Y CHROMOSOME RELATED ANOMALIES DETECTED WITH Y CHROMOSOME STR SYSTEMS

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ABSTRACT: Two rare cases of Y chromosome related anomalies are presented. In both cases anomalies were detected during routine PCR analysis of Y chromosome polymorphic loci. The first case regards the 18-years old female with 46, XY phenotype. In the second case DYS19 locus duplication was detected.

KEY WORDS: Y chromosome; STR; PCR.

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INTRODUCTION

The male specific Y chromosome polymorphism is very powerful tool in forensic genetics, especially in paternity testing and cases of sexual assault or mixed stains. The analysis of human Y chromosome is also useful in solving evolutionary and anthropological problems, due to its haploid nature and paternal inheritance [1].

Two rare cases of Y chromosome related anomalies detected during routine PCR analysis of Y chromosome polymorphic loci are presented.

METHODS

DNA was extracted from buccal swab (Case 1), blood stain and whole blood (Case 2) using Chelex-100 method [8]. DNA was quantified using Primate specific DNA probe with chemiluminescent detection (QuantiBlot™ kit, PE, USA). Amplification of Y chromosome STR loci DYS19, DYS390, DYS393, DYS392, DYS391, DYS389I, DYS389II, DYS385a/b was carried out as described earlier [2, 7]. Amplification of 10 loci included in Amp*Fl*STR ProfilerPlus™ system (PE, USA) was carried out according to the procedure described by manufacturer. Detection of PCR products using capillary electrophoresis was carried out in ABI PRISM™ 310 Genetic Analyzer. 1 μl of

PCR product was mixed with 12 µl of deionised formamide and 1 µl of internal lane standard: GeneScan®-500, labelled with TAMRA (PE, USA) or fluorescent ladder FL600 labelled with CXR (Promega, USA). Analysed samples were denatured in 95°C for 3 min and immediately snap cooled in water-ice bath. The samples were run on a capillary 47 cm in length, filled with a denaturing polymer POP-4™ (PE, USA). The separation was conducted for 24 minutes at 15 kV, 9 mA, 10 µW at 60°C for all products, except for DYS389 loci where run time of 30 min was used. The analysis of results was carried out using GeneScan™ 2.1 software (PE, USA). Y chromosome STR allelic ladders were kindly supplied by Dr. P. de Knijff, University of Leiden, Netherlands and Prof. B. Brinkmann, Institut für Rechtsmedizin, Münster, Germany.

RESULTS AND DISCUSSION

Case 1

The first case is 18 years old female whose DNA was collected for routine sex analysis. Amelogenin locus analysis of DNA sample showed the presence of X and Y chromosome specific fragments. In further studies we performed the amplification and analysis of Y chromosome specific STR loci. In all analysed Y chromosome STR loci PCR products were observed. The obtained DNA profile (haplotype) is presented in Ttable I. Figure 1 presents the electropherograms of capillary electrophoresis (CE) analysis of amelogenin and 9 Y chromosome specific STR loci.

TABLE I. CASE1: THE RESULT OF Y STR LOCI ANALYSIS

DYS19	DYS390	DYS393	DYS392	DYS391	DYS389I	DYS389II	DYS385a/b
15	25	12	15	10	13	29	12 14

It was shown [4] that 46,XY karyotype in phenotypicaly normal female may be the result of a deletion in sex-determining region of Y chromosome located on its short arm. This deletion could be especially located in HMG box, a highly conserved motif of *SRY* gene, what may affect the DNA-binding activity of SRY protein.

This case could be also the example of 46,XY sex reversal originating from point mutation in SRY gene. Such examples were reported earlier [5]. A point mutation e.g. introduction of stop codon in *SRY* gene may have an effect in no coded SRY protein or a synthesis of truncated, incomplete polypeptide chain (inactive SRY protein). The missense mutation causing

Fig. 1. Case 1: Electropherogram presenting the separation of amelogenin and Y-STR loci PCR products run under denaturing conditions using ABI Prism 310 Genetic Analyzer: a) – amelogenin, DYS19, DYS389I and DYS389II (5-FAM); b) – DYS390 (TET); c) – DYS393, DYS392, DYS391 (HEX); d) – DYS385a/b (TET).

the same effect was also described [6]. However the examples of 46,XX males containing active SRY were also reported, what suggests that the whole process is regulated by more different genes.

The third explanation for this anomaly may be "illegitimate" crossover, which results in loss of SRY gene from the Y chromosome and its transfer to the X chromosome. If the sperm containing Y chromosome lacking TDF region fertilises a normal egg it results in 46,XY female forming. 46,XX male is formed when a normal egg is fertilised by a sperm containing X chromosome with TDF region translocated [3].

In cases when Y chromosome STRs are analysed not only male, but as shows the above example, female reference samples should be tested.

Case 2

The second case regards male with DYS19 locus duplication. The duplication was detected in reference sample and a few bloodstains submitted to the case analysis. Further analysis of additional Y-STR loci and autosomal

loci (Profiler Plus system) excluded the presence of DNA mixture. This anomaly was observed once in population of 385 males and was reported as a very rare event [2]. Figure 2 presents the results of DYS19, DYS390, DYS393 loci analysis as well as autosomal loci profile.

Fig. 2. Case 2: Electropherogram presenting the separation of Y-STR loci and autosomal loci (Profiler Plus system) PCR products run under denaturing conditions using ABI Prism 310 Genetic Analyzer; a) – DYS393 (HEX), DYS19 (5-FAM) and DYS390 (TET); b) – autosomal loci PCR products (Profiler Plus). The observed Y STR haplotype is: DYS19 16,17; DYS390 22; DYS393 14.

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