AMH and AMH receptor defects in persistent Müllerian duct syndrome

Nathalie Josso1, Corinne Belville, Nathalie di Clemente and Jean-Yves Picard

Unité de Recherches sur l’Endocrinologie du Développement (INSERM), Université Paris XI Clamart, France

1To whom correspondence should be addressed at: INSERM u493, 32 rue des Carnets, 92140 Clamart, France. E-mail: nathalie.josso@inserm.ipsc.u-psud.fr

Anti-Müllerian hormone (AMH) produced by fetal Sertoli cells is responsible for regression of Müllerian ducts, the anlage for uterus and Fallopian tubes, during male sex differentiation. A member of the transforming growth factor-β (TGF-β) superfamily, AMH signals through two transmembrane receptors, type II which is specific and type I receptors, shared with the bone morphogenetic protein family. Mutations of the AMH and AMH receptor type II (AMHR-II) genes lead to persistence of the uterus and Fallopian tubes in males. Both conditions are transmitted according to a recessive autosomal pattern and are symptomatic only in males. Affected individuals are otherwise normally virilized, undergo normal male puberty; and may be fertile if testes, tightly attached to the Fallopian tubes, can be replaced in the scrotum. Approximately 85% of the cases are due, in similar proportions, to mutations of the AMH or AMHR-II gene. The genetic background does not influence the phenotype, the only difference is the level of circulating AMH which is normal for age in AMHR-II mutants and usually low or undetectable in AMH gene defects. This is due to lack of secretion, explained by the localization of the mutations in critical regions, based on the assumed 3D structure of the molecule. Similarly, lack of translocation to the surface membrane is responsible for the inactivity of AMHR-II molecules bearing mutations in the extracellular domain. In 15% of cases, the cause of the persistent Müllerian duct syndrome is unknown and could be related to complex malformations of the urogenital region, unrelated to AMH physiology.

Key words: anti-Müllerian hormone/mutations/persistent Müllerian duct syndrome/receptor/urogenital region

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, a member of the transforming growth factor-β (TGF-β) superfamily produced by Sertoli cells of the fetal testis from 7 weeks gestation, is responsible for the regression of Müllerian ducts in male fetuses, the first step of male sex differentiation. AMH signals through receptors located in the mesenchyme of the fetal Müllerian ducts (reviewed in Josso and di Clemente, 2003). Testosterone, produced by Leydig cells starting at 9 weeks gestation, is responsible for the virilization of the male internal and external genital tract, i.e. maintenance and male differentiation of the Wolffian ducts and morphogenesis of a male external genital phenotype (Figure 1).

It follows that genetic abnormalities of the AMH or its receptors will interfere only with the process of Müllerian duct regression, while differentiation of male accessory organs and external virilization will proceed normally under the influence of testosterone. Such is the definition of the persistent Müllerian duct syndrome (PMDS), the subject of this review. The other cause of persistence of Müllerian ducts, testicular dysgenesis, usually affects both Sertoli and Leydig cells; persistence of Müllerian derivatives is then associated with external genital ambiguity.

PMDS

Patients with PMDS have a normal male phenotype and are assigned to the male sex at birth without hesitation. However, thorough examination of the newborn shows genital abnormalities. One or both testes are not palpable in the scrotum and in the case of unilateral cryptorchidism, the contralateral scrotal sac may contain a hernia, in addition to the testis. The hernia may appear incarcerated, in spite of the lack of symptoms of intestinal obstruction, but this discrepancy is usually not correctly interpreted. Unless an elder brother has been diagnosed with the condition, the persistence of Müllerian derivatives, uterus and Fallopian tubes is discovered at surgery motivated by cryptorchidism eventually associated with inguinal hernia. The Müllerian derivatives are always tightly tethered to the testes.

Two clinical variants, not genetically determined (Guerrier et al., 1989) are possible. PMDS may present as bilateral cryptorchidism, if the uterus is in the pelvis and the testes are...
embedded in the broad ligament. Otherwise, in a condition known as hernia uteri inguinalis, one testis has descended and has dragged the Fallopian tube and more rarely also the other testis in its wake, a condition called transverse testicular ectopia. Because the testes are not anchored to the bottom of the processus vaginalis by a normal male gubernaculum (Hutson et al., 1994) they are abnormally mobile and prone to testicular torsion and subsequent testicular degeneration, a high proportion of which has been reported in the PMDS (Imbeaud et al., 1995).

Testes are normally differentiated and, in the absence of longstanding cryptorchidism, usually contain germ cells. However they are often not properly connected to male excretory ducts due to aplasia of the epididymis and upper part of the vas deferens (Imbeaud et al., 1996).

Treatment of PMDS is exclusively surgical and aims to correct cryptorchidism. Removal of the uterus is not necessary, apart from the fact that it is usually difficult to bring the testes down to a normal position, because the vasa deferentia are embedded in the mesosalpinx, lateral uterine wall and cervix. When this is the case, careful dissection is required to avoid harming the excretory ducts. Lack of proper communication between the testis and excretory ducts and lesions at orchidopexy probably explain why fertility is rare in PMDS patients, 11% according to a Kuwait study (Farag, 1993).

Few associated clinical abnormalities have been described, apart from testicular tumors, but these usually develop in cryptorchid gonads and may therefore not be specifically related to PMDS. The question arises because transgenic ‘PMDS’ mice with an inactivated AMH or AMH receptor gene are prone to Leydig cell hyperplasia and testicular tumorogenesis (Mishina et al., 1996). Associated defects are more frequent in patients with unexplained PMDS (see below). Serum testosterone and response to chorionic gonadotrophin are normal, except when testes have degenerated (Imbeaud et al., 1995). The level of circulating AMH is extremely variable and depends on the molecular basis of the condition (Figure 2).

**Biology and molecular basis of PMDS**

Patients with the PMDS are 46,XY genetic males, with no chromosomal abnormalities and normal testosterone production and responsiveness. The condition is transmitted according to a recessive autosomal pattern and is due either to lack of production of AMH, secondary to a mutation of the AMH gene, or to insensitivity of Müllerian ducts to AMH action, due to a mutation of the AMH receptor. In the great majority of subjects, mutations are detected on both alleles; however, in exceptional cases, extensive searches yielded mutations on only one allele (Josso et al., 2003b). Possibly, the other allele bears a splicing mutation (Hoshiya et al., 2003a), which went undetected because testicular mRNA was unavailable. The etiology is reflected in the level of circulating AMH, assessed by enzyme-linked immunosorbent assay (ELISA) (Figure 2). Mutations of the AMH gene are associated with extremely low or undetectable levels of serum AMH. Serum AMH is normal for age in patients with receptor mutations; however ELISA is discriminant only before puberty, since repression of AMH expression by the Sertoli cells occurs physiologically at that time under the influence of testicular androgens. We have completed molecular studies in a total of 82 families, 38 with AMH and 33 with AMH receptor mutations. In 11 additional families, both genes are normal and the origin of the syndrome is unknown.

**Mutations of the AMH gene**

The human AMH gene was cloned in 1986 (Cate et al., 1986), it is located on the tip of the short arm of chromosome 19, band
p13.3 (Cohen-Haguenauer et al., 1987). Only 2.75 kbp in length, it contains 5 exons, the 3' end of the fifth one is particularly GC-rich and codes for the bioactive C-terminal domain. The minimal promoter is only 200 bp (Giuli et al., 1997) and is flanked by a household gene, coding for a spliceosome protein (Dresser et al., 1995). The AMH promoter contains binding sites for various transcription factors, namely SF-1, SOX9 and GATA-1, the most important one being SOX9 (Arango et al., 1999). The AMH precursor protein, a homodimer linked by disulfide bonds, is proteolytically cleaved to yield a 110 kDa, inactive N-terminal fragment and a biologically active 25 kDa C-terminal fragment. The homology of AMH to other members of the TGF-β family is restricted to the C-terminus.

At the time of writing, 38 different mutations of the AMH gene, representing 46% of the total number of families, have been detected (Figure 3A). Most are homozygous, due to the predominantly Mediterranean and Arab origin of the patients, populations with a high incidence of consanguinity (Figure 4). Mutations, for the most part missense, are spread along the whole length of the gene except exon 4 and are particularly frequent in exon 1 and in the 3' end of exon 5 which codes the bioactive C-terminus, the site of recurrent mutations.

Seven AMH mutations, 3 in the N-terminus and 4 in the C-terminus (Figure 5), have been reproduced by site-directed mutagenesis, expressed in COS cells and studied in depth by Western blot analysis, pulse chase and confocal microscopy (Belville et al., 2004). Most mutant proteins were retained in the endoplasmic reticulum and failed to be secreted. A three-dimensional model of the C-terminus dimer by comparison with bone morphogenetic protein (BMP)2 and BMP7 (Figure 6) showed that mutations occurring in key structures such as α-helices or the small hydrophobic core were most likely to affect trafficking. 

Figure 3. Mutations of the AMH (A) and the AMHR-II (B) gene in PMDS. Missense mutations are shown on top, all other mutations below the affected exons. Purple-boxed mutations are recurrent.

Figure 4. Ethnic origin and allelic status of PMDS patients with AMH or AMHR-II mutations. homoz. = homozygous; heteroz. = heterozygous. Reproduced with permission from (Josso et al., 2003b).
Mutations of the AMH receptor

Transduction pathway of AMH

AMH is a member of the TGF-β family and shares the general characteristics of its signalling pathway, elucidated by Massagué et al. (1996). Two different types of membrane bound serine/threonine kinase receptors are involved. Characteristically, type II receptors bind the ligand and form a complex with type I receptors, which they phosphorylate. Activated type I receptors then bind cytoplasmatic effectors, the Smads, which enter the nucleus to bind to specific promoter elements of target genes, aided by effectors and activators. (Shi and Massagué, 2003).

Type II receptors are usually in a one to one relationship with a given ligand, in contrast to the promiscuous ligand/type I receptor relationship: the same type I receptor may serve different ligands and, depending on the environment, a ligand may use different type I receptors. AMH binds specifically to a AMH receptor type II (AMHR-II), cloned in 1994 by two different groups (Baarends et al., 1994; di Clemente et al., 1994b).

Mapped to chromosome 12 (13.q12), it has a total length of 8.7 kbp and is divided into 11 exons. The first three encode the extracellular domain, exon 4 codes for most of the transmembrane domain and the rest encodes the intracellular domain where the kinase consensus elements are located. All AMH biological effects transit through this receptor, (Mishina et al., 1999) expressed in Müllerian ducts and gonads of both sexes (Baarends et al., 1994; di Clemente et al., 1994b) and also in endometrium (Renaud et al., 2005), prostate and mammary gland (Hoshiya et al., 2003b).

The situation concerning type I receptors is more complicated. Gouéddar et al. (2000) were the first to point out that AMH signals through Alk6 and Smad1, elements 'borrowed' from the transduction pathway of the BMPs which also belong to the TGF-β family. Since then, other investigators (Clarke et al., 2001; Visser et al., 2001; Jamin et al., 2002) have demonstrated that the whole repertoire of type I receptors and Smad molecules used by the BMP family are also part of the AMH transduction pathway (Figure 7). The most important type I receptor is Alk3, because its targeted inactivation results in persistence of Müllerian ducts in transgenic mice (Jamin et al., 2002).

The main difference between the BMP and AMH transduction pathways appears to lie in the mode of ligand/receptor interaction. In the BMP family, apart from growth differentiation factor 9, the pathways appears to lie in the mode of ligand/receptor interaction. In the BMP family, apart from growth differentiation factor 9, the type I receptors are generally the high-affinity binding receptors, whereas type II receptors are dispensible (reviewed in Nohe et al., 2001; Jamin et al., 2004). Such does not appear to be the case for AMH which requires its type II receptor for transduction (Gouéddar et al., 2000).

Mutations of the AMHR-II

In thirty three families (40% of the total number) mutations of the AMHR-II have been identified (Figure 3B). Mutations of
various types are spread out along the entire length of the gene. A deletion of 27 bp, located on exon 10, del6331–6357, is particularly frequent and is present in the homozygous state or associated with a different mutation in approximately half of patients affected with an AMHR-II mutation (Josso et al., 2003a). It can be rapidly recognized by a simple PCR and its identification does not require sequencing of the gene. The level of circulating AMH is normal for age. Otherwise, patients with AMH or AMHR-II mutations are clinically similar.

Functional studies were undertaken in selected mutations, reproduced by targeted mutagenesis in COS cells (Figure 8). Three ‘soluble’ mutations, characterized by a truncation upstream of the transmembrane domain, are probably unstable since they are not detectable in the culture medium of recombinant cells. Otherwise, most mutations located in the extracellular domain impair the secretory process and prevent the insertion of the receptor into the cell membrane (Faure et al., 1996) precluding binding to AMH. A similar situation was described recently for an homozygous mutation in the extracellular domain of the LH receptor. Using structural predictions for the ligand-binding domain, Richter-Unruh et al. (2004) hypothesized that steric hindrance was responsible for the aberrant trafficking of the protein. At the present time, the AMHR-II receptor has not been modeled. Mutations located in the intracellular domain, for instance R406Q which is thought to disrupt the substrate-binding site of the kinase domain (Messika-Zeitoun et al., 2001), migrate normally to the cell surface but are unable to transduce the AMH signal. One stop mutation, truncating the receptor immediately after the transmembrane domain, binds AMH normally but, when overexpressed in vitro, exhibits dominant negative activity (Messika-Zeitoun et al., 2001), as expected when the mutant protein competes for binding with the wild-type.

‘Idiopathic’ PMDS

In eleven families (13% of the total number), sequencing of the AMH or AMHR-II gene revealed no abnormality in the exons, proximal intronic sequences or proximal promoter. Serum AMH concentration is normal for age. In half the cases, other severe congenital abnormalities were present (see Josso et al., 2003b for details). Assuming that a mutation in the distal promoter or intronic sequences (Hoshiya et al., 2003a) of AMHR-II has not been overlooked, mutations in a downstream component of the AMH transduction pathway is the next possibility. At first glance, a mutation in one of the candidate type I receptors does not appear likely, because these receptors are shared with the BMPs and are required for embryonic survival or at least for normal bone development. Indeed, sequencing of ALK2, 3 and 6 in these families revealed no abnormality (Belville, unpublished). The Smad molecules are probably not involved either, because mutations are usually associated with a high incidence of cancer, which has not been reported in these families. In view of the high incidence of associated congenital defects, ‘idiopathic’ PMDS could be part of a complex malformative syndrome, the etiology of which is not specifically related to AMH transduction. Similarly, the Mayer-Rokitansky-Küster-Hauser syndrome is not associated with abnormal activation of the AMH cascade (Oppelt et al., 2004; Zenteno et al., 2004). This syndrome is the mirror image of PMDS, consisting of the aplasia of Müllerian duct derivatives (uterus and upper vagina) in phenotypic females; ovaries are typically normal but many phenotypic variations have been described, including associated malformations, essentially renal aplasia (Simpson, 1999).

AMH-related mutations in females

AMH clinical research in females has recently exploded. Fauser and his colleagues were the first to stress the value of AMH measurements for the assessment of follicular reserve (de Vet et al., 2002; van Rooij et al., 2002; Weenens et al., 2004) and hence the prognosis of IVF. In contrast, the role of AMH in human ovarian function does not appear crucial. Animal studies have demonstrated that AMH represses the LH receptor and aromatase of granulosa cells (di Clemente et al., 1994a) and the recruitment of primary follicles (Durlinger et al., 1999) leading to premature cessation of ovarian cycling in AMH KO mice (Gruijters et al., 2003). Female relatives with the same genotype as the PMDS patients are normally fertile, however, the duration of follow-up is not sufficient to exclude that they will undergo early menopause.

References


Gene mutations in persistent Müllerian duct syndrome

Figure 8. Mutations of the AMHR-II receptor reproduced in COS cells. The boxed mutation is recurrent, representing 50% of receptor mutations, in heterozygous or homozygous form.


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