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ORIGINAL ARTICLE

Male Health

Surgical management and molecular diagnosis of persistent Müllerian duct syndrome in Chinese patients

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Persistent Müllerian duct syndrome (PMDS) is a rare clinically and genetically overlapping disorder caused by mutations in the anti-Müllerian hormone (*AMH*) gene or the anti-Müllerian hormone receptor type 2 (*AMHR2*) gene. Affected individuals present uterus and tubes in normally virilized males and are discovered unexpectedly during other surgeries. Since it is rare and complex, a definitive clinical diagnosis can be missed, and there are no guidelines regarding how to deal with the uterus. In the present study, exome sequencing and Sanger verification were performed for causal variants in 12 PMDS patients. Preoperative diagnoses were made by positive exome sequencing in 8 patients. Of them, 7 patients evoked on the basis of ultrasound indicating bilateral testes on the same side of the body. Twelve different *AMH* variants (2 frameshift/nonsense, 1 deletion, 8 missense, and 1 in-frame) in 9 patients and 6 different *AMHR2* variants (5 missense and 1 splicing) in 3 patients were identified. Seven variants were classified as "pathogenic" or "likely pathogenic", and 4 of them were novel. All but two patients with *AMH* defects showed low serum AMH concentrations, but all patients with *AMHR2* defects showed elevated AMH levels. During surgery, an abnormal vas deferens was observed in half of the patients. Eight patients underwent orchidopexy with uterine preservation. Of them, 2 patients presented complications including irreducible cryptorchidism, and 3 patients developed Müllerian remnant cysts. Three patients underwent subtotal hysterectomy. Of them, one patient had complication of injury to the vas deferens, and one had hemorrhage after operation. This is the first report of PMDS involving a large Chinese population. The present study not only expands the variation spectrum but also provides clinical experience about the management of the uterus.

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INTRODUCTION

Persistent Müllerian duct syndrome (PMDS; OMIM# 261550) is a rare recessive disorder characterized by the persistence of Müllerian remnants (MRs), uterus, and tubes, in normally virilized males.[1] The karyotype of individuals with PMDS is 46,XY, the urethra opens normally at the glans, and both gonads are testes. PMDS has three main clinical presentations: bilateral cryptorchidism, unilateral cryptorchidism with inguinal hernia, and transverse testicular ectopia (TTE).[2] During the corrective surgery for cryptorchidism and inguinal hernia, the presence of MRs is usually an unexpected discovery.[2] The accurate diagnosis can be missed during the initial surgery, leading to reoperations. The uterus is usually an obstacle to testicular descent and at risk of malignant degeneration.[2–5] However, removing the uterus almost unavoidably damages the vas deferens or the deferential artery.[2] Nowadays, it is difficult to identify PMDS preoperatively and the debate continues about how to deal with the uterus.

Biallelic loss-of-function variants in the anti-Müllerian hormone (*AMH*) gene and the anti-Müllerian hormone receptor type 2 (*AMHR2*) gene have been identified in patients with PMDS. The *AMH* gene is a 2.8-kb-long gene containing five exons located on chromosome 19p13.3, and its protein product is a glycoprotein dimer belonging to the transforming growth factor-beta (TGF-β) family that acts via its specific receptor (AMHR2). The *AMHR2* gene contains 11 exons located on 12q13 and encodes a serine/threonine kinase belonging to the TGF-β-related protein family. To date, 85 different variants in *AMH* and 84 variants in *AMHR2* have been identified according to the Human Gene Mutation Database (HGMD). However, few variants have been reported in Chinese individuals with PMDS.[6]

In this report, we studied the management experiences and genetic findings of 12 patients with PMDS in China. After a review of the literature, we presented procedures for the diagnosis and treatment of PMDS.

PATIENTS AND METHODS

The study included all patients with PMDS admitted to the Department of Urology at Children's Hospital, Zhejiang University School of

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Medicine (Hangzhou, China), from January 2012 to December 2020. The inclusion criteria were (1) normal male external genitalia; (2) a 46,XY karyotype; and (3) the presence of MRs ascertained by surgical exploration. The exclusion criteria included (1) a lack of molecular diagnosis, or (2) an absence of treatment or follow-up. Data on demographics, clinical presentations, molecular findings, AMH levels, surgical findings, and outcomes were retrospectively reviewed. This study was approved by the Ethical Committee of Children's Hospital, Zhejiang University School of Medicine (Approval No. 2018-IRB-076). Written informed consent was also obtained.

AMH levels were determined via the Immunotech AMH/Müllerian inhibiting substance (MIS) enzyme immunoassay (UniCel DXI800; Beckman Coulter, Fullerton, CA, USA). G-banding karyotyping was carried out using peripheral lymphocytes.

Targeted next-generation sequencing was performed. Genomic DNA was extracted from peripheral blood samples of patients using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Paired-end libraries were generated with the Agilent Sure Select Target Enrichment System and an XT Inherited Disease Panel containing 2742 genes (Agilent Technologies Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. Sequencing was performed on an Illumina HiSeq 2500 System (Illumina Inc., San Diego, CA, US). The analysis was carried out using an in-house pipeline adapted from BWA, GATK 4.0.0.0 and SAMtools 1.8. Sanger sequencing was used to confirm the identified variants in the patients and their parents. When a confirmed origin of the observed variants was lacking in the patients, their parents also underwent exome sequencing. *AMH/AMHR2* copy numbers were determined through CapCNV analysis followed by the CNVkit protocol (https://cnvkit.readthedocs.io/en/stable/pipeline.html).

To predict the possible impact of identified variants, multiple prediction tools such as ClinPred and REVEL were implemented. According to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) 2015 guidelines, the pathogenicity of the variants was classified as five levels: pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, and benign.[7]

All analyses were carried out using the SPSS software, version 20 (SPSS, Chicago, IL, USA).

RESULTS

Clinical findings

There were 12 patients with a median age of 10 (range: 2–49) months included during the study period (**Table 1**). All the parents of the patients were unrelated. Among the patients, 6 patients presented with unilateral cryptorchidism (50.0%; **Figure 1**), 3 patients with bilateral cryptorchidism (25.0%), and 3 patients with TTE (25.0%). The preoperative diagnosis of PMDS was evoked by ultrasound in 7 patients and another one patient presenting with bilateral unpalpable cryptorchidism was diagnosed preoperatively by exome sequencing. The preoperative diagnosis rate of PMDS was 66.7%, as shown in **Figure 2**. Notably, patient 1 and 2 are brothers. Patient 1 was diagnosed as PMDS during surgery for henia. Later, his elder brother,patient 2, was confirmed to had PMDS postoperatively by exome sequencing.

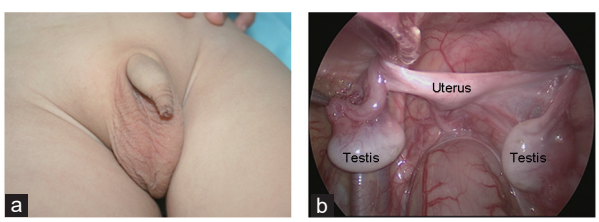

Figure 1: Normally virilized external genitalia and unexpected Müllerian remnants in the pelvic cavity. (**a**) The length and size of the penis are normal-for-age and its urethra opens at the glans. The scrotum is empty. (**b**) There is a uterus between two intra-abdominal testes.

Molecular findings and AMH levels

We identified 12 different *AMH* variants (2 frameshift/nonsense, 1 deletion, 8 missense, and 1 in-frame) in 9 patients and 6 different *AMHR2* variants (5 missense and 1 splicing) in 3 patients (**Figure 3** and **Supplementary Table 1**). The variants in *AMH* and *AMHR2* occurred in the compound heterozygous form in all patients except for patient 7, who harbored a homozygous variant in *AMH*. The same nonsense variant (p.E389X) in *AMH* occurred in patient 5 and patient 7, while the same missense variant (p.G101R) occurred in patient 3, 6, and 8. The numbers of detected and independent variants in the N-terminal region of *AMH* were 8 and 5, respectively, while those in the TGF-beta-like domain were both 3. Three missense variants occurred in the activin type I and II receptor domain of *AMHR2*, and two occurred in the protein kinases catalytic domain. Of the 12 identified variants in *AMH*, 8 variants are novel, and 4 missense variants have been reported previously. According to the ACMG 2015 criteria, 7 variants were classified as pathogenic, or likely pathogenic, which were c.301G>A, c.1165G>T, c.707G> A, c.992C>T, c.1447T>C, and c.102dupC in *AMH*, and c.160C>T in *AMHR2*. Of them, 4 variants were novel: c.707G> A, c.992C>T, c.1447T>C and c.102dupC in *AMH*. Three detected variants occurred in activin types I and II receptor domain of *AMHR2*, and two variants occurred in protein kinase catalytic domain. Five of the 6 identified variants in *AMHR2* were novel and VUS, and the other missense variant was classified as likely pathogenic.

All but two patients with *AMH* gene defects showed low or undetectable serum AMH concentrations. Patient 3 with 80.88 ng ml^{-1} AMH and patient 9 with more than 168.57 ng ml^{-1} AMH were the exceptions. All patients with *AMHR2* gene defects presented elevated AMH levels.

Intervention and follow-up

All patients underwent laparoscopic exploration and bilateral orchidopexy. Eight patients preserved the uterus until now. Three patients underwent subtotal hysterectomy, which was accompanied by complications of injury to the vas deferens in one patient and

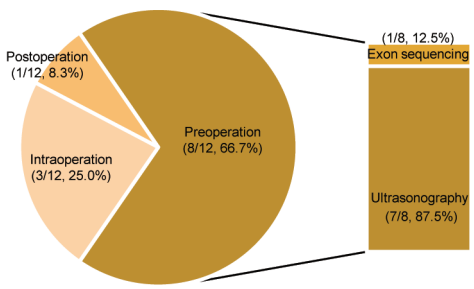

Figure 2: Timing of PMDS diagnosis and detection methods. A preoperative diagnosis was made in 66.7% (8/12) of cases, including 7 cases evoked by ultrasonography and confirmed by exome sequencing, and 1 case diagnosed by exome sequencing. The postoperative diagnosis obtained by exome sequencing was unilateral testis because of his PMDS brother. The last three cases were identified due to the presence of the uterus during corrective surgery for hernia or cryptorchidism. PMDS: persistent Müllerian duct syndrome.

Table 1 Clinical and genetic findings of patients with PMDS

Patient	*Age (month)*	*Presentation*	*Diagnosis method*	*Preoperatively diagnosed*	*AMH (ng ml^{-1})*	*Gene*	*Mutation*	*Amino acid change*	*Protein*	*Parental validation*	*Variant classification*	*Intervention*	*Vas*	*Outcome*
1	8	Left cryptorchidism, bilateral hernia	Müllerian structurs noted during surgery for hernia	No	5.18	*AMH*	c. 1522_1524dupGTG	p.V508dup	In-frame	F	VUS	Gonadal biopsy + bilateral orchipexy + bilateral hernia repair	Normal	Good
							c. 1637C>A	p.A546E	Missense	M	VUS			
2	49	Left orchiatrophy, right hernia	Exon sequencing was positive because his brother was patient 1	No	<0.06	*AMH*	c. 1522_1524dupGTG	p.V508dup	In-frame	F	VUS	Right orchipexy + right hernia repair	Abnormal	Good
							c. 1637C>A	p.A546E	Missense	M	VUS			
3	10	Bilateral cryptorchidism, left hernia	Both testes detected at left groin by ultrasound	Yes	80.88	*AMH*	c. 1352G>A	p.R451H	Missense	F	VUS	Gonadal biopsy + bilateral orchipexy	Abnormal	Recurrence of left cryptorchidism
							c. 301G>A	p.G101R	Missense	M	LP			
4	0.6	Left cryptorchidism, right hernia, TTE	Both testes detected at right scrotum by PE and ultrasound	Yes	0.91	*AMH*	c. 1604T>C	p.L535P	Missense	F	VUS	Gonadal biopsy + bilateral orchipexy + right hernia repair	Normal	Scrotum cyst
							c. 584A>G	p.Y195C	Missense	M	VUS			
5	16	Left cryptorchidism, right hernia	Müllerian structurs noted during surgery for hernia	No	<0.06	*AMH*	c. 1165G>T	p.E389X	Nonsense	F	P	Gonadal biopsy + bilateral orchipexy + right hernia repair	Abnormal	Recurrence of right cryptorchidism[a], bilateral TM[a]
							NA	NA	Deletion	M	P			
6	2	Bilateral cryptorchidism, bilateral hernia	Both testes detected at right groin by ultrasound	Yes	<0.06	*AMH*	c. 707G>A	p.G236D	Missense	F	LP	Gonadal biopsy + bilateral orchipexy	Abnormal	Recurrence of bilerteral hernia, bilateral TM
							c. 301G>A	p.G101R	Missense	M	LP			
7	2	Bilateral cryptorchidism	Gene sequencing was positive	Yes	0.05	*AMH*	c. 1165G>T	p.E389X	Nonsense	F	P	Cystoscopy + radiography + bilateral orchipexy + hysterectomy	normal	Good
							c. 1165G>T	p.E389X	Nonsense	M	P			
8	10	Right cryptorchidism, left hernia, TTE	Both testes detected at right scrotum by ultrasound	Yes	8.01	*AMH*	c. 992C>T	p.S331L	Missense	F	LP	Bilateral orchipexy + left hernia repair + hysterectomy	Normal	Injury to right side vas
							c. 301G>A	p.G101R	Missense	M	LP			
9	11	Left cryptorchidism	Müllerian structurs noted during surgery for cryptorchidism.	No	>168.57	*AMH*	c. 1447T>C	p.Y483H	Missense	F	LP	Bilateral orchipexy	Abnormal	Inguinal cyst
							c. 102dupC	p.S35Qfs*46	Frameshift	M	P			
10	2	Left cryptorchidism, right hernia	Left testis detected at groin by ultrasound	Yes	>24.5	*AMHR2*	c. 356A>G	p.N119S	Missense	F	VUS	Bilateral orchipexy + bilateral hernia repair	Abnormal	Inguinal and pelvic cyst
							c. 733G>A	p.A245T	Missense	M	VUS			
11	12	Left cryptorchidism, TTE	Both testes detected at right scrotum by ultrasound	Yes	214.67	*AMHR2*	c. 706T>A	p.S236T	Missense	F	VUS	Bilateral orchipexy	Normal	Good
							c. 160C>T	p.R54C	Missense	M	LP			
12	2	Left cryptorchidism, right hernia	Left testis detected at right internal ring by ultrasound	Yes	117.62	*AMHR2*	c. 337A>C	p.T113P	Missense	M	VUS	Gonadal biopsy + left orchipexy + hysterectomy	Normal	Hemorrhage; bilateral TM
							c. 1288 + 23C>A	NA	Intron variant	F	VUS			

[a]The testis on the normal side occurred cryptorchidism twice. PMDS: persistent Müllerian duct syndrome; P: pathogenic; LP: likely pathogenic; VUS: variants of uncertain significance; TTE: transverse testicular ectopia; TM: testicular microlithiasis; *AMH*: anti-Müllerian hormone; *AMHR2*: anti-Müllerian hormone receptor type 2; NA: not available

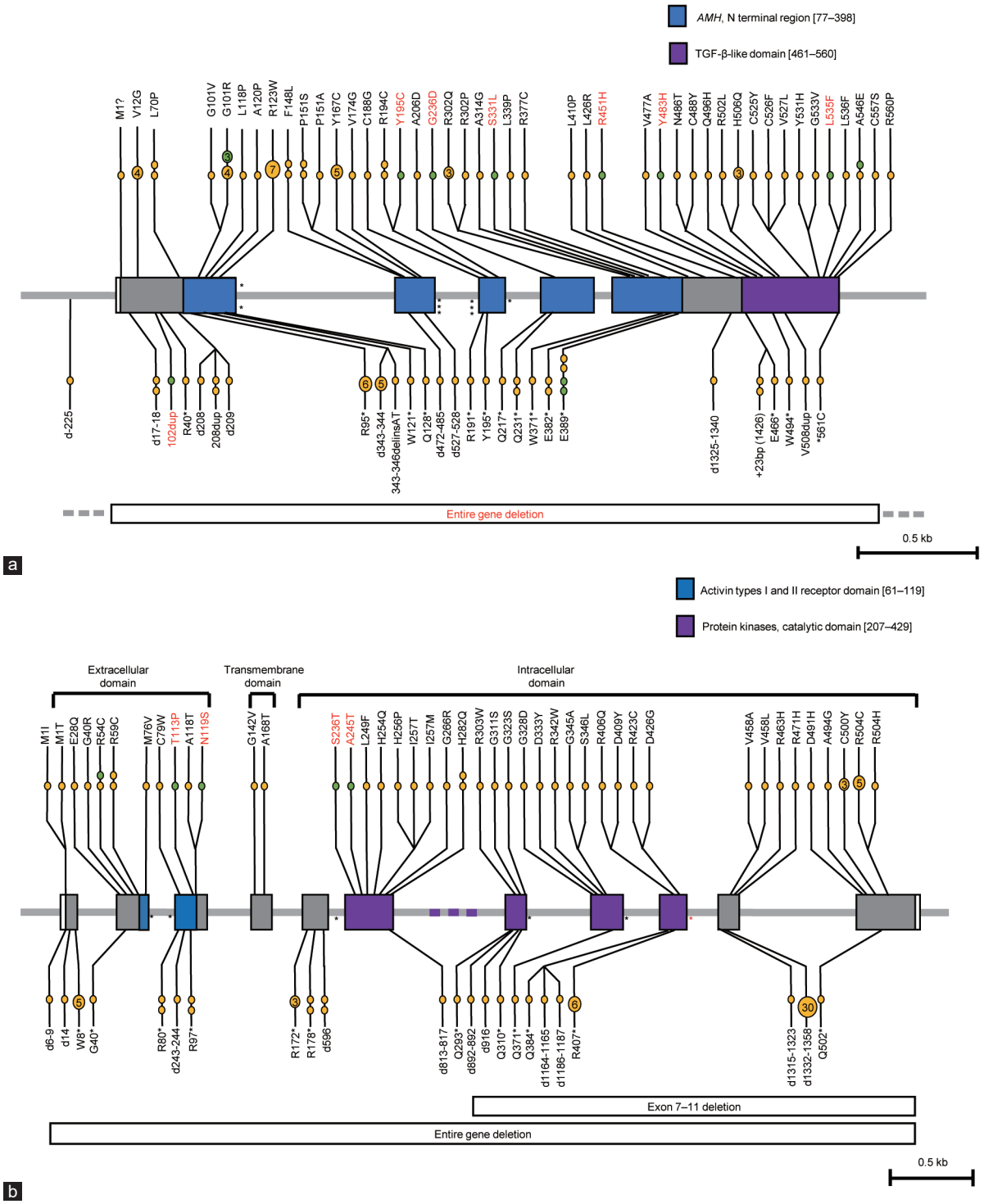

Figure 3: Summary of reported variants and novel variants in the (**a**) *AMH* gene and (**b**) *AMHR2* gene. Green dots represent variants found in the present study; yellow dots represent variants previously reported in the literature. Each point represents one affected family. The number in the circle represents the times of reports. Novel variants are indicated in red. Missense variants are indicated on the top, and others are indicated on the bottom. *Intron variants are indicated. Variants in each domain cluster together. PMDS: persistent Müllerian duct syndrome; *AMH*: anti-Müllerian hormone; *AMHR2*: anti-Müllerian hormone receptor type 2; TGF-β: transforming growth factor-beta.

hemorrhage in another patient. The uterus in the last patient could not be detected by laparoscopy or imaging. During surgery, the vas deferens was observed to be blind and dissociated from the testes in six patients (50.0%). For histopathology, testicular biopsies were performed in 6 patients, and the examination of sections confirmed testicular microlithiasis (TM) in 3 patients (25.0%), while the others presented normal infantile testes. Patient 7 underwent retrograde urethrography showing a long narrow prostatic utricle (**Figure 4a** and **4b**)

All patients were followed up, and the median follow-up time was 31 (range: 7–95) months. We observed complications in six patients. One patient suffered bilateral hernia recurrence, which was repaired during reoperation. Five of eight patients whose uterus was retained presented complications of irreducible cryptorchidism (2/8) and MR cysts (3/8). One patient with irreducible cryptorchidism was followed up without further intervention; the other underwent two additional surgeries to repair the normal-side testis. MR cysts occurred in the scrotum, groin, or pelvis. The size of the cyst in patient 4 was small and stable (2.3 cm × 0.8 cm), and the size in patient 9 was enlarged at one time point (6.3 cm × 4.0 cm) but has now been reduced (1.5 cm × 1.1 cm). Patient 10 exhibited a progressively enlarged cyst (6.2 cm × 1.6 cm) for 25 months after the operation and continued to undergo follow-up without further intervention (**Figure 4c** and **4d**).

Description of the variant spectrum of the literature

Based on a review of 157 cases in 2017,[8] nine published peer-reviewed articles were identified. Adding with our patients in China, there were 93 families with 78 different variants in *AMH* (**Supplementary Table 2**) and 94 families with 80 different variants in *AMHR2* have been reported (**Supplementary Table 3**).[6,8–16] The relationship between the phenotypes and genotype of all patients was analyzed statistically (**Supplementary Figure 1**). There was no significant difference in anatomy between patients with either *AMH* or *AMHR2* variants. A total of 56.6% of the patients presented with bilateral cryptorchidism, 20.8% with unilateral cryptorchidism, and 22.5% with TTE. Variants in *AMH* and *AMHR2* were detected in 87.9% of all cases. Meanwhile, all TTE with PMDS has possible causative variants.

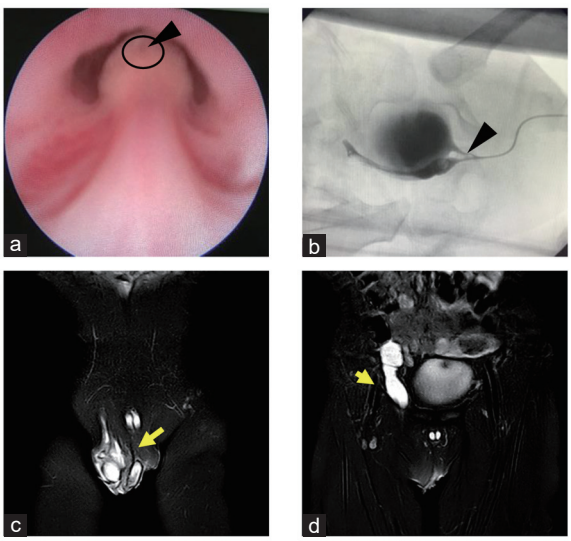

Figure 4: Images of Müllerian remnants. (**a**) Posterior urethral cystoscopy (patient 7). Cystoscopy demonstrated the verumontanum to be in a normal location with an edematous slit-like opening in its center (cursor). (**b**) Retrograde urethrography (patient 7). The 3F catheter was inserted into the opening, which was long and narrow in the oblique coronal plane in retrograde urethrography. The cursor points to the confluence of the vagina and urethra. (**c**) Pelvic MRI (patient 10). This T2 coronal view demonstrates that the Müllerian remnants (yellow arrow) extend from the scrotum. (**d**) Pelvic MRI (patient 10). This T2 coronal view demonstrates that the Müllerian remnants (yellow arrow) extend along the groin and extend within the pelvic cavity on the normal side. The area measured 6.2 cm × 1.6 cm. MRI: magnetic resonance imaging.

Variants occur along the whole length of *AMH* and *AMHR2* (**Figure 3**). The large N-terminal fragment is hit 70 times. The TGF-β like domain in C-terminus is hit 26 times. The variant rate in the N-terminal region was nearly 2.5 times that of the TGF-β-like domain. The activin types I and II receptor domain in *AMHR2* is an extracellular domain and is hit 8 times. The protein kinase catalytic domain in *AMHR2* is located in the intracellular domain and is hit 37 times and 4 times more than the receptor domain.

DISCUSSION

PMDS is one of the rarest causes of disorders of sex development (DSD) with clinical and genetic heterogeneity and is characterized by the presence of a bicornuate uterus, fallopian tubes, and the upper third of the vagina in normally masculinized 46,XY subjects.[1] The incidence has not been accurately determined. Since its initial description, the details of more than 250 cases have been published, and variants in *AMH* and *AMHR2* have been identified in these patients. Thus far, few studies from China have reported the variant spectrum and management experiences of PMDS patients. In the current study, we report the clinical experiences and genetic findings of 12 new patients from China.

In our literature review, 56.6% of the included patients presented with bilateral cryptorchidism, 20.8% with unilateral cryptorchidism, known as hernia uteri inguinalis, and 22.5% with TTE (**Supplementary Figure 1**). The data were updated and included newly reported patients and our Chinese patients.[2] However, in the current study, unilateral cryptorchidism present in more than half of the patients was the most common phenotype. We also found that the position of the testis was flexible and was always reset during laparoscopic exploration, although it was preoperatively detected on the contralateral side of the body (mostly in the groin) by ultrasound (**Table 1**). It is easy to confuse TTE with unilateral cryptorchidism, and it is possible that there is no need to distinguish them. Moreover, the high flexibility of the testes due to the abnormal mobility of the uterus facilitates their torsion and may lead to uni- or bilateral testicular degeneration.[17] The testis of patient 2 was ascertained to be atrophied.

In children, PMDS is usually diagnosed due to an accidental discovery of uterus during corrective surgery for inguinal hernia or cryptorchidism. Most surgeons may not see any patient with PMDS due to its low incidence. The clinical manifestations of PMDS are a common presentation compared with other cryptorchidism. Testicular histology is generally performed for diagnosis during the first surgery without therapeutic surgery, and the morphology is usually normal.[2] Therefore, preoperative diagnosis is critical and can help to avoid unnecessary testicular biopsy and reoperations. Unilateral or bilateral cryptorchidism is not particularly evocative, although unilateral cryptorchidism with contralateral hernia, particularly TTE, should evoke suspicion. In the present study, the rate of preoperative diagnosis was as high as 66.7%. Further assessment was carried out when suspicion of PMDS arose from ultrasound in 7 patients, which revealed that the testis crossed to the contralateral side of the body. The results of exome sequencing were positive in these 7 patients and one bilateral cryptorchidism, which enable us to make a preoperative diagnosis. Pelvic ultrasound is routinely used to describe the internal anatomy in these patients, but limited in the identification of MRs. The sensitivity and specificity of ultrasound in localizing MRs is 54% and 50%, respectively.[18] If physical examination and ultrasound show any sign of a testis crossing the body midline to the contralateral side, PMDS should be considered. Moreover, preoperative gene sequencing can contribute to an accurate diagnosis when a damaging variant of *AMH* or *AMHR2* is detected.

In our literature review, variants of *AMH* and *AMHR2* were reported in 87.9% of all patients and were approximately equally distributed among the genes coding AMH and its type II receptor, AMHR2.[6,8–16] Statistics indicated that *AMH* presented 78 different variants in 93 families, and 80 different alleles of *AMHR2* were discovered in 94 families. In our Chinese patients, mutational analyses revealed possible causative variants in all patients. *AMH* variants occurred more frequently than *AMHR2* variants, consistent with a previous report.[2] Variants are known to occur along the entire length of *AMH*, although exons 3 and 4 are very rarely involved (**Figure 3**). The short C-terminal fragment is a TGF-β-like domain with biological activity. The large N-terminal region is not thought to be essential for activity, but exerts the ability to stabilize the C-terminus,[19] and shows nearly 2.5 times the hit of the C-terminus (**Figure 3**). In our study, twelve variants, including eight new variants, were identified in *AMH*. The c.301G>A (p.G101R) and c.1165G>T (p.E389X) were recurrent in three and two unrelated families, respectively. The variant c.301G>A (p.G101R) has been previously described in four families, while c.1165G>T (p.E389X) has been reported in two families. The variant c.1637C>A (p.A546E) was also previously detected in one family. *AMHR2* encodes a membrane protein and has 11 exons, which are all affected by variants (**Figure 3**). Only two hits were observed in the transmembrane domain. The protein kinase catalytic domain was more conserved and showed nearly 5 times more hits than the activin types I and II receptor domains. In our Chinese patients, six variants in the *AMHR2* gene were found, including five new VUS. The variant c.160C>T (p.R54C) was previously described in one family.[20] Functional studies have not been reported, so further studies are needed to investigate the harmfulness of these variants.

Normally, AMH concentrations are maintained at high levels during childhood and decrease at puberty.[21] Patients with *AMH* gene defects show low AMH levels from birth, whereas patients with variants in *AMHR2* show elevated AMH levels, indicating insensitivity of the target tissues.[22] In contrast, patients 3 and 9 with variants in *AMH* presented increased AMH levels, indicating that the interactions between variants p.(R451H) and p.(G101R) and between p.(S35Qfs*46) and p.(Y483H) might affect hormone bioactivity but not the hormone secretion rate.[23]

The genotypes of *AMH* and *AMHR2* are not related to the observed phenotypes.[2,24] The phenotype can differ within the same family and shows no relationship to the type of genetic defect involved. The anatomical abnormalities common to all patients with PMDS result from the failure of the gubernaculum to anchor the testes at the base of the scrotum. The abnormal mobility of the uterus with the testes facilitates their torsion and may lead to uni- or bilateral testicular degeneration.[17] In the current study, the left testis of patient 1 was located in the left groin, while the testis of his brother, patient 2, was atrophied on the left side. Furthermore, neither laparoscopy nor imaging was able to detect Müllerian derivatives in patient 2, even though he harbored the same variants as his brother (patient 1) with PMDS. This suggests highly variable penetrance of the abnormal alleles and/or the existence of other genetic or epigenetic modifiers of gene expression.

The management of PMDS consists of the correction of cryptorchidism to prevent testis degeneration and preserve fertility.[2,25] Because of the high mobility of the uterus with the testes, bilateral orchidopexy is recommended, as we performed in the current study. The rate of testicular malignancy is up to 33.0% in PMDS patients older than 18 years, which is higher than the rate among general cryptorchidism cases. Close follow-up of the testes is necessary with age.[2]

There is no consensus regarding whether MRs should be excised in PMDS patients. Previous reports have advocated retaining MRs to prevent damage to the vas deferens, considering that MRs present no risk of malignancy.[26,27] In the current study, MRs were preserved in eight patients, but complications occurred in five patients. To our knowledge, this is the first study to report the emergence of MR cysts after MR preservation. The cysts were thought to be hydrocolpos caused by the accumulation of fluid due to congenital vaginal obstruction. In our experience, such cysts may decrease in size and remain stable, but long-term outcomes still need to be followed up. Irreducible cryptorchidism occurred in two patients following MR preservation, which may have been due to the mobilization of MRs.

Since 2002, three PMDS patients have been reported to have developed malignancy of MRs originating from the mucosa.[3–5] The preferred surgical procedure is to split the uterus in the middle, destroy the mucosal lining, and leave an intact pedicle of the myometrium.[25,28,29] This not only releases the testes to the ideal position but also protects the integrity and vascularity of the vas deferens and reduces the chance of malignancy. In the current study, three patients underwent partial hysterectomy, but two of them experienced side effects. One presented the complication of hemorrhage, indicating that complete hemostasis should be noticed. The vas deferens is injured in TTE because it is enclosed in MRs, so surgeons need to improve their surgical techniques and perform dissection carefully. In addition to malignant degeneration, retained MRs are known to cause hematuria, recurrent urinary tract infection, stones, and voiding disturbances,[29,30] but these conditions were not noticed in our series.

Infertility is another problem observed in PMDS patients. The possible causes include congenital malformations of excretory ducts, long-term cryptorchidism, and damage to the testicular blood supply or the vas deferens during hysterectomy. In the present study, the incidence of vas deferens malformation was high, occurring in half of the patients. We also noted a high incidence of TM (25.0%), which may contribute to another cause of infertility.[31]

Follow-up is extremely important for these patients, especially the monitoring of malignancies of the testes and uterus, with concern for their fertility. Close follow-up of all of our patients continues in our clinic.

CONCLUSION

We report 12 new Chinese PMDS patients with 13 novel possible causative variants in the *AMH* and *AMHR2* genes. Bilateral orchidopexy with destruction of the uterine mucosa may be the optimum surgical procedure for PMDS.

AUTHOR CONTRIBUTIONS

HJT and DXT conceived and designed this study. DXT and JFF supervised this study. HJT and DHW wrote the manuscript. HJT and CT extracted clinical data. DWW analyzed the genetic variants. WR and JNY performed statistical analysis of the results with assistance and/or inputs from other authors. CT and GJC provided necessary study supports. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Detailed genetic findings of patients

Patient	*Gene*	*Assembly ID*	*Transcript ID*	*Chromosomal location*	*rs*	*Mutation*	*a.a. change*	*Exon-intron*	*Variant type*	*Type*	*Parental validation*	*Mutation type*	*Evidence level*	*Variant classification*
1	*AMH*	GRCh37/hg19	NM_000479.3	chr19: 2251795-2251797	None	c.1522_1524 dupGTG	p.V508dup	Exon 5	In-frame	Het	Paternal inheritance	Novel	PM2 PP1 PP4	VUS
				chr19:2251910	rs748647281	c.1637C>A	p.A546E	Exon 5	Missense	Het	Maternal inheritance	Known	PM2 PP1 PP4	VUS
2	*AMH*	GRCh37/hg19	NM_000479.3	chr19: 2251795-2251797	None	c.1522_1524 dupGTG	p.V508dup	Exon 5	In-frame	Het	Paternal inheritance	Novel	PM2 PP1 PP4	VUS
				chr19:2251910	rs748647281	c.1637C>A	p.A546E	Exon 5	Missense	Het	Maternal inheritance	Known	PM2 PP1 PP4	VUS
3	*AMH*	GRCh37/hg19	NM_000479.3	chr19:2251625	rs1235377959	c.1352G>A	p.R451H	Exon 5	Missense	Het	Paternal inheritance	Novel	PM2 PM3 PP4	VUS
				chr19:2249632	rs778071215	c.301G>A	p.G101R	Exon 1	Missense	Het	Maternal inheritance	Known	PM2 PP1_strong PP3 PP4	LP
4	*AMH*	GRCh37/hg19	NM_000479.3	chr19:2251877	None	c.1604T>C	p.L535P	Exon 5	Missense	Het	Paternal inheritance	Novel	PM2 PP3 PP4	VUS
				chr19:2250679	None	c.584A>G	p.Y195C	Exon 3	Missense	Het	Maternal inheritance	Novel	PM2 PP4	VUS
5	*AMH*	GRCh37/hg19	NM_000479.3	chr19:2251438	rs1415701260	c.1165G>T	p.E389X	Exon 5	Nonsense	Het	Paternal inheritance	Known	PM2 PM3 PM4 PP1 PP3 PP4	Pathogenic
				chr19: 2114728-2456964	None	-	-	Whole gene	Gross deletion	Het	Maternal inheritance	Novel	PVS1 PM2 PP4	Pathogenic
6	*AMH*	GRCh37/hg19	NM_000479.3	chr19:2250890	None	c.707G>A	p.G236D	Exon 4	Missense	Het	Paternal inheritance	Novel	PM2 PM3 PP3 PP4	LP
				chr19:2249632	rs778071215	c.301G>A	p.G101R	Exon 1	Missense	Het	Maternal inheritance	Known	PM2 PP1_strong PP3 PP4	LP
7	*AMH*	GRCh37/hg19	NM_000479.3	chr19: 2251438	rs1415701260	c.1165G>T	p.E389X	Exon 5	Nonsense	Hom	Paternal inheritance	Known	PVS1 PM2 PP1 PP3 PP4	Pathogenic
				chr19: 2251438	rs1415701260	c.1165G>T	p.E389X	Exon 5	Nonsense	Hom	Maternal inheritance	Known	PVS1 PM2 PP1 PP3 PP4	Pathogenic
8	*AMH*	GRCh37/hg19	NM_000479.3	chr19:2251265	None	c.992C>T	p.S331L	Exon 5	Missense	Het	Paternal inheritance	Novel	PM2 PM3 PP3 PP4	LP
				chr19:2249632	rs778071215	c.301G>A	p.G101R	Exon 1	Missense	Het	Maternal inheritance	Known	PM2 PP1_strong PP3 PP4	LP
9	*AMH*	GRCh37/hg19	NM_000479.3	chr19:2251720	rs764585665	c.1447T>C	p.Y483H	Exon 5	Missense	Het	Paternal inheritance	Novel	PM2 PM3 PP3 PP4	LP
				chr19: 2249433	None	c.102dupC	p.S35Qfs*46	Exon 1	Frameshift	Het	Maternal inheritance	Novel	PVS1 PM2 PP4	Pathogenic
10	*AMHR2*	GRCh37/hg19	NM_020547.3	chr12:53818616	None	c.356A>G	p.N119S	Exon 3	Missense	Het	Paternal inheritance	Novel	PM2 PP3 PP4	VUS
				chr12:53819584	rs1439647673	c.733G>A	p.A245T	Exon 6	Missense	Het	Maternal inheritance	Novel	PM2 PP4	VUS
11	*AMHR2*	GRCh37/hg19	NM_020547.3	chr12:53819557	None	c.706T>A	p.S236T	Exon 6	Missense	Het	Paternal inheritance	Novel	PM2 PM3 PP4	VUS
				chr12:53818182	rs534999427	c.160C>T	p.R54C	Exon 2	Missense	Het	Maternal inheritance	Known	PS3 PM2 PP3 PP4	LP
12	*AMHR2*	GRCh37/hg19	NM_020547.3	chr12:53818597	None	c.337A>C	p.T113P	Exon 3	Missense	Het	Maternal inheritance	Novel	PM2 PP3 PP4	VUS
				chr12:53823785	rs571389839	c.1288 + 23C>A	-	Intron 9	Missense	Het	Paternal inheritance	Novel	PM2 PP4	VUS

AMH: anti-Müllerian hormone; *AMHR2*: anti-Müllerian hormone receptor type 2; LP: likely pathogenic; VUS: uncertain significance; PVS: pathogenic very strong; PM: pathogenic moderate; PP: pathogenic supporting; -:

Supplementary Table 2: Reported variants in AMH

Patient	*Origine*	*Exon-intron*	*Mutation*	*Effect*	*Alleles*	*Publication*
H089	China		whole gen deletion	?	Hetero	Current study
H001	Mexico		NC_000019.10:c.-225del	(SF1 response element)	Homo	Valeri *et al*. 2016
H002	Morocco	1	NM_000479.3:c.3G>T	NM_000479.3:p.Met1?	Homo	
H003	France N	1	NM_000479.3:c.17_18del	NM_000479.3:p.(Leu6Hisfs*17)	Hetero	
H004	Turkey				Homo	
H005	Italy	1	NM_000479.3:c.35T>G	NM_000479.3:p.(Val12Gly)	Hetero	Imbeaud *et al*. 1994
H006	France S				Hetero	
H007	Scotland				Hetero	
H008	Egypt				Homo	Mazen *et al*. 2011
H093	China	1	NM_000479.3:c.102dupC	NM_000479.3:p.(Ser35GInfs*46)	Hetero	Current study
H009	Germany	1	NM_000479.3:c.118C>T	NM_000479.3:p.(Arg40*)	Hetero	
H010	Egypt	1	NM_000479.3:c.208del	NM_000479.3:p.(Leu70Cysfs*7)	Homo	Mazen *et al*. 2017
H011	Maghreb	1	NM_000479.3:c.208dup	NM_000479.3:p.(Leu70Profs*11)	Homo	
H012	Netherlands				Homo	van der Zwan *et al*. 2012
H013	Tunisia	1	NM_000479.3:c.209T>C	NM_000479.3:p.(Leu70Pro)	Homo	
H014	Algeria				Homo	Zeller *et al*. 1994
H015	Tunisia	1	NM_000479.3:c.209del	NM_000479.3:p.(Leu70Argfs*7)	Homo	
H003	France N	1	NM_000479.3:c.283C>T	NM_000479.3:p.(Arg95*)	Hetero	
H017	Germany				Homo	
H018	France S				Homo	
H019	Brazil				Hetero	Nishi *et al*. 2012
H020	Egypt				Homo	Mazen *et al*. 2011
H083	Great Britain				Hetero	Hughes *et al*. 2019
H021	Pakistan	1	NM_000479.3:c.301G>A	NM_000479.3:p.(Gly101Arg)	Homo	
H022	Turkey				Homo	
H023	Turkey				Hetero	
H024	Afghanistan				Homo	
H087	China				Hetero	Current study
H090	China				Hetero	Current study
H092	China				Hetero	Current study
H025	France N	1	NM_000479.3:c.302G>T	NM_000479.3:p.(Gly101Val)	Hetero	
H026	France S	1	NM_000479.3:c.343_344del	NM_000479.3:p.(Leu115Thrfs*58)	Hetero	
H027	Switzerland				Homo	
H028	Germany				Hetero	
H029	Portugal/ Philippine				Hetero	
H030	Switzerland				Homo	
H031	Great Britain	1	NM_000479.3:c.343_346delinsAT	NM_000479.3:p.(Leu115Metfs*58)	Hetero	
H028	Germany	1	NM_000479.3:c.353T>C	NM_000479.3:p.(Leu118Pro)	Hetero	
H033	Denmark	1	NM_000479.3:c.358G>C	NM_000479.3:p.(Ala120Pro)	Hetero	
H034	Morocco	1	NM_000479.3:c.363G>A	NM_000479.3:p.(Trp121*)	Homo	
H035	Turkey	1	NM_000479.3:c.367C>T	NM_000479.3:p.(Arg123Trp)	Homo	
H036	Italy				Homo	
H037	USA				Homo	Loeff *et al*. 1994
H038	France S				Homo	
H039	France S				Hetero	
H019	Brazil				Hetero	Nishi *et al*. 2012
H041	Brazil				Homo	Nishi *et al*. 2012
H042	Israel	1	NM_000479.3:c.382C>T	NM_000479.3:p.(Gln128*)	Homo	
H043	Belgium	1st intr	NC_000019.10(NM_000479.3):c.412+3A>G	?	Homo	Guerrier *et al*. 1989
H044	Algeria	1st intr	NC_000019.10(NM_000479.3):c.412+3A>C	?	Homo	Gricourt *et al*. 2011
H026	France S	2	NM_000479.3:c.444C>G	NM_000479.3:p.(Phe148Leu)	Hetero	
H046	Great Britain				Hetero	
H009	Germany	2	NM_000479.3:c.451C>G	NM_000479.3:p.(Pro151Ala)	Hetero	
H048	Netherlands	2	NM_000479.3:c.451C>T	NM_000479.3:p.(Pro151Ser)	Hetero	
H049	Great Britain				Hetero	
H050	Italy	2	NM_000479.3:c.472_485del	NM_000479.3:p.(Pro158Alafs*11)	Hetero	Carré-Eusèbe *et al*. 1992

Contd...

Supplementary Table 2: Contd...

Patient	*Origine*	*Exon-intron*	*Mutation*	*Effect*	*Alleles*	*Publication*
H051	Great Britain	2	NM_000479.3:c.500A>G	NM_000479.3:p.(Tyr167Cys)	Homo	
H033	Denmark				Hetero	
H007	Scotland				Hetero	
H025	France N				Hetero	
H055	Scotland				Hetero	
H056	Greece	2	NM_000479.3:c.521T>G	NM_000479.3:p.(Val174Gly)	Hetero	
H057	Denmark	2	NM_000479.3:c.527_528del	NM_000479.3:p.(Val176Aspfs*206)	Hetero	
H058	Germany	2nd intr	NC_000019.10(NM_000479.3):c.555+1G>T	?	Hetero	
H059	Netherlands				Hetero	
H048	Netherlands				Hetero	
H061	Brazil	2nd intr	NC_000019.10(NM_000479.3):c.556-2A>G	?	Homo	Nishi *et al*. 2012
H062	Brazil				Homo	Nishi *et al*. 2012
H063	Brazil				Homo	Nishi *et al*. 2012
H064	Kurdistan	3	NM_000479.3:c.562T>G	NM_000479.3:p.(Cys188Gly)	Homo	
H050	Italy	3	NM_000479.3:c.571C>T	NM_000479.3:p.(Arg191*)	Hetero	Carré-Eusèbe *et al*. 1992
H066	Yugoslavia	3	NM_000479.3:c.580C>T	NM_000479.3:p.(Arg194Cys)	Homo	
H067	Pakistan				Homo	
H088	China	3	NM_000479.3:c.c.584A>G	NM_000479.3:p.(Try195Cys)	Hetero	Current study
H085	China	3	NM_000479.3:c.585C>A	NM_000479.3: p.(Try195*)	Hetero	Xu Yufei *et al*. 2019
H068	Great Britain	3	NM_000479.3:c.617C>A	NM_000479.3:p.(Ala206Asp)	Hetero	
H005	Italy	3rd intr	NC_000019.10(NM_000479.3):c.664+5G>A	?	Hetero	
H084	Great Britain	4	NM_000479.3:c.649C>T	NM_000479.3:p.(Gln217*)	Homo	Hughes *et al*. 2019
H070	Pakistan	4	NM_000479.3:c.691C>T	NM_000479.3:p.(Gln231*)	Hetero	
H071	Pakistan				Homo	
H090	China	4	NM_000479.3:c.707G>A	NM_000479.3:p.(Gly236Asp)	Hetero	Current study
H046	Great Britain	5	NM_000479.3:c.905G>A	NM_000479.3:p.(Arg302Gln)	Hetero	
H049	Great Britain				Hetero	
H083	Great Britain				Hetero	Hughes *et al*. 2019
H058	Germany	5	NM_000479.3:c.905G>C	NM_000479.3:p.(Arg302Pro)	Hetero	
H075	Morocco	5	NM_000479.3:c.941C>G	NM_000479.3:p.(Ala314Gly)	Homo	
H092	China	5	NM_000479.3:c.992C>T	NM_000479.3:p.(Ser331Leu)	Hetero	Current study
H059	Netherlands	5	NM_000479.3:c.1016T>C	NM_000479.3:p.(Leu339Pro)	Hetero	
H081	Australia	5	NM_000479.3:c.1112A>G	NM_000479.3:p.(Trp371*)	Hetero	Eggers *et al*. 2016
H077	Indonesia	5	NM_000479.3:c.1129C>T	NM_000479.3:p.(Arg377Cys)	Homo	
H078	Algeria	5	NM_000479.3:c.1144G>T	NM_000479.3:p.(Glu382*)	Homo	
H079	Morocco				Homo	Knebelmann *et al*. 1990
H080	Australia	5	NM_000479.3:c.1165G>T	NM_000479.3:p.(Glu389*)	Homo	
H085	China				Hetero	Xu Yufei *et al*. 2019
H089	China				Hetero	Current study
H091	China				Homo	Current study
H016	Algeria	5	NM_000479.3:c.1229T>C	NM_000479.3:p.(Leu410Pro)	Homo	
H032	Guatemala	5	NM_000479.3:c.1277T>G	NM_000479.3:p.(Leu426Arg)	Homo	
H040	Maghreb	5	NM_000479.3:c.1325_1340del	NM_000479.3:p.(Asp442Valfs*23)	Homo	
H087	China	5	NM_000479.3:c.1352G>A	NM_000479.3:p.(Arg451His)	Hetero	Current study
H045	Great Britain	5	NM_000479.3:c.1396G>T	NM_000479.3:p.(Glu466*)	Hetero	
H047	Kosovo	5	NM_000479.3:c.1425_1426ins1397_1419	NM_000479.3:p.(Val477Serfs*3)	Homo	Lang-Muritano *et al*. 2001
H056	Greece				Hetero	
H052	Italy	5	NM_000479.3:c.1430T>C	NM_000479.3:p.(Val477Ala)	Homo	
H093	China	5	NM_000479.3:c.1447T>C	NM_000479.3:p.(Tyr483His)	Hetero	Current study
H053	Japan	5	NM_000479.3:c.1457A>C	NM_000479.3:p.(Asn486Thr)	Hetero	Morikawa *et al*. 2014
H057	Denmark	5	NM_000479.3:c.1463G>A	NM_000479.3:p.(Cys488Tyr)	Hetero	
H054	Comores	5	NM_000479.3:c.1481G>A	NM_000479.3:p.(Trp494*)	Homo	
H060	Germany	5	NM_000479.3:c.1488G>T	NM_000479.3:p.(Gln496His)	Homo	
H065	Brazil	5	NM_000479.3:c.1505G>T	NM_000479.3:p.(Arg502Leu)	Homo	Nishi *et al*. 2012
H031	Great Britain	5	NM_000479.3:c.1518C>G	NM_000479.3:p.(His506Gln)	Hetero	

Contd...

Supplementary Table 2: Contd...

Patient	*Origine*	*Exon-intron*	*Mutation*	*Effect*	*Alleles*	*Publication*
H068	Great Britain				Hetero	
H081	Australia				Hetero	Eggers *et al*. 2016
H086	China	5	NM_000479.3:c.1522_1524dupGTG	NM_000479.3:p.(Val508dup)	Hetero	Current study
H069	Pakistan	5	NM_000479.3:c.1574G>A	NM_000479.3:p.(Cys525Tyr)	Homo	Carré-Eusèbe *et al*. 1992
H082	Turkey	5	NM_000479.3:c.1577G>T	NM_000479.3:p.Cys526Phe	Homo	Altincik *et al*. 2017
H053	Japan	5	NM_000479.3:c.1579G>T	NM_000479.3:p.(Val527Leu)	Hetero	Morikawa *et al*. 2014
H072	Turkey	5	NM_000479.3:c.1591T>C	NM_000479.3:p.(Tyr531His)	Homo	Nalbantoglu *et al*. 2015
H075	Morocco	5	NM_000479.3:c.1598G>T	NM_000479.3:p.(Gly533Val)	Homo	
H088	China	5	NM_000479.3:c.c.1604T>C	NM_000479.3:p.(Leu535Phe)	Hetero	Current study
H073	Great Britain	5	NM_000479.3:c.1606C>T	NM_000479.3:p.(Leu536Phe)	Hetero	
H070	Pakistan	5	NM_000479.3:c.1637C>A	NM_000479.3:p.(Ala546Glu)	Hetero	
H086	China				Hetero	Current study
H055	Scotland	5	NM_000479.3:c.1669T>A	NM_000479.3:p.(Cys557Ser)	Hetero	
H074	Comores	5	NM_000479.3:c.1679G>C	NM_000479.3:p.(Arg560Pro)	Homo	
H076	U. A. Emirates	5	NM_000479.3:c.1683A>T	NM_000479.3:p.(*561Cysext*?)	Homo	

Supplementary Table 3: Reported variants in AMHR2

Patient	*Origin*	*Exon-intron*	*Mutation*	*Effect*	*Alleles*	*Publication*
R001	Maghreb	1	NM_020547.2:c.2T>C	NM_020547.2:p.(Met1Thr)	Homo	
R002	Belgium	1	NM_020547.2:c.3G>A	NM_020547.2:p.(Met1Ile)	Homo	
R003	Guadeloupe	1	NM_020547.2:c.6_9del	NM_020547.2:p.(Gly3Leufs*40)	Hetero	
R004	Algeria	1	NM_020547.2:c.14del	NM_020547.2:p.(Leu5Trpfs*39)	Hetero	
R005	Turkey	1	NM_020547.2:c.24G>A	NM_020547.2:p.(Trp8*)	Homo	
R006	Turkey				Homo	Korkmaz *et al*. 2017
R090	Turkey				Hetero	E. Unal *et al*. 2020
R091	Turkey				Hetero	E. Unal *et al*. 2020
R094	Turkey				Hetero	E. Unal *et al*. 2020
R093	Turkey	2	NM_020547.2:c.71G>A	NM_020547.2:p.(Cys24Tyr)	Hetero	E. Unal *et al*. 2020
R092	Turkey	2	NM_020547.2:c.78del	NM_020547.2:p.(Phe27Leufs*17)	Homo	E. Unal *et al*. 2020
R007	Pakistan	2	NM_020547.2:c.82G>C	NM_020547.2:p.(Glu28Gln)	Homo	
R008	Marocco	2	NM_020547.2:c.118G>A	NM_020547.2:p.(Gly40Arg)	Homo	
R009	Italy	2	NM_020547.2:c.118G>T	NM_020547.2:p.(Gly40*)	Hetero	
R084	Indian	2	NM_020547.2:c.119G>C	NM_020547.2:p.(Gly40Ala)	Homo	Mónica *et al*. 2019
R010	Great Britain	2	NM_020547.2:c.160C>T	NM_020547.2:p.(Arg54Cys)	Hetero	
R086	China				Hetero	Current study
R011	Germany	2	NM_020547.2:c.175C>T	NM_020547.2:p.(Arg59Cys)	Hetero	
R012	Argentina				Hetero	
R013	France	2	NM_020547.2:c.226A>G	NM_020547.2:p.(Met76Val)	Hetero	
R014	Pakistan	2nd intr	NC_000012.12(NM_020547.2):c.232+1G>A		Homo	Imbeaud *et al*. 1995
R081	Turkey	2nd intr	NC_000012.12(NM_020547.2):c.233-1G>A		Homo	Unal *et al*. 2018
R076	Australia	3	NM_020547.2:c.237C>G	NM_020547.2:p.(Cys79Trp)	Homo	Eggers *et al*. 2016
R015	France N	3	NM_020547.2:c.238C>T	NM_020547.2:p.(Arg80*)	Hetero	Guerrier *et al*. 1989
R016	France N				Hetero	
R017	Italy	3	NM_020547.2:c.243_244 del	NM_020547.2:p.(Asp81Glufs*2)	Hetero	
R018	Italy	3	NM_020547.2:c.289C>T	NM_020547.2:p.(Arg97*)	Homo	
R078	Great Britain				Homo	Hughes *et al*. 2019
R087	China	3	NM_020547.2:c.337A>C	NM_020547.2:p.(Thr113Pro)	Hetero	Current study
R019	Argentina	3	NM_020547.2:c.352G>A	NM_020547.2:p.(Ala118Thr)	Hetero	
R085	China	3	NM_020547.2:c.356A>G	NM_020547.2:p.(Asn119Ser)	Hetero	Current study
R020	Denmark	4	NM_020547.2:c.425G>T	NM_020547.2:p.(Gly142Val)	Hetero	
R083	Spanish	4	NM_020547.2:c.502G>A	NM_020547.2:p.(Ala168Thr)	Hetero	Orós-Millán *et al*. 2017
R021	Turkey	5	NM_020547.2:c.514C>T	NM_020547.2:p.(Arg172*)	Homo	
R015	France N				Hetero	
R023	Maghreb				Homo	
R024	Netherlands	5	NM_020547.2:c.532C>T	NM_020547.2:p.(Arg178*)	Hetero	
R088	Dutch				Hetero	L.Tosca *et al*. 2020
R025	Great Britain	5	NM_020547.2:c.596del	NM_020547.2:p.(Glu199Glyfs*10)	Hetero	Messika-Zeitoun *et al*. 2001
R026	Switzerland				Hetero	
R027	USA	5th intr	NC_000012.12(NM_020547.2):c.622-51C>T		Hetero	Hoshiya *et al*. M 2003
R086	China	6	NM_020547.2:c.706T>A	NM_020547.2:p.(Ser236Thr)	Hetero	Current study
R085	China	6	NM_020547.2:c.733G>A	NM_020547.2:p.(Ala245Thr)	Hetero	Current study
R028	France S	6	NM_020547.2:c.745C>T	NM_020547.2:p.(Leu249Phe)	Hetero	
R029	Saudi Arabia	6	NM_020547.2:c.762C>G	NM_020547.2:p.(His254Gln)	Homo	Abduljabbar *et al*. 2012
R030	Egypt	6	NM_020547.2:c.767A>C	NM_020547.2:p.(His256Pro)	Homo	Mazen *et al*. 2016 abstr. ESPE
R031	Pakistan	6	NM_020547.2:c.770T>C	NM_020547.2:p.(Ile257Thr)	Hetero	
R028	France S	6	NM_020547.2:c.771T>G	NM_020547.2:p.(Ile257Met)	Hetero	
R033	Turkey	6	NM_020547.2:c.796G>C	NM_020547.2:p.(Gly266Arg)	Homo	
R034	Denmark	6	NM_020547.2:c.796G>A	NM_020547.2:p.(Gly266Arg)	Homo	
R077	Great Britain	6	NM_020547.2:c. 813_817del	NM_020547.2:p.(Leu272Trpfs*24)	Hetero	Hughes *et al*. 2019

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Supplementary Table 3: Contd...

Patient	*Origin*	*Exon-intron*	*Mutation*	*Effect*	*Alleles*	*Publication*
R035	Netherlands	6	NM_020547.2:c.846T>G	NM_020547.2:p.(His282Gln)	Hetero	Imbeaud *et al*. 1995
R036	Netherlands				Homo	
R089	Romanian	6th intr	Exon 7-11 deletion		Homo	L.Tosca *et al*. 2020
R083	Spanish	7	NM_020547.2:c.877C>T	NM_020547.2:p.(Gln293*)	Hetero	Orós-Millán ME *et al*. 2017
R037	Turkey	7	NM_020547.2:c.892_893del	NM_020547.2:p.(Trp298Glyfs*19)	Homo	
R038	USA	7	NM_020547.2:c.907C>T	NM_020547.2:p.(Arg303Trp)	Hetero	
R080	Colombian	7	NM_020547.2:c.916del	NM_020547.2:p.(Leu306Cysfs*29)	Homo	Acero *et al*. 2019
R039	Israel	7	NM_020547.2:c.928C>T	NM_020547.2:p.(Gln310*)	Homo	Elias-Assad *et al*. 2016
R077	Great Britain	7	NM_020547.2:c.931G>A	NM_020547.2:p (Gly311Ser)	Hetero	Hughes *et al*. 2019
R040	Brazil	7	NM_020547.2:c.967G>A	NM_020547.2:p.(Gly323Ser)	Homo	Nishi 2012
R041	Argentina-Sweden	7th intr	NC_000012.12(NM_020547.2): c.967+2T>G		Hetero	
R042	Brazil	8	NM_020547.2:c.983G>A	NM_020547.2:p.(Gly328Asp)	Homo	
R043	Canada	8	NM_020547.2:c.997G>T	NM_020547.2:p.(Asp333Tyr)	Hetero	
R044	Italy	8	NM_020547.2:c.1024C>T	NM_020547.2:p.(Arg342Trp)	Homo	
R045	Israel	8	NM_020547.2:c.1034G>C	NM_020547.2:p.(Gly345Ala)	Homo	
R046	France N	8	NM_020547.2:c.1037C>T	NM_020547.2:p.(Ser346Leu)	Hetero	
R047	Palestine	8	NM_020547.2:c.1111C>T	NM_020547.2:p.(Gln371*)	Homo	
R048	Africa	8th intr	NC_000012.12(NM_020547.2):c. 1140+1G>A		Homo	
R011	Germany	9	NM_020547.2:c.1150C>T	NM_020547.2:p.(Gln384*)	Hetero	
R050	Comores	9	NM_020547.2:c.1164_1165del	NM_020547.2:p.(Pro389Argfs*20)	Homo	
R082	China	9	NM_020547.2:c.1186-1187del	NM_020547.2:p.(Leu396Glyfs*13)	Hetero	Ren *et al*. 2017
R025	Great Britain	9	NM_020547.2:c.1217G>A	NM_020547.2:p.(Arg406Gln)	Hetero	Messika-Zeitoun *et al*. 2001
R052	Morocco	9	NM_020547.2:c.1219C>T	NM_020547.2:p.(Arg407*)	Homo	
R053	Saudi Arabia				Homo	Abduljabbar *et al*. 2012
R054	France S				Hetero	
R009	Italy				Hetero	
R017	Italy				Hetero	
R057	Brazil				Homo	Nishi 2012
R058	Denmark	9	NM_020547.2:c.1225G>T	NM_020547.2:p.(Asp409Tyr)	Homo	
R059	Spain	9	NM_020547.2:c.1267C>T	NM_020547.2:p.(Arg423Cys)	Hetero	
R060	Pakistan	9	NM_020547.2:c.1277A>G	NM_020547.2:p.(Asp426Gly)	Homo	
R087	China	9th intr	NC_000012.12(NM_020547.2):c. 1288+23C>A	?	Hetero	Current study
R061	Iran	10	NM_020547.2:c.1317_1325del	NM_020547.2:p.(Tyr440_Ala442del)	Homo	
R094	Turkey	10	NM_020547.2:c.1319A>G	NM_020547.2:p.(Tyr440Cys)	Hetero	E. Unal *et al*. 2020
R062	USA	10	NM_020547.2:c.1332_1358del	NM_020547.2:p.(Gly445_ Leu453del)	Hetero	Imbeaud *et al*. 1996
R063	Sweden				Homo	
R054	France S				Hetero	
R065	France S				Hetero	
R066	Netherlands				Hetero	
R067	France N				Hetero	
R068	France N				Homo	
R069	Germany				Homo	
R019	Argentina				Hetero	
R071	Russia				Hetero	
R013	France				Hetero	
R020	Denmark				Hetero	
R074	Germany				Hetero	
R075	Sweden				Homo	
R022	Portugal				Homo	
R026	Switzerland				Hetero	
R032	France N				Hetero	
R049	Sweden				Homo	
R051	Reunion				Homo	

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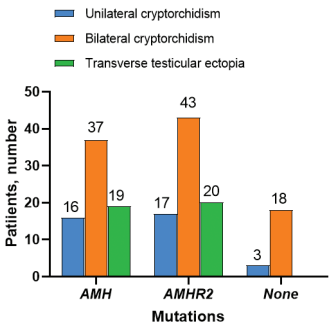
Supplementary Table 3: Contd...

Patient	*Origin*	*Exon-intron*	*Mutation*	*Effect*	*Alleles*	*Publication*
R046	France N				Hetero	
R059	Spain				Hetero	
R012	Argentina				Hetero	
R055	Switzerland				Homo	
R010	Great Britain				Hetero	
R035	Netherlands				Hetero	Imbeaud *et al*. 1995
R056	Netherlands				Homo	
R041	Argentina-Sweden				Hetero	
R016	France N				Hetero	
R027	USA				Hetero	Hoshiya *et al*. 2003
R064	Portugal				Homo	Rosal-Goncalves *et al*. 2010
R079	Turkey	10	NM_020547.2:c.1372G>T	NM_020547.2:p.(Val458Leu)	Homo	Çakir *et al*. 2017
R062	USA	10	NM_020547.2:c.1373T>C	NM_020547.2:p.(Val458Ala)	Hetero	Imbeaud *et al*. 1996
R082	China	10	NM_020547.2:c.1388G>A	NM_020547.2:p.(Arg463His)	Hetero	Xiaoya Ren *et al*. 2017
R070	Italy	10	NM_020547.2:c.1412G>A	NM_020547.2:p.(Arg471His)	Hetero	Avolio *et al*. 2003
R093	Turkey	11	NM_020547.2:c.1460dup	NM_020547.2:p.(Cys487Trpfs*13)	Hetero/homo	E. Unal *et al*. 2020
R032	France N	11	NM_020547.2:c.1471G>C	NM_020547.2:p.(Asp491His)	Hetero	
R043	Canada	11	NM_020547.2:c.1481C>G	NM_020547.2:p.(Ala494Gly)	Hetero	
R004	Algeria	11	NM_020547.2:c.1499G>A	NM_020547.2:p.(Cys500Tyr)	Hetero	
R038	USA				Hetero	
R072	France N				Homo	
R031	Pakistan	11	NM_020547.2:c. 1504C>T	NM_020547.2:p.(Gln502*)	Hetero	
R074	Germany	11	NM_020547.2:c. 1510C>T	NM_020547.2:p.(Arg504Cys)	Hetero	
R073	Turkey				Homo	
R070	Italy				Hetero	Avolio *et al*. 2003
R090	Turkey				Hetero	E. Unal *et al*. 2020
R091	Turkey				Hetero	E. Unal *et al*. 2020
R066	Netherlands	11	NM_020547.2:c. 1511G>A	NM_020547.2:p.(Arg504His)	Hetero	



Supplementary Figure 1: Bar chart of the relationship between the genotypes and phenotypes of 173 reported PMDS cases. The number in each category is indicated at the top of the bar. There was no significant difference in anatomy among the patients with either *AMH* or *AMHR2* gene variants. All TTE with PMDS cases had possible causative variants. PMDS: persistent Müllerian duct syndrome; TTE: transverse testicular ectopia; *AMH*: anti-Müllerian hormone; *AMHR2*: anti-Müllerian hormone receptor type 2.