Brief Communication

The impact of chimerism in DNA-based forensic sex determination analysis

Renjith George, Preethy Mary Donald, Sumanth Kumbargere Nagraj, Jose Joy Idiculla, Rashid Hj Ismail

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Department of Oral Medicine, Oral Diagnosis and Oral Pathology, Faculty of Dentistry, Melaka Manipal Medical College, 75150 Melaka, Malaysia

Abstract

Sex determination is the most important step in personal identification in forensic investigations. DNA-based sex determination analysis is comparatively more reliable than the other conventional methods of sex determination analysis. Advanced technology like real-time polymerase chain reaction (PCR) offers accurate and reproducible results and is at the level of legal acceptance. But still there are situations like chimerism where an individual possess both male and female specific factors together in their body. Sex determination analysis in such cases can give erroneous results. This paper discusses the phenomenon of chimerism and its impact on sex determination analysis in forensic investigations.

Keywords: *DNA, chimerism, forensic sciences, polymerase chain reaction, sex determination analysis*

Introduction

Forensic science aids in the personal identification process by comparative methods or by enabling a profile of the individual regarding age at death, and sex, in crime investigations involving biological evidence, such as murder, sexual assault, and disaster victim identification. Investigation of violent crime has always been a driving motivation for law enforcement agencies in any country, and more so in developing countries. Biological specimens recovered from the scene of crime provide remarkably valuable information about the crime and the personnel involved. Deoxyribonucleic acid (DNA) technology has gained wide acceptance in crime investigations involving biological evidence, such as murder (blood evidence), sexual assault (semen evidence), murder with sexual assault (blood and semen evidence), or in cases of mass disasters (saliva evidence and body tissues), and in identification of mutilated bodies and exhumed skeletons (tissues and bones). Variability in biomolecules has been exploited for identification of the source of the biospecimen. Earlier, identification procedures employed for the characterization of biological specimens were protein or 'classical' markers such as the ABO blood group antigens, serum proteins, and RBC enzymes. However, these suffered from low polymorphism, poor stability and restricted activity of the molecules, and the limited resolution of the detection methods. The advent of DNA markers allowed for greater precision and higher discriminatory power in forensic testing.

DNA is the vehicle for generational transfer of heritable traits. It encodes the genetic information in most organisms and is identical in every cell of an individual. Chemically, the DNA molecule is a highly stable polymer composed of subunits known as nucleotides, and in humans makes up the 22 pairs of autosomal and single pair of sex chromosomes. Each parent contributes a chromosome to the pair an individual inherits. The information content of DNA resides in the sequence of bases, and although the DNA sequence in different individuals is more similar than different, many regions of human chromosomes exhibit a great deal of diversity. Such variable sequences are termed 'polymorphic' (meaning 'many forms') and are used for human identification, paternity testing, and diagnosis of genetic diseases. Most polymorphisms are located in the estimated 95% of the human genome that does not encode for proteins (1).

Various methods are employed for sex determination analysis in forensic investigations, and include visual, clinical, microscopic, and advanced methods. Visual methods are reliable, but are subject to inter-observer variation and lack accuracy. Microscopic methods, such as identifying sex by means of Barr bodies, show diminishing accuracy with the time elapsed after death (2). Amongst the above mentioned methods, sex determination analysis by advanced

methods is found to be accurate with reproducible results. For instance, DNA analysis for male and female specific sex-typing markers in X and Y chromosomes is used in sex determination analysis (3). DNA is the molecule of choice for forensic analysis because of its discrimination, genetic continuity, sensitivity, and stability (4).

The introduction of polymerase chain reactions (PCR) has helped DNA-based analysis by amplifying identification markers even if the available samples are of limited quantity. Conventional PCR has its own limitations, such as poor precision, limited dynamic range of detection, low sensitivity, the need for post-PCR processing, and allowing for only size-based discrimination. Further advancements such as real-time quantitative PCR are highly sensitive, and do not require post-PCR processing. This allows for real-time visualisation of amplicons with an increased dynamic range of detection and has made DNA analysis much easier, with accurate results. Compared to other methods of sex determination analysis, the results are much more reliable and reproducible, and it is considered one of the gold standard techniques for sex identification in forensic investigations. But, as with other methods, there can be situations where DNA-based sex typing can yield erroneous results. This paper discusses 'chimerism', that is occurrence in an individual of two or more cell populations of different chromosomal constitutions, derived from different individuals, which is critical in DNA-based personal and sex identification (5).

Sex typing markers

There are various sex typing markers used in DNA-based identification such as amelogenin, zinc finger genes, the sex determining region on the Y chromosome, and the like.

Amelogenin is the most common gene used for sex determination analysis in the forensic field. In humans there are two amelogenin genes, located on the sex chromosomes (AMELX and AMELY). The difference in the length of the first introns of the AMEL genes, located on both X and Y chromosomes, differentiates males from females. AMEL genes in females are located on both X chromosomes and are homozygous. In males, the AMEL gene is present on both the X and Y chromosome, but they are heterozygous. If a sample yields amelogenins with the same molecular weight, it can be concluded to be a female sample, whereas different molecular weights represent a male sample (6).

The Sex-determining Region on the Y chromosome (SRY) is a gene that causes development of male characteristics. The SRY gene is located on the short (p) arm of the Y chromosome at position 11.3. In humans, where there are two distinct sex chromosomes, the X and Y chromosomes; it is the presence of the Y chromosome that specifies male development. The SRY gene on the Y chromosome causes the embryo to develop as a male. Female development seems to be the default pathway, and in the absence of SRY, the urogenital tract develops as that of a female. Detection of the SRY sequence would distinguish an authentic male DNA sample from a female DNA sample, as female DNA lacks the SRY gene. The detection of the SRY gene in DNA from a forensic sample can be confirmatory to type the sex as male (7).

The current methods of DNA forensic sex typing involve multiplex X and Y STR analysis, Single Nucleotide Polymorphism (SNP) pyro-sequencing, or Y STR haplotype analysis. Y-STRs are short tandem repeats found on the male-specific Y chromosome. The coding genes, mostly found on the short arm of the Y chromosome, are vital to male sex determination, spermatogenesis, and other male-related functions. Y-STRs are polymorphic among unrelated males and are inherited through the paternal line with little change through generations.

Y-STRs are useful in examining sexual assault evidence, such as vaginal swabs, that contains both female and male DNA. It is necessary to separate the male component (sperm cell DNA) from the female component DNA by differential extraction, but sometimes the male and female components cannot be separated completely. As a result, the female component could exist predominantly in the male component even after differential separation. This can lead to masking of the male DNA by the female DNA when they are co-amplified, and will lead to erroneous results.

Masking effects by female DNA do not occur when Y-STRs are examined. Since females do not possess a Y chromosome, any Y-STR data obtained can only be contributed by males and will be easily detected, since only the male component of the DNA mixture will be amplified, not the female component. The Y-STR system is especially helpful when there is more than one male assailant. The mixed male pattern in the evidence can help to identify those males responsible for the assault. Y-STR analysis is useful to detect the presence of minimal male DNA where there is a masking effect of female DNA with regular STR analysis (8).

In all of the above mentioned methods, the X or Y specific marker is analysed, and based on the presence or absence or the relative proportion of amplified products from the sample, the sex determination is done. However, in situations such as an individual possessing both male and female specific factors in their body, sex determination may be misleading, especially while analysing forensic samples.

The impact of chimerism on DNA-based sex determination analysis

A condition where there is more than one set of cell lines with different sets of chromosomes making up the body is known as chimerism. A 'chimera' is an individual with at least two different populations of cells, which are genetically distinct and originate in different zygotes (5).

Chimerism can be classified as:

1. Artificial
2. Transplacental
3. Tetragametic

Artificial chimerism

Artificial chimerism is caused by the transplanted blood stem cells via blood transfusion or bone marrow transplantation (5). After transplantation, the short tandem repeats (STRs) used for personal identification in forensic investigations will be identical for the donor and the receiver, leading to erroneous identification. If the donor is of the same genetic pattern as the receiver but of different sex, the samples from the receiver may be misidentified in sex identification. Samples such as blood, buccal swabs, or fingernails are not exempted from infiltration by the donor's cells; the exception is hair root cells (9).

Transplacental chimerism

A female bearing a male foetus is an example of a transplacental chimera. At a certain time period in the pregnancy, there is some cell traffic between the mother and the foetus, and these cells from the foetus can persist in mother's blood for decades (10). Microchimerism refers to a small population of cells or DNA in one individual derived from another genetically distinct individual (11).

Microchimerism can occur as foetal microchimerism, where there is free passage of blood between mother and child, or as twin chimerism, or blood chimerism, where blood passes between twin foetuses. In either situation, if the involved subjects are of different sex, it can result in chimeras whose samples may yield erroneous results on DNA-based gender analysis. A blood sample from a mother or twins bearing alleles of the opposite sex can yield ambiguous results in DNA-based sex determination analysis.

Costa et al., were able to detect the presence of SRY in maternal plasma. They stated that foetal SRY genes can be found in maternal plasma as early as 42 days of gestation, and the number of foetal DNA increases with gestational age (12).

The foetal cells can migrate into the female body during pregnancy and can persist for decades. The body of a woman after a pregnancy with a male embryo can therefore display a small fraction of foetal cells with Y chromosomes (13).

Bayes-Genis et al., stated that foetal progenitor cells may cross the placenta during pregnancy, persist for decades in the maternal bloodstream, and can colonise in maternal solid organs. This study identified male cardiomyocytes of extra-cardiac origin, presumably foetal, in the hearts of two women with male progeny (14).

Rao et al., reported foetal microchimerism showing male DNA of foetal origin in maternal circulation several years after delivery. They identified SRY sequences in normal women with a male child as evidence of the existence of foetal progenitor cells in the maternal circulation, which is completely absent in normal women with a female child (15).

Hence the DNA-based analysis of a sample from a female who has carried a male foetus may identify the source as a male, which can affect the investigation in a negative manner. The situation may be similar in the case of a surrogate mother who receives a male foetus.

Tetragametic chimerism

Fertilization of two oocytes by two spermatozoa and fusion, or the resulting two embryos leading to development of one organism, may result in tetragametic chimerism (5). A tetragametic chimera can have two genetic profiles in their blood, and distinct DNA markers in different parts of their body (11). A hermaphrodite chimera is a variant of a tetragametic chimera where a female embryo is merged with a male embryo, and the resultant chimera will have both male and female specific markers in their body. To a greater or lesser degree, they will also possess ambiguous genitalia. This can occur in in vitro fertilisation where more than one fertilised egg

is placed in the uterus for a better success rate. If the fertilised eggs used are of opposite sex, it can lead to the development of a hermaphrodite chimera. Twin chimerism is comparatively rarer than artificial and transplacental chimerism.

A forensic sample obtained from a chimera that possesses both male and female cell lineages can result in ambiguous results with DNA-based sex typing. Gender analysis based on detection of male or female specific markers, will not be applicable for a chimera that possesses cell populations of both genders, and for that reason it is advisable to include differential extraction for such samples so as to detect the ambiguous conditions.

Conclusion

Although DNA-based sex determination methods are a comparatively better approach for forensic sex determination analysis, there are enigmatic conditions where the analysis can go wrong. Apart from simple procedures of gender determination using X or Y specific markers, detailed analysis may be necessary to identify a sample from a chimera. Sex typing procedures involving a single marker such as amelogenin or SRY are not reliable, and multiplex STR analysis is preferable to single marker procedures. To reduce the risk of error in situations such as chimerism, the suspected sample can be subjected to highly discriminating procedures such as multiplex Y chromosome STR typing, or SNP pyro-sequencing (16). Most of the time, however, the forensic analyst will be receiving the sample without any details of the donor. As the first and most important step in personal identification, it is of great importance to avoid the possible pitfalls in forensic sex determination analysis. An investigator must be aware of such situations where sex determination analysis can be ambiguous and neither caused by his/her mistake nor the technology.

Authors' Contributions

Conception and design: RG
Analysis and interpretation of the data: RG, PMD, SKN
Drafting of the article: PMD
Critical revision of the article for the important intellectual content: SKN
Final approval of the article: JJI, ARHI

Correspondence

Dr Renjith George
MDS (India)
Assistant Professor
Department of Oral Medicine, Oral Diagnosis and Oral Pathology
Faculty of Dentistry
Melaka Manipal Medical College
Melaka, 75150, Malaysia
Tel: +6010 790 1963
Fax: +606 289 6669
Email: drrenjithgeorgep@gmail.com

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