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Sex determination using biochemical methods from dentition

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Abstract

PCR is a method of amplifying small quantities of relatively short target sequences of DNA using sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase. The teeth can withstand high temperature and are used for personal identification in forensic medicine. In the case of few teeth or missing dental records, there is not enough information to identify the person. The dental pulp enclosed by the hard tissue is not influenced by temperature unlike the buccal mucous membrane, saliva, and calculus. Barr bodies, F-bodies, SRY gene, AMEL gene can be studied to determine sex from these samples. A thorough knowledge and usage of the appropriate evidence from forensic scene enables proper identification of the individual.

Introduction

PCR is a method of amplifying small quantities of relatively short target sequences of DNA using sequence-specific oligonucleotide primers and thermostable Taq DNA polymerase.[1]

The teeth can withstand high temperature and are used for personal identification in forensic medicine. In the case of few teeth or missing dental records, there is not enough information to identify the person. The dental pulp enclosed by the hard tissue is not influenced by temperature unlike the buccal mucous membrane, saliva, and calculus.[2]

In a study by Tsuchimochi *et al.*, they used Chelex method to extract DNA from the dental pulp and amplified it with PCR and typing at Y chromosomal loci to determine the effects of temperature on the sex determination of the teeth.

Hanaoka and Minaguchi conducted a study to determine sex from blood and teeth by PCR amplification of the alphoid satellite family using amplification of X-specific (131 bp) and Y-specific (172 bp) sequences in males and Y-specific sequences in females. It was shown to be a useful method in determining the sex of an individual.[3]

Sivagami *et al.* prepared DNA from teeth by ultrasonication and subsequent PCR amplification and obtained 100% success in determining the sex of the individual.[4]

Sex determination from the f-bodies

Y chromosome contains F-bodies. These F-bodies can be used to identify sex. Various studies have been undertaken to identify F-bodies from pulpal tissue. Caspersson *et al.* [5] suggested that F-bodies can be applicable in forensic for sex determination. Dried blood stains, saliva, hair, and extracted dental pulp can serve as a sample for the test. Seno and Ishizu [6] carried out the detection of Y chromosome in the nuclei of dental pulp. Their study result was that over 30% of the male pulpal tissue showed positivity for F-bodies. F-bodies could be examined even in teeth as old as 5 months after extraction. Nayar *et al.* [7] in their study of pulp tissue in sex determination using fluorescent microscopy concluded that sex determination by fluorescent staining of the Y chromosome is a reliable technique in teeth with healthy pulps or caries within enamel or up to half the way of dentin. Teeth with caries involving pulp cannot used for sex determination.

Sex determining region "Y" gene

The abbreviation of SRY is the sex determining region "Y" gene. These gene codes for the sex-determining region Y protein, which is responsible for further development as male. Females have 2X chromosomes (46XX), and males have 1X and 1Y chromosome (46XY). SRY is located on the short (p) arm of the Y chromosomes at the position 11.3. Therefore, SRY gene can be used as a sex-typing marker in forensic samples. Many studies have shown the amplified SRY gene in various samples to determine sex. False positive results can be attained in certain syndromes, maternal-fetal microchimerism, and dissimilar sex between donor and recipient during transplantation (chimerism). George *et al.* [8] identified gender by amplification of SRY gene using real-time PCR from isolated epithelial cells of the removable partial denture. They concluded that saliva-stained acrylic dentures

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can act as a source of forensic DNA and co-amplification of SRY gene with other routine sex-typing markers will give unambiguous gender identification. Reddy *et al.* [9] studied the epithelial cells adherent to toothbrush as a source of DNA for sex determination using real-time PCR. All male sample in their study showed positive results and out of 15 female samples four were wrongly identified as males.

Sex determination from the enamel protein - amelogenin gene

Amelogenin (AMEL) is a major matrix proteins found in the human enamel involving Amelogenesis. Developing human enamel has about 30% protein, 90% of which are AMELs. AMEL gene is involved in the formation of AMEL. AMEL X gene is present in 106 bps and AMEL Y is present in 112 bps of the DNA. It has a different signature (or size and pattern of the nucleotide sequence) in male and female enamel. The AMEL gene that encodes for female AMEL is located on the X chromosome and AMEL gene that encodes for male AMEL is located on the Y chromosome. The female has two identical AMEL genes or alleles, whereas the male has two different AMEL genes. This can be used to determine the sex of the remains with very small samples of DNA.

Sex determination from barr bodies

Sex can also be determined by the study of X and Y chromosomes in the cells that are not undergoing active division. Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp. After death, it persists for variable periods depending upon the humidity and temperature in which tissue has remained. X chromatin and intra-nuclear structure is also known as Barr body as it was first discovered by Barr *et al.* It is present as a mass usually lying against the nuclear membrane in the females.[10] In a study carried out by Das *et al.* [11] it has been shown that up to a period of 4 weeks after death we can determine the sex accurately from the study of X and Y chromosomes keeping in view the variation of temperature and humidity.

Whittaker *et al.* [12] determined sex from necrotic pulp tissue stained by quinacrine mustard using fluorescent Y chromosome test for maleness and claimed that up to 5 weeks sex determination can be done with high degree of accuracy. It was found that in cases after fires, high impact crashes and explosions fragmentation and thermal trauma renders other methods impossible to determine the sex of the remains except the above said method from pulp. Pulp tissue cells become embedded firmly into the dried fibrosis matrix. Duffy *et al.* [13] have shown that Barr bodies and F bodies of Y chromosomes are preserved in dehydrated pulp tissues up to 1-year and pulp tissues retain sex diagnostic characteristics when heated up to 100C for 1 h.

Conclusion

Barr bodies, F-bodies, SRY gene, AMEL gene can be studied to determine sex from these samples. A thorough knowledge and usage of the appropriate evidence from forensic scene enables proper identification of the individual.

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