Testicular Anti-Müllerian Hormone: Clinical Applications in DSD

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Abstract

Male fetal sexual differentiation of the genitalia is driven by Leydig cell-secreted androgens and Sertoli cell-secreted anti-Müllerian hormone (AMH). Disorders of sex development (DSD) may be due to abnormal morphogenesis of genital primordia or to defective testicular hormone secretion or action. In dysgenetic DSD, due to an early fetal-onset primary hypogonadism affecting Leydig and Sertoli cells, the fetal gonads are incapable of producing normal levels of androgens and AMH. In non-dysgenetic DSD, either Leydig cells or Sertoli cells are affected but not both. Persistent Müllerian duct syndrome (PMDS) may result from Sertoli cell-specific dysfunction due to mutations in the AMH gene; these patients have Fallopian tubes and uterus, but male external genitalia. In DSD due to insensitivity to testicular hormones, fetal Leydig and Sertoli cell function is normal. Defective androgen action is associated with female or ambiguous genitalia whereas insensitivity to AMH results in PMDS with normal serum AMH. Clinical and biological features of PMDS due to mutations in the genes coding for AMH or the AMH receptor, as well as genetic aspects and clinical management are discussed.

Keywords
- testis differentiation
- steroidogenesis
- ambiguous genitalia

Fetal sexual differentiation of the internal and external genitalia is driven by testicular androgens and anti-Müllerian hormone (AMH). Androgens, secreted by the Leydig cells present in the interstitial tissue, are responsible for the stabilization of the Wolffian ducts and their differentiation into the epididymides, vasa deferentia and seminal vesicles, as well as for the virilization of the urogenital sinus and the external genitalia. AMH, also called Müllerian inhibiting substance, plays a biological major role in shaping the male reproductive tract by triggering the regression of male fetal Müllerian ducts from 8 to 10 weeks in the human fetus. In rodents of both sexes, AMH has been shown to play a role in Leydig cell function[1] and follicle development[2] but these results were usually obtained using transgenic mice and have not been translated to humans. Patients lacking AMH are normal, apart from the persistence of Müllerian derivatives in males. It follows that clinical applications of AMH in DSD are essentially diagnostic and are based upon the assay of serum AMH. Several AMH ELISA kits are commercially available. Normative values[3–7] may differ according to the kit used.[8] AMH assay in women is an essential tool for assisted reproduction, because it is considered a reflection of ovarian reserve but will not be considered further in this review, since it has no bearing on disorders of sex development.

Ontogeny and Regulation of Testicular AMH Production

AMH is a homodimeric glycoprotein member of the TGF-β family.[9] It is initially secreted as a precursor, subsequently cleaved to yield 110-kDa N-terminal and 25-kDa C-terminal homodimers, which remain associated in a biologically active

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noncovalent complex.[10] Dissociation of non-covalent complex occurs at the time of binding to the type II AMH receptor and is required for biological activity.[11] The major site of AMH production is the fetal Sertoli cell. In the late fetal and postnatal ovary, it is also produced by granulosa cells of developing follicles, essentially preantral and small antral follicles.[12–14]

In the male, AMH is a specific functional marker of the immature Sertoli cell. AMH expression is initiated at the time of fetal differentiation of the seminiferous cords, by the end of the 7th embryonic week, and remains at high levels until the onset of puberty, except for a transient decline in the perinatal period.[3,5] AMH expression is triggered by SOX9, which binds to the AMH promoter[15,16]; subsequently, SF1,[15,17,18] GATA4,[18,19] and WT1[20] further increase AMH promoter activity. The onset of AMH expression and its basal expression level throughout life are independent of gonadotropins. However, FSH stimulates testicular AMH production by both inducing Sertoli cell proliferation and upregulating AMH transcription.[21] The latter is mainly mediated by the classical pathway involving Gsα and adenylyl cyclase increase of cyclic AMP levels,[21,22] which stimulates protein kinase A (PKA) activity, subsequently involving transcription factors SOX9, SF1, GATA4, NFκB and AP2.[21,23] During puberty, AMH production is inhibited by the increase of intratesticular testosterone concentration and the onset of meiosis.[24,25] The inhibitory effect of androgens on AMH overcomes the positive effect of FSH after pubertal onset (reviewed in ref.[26]) except in the fetal and neonatal testis, where Sertoli cells do not yet express the androgen receptor.[27–29]

Classification of Disorders of Sex Development (DSD)

The development and differentiation of the sex organs during fetal life involves three successive steps: 1) the early morphogenesis of the gonadal and genital primordia, which is identical in XY and XX embryos; 2) the differentiation of the gonadal ridge into a testis or an ovary; 3) the differentiation of the primordia of the internal and external genitalia, which are virilized by the action of androgens and AMH or feminized in their absence.

Based on the recognition of the cause of abnormal sex organ development in patients bearing a Y chromosome (►**Table 1**), DSD may be divided into: a) malformative DSD, where abnormal morphogenesis of the genital primordia occurs in early embryonic life; b) dysgenetic DSD, due to abnormal gonadal differentiation resulting in insufficient secretion of androgens and AMH; and c) non-dysgenetic DSD, in which the abnormal sex hormone-dependent genital differentiation results from specific defects in the production or action of androgens or AMH.

AMH IN DSD

AMH in Malformative DSD

Defects in the early morphogenesis of the Müllerian or Wolffian ducts, the urogenital sinus or the primordia of the external genitalia, e.g., cloacal malformations, isolated hypospadias or aphallia, usually occur in eugonadal patients. Therefore, serum AMH and testosterone levels are within the expected range for sex and age. From a practical standpoint, non-endocrine related DSD should be considered when there is inconsistency in the development of the different elements of the genitalia. For instance isolated hypospadias, with no other signs of hypovirilization, in patients with normal AMH and androgen levels are most probably due to early morphogenetic defects.[30,31] In most cases, endocrine-unrelated malformations of the genitalia are associated with other somatic dysmorphic features, like in Robinow syndrome due to ROR2 mutations, Pallister-Hall syndrome due to GLI3 mutations, or many other polymalformative associations of unknown etiology. "Idiopathic"persistence of Müllerian derivatives (PMDS) in patients with a normal AMH level and mutation-free AMH and AMH receptor genes may belong to the same category (see below).

AMH in DSD due to Fetal-onset Primary Hypogonadism

Dysgenetic Primary Hypogonadism

Gonadal dysgenesis established in the first trimester of fetal life represents the earliest form of primary hypogonadism and prevents the normal hormone-driven differentiation of the sex organs. In the fetus carrying a Y chromosome, gonadal dysgenesis results in female or ambiguous genitalia, reflecting the degree of testicular hormone deficiency. If testicular tissue vanishes during the second half of fetal life, when external genitalia are already virilized, the growth of the penis and scrotum is impaired. In all cases, serum AMH is low or undetectable, depending on the amount of testicular tissue remaining[32–35] (►**Table 1** and ►**Fig. 1**). Serum AMH observed in a newborn with ambiguous genitalia should be compared with reference levels for the adequate age period to avoid overdiagnosis of dysgenetic DSD. AMH levels are transiently lower during the first 2–3 weeks after birth in the normal newborn[3–6]; when in doubt, a repeat measurement to assess the evolution of serum AMH may be helpful.[36]

In 45,X or 45,X/46,XX patients, gonads are reduced to fibrous streaks or develop into dysgenetic ovaries. Serum AMH levels reflect the amount of small follicles present in these gonads and predict the occurrence of spontaneous pubertal onset.[37]

Ovotesticular DSD is a particular type of gonadal dysgenesis where both testicular and ovarian tissues are present. The most frequent karyotypes are 46,XX or mosaicisms including at least one XY lineage. The degree of virilization is usually commensurate with the amount of testicular tissue. In XX patients, the differential diagnoses are congenital adrenal hyperplasia, aromatase deficiency and androgen-secreting tumors. An increased level of serum AMH is specific of ovotesticular DSD,[34,35] in the other conditions serum AMH is in the female range. In contrast, androgen assay is not useful for diagnosis, since androgens are always above normal female levels.

Non-dysgenetic Primary Hypogonadism

While gonadal dysgenesis affects the production of both androgens and AMH, non-dysgenetic DSD may result from

Table 1 Etio-pathogenic Classification of Disorders of Sex Development (DSD) in Patients with a Y Chromosome and Classification according to the Chicago Consensus [ref.[94]]

Etio-pathogenic classification	Chicago consensus	Serum AMH	Serum T
A. Malformative DSD			
Defective Morphogenesis of the Wolffian ducts Congenital Absence of the Vas Deferens (Cystic Fibrosis)	46,XY DSD	Normal	Normal
Defective Morphogenesis of the Urogenital Sinus and of the Primordia of the External Genitalia Cloacal malformations, Aphallia, Isolated hypospadias	46,XY DSD	Normal	Normal
B. PRIMARY HYPOGONADISM (EARLY FETAL-ONSET)			
B.1. DYSGENETIC DSD: Whole testicular dysfunction			
Complete gonadal dysgenesis Y-chromosome aberrations DSS duplications, 9p deletions (DMRT1/2?), 1p duplication (WNT4?) Gene mutations: SRY, CBX2, SF1, WT1, SOX9, DHH, MAMLD1, TSPYL1, DHCR7, etc.	 Sex-chromosome DSD 46,XY DSD	Undetectable	Undetectable
Partial gonadal dysgenesis Same as complete gonadal dysgenesis	 Sex-chromosome DSD 46,XY DSD	Low	Low
Asymmetric gonadal differentiation 45,X/46,XY, other mosaicisms or Y-chromosome aberrations	 Sex-chromosome DSD	Low	Low
Ovotesticular gonadal differentiation 46,XX/46,XY; other mosaicisms	 Sex-chromosome DSD Ovotesticular DSD	Low	Low
B.2. NON-DYSGENETIC DSD: Cell-specific dysfunction			
Leydig cell dysfunction Mutations in LH/CG-R, StAR, P450scc, P450c17, POR, Cytochrome b5, 3β-HSD, 17β-HSD	 46,XY DSD	High in neonates/pubertal age Normal in childhood	Low/Undetectable
Sertoli cell dysfunction AMH gene mutations	 46,XY DSD	Low/Undetectable	Normal
C. END-ORGAN FAILURE			
C.1. ANDROGEN END-ORGAN FAILURE			
Impaired DHT production 5α-reductase gene mutations	46,XY DSD	Normal	Normal
Androgen insensitivity syndrome (AIS) Androgen receptor mutations	 46,XY DSD	Partial AIS: High in neonates, normal in childhood, inadequately high at pubertal age Complete AIS: Normal/low in neonates, normal in childhood, very high at pubertal age	Normal/High
C.2. AMH END-ORGAN FAILURE AMHR-II mutations	46,XY DSD	Normal	Normal

Abbreviations: 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; AGD, asymmetric gonadal differentiation; AMH, Anti-Müllerian hormone; AMHR2, Anti-Müllerian hormone receptor type 2.

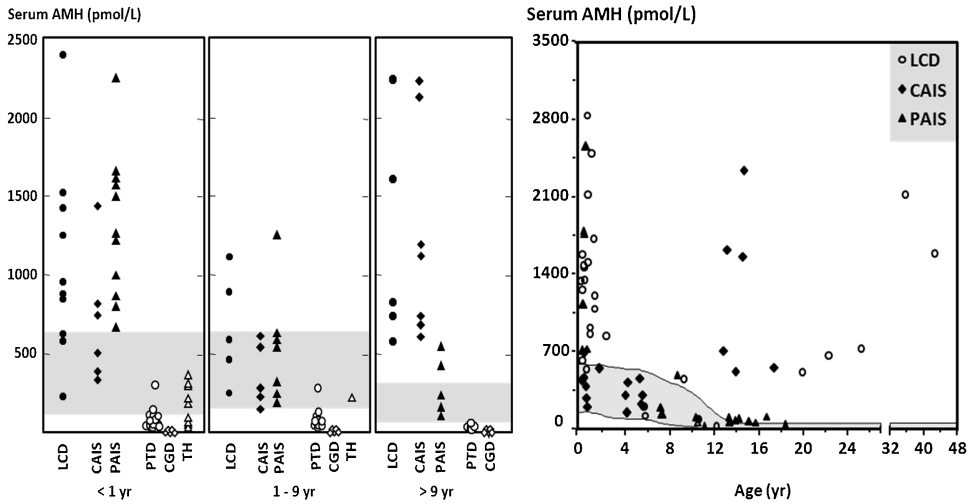

Figure 1 Left: Serum AMH levels in patients with DSD. LCD, Leydig cell defects, including Leydig cell aplasia or hypoplasia and steroidogenic enzyme mutations; CAIS, Complete Androgen Insensitivity Syndrome; PAIS, Partial Androgen Insensitivity Syndrome; PTD, Parttial Testicular Dysgenesis, including assymetrical gonadal differentiation; CGD, Complete Gonadal Dysgenesis; TH, True hermaphroditism or Ovotesticular DSD. The shaded areas represent the normal levels. Data obtained from: Rey R et al. Evaluation of gonadal function in 107 intersex patients by means of serum antimüllerian hormone measurement. J Clin Endocrinol Metab 1999;84:627–631. Copyright, The Endocrine Society, 1999. Right: Serum AMH levels in patients with DSD due to defects in androgen production (Leydig cell defects, LCD) or action (Complete or Partial Androgen Insensitivity Syndrome, AIS). The shaded area represents the normal levels. Data obtained from: Rey R, Mebarki F, Forest MG, Mowszowicz I, Cate RL, Morel Y, Chaussain JL, Josso N. Anti-Müllerian hormone in children with androgen insensitivity. J Clin Endocrinol Metab 1994;79:960–4. Copyright, The Endocrine Society, 1994.

a specific defect impairing the endocrine function of either Leydig cells or Sertoli cells, but not both. These cases represent "dissociated" or "cell-specific" forms of fetal-onset primary hypogonadism,[26] as opposed to gonadal dysgenesis leading to whole gonadal failure. Deficiency of androgen synthesis results in the occurrence of female or ambiguous external genitalia and no uterus, whereas defective AMH production is characterized by the existence of uterus and Fallopian tubes in an otherwise normally virilized patient, a condition known as PMDS.

Androgen Deficiency: Leydig Cell Hypoplasia and Steroidogenic Protein Mutations

Leydig cell aplasia, due to inactivating mutations of the LH/CG receptor, and defects in proteins or enzymes involved in testicular steroidogenesis result in complete lack or insufficient androgen production by the testes. Consequently, hypovirilization or feminization of genitalia occurs as in dysgenetic DSD. Both dissociated primary hypogonadism specifically affecting Leydig cells and dysgenetic DSD have low testosterone levels in serum, yet it is possible to distinguish them by measuring AMH. While AMH is low or undetectable in dysgenetic DSD, as described above, it is normal/high in steroidogenic defects because the androgen inhibitory effect on AMH is lacking and the elevation of serum FSH upregulates AMH secretion,[34,35]particularly in the first 3–6 months after birth and at pubertal age in those cases where gonadectomy has not yet been performed (►**Table 1** and ►**Fig. 1**). It should be noted that AMH may be within the normal male range in these patients during childhood.

AMH Deficiency: PMDS due to AMH Gene Mutations

Another example of dissociated or cell-specific primary hypogonadism is represented by PMDS. PMDS is characterized by the persistence of Müllerian duct derivatives, uterus, Fallopian tubes and upper vagina, in 46,XY males. Approximately 85% of cases are due to mutations of the AMH or AMHR-II gene, in roughly equal proportions, 15% are idiopathic. All the information provided is current up to April 2012.

Clinical features. Because of their normal external male phenotype, patients are assigned at birth to the male gender without hesitation, in spite of the fact that one or both testes are not palpable in the scrotum. When cryptorchidism is unilateral, the contralateral scrotal sac contains a hernia, in addition to the testis. Preoperative diagnosis of PMDS can be reached by ultrasonography[38,39] or better still laparoscopy.[40,41] However, unless an elder brother has been diagnosed with the condition, persistence of Müllerian derivatives is usually discovered unexpectedly during a surgical procedure for cryptorchidism and/or hernia repair. Testicular,[42–46] or more rarely uterine[47–49] tumors are a frequent mode of presentation of PMDS in older patients, particularly in settings where cryptorchidism may have been neglected in childhood. Exceptionally, gynecomastia and hemospermia has led to the discovery of PMDS in a 60-year old Algerian patient with low testosterone and high estrogen secretion.[50]

Anatomy. Testes and the vasa deferentia are always tightly attached to the Müllerian derivatives and adhere to the walls of uterus and vagina.[51] Their location depends upon the mobility of the Müllerian structures. Often, the broad ligament which anchors the uterus to the pelvis is abnormally

thin and does not attach to the abdominal wall,[52] allowing the Müllerian derivatives to follow one testis through the inguinal canal and into the scrotum, resulting in "*hernia uteri inguinalis*." The testis on the opposite side can be drawn into the same hemiscrotum by gentle traction or may already be present there, a condition known as "*transverse testicular ectopia*"; this rare condition is associated with PMDS in 30% of cases.[53] Very rarely, transverse testicular ectopia may be the only anatomical abnormality observed in patients homozygous for an AMH or AMHR-II mutation, no Müllerian derivatives can be detected.[54]

Even when descended, the PMDS testis is only loosely anchored to the bottom of the processus vaginalis, the gubernaculum is long and thin, resembling the round ligament of the uterus and exposing the mobile testis to an increased risk of torsion[55] and subsequent degeneration.[56] Alternatively, the Müllerian derivatives may remain anchored in the pelvis, preventing testicular descent[57] and giving rise to bilateral cryptorchidism. The presence of these midline structures may be missed if cure is attempted through inguinal incisions. The apparent rise in the incidence of PMDS over recent years may be due to the increased use of laparoscopy in patients presenting with bilateral impalpable testes.

Fertility and surgical management. Pubertal development is normal, spermatogenesis is not unheard of[51] and even fertility has been reported[58]; however, incontrovertible evidence of paternity is lacking. Several causes contribute to infertility: i) the excretory ducts may not be properly connected to the testis, due to aplasia of the epididymis, ii) The germinal epithelium is damaged by long standing cryptorchidism. However, excising the uterus to allow abdominal testes to descend into the scrotum carries significant risks. The primary testicular blood supply is through the internal spermatic and the deferential arteries. Often the spermatic vessels are too short and must be divided to allow orchiopexy, the viability of the testis then becomes wholly dependent upon the deferential artery, which is closely associated with the Müllerian structures and may be severely damaged by attempts to remove them.[59] On the other hand, if they are left in place, malignant degeneration may occur.[47,49] Most authors recommend partial hysterectomy, limited to the fundus and proximal Fallopian tubes or the simple division of Müllerian structures in the midline. If the length of the gonadal vessels is the limiting factor, a Fowler-Stephens orchidopexy[60] or microvascular autotransplantation[61] may be indicated. Later, in the case of ejaculatory duct defects, intracytoplasmic sperm injection may be helpful. Orchidectomy is required if the testis cannot be brought down because of a 15% risk of cancer, an incidence apparently not higher than for other abdominal undescended testes (reviewed in refs.[59,61]).

AMH mutations are asymptomatic in females, the mother of one patient, who happened to be homozygous for her son's mutation, is normally fertile and puberty is normal in the sisters of PMDS patients with the same genetic abnormalities. It is too early to know whether these women will undergo premature ovarian failure, as might be suspected from animal studies.[2]

Biological features. Testosterone and gonadotropin levels are normal for age, unless testicular degeneration has occurred (see above).[56] Older patients may experience an imbalance between androgen and estrogen production, leading to clinical feminization.[50] Serum AMH levels are generally very low or undetectable in prepubertal patients.[62] Belville et al[63] have shown that this is due to instability of the mutant protein and is not restricted to mutations of the bioactive C-terminus: mutations in the N terminus may have profound effects upon protein stability and folding. A 3D model of the C-terminus has been generated, using BMP2 and BMP7 as templates, providing insights into the impact of AMH mutations upon secretion and action. For instance, in a patient with mutation Q496H, serum AMH level was normal and secretion was not impaired in vitro, the mutation is believed to disturb the interaction of the molecule with its type I receptor, ALK3.[63] Thus, a normal serum AMH, albeit very rare, does not necessarily rule out the possibility of a pathogenic AMH gene mutation, however this hypothesis cannot be entertained unless the AMHR-II gene has been totally exonerated.

Molecular genetics. The human AMH gene, first cloned in 1986,[9] contains 5 exons. The 3′ end of the last one is extremely GC rich and shows homology to other members of the TGF-β family,[9] it codes for the bioactive C-terminal domain of the AMH molecule. The gene is located on the short arm of chromosome 19.[64] PMDS is usually transmitted as an autosomal recessive trait; X-linked inheritance has been suggested in two pedigrees of unknown genetic make-up.[65,66] AMH mutations are responsible for 52% of the PMDS cases in which genetic defects have been detected. The first reported AMH mutation, a nonsense mutation of the 5th exon, was discovered in 1991 in a Moroccan family.[67] At the time of writing, April 2012, 64 families with AMH mutations, including two reported by Mazen et al,[68] representing a total of 53 different alleles have been identified (►**Fig. 2**). Not included are three AMH sequence variations associated with a normal level of serum AMH because a receptor gene mutation was not ruled out.[69] All exons, except exon 4, coding both the

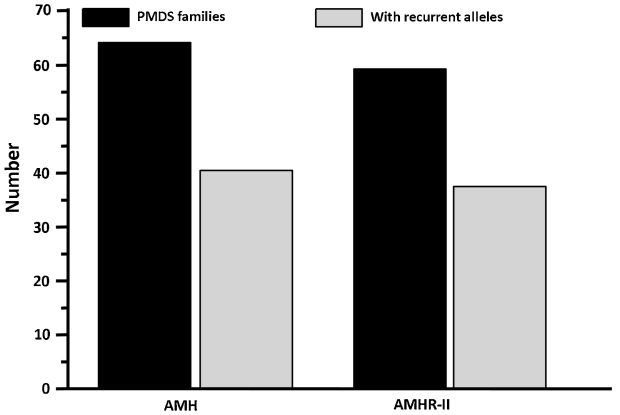

Figure 2 Black columns: number of PMDS families. Gray columns: number of families harboring recurrent alleles. PMDS, persistent Müllerian duct syndrome; *AMH*, anti-Müllerian hormone gene; *AMHR-II*, anti-Müllerian hormone receptor type II gene.

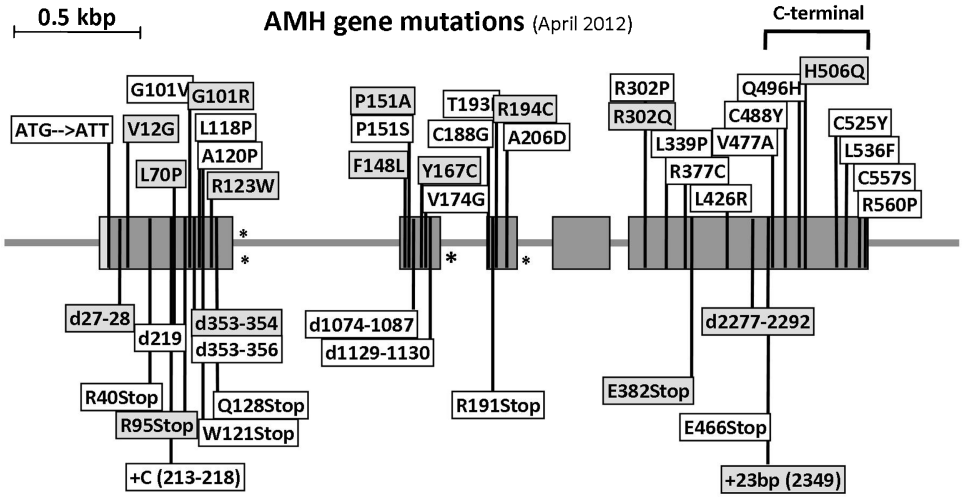

Figure 3 Mutations of the AMH gene in PMDS. Exons are shaded. Missense mutations are represented above, note that the first mutation destroys the translation initiation site. Asterisks represent splice mutations; the bold asterisk at the beginning of the second intron indicates a mutation detected in three different families all from Northern Europe. Deletions (marked "d"), insertions (marked " + ") and nonsense mutations are shown below the gene. All recurrent mutations are shaded. A deletion mutation in the promoter is not shown. C-terminal, coding for bioactive C-terminal domain of the AMH molecule.

inactive N-terminal pro-region and the bioactive C-terminal mature protein, are affected. All types of mutations are represented, 64% are homozygous. There is no true hotspot, though 16 abnormal alleles have been detected in more than one family (►**Fig. 3**). G101R has been observed in 4 patients from the Middle East; two other mutations, R123W and a deletion of bases 353–354, have occurred 5 times essentially in Europe. Y167C, in exon 2, has been identified in 5 patients, all from Northern Europe. A recurrent splice mutation in intron 2 was present in 3 Northern European families. Recurrence of other mutations is less frequent; most have been detected only in two families each.

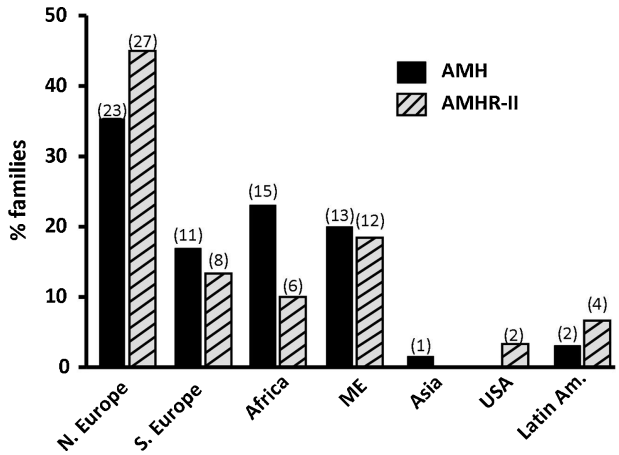

Figure 4 Ethnic origin of PMDS families. Results are expressed as percentages of total number of families with respectively AMH or AMHR-II mutations. The number of families is shown between parentheses. Differences between *AMH* or *AMHR-II* mutations are not statistically significant; the predominance of Northern Europe merely reflects a recruitment bias. N. Europe: Northern Europe (including Northern France), S. Europe: Southern Europe (including Southern France), Africa: (mostly Maghreb), ME: Middle East (includes Turkey, Afghanistan and Pakistan, as per Wikipedia definition) Latin Am.: Latin America (includes Mexico, Central and South America).

The ethnic origin of patients with AMH mutations is shown on ►**Fig. 4**. The high proportion of European families is certainly due to a recruitment bias. Over the past 10 years, PMDS cases have been reported in Japan,[47,70,71] China,[72] Taiwan,[46] India,[39,42,45] Turkey,[73] Nigeria,[74] Tunisia,[53,55] Brazil[49,75] and the United States[61,76] but have not been included, due to the lack of molecular characterization.

AMH in DSD due to End-Organ Defects

Alternatively in XY patients, development of female or ambiguous genitalia may occur in eugonadal states, i.e., with adequate testicular secretion of androgens and AMH, when end-organ defects are present.

Deficiency of 5α-reductase

Steroid 5α-reductase is the key enzyme for the conversion of testosterone to dihydrotestosterone (DHT). The androgen receptor has a higher affinity for DHT than for testosterone. In the absence of 5α-reductase activity, the Wolffian ducts differentiate normally because the adjacent testes supply sufficiently high local testosterone concentrations. Conversely, more distant androgen-dependent organs, like the urogenital sinus and the external genitalia, need testosterone conversion to DHT for adequate virilization (see Costa et al in this issue).[77] The Müllerian ducts regress normally because Sertoli cell AMH production is not affected. Testosterone levels are normal, and serum AMH is also within the normal male range. Because there is normal testicular androgen concentration and androgen receptor expression and FSH is not elevated, serum AMH is not increased in these patients[78] (►**Table 1**).

Androgen Insensitivity Syndrome (AIS)

Androgen insensitivity due to mutations in the androgen receptor is the most frequent cause of lack of virilization in

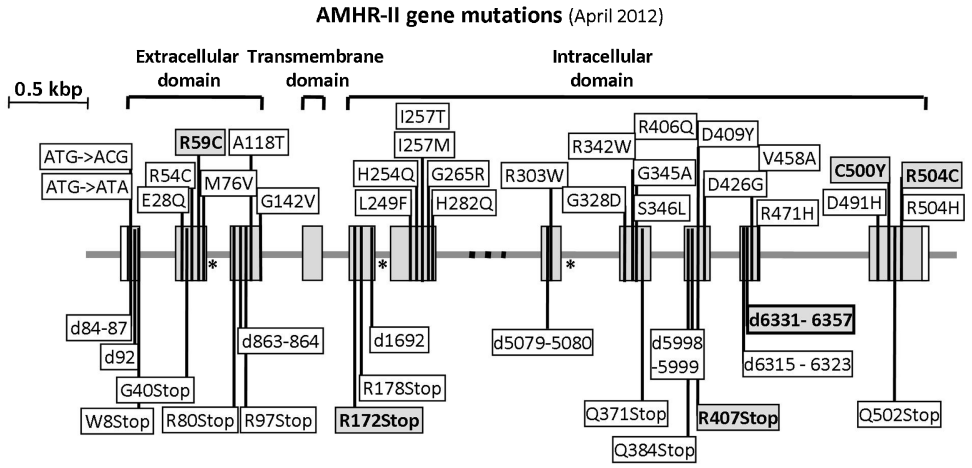

Figure 5 Mutations of *AMHR-II* gene in PMDS. Same representation as in ►**Figure 2**. Missense mutations are represented above. Deletions (marked "d") and nonsense mutations are shown below the gene. All recurrent mutations are shaded. The deletion of 27 bases between bases 6331 and 6357 (d6331–6357 in exon 10) is extremely frequent: it is present in 21% of all PMDS families and in 44% of those with receptor mutations.

eugonadal XY patients (see Hughes and Hiort in this issue).[79] The testes differentiate normally, and both Sertoli and Leydig cells are functionally normal from an endocrine standpoint. Owing to end-organ insensitivity to androgens, Wolffian ducts regress, and the urogenital sinus and the external genitalia fail to virilize. Müllerian ducts do not develop, reflecting normal AMH activity. Complete AIS result in a female external phenotype, whereas partial AIS presents with ambiguous genitalia.

The pituitary-gonadal axis shows different features during the first three months of life in complete and partial AIS. In the newborn with complete AIS, FSH remains low, which probably explains why serum AMH is not as high as expected.[80] Conversely, in partial AIS, gonadotropins as well as AMH are elevated (►**Table 1** and ►**Fig. 1**).[34,35,80,81] As in DSD due to defects of steroid synthesis, serum AMH remains within the normal male range during childhood.[34,35,81] At pubertal age, provided gonadectomy has not been performed, a difference is again observed between partial and complete AIS. In complete AIS, serum AMH increases to abnormally high levels, whereas in partial AIS the elevation of intratesticular testosterone concentration is capable of inducing an incomplete inhibition of AMH expression. Nonetheless, AMH levels are inadequately high for the concomitant circulating testosterone.[34,35,81]

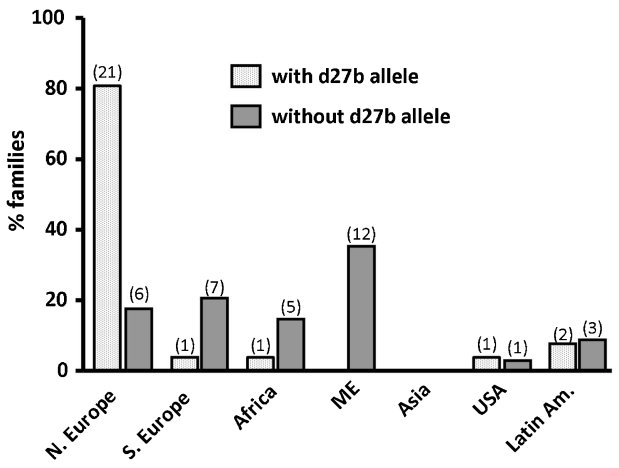

Figure 6 Ethnic origin of families with the recurrent 27 base deletion allele. Results are expressed as percentage of total number of families with AMHR-II mutations, the number of families is shown between parentheses. The predominance of Northern European families with the recurrent deletion, suggests a founder effect. d27b: deletion of bases 6331 to 6357 in exon 10. Differences are statistically significant by a chi square test ($p < 0.001$).

Insensitivity to AMH: PMDS due to AMH Receptor Mutations

Like other members of the TGF-β family, AMH uses two types of membrane-bound serine/threonine kinase receptors for signal transduction. The AMH type II receptor, cloned in 1994,[82,83] binds specifically to AMH and then recruits a type I receptor, probably ALK3,[84] which phosphorylates intracytoplasmic proteins, the SMADs, allowing them to enter the nucleus to interact with target genes. ALK and SMAD molecules also transmit BMP signals and their integrity is absolutely required in early fetal life. Mutations of the type II receptor, AMHR-II, are responsible for 48% of PMDS cases with genetic abnormalities (►**Fig. 2**). Clinical and biological features do not differ from those described above for AMH mutations, apart from the fact that serum AMH level is normal. AMH assay cannot discriminate between AMH and AMHR-II mutations in adulthood, because in both instances, AMH levels are low. Even in childhood, a normal AMH level is not specific for AMHR-II mutations, since ~15% of PMDS cases are not associated with either AMH or AMHR-II mutations.

The AMHR-II gene is composed of 11 exons, the first 3 coding for the receptor extracellular domain, exon 4 for most of the transmembrane domain and the rest for the intracellular domain, where the kinase consensus elements are located. The gene has been mapped to the long arm of

chromosome 12.[85] The first AMHR-II mutation in PMDS, a splice mutation, was reported in 1995.[85] Since then, 59 families, harboring a total of 49 abnormal AMHR-II alleles, have been studied in our laboratory, an additional one has been reported in Boston[86] (►**Fig. 5**). All exons except exon 4 may be affected. A 27-base deletion, from 6331 to 6357 in exon 10, which can be easily detected by PCR, is present in 26 out of 59 families with receptor mutations, nearly all of Northern European origin (►**Fig. 6**), suggesting a founder effect; 30.8% of the families are homozygous for the deletion. Other recurrent mutations are much less frequent, apart from the nonsense R407Stop in exon 9, detected in 5 cases, essentially around the Mediterranean and in the Middle East.[54]

In ~15% of PMDS cases, all with a normal level of serum AMH, both the AMH and AMHR-II genes, including their proximal promoters and intronic sequences, are free of mutations. Several were born small and/or presented with various other congenital defects, such as jejunal atresia.[87] Mutations of the AMH type I receptors or cytoplasmic downstream effectors[88] are unlikely since these are shared with the BMPs and required for normal embryonic development. Inactivation[89] or dysregulation[90] of β-catenin or dysfunction of other factors capable of interfering with AMH action might be involved. Many patients with unexplained PMDS present with various other congenital defects, for instance jejunal atresia,[87] lymphangiectasia,[91] or vitamin D-resistant rickets[92]; these syndromes are probably heterogeneous and not directly related to the AMH pathway. New techniques for genetic investigation of disorders of sexual development[93] will be useful in such cases.

Concluding Remarks

DSD may occur in patients with eugonadal or hypogonadal states. The assessment of both serum AMH, a marker of Sertoli cell function, and serum testosterone, reflecting Leydig cell function, is a simple and useful tool for the clinician to classify DSD cases. When both hormones are below the normal male range, dysgenetic forms of DSD should be suspected. AMH in the male range and low testosterone indicate Leydig cell-specific disorders. When both hormones are within or above the male range, androgen target organ defects are most likely. Finally, PMDS is a rare cause of cryptorchidism in boys with virilized external genitalia: in these cases, low or undetectable serum AMH predicts mutations in the AMH gene while normal serum AMH drives attention to the AMHR-II gene.

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