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Heteropaternal superfecundation: Implicancies in forensic genetics

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ABSTRACT

A marked increase in multiple pregnancies has been observed in recent years, this suggests an increase of twin brothers involved in paternity cases. Occasionally, it has been seen in many court cases involving twins' participation, the attempt to prove that tested paternity to the alleged father for one of them, necessarily implies the same result for the other.

This article describes a rare event called heteropaternal superfecundation (HS) detected in a case of paternity investigation which involves a set of twins conducted in our laboratory.

The probability of paternity (PP) of twin 2 was 99.999% and the combined paternity index (CPI) obtained was 1×10^7. Whereas that for twin 1 was excluded in 14 out of 17 Y-chromosome DNA markers and in 11 out of 15 autosomal short tandem repeats (STR) markers tested.

Although the frequency of these events is not high, its likelihood is possible, as showed in this study. Therefore, assuming that sometimes it is intended that the same result can be shared by two brothers, by the mere fact of being twins would have meant a great negligence.

1. Introduction

A marked increase in multiple pregnancies has been observed in recent years with significant variations between countries worldwide, mainly due to the expansion of assisted reproduction techniques and, to a lesser extent, delayed motherhood onset [1–3]. In cases of paternity research, different biological ties, and cases regarding human identification, this suggests an increase of twin brothers involved in this type of study. Occasionally, it has been seen in many court cases involving twins' participation, the attempt to prove that tested paternity to the alleged father for one of them, necessarily implies the same result for the other. This usually occurs when one of the twins is not available for affiliation studies due to different reasons, such as death, missing, illness or other. This article describes a rare event called heteropaternal superfecundation detected in a case of paternity investigation conducted in our laboratory.

HS is a phenomenon that occurs as a result of fertilization of two eggs in the same cycle of ovulation, either during the same sexual intercourse when ovulations are simultaneous or, with intervals between 3 and 9 days when ovulations are discordant, at the same menstrual cycle, but containing sperm from different men, resulting into fraternal or dizygotic twins who, unlike full siblings that share about 50% of their genome, only share an average of 25% of it [4].

In 1978 Terasaki et al. described this phenomenon by histocompatibility antigens typing [5]. Thereafter, it has been sporadically seen the occurrence of similar events in different populations [6,7].

The high discriminative power that characterizes the DNA STR (short tandem repeats) markers, due to its highly polymorphic nature, provides a fundamental genetic tool to solve these cases of paternity and other biological links [8]. The application in this case has allowed by identifying a rather exceptional event like the SFH, to demonstrate that the adjudication of the same result to both brothers, despite being twins, when only one of them has gone through a genetic expertise, may involve an erroneous result.

2. Materials and methods

2.1. Case presentation

In order to establish the link paternity between a pair of dizygotic twins, four months old and an alleged father, our laboratory was consulted about the possibility of conducting the study with the participation of only one of the minors as it would

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be less difficult for the mother to bring one of the babies, and especially because in that way the cost of the study would be lower. The mother believed that the result of one of the babies, since they were twins, could be extended to the other child. After explaining the reasons why this was not possible, she agreed to do it with the participation of both. The MANLAB Laboratory Medicine Department of Genomics, located at the Autonomous City of Buenos Aires, received blood samples from the alleged father and the children biological mother, and swabs from the twins' buccal mucosa for analysis, derived from a laboratory located at General Belgrano City, Buenos Aires. The study was conducted with the written consent from both the twins' mother and the alleged father. They also gave their consent for the publication of this work.

2.2. Genetics profiles

The automated extraction of genomic DNA was performed with blood samples from the mother and alleged father and from the twins' buccal swabs, by using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche). To define the genetic profiles, they were amplified in the same reaction by the PCR technique (Polymerase chain reaction) 15 microsatellite autosomal STR and amelogenin, as a marker for sex with Identifiler®PCR AmpFLSTR® Amplification Kit (Applied Biosystems). In another reaction, 17 Y-chromosome STR markers were amplified simultaneously with PCR Amplification Yfiler® AmpFlSTR® Kit (Applied Biosystems). PCR reactions were carried out in the Veriti® thermalcycler (Applied Biosystems). The amplification products were subjected to capillary electrophoresis on an ABI 3500 Genetic Analyzer (Applied Biosystems) and genotypes were assigned using the analysis software GeneMapper® ID-X v 1.2 (Applied Biosystems). Statistical analysis was performed with the program "Familias" available at http://familias.name/download.html. The Y-chromosome haplotype was added to the Y-Chromosome Haplotype Reference Database (YHRD), freely available at www.yhrd.org.

3. Results

3.1. Alleged father-twin 1

Discrepancies in 14 STR loci for Y chromosome and in 11 autosomal STR markers between the alleged father and twin 1 were observed.

3.2. Alleged father-twin 2

The genetic profile in twin 2 showed a 50% match with her mothers' and a 50% with the alleged fathers', for all autosomal STR analyzed.

The combined paternity index (CPI) obtained was 1×10^7 and the probability of paternity (PP) of 99.999%.

The Y-chromosome haplotype of twin 2 matches the alleged father.

4. Discussion

The heteropaternal superfecundation seems to be an anecdotal and rare event. Wenk et al. reported that the prevalence of dizygotic twins with different fathers is one in 13,000 cases of paternity [9], while James has estimated that the incidence of HS in American Caucasian women is one pair every 400 twin births [10].

There are few cases reported worldwide, probably because they have not participated in studies of paternity. Nevertheless, it has been suggested that this event will be described with increasing frequency due to the availability of molecular techniques which provide a fundamental tool for the correct resolution of these cases and the marked increase in twin births observed in recent years. There is a tendency in some court cases to attempt to assign the same result to both twins even though only one of them participated in the study of DNA. In these cases, is usually observed the intention to prove fatherhood for both of the children, by providing different arguments. An example can be seen in the court case "R., AM C/G, RO s/filiation (Family Court and Succession of the III Nomination)" Judicial Power of the Province of Tucumán, Argentina, on a judgment of the Trial Chamber II of the Family Inheritance, vocals: Maria Lucia Stefanini of Trabadelo and Pedro Alfonso Parra.

In this case, only one of the brothers attended the paternity study manifesting health problems. One party attempted to show that both children were twins by presenting birth certificates showing they were born from the same mother with a difference of only a few minutes and in the same place. Therefore, they continued to argue that the result of DNA test for one of them should be the same for both.

There is evidence that shows two individuals, even when they are not biologically related, may present strong similarities in their genetic profiles [11]. These similarities, when one or both of the alleles match for each one of the analyzed STR systems, are used to be found with some frequency between people who actually are related.

These observations prove, therefore, the importance of including the mother, when available, in the study, the need to consider each of the sons separately, and the great contribution of Y chromosome STR analysis.

In our investigation case of paternity, the coincidences obtained between Y chromosome haplotypes of the alleged father and twin 2, in addition to the observed discrepancies between haplotypes of the alleged father and twin 1, permitted to include twin 2 in the same paternal lines than the alleged father, and thus excluding twin 1.

At the same time, the analysis of polymorphisms present in autosomal markers allowed on one hand to establish the biological link paternity of the alleged father regarding twin 2, and on the other hand, to exclude him as the biological father of twin 1. In addition, the analysis of genetic profiling also enables the confirmation of the biological bond between the mother and both twins, thus ruling out any possibility of interchanging any of them.

Additionally, the mother admitted to have sex with the alleged father and another man afterwards, in a very short period of time.

The results allowed firstly detecting an infrequent event, such as the paternity of dizygotic twins from different fathers. Secondly they also show that although the frequency of these events is not high, its likelihood is possible, as showed in this study. Therefore, assuming that sometimes it is intended that the same result can be shared by two brothers, by the mere fact of being twins would have meant a great negligence.

Conflict of interest

None for declare.

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