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HUMAN GENETICS

A Comparative Study on the Frequency of Amelogenin Y Deletion in a Brahmin Population of Haryana and Rajasthan State with Other Indian and Global Populations

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Abstract—In forensics, DNA plays an important role to link the victim and accused to the scene of a crime. It also identifies the gender in missing person identifications, mass disaster cases, and helps in the analysis of samples of sexual assault. The presence of the Y chromosome in a sample determines that it is of a male person. Modern multiplex STR kits have amelogenin locus as a gender-determining marker. Two single-copy genes located on Xp22.1–Xp22.3 (AMELX) and Yp11.2 (AMELY) encode the amelogenin locus. Although, dropout of Amelogenin Y in a known male, may falsely be exhibited as female with these kits. In this case, confirmation of Y amelogenin is done with Y chromosome-specific kits. This type of dropout at amelogenin Y was observed globally, especially in Asian populations. In our study, out of 102 and 100 known male samples from Haryana and Rajasthan state, three and one samples showed amelogenin Y deletion with a frequency of 2.94 and 1.00%. Haryana Brahmin has the highest amelogenin Y deletion among the studied Indian populations. Hence, in this study, a comparison was discussed with other Indian and worldwide populations.

Keywords: frequency, amelogenin, Y-deletion, Haryana Brahmin population

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INTRODUCTION

In forensics, sex determination is critical and inescapable step in DNA profiling of cases such as mass disaster victim identification, investigation of missing persons, solving murder mysteries and sexual assault cases [1]. Previously, sex determination of skeletal remains was done on the basis of its morphological examination. Nevertheless, this technique of gender determination fails while examining fragmentary remains especially in case of mass disaster victim identification. However, with the evolution of molecular biology, a gender can be determined from the traces of skeleton with DNA profiling [2]. In gender identification through DNA profiling, Amelogenin is most common sex determining marker. Amelogenin is encoded by two single copy genes i.e. AMELX (Xp22.1–Xp22.3) and AMELY (Yp11.2) [3]. For amelogenin typing, the universal set of primers is available which depicts six base pair deletion on AMELX [4–6]. In 1998, first time sex determination based on the amelogenin marker was questioned [7]. Afterword various studies have shown AMELY deletion in many of the population studies around the globe [8, 9]. AMELY is now been well characterized and its deletion mapping data is available [10, 11]. In present study, we also experienced AMEL Y deletion in the Brahmin population of Rajasthan and Haryana state, India. Further, we also compare our findings with the other available population's data.

MATERIALS AND METHODS

Blood samples of 102 and 100 unrelated Brahmin males from Haryana and Rajasthan states were collected on FTA Cards with informed consent after ethical approval. DNA was extracted from these samples using a novel DNA purification buffer given by Sahajpal et al. [12]. Autosomal STR and Amelogenin were typed using Powerplex 21 Autosomal kit (Promega, Madison, WI, USA) according to the corresponding manufacturer's recommendations. Further Global filer PCR amplification was performed on these samples as it contains the 3 markers of Y chromosomes. In addition to this, further amplification was carried out with Y Chromosome specific Kits like Y Filer, Powerplex Y23 (Promega, Madison, WI, USA), and Y Filer plus PCR amplification kit (Applied Biosystem) to know the number of markers deleted in the null Y allele males. PCR was done according to the recommended protocol using GeneAmp® PCR System 9700 (Applied Biosystems). The amplified products of Powerplex 21 Autosomal STRs, Y filer and Powerplex

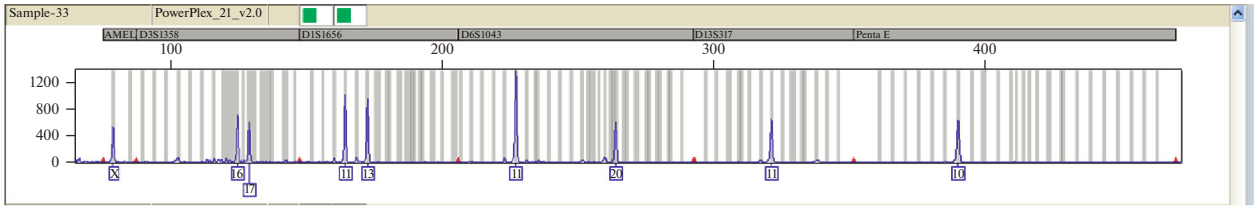

Fig. 1. Male Y missing profile (AMELY) with Powerplex 21.

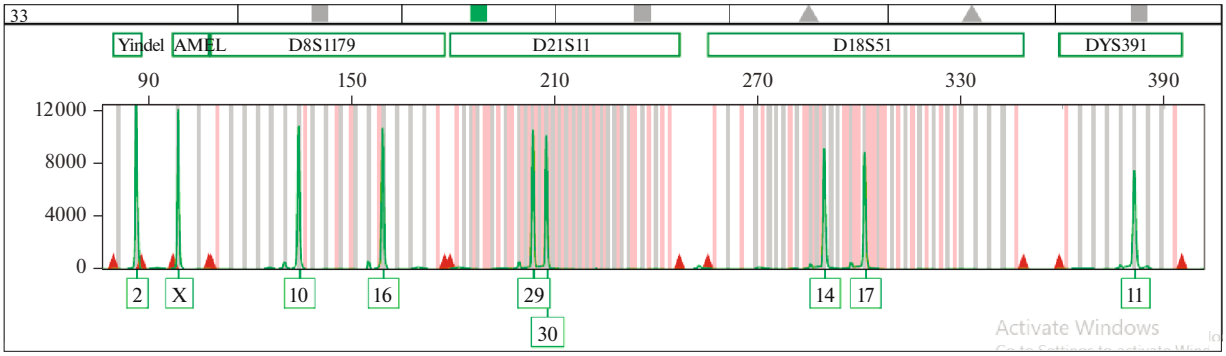

Fig. 2. Male Y missing profile (AMELY) with Global Filer.

Y23 were detected through capillary electrophoresis on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) while Global Filer and Y filer Plus amplified products were separated on a 3500xl Genetic Analyzer (Applied Biosystems).

Statistical Analysis

The analysis was performed using GeneMapper ID v3.2 and GeneMapper ID-X (Applied Biosystems) to generate DNA profiles. Alleles were assigned according to the International Society of Forensic Genetics (ISFG) guidelines for STR analysis.

RESULTS

In amplification with Powerplex 21, three samples out of 102 of Haryana Brahmin and one out of 100 of Rajasthan Brahmin showed Y deletion at amelogenin and exhibited a female genotype as shown in Fig. 1. All these samples were further typed with Global Filer autosomal STRs kit, which contains three respective gender-determining markers like amelogenin, Y Indel, and DYS390. Except for amelogenin Y, the other two markers showed their presence as shown in Fig. 2.

DISCUSSION

This study analyzed the Brahmin population of Rajasthan (100) and Haryana (104) state. The observed rate of amelogenin Y failure was high in Haryana Brahmin population compared to various other published studies. In earlier Indian population studies, the samples represented the state's whole population, but our study is specially focused on the state's Brahmin population. In addition to this, these samples were typed with a different increased number of Y chromosome-specific marker kits to check the number of loci missing in a Y amelogenin deletion male. In genotyping with Y filer marker DYS458 was missing in all three samples and similar results were found in the Japanese population [3]. With Powerplex Y 23, kit markers DYS458, DYS481, DYS570, and DYS576 were missing while Yfiler plus showed two more dropout markers DYS449 and DYS627. Dropout of markers with Powerplex Y23 was concordant with the global study conducted on 19300 samples by Purps et al [13]. In this study, only nine samples showed deletion at Yp11.2 AMELY with the same four markers dropout and all the samples were of Asian ancestry, namely Tamils from Southern India, Indians from Singapore, and British Asians with India or Pakistan origin. A similar various study conducted around the world and India is given in Tables 1 and 2. In comparison with 104 endogamous populations tested [4] and the Delhi population [14]. Brahmins of the Haryana population showed the highest amelogenin Y deletion frequency. In comparison with other Brahmin population, only Desasth Brahmin from Maharashtra and Iyenger Brahmin from Karnataka showed Y deletion.

Table 1. Frequency of amelogenin Y deletion in different Indian populations

Sr. no.	State	Sample size	No. of Y deletion	Frequency, %	References
1.	Haryana Brahmin	102	3	2.94	Current study
2.	Rajasthan Brahmin	100	1	1.00	Current study
3.	Jammu and Kashmir	312	0	–	4
4.	Himachal Pradesh	50	0	–	4
5.	Uttaranchal	132	0	–	4
6.	Uttar Pradesh	286	1	0.349	4
7.	Bihar	374	0	–	4
8.	Gujarat	45	0	–	4
9.	Maharashtra	630	2	0.317	4
10.	Chhattisgarh	298	1	0.335	4
11.	Jharkhand	435	0	–	4
12.	West Bengal	640	0	–	4
13.	Orrisa	404	2	0.495	4
14.	Andhra Pradesh	1100	0	–	4
15.	Tamil Nadu	401	2	0.498	4
16.	Kerala	87	0	–	4
17.	Karnataka	451	2	0.443	4
18.	Sikkim	233	0	–	4
19.	Mizoram	459	0	–	4
20.	Arunachal Pradesh	203	0		4
21.	Manipur	523	0	–	4
22.	Andaman and Nicobar Islands	151	0	–	4
23.	Delhi	708	2	0.282	14

CONCLUSIONS

DNA is the workhorse for sex identification for many years, but sex determination based on amelogenin marker has some discrepancies. In present study, confirmed known males were mistyped as female samples because of Y amelogenin deletion. Modern multiplex PCR amplification kits with single sex-determining marker even fail to identify the gender of a person if the identity of the sample is unknown. Failure of sex determination based on Amelogenin is the most common in forensic science laboratories throughout the world. Therefore, whenever any case shows deletion or negative result with AMELY typing can never be conclusive to confirm sample of female origin. It is therefore, Y-STR typing becomes mandatory in such cases. The deletion of DYS458 locus in Y-STR profile also serve as indication for AMELY deletion. In the various studied Indian populations, Brahmin of Haryana showed the maximum AMELY deletion. Although the frequency of AMELY deletion is less in other, worldwide population groups in comparison to Indian population. That is why gender testing in human identification needs high reliability and other methods like sex-determining region Y (SRY), Y-encoded testis-specific protein (TSPY), locus DXYS156, and steroid sulfatase (STS). All these markers have properties that could be used for developing more rigorous methods of testing forensic DNA samples for a Y chromosome. In this connection, advanced multiplex STRs kits like Global Filer, PowerPlex Fusion 6C and Investigator 24Plex QS should be used for cases like unidentified dead bodies or mass disaster victim identification. In Indian population, where AMEL Y deletion is present, use of these advanced multiplex PCR amplification kit is must.

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Table 2. Frequency of globally observed Amelogenin Y deletion populations

Sr. No.	Population	Sample size	Frequency, %	Reference
1.	Haryana Brahmin	102	2.94	Current study
2.	Rajasthan Brahmin	100	1.00	Current study
3.	Indian	4257	2.34	4
4.	India	4257	1.852	15
5.	India	708	0.282	14
6.	Malaysian Indians	315	3.175	9
7.	Singapore Indian	175	1.714	8
8.	Nepal	769	1.170	16
9.	Nepal	77	6.49	17
10.	Chinese	8087	0.037	18
11.	Malaysian Chinese	331	0.000	9
12.	China	12915	0.039	32
13.	Chinese	79304	0.023	19
14.	Singapore Chinese	210	0.000	8
15.	Malaysian Malays	334	0.599	9
16.	Singapore Malay	182	0.549	8
17.	Japan	500	0.200	3
18.	Sri Lanka	24	8.333	20
19.	China	12735	0.236	26
20.	China	10526	0.019	29
21.	Austrians	28182	0.018	21
22.	Australia	109000	0.020	22
23.	Libya	238	0.84	28
24.	Israel	96	1.042	23
25.	Italy	13000	0.008	24
26.	China	8850	0.042	25
27.	Belarus	30000	0.03	27
28.	Globally	19300	0.046	13
29.	Nepal	200	0.045	31
30.	Mexico	1230	0.081	30
31.	Taiwan	80000	0.00625	32

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

AUTHOR CONTRIBUTIONS

RY and VS conceived the idea and designed the experiments. SS and LK performed all the wet lab experiments. SS and VS analyzed data and wrote the manuscript. AS contributed in providing materials/analysis tools. All the authors participated in the discussion and provided inputs

to improve the content of the manuscript. All authors read and approved the final manuscript.

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