Forensic Science International: Genetics xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Letter to the Editor

A molecular analysis of three amelogenin negative males in two routine paternity tests

Dear Editor,

The gender marker amelogenin is incorporated in almost all commercially available multiplex STR kits. It is widely used in forensic casework, DNA databasing, blood sample storage, for prenatal sex determination and in pre-implantation genetic diagnosis. As shown by several publications [1–13] amplification of the homologous amelogenin part on the X and Y chromosome using these STR kits is not fully reliable and sometimes fails for different reasons, either due to translocation or deletion events or due to mutations in the amelogenin primer binding site. During routine paternity testing in our laboratories we observed two cases of amelogenin Y negative males. Further investigations revealed one XX male and a XY father son pair with a failure to amplify the homologous amelogenin part on the Y chromosome. Wrong sex determinations can result in misinterpretations and their forensic relevance should not be underestimated [14].

DNA samples were obtained from buccal swabs. Extractions were carried out using QIAamp DNA mini kit or Macherey and Nagel Blood Core Kit respectively following the manufactures instructions. Subsequent PCR was done with the commercial kits AmpFlSTR® Identifiler™ Kit, Mentype Argus X-8 Kit (Biotype AG, Dresden) the Powerplex Y Kit and the PowerPlex 16 Kit (Promega Corp., Mannheim). The amplicons were analyzed on an Applied Biosystems 310 or 3130 Genetic Analyzer using Genescan and Genotyper software.

In a routine paternity trio-test the alleged father only showed an amelogenin X result using the AmpFlSTR® Identifiler™ PCR Amplification Kit. Furthermore, in 12 STR systems he was excluded being the father of the child. The man was invited to provide a second sample and the results of this sample confirmed the first results excluding any laboratory errors. In a personal conversation with him, he told us that he knew from former clinical check-ups that he is an XX man. During his marriage the desire for children was growing and therefore the couple went to a children hospital and through several medical check-ups by the clinical genetic department his sterility and XX status was confirmed. His phenotype is male and a karyogram proved the XX status. We assume that this man has the so-called SRY positive XX male syndrome, because his phenotype looks male and twin pregnancy, blood transfusion, bone marrow or stem cell transplantation could be excluded in an interview. The ongoing paternity case could be clarified with the DNA profile from another man, which was named by the child's mother. The DNA from the XX man was typed with the AmpFlSTR® Yfiler Kit with negative results. Profiling with Mentype Argus X-8 PCR Amplification Kit revealed a heterozygous genotype for 6 of the 8 loci supporting the assumption of a XX male.

The second case is a paternity case without mother. We tested an alleged father and the male putative child. Using the Powerplex 16 Kit, paternity could be proven, but both the father and the son showed no amelogenin Y allele, only a X was present. With the Powerplex Y Kit, a full and identical Y profile was obtained for both males, further supporting the paternity of the alleged father. With the Mentype Argus X-8 PCR Amplification Kit, both the alleged father and the son exhibited again the amelogenin X finding and a hemizygous profile with only one allele at each X-STR locus. These findings support the assumption of the presence of Y chromosome with either a primer binding site mutation at the amelogenin locus or a deletion of (parts of) the amelogenin locus.

Human sex determination based on the amplification of homologous parts of the X and the Y chromosome can fail because of several reasons. We observed in our routine paternity testing one male with the so-called XX male syndrome and two men – father and male putative child – lacking of Y-specific amelogenin amplification.

XX male syndrome (XX testicular disorder of sex development) is very rare, it occurs 1:20,000 to 1:30,000 and was first described by de la Chapelle et al. [15] in 1964. Since then estimations [16,17] conclude that up to the year 2006 approximately 250 cases were published in the world literature.

XX male syndrome finds its expression in different phenotypes [17–19] and phenomena like Swyer syndrome (XY females), Reifenstein syndrome, chimersim, mosaicism, intersex and transsexualism can lead to false results in forensic sex and gender analysis [14].

The other findings concerned two men, which are a father–son pair. The observed deletion or primer binding site mutation of the amelogenin locus is not a de novo mutation but was segregated from father to son.

Misinterpretation and false results, especially from samples from crime scene can be overcome with the new diagnostic approach from Esteve et al. [20]. They suggest carrying out the so-called GenderPlex, which co-amplifies two different regions of the amelogenin gene, a 93-bp sequence stretch of the SRY gene and four mini-X-STR loci. Kastelic et al. [21] amplify the SRY gene for the same reason. However, these approaches cannot solve complex constellations like XX male SRY negative or Swyer syndrome samples.

Although our observed constellations are very rare, a careful and responsible working style connected with a well-established laboratory quality management system helps to clarify even such rare events. Discrepancies between DNA profiles, records, data on ID-forms or medical history papers should make lab investigators suspicious and let them analyze the case even more carefully.

Acknowledgement

The authors thank Catherine Zehethofer for language editing of the manuscript.

1872-4973/$ – see front matter
doi:10.1016/j.fsigen.2010.04.006

Please cite this article in press as: Zehethofer, K. and Rolf, B. A molecular analysis of three amelogenin negative males in two routine paternity tests, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.04.006

References

[1] A. Maciejewska, R. Pawłowski, A rare mutation in the primer binding region of the Amelogenin X homologue gene, Forensic Sci. Int. Genet. 3 (2009) 265–267.
[2] E. Plöchl, I. Vlasak, O. Rittinger, E. Bergendi, M. Stopar, P. Kurnik, M. Nachtigall, H. Zierler, G.A. Rappold, K. Schiebel, Clinical, cytogenetic and molecular analysis of three 46,XX males, J. Pediatr. Endocrinol. Metab. 12 (1999) 389–395.
[3] T. Takayama, N. Takada, R. Suzuki, S. Nagaoka, Y. Watanabe, R. Kumagai, Y. Aoki, J.M. Butler, Determination of deleted regions from Yp11.2 of an amelogenin negative male, Leg. Med. (Tokyo) 11 (Suppl. 1S) (2009) 578–580.
[4] M.A. Jobling, I.C. Lo, D.J. Turner, G.R. Bowden, A.C. Lee, Y. Xue, D. Carvalho-Silva, M.E. Hurles, S.M. Adams, Y.M. Chang, T. Kraaijenbrink, J. Henke, G. Guanti, B. McKeown, R.A. van Oorschot, R.J. Mitchell, P. de Knijff, C. Tyler-Smith, E.J. Parkin, Structural variation on the short arm of the human Y chromosome: recurrent multigene deletions encompassing Amelogenin Y, Hum. Mol. Genet. 16 (2007) 307–316.
[5] R. Kumagai, Y. Sasaki, T. Tokuta, H. Biwasaka, Y. Aoki, DNA analysis of family members with deletion in Yp11.2 region containing amelogenin locus, Leg. Med. (Tokyo) 10 (2008) 39–42.
[6] Y.M. Chang, R. Perumal, P.Y. Keat, R.Y. Yong, D.L. Kuehn, L. Burgoyne, A distinct Y-STR haplotype for Amelogenin negative males characterized by a large Y(p)11.2 (DYS458-MSY1-AMEL-Y) deletion, Forensic Sci. Int. 166 (2007) 115–120.
[7] Y.M. Chang, L.A. Burgoyne, K. Both, Higher failures of amelogenin sex test in an Indian population group, J. Forensic Sci. 48 (2003) 1309–1313.
[8] R.J. Mitchell, M. Kreskas, E. Baxter, L. Buffalino, R.A. Van Oorschot, An investigation of sequence deletions of amelogenin (AMELY), a Y-chromosome locus commonly used for gender determination, Ann. Hum. Biol. 33 (2006) 227–240.
[9] A. Michael, P. Brauner, Erroneous gender identification by the amelogenin sex test, J. Forensic Sci. 49 (2004) 258–259.
[10] M. Steinlechner, B. Berger, H. Niederstätter, W. Parson, Rare failures in the amelogenin sex test, Int. J. Legal Med. 116 (2002) 117–120.
[11] B. Shadrach, M. Commane, C. Hren, I. Warshawsky, A rare mutation in the primer binding region of the amelogenin gene can interfere with gender identification, J. Mol. Diagn. 6 (2004) 401–405.
[12] S. Caratti, G. Voglino, V. Cirigliano, A. Ghidini, R. Taulli, C. Torre, C. Robino, Amplification failure of the amelogenin gene (AMELX) caused by a primer binding site mutation, Prenat. Diagn. 29 (2009) 1180–1182.
[13] A.P. Chen, Y. Chen, H.P. Wang, W.H. Chen, H. Chen, L.X. Chen, H.Y. Sun, Types and frequencies of variants in Amelogenin gene in Chinese population, Zhonghua Yi Xue Yi Chuan Xue Za Zhi 24 (2007) 615–619 (Chinese).
[14] N. von Wurmb-Schwark, H. Bosinski, S. Ritz-Timme, What do the X and Y chromosomes tell us about sex and gender in forensic case analysis? J. Forensic Leg. Med. 14 (2007) 27–30 (review).
[15] A. de la Chapelle, H. Hortling, M. Niemi, J. Wennstroem, XX sex chromosomes in a human male. First case, Acta Med. Scand. 175 (Suppl. 412) (1964) 25–28.
[16] A. Rego, E. Margarit, X. Estivill, M. Regal, R.V. García-Mayor, Development in a 46 XX boy with positive SRY gene, J. Pediatr. Endocrinol. Metab. 9 (1996) 623–626.
[17] E. Vorona, M. Zitzmann, J. Gromoll, A.N. Schüring, E. Nieschlag, Clinical, endocrinological, and epigenetic features of the 46,XX male syndrome, compared with 47,XXY Klinefelter patients, J. Clin. Endocrinol. Metab. 92 (2007) 3458–3465.
[18] B. Ergun-Longmire, G. Vinci, L. Alonso, S. Matthew, S. Tansil, K. Lin-Su, K. McElreavey, M.I. New, Clinical, hormonal and cytogenetic evaluation of 46,XX males and review of the literature, J. Pediatr. Endocrinol. Metab. 18 (2005) 739–748.
[19] A.A. Rizvi, 46 XX man with SRY gene translocation: cytogenetic characteristics, clinical features and management, Am. J. Med. Sci. 335 (2008) 307–309.
[20] C.A. Esteve, H. Niederstätter, W. Parson, "GenderPlex" a PCR multiplex for reliable gender determination of degraded human DNA samples and complex gender constellations, Int. J. Legal Med. 123 (2009) 459–464.
[21] V. Kastelic, B. Budowle, K. Drobnic, Validation of SRY marker for forensic casework analysis, J. Forensic Sci. 54 (2009) 551–555.

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7 April 2010

Please cite this article in press as: Zehethofer, K. and Rolf, B. A molecular analysis of three amelogenin negative males in two routine paternity tests, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.04.006