A Novel AMH Missense Mutation in a Patient with Persistent Müllerian Duct Syndrome

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Key Words
Anti-Müllerian hormone · Missense mutation · Persistent Müllerian duct syndrome

Abstract
Persistent Müllerian duct syndrome (PMDS) is characterized by the presence of a uterus, fallopian tubes, and the upper part of the vagina in phenotypic normal male patients. Here, we report a patient diagnosed with PMDS with a novel homozygous missense mutation in the anti-Müllerian hormone (AMH) gene (single nucleotide insertion (C) at position 208 (c.208dup, p.Leu70fs)) leading to a frameshift and the introduction of a premature stop codon. Biopsy of both gonads revealed that germ cells were present in an irregular distribution. However, the absence of OCT3/4, PLAP and c-KIT expression indicated physiological maturation.

Sexually dimorphic development of the reproductive system is the result of 3 sequential processes: initial or chromosomal sex determination, differentiation of the bipotential gonad into either testis or ovary, and finally sex-specific development of the reproductive tracts and external genitalia under influence of hormones produced by the gonads [Wilhelm et al., 2007; Franco and Yao, 2012]. Testosterone produced by testicular Leydig cells will induce the differentiation of the male reproductive tract, i.e. the Wolffian ducts, into epididymis, vas deferens, and seminal vesicles. Anti-Müllerian hormone (AMH), produced by the testicular Sertoli cells, is responsible for the regression of the Müllerian ducts. In the absence of AMH the Müllerian ducts will develop into normal female internal organs [Behringer et al., 1994]. Persistent Müllerian duct syndrome (PMDS) is characterized by the presence of a uterus, fallopian tubes and the upper part of the vagina in phenotypic normal male patients, and is usually discovered at surgery for cryptorchidism or inguinal hernias. AMH, a member of the transforming growth factor β (TGFβ) family, signals through a heterodimeric receptor complex consisting of a specific type II receptor (AMHRII) and shared type I receptors (ALK2, 3, and 6) [Visser, 2003; Orvis et al., 2008]. In approximately 85% of the cases mutations within the AMH and AMHR2 genes are responsible for this error in sex differentiation [Josso et al., 2005]. Mutations in the 3 type I receptor genes have not been detected [Josso et al., 2005], and thus for the remaining 15% of the cases the causative genes remain to be identified. Patients with mutations in
AMH or AMHR2 present with a similar phenotype. However, when assessed before puberty, levels of circulating AMH are usually extremely low or undetectable in patients with AMH mutations, whereas normal levels are observed in patients carrying AMHR2 mutations [Josso et al., 2005]. We report here a novel missense mutation within AMH due to a single base pair insertion leading to a premature stop codon. The mutation was homozygously present in the patient due to inheritance from both parents.

Materials and Methods

Case Report

A phenotypically normal male presented at birth with bilateral nonpalpable testes. There was no parental consanguinity. Ultrasonography showed that the testes were high scrotal and inguinal, respectively. Therefore, the policy was to wait and see if descent would occur spontaneously. At the age of 2, ultrasound showed that both testes (0.5 cc in volume) were abdominally located. Karyotyping of peripheral blood lymphocytes showed a normal 46,XY karyotype.

Laparoscopic exploration at the age of 2 revealed a uterus and tubae next to the testes and epididymides at the level of the internal abdominal ring. A biopsy was taken from both gonads and epididymides. The Müllerian structures were left in situ.

Histology and Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed paraffin-embedded slides of 3 μm thickness. The antibodies directed against VASA, TSPY, OCT3/4, c-KIT and PLAP have been described before [Hersmus et al., 2008; Stoop et al., 2008]. Briefly, after deparaffinization and 5 min incubation in 3% H2O2 to inactivate endogenous peroxidase activity, sections were subjected to heat-induced antigen retrieval under pressure of up to 0.9 bar in the appropriate buffer. After blocking endogenous biotin using the Avidin/Biotin Blocking Kit (SP-2001, Vector Laboratories, Burlingame, Calif., USA), sections were incubated either overnight at 4°C (VASA, TSPY, PLAP, c-KIT) or for 2 h at room temperature (OCT3/4). Next, sections were incubated with the appropriate biotinylated secondary antibodies, followed by avidin-biotin complex. Peroxidase activity was developed with 3,3'-diaminobenzidine (DAB) and alkaline phosphatase activity was developed with New Fuchsine (Vector Laboratories).

Hormonal Follow-Up

Serum determinations of inhibin B, AMH, testosterone, LH and FSH were performed in the endocrine laboratory of Erasmus MC (Rotterdam) as described previously [Pierik et al., 2009]. Serum FSH and LH were determined with the Immulite assay (Diagnostic Products Corporation, Los Angeles, Calif., USA). Serum testosterone was determined using a Coat-a-Count radioimmunoassay purchased from Siemens (Los Angeles, Calif., USA). Inhibin B was measured by ELISA purchased from Serotec Ltd (Oxford, UK). An in-house AMH ELISA with a detection limit of 6.3 pg/ml (commercially available through Beckman-Coulter, Marseille, France) was used to measure AMH [Kevenaar et al., 2006].

Molecular Analysis

DNA from the patient and his parents was isolated from peripheral blood leukocytes using Magnetic Separation Module 1 from Chemagen (Baesweiler, Germany), after informed consent from both patient and his parents was received. All 5 exons of the AMH gene, including flanking sequences, were amplified by PCR using specific primers (table 1). Direct sequencing was performed on an ABI prism 3100 automatic capillary sequencer (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). In the laboratory of Clinical Genetics the mutation was confirmed, and parental DNA was analyzed for the presence of the pathogenic variant by direct sequence analysis using an independent primer pair (table 1) (ABI 3730XL automated sequencer). The AMH sequence with accession number NG_012190.1 was used as a reference sequence. For nucleotide numbering we followed the recombinant 46,XY karyotype.

Table 1. PCR primers for the amplification of the AMH gene (including intron-exon boundaries)

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward 5′→3′ (location)</th>
<th>Reverse 5′→3′ (location)</th>
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<tbody>
<tr>
<td>1</td>
<td>CACTGTCCCCCCAAAGGTCGAG (−112)</td>
<td>CTTGCTCATAGGGCGTTAGAG (224)</td>
</tr>
<tr>
<td>2</td>
<td>TTGGAGGAAAGGGAGCCGTTAG (105)</td>
<td>CGCAACCGATCTAGGAAACA (530)</td>
</tr>
<tr>
<td>1*</td>
<td>GTCCAGGCGGCAGGAG (−97)</td>
<td>CTTGAGCCCGCTGATCTT (480)</td>
</tr>
<tr>
<td>2</td>
<td>CCTCAATGGTCAGGCCTCCC (966)</td>
<td>CAGGGTCGATGACGGCTTACC (1278)</td>
</tr>
<tr>
<td>3</td>
<td>TGGGAGGAAGGGAGCCGTTAG (1247)</td>
<td>GAAGAACGTGTTGACTAGGC (1560)</td>
</tr>
<tr>
<td>4</td>
<td>CCTCAGCCCCCGCGAGAG (1409)</td>
<td>GGCCTGGAGGGATCGCC (1749)</td>
</tr>
<tr>
<td>5</td>
<td>CAGGCAACCCGAGACAG (1684)</td>
<td>CTTGAGCCGAGGTGACAG (1919)</td>
</tr>
<tr>
<td>2</td>
<td>CCCCTTCCGAGGCGTCCAGG (1807)</td>
<td>CAGGGTCGAGGGATCGCC (2199)</td>
</tr>
<tr>
<td>5</td>
<td>GCTGCGAAGCCTCCCCGAT (2109)</td>
<td>CAGCGACACGTGTGACAG (2452)</td>
</tr>
<tr>
<td>5</td>
<td>CAACAATTGCCAGGCGTGTG (2397)</td>
<td>GTCCCCAGGCCCCCTAA (2759)</td>
</tr>
</tbody>
</table>

* Primers used for confirmation of the mutation.
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Results

At the age of 2 the patient showed a normal basal testosterone (0.10 nmol/l) and inhibin B (91 ng/l) production. However, AMH levels were extremely low (0.2 μg/l). Measurements at the age of 10 years showed normal basal prepubertal levels of LH (0.2 U/l), FSH (1.7 U/l), and testosterone (0.10 nmol/l). Inhibin B levels were also in the normal prepubertal range (149 ng/l), whereas AMH levels remained extremely low (0.2 μg/l).

Since AMH levels were extremely low, a mutation in the AMH gene was suspected. Direct sequencing revealed the presence of a novel homozygous single base pair insertion at position 208 in exon 1 (c.208dup, p.Leu70fs) (fig. 1). The insertion of an additional C leads to a frameshift and the introduction of a premature stop codon. Both parents were heterozygous for this mutation (fig. 1). Histological examination of testicular biopsies showed the presence of normal testicular tissue in both gonads (fig. 2A). Germ cells were present but in an irregular pattern, confirmed by immunohistochemistry for VASA (fig. 2B). There were tubules that contained germ cells but also tubules that were empty and therefore showed no VASA-positive staining. Comparison with tissues of normal descended testis of the same age group as the patient showed no difference in number and distribution of spermatogonia. Healthy boys show remarkable variation in the number of spermatogonia until puberty. Immunohistochemistry for the markers OCT3/4, PLAP, and c-KIT was negative, thus there were no indications for the presence of an invasive germ cell tumor or an in situ lesion in the patient.

Discussion

Here, we describe a patient with PMDS with a novel pathogenic mutation in the AMH gene. An insertion of an additional C at base pair position 208 results in a frameshift and consequently in the introduction of a premature stop codon. AMH maps to chromosome 19p13.3 and consists of 5 exons, encoding a glycoprotein of 535 amino acids [Cohen-Haguenauer et al., 1987]. A number of pathogenic mutations spanning the AMH gene have been described. Most of the mutations, including the novel mutation we identified, are located in exon 1 or exon 5 [Josso et al., 2005], although hotspots for mutations have not been observed. Interestingly, at the position where we detected the novel mutation, a previous study has reported a base pair change T>C, leading to a L70P amino acid change [Imbeaud et al., 1994], which suggests that also in the AMH gene certain positions may be more prone to mutations. Furthermore, most of the reported mutations were homozygously present due to parental consanguinity. In our patient, the mutation was
present in a homozygous pattern due to inheritance from both non-consanguineous parents.

Surgical procedures in men with PMDS are still under debate. Odi et al. [2010] claim that surgery should be conducted in 2 separate procedures: (1) testis reposition into the scrotum with hernia repair and testis biopsy; (2) orchiectomy upon indication for atrophic testis or when orchidopexy cannot be performed.

Germ cell tumors, mostly seminomas, have been reported in patients with PMDS, although the incidence is similar to that in non-affected males with cryptorchidism [Berkmen, 1997]. Manassero et al. [2004] describe a patient with PMDS and transverse testicular ectopia with the presence of a mixed germ cell tumor (teratoma and embryonal carcinoma) in a testis in the scrotal position after orchidopexy at the age of 5 years, suggesting that orchidopexy early in life does not decrease the risk of malignancy in this patient group. Therefore, it has been suggested that orchidectomy should be performed [Berkmen, 1997].

Our patient had normal testicular tissue in both biopsies. Germ cells were present but showed an irregular distribution pattern. However, comparison with normal descended testis tissue of the same age group as the patient showed no difference in number and distribution of spermatogonia, due to the remarkable variation in number of spermatogonia in this age group. Bilateral orchidectomy is a definite procedure leading to life-long androgen substitution, induction of puberty and loss of fertility. We suggest that with the markers for germ cell tumors known to date [Cools et al., 2006] a more individually tailored approach is possible. Therefore, evaluation of both testes and of potential fertility problems will be part of clinical follow-up [Berkmen, 1997; Gutte et al., 2008].

Acknowledgements

This work was financially supported by the European Society for Pediatric Endocrinology Research Fellowship (Y.Z.). The authors would like to thank Anke McLuskey for technical support.

References


Fig. 2. Morphological analysis of testicular tissue of the patient at the age of 2. A Representative hematoxylin and eosin staining showing normal testicular tissue. B Germ cells (VASA-positive) are present and show an irregular distribution pattern. Magnification in A 100×, in B 200×.
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Erratum

In the article by Balsamo A, Baldazzi L, Menabò S, Cicognani A: Impact of Molecular Genetics on Congenital Adrenal Hyperplasia Management. Sex Dev 2010;4:233–248 (DOI: 10.1159/000315959), the ‘Acknowledgement’ statement (see below) was missing:

Acknowledgement

The present paper was partially supported by PRIN No. 20083ENLWJ.

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Sex Dev 2012;6:279–283

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