THE USE OF ALPHOID REPEATS IN SEX DETERMINATION FROM HUMAN SKELETAL FRAGMENTS AND TEETH SPECIMENS

Zagga AD,¹ Ismail SM,² Tadros AA¹ and Ahmed H.³

¹Department of Anatomy, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

²Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt.

³Department of Paediatrics, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Correspondence: Dr. A.D. Zagga,

Department of Anatomy, College of Health Sciences, Usmanu Danfodiyo University,

P. M. B. 2346, Sokoto, Sokoto State, Nigeria.

GSM: +234 (0)8035 175 780, Fax: +234-60-231514. E-mail: adauduzagga@gmail.com

ABSTRACT

Background: The advent of molecular techniques has revolutionized the ability of scientists to determine the sex of individuals. The genetic difference between males and females is defined by the presence or absence of the Y-chromosome. The only repetitive DNA family likely to exhibit the properties of significant chromosome specificity is the alphoid (alpha) satellite family located in pericentromeric regions of all human chromosomes.

Materials and Methods: A review composed via Medline Internet search of literature and contributions from our experiences as well as experiences of colleagues. The literature search for review was based on the aim and objectives of the original research work I conducted for my Ph.D thesis. The terms used to hook on internet include “alphoid repeats in sex identification from human bones and teeth”, “sex identification from burnt bones and teeth specimens using alphoid repeats primers”.

Results: The use of alphoid repeat primers in sex determination from human skeletal fragments and teeth specimens gives accurate result.

Conclusion: Alphoid repeats is efficient and reliable in sex determination from human skeletal fragments and teeth specimens. Hence, sex identification with alphoid repeats, of subjects for forensic purposes can be obtained with little or no involvement of morphological approach.

However, a large sample size coming from different sources may need to be considered to test the reliability and efficiency of these primers so that success rate in sex determination using these primers may be optimised.

Key words: Alphoids, sexing, bone, teeth, review.

INTRODUCTION

Despite its importance in cell biology and evolution, the centromere has remained the final frontier in genome assembly and annotation due to its complex repeat structure. However, isolation and characterization of the centromeric repeats from newly sequenced species are necessary for a complete understanding of genome evolution and function¹. Alpha satellite DNA covers the centromeric region of all human chromosomes over distances often as large as several megabases. It is composed of a basic 171 bp long unit that is organized into tandemly arranged higher order repeats (HORs); HORs contain variable numbers of basic repeats, from 4

(chromosome 2) to as many as 34 (chromosome Y). The HORs of a given chromosome are highly homogeneous, with sequence identity often exceeding 99% along the blocks they constitute. The length of the *alphoid* blocks also varies substantially².

Probes specific for repeat sequences derived from the heterochromatic region of the long arm of the Y chromosome were used to develop the first simple screening test for sex diagnosis through dot blot hybridization in small samples of unrestricted genomic DNA^{3,4}. These probes have also been used for in situ hybridization⁵. However, the use of a probe specific to the heterochromatic region of the Y chromosome

may be of limited reliability because of its inability to detect Y sequences in individuals lacking this region of the Y chromosome, as demonstrated in some normal, fertile males⁶. Conversely, this region of the Y may be carried as a translocation in 1 of 3, 000 apparently normal females⁷. Fragments of Yq may also be absent in Turner syndrome mosaics whose marker chromosome is a Y derivative. Thus, probes for the heterochromatic region of the long arm of the Y chromosome have an increased risk of an incorrect diagnosis of sex⁸.

From a forensic perspective, fire is arguably one of the most destructive forces in our society⁹. A physical fire can result in soft tissue damage so extensive that conventional methods of identification are precluded. Because of the durability of the mineralized bone and dental structures and the protection afforded by the soft tissues for the bones and hard tissue mass of the head, bones and teeth resist post-mortem degradation and can withstand extreme environmental insult¹⁰ and incineration^{11,12}. Usually cremation takes place at temperatures of over 1000°C for more than 70 minutes, destroying the corpse and only leaving severely burned teeth and some fragments of larger bones¹³. Even in severe conflagrations, such as vehicle fires, explosions, and aircraft crashes, preservation of the bone and dentition is common and often

used as a primary or contributory method of identification^{14,15}.

In instances where ante mortem records are not available for comparison, or where destruction of the bone or dentition by fire leaves too little bone or dental data for comparison, alternative methods of identification are required. A valuable source of DNA evidence is contained within bone and dentition, and this can be extracted and profiled to confirm identity by comparison to a known ante mortem sample or

to DNA profiles from relatives of the individual¹⁶. With the development of the polymerase chain reaction (PCR)¹⁷, it has become possible to analyze DNA that is severely degraded, of low copy number, and present only in minute quantities¹⁸⁻²⁰.

The review is aimed at bringing out latest works and challenges experienced in using alphoid repeats primers

in sex determination from human skeletal fragments and teeth specimens.

The Use of Alphoid Repeats Primers (X1, X2 and Y1, Y2) in Sex Determination from Human Bone Fragments and Teeth Specimens

The use of *alphoid repeats* primers in sex determination was first applied on dried blood by Witt and Erickson in 1989²¹. The use of *alphoid* repeat primers in sex determination is

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expected to allow more accurate result since the X chromosome-specific *alphoid* repeat sequence can be detected along the Y chromosome-specific repeat sequence²².

From Dalian Medical University, China, Lin *et al.* (1995)²³ reported that with *alphoid* repeat primers sex determination was possible even with very ancient human bone samples > 1300 years. In two adult cases where sex estimation was externally impossible, the sex was determined to be male because both X-specific and Y-specific bands were clearly detected. Amplification of rib samples from a child mummy showed that it was definitely male²³.

Cipollaro *et al.*, (1999)²⁴ reported from the Università degli Studi di Napoli "Federico II," Italy, on the successful amplification of ancient (79 A.D) DNA and gender determination of 8 femuri, 4 tibia and 1 humerus from Pompeii Archaeological Site, using Y-specific *alphoid* repeats. However, there was no amplification in 1 tibia due to very poor preservation of the specimen. The results suggest that the remains contain endogenous human DNA that can be amplified and analyzed²⁴.

From the Okayama University Medical School, Japan, Murakami *et al.*, (2000)²²,

reported on the sensitivity of PCR for detection of the Y chromosome-specific *alphoid* repeat and the X chromosome-specific *alphoid* repeat sequences. Sex could be determined by PCR of DNA extracted from the pulp of 16 freshly extracted permanent teeth and dentine including the surface of the pulp cavity of 6 freshly extracted milk teeth. Sex could be determined using the pulp in all 20 teeth (10 males 10 females) preserved at room temperature for 22 years. For the pulp of teeth stored in sea water, the sex could be determined

in all 8 teeth immersed for 1 week and in 5 of 6 teeth immersed for 4 weeks. In the remaining 1 tooth, in which sex determination based on the pulp failed, the sex could be determined correctly when DNA extracted from the hard tissue was examined. For teeth stored in soil, the sex could be determined accurately in all 8 teeth buried for 1 week, 7 of 8 teeth buried for 4 weeks, and in all 6 teeth buried for 8 weeks. The sex of a mummified body estimated to have been discovered half a year to 1 year after death could be determined readily by examination of the dental pulp. In the skeletons of 2 bodies placed under water for approximately 1 year and approximately 11 years 7 months, pulp tissues had been dissolved and lost, but sex determination was possible using DNA extracted from hard dental tissue²².

Matheson and Loy (2001)²⁵ reported from the University of Queensland, Brisbane, Australia, on the *alphoid* repeats for the genetic sex identification of 9400-year-old ten human skull samples from Çayönü Tepesi, Turkey. The X, Y *alphoid* repeats method produced a male identification in three of the 10 samples of the Çayönü Tepesi skull samples. The six other samples amplified with the X *alphoid* repeat primers but not with the Y *alphoid* repeat primers. Generally, the results of the X, Y *alphoid* repeats blind test attest to the accuracy of genetic testing, and also point the potential for occasional error in morphometric sexing²⁵.

Holland *et al.*, (2003)²⁶, reported from The Bode Technology Group, Springfield, Va, United States of America, on the DNA analysis of the skeletal remains of the World Trade Centre (WTC) Towers victims of September 11, 2001 attack. Approximately 13,000 skeletal fragments were analyzed at least once,

for a total of more than 18,000 analyses, and greater than 8,000 of the skeletal samples produced STR results (65%). The percentage of successful results was low in relation to previous mass fatality incidents involving airline disasters. However, when this same process was applied to the analysis of skeletal remains from the American Airlines Flight 587 disaster that occurred on November 12, 2001, the success rate was in line with expected results (ie, greater than 92% of the skeletal remains produced results)²⁶. However, no explanation was given for the observed variation in the success rates.

A study by von Wurmb-Schwark *et al.* (2004)¹³ from the Institute of Legal Medicine, Christian-Albrechts-Universität, Kiel, Germany, used the method of typing of short tandem repeats (STR) to identify the genetic sex of ten cadavers after cremation. The genetic profiles obtained from the remains after the burning of a corpse did in no case match the alleles from buccal swabs taken prior to cremation. A reliable post-cremation identification is unlikely¹³ (von Wurmb-Schwark *et al.*, 2004).

Several studies have looked at the effects of incineration on the extraction and amplification of DNA from the pulp of human teeth using PCR-based analysis²⁷⁻²⁹.

Rees and Cox, 2010³⁰ from the Centre for Forensic Sciences, Bournemouth University,

Talbot Campus, Fern Barrow, Poole Dorset, United Kingdom, carried out a comparative analysis of the effects of heat on the PCR-amplification of various sized DNA fragments extracted from *Sus Scrofa* (an animal) molars. A total of 88 molars were subjected to a range of temperatures for 15 minutes, by encasing

them in segments of defleshed maxilla and mandible. Amplifications using three-primer and four-primer multiplexes showed no degradation of the largest fragment of DNA following exposure to 450°C. Amplifications in the three-primer multiplex (283 bp) were successful following exposure to 525°C in maxillary samples only³⁰.

A recent study by Zagga *et al.*, 2013³¹, on the forensic study for genetic sex determination of burnt powdered skeletal fragments from Sokoto, North-western Nigeria, using *alphoid repeats* primers, yielded a success rate of 50% for correct genetic sex identification. PCR Sensitivity = 100%, Specificity = 33%, Predictive value of positive test = 60%, Predictive value of negative test = 100%, False positive rate = 200%, False negative rate = 0%, Efficiency of test = 83%. Fisher's exact probability test **P = 1**. Z-test: **z-value = -2.02837, p<0.05; statistically significant**.

Conclusion: *Alphoid repeats* is efficient and reliable in sex determination from human skeletal fragments and teeth specimens. Hence, sex identification with *alphoid repeats*, of subjects for forensic purposes can be obtained with little or no involvement of morphological approach. However, a large sample size coming from different sources may need to be considered to test the reliability and efficiency of these primers so that success rate in sex determination using these primers may be optimised.

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