CLINICAL STUDY

Partial deletion of DMRT1 causes 46,XY ovotesticular disorder of sexual development

Susanne Ledig, Olaf Hiort, Lutz Wünsch and Peter Wieacker

Institute of Human Genetics, Westfälische Wilhelms Universität Münster, Vesaliusweg 12-14, D-48149 Münster, Germany, 1Department of Pediatric and Adolescent Medicine, University of Lübeck, 23562 Lübeck, Germany and 2Department of Pediatric Surgery, University of Lübeck, 23538 Lübeck, Germany

(Correspondence should be addressed to P Wieacker; Email: wieacker@uni-muenster.de)

Abstract

Objective: Ovotesticular disorder of sexual development (DSD) is an unusual form of DSD, characterized by the coexistence of testicular and ovarian tissue in the same individual. In a subset of patients, ovotesticular DSD is caused by 46,XX/46,XY chimerism or mosaicism. To date, only a few monogenetic causes are known to be associated with XX and XY ovotesticular DSD.

Design and methods: Clinical, hormonal, and histopathological data, and results of high-resolution array-comparative genomic hybridization (CGH) were obtained from a female patient with 46,XY ovotesticular DSD with testicular tissue on one side and an ovary harboring germ cells on the other. Results obtained by array-CGH were confirmed by RT-quantitative PCR.

Results: We detected a deletion of ~35 kb affecting exons 3 and 4 of the DMRT1 gene in a female patient with 46,XY ovotesticular DSD. To the best of our knowledge, this is the smallest deletion affecting DMRT1 presented to this point in time.

Conclusions: We suggest that haploinsufficiency of DMRT1 is sufficient for both XY gonadal dysgenesis and XY ovotesticular DSD. Furthermore, array-CGH is a very useful tool in the molecular diagnosis of DSD.

European Journal of Endocrinology 167 119–124

Introduction

Ovotesticular disorder is a rare cause of disorder of sexual development (DSD) and is characterized by the coexistence of both differentiated ovarian and testicular tissues in one or both gonads. Lateral, unilateral, and bilateral forms of ovotesticular DSD can be delineated. In the lateral form (30%), a testis is present on one side and an ovary or ovotestis can be detected on the other side. In the unilateral form (50%), a testis or ovary is present on one side whereas the other side shows a testis or ovary or an ovotestis. In bilateral ovotestis, ovarian and testicular tissues are present on both sides. Interestingly, gonads with testicular tissue are more frequent on the right-hand side, while pure ovarian tissue is more common on the left-hand side (1).

The cytogenetic classification delineates ovotesticular DSD with a 46,XX/46,XY or various combinations (46,XX/47,XXX, 45X/46,XY) in 33% of the patients, a 46,XX (60%) or a 46,XY karyotype (7%).

While the presence of chimerism or mosaicism in XX/XY chimera (2, 3) easily explains the coexistence of both gonads, the origin of an ovotestis is more difficult to explain in patients with a normal karyotype. Molecular analyses of peripheral lymphocytes have revealed that in ~10% of the cases with 46,XX ovotesticular DSD, a translocated SRY is cause for the phenotype (4). Furthermore, low-level hidden mosaicism for Y chromosomal sequences restricted to the gonad has been identified in a subset of patients with 46,XX (5, 6). In a patient with SRY-negative XX ovotesticular DSD, an inverted duplication affecting the long arm of chromosome 22 has been detected (7).

The only known monogenic cause for a syndromic form of SRY-negative XX ovotesticular DSD are mutations of RSPO1 (8). However, mutations of RSPO1 have also been reported in XX males, suggesting that 46,XX ovotesticular DSD and 46,XX testicular DSD are different phenotypic outcomes of the same condition (9).

So far only a few causes of 46,XY ovotesticular DSD have been described, such as mutations of the SRY gene (10, 11, 12). Mutations of this Sertoli cell-expressed gene are normally associated with nonsyndromic XY gonadal dysgenesis. Typically, SOX9 mutations are associated with skeletal malformations and are found in two-thirds of karyotypic males with partial or complete XY gonadal dysgenesis. But the phenotypic outcome of SOX9 mutations seems to be variable, as in a family with three siblings affected by campomelic dysplasia, the same heterozygous SOX9 mutation
caused in one sibling 46,XY ovotesticular phenotype, while the other two siblings had bilateral ovaries with normal female genitalia in the presence of a XY and a XX karyotype respectively (13).

Deletions of the terminal part of chromosome 9p are associated with monosomy 9p syndrome characterized by mental retardation, delayed motor development, trigonocephaly, and other dysmorphic features (14, 15), and in patients with a XY karyotype by a high frequency of partial to complete XY gonadal dysgenesis (16, 17, 18). The region associated with the sex reversal has been narrowed down to the region 9p24.3 including the three DMRT genes (DMRT1–3). The DMRT genes belong to a gene family with a unique zinc finger DNA-binding domain called DM. Among them, DMRT1 is the strongest candidate for XY gonadal dysgenesis as it is expressed in the human embryonic genital ridges of both sexes. Furthermore, it is related to sex-determining genes in nematodes and insects (19). Mutational analysis in all three genes resulted in only two potential pathogenic mutations of DMRT1 (20, 21). Recently, we showed that deletions of only DMRT1 can cause XY gonadal dysgenesis (22).

Materials and methods

Case report

Informed consent was obtained and the study was approved by the regional ethics committee. As the first child of healthy, nonconsanguineous parents, the patient initially presented with ambiguous genitalia at birth and was assigned as male and reassigned as female within 24 h. Her birth weight was 3.050 g, length 51 cm, and the initial investigation was normal apart from the genital appearance. The karyotype was found to be 46,XY. At the age of 12 weeks, she was presented to our institutions (O H; L W). At this time, LH was not detected. At this time, LH was 0.98 U/l, FSH 3.56 U/l, and both testosterone (0.05 μg/l) and estradiol (<5 ng/l) were very low. On laparoscopic evaluation, a biopsy of the left gonad was not biopsied but was seen by both ultrasound and gross examination as typical testicular tissue. Laparoscopy further revealed a hemiuterus with a Fallopian tube on the left side. On the right side, a ductus deferens was present.

Methods

Array-comparative genomic hybridization For array-comparative genomic hybridization (CGH) analysis, genomic DNA was extracted from peripheral blood of the patient by a standard method. The patient’s DNA was analyzed using the commercially available Human Genome CGH Microarray Kit 105K (Agilent Technologies, Santa Clara, CA, USA) comprising 99,000 60-mer oligonucleotide probes with a median probe spacing of 21.7 kb. Labeling and hybridization of patient’s DNA and ten pooled male control DNAs was performed according to the manufacturer’s protocol (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, version 4.0; June 2006; Agilent Technologies). Briefly, 1 μg of patient’s DNA and the pooled control DNAs were double-digested with Alul and Rsal (Promega) and subsequently differentially labeled with Cy5-dUTP and Cy3-dUTP respectively, using the Genomic DNA Enzymatic Labeling kit (Agilent Technologies). After purification of the labeled DNAs by filtration (Microcon YM-30; Millipore, Billerica, MA, USA), the patient’s and the control DNAs were pooled and hybridized with 25 μg of human Cot DNA (ArrayGrade KREA Cot DNA; Kreatech, Amsterdam, The Netherlands) for 40 h at 65 °C in the hybridization oven (Agilent Technologies). After post-hybridization washes, the array was scanned using a Microarray Scanner (G2565BA; Agilent Technologies), and the spot intensities were measured by Feature Extraction Software (version 9.5.31; Agilent Technologies). Analyses and visualization were performed with CGH Analytics (version 3.5.14 by using the following parameters: aberration algorithm ADM-1, threshold 6.0, fuzzy zero, centralization, moving average window 1 Mb, three-point aberration filter; Agilent Technologies). Aberrant signals including three or more adjacent probes were considered as genomic aberrations and were further evaluated by real-time quantitative PCR (RT-qPCR), if they were not listed as a frequent variant in the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/). Coordinates of copy number variations (CNVs) are based on the National Center for Biotechnology Information (NCBI) Human Genome Build 36.

RT-qPCR The microdeletion in chromosomal region 9p24.3 and the microduplication affecting chromosomal regions 11q24.2 and 19p13.3 detected by array-CGH in the patient were checked by RT-qPCR with SYBR Green detection (SensiMix Plus SYBR Kit; Quantace, London, UK) using two nonpolymorphic pairs of primers evenly distributed within the affected region. RT-qPCR primers were designed using Primer3 Software online (http://frodo.wi.mit.edu/primer3/) with the following criteria: amplicon size 60–120 bp, GC content of 20–80%, and melting temperature of 59–61 °C. The primers were checked with MFOLD (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=mfold) and SNPCheck.
Real-time detection was performed using the LightCycler 480 (Roche). Absolute quantification of target amplicons in the patient’s DNA was performed by interpolation of the crossing point against the corresponding standard curve obtained by amplification of a male control DNA pool. We used 10 ng of genomic DNA from the patient as well as from the pooled male control DNAs. In this manner, values of 10 ng indicate a diploid situation, while values of 5 or 15 ng indicate a deletion or duplication respectively. The RT-qPCRs were performed in triplicate for each reaction.

Results

We identified a total of 13 CNVs by array-CGH. Of these 13 rearrangements, ten have been excluded as they are frequently listed in the DGV. The residual three non-polymorphic CNVs, the deletion in 9p24.3 (see Fig. 1), and the duplications in 11q24.2 and 19p13.3 respectively, could be confirmed by RT-qPCR (arr cgh 9p24.3(883300-918342)x1,11q24.2(125609874-125895030)x3,19p13.3(2800185-2842101)x3) (see Fig. 2).

The deletion in chromosomal region 9p24.3 affects ~35 kb and causes a partial deletion of exons 3 and 4 of the DMRT1 gene (see Fig. 1). To the best of our knowledge, this is the smallest deletion affecting the DMRT1 gene, which has been reported in a patient with DSD.

Discussion

We describe here the unusual case of a partial deletion of DMRT1 in a patient with XY ovotesticular DSD. Haploinsufficiency of DMRT1 is typically associated with partial or complete XY-gonadal dysgenesis but not with ovotesticular DSD. Furthermore, the patient carries two additional uncharacterized duplications. The pathogenic relevance of duplications is less likely than that of deletions. The duplication in 11q24.2 affects the following genes: FAM118B, SRPR, FOXRED1, TIRAP, DCPS, FLJ39051, ST3GAL4, and KIRREL3. Only two of these genes are known to be associated with diseases: loss-of-function mutations of FOXRED1 are associated with infantile-onset mitochondrial encephalopathy (MIM#252010) and Leigh syndrome (MIM#256000), while heterozygous missense mutations of KIRREL3 cause mental retardation (MIM#612581). None of these syndromes are correlated with impaired gonadal development. To the best of our knowledge, for the other genes located in the duplication interval, no connection with gonadogenesis is known.

The duplication affecting chromosomal region 19p13.3 encompasses the two zinc-finger protein encoding genes ZNF555 and ZNF556. The function of the corresponding proteins is still unknown. Because of all these findings, it is very unlikely that the duplications

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Figure 1 Deletion of ~35 kb affecting exons 3 and 4 of DMRT1 gene in a patient with 46,XY ovotesticular DSD. The left part of the graph shows whole chromosome 9, and the right part of the graph illustrates genetic view in detail. The dots demonstrate the 60-mer oligonucleotide probes of the array. Deletions show values of −1, while unaffected oligonucleotides are localized on the base line. In case of gains, the dots have values of +0.5. But only signals including three or more adjacent probes were considered as genomic aberrations.

Figure 2 RT-qPCR results for the three aberrations identified in the patient. Results of RT-qPCR are represented by histograms with error bars. In the data lettering, first the kind of imbalance and second the chromosomal location of the aberration is mentioned. Absolute quantification of target amplicons in the patient’s DNA was performed by interpolation of the crossing point (Cp) against the corresponding standard curve obtained by amplification of a male control DNA pool. We used 10 ng of genomic DNA from the patient as well as from the pooled male control DNA. In this manner, values of 10 ng would indicate a diploid situation, while values of 5 or 15 ng indicate deletion or duplication respectively.
The authors suggest that mouse Dmrt1 expression in the testis characterized by reprogramming of Dmrt1 in mouse Sertoli cells induces postnatal feminization of multiple female-promoting genes as a result of male sex determination in the adult by repressing similar but different control mechanisms in humans.

In spermatogonia, Dmrt1 regulates genes required for Sertoli cell differentiation, cell cycle control, tight junction dynamics, germ cell differentiation, and pluripotency in vivo. In fact, Dmrt1-null mutant mice are born as males, but they show defects in early postnatal testis differentiation like failure of radial gonia migration, gonocyte proliferation, meiotic initiation, and abnormal Sertoli cell differentiation and proliferation. Recently, Matson et al. showed that fetal loss of Dmrt1 in mouse Sertoli cells induces postnatal feminization of the testis characterized by reprogramming of differentiated Sertoli cells into apparent granulosa cells. The authors suggest that mouse Dmrt1 maintains the male sex determination in the adult by repressing multiple female-promoting genes as Foxl2 on the one hand and by inducing male-promoting factors on the other hand. In contrast, in humans, gross deletions of DMRT1 as a result of 9p deletion syndrome are associated with congenital feminization, suggesting a similar but different control mechanism in humans.

In spermatogonia, Dmrt1 blocks the precocious entry into meiosis by directly and indirectly blocking retinoic acid-signaling pathways essential for meiosis entry. A conditional knockout of Dmrt1 in undifferentiated spermatogonia leads to uncontrolled premature meiosis and subsequently to a depletion of differentiating spermatogonia and germ cells. On the other hand, Dmrt1 promotes directly the expression of Sohlh1, a factor essential for spermatogonial differentiation. Although Dmrt1 is expressed in female germ cells during transition from mitosis to meiosis, Dmrt1-null females are fertile.

Furthermore, DMRT1 acts as a dosage-sensitive tumor suppressor by directly regulating transcription of the pluripotency regulator Sox2; therefore, loss of function can result in testicular teratoma independent of the genetic background.

Further insights come from some findings in moles. The gonads of female moles of the family of Talpidae are unique among mammals as they develop ovotesticular tissues instead of ovaries. These are composed of only a small portion of normal ovarian functional tissue and of a gross portion of dysgenetic testicular tissue, which lacks germ cells from a certain developmental stage. In contrast to most of the other eutherian animals, female germ cells from the mole enter meiosis postnatally. It is thought that the late initiation of meiosis of female germ cells in the mole is causative for the development of an ovotestis, as meiotic but not premeiotic female germ cells antagonize testis organogenesis during early gonadal development in the mouse. Interestingly, expression of DMRT1 coincides with premeiotic stages of female germ cells in the mole and downregulation occurs just before the onset of meiosis. While the ovarian portion of ovotestis usually appears normal with follicular growth, the testicular section shows degeneration with poor germ cell development.

In conclusion, we suggest that haploinsufficiency of DMRT1 is sufficient for both XY gonadal dysgenesis and XY ovotesticular DSD. We hypothesize that ovotestis formation is caused by disturbed action of DMRT1 in germ cells as well as in Sertoli cells. Loss of DMRT1 in germ cells may cause precocious entry of germ cells into meiosis and, in Sertoli cells, female reprogramming of the testis.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived a prejudicing the impartiality of the research reported.

**Funding**

This work was supported by EuroDSD (in the European Community’s Seventh Framework Programme FP7/2007-2013 under grant agreement no. 201444).

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R-spondin1 is a sporadic case.

A novel germ line mutation with 9p deletions in the absence of monosomy-9p syndrome.

DMRT1 deletion causes XY ovotesticular DSD


Received 14 February 2012
Revised version received 23 April 2012
Accepted 9 May 2012