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Patient report

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Two heterozygous mutations of the *AMH* gene in a Japanese patient with persistent Müllerian duct syndrome

Abstract: Persistent Müllerian duct syndrome (PMDS) is an autosomal recessive disorder of sex development (DSD) characterized by the presence of Müllerian duct derivatives in 46, XY phenotypic males. To date, more than 50 different mutations of the anti-Müllerian hormone gene (*AMH*) have been reported. Here, we report two novel mutations of *AMH* in a Japanese patient with PMDS. A 1-year-old male presented with bilateral cryptorchidism and normal male external genitalia. A laparoscopic surgery revealed a uterus and fallopian tubes. Serum AMH was very low. The patient's elder brother was also diagnosed as having PMDS at another hospital. Genetic analysis of *AMH* showed two novel mutations of p.N486T and p.V527L. Given that these two amino acids are well conserved among different species of AMH, the substitution of two amino acids might affect the normal function of AMH. In conclusion, PMDS should be included in differential diagnoses of cryptorchidism.

Keywords: *AMH*; cryptorchidism; mutations; persistent Müllerian duct syndrome (PMDS).

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Introduction

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance (MIS) or Müllerian inhibiting factor (MIF), is a member of the transforming growth factor- β

(TGF- β) superfamily. At 8 weeks of gestation in a male fetus, testicular Sertoli cells begin to produce AMH, which leads to the regression of the Müllerian ducts. In the absence of AMH, the Müllerian ducts differentiate into the fallopian tubes, uterus, and upper vagina. AMH signals through two transmembrane receptors, namely, type I and type II (1, 2). AMH binds specifically to AMH receptor type II (AMHR-II), which then forms a complex with type I, leading to its phosphorylation and activation. AMHR-II is expressed in the Müllerian ducts, the gonads of both sexes, the endometrium, prostate, and in the mammary glands (2–6).

Persistent Müllerian duct syndrome (PMDS) is an autosomal recessive disorder of sex development (DSD) characterized by the presence of Müllerian duct derivatives in 46, XY phenotypic males. This condition is mostly due to mutations of the gene encoding AMH or the AMHR-II. In total, 82 families with PMDS have been identified, and of these, 46.3% (38 cases) can be attributed to mutations in *AMH* and 40.2% (33 cases) to mutations in the AMHR-II gene (*AMHR-II*) (2). To date, more than 50 mutations in AMH have been reported as the causes of PMDS (7).

Methods

Stimulation test

Serum level of total testosterone was determined in response to three intramuscular injections of hCG (3000 IU/m²/dose) given at intervals of 24 h.

Sequence analysis of the *AMH* gene

The Institutional Review Board Committee approved this study. The patient's parents provided written informed consent. Genomic DNA was extracted from peripheral blood leukocytes. *AMH* exon was amplified by polymerase chain reaction (PCR) using five pairs of primers as reported previously (8), and PCR products were purified and sequenced directly using an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

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Case report

A 1-year-old Japanese boy was admitted to our hospital for orchiopexy to treat bilateral cryptorchidism. He was born at 39 weeks of gestational age by normal vaginal delivery and weighed 3122 g with no complications during pregnancy. At birth, his physical examination showed a normal phallus, a good scrotal fold on both sides, and normal penile length. Although the left testis was palpable in the left inguinal region, the right testis could not be identified by physical examination. Karyotyping of peripheral blood lymphocytes showed a normal 46, XY karyotype.

When laparoscopy was performed at the age of 1 year, the left testis was found at the internal left inguinal ring, and the right testis was found in the abdominal cavity. During the procedure, a uterus and an epididymis were found. Both testes were connected to the uterus and no spermatic duct was detected. Thereafter, endocrine evaluation of the patient was performed. Serum AMH level was measured at 62.2 pmol/L (Gen II by Beckman Coulter, Brea, CA, USA) and 46.0 pmol/L (IOT by Beckman Coulter, Brea, CA, USA) (9). The normal serum AMH range (95% CI) at 1 year is 684–2329 pmol/L (IOT) (10). An hCG stimulation test showed normal response of serum testosterone (Table 1). Based on these clinical and endocrinological findings, the patient was diagnosed with PMDS. There was no consanguineous marriage in his family and he had two elder brothers. The eldest brother had also been diagnosed with PMDS when he had undergone surgery for bilateral cryptorchidism at another hospital. However, detailed medical records were not available.

The patient's extremely low serum AMH level prompted us to investigate the existence of an *AMH* mutation. As a result, sequence analysis of *AMH* revealed the presence of two novel heterozygous missense mutations in exon 5 (Figure 1A). One was an A to C transversion at

position 1457, resulting in a substitution of threonine for asparagine at amino acid position 486 (c.1457A>C, p.N486T). The other was a G to T change at position 1579, resulting in a change of valine to leucine at amino acid position 527 (c.1579G>T, p.V527L) (Figure 1B). These amino acid changes have not been identified in 100 normal Japanese males. Analysis using the software Polyphen2 (11) evaluated these changes as “probably damaging”. The parents of the patient did not want further genetic analysis of themselves or the patient's elder brother.

Discussion

PMDS is usually caused by mutations of the gene encoding AMH or AMHR-II (1, 2). To date, more than 50 mutations in *AMH* have been reported as the cause of PMDS (7). In our patient, the low serum level of AMH suggested that the cause of disease in this patient was probably a genetic defect of AMH. Indeed, we identified two novel missense mutations in this gene (p.N486T and p.V527L). We did not determine the functional consequences of these two mutations in vitro; however, they are likely to be pathogenic for several reasons. First, these two amino acid substitutions were not present in 100 normal Japanese males. Second, N486 and V527 are located in the C-terminal region of AMH in a region which is well conserved among mammalian species (Figure 1C). In addition, several missense mutations (p.L426R, p.V477A, p.C488Y, p.Q496H, p.H506Q, p.C525Y, p.L536F, p.C557S, and p.R560P) in this region of *AMH* have previously been reported in PMDS (7, 12). Due to the fact that the C-terminal region of AMH is critical for its biological activity (1, 2), our mutations are likely to impair normal AMH functions. Third, PolyPhen2, a computational tool for the identification of potentially-functional amino acid changes, identified both amino acid changes as probably damaging with a position-specific independent count (PSIC) of 1.0.

Most reported mutations of *AMH* are homozygous and arise due to consanguineous marriage. Missense mutations are frequent in exons 1 and 5, but no hotspot has been identified. In our familial cases, we could not prove the compound heterozygous in the patient, because we were unable to determine the genotype of the father, mother, or the elder brother.

The persistent Müllerian structures often tightly tether the testis and impede their descent, thus causing unilateral or bilateral undescended testis. In addition, PMDS has been reported in association with transverse testicular ectopia, a rare anomaly in which the testis is seen in the

Table 1 Results of hCG stimulation test and serum AMH.

	Pre-stimulation	Post-stimulation (72 h)
Testosterone, nmol/L	0.07	6.62
Serum AMH, pmol/L	62.2 ^a	ND

^aSerum AMH was measured by Gen II (Beckman Coulter, Brea, CA, USA) and corresponds to 46.0 pmol/L (same IOT as Beckman Coulter), the reference value for testosterone after hCG stimulation test (mean±SE); pre-stimulation 0.624±0.138 nmol/L, post-stimulation (72 h) 5.99±0.936 nmol/L at Tanner stage I, the normal serum AMH range (95% CI) at 1 year is 684–2329 pmol/L (IOT); AMH, anti-Müllerian hormone; ND, not done.

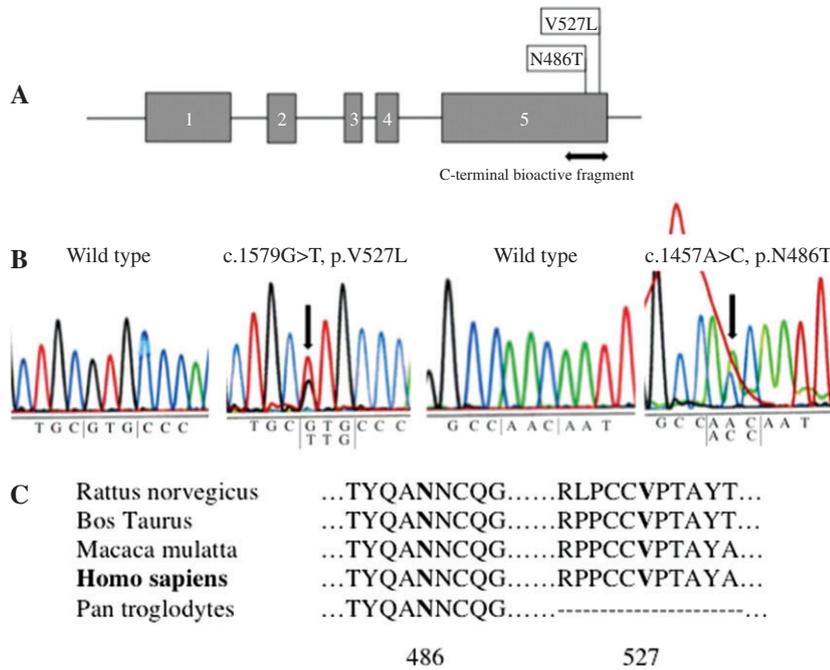


Figure 1 (A) Schematic representation of *AMH* and the location of mutations identified in our study. Exons are numbered 1–5. The C-terminal bioactive region of *AMH* is indicated in two-headed arrow. Two flagged arrows represent sites of p.N486T and p.V527L. (B) Sequencing chromatograms of *AMH* in the patient. One base change of A to C at position 1457 was identified. This change resulted in a substitution of threonine for asparagine at amino acid position 486 (p.N486T). The other change of a G to T at position 1579 results in a change of valine to leucine at amino acid position 527 (p.V527L). Arrows indicate the sites of mutations. (C) Partial amino acid alignment of C-terminal region of *AMH* among different mammalian species. Bold characters indicate mutant residues and corresponding amino acids of the aligned sequence.

contralateral inguinal canal or in the hemiscrotum (1, 2, 13, 14). Among these phenotypes of PMDS, unilateral cryptorchidism is most common, and only about 10% of patient with PMDS have bilateral undescended testes located in an area analogous to the ovaries in women (13, 14). This was the case in our patient. However, the exact reason for such phenotypic differences is not clear. Given that the genotype of *AMH* and *AMHR-II* is not related to the phenotypes (15–18), other environmental or genetic factors might influence the phenotypic manifestations.

In conclusion, we report two novel mutations in *AMH* as a cause of PMDS. PMDS is a rare condition; however, it must be considered in differential diagnosis of cryptorchidism with normal male genitalia.

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