Abstract

Introduction. During normal female (46,XX) sex development one X-chromosome is inactivated for dosage compensation while normal males (46,XY) have only one X-chromosome active. A 46,XX testicular disorder of sex development (DSD) is a rare condition (1:20,000) in which individuals with two X have male external genitalia, also called Chapelle syndrome. The study of the pattern of X-chromosome inactivation (XCI) by methylation using Human Androgen Receptor Assay (HUMARA) has become an important molecular tool in X-linked diseases and DSD syndromes. Objectives. To verify the presence of sex-determining region Y protein (SRY) together with the pattern of XCI in three 46,XX males presenting with DSD. Materials and methods. HUMARA was performed using restriction endonucleases sensitive to DNA methylation in the first exon of Androgen Receptor (AR) gene that comprises a short tandem CAG repeat region, allowing the use of fluorescent-labeled primer PCR to identify XCI pattern. HpaII and HhaI that cleave only the X-chromosome active were used. After DNA digestion, two PCRs were performed using specific primer pairs for AR-exon1. Results were obtained by capillary electrophoresis using 3130xl Genetic-Analyzer and analyzed with GeneMapper. Results. One out of three studied males with 46,XX testicular DDS is in fact SRY positive. Two SRY-negative male XX who undergone gonadal biopsy confirmed ovotestis, reclassifying them as DSD ovotesticular, formerly called hermaphrodites. HUMARA analysis showed that Psup (major and minor allele/digestion) ratio varied within normal range in case 1, 2 and 3: 39:61, 29:71, and 72:28 respectively. For the first time we were able to verify that in all three cases of male XX followed with DSD, independently of being SRY positive, the pattern of XCI was random, as observed in normal 46,XX female controls. Conclusion. The normal pattern of random XCI in male 46,XX suggests that it does not either play a role or explain the molecular physiopathology of DDS ovotesticular.

Key Words. Human Androgen Receptor Assay, X-chromosome inactivation, Disorder of sex development.