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REVIEW

Molecular technique for gender identification: A boon in forensic odontology

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Identification of an individual is essential both for legal and humanitarian purposes. Gender identification is an essential criterion required to establish the identity of an individual. The use of molecular techniques in forensic odontology is an emerging field that widens the avenue even in challenging cases of identification. Gender determination using molecular techniques in forensic odontology shows prodigious outcomes and hence befits a frontier in forensic odontology. Identification of an individual in mass disaster and crime investigation cases in the current scenario is an arduous but highly desirable task. Thorough knowledge of forensic genetics is essential and is the need of the hour. Recent advancements in molecular techniques for DNA typing useful in gender determination are both economical and time-saving. These advancements will bring justice to people and large benefits to the society. An exhaustive internet-based literature search was carried out in PubMed and Google Scholar databases using terms 'gender identification' OR 'individual identification' AND 'DNA fingerprinting'. The search yielded 1,848 articles. Inclusion criteria included relevance to the context determined by reading the abstract and availability of the full text. A total of 169 articles were selected. After reading the full texts, only articles pertaining to the oral cavity along with relevant articles from the references of the selected articles were considered. A total of 90 articles were considered in the final review. The current article aims to comprehensively review the gender determination molecular techniques useful in forensic odontology. It emphasizes the various sources, techniques, profiling systems, and genes employed for gender identification from the oral cavity.

Keywords: gender determination, forensic odontology, molecular techniques, amelogenin, alphoid repeats

Introduction

Personal is a prime requisite in several instances, including mass disasters resulting from natural calamities (earthquakes and floods), terrorist attacks or mass murders, and other situations where the biological remains are insufficient or decomposed enough to cause difficulty in identification (1).

Personal identification relies on features aiding in tentative identification described as 'big fours' (determination of age, gender, stature, and ethnicity). This is required for legal, social, and humanitarian purposes. Gender determination forms the pillar of personal identification. The conventional method for gender determination includes analysis of morphological differences in the various anatomical parts such as bone, mandible, clavicle, and skull (2). Though the efficiency of individual or gender identification using morphological features is appreciable, sole dependence on the same is hampered in cases where a complete tooth or desired structure is unavailable. In such cases, employing molecular techniques proves a boon. Decoding individual DNA via DNA fingerprinting or DNA profiling aids in individual and gender identification.

This review aims to comprehensively review the gender determination molecular techniques useful in forensic odontology. It emphasizes the various sources, techniques, profiling systems, and genes employed for gender identification from the oral cavity.

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Method of data collection

An exhaustive internet-based literature search was carried out in PubMed and Google Scholar databases using terms 'gender identification' OR 'individual identification' AND 'DNA fingerprinting'. The search yielded 1,848 articles. Inclusion criteria included relevance to the context determined by reading the abstract and availability of the full text. A total of 169 articles were selected. After reading the full texts, only articles pertaining to the oral cavity along with relevant articles from the references of the selected articles were considered. A total of 90 articles were considered in the final review.

Morphological and molecular basis for gender determination

Gender determination plays a key role in personal identification. Several morphological characteristics exhibit sexual dimorphism thereby aiding in identification of the gender of the individual. Forensic dentists analyze key morphological differences in bones, particularly skull, including mandible depending on the reported literature findings (see **Table 1**).

It is well known that tooth escapes most insults in adversities like fire, explosions, and decomposition of bodies or situations with minimal forensic biological sample availability. Forensic odontology comes into play to support human identification in such scenarios (see **Table 2**).

Gender identification via differentiating morphological features is quite efficacious but in cases with incomplete or destructed dental structures, amalgamation of Forensic odontology with molecular techniques comes to rescue. Decoding of individual DNA via DNA fingerprinting or DNA profiling aids in individual and gender identification (3).

Analysis of DNA is relatively easy to work with owing to its biologically robust molecular structure being capable of procurement and analysis even from archival specimens like hair, dried blood, fixed tissue sections, or even exfoliated cells into secretions such as saliva.

Also, the unique nucleotide sequences present in the DNA of biological tissue result in differential genetic expression and variations in protein levels leading to physical and functional variations between individuals revealing an individual's DNA constitution and therefore reveal person's identity. The DNA profile tests are completely consistent and are authentic legal evidence required in paternity disagreements as well as identification of individuals (4–6).

TABLE 1 | Differentiating features in the skull for sexual dimorphism.

Characteristic feature	Male	Female
Skull Architecture	Big and rugged	Sloped and smooth
Cranial mass	More blocky And massive	Rounder and tapers at the top
Temporal Ridge	More prominent	Less prominent
Supraorbital Margin	Round and dull	Sharper
Eye Orbit	Sharp superior margin	Circular, higher and larger
Frontal lobe	Low and sloped	Higher and rounded
Zygomatic Bone	More pronounced	Less pronounced
Superciliary Arch	Larger and pronounced	Smaller
Gonion	Flared and sharply angled	Less flared
Teeth	Larger	Smaller
Mastoid	Medium to large	Small to medium
Nasal Aperture	High and sharp	Lower and round

Role of DNA fingerprinting in identification

After its discover by Watson and Crick (7, 4) the double-helix structured DNA has been enormously studied in almost all field of sciences since it provides the blueprint of the genetic make-up of every species. DNA has exons (protein-coding segments constituting 2–5% of total cellular DNA) and introns (non-coding DNA comprising the remaining 95%). The introns owing to no other known function apart from acting as a spacer DNA between exons are referred as junk DNA. It generally exists in multiple copies leading to repetitive DNA (20–30%) (5). These regions are unique and highly polymorphic (different in each individual). According to their size, these repeats are labeled as variable number of tandem repeat (VNTR) also called as mini-satellites (9–80 base pairs), and short tandem repeats (STR) popularly known as microsatellites (2–5 bp) (6). In addition to this, single base variations in DNA sequence called polymorphisms also exist.

Alec Jeffrey described 'DNA fingerprinting' or 'DNA typing' (profiling) for deciphering these variations and confirming an individuality (8). DNA fingerprinting depends upon prompt recognition by the probe along with a constant Mendelian pattern of inheritance. DNA profiling is sensitive, reproducible, and amenable to computer data basing, and therefore forensic DNA technology is a forerunner in deciphering complex criminal cases and also solving paternity disputes all over the world (9).

Extraction of DNA from oral cavity

Although whole blood is the highest yielding source of DNA, it may not be accessible in certain adverse circumstances. In

TABLE 2 | Characteristic dental features for sexual dimorphism.

	Characteristic	Male	Female
METRIC ANALYSIS	Mesio-distal and Bucco-lingual Dimension.	More	Less
	Canine dimorphism -Inter-canine width -Mesio-distal width	More	Less
	Dental Index - Incisor index -Canine Index (Mandibular Canine)	More	Less
NON-METRIC	Distal Accessory ridge of canine	More pronounced	Less pronounced
	Number of cusps in mandibular first molar	4 cusp molar	5 cusp molar
	Shape of mandible	Square	Round
	Maxillary Sinus	Large volume and width	Small volume and width
RUGAE ANALYSIS	Rugae length	Short	Long
	Fragmentary Rugae	Less	More
LIP PRINTS		Type III, IV, V	Type I, I', II

such cases, DNA can be extracted effectively from a variety of sources in the oral cavity, for example, teeth, saliva or saliva-stained samples, and buccal swabs. Also, DNA from cells attached to a toothbrush and oral prostheses such as dentures has been successfully obtained.

Lessig and Edelmann demonstrated the use of dental parameters and extracted DNA from dental tissues for individualization, and later Malaver and Yunis studied samples from cadavers buried for more than 5 years and extracted DNA from pulp, dentin, and cementum from the same. DNA can be obtainable from pulp of the teeth that had been kept in various environmental conditions (10, 11). An appreciable amount of DNA can be isolated from teeth, buccal swabs, saliva, and saliva-stained samples. Isolation of DNA from buccal swabs is quite cost-effective method due to ease in sample collection, long-term storage capability, and minimal amount of sample requirement to obtain sufficient DNA (12). Ghatak et al. established a simplified phenol-chloroform method for DNA extraction using buccal swabs (13).

Walsh et al. successfully extracted DNA from saliva for the first time. Since then, several studies have demonstrated saliva and salivary staining samples as suitable sources of DNA. Saliva and salivary-stained samples contain desquamated epithelial cells from the lips and oral mucosa, are painless and non-invasive, and are relatively easy to collect (14, 15).

Gender determination via DNA from oral cavity

Highly degraded human remains present a great challenge in identification. The classical method of gender identification includes a visual analysis of the bones, which have a characteristic pattern for each gender. But such techniques are unsuitable in situations where the samples are insufficient or extremely damaged. Therefore, alternative technology for sex determination with molecular biology has emerged as one of the best methods and, hence, garnered immense attention.

As discussed earlier, DNA extraction from the oral cavity has been the most common method employed for gender identification. Literature reveals the integration of newer protocols and technologies that have led to better results for DNA extraction. Anzai-Kanto et al. recovered DNA followed by its typing from saliva that was deposited on the skin of the volunteers in order to assess its potential for a forthcoming case analysis (16).

Identification of gender employing SRY gene amplification using real-time PCR from isolated epithelial cells of removable partial dentures has also been attempted, and dentures stained with saliva proved to be an excellent source for forensic DNA (17). However, limitations with saliva include the small amount usually deposited on the skin, particularly in bite impressions. Moreover, there are potential chances of cross-contamination and mixing leading to aberrant results.

Apart from saliva, buccal swabs could be another potential source of DNA. But limitations include unavailability of buccal swabs in people who died a long time ago, the possibility of cross–contamination, and vulnerability to microbial action or decay.

The epithelial cells adhering to the toothbrush were also studied and proved as a promising source of DNA for sex determination (18). Tanaka et al demonstrated a 10.430 μg/μl DNA yield from toothbrushes (19). Teeth are the best material for forensic studies due to their resistant nature against environmental assaults such as burn, dipping, trauma, damage, decay, and microbial degradation; teeth epitomize as an outstanding source of DNA (8).

Sweet et al used cryogenic grinding of teeth to obtain a DNA yield of 18.4 μg per tooth (20). They exposed the identity of a homicide victim who was burned to death. DNA was retrieved from dental pulp (1.35 μg) extracted from an intra-osseous third molar. Potschet et al obtained 6–50 μg of genomic DNA from a dental sample (8). Prachi et al retrieved 25–27 ng/μl from 20-teeth samples (21). With a higher yield of DNA integrated into modern analysis techniques, gender can be identified from tooth samples with DNA from as few as 10 cells.

Amelogenin primer from bones and teeth has also been used to identify gender. Meyer et al evaluated gender using the amelogenin primer of 4000- and 7000-year-old ancient

bodies (22). Similarly, Faerman et al determined gender using amelogenin primer from bones and teeth in 200- to 8000-year-old bodies (23). After the 9/11 attacks (2005), about 20,000 human remains were examined and advanced STR-based technology was employed, aiding in the identification of even scarce samples of bone and teeth (21).

DNA profiling systems

Wyman and White (24) pioneered the discovery of polymorphic loci in the human genome using a DNA probe and advanced techniques that evolved with time. DNA profiling includes a range of typing systems. The following molecular ways assist a forensic odontologist in determining gender from DNA.

Polymerase chain reaction (PCR)

Polymerase chain reaction was demonstrated first by Saiki et al and its application in DNA synthesis by Mullis and Faloona (25, 26). Currently, PCR has become an indispensible tool in forensics, especially for the amplification of victim/suspect nucleic acid sequences and their subsequent matching in minimal sample cases. PCR is an *in vitro* technique for multiplication of particular DNA sequences using enzymes by concurrent primer extension of the complementary strands of DNA. The use of PCR was limited until heat-stable polymerases became available. Most PCR-based techniques ease standardization, increase sensitivity of results, and analyze old or badly degraded samples easily (27). The major limitation of the PCR technique is the difficulty of DNA quantification (28). This limitation was overcome by the evolution of real-time PCR (29). With advancements in reagents like the 'AmpFlSTR MiniFiler' PCR amplification kit, promising results even in cases of degraded or adulterated profiling DNA samples become possible (30).

Southern blotting with single locus and multi-locus DNA probes is the conventional technique, which is still in use in a few research setups (31). Initially, multi-locus probes were employed for forensic genetic analysis, but gradually, these were substituted by VNTR using single locus polymorphisms under highly stringent conditions.

Recently, the advent of STR analysis has overpowered VNTR as the method of choice for forensic identification.

Restriction fragment length polymorphisms (RFLP)

This method employs a "restriction endonuclease" enzyme, which cleaves DNA at specific sites known as 'Palindromes' and then analyzes the variable lengths of the DNA fragments thus obtained. The drawback with this typing system is that it requires a large amount of DNA samples, takes a long time, and cannot be used with polluted samples. It is an older DNA typing system, and with the development of more efficient typing systems, it is no longer used (32).

Short tandem repeat (STR) typing

Short tandem repeat is described as repetitive short sections of DNA at variable sites in the entire human genome. STR typing intends to assess specific loci within nuclear DNA, and owing to individual's variability in the number of repeats, STR analysis is considered as the most promising genetic typing method, particularly for ancient samples (21, 33, 34). Routinely, 9–16 sequences in a sample are assessed, and D21S11, D7S820, TH01, D13S317, and D19S433 are common markers used in the cases of doubtful parentage claims (35). The accuracy rate increases with the length of a sample.

Tierney and Bird integrated amelogenin assay into multiplex STR typing system facilitating simultaneous amplification of X and Y correlative amelogenin gene with various other STRs in single reaction leading to gender and STR genetic fingerprint of an individual to be concurrently acquired (36).

Short tandem repeat was first analyzed by electrophoretic system followed by fluorescent-based technology and the use of DNA sequencers. This allows typing of large multiplexes (upto10 systems) and automation of the technique (27). Several commercial multiplexes are now available like SGM Plus (Applied Biosystems) having 10 loci including amelogenin, Promega (Madison, WI, USA) multiplexes using manual electrophoretic systems or monochromatic sequencing platforms and the recent 15-plex systems. The Poweplex16 (Promega) and the Identifier (Applied Biosystems) are the more frequently used systems (27).

Y-Chromosome STR analysis

Y-chromosome analysis can especially be used in paternity disputes since the Y chromosome passes directly from father to son. It can also be used in criminal cases when male suspect is involved in sexual assault cases. Y-STR evaluations are predominantly useful to identify the male DNA segment.

Joint Y chromosome (STR) plus MiniSTR technique was employed for the identification from the DNA isolated from the teeth and bone remnants of World War II casualties (37). Y STR technique that has been successfully commissioned in human skeletal remains identification unearthed from the corpus in Croatia, Bosnia, and Herzegovina (38).

However, there are limitations of Y-chromosome analysis. Firsty, similar Y STR silhouette will be seen in all the male relatives for generations. Second, such strikingly similar profile is inherited exclusively patrilinearly and that too

Locus	Primers	Product Size
Amelogenin	5'-CCCTGGGCTCTGTAAAGAATAGTG-3' 5'-ATCAGAGCTTAAACTGGGAAGCTG-3'	X = 106 bp Y = 112 bp
Amelogenin	5'-ACCTCATCCTGGGCACCCTGG-3' 5'-AGGCTTGAGGCCAACCATCAG-3'	X = 212 bp Y = 218 bp
Amelogenin	5'-CTGATGGTTGGCCTCAAGCCTGTG-3' 5'-TAAAGAGATTCATTAACTTGACTG-3'	X = 977 bp Y = 788 bp
Amelogenin	5'-CCCTTTGAAGTGGTACCAGAGCA-3' 5'-GCATGCCTAATATTTTCAGGGAATA-3'	X = 80 bp Y = 83 bp
Centromeric alphoid repeat	5'-TATTTGGACTCTCTCTGAGGA-3' (X3) 5'-TTCTACTACAAGGGTGTTGCA-3' (X4) 5'-GTGTATTCACCTCCGGGAG-3' (Y3) 5'-ACAAAAGGTTCAATTCTGTGAG-3' (Y4)	X = 157 bp Y = 200 bp
Centromeric alphoid repeat	5'-AATCATCAAATGGAGATTTG-3' (X1) 5'-GTTCAGCTCTGTGAGTGAAA-3' (X2) 5'-ATGATAGAAACGGAAATATG-3' (Y11) 5'-AGTAGAATGCAAAGGGCTC-3' (Y22)	X = 170 bp Y = 130 bp
ZFX/ZFY zinc finger gene	5'-CTGGAGAGCCACAAGCTGAC-3' 5'-TTGCTGTGGACTGCCAAGAG-3' X/Y = 209bp after *Hae*III cuts	X = 172 +37 Y = 88 +84 +37
SRY	5'-ATAAGTATCGACCTCGTCGGAAG-3' 5'-GCACTTCGCTGCAGAGTACCGAAG-3'	Y = 93 bp

FIGURE 1 | Sequence for primers in amplifying amelogenin, centromeric alphoid repeats, ZFX/ZFY, and the SRY gene (adapted from https://strbase.nist.gov/sextype.html).

in haploid fashion. The Y-PLEX 12, a commercial kit system, allowing co-amplification of 11 polymorphic STR loci, residing on the Y chromosome including amelogenin, permits easier identification (39).

X-chromosome STR (X-STR) analysis

X-STR are easily amplifiable and highly sensitive alleles that form an approved system, particularly in doubtful parenthood examination (40).

Single nucleotide polymorphism (SNP)

Single nucleotide polymorphisms (SNP) are the utmost prevalent type of genetic deviation between individuals. Each SNP represents a different nucleotide in place of normal (41). Small size, the ability to analyze highly degraded samples, and a lower mutation rate are the reasons SNPs are gaining importance (42).

In recent years, the SNP technique has been effectively employed to identify 9/11 and tsunami victims (43). Pakstis et al have formulated worldwide accepted 92 SNPs for individual identification (27). The limitations of SNPs comprise, first, that there are no widely established core loci, and, second, the prerequisite of huge multiplexing assays for the analysis of multiple SNPs simultaneously (44). Though SNPs are the most promising and future of DNA technology, they are improbable to substitute STRs presently utilized in national DNA databases. Primarily, owing to the small mutation rate and, second, due to the use of manifold typing programs, worldwide acceptable SNP assortments are challenging (38). Attempts are being made to explore the possibility of making SNP a substitute to STR (38).

Genes for gender determination

DNA-based sex assessment can be executed by numerous sex typing genes and markers like zinc finger genes ZFX/ZFY, SRY, centromeric alphoid repeats, and the amelogenin gene (45). Amelogenin is the most widely used genetic marker for gender determination (Refer **Figure 1**).

Zinc finger genes

These sex-determining genes located on short arm of Y chromosome codes for zinc finger protein. It comprises 30 amino acids repeating region and acts as transcriptional activator proteins, which get attached to the particular DNA segment. The zinc-finger structure is reinforced by the zinc ion that synchronizes cysteine and histidine in diverse amalgamations. Crystallographic studies of conventional zinc-finger domains revealed the presence of two β-sheets and one α-helix (46).

These are also referred as testis-determining factor (TDF) located in the genome of the heteromorphic individual and cause the bi-potential gonadal primordia to differentiate into testes. In addition, a homologue of ZFY is present on the X chromosome and referred to as ZFX, which codes for zinc finger protein and cross-hybridizes to ZFY under stringent conditions (47). ZFY could be a useful marker for the Y chromosome for gender screening (27, 48). Pilly and Kramer

TABLE 3 | Gender determination studies using molecular techniques from oral cavity samples.

References	Study samples, design and description	DNA Primer used	Outcome
Yamada et al. (57)	Tooth samples (Dental Pulp)	Using 32 P labeled DNA probe 'Myo' and Y-chromosome specific DNA Probe	DNA extracted from dental pulp is useful for identification of individuals, paternity testing, and sex determination.
Potsch et al. (8)	Freshly extracted teeth stored for 4 years and 6 weeks at room temperature (*n* = 30); teeth from jaw fragments stored for 15 years; teeth extracted from actual identification cases (*n* = 8)	Genomic dot blot hybridization using biotinylated repeated DNA probe PHY2.1	Sex was accurately identified in all cases using 50–100 ng target DNA.
Chen et al. (65)	Human extracted teeth subjected to different conditions; varying pH solution; 10% formalin, 75% ethanol; different temperature (−20 degrees C, 4 degrees C, 25 degrees C, 37 degrees C, boiling, burning); humidity (20%, 66%, water submergence); burying the teeth outdoors; and aging (teeth stored at room temperature for 1.5, 3.5 and 8 years).	Gender determination using probe Y1.1 and Y 1.2	154 bp fragment was obtained in all male teeth, No 154 bp fragment was found in a female fresh tooth. 154 bp fragment bands were not observed in case of teeth subjected to burial outdoors for 10 weeks, submergence in water for 10 weeks, soak in pH 2 solution for 10 weeks, and combustion for 10 minutes; only faint bands were observed in case of teeth subjected to 66% humidity and pH 10 solution for 10 weeks and combustion for 5 minutes; obvious bands were observed in the rest cases. The quality of DNA from dental pulp affected PCR amplification result.
Pawlowski (63)	Gender determination from Fomalin fixed paraffin embedded tissues (FFPE)	Amelogenin	It is possible to identify using 788 bp and 977 bp amelogenin fragment from soft tissues stored for 2 years.
Yomamato (64)	DNA profile typing from dental pulp	Y specific Probe	DNA typing useful for accurate sex determination from extracted teeth upto 21 months
Hanaoka and Minaguchi (53)	Sex determination from blood and teeth	Alphoid repeat primers	Gender determination could be done with precision
Pilly and Kramer (49)	Sex determination from pulp from 21 male and 24 female using third molar teeth	ZFX and ZFY	Males distinguished from female by 317 base pairs
Jiang and Guo (66)	Gender determination using DNA obtained from various samples (including saliva)	Amelogenin	Sensitive, rapid and reliable gender determination from various sample could detect gender in as little as 50 pg DNA
Ouchinniko et al. (67)	Archaeological samples from teeth samples without any cavities (*n* = 15)	Amelogenin (DYZ1, DYZ3, DXZ3 loci)	Successfully determined gender from all samples.
Komuro et al. (60)	Gender determination from dental pulp by capillary gel electrophoresis (*n* = 10 males and 10 females)	Amelogenin	Correct Gender determination from all cases
Urbani et al. (68)	To determine effect of temperature on sex determination from DNA PCR analysis from human dental pulp (*n* = 94)	Amelogenin	PCR analysis was 100% reliable, Gender assessment from teeth heated at 100^{0} C for 15 minutes but less reliable when the teeth were heated at higher temperatures for longer periods of time.
Meyer et al. (22)	Gender determination from three tooth samples (ancient)	Amelogenin	Success rate > 90%
Inoue et al. (69)	They determined gender from 55 resin prosthesis	Amelogenin (184 bp in D4S43 locus)	Resin prosthesis used in oral cavity and left at room temperature for 200 days can be used for DNA extraction and analysis.
Murakami et al. (51)	Freshly extracted primary (*n* = 6) and permanent teeth (*n* = 16); pulp stored in seawater (*n* = 8); Teeth stored in soil 8 teeth for 1 week;8 teeth for 4 weeks and 6 teeth for 8 weeks along with 3 cases	Alphoid repeat primers	100% result; 1/6 immersed teeth in sea water for 4 weeks, successful gender determined through DNA hard tissue
Sivagami et al. (70)	10 known (6 males and 4 females) and 10 unknown samples	Amelogenin	Despite Ultrasonic treatment of teeth samples, sufficient yield amount with good quality DNA obtained AMEL gene proves to be good primer for gender determination in Indian population

(Continued)

TABLE 3 | *(Continued)*

References	Study samples, design and description	DNA Primer used	Outcome
Tsuchimochi et al. (71)	Gender determination from human dental pulp incinerated to various temperature (200, 300, 400 and 500 degrees Celsius)	Alphoid repeat primers, STR and Y loci (DYZ3, DYS19, DYS390, DYS393)	All markers typed in samples incinerated upto 300 ^{0} C for 2 min.
Andreasson and Allen (72)	DNA extraction from teeth using Real time DNA Quantification Assay; STR analysis	Amelogenin	
Bilge et al. (73)	Case report study of age and gender through DNA profiling	Amelogenin	Male gender identification could be done successfully
Chang et al. (74)	Gender analysis in 338 male individuals in a Malaysian population comprising Malays, Chinese and Indians, using the AmpFlSTR Profiler Plus kit	Amelogenin	The amelogenin test gave a significant proportion of null alleles in the Indian ethnic group (3.6% frequency) and 0.88% frequency in the Malay ethnic group due to a deletion of the gene on the Y chromosome. This sex test also failed in a forensic casework sample. Inclusion of amelogenin with MSY1 loci on short arm of Y chromosome is recommended for accurate result.
William et al. (75)	Gender typing from deciduous teeth exposed to incineration (100–500 degrees temperature)	Amelogenin	Successful identification from deciduous teeth incinerated at 200 degrees Celsius and below. Greater sensitivity using fragment gel analysis by laser induced fluorescence which achieved sex identification from same human teeth to 400 degrees Celsius
Yoshida et al. (76)	80 fresh and 15 old teeth which were preserved less than 186 days through VNTR	Amelogenin (DYZ1, DYZ3 combined with DXZ1)	Successful sex determination from fluorescent probes and amelogenin even with low quantity DNA
Kumar and Hegde (77)	Gender identification from Exfoliated primary teeth using phenol-chloroform method	Alphoid repeat primers (X and Y specific sequences, 131 bp and 172 bp)	Inferred that PCR analysis not effective after 6 months of extraction.
Anzai et al. (16)	Twenty saliva samples collected;5 of these samples were deposited on skin and then collected subjected to STR analysis	Multiple loci (A set of 15 STRs)	Amount od DNA recovered when deposited on skin was 14 times less than salivary DNA Standardized protocol for DNA collection and extraction from skin-deposited saliva can be used as a method to recover salivary DNA in criminal cases
Zhang et al. (78)	Archaeological samples (n = 8 graves; total of 16 incinerated teeth)	Amelogenin	7/16 samples gave positive result; Ancient DNA from teeth are more marked than bone
Kovatsi et al. (79)	Effectiveness of DNA repair protocol in improving genetic testing in ancient teeth sample (n = 10)	Amelogenin	Successful in 8/10 cases
Gharib (80)	Sex determination in tooth samples in Iraqi population (10 male; 10 female)	Amelogenin	Amelogenin proved as good marker for sex determination in Iraqi population
Reddy et al. (18)	Determined gender from epithelial cells from tooth brush samples using real time PCR (n = 30)	SRY	100% sensitivity and 73.3% specificity; All male samples gave positive result while 4 out of 15 female sample gave false positive result
Raimann et al. (81)	Gender determination from unidentified cadaveric samples (n = 26) samples included premolar and molar with different post-mortem interval	Y- STR	Successfully determined gender in all samples.
Vemuri et al. (82)	Extracted teeth subjected to various temperature (100, 200, 300 and 400 degree Celsius); seawater (20–36) and burying at 30 cm depth (n = 15)	Alphoid repeat primers	Out of 15 samples, 13 samples were successfully identified. No DNA yield at high temperature
Khare et al. (83)	Saliva and blood sample from 20 subjects were collected	Amelogenin	Quantity of salivary DNA was less compared to blood DNA but sufficient for gender determination
Laverde (84)	Retrospective study of 300 forensic cases were devised (n = 220 males; 80 females)	Amelogenin	Male component in amelogenin marker may not always be successful in correct gender determination.
Zapico and Ublekar (85)	Teeth samples from Dentin and pulpusing silica based DNA extraction technique	Amelogenin	DNA yield depends on type of tooth (smaller teeth with smaller yield)

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TABLE 3 | *(Continued)*

References	Study samples, design and description	DNA Primer used	Outcome
Zagga et al. (54)	Dry teeth samples from cadavers, primary and permanent teeth (*n* = 3 each)	Alphoid repeat	Successful use of alphoid repeat primers in dry teeth in Sokoto, Northwestern Nigeria population
Marjanovic et al. (86)	Teeth samples from skeletal remains of World War II victims (*n* = 9); 12 buccal swabs from potential living relatives were collected.	PowerPlex Y STR kit (Including Amelogenin)	Profile success rate was 90% Referent samples success rate was 100%
Thangraj and Aswath (87)	A total of 40 samples were subjected for gender determination through molecular technique (20 male; 20 female)	Amelogenin	100% specificity and sensitivity
George et al. (88)	Gender estimation from epithelial cells adhered to acrylic dentures (*n* = 38; 21 males and 17 females)	SRY	100% accuracy
Datta et al. (89)	Teeth subjected to various environmental conditions (*n* = 50)	Amelogenin	
Kholief et al. (90)	Freshly extracted teeth were subjected for PCR analysis (*n* = 40)	Amelogenin	Ultrasonication of teeth samples yield sufficient amount of good quality. Amelogenin gene is good primer for sex determination in Iraqi population.
Muthuswamy (55)	Maxillary and mandibular premolar (*n* = 10; 5 male and 5 females)	SRY	Prediction rate was 100% for male and 80% for female
Chowdhury et al. (90)	130 extracted premolars put in different environmental conditions	Amelogenin	Steady decrease in the amount of DNA obtained on increasing the time period at which teeth were exposed, irrespective of the environmental condition. Successful gender determination by AMEL gene with complete sensitivity and specificity, except in temperatures of 350°C
Bharath et al. (91)	30 partial dentures	SRY	Sex determination was possible from all samples with 100% accuracy
Lim et al. (92)	17 teeth samples was subjected to burning at different temperatures ranging from 100°C to 500°C, at 2 to 10 minutes	Amelogenin	76.47% accuracy; 63.64% sensitivity

successfully determined gender from pulp for 21 men and 24 women using ZFX and ZFY genes (49).

Centromeric alphoid repeats

In all human chromosomes, Alpha satellite DNA is located in the peri-centromeric region of all human chromosomes, which are spaced apart. It is made up of a basic 171 base pairs long unit that is structured into tandemly arranged higher order repeats (HORs); HORs are made up of varying amounts of basic repeats, ranging from 4 (chromosome 2) to 34 (chromosome Y) (50). The HORs of a given chromosome are highly similar. The lengths of the alphoid repeats are variable. The alphoid repeat primers are more accurate in sex estimation because both the X chromosome-specific alphoid repeat sequence and the Y chromosome-specific repeat sequence can be concurrently spotted (51).

Witt and Erickson for the first time used the alphoid repeat primers for gender determination on dried human blood samples (52). Hanaoka and Minaguchi also determined gender from the alphoid repeat sequences from teeth (53). Murakami et al determined gender-employing DNA extraction from freshly extracted permanent teeth pulp and dentin using X and Y chromosome-specific alphoid repeat sequences. These teeth were preserved at room temperature for about two decades. They also analyzed pulp from teeth that were immersed in seawater; the gender was successfully assessed in all eight teeth submerged for 1 week and in five out of six teeth submerged for 4 weeks. Similar interesting results were obtained for teeth stored in soil and exhibiting positive gender assessment from all eight teeth buried for 1 week, seven out of eight teeth submerged for 4 weeks, and all six teeth buried for 8 weeks.

In another experiment, the gender of a mummified body could be determined even approximately 1 year after death using dental pulp. Also, sex determination was successfully achieved through the analysis of dental hard tissue in the carcasses of 2 bodies kept under water for about 1 year and approximately 11 years, wherein the pulp tissues had been dissolved and lost (44). Zagga et al studied molecular sex determination on dry teeth specimens from cadaveric,

primary, and permanent teeth samples using alphoid repeat primers and obtained a 100% result (54).

SRY genes

The *SRY* gene located on Yp11.2 from base pairs 2,786,855 to 2,787,741 encodes 'sex-determining region Y' protein that plays a role in the sexual development of males (50). This protein is a transcription factor and modulates the activity of specific genes to initiate the development that instigates a fetus to grow male gonads and avert the growth of female reproductive structures.

Muthuswamy determined gender from incinerated teeth using the SRY gene (55). However, certain syndromes such as maternal-fetal microchimerism, Turner syndrome, Klinefelter syndrome, Swyer syndrome, or partial gonadal dysgenesis can generate false positive results (50). Nicole et al also revealed that sex determination could be inaccurate in samples acquired from bone marrow transplanted subjects or in pregnant female carrying a male fetus (52, 56).

Amelogenin

Amel gene encodes amelogenin proteins, which comprise 90% of the total enamel matrix proteins and perform a pivotal role in enamel morphogenesis and mineralization. The location of human amelogenin gene is on the X and Y chromosomes at Xp22.1–p22.3 and Yp11.2, respectively, (57). On X chromosome, it is 2,872 base pairs long, while on the Y chromosome it has a size of 3,272 base pairs. Almost 90% of the *amel* gene transcripts are articulated from the X chromosome, while the remaining from the Y chromosome (58). The X and Y copies of the amelogenin gene do not undertake recombination that is correlative and that is why, it is the most favored genetic marker for gender assessment in the current scenario (54). Nakahori et al sequenced amelogenin gene for the very first time (59). Komuro T et al used the capillary gel electrophoresis method to determine gender through the X and Y loci to DNA obtained from dental pulp tissue (60). Many marketing kits that provide promising results are now accessible for gender assessment (61). However, false positive results using amelogenin primer can be seen in cases of chimerism, that is, bone marrow transplants or microchimerism, such as pregnant women with a male fetus, genetic miscellany cases, intersex conditions, transsexualism, or aneuploidy in the sexual chromosome (62).

Young et al exposed an aberration with Klinefelter syndrome in two undiagnosed males while using amelogenin loci and X and Y STRs. Michael and Brunner reported the first case of failure of an amelogenin gender test on a phenotypically normal male in the Israeli population (63). Since single genes gave erroneous results, Yamamoto and Morikawa proposed a novel technique for gender assessment based on the findings of SRY, STS, and amelogenin gene regions with simultaneous amplification of their similar sequences by using multiplex PCR (64).

Table 3 highlights studies employing molecular techniques in samples from the oral cavity to determine gender.

Conclusion

Gender determination in forensic odontology using molecular techniques shows prodigious outcome and hence has befitted as frontier in forensic odontology. Identification of an individual in mass disaster and crime investigation cases in the current scenario is an arduous but highly desirable task. Thorough knowledge of forensic genetics is essential and is the need of the hour. Recent advancements in molecular techniques for DNA typing useful in gender determination are both economical and time-saving. These advancements will bring justice to people and large benefits to the society. However, there are still issues with technical expertise and the dissemination of knowledge. Moreover, technologies like Next-Generation Sequencing and nanotechnology are bringing revolutions in the human sciences, but forensic odontology is yet to see their ride.

Author contributions

RP and SS contributed in acquisition of data, analysis and interpretation of data, drafting of the article, critical revision, and final approval of the submitted version. RD, EA, DM, and SC contributed in drafting of the article, critical revision, and final approval of the submitted version. All authors contributed to the article and approved the submitted version.

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