

Case Report

A novel mutation of *AMHR2* in two siblings with persistent Müllerian duct syndrome

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Abstract

Persistent Müllerian Duct syndrome (PMDS) develops due to deficiency of anti-Müllerian hormone (AMH) or insensitivity of target organs to AMH in people with 46, XY karyotype. PMDS is characterized with normal male phenotype of external genital associated with persistence of Müllerian structures. The 2.5 years old male patient presented due to bilateral undescended testis. Karyotype was 46, XY. The amount of increase in testosterone following human chorionic gonadotropin (hCG) stimulation test was normal. The patient was referred to our clinic after uterine, fallopian tube and vaginal remnants were recognized in the orchiopexy surgery. The family reported that the 8 years old elder brother of the patient was operated on for right inguinal hernia and left undescended testis at age 1. Right transverse testicular ectopia was found in the elder brother. For both cases with normal AMH level, *AMHR2* gene was analyzed and a homozygous NM_020547.3:c.233-1G>A mutation was found that was not identified previously. In conclusion, we determined a novel mutation in the *AMHR2* gene that is identified for the first time and leads to different phenotypes in two siblings.

Keywords: Undescended testis, anti-Müllerian hormone, persistent Müllerian duct syndrome.

Introduction

Persistent Müllerian duct syndrome is a rare disorder of 46, XY sex development. The condition is characterized by normal length of penis in association with unilateral or bilateral undescended testis and persistence of Müllerian structures in people with 46, XY karyotype. PMDS develops mostly due to deficiency of AMH or insensitivity of target organs to AMH. Mutations of either the *AMH* or *AMHR2* gene have been detected in 88% of cases (1). PMDS has autosomal recessive inheritance and the incidence is not clearly known. However, it is reported that the published number of cases increases as the cryptorchidism is recently investigated at earlier stage of the life. Laparoscopic examination is introduced to routine clinical use and surgeons are more aware of this condition in comparison to the past (1).

In this study, we present two siblings with homozygous NM_020547.3:c.233-1G>A mutation that is identified for the first time in the *AMHR2* gene; one sibling presented with bilateral undescended testis, while the other presented with transverse testicular ectopia.

Case Report

Case 1

The 2.5 years old male patient presented to our outpatient clinic with bilateral undescended testis. Family history is notable for 3rd degree consanguineous marriage in parents. It is understood that bilateral undescended testis was recognized by the family immediately after the birth, but the family did not visit a doctor. Physical examination; weight: 13.5 kg (SDS score: -0.18), height: 94.0 cm (SDS score: 0.36), stretched length of penis: 4 cm; and bilateral testes could not be palpated. A pelvic ultrasound detected, a formation, suggestive of testis, was present in the proximal segment of bilateral inguinal canal, including one measuring 7x5x7 mm in size at the right side and one measuring 7x5x9 mm in size at the left side. Uterus, Fallopian tubes and ovaries could not be visualized. Laboratory tests; FSH: 1.2 mIU/ml, LH: 0.1 mIU/ml, total testosterone: 0.03 ng/ml, 17-hydroxyprogesterone: 0.48 ng/ml, AMH 35.1 ng/ml (5-265). HCG stimulation test was done, as the patient had

bilateral undescended testis and the hormone levels were at prepubertal level. Testosterone response was normal in the test and the patient was referred to pediatric surgery clinic for orchiopexy. Rudimentary uterine, fallopian tube and vaginal remnants were seen while orchiopexy was carried out by the pediatric surgery clinic; it was also reported that bilateral gonads were resembling testes, and gonads were biopsied and the operation was terminated. The patient was referred to our outpatient clinic again due to presence of Müllerian structures that were identified during the surgery. Biopsy specimens were consistent with bilateral testicular tissue. Persistent Müllerian duct syndrome was considered for the patient in the light of current findings. Since AMH level was normal, *AMHR2* gene mutation was considered. *AMHR2* gene mutation analysis was performed by sequencing of the coding exons and the exon-intron boundaries of the genes. Genomic DNA was isolated from peripheral blood cells with QIAGEN DNA Blood Mini Kit according to protocol provided with the kit. Sequencing was performed with Miseq V2 chemistry on MiSeq instrument (Illumina California, USA). Analysis was performed with IGV software. Homozygous NM_020547.3:c.233-1G>A mutation was found in the *AMHR2* gene in the genetic analysis (figure 1). This mutation was not identified in databases previously. Analyses made with MutationTaster, and splicing modeling softwares (NNSPLICE, GeneSplicer and SpliceSiteFinder) showed that the mutation can cause the disease. According to the ACMG 2015 criteria, this mutation was classified as “Pathogenic” (2).

Case 2

After Case 1 was diagnosed with PMDS, the elder sibling (8 years old) presented due to left undescended testis. From the patient history it was learned that the family recognized a swelling in the right groin when the child was 2 months old and that the child was operated on by pediatric surgeon at age 1. Right inguinal hernia and presence of both testes in the right scrotum were reported after the surgery. Right inguinal hernia was repaired, but Müllerian structures were not mentioned in the operative note. Physical examination; weight: 23.5 (SDS:-0.65) Height: 124 (SDS:-0.54), left testis could not be palpated. However, both testes was palpated in the right scrotum. The stretched length of penis was 5 cm. The scrotal ultrasound pointed to 2 testes in the right scrotal cavity, measuring 15x7 mm and 17x7 mm in size. A formation, consistent with uterus measuring 8x4 mm in size, was found posterior to the urinary bladder. FSH: 0.72 mIU/ml, LH: 0.1 mIU/ml, total testosterone: 0.03 ng/ml AMH: 35.1 ng/ml (3.2-182.4). Since AMH level was normal, *AMHR2* gene mutation was considered and homozygous NM_020547.3:c.233-1G>A mutation was found in the *AMHR2* gene in the genetic analysis. Written informed consent was obtained from the patient's family for publication of this Case report.

Discussion

Anti-Müllerian hormone is synthesized by immature Sertoli cells in men and ovarian granulosa cells in women. It is responsible for total regression of Müllerian structures in the Week 10 of the fetal development in the male fetus. External genitalia are completely normal in men with AMH deficiency. However, it causes persistence of Müllerian structures along with testes and male excretory ducts (1,3). PMDS usually originates from gene mutations in *AMH* or *AMHR2* (1,4). It is recognized during conventional surgery or laparoscopic examination of undescended testis alone or in combination with the inguinal hernia (1). PMDS has three main clinical presentations. 1. Bilateral cryptorchidism. This presentation accounts for approximately 55% of AMH pathway mutations and 86% of idiopathic cases. 2. Unilateral cryptorchidism. A testis and the accompanying fallopian tube and uterus case an inguinal hernia. This presentation is known as “hernia uteri inguinalis”. This presentation accounts for approximately 20% of AMH pathway mutations and 14% of idiopathic cases. 3. Transverse testicular ectopia. It refers unilateral herniation of both tests and a part of Müllerian structures through the processus vaginalis. This condition is the most specific anatomic situation of PMDS and it is found in 25% of cases with *AMH* or *AMHR2* gene mutation. However, it is never seen in idiopathic cases (5). Our first case was also diagnosed with this condition, after Müllerian structures were recognized during surgical treatment of bilateral undescended testis.

AMH is a member of TGF- β family. It contains fixe exons and it is 2.8 kb long (6). Cohen et al. determined that the *AMH* gene is located in the short arm of the chromosome 19 (p13.3) (7). It is found that *AMHR2* gene is located on the long arm of the chromosome 12 and it contains 11 exons (8). Jean-Yves Picard et al. (1) conducted a study on 157 families with PMDS from 1990 to 2016 and they found mutation of *AMH* or *AMHR2* gene in 88% of the cases. The same study demonstrated 64 different mutations in the *AMH* gene in 80 families and the authors found that mutations are more commonly located in Exon 1, 2 and 5. Similarly, it is reported that *AMHR2* gene mutations are discovered in 75 families, which represented 58 different alleles. No mutation is found in *AMH* or *AMHR2* gene approximately in 12% of the cases that are referred to as idiopathic PMDS (1). Serum AMH levels are undetectable or low in *AMH* gene mutations, while normal or high in *AMHR2* mutations (4,9). Since serum AMH levels were normal in our patient, *AMHR2* gene mutation was considered and this diagnosis was made. There is no significant anatomic difference between patients with *AMH* or *AMHR2* gene mutations. Previous studies demonstrated that position of testes and Müllerian structures may vary between siblings with PMDS and same mutation (10). Our study showed that a mutation causes bilateral undescended testis in one patient and transverse testicular ectopia in the sibling.

Recently, early orchiopexy is recommended, if and whenever possible, in order to prevent damage of the germ cells in patients with cryptorchidism. Previously, it was estimated that the incidence of testicular cancer in PMDS was not high than the testes with general cryptorchidism and the incidence was around 18 percent (11). However, Jean-Yves Picard et al. conducted a review in 2017 (1) and showed that unilateral or bilateral malignant testicular degeneration develops in 33% of patients with PMDS at age 18 or above. It is reported that the most common malignant degeneration is seminoma (1). Malignant degeneration of Müllerian derivatives is less common. Farikullah et al. (12) reported that degeneration is found Müllerian structures in relation with the PMDS only in 3 cases.

The most common complication of PMDS is infertility. Although fertility is rare in PMDS, it is also possible if 2 conditions are met. There should be minimum one testis and the excretory ducts should be intact (1). A comprehensive literature review showed that 19% of adult patients have 1 or more child (1). Farag et al. (13) reported the rate of fertile patients as 11 percent. There are many causes of the infertility and the azoospermia. Late orchiopexy, damage of testis and vas deferens during the surgery and abnormal anatomic connection of testes to the excretory ducts are some of those causes (13,14,15). Testes are usually not properly connected to the male excretory ducts due to aplasia at the upper part of vas deferens and epididymis or there is no connection between the testis and the epididymis (16).

In conclusion, PMDS is a rare condition that is usually seen in men who present with cryptorchidism and/or inguinal hernia. It should be diagnosed early for both protecting the fertility and preventing the potential malignant degeneration. Considering the possibility of damage to the vas deferens and testis during surgical procedure, the patient should always be referred to experienced surgeons.

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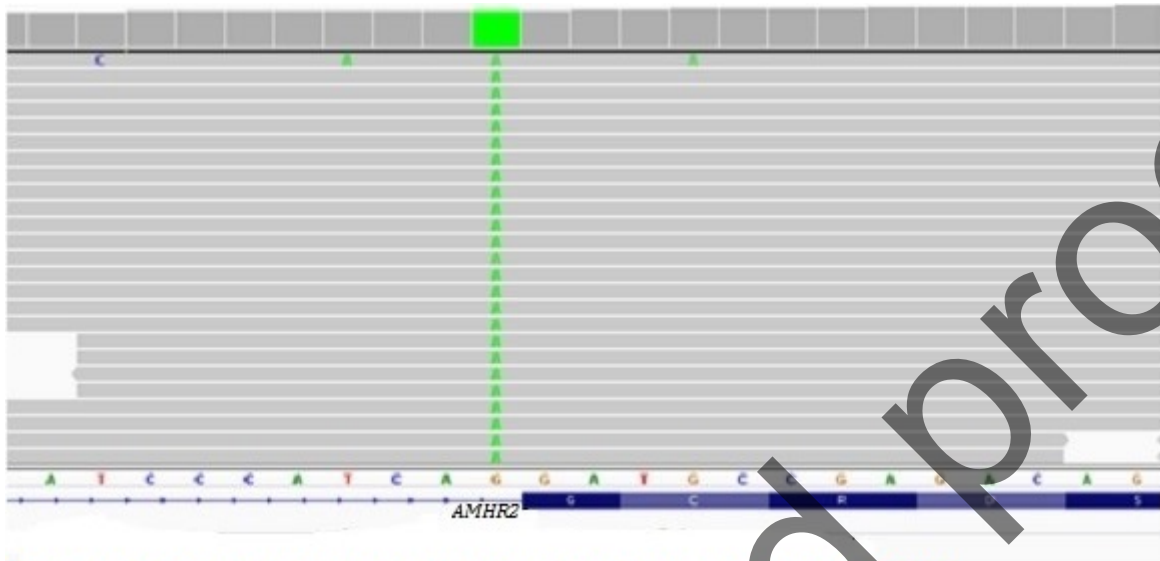


Figure 1. Homozygous mutation (NM_020547.3:c.233-1G>A) in intron 2 of the AMHR2 gene

Uncorrected proof