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Mosaicism as a possible reason for poor amplification of amelogenin-Y in three human male individuals

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Abstract. Unusual peak height ratios of the two amplified fragments in the X–Y homologous amelogenin gene in two multiplex STR kits (16 loci) required further investigations in three male Caucasoid individuals. Mosaicism is discussed as a possible reason for these findings.

Keywords: Amelogenin; Mosaicism; Peak height ratio; Peak imbalance

1. Introduction

A complete loss of the Y-specific PCR product of the X and Y homologous amelogenin gene [1] has already been detected in 0.018% of the males in the Austrian national DNA database. This loss was suggested to be due to a deletion on the Y-encoded gene [2]. Further failures of the amelogenin sex test have been found in Indians, which were found to be due to large deletions on the p-arm of the Y-chromosome [3].

This study reports three cases of unusual peak height ratios of the two amplified fragments of the amelogenin gene in three male Caucasoid individuals and suggests possible reasons for this phenomenon. The first case reported is a monozygotic twin suffering from a malign lymphoma (B-CLL). STR loci were investigated in our laboratory to find out whether the patient (*421) and his healthy brother (*420) were monozygotic or dizygotic twins, which is, in addition to HLA typing, relevant for bone marrow transplantation. Further two healthy, unrelated male Caucasoid individuals (*1514, *1703) showed the same phenomenon. All persons investigated were of normal male appearance.

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2. Materials and methods

DNA was extracted from blood samples using the salting out or the Chelex method. Multiplex PCR was carried out with the AmpFlSTR Identifiler PCR Amplification Kit (Applied Biosystems) and the Geneprint Powerplex 16 System (Promega), which applies published primer sequences [4]. A singleplex amelogenin PCR with the original primers [1], a DYS19 PCR [5] and the amplification of a male-specific SRY marker situated in the Y-chromosomal sex determining region [6] were added. The singleplex PCR products were detected by horizontal native polyacrylamide gel electrophoresis and silver staining. More Y-chromosomal STR loci including another amelogenin sex test were investigated with the genRES DYSplex-1 (Amelogenin, DYS390, DYS385 (I/II), DYS389 (I + II), DYS391) and the genRES DYSplex-2 (DYS393, DYS19, DYS389 (I/II), DYS392) kit (Serac, Germany).

Modified amelogenin-Y specific primers (GenBank acc. no. M55419) were designed using the Primer Express software (Applied Biosystems): the modified forward primer (5′ TTG CAT TAG CAG TCC CCT GG 3′) partially overlapped (6 bp) the 5′ end of the original forward primer, while the modified reverse primer (5′ GAG CAA CAC AGG CTT GAG GC 3′) binded downstream of the original reverse primer binding site. The PCR products were sequenced with the same primers (Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems).

In a next stage of analysis, a PCR applying an alternative amelogenin primer pair binding to the X- and Y-homologue was carried out [2]. Fragment analysis of multiplex PCR kits, the singleplex amelogenin sex test applying alternative primers and sequencing were performed on an ABI Prism 310 Genetic Analyzer.

3. Results and discussion

The AmpFlSTR Identifiler PCR Amplikation Kit and the Geneprint Powerplex 16 System revealed reproducible peak imbalances of the X- and Y-fragment at the amelogenin locus in three males. The ratios of the peak height values are given in Table 1. If possible, these findings were confirmed by PCR analysis from another DNA sample (*1514, *1703). A singleplex PCR of the amelogenin gene gave the same results. At the DYS-19 locus, an allele was detectable in all cases.

Amplification of the male-specific SRY gene and two multiplex Y-STR Kits (genRES DYSplex-1 and genRES DYSplex-2, Serac) including amelogenin revealed normal results

Table 1
Ratios of the peak height values (quotient of relative fluorescence units Y-fragment/X-fragment) of samples *421, *1514, *1703, showing abnormal results of amelogenin-Y, and *420, the monozygotic twin of *421, showing normal results

Sample	% rfu AmY/X Idfiler	% rfu AmY/X Powerplex16	% rfu AmY/X DYSplex-1	% rfu AmY/X alternative
*420	0.98	1.12	0.90	1.02
*421	0.08	0.16	0.09	0.07
*1514	0.19	0.30	0.14	0.16
*1703	0.54	0.61	0.54	0.58

(3 different Y-STR haplotypes), except for the imbalanced amelogenin peaks (Table 1). In the case of *421, both twins were identical in all STR loci with exception of the abnormal amelogenin results. Sequencing with the modified Y-specific primers revealed no differences to the GenBank sequence (acc. no. M55419). Therefore, a base substitution between the non-overlapping part of the modified forward primer and the modified reverse primer could be excluded as a single reason for the observed phenomenon. To rule out a sequence variation in the overlapping part of the primer sites, too, another amelogenin PCR was carried out, which applies an alternative primer pair, binding to the X- and Y-homologue. It revealed a poor amplification of the Y-specific peak, too, which resulted in the same peak imbalances as they were observed initially. Therefore, the insufficient amplification of the Y-fragment could not be explained by a mutation in a primer annealing site.

A possible explanation for these findings might be mosaicism: the coexistence of at least two cell lines, a normal cell line and a somatically mutated cell line. A deletion including the Y-chromosomal amelogenin gene, as it was already suggested in cases of a complete loss of the Y-fragment, might be the difference between the two cell lines. In this case, the mutated cell line might not have been amplified at all, while the normal cell line was amplified to a lower extent compared with the X-fragment. As males possess one X and one Y chromosome, the weaker intensity of the Y-specific signals compared with the signals of the X-fragment corresponds to the proportion of the normal to the mutated cell line. The malign disease of one of the monozygotic twins (*421), who could possess a somatic mutation, speaks also in favour of this theory. Furthermore, gonosomal mosaicism leading to different ratios of X- and Y-chromosomes cannot be ruled out as a reason for the observed phenomenon.

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