CASE REPORT

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The crucial role of *SRY* gene in the determination of human genetic sex: 46,XX disorder of sex development

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Abstract

Prenatal diagnosis of disorder of sex development (DSD) is very rare and is estimated to occur in 1/2500 pregnancies. A group of DSDs are the 46,XX testicular DSD. Today, the incidence of 46,XX testicular DSD is estimated at 1/20 000 newborn males. A majority of males with DSD have an unbalanced X;Y exchange involving the pseudoautosomal region, with translocation of the sex-determining region of the Y (*SRY*) gene onto Xp23.3. We present a rare case of very early prenatal diagnosis and management of a fetus with SRY-positive 46,XX testicular DSD.

Keywords: prenatal diagnosis, disorder of sex development, *SRY* gene, karyotype, ultrasound, genetic counseling.

Introduction

In humans, the process of sex determination has not yet been completely understood, but one of the most important gene is sex-determining region of the Y (*SRY*) gene, located on Y chromosome [1, 2].

SRY gene is thought to have developed approximately 300 million years ago due to duplication of SRY-box transcription factor 3 (*SOX3*) gene, resulting in the primary sex determinant present in mammals today [3].

The testis-determining factor (*TDF*) gene is responsible for initiating the process of sex determination on males [4]. Sex determination in males and spermatogenesis is controlled by genes located on the Y chromosome [5]. SRY protein plays a key role to initiate the cascade of male differentiation [6] and prevents the development of female reproductive structures.

Aim

The purpose of our case report is to highlight the relationship between the sonographic phenotypic aspects and cytogenetic aspects of a fetus diagnosed with SRY-positive 46,XX testicular disorder of sex development (DSD).

Case presentation

A 32-year-old woman, pregnant for the third time, comes to our Clinic for an ultrasound (US) investigation, as part of the routine pregnancy control.

The US examination is performed using a Voluson E10 US, which showed a single pregnancy, 10 weeks of gestation, normally developed.

A second ultrasonographic scan was performed after one month, and indicates a monofetal pregnancy, 14 weeks of gestation, with male phenotypic external genitalia.

The current pregnancy is the third pregnancy of the couple. We have to mention that it is a Caucasian couple, non-consanguineous, and there was no family history of sex discordance.

From previous medical history, we mention that the first two pregnancies were stopped in evolution at 12 weeks of gestation, for unknown causes.

Knowing the couple's medical history, following a discussion with a maternal–fetal medicine specialist, it was decided to perform additional specific prenatal genetic investigations, including amniocentesis, with amniotic fluid collection and fetal karyotype to prevent a new affected pregnancy.

Amniocentesis was performed at 18 weeks of pregnancy, with 18–20 mL of clear amniotic fluid collected, which was sent for quantitative fluorescent–polymerase chain reaction (QF–PCR) analysis for chromosomes 13, 18, 21, X and Y and fetal karyotype.

The intervention proceeded normally, without post-procedural complications.

Genomic deoxyribonucleic acid (DNA) was extracted from 5 mL amniotic fluid using a commercial kit (Favorgen, Biotech Corp, Hong-Kong).

The detection of trisomies 13, 18, 21 and sex chromosomes aneuploidies was performed by multiplex QF–PCR targeting 26 markers.

We analyzed five short tandem repeats (STRs) from chromosomes 21, six STR markers from chromosomes 18, five STR markers from chromosomes 13 and nine molecular markers from specific regions located in sex chromosomes.

To increase the possibility of detecting unbalanced chromosome rearrangements, the location of the selected markers covered most of the length of the targeted chromosomes [7].

The detection and quantification of the PCR products was performed by capillary electrophoresis, using fluorescence-labeled primers, analyzing the size and peak areas of alleles (GeneMapper® v.3.2, ABI Prism 310, by Applied Biosystems, USA).

For sex chromosomes, we analyzed six STR markers, of which four chromosome X-specific markers (*i.e.*, *DXS1187*, *DXS981*, *DXS1283*, *DXS7423*), one pseudo-autosomal marker (*DXYS267*) and one chromosome Y-specific marker (*DYS448*). In addition, we analyzed three non-polymorphic markers, one present in both sex chromosomes (*AMEL*) but size-specific to each sex, one chromosome Y-specific marker (*SRY*), and one chromosome X-specific marker (*XHPRT*) (Table 1).

Table 1 – ***Details of the molecular markers: name of the marker, citogenetic localization, fluorescent label, and the range of the allele resulted from the PCR***

Name	Location	Allele size range (bp)	Sequence (5'–3')
DXS1283	Xp22.3	295–340	NED–AGTTTAGGAGATTATCAAGCTG
			CCCATACACAAGTCCTCAAAGTGA
DXS981	Xq13.1	225–260	6-FAM–CTCCTTGTGGCCTTCCTTAAATG
			TTCTCTCCACTTTTCAGAGTCA
DXS1187	Xq26.2	125–170	VIC–CAGCTACTCAATGAAAAGCC
			TGATGGAGAAAGTCACTGAAC
XHPRT	Xq26.2	265–300	VIC–ATGCCACAGATAATACACATCCCC
			CTCTCCAGAATAGTTAGATGTAGG
DXS7423	Xq28	350–420	VIC–TACTGGAGGTGAGGGTTGTG
			TGGGCTGCCCAGATACAACT
DXYS267	Xq21.31Yp11.31	240–280	PET–ATGTGGTCTTCTACTTGTGTCA
			GTGTGTGGAAGTGAAGGATAG
AMEL	Xp22.2/Yp11.2	106/112	NED–CCCTGGGCTCTGTAAAGAATAGTG
			ATCAGAGCTTAAACTGGGAAGCTG
SRY	Yp11.31	248	NED–AGTAAAGGCAACGTCCAGGAT
			TTCCGACGAGGTCGATACTTA
DYS448	Yp11.223	323–370	PET–CAAGGATCCAAATAAAGAACAGAGA
			GGTTATTTCTTGATTCCCTGTG

PCR: Polymerase chain reaction; 6-FAM: 6-Carboxyfluorescein; PET: Paired-end-tag.

QF–PCR analysis did not indicate the presence of any aneuploidy of fetal chromosomes 13, 18 and 21.

In the case of sex chromosomes, 10 genetic markers were investigated (Figures 1 and 2):

- Four chromosome X-specific markers are heterozygous, and present normal values (1:1);
- One chromosome X-specific marker is homozygous (non-informative);
- The *AMEL* non-polymorphic marker indicates only the presence of X chromosome;
- The *TAF9L* paralogous marker (3p24.2/Xq21.1) was present in 1:1 ratio, and indicates the presence of two X chromosomes;
- The *DXYS267* pseudoautosomal marker showed the presence of three alleles in 1:1:1 ratio;
- The *SRY* marker was homozygous and indicated the presence of Y chromosome;
- The *DYS448* marker, Y-specific marker, was not present.

The electropherograms of the amplification products shown the presence of Y chromosome fragments in normal XX sample (Figures 1 and 2). For each marker, GeneMapper® software provides a short description that includes the following: marker name, fragment size, peak area and the height of the peak. On the 0Y axis are shown the fluorescence units and on the 0X axis are shown the fragment size (base pair length).

The analysis of the 10 genetic specific markers of the sex chromosomes (X and Y) indicated the presence of two X chromosomes in a 1:1 ratio, and the presence of nucleotide sequences characteristic of the Y chromosome.

Cytogenetic analysis of fetal chromosomes in amniocytes had revealed a 46,XX karyotype (Figures 3 and 4). No abnormalities of the number or structure of fetal chromosomes have been detected.

The US examination was repeated at 20 weeks of gestation and has confirmed the male phenotypic external genitalia (Figures 5 and 6). No other abnormalities were detected (Figures 7 and 8). The nuchal fold of 1.75 mm is within the normal range (Figure 9).

After the corroboration of all prenatal investigations, the final diagnosis was: SRY-positive 46,XX testicular DSD.

The parents were informed of the SRY-positive 46,XX testicular DSD prenatal diagnosis and they decided to stop the pregnancy at 21 weeks.

During the genetic consultation, the parents were supported by a multidisciplinary team including clinical geneticists, neonatologists, pediatricians, endocrinologists, urologists and psychologists with experience in this field.

Postnatal examination confirmed the prenatal findings.

Figure 1 – ***Electropherogram of the amplification products for* DXS7423, DXY267 *and* SRY *markers. The X chromosome specific marker* – DXS7423 *presents a 1:1 ratio. The pseudoautosomal marker* – DXY267 *has a three-allelic pattern in a 1:1:1 ratio. Only one peak was observed for the non-polymorphic Y marker* – SRY.

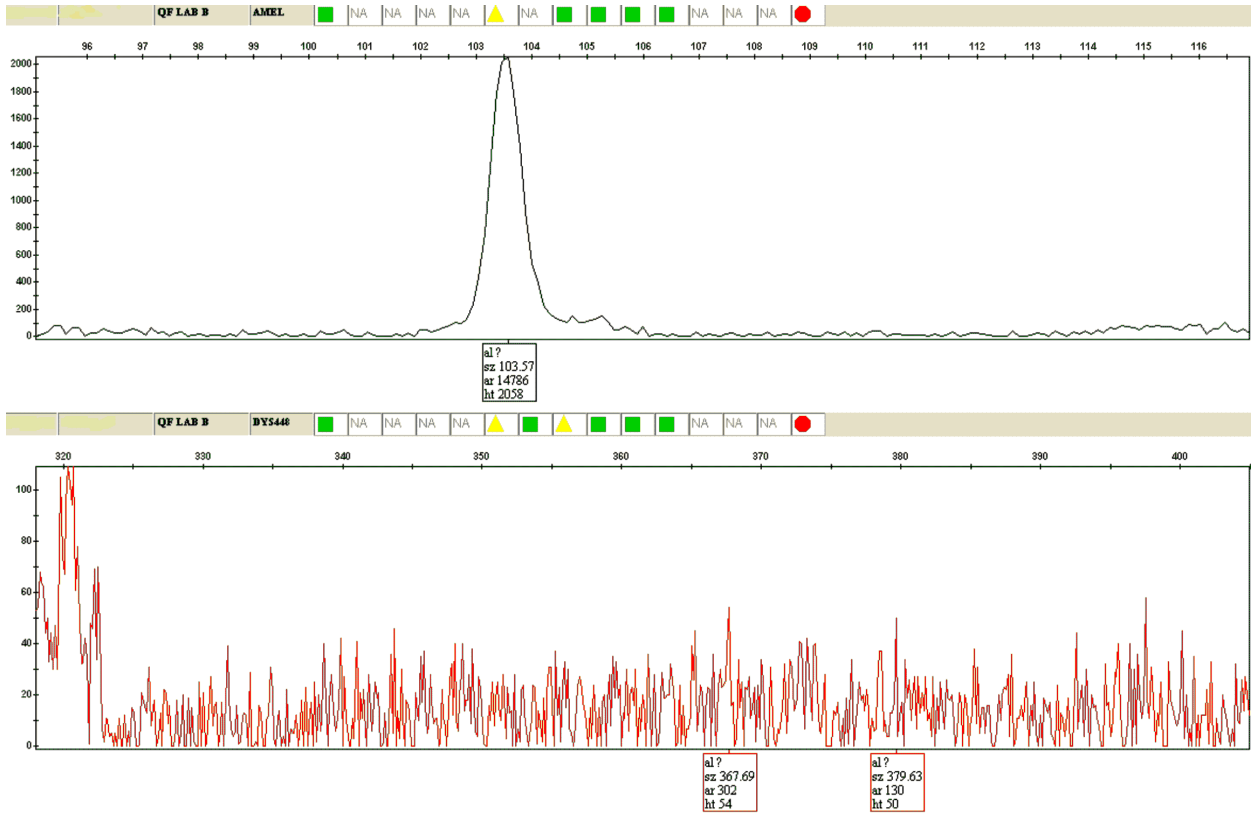

Figure 2 – ***Electropherogram of the amplification products* AMEL *and* DYS448 *markers. For the* AMEL *marker, the female sex (103 bp) is present. The Y chromosome specific marker* – DYS448 *has not been detected.***

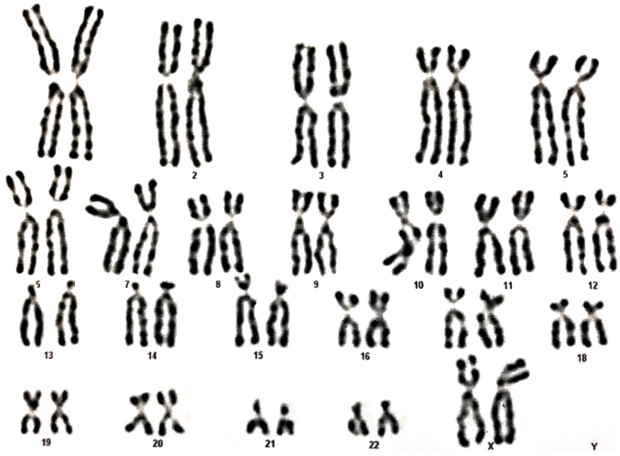

Figure 3 – ***Fetal karyotype: 46,XX (slide 1). Specimen type: amniotic fluid, 18 weeks of pregnancy.***

Figure 4 – ***Fetal karyotype: 46,XX (slide 2). Specimen type: amniotic fluid, 18 weeks of pregnancy.***

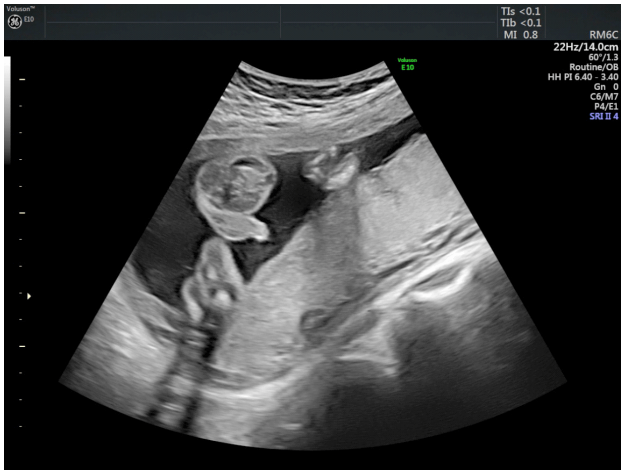

Figure 5 – ***Male phenotypic external genitalia (20 weeks of gestation, ultrasound examination).***

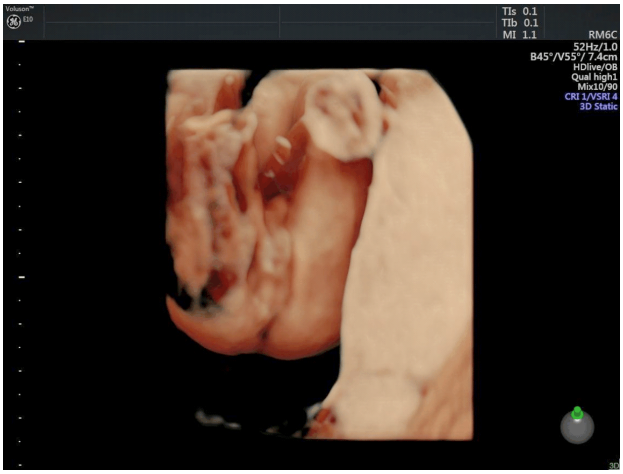

Figure 6 – ***Male phenotypic external genitalia (20 weeks of gestation, 3D static ultrasound examination). 3D: Three-dimensional.***

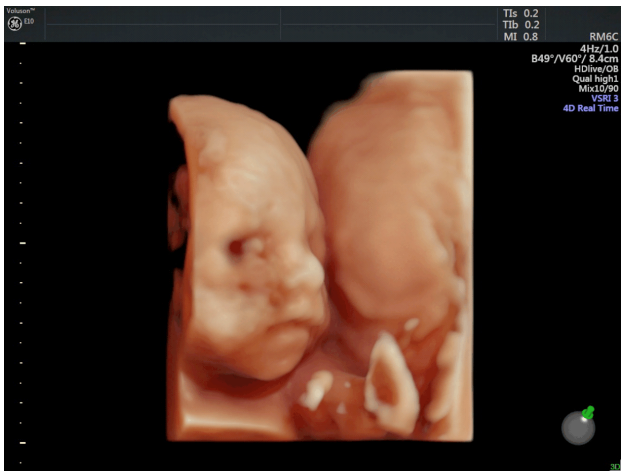

Figure 7 – ***Fetal half-profile (20 weeks of gestation, 4D real-time ultrasound examination). 4D: Four-dimensional.***

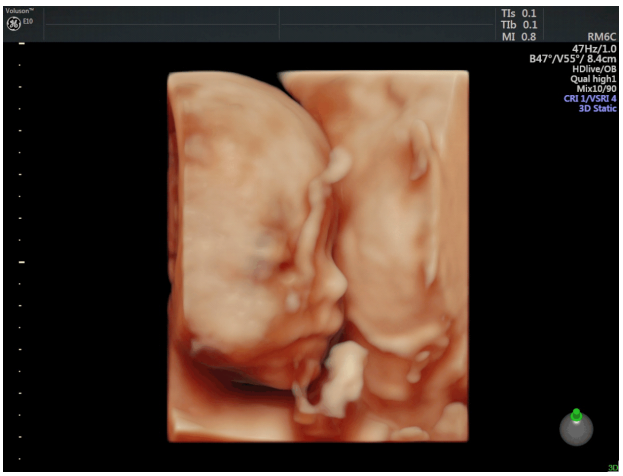

Figure 8 – ***Fetal profile (20 weeks of gestation, 3D static ultrasound examination).***

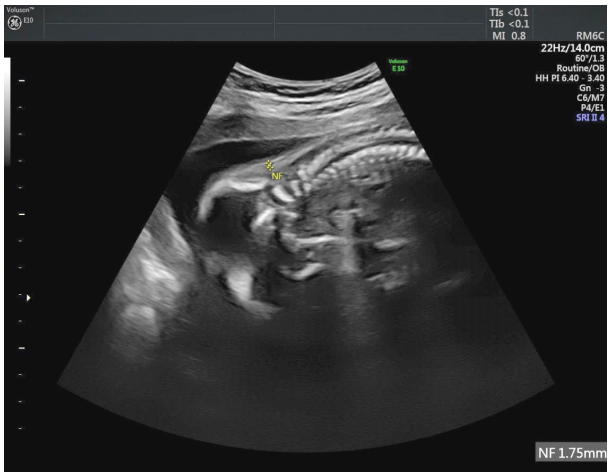

Figure 9 – ***Nuchal fold 1.75 mm (20 weeks of gestation, ultrasound examination).***

Discussions

During pregnancy, in most cases of 46,XX testicular DSD, the fetal karyotype is discordant with the screening analysis (US examination) [8, 9]. The discordance between phenotypic sex observed by US findings and prenatal cytogenetic results is not frequently encountered, but gender discordance is detected in approximately 1/2500 analyzed gestations [10–12].

DSDs contain a group of congenital conditions with various atypical development [12, 13].

Today, by the patient's karyotype, DSDs are subdivided in three main categories: 46,XY DSD, 46,XX DSD and sex chromosomal DSD [13–15].

Human males with a 46,XX karyotype are rare [10].

The reported incidence for 46,XX males varies from 1/9000 to 1/20 000 in newborn males [16–18].

Today, the frequency of 46,XX testicular DSD is estimated at 1/20 000 males [10, 19]. There are not studies to evaluate the risk for this disorder [8].

Based on molecular analysis (detection of *SRY* gene) and karyotype analysis, the 46,XX male patients can be classified in two categories: the SRY-positive 46,XX testicular DSD (80% of 46,XX testicular DSD), and the SRY-negative 46,XX testicular DSD (20% of 46,XX testicular DSD) [20].

The cause of SRY-positive 46,XX testicular DSD is a translocation of a fragment of Y chromosome during spermatogenesis. The abnormal interchange between X chromosome and Y chromosome is *de novo* translocation and is not inherited.

The cause of SRY-negative 46,XX testicular DSD is often unknown, but in some cases the inheritance is autosomal recessive [8].

The patients with SRY-negative 46,XX testicular DSD are more likely to have ambiguous genitalia, than the patients with the SRY-positive 46,XX testicular DSD. Because early diagnosis of DSD is important, the diagnostic procedures (US examination, fetal karyotype and molecular analysis) is necessary to make [11, 21–23].

In this case report, the early prenatal detection of the SRY marker justified the presence of male US phenotypic external genitalia.

The *AMEL* non-polymorphic marker indicates only the presence of X chromosome, according to a female pattern, justified the 46,XX karyotype.

In addition, the *TAF9L* paralogous marker (3p24.2/Xq21.1) was present in 1:1 ratio, indicating the presence of two X chromosomes.

The *DXYS267* pseudoautosomal marker showed the presence of three alleles in 1:1:1 ratio, and the *DYS448* marker, Y-specific marker, was absent.

Conclusions

DSDs can be diagnosed prenatally by the discrepancy between fetal karyotype and US phenotypic sex. In the present case report, the complex molecular genetic analysis was very useful in the early prenatal diagnosis, management and prognosis of the fetal disease. Couple's genetic counseling was essentially, regardless of the decision taken.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Ştefan-Dimitrie Albu has equal contributions to this paper as the first author.

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