where extension begins, are thought to be particularly negative to PCR amplifications. Additionally, differential amplification of size-variant alleles can generate null alleles. Due to the competitive nature of PCR, long alleles often amplify less efficiently than shorter ones, such that only the smaller of two alleles might be detected from a heterozygous individual. Then, another source of null alleles is related to inconsistent DNA template quality or low template quantity. Hence, these troubles are challenging because in some cases only one or a few loci (or alleles) fail to amplify, whereas others amplify with relative ease from the same DNA preparation. [1–4] In this case, a woman (ZAM) requested a paternity test to ensure whether a man (PGAF) was the father of her child (ZGC). Unfortunately, during the court settlement, PGAF died. Initially, only PGAF'S sister (PTFS) was open to undergo DNA investigation. PGAF's parents denied the access to biological fragments from their dead son and refused to undergo DNA investigation. Hence, we obtained genetic profiles only from ZAM, ZGC and PGAF's paternal sister (PTFS) – this is what we did call Pedigree #1. PTFS and ZGC showed homozygosis at D8S1179 locus. In this scenario, only the study of autosomal profiles could be useful, but the kinship assignment turned out to be inconclusive, with a low LR. Subsequently - following our requests - PGAF's parents (PRGF and LLGM) led us to obtain their DNA specimens, but not that of their dead

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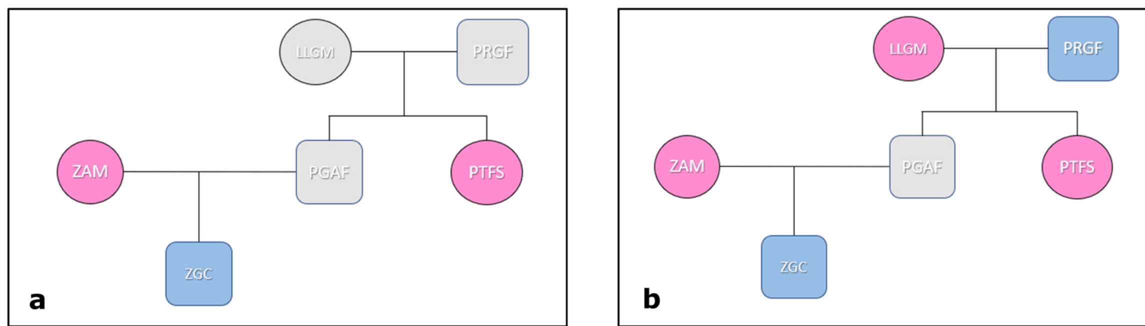

Fig. 1. a. Pedigree number one. b. Pedigree number two. Later, to solve the kinship assessment, even the grandparents did give their consensus to perform a DNA testing. The gray boxes mean that the person was not willing to undergo DNA testing or that their mortal remains were not available.

son – this is what we did call Pedigree #2(Fig. 1). Under this circumstance, even the Y-STRs profile was usable to establish a possible paternity.

2. Materials and methods

DNA extracted from buccal swabs – with QIAGEN spin column kit - was quantified with PowerQuant® System on Applied Biosystems™ 7500 Real-Time PCR System, while autosomal profiles were obtained with AmpFLSTR™ NGM Select™ Amplification kit, amplified on an Applied Biosystems™ GeneAmp® PCR System, while fragments analysis was performed on an Applied Biosystems™ 3500 genetic analyzer. Moreover, PRGF and ZGC Y-profiles were obtained by AmpFLSTR™ Yfiler™ PCR Amplification Kit. Amplification and fragments analysis were conducted in the same conditions as just described above. The analyses were performed in double. According to ISFG guidelines, the biostatistical evaluation in kinship analysis was based on a likelihood ratio approach [5], using the software Familias 3.

3. Results

The Pedigree #1 was evaluated with the software Familias 3 and the LR value was not useful to define the parentage assessment. The non-homozygosis of ZGC and PTFS at D8S1179 were not soon evident. They were part of a scenario that did lead to an expected inconclusive outcome (#1). Therefore, we did develop the hypothesis we were dealing with silent alleles. Hence, the samples were re-amplified, lowering the annealing temperature [6], but no more allele was detected at D8S1179. Then, we did re-analyze (with Familas 3) using the null allele frequencies, the pedigree #1, but the outcome was ever unconclusive. Instead, the Pedigree #2 was highly informative on the parentage assessment. The study of autosomal genetic profiles and Y-STRs showed a total LR value of 1,26 $*10^{24}$ that was consistent with a paternity probability of 99,9999999999992%.

4. Discussion

The null allele presence can be highlighted during population studies by observing a heterozygote deficit. Instead, this scenario is not easily evident when you are dealing with a de novo mutation studying a kinship relation between few people. The non-homozygosis of ZGC and PTFS did gain relevance only when we did obtain the full family Pedigree #2 (except the DNA from PGAF). Indeed, the autosomal analysis – carried on LLGM and PRGF - showed another homozygosis at D8S1179 for LLGM. Hence, merely comparing PTFS, LLGM and ZGC autosomal genetic profiles it was possible to underline that they were three different homozygous at D8S1179 locus. Then, our attention did start to focus on the peak heights of alleles. It was soon clear that (for green dye loci) the peak height of allele at D8S1179 was always as half as high of alleles peak heights in homozygote loci, while it was as high as those peaks in the heterozygote loci.

5. Conclusion

In conclusion, silent alleles are microsatellite DNAs that fail to amplify via the polymerase chain reaction (PCR). This situation could bring about mistaken assessment of parentage. In our scenario, the null allele did not bring to interpretative complications. The pedigree #1 could not lead to a conclusive result even though we did realize we were dealing with a null allele. ZGC and PTFS shared few alleles. A *posteriori* analysis performed on Familias 3 – using the frequencies of null alleles – did not change the status of inconclusive outcome. Although, if ZGC and PTFS shared more alleles, the presence of this silent allele could influence the result of the analysis. Moreover, the future perspective of this study is performing the sequencing of this region to determine the sequence of the silent allele.

Conflict of interest

None.

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