Anomalous Gender Assignation by the Amelogenin Sex Gene Test

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Abstract :

Accurate human gender identification, based on the amelogenin sex gene/locus, has crucial applications in many scientific disciplines such as medical/prenatal diagnosis of sex-linked disorders and forensics. Molecular genetics techniques, especially typing for a length variation in the X–Y homologous amelogenin gene (AMELX and AMELY), are used for human gender assignation. Amelogenin sex gene/locus test is an integral part of most PCR multiplex kits that are employed for DNA profiling. However, it was reported that normal males being typed as females with this test. It was also reported a number of amelogenin negative (or AMELY null) males in various populations. Therefore, the current study was taken to determine the frequency of AMELY null/ negative males in Libya. Forensic anthropology analysis showed that human remains are for males. Here we report on the first known case, in the Libyan population, of an amelogenin sex test failure on a phenotypically normal male. The frequency of AMELY negative is rare in Libya, with an overall frequency of 0.1175%. These results suggest that the determination of gender, based on the amelogenin test, should be interpreted cautiously. Although AMELY null males are extremely rare in most populations, typing an additional gender-determining

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locus should be considered in forensics where the reference sample is of unknown gender.

Keywords: Forensic science; Gender identification; Sex typing; Amelogenin gene/locus; FTA; STR; PCR.

Introduction:

The value of the Y chromosome in forensic DNA testing is that it is found only in males. The SRY (sex-determining region of the Y) gene determines maleness. Since a vast majority of crimes where DNA evidence is helpful, particularly sexual assaults, involve males as the perpetrator, DNA tests designed to only examine the male portion can be valuable. With Y chromosome tests, interpretable results can be obtained in some cases where autosomal tests are limited by the evidence, such as high levels of female DNA in the presence of minor amounts of male DNA [10].

The ability to designate whether a sample originated from a male or a female source is useful in sexual assault cases, where distinguishing between the victim and the perpetrator's evidence is important [14]. Likewise, missing persons and mass disaster investigations can benefit from gender identification of the remains. Over the years a number of gender identification assays have been demonstrated using polymerase chain reaction (PCR) technique [5]. By far the most popular method for sex-typing today is the amelogenin system, as it can be performed in conjunction with short tandem repeat (STR) technique [3].

Amelogenin gene, located on the X and Y chromosomes in humans [1], produces a protein important in the development of the tooth enamel matrix [2,3]. The British Forensic Science Service was the first to describe the particular PCR primer sets that are used so prevalently in forensic DNA laboratories today [14]. These primers flank a 6 base pair (bp) deletion within intron 1 of the amelogenin gene on the X homologue. PCR amplification of this area with their primers results in 106 bp and 112 bp amplicons from the X and Y chromosomes, respectively. Primers, which yield

a 212 bp X-specific amplicon and a 218 bp Y-specific product by bracketing the same 6 bp deletion, were also described in the original amelogenin research article [14] and have been used in conjunction with the D1S80 variable number tandem repeat (VNTR) system [3]. While amelogenin is an effective method for sex-typing biological samples in most cases, the results are not foolproof either due to primer binding sites that lead to null alleles or chromosomal deletions [3]. Amelogenin Y allele dropouts have been observed due to loss of portions of the Y chromosome in some population groups. Amelogenin X allele dropouts have been seen primarily due to primer binding site mutations. Prior study has been reported that a rare deletion of the amelogenin gene on the Y chromosome can cause the Y chromosome amplicon to be absent [11]. In such a case, a male sample would falsely appear as a female with only the amelogenin X allele being amplified. However, it was reported that two males were typed as females in 1998 by PCR amplification of amelogenin. It appears that this deletion of the Y chromosome amelogenin region is more common in Indian populations [15] than those of European or African origins. A study of almost 30,000 males in the Austrian National DNA database revealed that only six individuals lacked the amelogenin Y-amplicons [15]. Those individuals were verified to be male with Y-STRs and amplification of the SRY region [3].

However, to our knowledge, there is no study has addressed the aspects of anomalous amelogenin results on the identifying human gender in Libya. Thus, the current study was designed to study the frequency of AMELY null/negative males in Libyan population in order to widen our knowledge of this technique's possible sources of error. Here we report the a controversial case for male of human gender determination by amelogenin sex gene test in this study.

Materials and Methods:

Individuals and DNA Isolation:

Samples from 1147 individuals (50% from each sex [628 males and 519 females]). Peripheral blood samples were obtained from each participant in order to extract their DNA. Informed consent from each participant was also obtained. Blood samples were deposited on fast technique assay (FTA card). In addition, genomic DNA from 223 bone samples was extracted using a phenol/chloroform extraction method. The samples were collected from human remains in different mass graves in Libya after taking relative written consent. The extracted DNA from blood samples was quantified by real-time PCR using Plexor®HY system (Promega). The bone samples were directly amplified without quantification using the PowerPlex®16 HS System (Promega) kit instructions.

Amelogenin Multiplex Typing:

Deoxyribonucleic acid from the 1-mm diameter circle FTA® card was amplified using the PowerPlex® 16 HS System (Promega), in accordance with the manufacturer's recommendations (The-Perkin-Elmer-Corporation 1999). Amplified PCR products were analyzed by ABI PRISM® 310 Genetic Analyzer (Applied BioSystems).

Results:

The amelogenin test were undertaken on the 1370 Libyan individuals (37.88% females 519/1370 and 62.12% males 851/1370). The results of amelogenin sex gene/locus test are showed in Table 1. The results of amelogenin test showed that 54.75% (628/1147) of all tested blood samples were males with X.Y profile. However, 45.25% (519/1147) of the tested blood samples were females with X.X profile. These results matched with all individual's phenotypes (Table 1). Moreover, the results showed that ~ 99.5% (222/223) of all tested bone samples were males with X.Y profile. These results which obtained by sex typing test were matched with the data of anthropometry (Table 1).

After undertaking the amelogenin sex test (sex typing) and testing the concordance between genotype and referred sex in the database, we found only one non-concordant result in this study. This sample was codified as male in phenotype in the database (anthropological profile/ anthropometry parameters), whereas the results of amelogenin sex test revealed a female with X.X. profile. On repeating the amelogenin test in this sample, it showed again non-concordant results. No error could be discovered after checking the sex-specific variables in the database due to the fact that its masculine anthropological profile was concordant with the gender variable in the database (male), illustrating that this non-concordant result was not due to a database codification error. Therefore, we attributed this discrepancy to non-genotyping errors.

Table 1. Results of human gender determination by amelogenin sex gene test.

Samples	Referred phenotype*	Anthropometry	Amelogenin test
	· 628 males	----	· 628 X.Y
1147 blood	· 519 females	----	· 519 X.X
223 bone	· 223 males	· 223 males	· 222 X.Y
			· 1 X.X

***Phenotypes were identified according to the records of identification cards.**

Discussion:

In the current study we have explored the possible causes of eventual discrepancy between referred phenotype and amelogenin test. Using genetic tests as well as forensic medicine approach, we have found one non-concordant results when comparing the amelogenin outcome and the registered sex in the database. No apparent database codification error was discovered for this sample.

A male sample would falsely appear as a female with only the amelogenin X allele being amplified. Suggesting this case is anomalous amelogenin, this indicate to presence of deletion Y chromosome (Y chromosome drop-out) [3], by comparing this DNA profile with his parents' profiles we noted that parent's DNA profile were normal in amelogenin locus, that is mean the mutation was not inherit from parents to their offspring.

However, gender identification by amplification of amelogenin gene showed controversially case of human gender identification with amelogenin test, so the amplification of amelogenin gene will be adopted to confirm the gender, this suggest to significance uses of anthropology to determine the gender of human remains. In contrast, prior study [7] showed that the frequency of anomalous cases was about 0.00625% (5/80.000) for males, and 0.015% (3/20.000) for females. For Indian, Malay and Austrian populations, they were 3.2-3.6 %, 0.6-0.88 % and 0.02 % for males respectively [13,4] and the deletion of the Y chromosome amelogenin region is more common in Indian populations [15] than those European or African origins. In the current study, only one non-concordant result of DNA of the unknown male was seen. There was no matching between referred phenotype and amelogenin test. The frequency of anomalous amelogenin cases in this study was ~ 0.073% (1/1370) for males in this study. However, overall, the concordance between the amelogenin sex test and the database codified sex was 1369/1370 (~ 99.93%).

Many failures of this technique have been previously reported, resulting in a female amelogenin test outcome in a phenotypically normal male, due to the absence of the Y fragment [11,15,4,12,8,4,9]. One of the causes of this phenomenon is the presence of deletions of different sizes involving the AMEY gene that range from between 2.5 Mb and 304 Kb (Lattanzi 2005; Mitchell 2006). These deletions are especially frequent in the Indian population, having frequencies ranging from 1.8% to 3.6%

[11,15,4]. Another source of failure in the male sex diagnostic using the amelogenin test is the presence of mutations in sequences homologous to those of the primers used in the test, which may inhibit hybridization and amplification of the AMEY sequence [15]. In the present study, we did not find any genetic cause of amelogenin failure in the genetic sex determination. However, we cannot assess the impact of these potential genetic causes in the Libyan population because, at the moment, there are no studies that have assessed the frequency of these genetic anomalies (both deletions and mutations). However, taking in mind that prior study [13] studied 29,432 phenotypic male individual from the Austrian National DNA Database, and only found 6 individuals (0.018%) lacking the AMEY gene, we can hypothesize that in our Caucasian population the frequency would also have to be very low. It has been reported that another cause of discrepancy between referred sex and amelogenin test is the existence of identification errors in the samples during the different stages of its handling: DNA extraction, sample labeling, storage, as well as during its database codification.

It was precisely this kind of error that was not found in this study, suggesting that this source of amelogenin database discrepancy is, by far, the most frequent in Caucasian populations. It is for this reason that it has been proposed that the amelogenin test as a quality control tool to detect misclassification of individuals in the database and subsequent mistakes in statistical analysis [6]. The risk of misclassification is greater when very large sample sizes are used and even more in multicenter studies with a dense sample traffic.

To the best of our knowledge, this is the first time that this specific amelogenin sex gene used in identifying human gender in the field of forensics. We applied the DNA profiling, consisting of a panel of 15 different short tandem repeats (STRs) plus

amelogenin that has been employed for routine identity testing in forensics.

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الكشف عن طفرات في الصبغي المسؤول عن تحديد الجنس بواسطة اختبار جين تحديد الجنس (الأميلوجينين)

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المستخلص:

التحديد الدقيق لجنس الشخص يعتمد على موقع (جين) يقع على الحمض النووي، يعرف هذا الجين بـ (Amelogenin) والذي له تطبيقات حاسمة في العديد من التخصصات العلمية، مثل تشخيص ما قبل الولادة للكشف عن الأمراض الوراثية ذات العلاقة بهذا الجين. تستخدم تقنيات علم الجينات الجزيئي بشكل خاص في تحديد التغيير في طول كلاً من الصيغي (X) و الصبغي (Y) وذلك لتحديد جنس الشخص بشكل خاص. إن اختبار جين الجنس هذا يعتبر جزء متكامل من المواد الكاشفة التي تستخدم في عملية تفاعل انزيم البلمرة (PCR) وهي عملية تضخيم مواقع معينة من الحمض النووي لإنتاج ملف الحمض النووي للشخص (DNA profile). على كل حال أظهر عدد من النتائج في هذا الغرض نوع من اللبس (شذوذ) وذلك بغياب الصبغي الذكوري (Y chromosome) في عدد من الذكور، والتي ظهرت في النمط

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الجيني بالصبغي الأنثوي فقط (X chromosome) وكما هو معلوم ان الذكر في النمط الجيني يحتوي على نوعين من الصبغيات في موقع تحديد الجنس وهما (X,Y).

تهدف هذه الدراسة لتحديد نسبة الشذوذ او ما يسمى بفقدان الصبغي Y (AMEL Y null) في المجتمع الليبي. في هذه الدراسة تبيّن وجود طفرة في الموقع الخاص بتحديد الجنس (Amelogenin) بنسبة 0.1175% في المجتمع الليبي، والتي تمثلت في فقدان الكروموسوم Y الخاص بالذكور. تُظهر هذه النتائج أنه يجب التعامل بحذر في تحديد الجنس بهذه التقنية وخاصة في الحالات الجنائية والتي تكون فيها العينة المتحصل عليها من مسرح الجريمة مجهولة الجنس.

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