A Case of Two Sisters Suffering from 46,XY Gonadal Dysgenesis and Carrying a Mutation of a Novel Candidate Sex-Determining Gene STARD8 on the X Chromosome

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Abstract
Identification of novel genes involved in sexual development is crucial for understanding disorders of sex development (DSD). Here, we propose a member of the START domain family, the X chromosome \textit{STARD8}, as a DSD candidate gene. We have identified a missense mutation of this gene in 2 sisters with 46,XY gonadal dysgenesis, inherited from their heterozygous mother. Gonadal tissue of one of the sisters contained Leydig cells overloaded with cholesterol droplets, i.e., structures previously identified in 46,XY DSD patients carrying mutations in the \textit{STAR} gene encoding another START domain family member, which is crucial for steroidogenesis. Based on the phenotypes of our patients, we propose a dual role of \textit{STARD8} in sexual development, namely in testes determination and testosterone synthesis. However, further studies are needed to confirm the involvement of \textit{STARD8} in sexual development.

Development of male sex characteristics in 46,XY embryos depends on the expression of specific genes critical for testes determination as well as genes necessary for the synthesis and action of testicular hormones. Disruption of those genes may cause a spectrum of phenotypes of 46,XY disorders of sexual development (DSD). Despite a significant progress in understanding the mechanisms of male sexual development, the genetic background of \textasciitilde{}50% of 46,XY DSD cases remains to be uncovered [Eggers et al., 2016]. Therefore, identification of novel genes
involved in those pathways is of great importance to get a more complete insight into the genetic basis of human sex determination.

The STAR-related lipid-transfer (START) protein family encompasses a wide range of proteins characterized by the presence of the lipid-binding START domain. These proteins are responsible for proper lipid distribution in cells [Clark, 2012]. A founder member of this family, the steroidogenic acute regulatory protein (STAR), is known to be involved in male sex development since it plays a critical role in testosterone biosynthesis. STAR is expressed primarily in steroid-producing cells, including Leydig cells of the testes, theca, and luteal cells of the ovaries, as well as adrenal cortex cells. The START domain of STAR binds cholesterol and transfers it to the inner membrane of mitochondria, where the first enzymatic step of all steroid syntheses takes place [Clark et al., 1994; Sugawara et al., 1995]. STAR gene mutations lead to blockage of the synthesis of testosterone and other steroids, causing lipid congenital adrenal hyperplasia, which in addition to adrenal features is characterized by DSD in 46,XY individuals [Lin et al., 1995; Bose et al., 1996; Bhangoo et al., 2005]. At the cellular level, STAR mutations cause an abnormal accumulation of cholesterol droplets within the cytoplasm of Leydig and adrenal cortex cells [Bose et al., 1996]. Beside the STAR protein, no other START family member has been described to be implicated in sexual development.

Here, we describe a mutation of the X chromosomal STARD8 gene (STAR-related lipid transfer domain containing 8) in 2 sisters suffering from 46,XY gonadal dysgenesis, which they inherited from their heterozygous mother. Based on the phenotypes of our patients, we propose a dual role of STARD8 in sexual development, namely in testes determination and testosterone synthesis.

Case Report

Two sisters suffering from 46,XY DSD were analyzed in the present study.

Patient 1 was diagnosed at the age of 17 years because of a lack of signs of puberty and menstruation. She was characterized by female-type external genitalia, oviducts, a normal prepubertal vagina, and uterus in spite of a 46,XY karyotype. Small gonads located at the normal ovarian position were detected by ultrasonography. Hormonal analysis revealed a very low level of testosterone and estradiol, despite a high level of gonadotropins, which is typical for hypergonadotropic hypogonadism. The patient was subjected to bilateral gonadectomy.

Patient 2 was diagnosed at the age of 8 months because of ambiguous external genitalia (labioscrotum, perineal urethral orifice and vaginal opening, phallic tubercle ca. 150 mm long) on the background of a 46,XY karyotype. The patient had oviducts, normal prepubertal vagina, and uterus. Both gonads were located in the ovarian position. Gonadotropin and testosterone serum levels were low, typical for the prepubertal period, and a hCG test revealed impaired synthesis of testosterone. The child was subjected to bilateral gonadectomy.

Methods

Array Comparative Genome Hybridization

Genomic DNA samples from the probands, their parents, and their healthy brother were isolated from blood samples by using the DNA mini kit (Qiagen, Hilden, Germany) and were tested using the Agilent SurePrint G3 CGH Microarray Kit, 1x1M (Agilent Technologies, Santa Clara, CA, USA). Labeling and hybridization were performed according to the manufacturer’s protocol. Data were analyzed using Agilent Genomic Workbench, and probe positions were determined according to NCBI37/hg19 build.

Whole Exome Sequencing

Exome capture was performed on DNA from the patients, their parents, and a healthy brother by using the SureSelect Human All Exon v3 kit (Agilent Technologies). Sequencing was performed on an Illumina HiSeq 2000 device, and Fastq files were obtained using the Illumina CASAVA v1.8.1 software. The raw data were analyzed using our bioinformatic pipeline hosted on the Vital-IT Center of the Swiss Institute of Bioinformatics (SIB; http://www.vital-it.ch) as described previously [Callier et al., 2014]. Whole exome sequencing (WES) data were analyzed using the VariantMaster software [Santoni et al., 2014] in order to identify de novo variants as well as variants with different Mendelian inheritance models (dominant with reduced penetrance, recessive, and X-linked). The mutation identified in the STARD8 gene was validated by Sanger sequencing in the patients and compared to their parents and their healthy brother.

Histochemistry

Histological characteristics were examined in paraffin-embedded sections of gonadal tissue specimens. The general structure of the gonadal tissues was assessed by standard hematoxylin-eosin staining.

Accession Numbers

Accession numbers of Homo sapiens STARD8 used are NM_014725 and NP_055540.

Results

Identification of the p.Ser913Asn STARD8 Mutation

While searching for the genetic background of 46,XY DSD in our patients, no mutations were identified in the SRY gene (not shown). Therefore, we performed WES to identify some single nucleotide variants (SNVs) underlying the disease, as well as array comparative genomic hybridization (aCGH) to detect potential causative copy number variations. aCGH revealed only several copy
Among the 22,645 and the 22,675 SNVs identified by WES in patient 1 and patient 2, respectively (online suppl. Table 1; see www.karger.com/doi/10.1159/000489692 for all online suppl. material), we focused on the p.Ser913Asn substitution caused by a c.2738G>A transition in the \textit{STARD8} gene on the X chromosome, which was detected in both patients. Importantly, this mutation was also present in the heterozygous mother but absent in the father as well as in the healthy brother (Fig. 1). Moreover, this mutation was located within the STAR functional domain. Notably, the position of the p.Ser913Asn substitution is conserved in vertebrates (online suppl. Fig. 1). No mutations in other genes supposed to play a role in DSD were identified (online suppl. Table 2).

\textbf{Histological Phenotypes of Gonads of the Patients}

Since the \textit{STARD8} gene belongs to the same family as the \textit{STAR} gene, mutations of which were previously described as causing 46,XY DSD, we sought to perform histopathological analyses of our patients to compare them with histological phenotypes of the previously reported patients carrying \textit{STAR} gene mutations [Khoury et al., 2016].

A histological analysis of gonads of patient 1 revealed dysgenetic gonads composed of ovarian-like connective

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{histological_analysis.png}
  \caption{Histological analysis of the patients' gonads stained with hematoxylin-eosin. \textbf{a} Numerous clarified Leydig-like cells (LI) reminiscent of cells overloaded with cholesterol droplets are visible below the ovarian-like stroma (Os) in patient 1. White rectangle encompasses Leydig-like cells. Scale bar, 20 μm. \textbf{b} Magnified image of clarified Leydig-like cells from \textbf{a}. Scale bar, 10 μm. \textbf{c} Seminiferous tubules containing immature Sertoli cells (SC), gonocytes (G), spermatogonia (Sg), while fibroblast-like cells (Fl) are present in the intertubular space in patient 2. Scale bar, 20 μm. \textbf{d} Fibroblast-like cells (Fl) and single seminiferous tubules containing immature Sertoli cells (SC) and gonocytes (G). Scale bar, 20 μm.}
  \end{figure}
tissue, but lacking the ovary or testis structures. Below the gonadal cortex, large aggregations of Leydig-like cells were present, and a significant number of them had a clarified cytoplasm characteristic of cholesterol accumulation (Fig. 2a, b). Finally, 46,XY complete gonadal dysgenesis was diagnosed in this patient.

A histological analysis of the gonads of patient 2 revealed that the right gonad was a streak of connective tissue, lacking testicular or ovarian structures but containing numerous fibroblast-like cells. The left gonad instead had a partly testicular structure. There were 2 types of adjacent tissues: (a) testicular tissue similar to the normal equivalent (considering the age of the patient), composed of tubules with slightly larger diameter (95–125 μm, mean 113.7 μm) and slightly thicker tubular membranes (3.1–5.6 μm, mean 3.7 μm), containing immature Sertoli cells, fetal germ cells (gonocytes), single spermatogonia, and intertubular space containing fibroblast-like cells (Fig. 2c), and (b) an abnormal testicular tissue composed of fibroblast-like cells and rare seminiferous tubules of smaller diameter (43.1–92.3 μm, mean 76.5 μm), containing immature Sertoli cells and single gonocytes (Fig. 2d). Finally, 46,XY asymmetric gonadal dysgenesis was diagnosed in this patient.

Discussion

In this work, we describe the p.Ser913Asn STARD8 gene mutation in 2 sisters manifesting with 46,XY gonadal dysgenesis. The role of STARD8 is not established yet, since no mutations of this gene related to any pathological phenotype have been described so far. STARD8 belongs to the START domain protein family, which contains a conserved lipid-binding START domain responsible for proper lipid distribution in cells [Clark, 2012]. Importantly, the p.Ser913Asn amino acid substitution in STARD8 is located within this domain and, notably, at a position that is conserved among vertebrates (online suppl. Fig. 1). This may suggest a causative effect of this serine amino acid substitution for the STARD8 dysfunction. Moreover, the p.Ser913Asn STARD8 substitution is a very rare variant (rs201005000) in populations worldwide, the highest minor allele frequency being <0.01 [McLaren et al., 2016].

The presence of dysgenetic gonad(s) in both 46,XY patients carrying the STARD8 mutation strongly suggests a role of this gene in testicular determination. The male sex-specific Star8 expression in developing murine gonads during the time critical for sex determination in mice is an argument supporting STARD8 function in gonadal sex determination [Nef et al., 2005; Jameson et al., 2012].

The STARD8 mutation occurred within the START domain. Interestingly, mutations within the START domain of the STAR protein (which is another member of the START family) cause 46,XY DSD, coexisting with lipid congenital adrenal hyperplasia [Lin et al., 1995; Bose et al., 1996; Bhangoo et al., 2005]. This phenotype is due to a deficient steroidogenesis in gonads and adrenal glands, as STAR controls a crucial step for steroid biosynthesis, namely the transfer of cholesterol from the outer to the inner mitochondrial membrane. The blockage of cholesterol transport due to STAR gene mutations causes cholesterol accumulation in the cytoplasm in the form of droplets [Sugawara et al., 1995; Bose et al., 1996]. This cholesterol overloading of Leydig cells is manifested by clarified cytoplasm [Khoury et al., 2016]. It is noteworthy that we observed such Leydig cells with clarified cytoplasm in patient 1 (Fig. 2a, b). This abnormal phenotype of Leydig cells, suggesting testosterone synthesis blockage, is in line with the low testosterone level in patient 1, which was measured before gonadectomy. However, in the gonads of patient 2 we did not observe Leydig cells overloaded with cholesterol, probably because of her young age (8 months) when gonadectomy was performed, as gonads are not hormonally active in this period of life. Nevertheless, coexistence of 3 features—the STARD8 mutation, the Leydig cell phenotype characteristic for testosterone synthesis blockage in patient 1, and the low testosterone levels in patient 1 (in pubertal age) and patient 2 (in infancy, as measured by the hCG test) — may suggest a causative nature of the p.Ser913Asn STARD8 mutation described here as well as the role of STARD8 in gonadal steroidogenesis, possibly synergistic to STAR. In addition, its similar cytoplasmic/membranous location within steroidogenic Leydig cells of adult males supports a synergistic role of those 2 proteins in testosterone biosynthesis [Uhlen et al., 2015]. Interestingly, in contrast to patients carrying STAR mutations, our patients had no features of adrenal failure. Since our report is the first one describing a STARD8 mutation in 46,XY DSD patients, we cannot exclude that there are other types of STARD8 mutations associated also with adrenal insufficiency. Such only partially overlapping phenotypes could exist, like in patients carrying mutations of another sex determination gene, NR5A1. In some patients, NR5A1 gene mutations caused 46,XY DSD with adrenal failure, while in other 46,XY DSD patients, various anomalies of testis development were identified, but with no evidence of ad-
renal failure. The latter group includes a majority of cases carrying NR5A1 mutations [Domenice et al., 2016].

Altogether, the coexistence of the STARD8 p.Ser913Asn hemizygous mutation in association with 46,XY DSD in 2 patients, the location of the mutation at a conserved position within the functional START domain, gonadal dysgenesis in both patients, and sex-specific expression of Stard8 in differentiating gonads of mice suggest that the STARD8 gene may play a role in male determination of primary gonads. On the other hand, the presence of large numbers of Leydig cells or Leydig cell precursors in combination with low testosterone levels and the pathological phenotype of Leydig cells indicating cholesterol accumulation suggest a role of STARD8 in testosterone synthesis. Identification of other 46,XY DSD patients with STARD8 mutations is necessary to confirm the significance of this gene in sexual development.

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Statement of Ethics

All clinical investigations were performed according to the Declaration of Helsinki principles. The study was approved by the Bioethics Committee at Poznan University of Medical Sciences (authorization number 817/13) and the Genova Ethical Committee (CCER, authorization number 14-121).

Disclosure Statement

The authors have no conflicts of interest to declare.

References


