SHORT COMMUNICATION

MADRID, SPAIN

Prenatal diagnosis of 46, XX male fetus

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Prenatal diagnosis of sex differentiation disorders is extremely rare and is estimated in 1/2500 analyzed gestations. A group of these disorders are the 46, XX males and its incidence is estimated in 1/20000 male neonates. We report a male XX fetus in which the diagnosis of sex determination was requested at 20 gestation weeks to clarify the real gender of the fetus. Discrepancy between cytogenetic and ultrasonographic was detected.

KEY WORDS: AMEL gene; prenatal diagnosis; SRY gene; 46, XX male.

INTRODUCTION

The cytogenetic prenatal diagnosis includes fetal sex determination, which is always confirmed by the ultrasonographic scan as part of the routine pregnancy control. In those cases with a discrepancy between both results appeared, additional analysis have to be performed in order to find out the real fetal sex as well as to exclude a possible mistake or change between amniotic fluid samples. The molecular approach of fetal DNA analysis is useful in these cases.

The SRY gene has been proven to be determinant in the male sexual differentiation pathway during fetal life, initiating a genetic cascade leading to testicular development (1). This gene is located on the short arm of chromosome Y, but might be transferred to the X chromosome by unequal interchange between short arms of the sex chromosomes during paternal meiosis (2). This is the cause of the majority of the XX male cases.

We present a case in which a prenatal diagnosis of an XX male after a karyotype/phenotype discrep-

ancy was performed using the molecular analysis of SRY and Amelogenin gene.

MATERIALS AND METHODS

Fetal blood and amniotic fluid samples from a pregnant woman at 20 gestation weeks were sent to our laboratory in order to clarify the discrepancy of fetal sex. Cytogenetic analysis in amniocytes had revealed a 46, XX karyotype, while ultrasonographic scan at 20 weeks of gestation had showed male external genitalia.

Genomic DNA for molecular studies was obtained from amniotic fluid and fetal blood. DNA from 3-ml of amniotic fluid was isolated using Chelex resin (Instagen Matrix, Biorad). Fetal blood DNA was isolated by two different methods, Chelex resin and DNA BOX 500 kit (Protrams). The oligonucleotids used and PCR conditions were those previously reported (<http://www.gdb.org/>) (3).

RESULTS AND DISCUSSION

The results of the molecular analysis of SRY gene detected the presence of this gene in all three samples (Fig. 1a). The analysis of the Amelogenin gene showed a female pattern (Fig. 1b) corresponding to the absence of the Y chromosome.

The parents were informed of the XX male diagnosis and they decided to continue the pregnancy.

The prenatal karyotype/phenotype discrepancy occurs in approximately one out of 2500 analyzed gestations (4), leading usually to a high parental anxiety. The molecular approach of fetal DNA analysis is useful in most of these cases (5).

In the present report, the molecular analysis confirmed the previous results of karyotype and ultrasound. The detection of the SRY gene justified the presence of male genitalia. The amplification of the Amelogenin gene according to a female pattern justified the absence of the Y chromosome and therefore the 46, XX karyotype. In addition, the analysis with both genes simultaneously allowed a rapid diagnosis of an XX male.

The incidence of 46, XX males is estimated in 1/20000 male neonates (6). SRY sequences are present in at least 80% of these cases (7). Some of the XX male, as well as other cases with sexual differentiation disorders, implies other genes, some of them are not known yet (8). Nevertheless, the molecular analysis of fetal DNA using both genes (Amelogenin

Fig. 1. DNA was obtained from amniotic fluid and fetal blood was isolated using Chelex and a commercial kit. Positive control (male) and negative controls (female, water) are indicated. (a) Amplification of the SRY gene; primers used amplified a 472 pb resolved in a 3% agarose gel electrophoresis. Lane 1: negative control (female DNA); Lanes 2, 3, 4: amniotic fluid (AF); Lanes 5, 6, 7: fetal blood (FB); Lanes 8, 9: positive control (male DNA); Lane 10: water (W). (b) Amplification of the Amelogenin gene; primers used amplified a part of first exon. 106 and 112 pb PCR products were generated from X and Y homologues respectively and they were resolved by 9% acrylamide:bisacrylamide (19:1) gel electrophoresis. Lane 1: amniotic fluid (AF); Lane 2: fetal blood (FB); Lane 3: male DNA control; Lane 4: female DNA control.

and SRY) is a rapid, simple and in many cases useful method to a definite prenatal diagnosis, which allows early parental counseling.

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**M. J. Trujillo-Tiebas,^{1,3} C. González-González,¹
I. Lorda-Sánchez,¹ M. E. Querejeta,² C. Ayuso,¹
and C. Ramos¹**

¹ Department of Genetics, Fundación Jiménez Díaz, Madrid, Spain.

² Department of Genetics, Policlínica Guipuzcoa, San Sebastián, Spain.

³ To whom correspondence should be addressed at Department of Genetics, Fundación Jiménez Díaz, Madrid, Spain; e-mail: mtrujillo@fjd.es.