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# **The persistent Müllerian duct syndrome : an update based upon a personal experience of 157 cases**

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## ABSTRACT

Male sex differentiation is driven by two hormones, testosterone and anti-Müllerian hormone (AMH), responsible for regression of Müllerian ducts in male fetuses. Mutations inactivating AMH or its receptor AMHRII lead to the Persistent Müllerian Duct Syndrome (PMDS) in otherwise normally virilized 46,XY males. Our objective was to review the clinical, anatomical and molecular features of PMDS, based upon a review of the literature and upon 157 personal cases. Three presentations exist: bilateral cryptorchidism, unilateral cryptorchidism with contralateral hernia, and transverse testicular ectopia. Abnormalities of male excretory ducts are frequent. Testicular malignant degeneration occurs in 33% of adults with the disorder. Cancer of Müllerian derivatives is less frequent. Fertility is rare but possible if at least one testis is scrotal and its excretory ducts are intact. Eighty families with 64 different mutations of the AMH gene have been identified, mostly in exons 1, 2 and 5. AMHRII gene mutations representing 58 different alleles have been discovered in 75 families. The most common mutation, a 27 bp deletion in the kinase domain, was found in 30 patients of mostly Northern European origin. In 12% of cases, no mutation of AMH or AMHRII has been detected, suggesting a disruption of other pathways involved in Müllerian regression.

## INTRODUCTION

Male sex differentiation is driven by two separate hormones [Jost, 1953], each produced by a separate cell compartment of the fetal testis. Testosterone produced by fetal Leydig cells maintains Wolffian ducts and promotes virilization of external genitalia. Anti-Müllerian hormone (AMH), a member of the TGF- $\beta$  family [Cate et al., 1986] synthesized by fetal Sertoli cells [Josso, 1973], is responsible for the regression of fetal Müllerian ducts, which in its absence would differentiate into uterus, Fallopian tubes and upper vagina. Disorders of sex development (DSD) in males may affect either one or both hormones. The persistent Müllerian duct syndrome (PMDS) belongs in the first category: AMH is either not secreted or inactive, while testosterone is normally produced and metabolized. External virilization is complete but, due to AMH deficiency, Müllerian ducts do not regress and coexist with testes and male excretory ducts. Nilson [1939] is often credited with the description of the first case but earlier publications can be found in the world literature [Jordan, 1895; Webster, 1906].

PMDS is defined as the presence of Müllerian derivatives, uterus and Fallopian tubes in otherwise normally masculinized 46,XY subjects. The PMDS patient is outwardly completely male, the urethra opens at the tip of the penis (there is no hypospadias). The absence of external genital ambiguity differentiates PMDS from mixed gonadal dysgenesis, a completely different type of DSD that affects both Leydig and Sertoli cells. Until 1989, diagnosis was based exclusively upon clinical data but following the cloning of the AMH [Cate et al., 1986; Picard et al., 1986] and AMHRII [Baarends et al., 1994; di Clemente et al., 1994] cDNA and genes, it has emerged that there is not always a perfect agreement between molecular and clinical data. A few individuals with AMH or AMHRII mutations may lack Müllerian remnants, whereas in others with clinical features of the condition, no mutations can be detected.

**The incidence** of PMDS has not been accurately determined. It has the reputation of being a very rare condition. In fact, prior to the advent of Medline, approximately 60 publications had been collated by various authors [Royer et al., 1961; Scrivere et al., 1976; Golladay and Redman, 1982]. Two hundred more have been published between 1964 and 2012 [reviewed by Farikullah et al., 2012]. Since then, the rate has greatly accelerated with 34 new cases published over the last 4 years [reviewed by Elias-Assad et al., 2016]. The apparently increasing incidence may be due to the fact that cryptorchidism, the usual presenting symptom, is now investigated early in life, laparoscopy has become routine and more surgeons are aware of the condition now than in the past. Between 1990 and 2016 our group has performed molecular studies in 157 families with PMDS. Mutations of either the AMH or the AMH receptor type II (AMHRII) genes have been detected in 88% of

cases. This review is based on our personal experience and upon the literature accessible to us in complete or abstract form. A chi-square test was used to compare the characteristics of patients with different genotypes.

## ANATOMICAL FEATURES

**Position of the testes** During normal male embryological development, under the influence of AMH, Müllerian ducts have totally disappeared at 10 weeks of fetal development, releasing the testes from their initial position in the pelvis and allowing them to reach the scrotum, provided the cord is of sufficient length. If the Müllerian ducts fail to regress, the testes remain suspended in the broad ligament. In females the broad ligament is solidly attached to the pelvis, but in PMDS, the connection is usually flimsy or non-existent [Miller et al., 2004], allowing the testes, still attached to the Fallopian tubes, to descend towards the inguinal canal and scrotum, dragging the uterus in their wake.

The degree of mobility of Müllerian derivatives determines the position of the testes (Fig 1). Schematically, if the uterus is fixed in the pelvis, the testes retain a high “ovarian-like” location; alternatively one or both testes may make it into the inguinal canal or the scrotum, dragging the Müllerian derivatives along. Often, one testis is contained in an inguinal hernia together with the uterus and one Fallopian tube, hence the name of “*hernia uteri inguinalis*” given to this condition. Inguinal hernias are very common in all forms of PMDS and may develop at any time postnatally. The anatomical condition most specific of PMDS is transverse testicular ectopia. Both testes have descended into the same hemi-scrotum and are contained in the same hernial sac, together with the uterus and tubes. Testicular fusion has been reported in this condition [Zhapa et al., 2010]

The scrotal PMDS testis is only loosely anchored to the bottom of the processus vaginalis by a thin gubernaculum resembling the round ligament of the uterus [Hutson et al., 1994]; it is abnormally mobile and easily drawn into the contralateral scrotal pouch. In two of our cases, transverse testicular ectopia was the only sign of an AMH mutation, as Müllerian derivatives had regressed normally. Rarely, one or both testes are missing in patients fulfilling the criteria for PMDS [Imbeaud et al., 1995b; Souto et al., 1995; El-Gohary, 2003]. Testicular torsion, facilitated by the abnormal mobility of the testes, has been incriminated [Chaabane et al., 2010].

There is no significant difference in anatomy between patients with either AMH or AMHR11 gene mutations, and slightly more than half the patients present with bilateral cryptorchidism, a fifth with unilateral cryptorchidism and contralateral hernia and the rest with transverse testicular ectopia. In contrast, the characteristic transverse testicular ectopia has never been observed in patients with “idiopathic” PMDS, i.e. those with apparently intact AMH and AMHR11 genes (Fig 2). The position of the testes/Müllerian duct complex in PMDS may differ between brothers with the same genotype [Knebelmann et al., 1990; Abduljabbar et al., 2012; Nalbantoglu et al., 2015]. In patients described in the literature in whom molecular analysis has not been performed, the proportions are not very different: 41% for bilateral cryptorchidism, 32% for *hernia uteri inguinalis* and 27% for transverse testicular ectopia.

**Male excretory ducts** The anatomy of the male excretory ducts deserves special attention. Initially the vas deferens is included in the mesosalpynx, and then reaches the uterine wall, eventually penetrating it to open at the top of the vagina, the anatomical equivalent of the prostatic utricle (Fig 1). The spermatic vessels are usually very short and must be divided at surgery to allow placement of the testis in the scrotum. This leaves the vascularization of the testis at the mercy of the deferential artery, in close proximity to the Müllerian derivatives and therefore easily damaged by attempts to remove them (see the section on Treatment). In PMDS, the vas is often abnormal, narrow, blind or even absent. Epididymal dissociation from the testis is common. Abnormalities of male excretory ducts are often reported in the literature [Bhatnagar, 1962; Binns and Cross, 1967; Scriver et al., 1976; Sloan and Walsh, 1976; Mouli et al., 1988; Morikawa et al., 2014]. Ductal and/or epididymal abnormalities were specifically mentioned in 9 and 13 of patients with respectively AMH and AMHR11 mutations and in 2 idiopathic cases with no detected molecular defects. However, ductal and

epididymal anomalies are commonly associated with cryptorchidism, particularly its abdominal variant, even in the absence of PMDS [Mollaeian et al., 1994; Sharma and Sen, 2013].

**Müllerian derivatives** The development of Müllerian derivatives is extremely variable, even between brothers. The uterus may be comparable in size to that of normal females but it is often much smaller. Uterus and tubes are often contained in an inguino-scrotal hernia where they are easily detectable at surgery. However, when the uterus is retained in the pelvis, its presence can be missed [El-Gohary, 2003; Farikullah et al., 2012; Goto et al., 2012] as seen in several of our patients. The Müllerian ducts enter the upper urethra at the level of the prostate but the communication is often obliterated. Sometimes, Müllerian derivatives are not found in subjects bearing an AMH or AMHR11 mutation. They may be totally asymptomatic [Abduljabbar et al., 2012] or present with isolated transverse testicular ectopia.

## CLINICAL FEATURES

In children, PMDS is usually discovered at surgery or laparoscopy motivated by cryptorchidism with or without inguinal hernia (Fig 3). Unilateral or bilateral cryptorchidism are not particularly evocative although unilateral cryptorchidism with contralateral hernia and particularly transverse testicular ectopia should awake suspicion. If a sibling has been diagnosed with the condition, imaging investigations should be scheduled [Di et al., 1998; Di Cesare et al., 1998; Dekker et al., 2003]. Ultrasonography of the scrotum and pelvis is generally sufficient to suspect PMDS, but magnetic resonance imaging could be necessary to recognize the different structures. Occasionally, the patient has a history of a previous surgical procedure for hernia or inguinal cryptorchidism. Adults in whom cryptorchidism or inguinal hernia have been disregarded may come to medical attention because of hematuria due to hormonal imbalance in aging patients whose testes produce less androgens and an excessive amount of estrogens [Gricourt et al., 2010]. Cyclic hematuria led to the discovery of PMDS in a 27-year old patient with no apparent hormonal abnormalities [Smith-Harrison et al., 2015]. More often, discovery of PMDS is contingent upon the development of testicular or Müllerian malignancies.

**Malignant degeneration.** Nowadays in developed countries, cryptorchidism is treated as early as possible to avoid damage to germ cells but formerly, this was usually not the case. Adult PMDS patients were often diagnosed because of malignant degeneration of the testes or the Müllerian remnants. The incidence of testicular cancer in PMDS has been formerly estimated at 18%, no higher than the risk for cryptorchid testes in general [Bucci et al., 2002; Shamim, 2007]. Our own perusal of the literature yields a much higher figure: 33% of PMDS patients 18 years and older experienced some form of unilateral or bilateral malignant testicular degeneration. Seminomas are the most frequent, but choriocarcinomas [Giri et al., 2004; Aboutorabi et al., 2005], mixed germ cell tumors [Eastham et al., 1992; Manassero et al., 2004; Jaka and Shankar, 2007; Mohapatra and Subramanya, 2016] embryonal cell carcinoma [Melman et al., 1981; Carré-Eusèbe et al., 1992; Barad et al., 2016] gonadoblastomas [Morillo-Cucci and German, 1971] or yolk sac tumor [Snow et al., 1985] have also been described. In our own cohort, we encountered 3 testicular tumors (H069 and H050) [Carré-Eusèbe et al., 1992] and H009, all adults with AMH mutations (see Suppl. Table 1). Young patients may be affected by germ cell neoplasia *in situ*, as seen by Williams et al [1994] in a 17-year-old patient and by ourselves in a 15 month-old boy with a receptor defect (R031) (Suppl. Table 2). Early orchidopexy is recommended to preserve against testicular degeneration but is not necessarily 100% effective. Melman et al [1981] have reported embryonal carcinoma in a 16-year-old boy who had undergone bilateral orchidopexy at 2 months of age. Likewise, Manassero et al [2004] observed a mixed germ cell tumor in a 23-year old patient operated at 5 years of age for transverse testicular ectopia. These observations, in addition to the abnormally high incidence of testicular cancer in PMDS compared to simple cryptorchidism, suggests that misplacement of the testis may not be the only factor favoring malignant degeneration in this disorder. In mice, deletion of the AMH [Behringer and Cate, 1994; Matzuk et al., 1995] or AMHR11 gene [Tanwar et al., 2012] facilitates testicular tumors induced by respectively inhibin suppression or WT1/ $\beta$ -catenin imbalance. Prognosis depends upon the

histological type of the tumor, choriocarniomas [Giri et al., 2004; Aboutorabi et al., 2005] and mixed germ cell tumors [Manassero et al., 2004; Jaka and Shankar, 2007] share a poor prognosis.

Malignant degeneration of Müllerian derivatives is much less frequent. Farikullah et al. [2012] found 11 cases of male Müllerian duct degeneration in the literature but only 3 qualified as PMDS. The rest were so-called “Müllerian” cysts, i.e. prostatic utricle cysts [Kato et al., 2002] which arise at the site of confluence between the urogenital sinus, the Müllerian and the Wolffian ducts [Glenister,1962]. Hematuria is often the presenting sign [Thiel and Erhard, 2005]. Two young men with uterine adenocarcinoma died from metastatic disease one at 14 years of age [Thiel and Erhard, 2005] and the other at age 39 [Romero et al., 2005]. An additional patient [Kovachev et al., 2014] was diagnosed with a benign uterine leiomyoma.

**Infertility** is the most frequent complication of PMDS. In our personal experience, only one patient with an AMH mutation, (H071), fathered a child after testicular sperm extraction motivated by oligo and asthenozoospermia. We noted similar sperm defects in two other patients, one with an AMH and two with an AMHR11 mutation [Zeller et al., 1994]. In the literature, sterility is indeed the rule but normal spermatogenesis has been reported. A thorough literature search found that 19% of reported adult patients fathered one or more children [Karimi-Nejad et al. 1988; Farag, 1993; Berkmen, 1997; Aboutorabi et al., 2005; Liang et al., 2006; Jaka and Shankar, 2007; Prakash et al., 2009; Sichani et al., 2009; Inuganti et al., 2011; Kaore and Kaore, 2012; Kumar and Mohan, 2012; Kovachev et al., 2014; Sherwani et al., 2014; Agrawal and Kataria, 2015; Modi et al., 2015]. Farag et al [1993] reported an 11% proportion of fertile patients in Kuwait and neighboring populations. All these patients except one [Modi et al., 2015] presented with either transverse testicular ectopia or *hernia uteri inguinalis*, i.e. at least one testis was in a normal scrotal position. Proof of paternity was not provided but it is unlikely that all births could be the result of extra-conjugal relationships, particularly since many of the reports originated from countries where contact between the sexes is strongly discouraged outside of matrimony. We conclude that fertility is rare but possible in PMDS provided two conditions are met : at least one testis should be normally descended and the excretory ducts should be intact. All fertile PMDS patients fathered children before their condition had been diagnosed. Late diagnosis carries a price, however: many of these patients developed testicular cancer. PMDS dogs with uni or bilateral scrotal testes are also fertile [Meyers-Wallen et al., 1989].

**Congenital malformations associated with PMDS.** Various congenital abnormalities have been described in association with PMDS. Intestinal defects such as atresia or lymphangectasia (Urioste syndrome) were described in 4 cases [Klosowski et al., 1997; Bellini et al., 2001; vanHaelst et al., 2001]. Hirschsprung’s disease [Cass and Hutson, 1992], horseshoe kidney [Barad et al., 2016] or mental deficiency [Snow et al., 1985], lipodystrophy and vitamin D-resistant rickets [Van Maldergem et al., 1996], renal polykystosis, hydronephrosis or deafness may also be present, as well as prematurity or small for gestational age syndrome. In our experience, in patients with associated malformations AMH or AMHR11 mutations are seldom detected.

## ENDOCRINE INVESTIGATIONS

Endocrine investigations in PMDS usually show that Leydig cell function is normal, except in patients with testicular degeneration [Imbeaud et al., 1995b]. The AMH concentration in serum depends upon the molecular origin of the disorder. Very low or undetectable serum AMH concentrations in prepubertal boys are characteristic of mutations of the gene coding AMH. Low AMH concentration in post-pubertal and, to a lesser degree, newborn males is physiological [Grinson et al., 2011] and should not be interpreted as pointing to an AMH mutation. AMH serum concentration may be decreased immediately after surgery. In patients with virilizing defects such as hypospadias, low AMH reflects testicular dysgenesis, not to be confused with PMDS. Normal values of serum AMH in developing boys are shown in the Table. AMH was measured in the serum of all our cases by the referring physician. The results are not comparable because they were obtained with different methods [Nelson et al., 2015].

Normal AMH levels usually exclude an AMH mutation, with the exception of AMH mutation p.(Gln496His) which is thought to affect receptor binding [Belville et al., 2004]. The sequence variations reported in an Italian patient [Menabo et al., 2008] with a normal serum AMH level could be innocuous, and an AMHR11 mutation cannot be ruled out because the gene was not sequenced. Normal for age levels of circulating AMH are characteristic of AMHR11 mutations, and circulating levels of AMH are usually not increased, as is the case in androgen insensitivity. However, receptor mutations are not always detectable in PMDS patients with normal levels of serum AMH. Idiopathic PMDS is described below.

## INHERITANCE

PMDS is an inherited disease transmitted as an autosomal recessive trait explained by the location of the AMH gene on the short arm of chromosome 19 [Cohen-Haguenauer et al., 1987] and that of the AMHR11 gene on the long arm of chromosome 12 [Imbeaud et al., 1995a]. The rate of consanguinity is high: 40% and 33% in families with respectively AMH and AMHR11 mutations but only 10% in idiopathic PMDS. The rate of homozygosity varies in different parts of the world but is grossly the same for AMH and AMHR11 mutations, respectively 65% and 57% (Fig 4).

Two reports [Sloan and Walsh, 1976; Naguib et al., 1989] raise the possibility that PMDS may sometimes be transmitted as an X-linked condition but they are difficult to interpret in highly inbred communities [Naguib et al., 1989] since many family members might carry the pathological allele. Heterozygous subjects with one normal allele are clinically normal. There is no phenotype in females. Homozygous sisters and, in one instance, the homozygous mother of a PMDS propositus (H066), are normal and fertile. AMH-null female mice experience early depletion of their follicular pool [Durlinger et al., 1999]. Possibly, women homozygous for AMH or AMHR11 mutations will undergo premature menopause, but this cannot be verified at the present time.

## TREATMENT

Treatment aims at the prevention of the two main complications of PMDS: infertility and cancer. Paradoxically, as mentioned above, the best chances of fertility are sustained by patients having escaped treatment altogether but this is no longer likely because cryptorchidism and inguinal hernia are now surgically corrected in early childhood. The surgeon should bear in mind that the male excretory ducts are in close apposition to or even enclosed in the walls of the Müllerian derivatives thus any attempt to remove the Müllerian organs *in toto* will automatically damage the vas deferens or the deferential artery. Why then not leave them in place? For several reasons: they may, although rarely, undergo malignant degeneration (see above) or cause discomfort or hematuria in the event of estrogen excess [Gricourt et al., 2010]. However, the main reason for hysterectomy is mechanical. When both testes are in an abdominal position, the uterus blocks testicular descent and orchidopexy is impossible. To overcome this difficulty, the fundus of the uterus can be split in the midline [Brandli et al., 2005; Manjunath et al., 2010] and the uterine walls carefully dissected to free the vas. The magnification provided by laparoscopy is helpful in this respect [Farikullah et al., 2012]. Alternatively, the uterus can be stripped of its mucosal lining to reduce the risk of malignant degeneration [Manjunath et al., 2010]. Abnormalities of male excretory ducts, whether congenital or iatrogenic, are treatable by testicular sperm extraction followed by intracytoplasmic sperm injection if fertility is desired, this procedure was successful in patient H071 (Suppl. Table 1)

The uterus is not the only obstacle to testicular descent. When testes are in a high abdominal position, the cord is often much too short to allow placement of the testis in the scrotum. The internal spermatic artery must then be divided and testicular viability becomes dependent on the deferential artery. To minimize the risk to this vessel, pedicles of myometrium should be left adhering to the vasa and the fimbriae of the Fallopian tube should not be dissected from the testis [Vandersteen et al., 1997]. As an alternative to the Fowler-Stephens procedure, microvascular autotransplantation of the testis has been successfully performed [Brandli

et al., 2005]. Most of these procedures can be carried out by laparoscopy [Bowen et al., 2016] or robotic surgery [Smith-Harrison et al., 2015].

Testicular biopsy is unnecessary provided the diagnosis of PMDS is not in doubt. In children, testicular morphology is normal [Loeff et al., 1994; van der Zwan et al., 2012], and this is also our experience. In older patients, testes are often hypoplastic with tubular fibrosis, thickened basal membrane and no spermatogenesis [Mouli et al., 1988]. If the testes cannot be brought down, orchidectomy is recommended to avoid future malignant degeneration but should be considered only as a last resort. Obviously, there is no justification for castration in children without any attempt at orchidopexy [Guell-Gonzalez et al., 1970; Armendares et al., 1973]. A horrifying report from Pakistan [Kaore and Kaore, 2012] relates an *en bloc* removal of uterus and scrotal testes in a fertile 35-year old man with transverse testicular ectopia; such mutilations are thankfully exceptional.

## MOLECULAR STUDIES

In our experience, mutations of the AMH or the AMHRII gene are responsible for 88% of PMDS cases; the others have no identified molecular cause and are presently labelled “idiopathic”.

### AMH GENE MUTATIONS

AMH, a glycoprotein dimer belonging to the TGF- $\beta$  family [Cate et al., 1986] is coded by a 5 exon gene 2.8 kbp long, located on the short arm of chromosome 19 p.13.3 [Cohen-Haguenaer et al., 1987]. AMH, like other members of the family is translated as a dimeric precursor comprising two polypeptide chains, each containing a large N-terminal pro-region and a much smaller C-terminal mature domain which shows homology with the other members of the family and carries the bioactivity of the molecule. Cleavage at arginine 451 yields 110 kDa N-terminal and 25 kDa C-terminal dimers which remain associated in a bioactive non-covalent complex [Pepinsky et al., 1988]. While cleavage is obligatory, dissociation of the two fragments is not required for binding to the receptor and subsequent biological activity [di Clemente et al., 2010] (Figure 5). In the absence of the N-terminal fragment the C-terminus cannot fold properly and is rapidly degraded prior to secretion, explaining the pathogenicity of N-terminal mutations.

Patients with AMH mutations are described in Supplemental Table 1. The first one, a Stop mutation of the 5th exon affecting the N-terminal domain, was detected in 1989 in three brothers belonging to a Moroccan family [Knebelmann et al., 1990]. Since then, we ourselves have identified 68 families with AMH mutations; 12 have been described by other groups [Mazen et al., 2011; Nishi et al., 2012; van der Zwan et al., 2012; Morikawa et al., 2014; Nalbantoglu et al., 2015; Mazen et al., 2016]. 65% of mutations are homozygous reflecting a high rate of inbreeding in some parts of the world (Fig 4). Given the high value placed on fertility in the culture of these populations, we recommend genetic testing in the relatives of PMDS patients with identified AMH or AMHRII mutations and their prospective partners, if marriage is contemplated. In six instances, only one abnormal allele could be identified. The most likely explanation is that the other escaped detection, perhaps because it is located in the center of an intron or for technical reasons. The AMH gene is difficult to sequence because it is very rich in guanine and cytosine bases.

Altogether, a total of 64 different pathogenic AMH alleles have been identified in 80 families: 37 missense, 10 Stop and one non-stop p.(<sup>\*</sup>561Cys<sup>ext</sup>\*?), 9 deletions (including one insertion/deletion (H031), 2 insertions and 5 splicing mutations (Suppl. Fig. 1). Mutations occur along the whole length of the gene though exon 4 is very rarely involved (Fig. 6A); exons 1 and 2 are proportionally the most affected. In exon 5, the bases coding the biologically active C-terminus are hit at nearly three times the rate of the N-terminal ones. There are no significant “hotspots” though many mutations are recurrent (**Fig. 7A**). Six have been found in 4 or more families while 13 others have occurred less frequently. Intron 2 is the target of two distinct recurrent splicing mutations, the first affecting the donor and the second one the acceptor site. The latter, a A>G change at the



penultimate base of the 2<sup>nd</sup> intron, was found in three unrelated Brazilian patients and may represent a founder effect [Nishi et al., 2012]. Similarly, the four families affected by mutation c.301G>A, p.(Gly101Arg) all originate from the Middle East or Pakistan [Abduljabbar et al., 2012]. The five families affected by mutation c.500A>G, p.(Tyr167Cys) are Northern European.

Belville et al [2004] have studied the biosynthesis and secretion of seven mutant AMH proteins. Those lacking a C-terminal domain are secreted more rapidly whereas single amino acid changes in either the C- and the N-terminal domain have significant effects upon protein stability and folding. Addition or elimination of cysteines are particularly damaging.

Alleles deserving a special mention are shown on Fig. 7A.

A deletion within the promoter, 225 bp upstream from the initiating ATG, results in the loss of the 4<sup>th</sup> base of the SF1 response element, TCAAGGACAG [Valeri et al., 2016]. The AMH promoter carries two SF1 response elements, a proximal one at -102 from the ATG [Shen, 1994] and a distal one at -228 [Watanabe et al., 2000]. Both sites are involved in the upregulation of AMH gene expression in cultured Sertoli cells [Shen et al., 1994; Lasala et al., 2011]. In transgenic mice Arango et al [1999] found that inactivation of the proximal site did not prevent Müllerian duct regression. The persistence of Müllerian derivatives in our patient shows that the distal SF1 response element is absolutely required for significant human AMH expression.

Another mutation, c.3G>T, observed in a Moroccan patient, H002, changes the translation initiation codon ATG for methionine into ATT, coding for isoleucine. The molecular effect of the mutation is not known. Kozak [Kozak, 2002] has shown that proximity to the 5' end plays a dominant role in identifying the start codon. If the canonical start site is destroyed, translation may initiate at the next available ATG. However, in the AMH protein the next ATG at position 191 is not in frame and the next in frame ATG is far downstream in exon 4. It is highly unlikely that either could allow translation of a functional protein [Mentrup et al., 2011]. Most start codon mutations result in complete absence of the affected protein [Sargiannidou et al., 2015; Jinda et al., 2016].

The “nonstop” mutation c.1683A>T changes the stop codon TGA into TGT the cysteine codon. No downstream in frame stop codon is present in the 3' untranslated region before the polyadenylation site, suggesting that the translated protein must be significantly extended. However, such extra large proteins are usually not produced because of ribosome stalling at the 3' end of the mutated mRNA, which blocks translation before the full-length polypeptide can be synthesized [Hamby et al., 2011]. A recurrent mutation c.35 T>G [Imbeaud et al., 1994] leads to the replacement of valine by glycine at position 12 p.(Val12Gly), in the midst of the signal sequence.

The DNA of patient H075 carries two homozygous mutations, p.(Ala314Gly) and p.(Gly533Val), none of which correspond to recognized polymorphisms. His serum AMH level was very low. Gly533 is located in the  $\beta$ 6 strand of the mature domain (Fig. 8). The  $\beta$  sheet structure would probably not be disrupted by a valine substitution, in fact quite the opposite could occur. Glycine is an intrinsically destabilizing residue in  $\beta$  sheets [Merkel and Regan, 1998] and its presence in the  $\beta$  strands of several natural proteins might be due to its ability to inhibit aggregation and formation of amyloid fibrils [Parrini et al., 2005]. Thus the Gly533Val mutant protein might be less stable and prone to aggregation. The fact that Gly533 is conserved in AMH across species provides support for this hypothesis. The role, if any, of the Ala314Gly mutation, located in the  $\beta$ 7 strand of the pro-region, is not clear. The replacement of Ala314, which is strongly conserved across species, by glycine could destabilize the  $\beta$  sheet and thereby add to the instability of the double mutant protein, but no conclusions can be drawn in the absence of experimental data. By comparison to a molecular model of the BMP9 noncovalent complex [Mi et al., 2015], it can be inferred that the  $\beta$ 7 strand in the pro-region is too far away from the cleavage site at Arg451 to interfere with processing.

The impact of splicing mutations cannot be assessed in the absence of mRNA. In the male, AMH is expressed nearly exclusively in testicular Sertoli cells. When tissues normally expressing a protein of interest are not

available, “illegitimate” transcription may be successful [Chelly et al., 1989]. In patient H044, we were able to obtain AMH mRNA by amplifying a discrete RT-PCR product obtained from uterine mRNA primed for reverse transcription. Sequencing of AMH mRNA revealed retention of the first 46 bases of intron 1 and use of a downstream cryptic donor site at position +47 in intron 2 [Gricourt et al., 2010].

The polymorphism c.146T>G, p.(Ile49Ser) seen in 21% of normal subjects is identical to the sequence of bovine AMH. For this reason, it is not expected to affect AMH bioactivity. In normal women it is associated with higher levels of estradiol at the follicular phase of the menstrual cycle, suggesting a role in ovarian sensitivity to FSH [Kevenaar et al., 2007a].

## AMH RECEPTOR MUTATIONS

Like other member of the TGF- $\beta$  family, AMH signals through two membrane-bound serine/threonine kinase receptors type I and type II. Paradoxically the type II receptor, AMHRII, is the primary one and is AMH-specific. AMHRII binds AMH after the latter has undergone proteolytic cleavage at Arg 451, and then activates a type I receptor, ALK 2, 3 or 6. This leads to the phosphorylation of R-Smad proteins 1, 5 or 8, its interaction with Smad4 and the translocation of the R-Smad/Smad4 complex to the nucleus where it promotes or more likely represses the transcription of target genes (Fig. 9). AMH shares its type I receptors, and its R-Smad effectors, with the bone morphogenetic proteins (BMPs) [reviewed in Orvis et al., 2008]. Since the BMP pathway is required for early embryonic development, it cannot be involved in isolated PMDS and so the only possible culprit is the specific AMH type II receptor, AMHRII. AMHRII is a 573 amino acid membrane protein containing an N-terminal extracellular domain that binds AMH, a single transmembrane domain, and an intracellular domain with serine/threonine kinase activity. The AMHRII gene, located at 12q13 [Imbeaud et al., 1995a] contains 11 exons spread over 8 kbp. The first three encode the extracellular domain which binds cleaved AMH [di Clemente et al., 2010], the fourth encodes the transmembrane domain and the last seven exons encode the catalytic intracellular serine/threonine domain (Fig. 7B). The level of bioactive AMH in biological fluids can be assessed by monitoring their capacity to bind to AMHRII [Pierre et al., 2016].

There is no phenotypic difference between patients with either AMH or AMHRII mutations (Fig. 2), confirming the monogamic relationship between AMH and its type II receptor [Mishina et al., 1999]. Patients with AMH receptor mutations are described in Supplemental Table 2. The first one was described in 1995 [Imbeaud et al., 1995a]. Since then a total of 75 families with PMDS due to AMHRII mutations have been identified, 68 by our group. The other cases were reported in the USA [Hoshiya et al., 2003], Brazil [Nishi et al., 2012], Egypt [Mazen et al., 2016], Portugal [Rosal-Gonçalves et al., 2010], Turkey [Korkmaz et al., 2017] and Israel [Elias-Assad et al., 2016]. A total of 58 different alleles have been identified: 35 missense, 11 stop, 8 deletions, and 4 splicing defects (Suppl. Fig. 2). All 11 exons are affected (Fig 6B). Two different mutations of the initiation codon were observed (R001 and R002) (Fig 7B) with unknown consequences. The next methionine is at position 76, probably too far downstream to initiate translation (see section on AMH mutations). Patient R024 was initially considered to be homozygous for c.532C>T, p.(Arg178\*). However, while the father was heterozygous as expected, surprisingly, only the normal allele could be detected in the mother. To explain this paradox, we hypothesized that she was heterozygous for a large deletion of all or part of the AMHRII gene. This hypothesis has been confirmed [Lucie Tosca and Gérard Tachdjian, personal communication]; studies are in progress to determine the extent of the deletion.

Ten recurrent mutations have been identified (Fig 7B). The most frequent, a deletion of 27 base pairs in exon 10, c.1332\_1358del, p.(Gly445\_Leu453del) according to the present official nomenclature, was initially considered to result in p.(Leu444\_Glu452del) [Imbeaud et al., 1996]. It has been observed in 30 patients, 67% of whom are Northern Europeans, a proportion significantly different from the 36% of that origin in the 75 patients with AMHRII mutation ( $p < 0.05$ ), suggesting a founder effect. Twenty out of 27 Northern European patients carry the deletion, compared to only one out of 11 Southern Europeans. The deletion can be recognized by PCR (Fig. 10). When a receptor mutation is suspected, we recommend performing this procedure

as a first line investigation. Other recurrent mutations are much less prevalent. The c.1219C>T mutation, resulting in the change of arginine at position 407 to a Stop codon, has been observed in 6 families, 4 from Mediterranean countries, 1 from Saudi Arabia and one Brazilian patient of undisclosed ancestry. The number of families affected by the other recurrent mutations is too small to allow geographical analysis.

Belville et al. [2009] have studied the impact of receptor mutations upon expression, stability and secretion. Interestingly, in contrast to other receptors of the TGF $\beta$  family, mutations of the extracellular domain which truncate the transmembrane domain just upstream of the transmembrane domain are not secreted, unless the endogenous signal sequence is replaced with that of the TGF $\beta$  receptor type II, indicating that the AMHRII signal sequence is defective. This likens AMHRII to a type III membrane protein, whose transmembrane domain acts like a signal anchor directing the N-terminal end of the protein to the outside of the cell [Goder and Spiess, 2001]. Like similar receptors of the TGF $\beta$  family, these receptors are dominant negative when overexpressed but *in vivo*, only siblings carrying the mutation on both alleles are affected by PMDS, presumably because the mutant and wild-type allele are expressed at a one to one ratio [Messika-Zeitoun et al., 2001].

Missense mutations in the extracellular domain cannot be analyzed reliably because of low sequence identity (<20%) between AMHRII and the receptor templates used to construct the three-dimensional model [Belville et al., 2009]. In the intracellular domain, two AMHRII mutants, c.596delA and the frequent allele c.1332\_1358del, p.(Gly445\_Leu453del) lack all of the kinase domain or contain a critical deletion. The p.(Gly445-Leu453del) mutation results in a deletion of nine amino acids that constitute part of the  $\alpha$ G helix and the loop that precedes it (Fig. 11A). In eukaryotic protein kinases, helices  $\alpha$ G,  $\alpha$ H and  $\alpha$ I have coevolved together with the Activation segment (Fig. 11B) and contribute to an important regulatory mechanism [Taylor and Kornev, 2011]. Mutations that disrupt communication between these two conserved elements have been shown to have severe effects on catalytic activity [Yang et al., 2012]. p.(Arg406Gln), p.(Asp491His) and p.(Arg504Cys) or p.(Arg504His) affect residues conserved in all type II receptors of the TGF $\beta$  family.

Incidentally, in women, a promoter A>G polymorphism, -482 bp from the ATG, is associated with follicular estradiol levels [Kevenaar et al., 2007a] and age at menopause [Kevenaar et al., 2007b]. A receptor mutation in exon 3, p.Arg81Stop, is responsible for PMDS in miniature Schnauzer dogs [Pujar and Meyers-Wallen, 2009; Wu et al., 2009], but the molecular defect in the Basset Hound with PMDS has not been elucidated [Nickel et al., 1992]. AMH and AMHRII mutations have also been identified in European bisons with persistent Müllerian derivatives [Panasiewicz et al., 2015].

## IDIOPATHIC PMDS

In approximately 12% of our PMDS cases, we have been unable to detect mutations in either the AMH or the AMHRII genes. We cannot exclude that some escaped our notice, particularly in patients studied several years ago with less effective sequencing methods. Mutations in the distal promoter or central introns would not have been detected either, for instance the receptor splice mutation described by Hoshiya [Hoshiya et al., 2003] would not have been identified in our laboratory. Another possibility is that “idiopathic” PMDS represents a separate entity. We favor this hypothesis because of the marked clinical differences between it and the syndrome due to AMH or AMHRII mutations. Associated malformations are frequent, the characteristic transverse testicular ectopia is not observed (Fig. 2) and the rate of consanguinity is low. Screening for other genetic alterations is planned in the few cases with sufficient DNA left for study.

Multiple genetic pathways implicated in Müllerian development have been invoked [Mullen and Behringer, 2014], namely the Wnt pathway. Wnt4 is required for Müllerian duct development in both sexes [Vainio et al., 1999]; Wnt7 triggers the expression of AMHRII in the Müllerian duct mesenchyme, in its absence Müllerian ducts do not regress in the male [Parr and McMahon, 1998]. AMH signaling induces the translocation of  $\beta$ -catenin and the accumulation of lymphocyte enhancer factor 1 (LEF1) in the nucleus of mesenchymal cells [Allard et al., 2000]. Normal AMH regression does not occur in male mice in which  $\beta$ -catenin has been

inactivated [Kobayashi et al., 2011]. The postulated mechanisms of interaction between the Wnt and AMH pathway are shown in Figure 9. Modern methods of genetic investigation [Bashamboo et al., 2010; Eggers et al., 2016] applied to idiopathic PMDS will probably produce unexpected results.

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## Legend of figures

**Figure 1:** The three main clinical presentations of persistent Müllerian duct syndrome. A) Bilateral cryptorchidism: the testes are in the pelvis, in the position of normal ovaries. This presentation is found in approximately 55% of cases with mutations in the AMH pathway and in 86% of idiopathic cases. B) Unilateral cryptorchidism: one testis is in an inguinal hernia along with its attached tube and uterus. This presentation is known as '*hernia uteri inguinalis*' and occurs in approximately 20% of cases with mutations of the AMH pathway and in 14% of idiopathic cases. C) Transverse testicular ectopia: both testes and part of the Müllerian organs have herniated into a single processus vaginalis. This presentation is very evocative of PMDS and is seen in approximately 25% of cases with AMH or AMHRII mutations but never in idiopathic cases ( $p < 0.001$ ). Permission obtained from Nature Publishing group Hutson JM et al Malformation syndromes associated with disorders of sexual development Nat. Rev. Endocrinol. 10, 476–487 (2014).

**Figure 2:** Position of the testes in PMDS patients with or without detectable mutations in the AMH pathway. Relevant data were available in 151 patients; the number in each category is indicated at the top of the bar.

**Figure 3:** Operative findings in a patient with a mutation of the AMH receptor, showing the adherence of the testes to the Fallopian tubes. Before surgery, this patient had transverse testicular ectopia. Reprinted with permission from Abduljabbar M, Taheini K, Picard JY, Cate RL and Josso, N Mutations of the AMH Type II receptor in two extended families with persistent Müllerian duct syndrome: lack of phenotype/genotype correlation Horm. Res. Paed. 77:291-297 2012

**Figure 4:** Homozygosity in PMDS patients in various parts of the world. AF: Africa (12 out of 17 patients are from Maghreb); AM: America (mostly South America); NE: Northern Europe (including France North of the Loire) SE: Southern Europe (including France South of the Loire); ME: Greater Middle East according to Wikipedia. The number of patients is indicated at the top of the bar.

**Figure 5:** AMH processing. Full length homodimeric AMH is cleaved at arginine 451 into a long N-terminal fragment and an short C-terminal fragment, carrying the biological activity The two fragments are associated in a non-covalent complex which is able to interact with the receptor. Separation of the C and N terminal fragments occurs only after binding to the receptor. Reprinted with permission from di Clemente N, Jamin SP, Lugovskoy A, Carmillo P, Ehrenfels C, Picard JY, Whitty A, Josso N, Pepinsky RB, Cate RL Processing of anti-Müllerian Hormone regulates receptor activation by a mechanism distinct from TGF. Mol Endo 24:2193-2206

**Figure 6:** Recurrent mutations (red print) and those of particular interest (black print) detected in the AMH gene (A) and the AMHRII gene (B). n: number of affected families. Type of mutation: missense, yellow box; nonsense, white box; deletion, green box; insertion, blue box; splicing mutation, star.

**Figure 7:** Schematic representation of the AMH gene (A), and the AMHRII gene (B). The number of total and different mutations and the number of mutations per 100 base pairs are shown for each exon. N-terminal: coding the pro-region of the AMH protein; C-terminal: coding the mature, biologically active region of the protein. Note that the rate of mutations for 100 base pairs is much higher for the C-terminus.

**Figure 8:** Molecular model of the C-terminal mature AMH dimer showing the p.(Gly533Val) mutation. To allow a comparison, one monomer is shown in green and contains the wild type residue Gly533, while the other monomer is shown in blue and contains the mutant residue Val533. Disulfide bonds which form the cysteine knot motif are shown as sticks in yellow. The residues at position 533 are shown as spheres.

**Figure 9:** Cross-talk between AMH and Wnt/ $\beta$ -catenin pathways. Upon AMH binding to AMHRII, type I receptors ALK2 and ALK3 are recruited, resulting in activation of SMADS 1/5/8, which translocate to the nucleus in concert with Smad4 to regulate the expression of target genes, including Wnt4 or 5A and others. These factors stabilize  $\beta$ -catenin which associates with T-cell factor (TCF)/lymphocyte enhancer factor 1 (LEF1)

to activates transcription of gene products involved in regression of the Müllerian duct epithelium (e.g. apoptosis induced by caspase-3 cleavage).

**Figure 10:** Detection of the 27 bp deletion in exon 10 by PCR. SM: DNA size markers. c/ control, F father, M mother; 1-3 phenotypically normal siblings. 4 PMDS propositus; The figure shows the segregation of a normal allele (382 bp) and a smaller, deleted one (355 bp) which migrates faster. Brother 2 has 2 normal alleles represented by a single, slow migrating band. Sister 1 and the propositus are homozygous for the deleted allele, represented by a single, fast migrating band. Consanguineous parents and brother 3 are heterozygous for the mutations, as shown by the presence of both the slow and fast migrating bands. Reprinted with permission from Josso N, Cate RL, Picard JY The Persistent Müllerian Duct syndrome in Genetic Steroid Disorders, MI New, A Parsa, TT Yuen, B O'Malley, and GD Hammer, eds. (New York: Elsevier) 2013.

**Figure 11:** Molecular models of the kinase domain of AMHRII showing the location of the amino acids affected by the p.(Gly445\_Leu453del) mutation. A) A close up view showing the amino acids affected by the deletion mutation as sticks; the residues are located in the  $\alpha$ G helix and preceding loop. B) A view of the entire kinase domain showing the proximity of the  $\alpha$ G helix to the Activation segment (shown in magenta), two conserved features within eukaryotic protein kinases that have coevolved as part of a regulatory mechanism

**Supplemental figure 1:** All the mutations detected in the AMH gene by ourselves and others. Recurrent mutations are shown in red. Type of mutation: missense, yellow box; nonsense, white box; deletion, green box; insertion, blue box; splicing mutation, star.

**Supplemental figure 2:** All the mutations detected in the AMHRII gene by ourselves and others. Recurrent mutations are shown in red. Type of mutation: missense, yellow box; nonsense, white box; deletion, green box; insertion, blue box; splicing mutation, star.

**Legend of Table:** Normal values for serum AMH (pmol/L) in developing boys assayed with the ultrasensitive EIA Beckman-Immuntotech AMH/MIS kit (reference A18893). Results according to Grinspon et al. [2011]. To obtain values in ng/ml, divide by 7.14. The EIA kit is no longer commercially available, pediatric normograms have not been published for the kits now on sale. An international standard of measurement of AMH has yet to be defined. G1-G5: genitalia development stages according to Lindgren [1996].

**Supplemental table 1:** PMDS patients with AMH mutations detected by ourselves (in black) or by others (in blue). Mutations are arranged by their coding position on the gene, according to HGVS recommendations. In our previous publications, numbering was genomic from the transcription site. Recurrent mutations are boxed and specified only once. Homo: homozygous mutation, Hetero: 2 different mutations, Hemi: hemizygous with only one detected mutation. France N: North of the river Loire; S: South of the Loire.

**Supplemental table 2:** PMDS patients with AMHRII mutations detected in by ourselves (in black) or others (in blue). Mutations are arranged by their coding position on the gene, according to HGVS recommendations. In our previous publications, numbering was genomic from the transcription site. Recurrent mutations are boxed and specified only once. Homo: homozygous mutation, Hetero: 2 different mutations, Hemi: hemizygous with only one detected mutation. France N: North of the river Loire; S: South of the Loire.

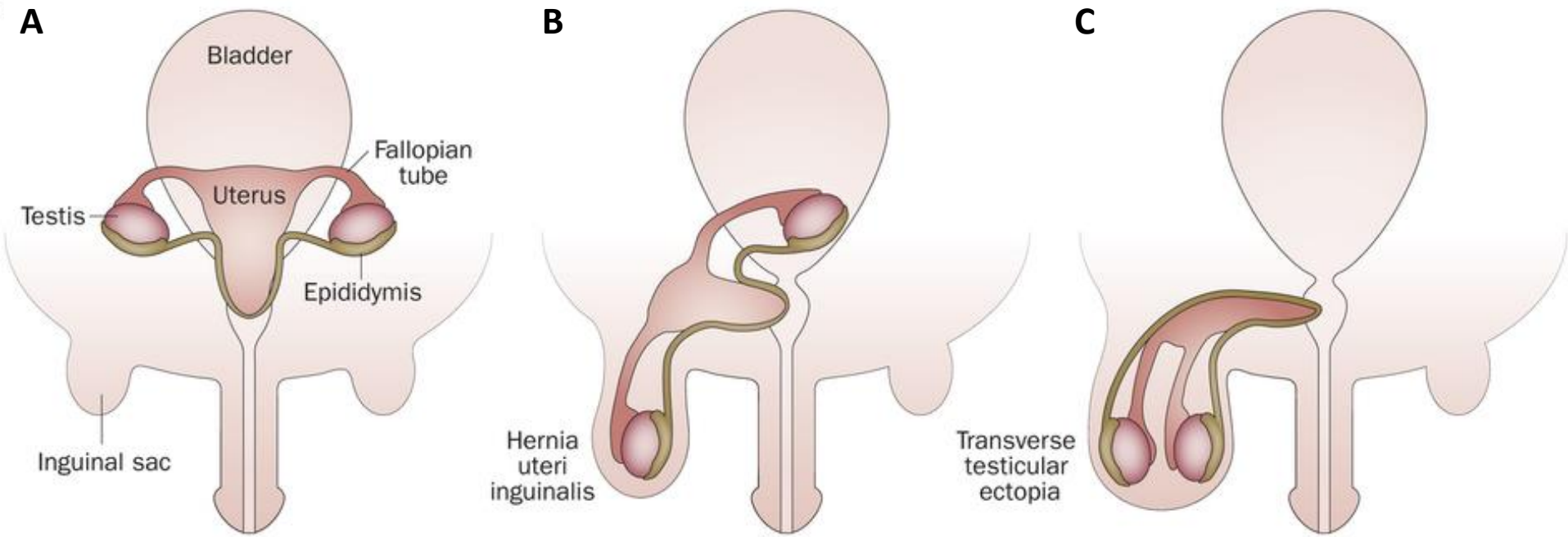


Fig. 1



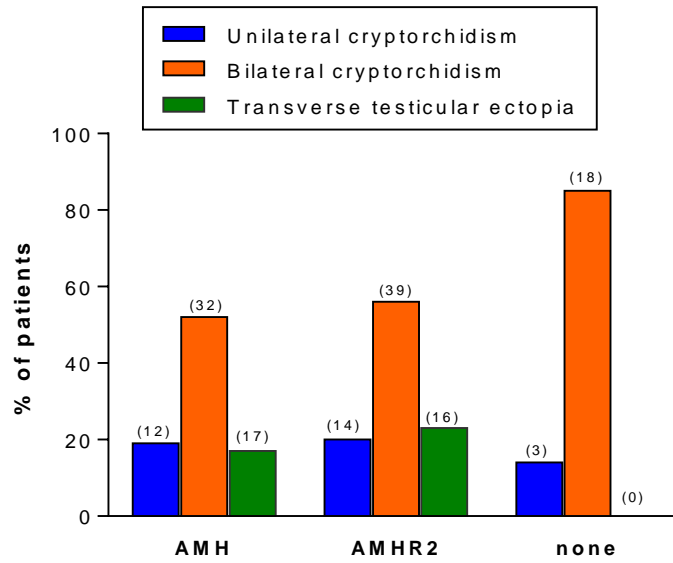


Fig. 2



Fig. 3

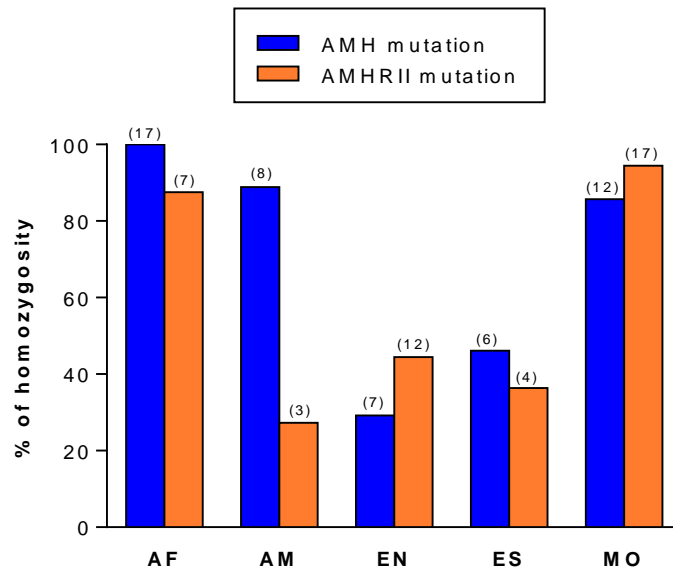


Fig. 4

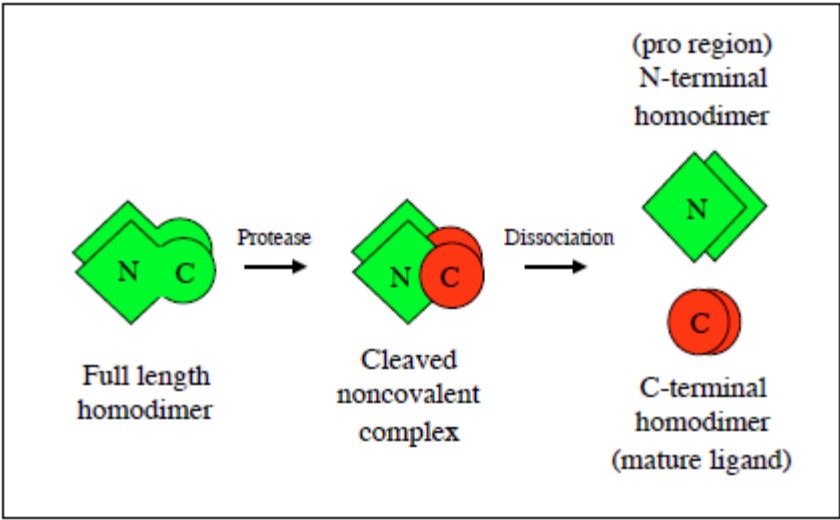


Fig. 5

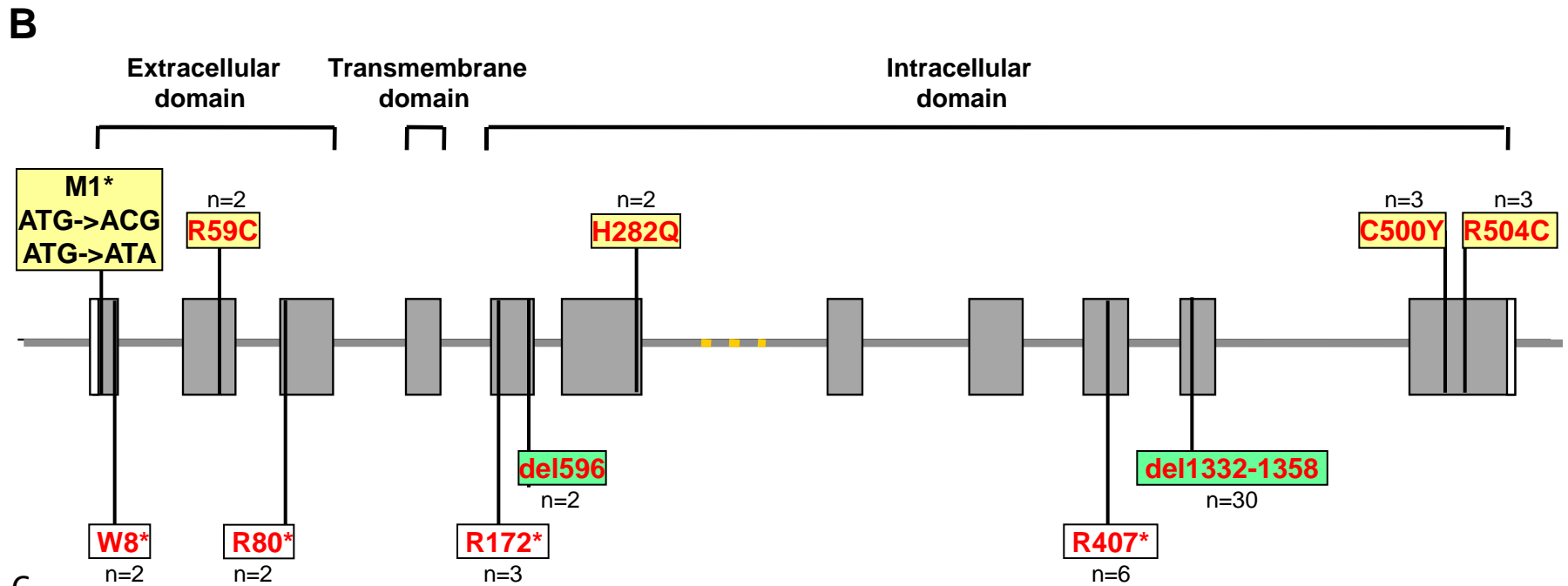
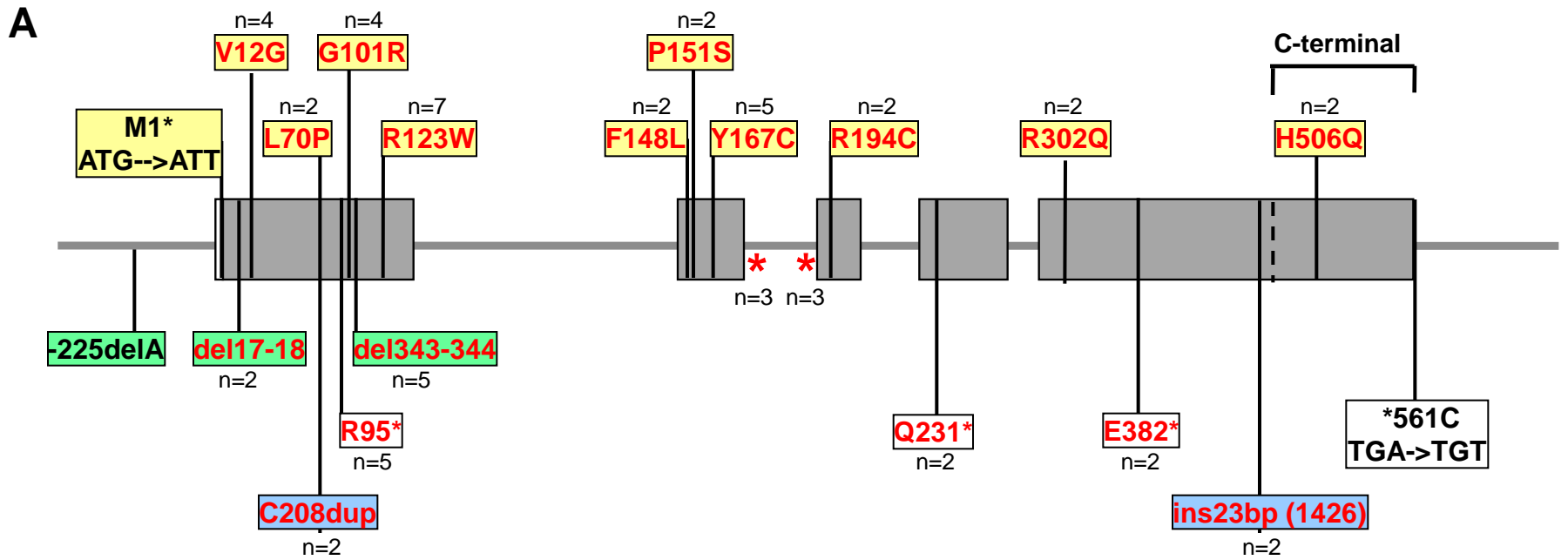


Fig. 6

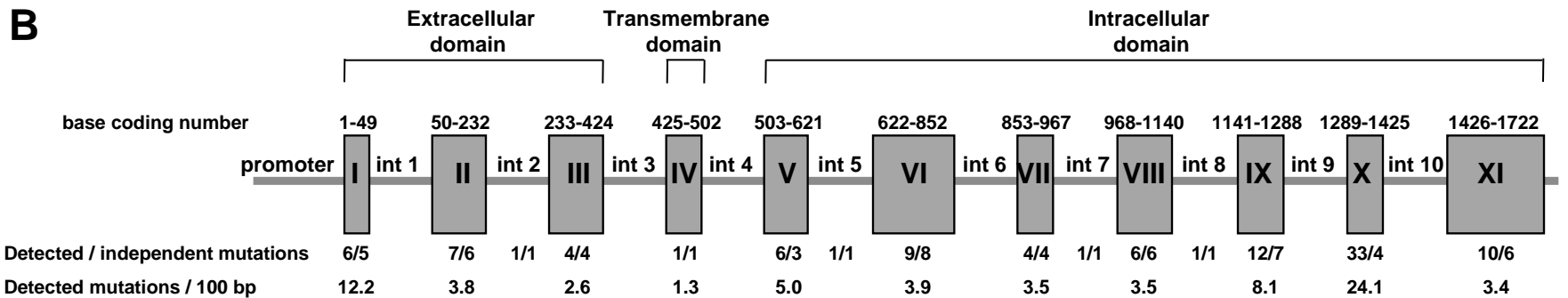
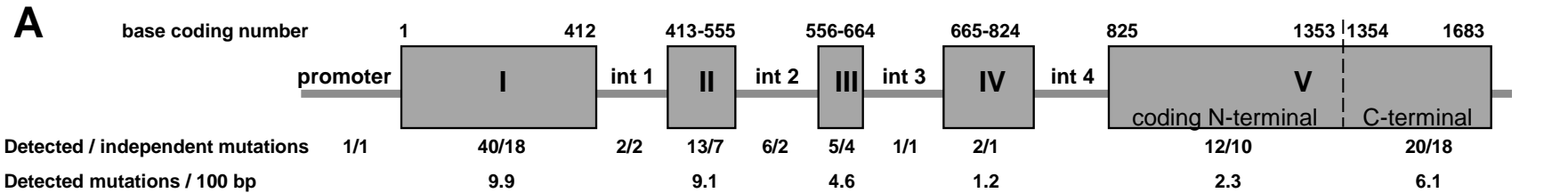


Fig. 7

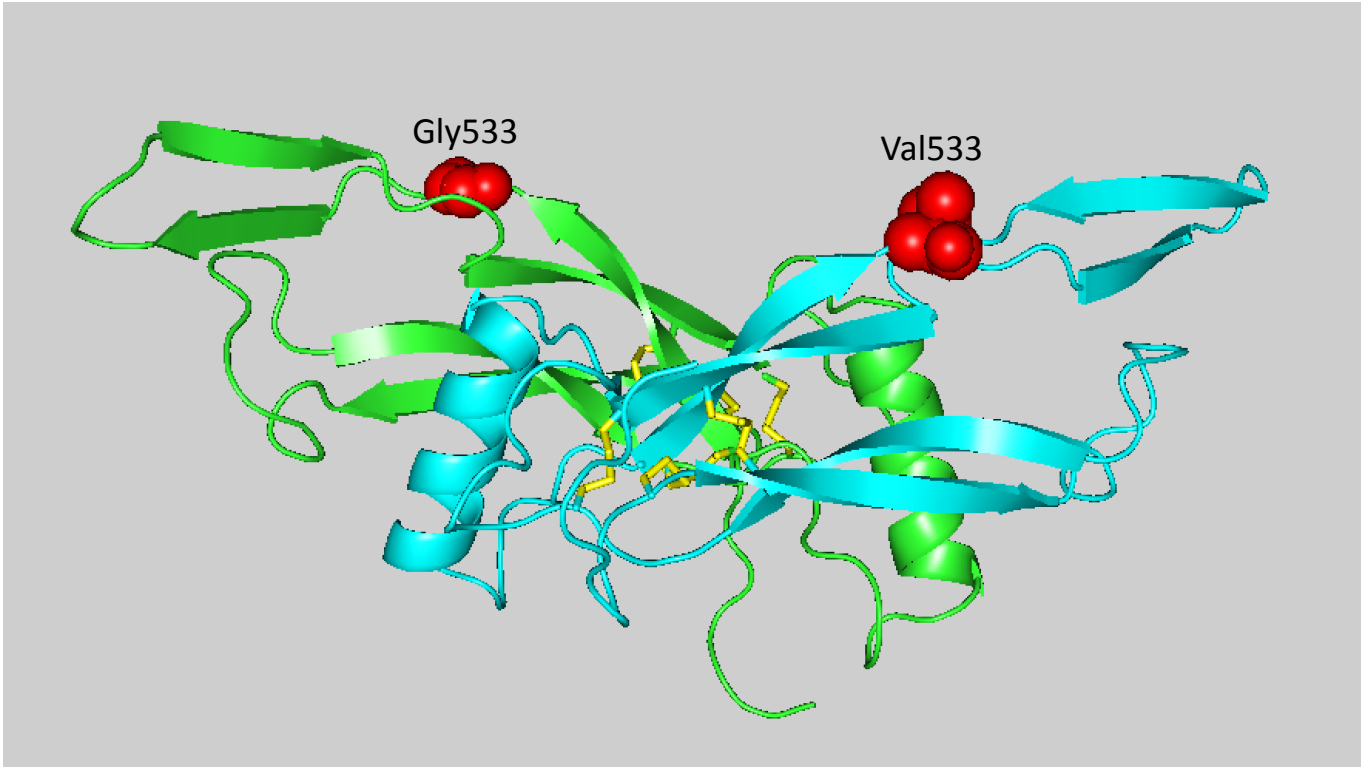


Fig. 8

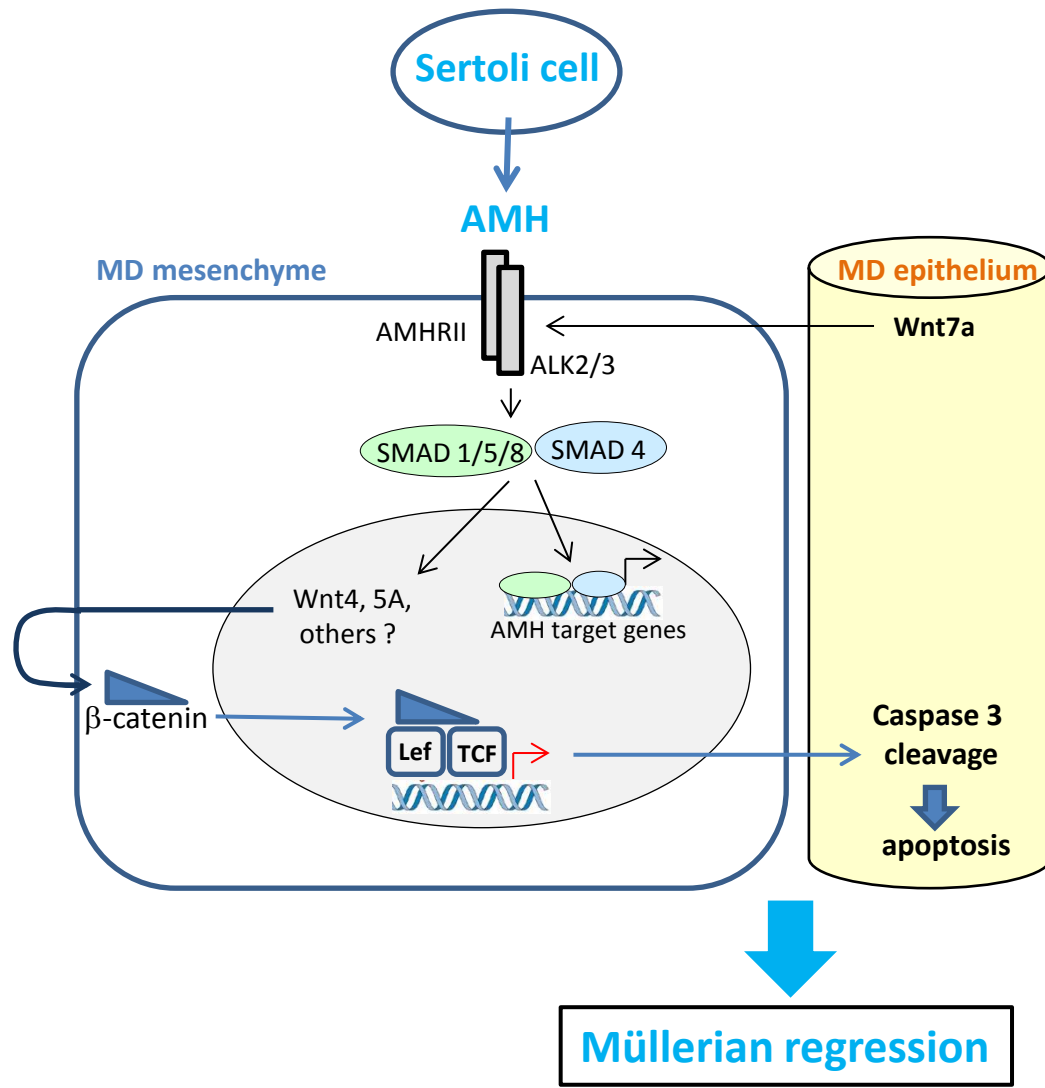


Fig. 9



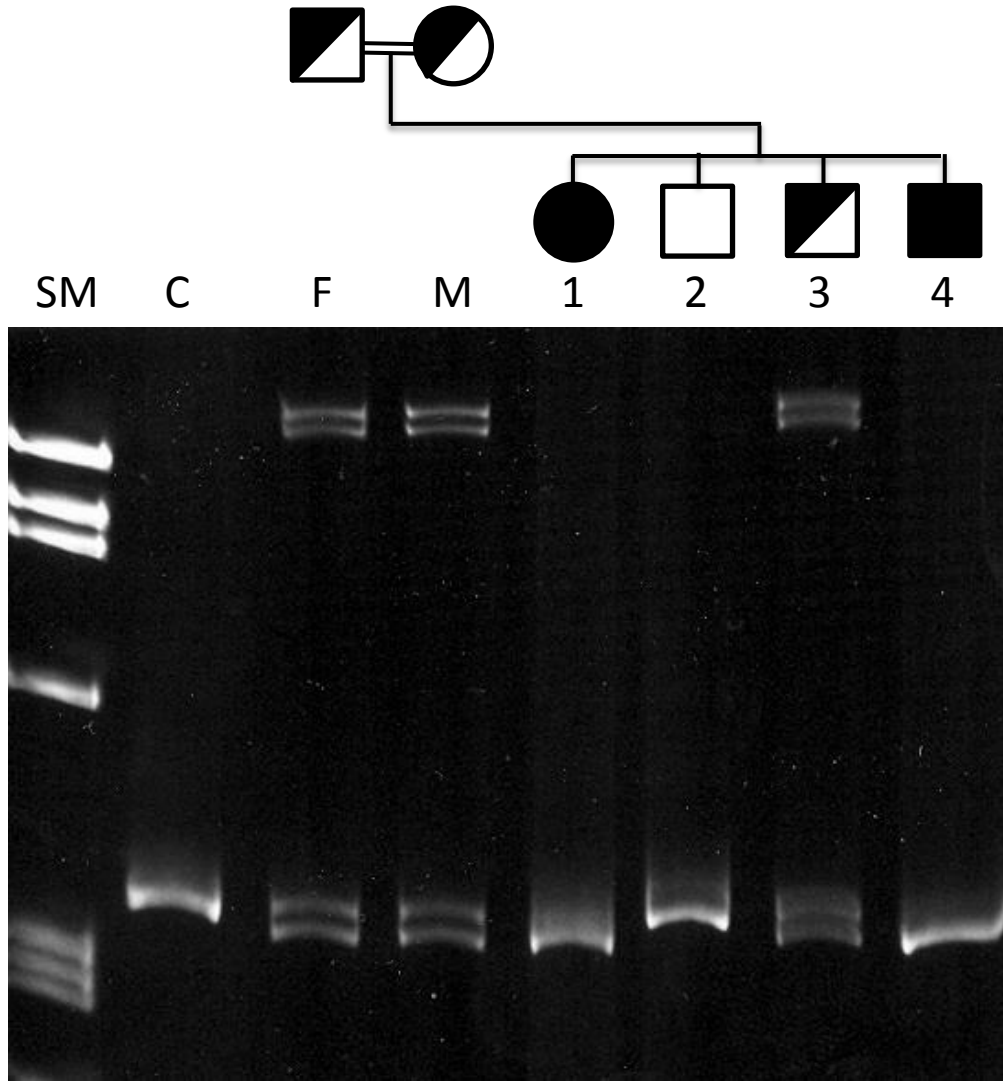


Fig. 10

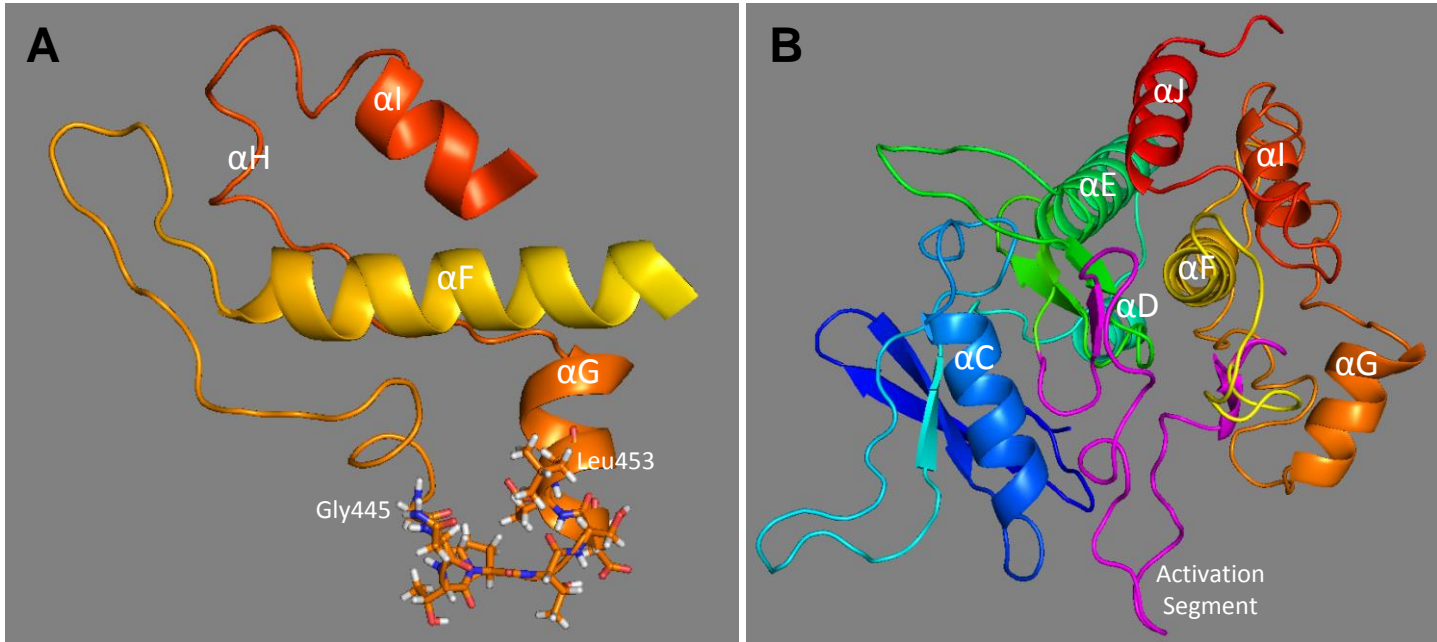
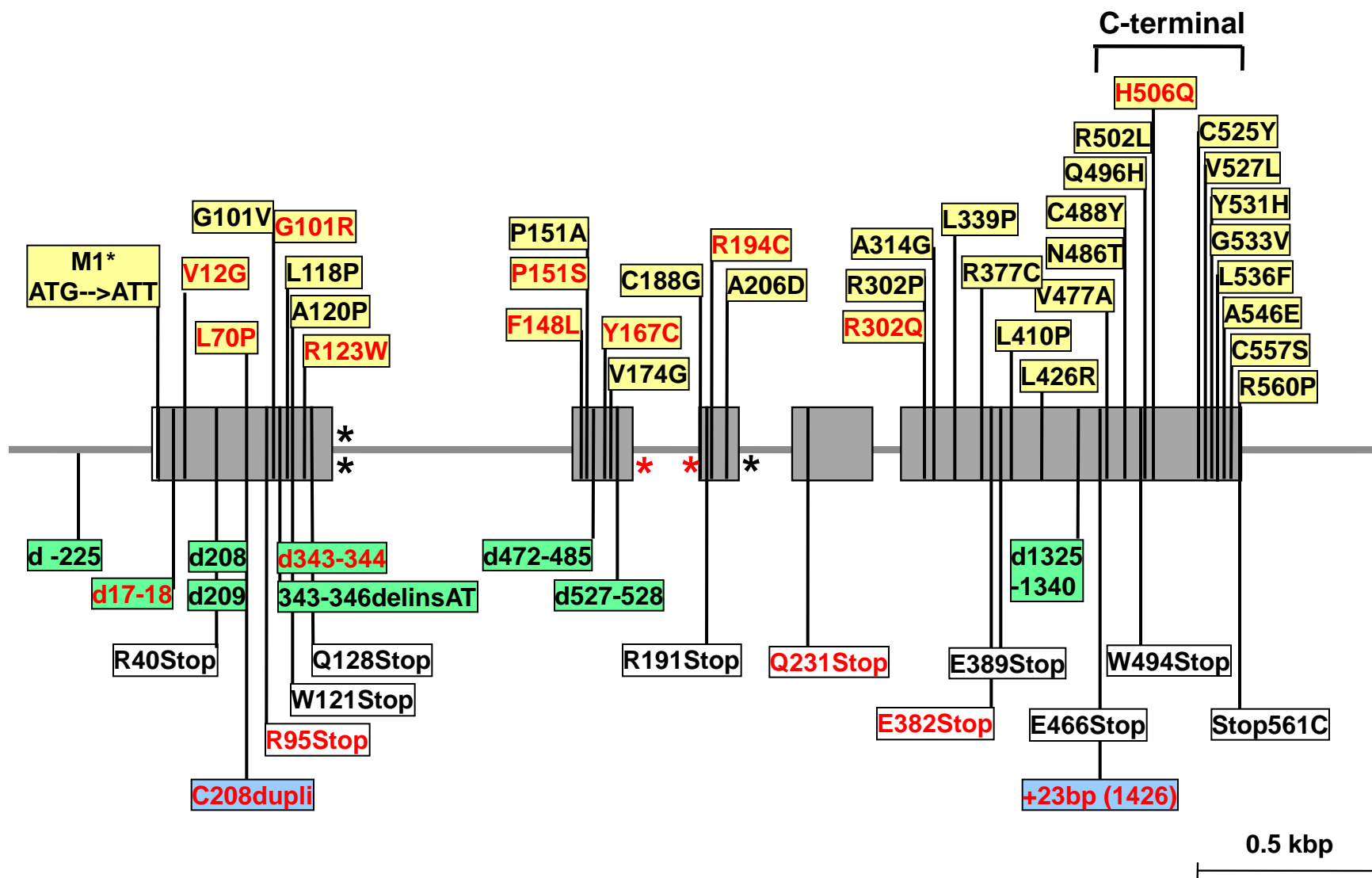


Fig. 11

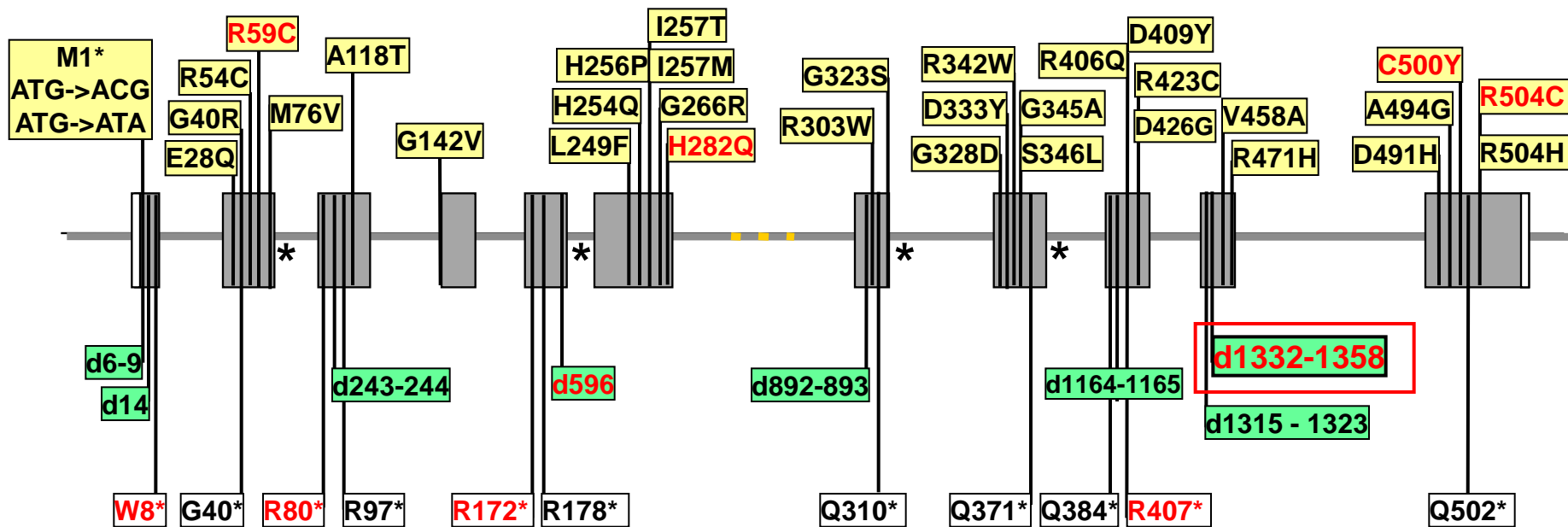


Suppl Fig. 1

Extracellular domain

Transmembrane domain

Intracellular domain



0.5 kbp

**Table**

<b>Age</b>	<b>n</b>	<b>Genitalia development stages</b>	<b>Mean ± SD</b>	<b>Median</b>	<b>3<sup>rd</sup>-97<sup>th</sup> percentile</b>
0-14 days	39		621 ± 271	584	253-1038
15 days to 6 months	26		761 ± 357	697	421-1470
6-21 months	26		1253 ± 502	1132	684-2329
2 to 8.9 years	95		782 ± 461	684	236-1831
9-18 years	34	G1	741 ± 327	713	257-1371
	34	G2	419 ± 301	295	69-1017
	42	G3	121 ± 114	71	30-423
	41	G4	75 ± 40	65	33-164
	60	G5	92 ± 50	82	38-195
> 18 years	24		62 ± 35	56	25-137

Supplemental table 1

Patient	Origine	Exon-intron	Mutation	Effect	Alleles	Publication
H001	Mexico		NC_000019.10:g.2249108delA	? (SF1 response element)	Homo	Valeri et al 2016 abstr. ESPE
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	<a href="#">Imbeaud et al 1994</a>
H006	France S				Hémi	
H007	Scotland				Hetero	
H008	Egypt				Homo	<a href="#">Mazen et al 2011</a>
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	<a href="#">Mazen et al 2017</a>
H011	Maghreb	1	NM_000479.3:c.208dup	NM_000479.3:p.(Leu70Profs*11)	Homo	
H012	Netherlands				Homo	<a href="#">van der Zwan et al 2012</a>
H013	Tunisia	1	NM_000479.3:c.209T>C	NM_000479.3:p.(Leu70Pro)	Homo	
H014	Algeria				Homo	<a href="#">Zeller et al 1994</a>
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	<a href="#">Nishi et al 2012</a>
H020	Egypt				Homo	<a href="#">Mazen et al 2011</a>
H021	Pakistan	1	NM_000479.3:c.301G>A	NM_000479.3:p.(Gly101Arg)	Homo	
H022	Turkey				Homo	
H023	Turkey				Hemi	
H024	Afghanistan				Homo	
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				Hemi	
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	<a href="#">Loeff et al 1994</a>
H038	France S				Homo	
H039	France S				Hemi	
H019	Brazil				Hetero	<a href="#">Nishi et al 2012</a>
H041	Brazil				Homo	<a href="#">Nishi et al 2012</a>
H042	Israel	1	NM_000479.3:c.382C>T	NM_000479.3:p.(Gln128*)	Homo	
H043	Belgium	1st intr	NC_000019.10:g.2249747A>G	?	Homo	<a href="#">Guerrier et al 1989</a>
H044	Algeria	1st intr	NC_000019.10:g.2249747A>C	NM_000479.3:r.412_413ins412+1_412+46 NM_000479.3:p.(Val138Glyfs*51)	Homo	<a href="#">Gricourt et al 2011</a>
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	<a href="#">Carré-Eusèbe et al 1992</a>
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:g.2250480G>T	?	Hetero	
H059	Netherlands				Hetero	
H048	Netherlands				Hetero	
H061	Brazil	2nd intr	NC_000019.10:g.2250650A>G	?	Homo	<a href="#">Nishi et al 2012</a>
H062	Brazil				Homo	<a href="#">Nishi et al 2012</a>
H063	Brazil				Homo	<a href="#">Nishi et al 2012</a>

Supplemental table 1

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	<a href="#">Carré-Eusèbe et al 1992</a>
H066	Yugoslavia	3	NM_000479.3:c.580C>T	NM_000479.3:p.(Arg194Cys)	Homo	
H067	Pakistan				Homo	
H068	Great Britain	3	NM_000479.3:c.617C>A	NM_000479.3:p.(Ala206Asp)	Hetero	
H005	Italy	3rd intr	NC_000019.10:g.2250765G>A	?	Hetero	
H070	Pakistan	4	NM_000479.3:c.691C>T	NM_000479.3:p.(Gln231*)	Hetero	
H071	Pakistan				Homo	
H046	Great Britain	5	NM_000479.3:c.905G>A	NM_000479.3:p.(Arg302Gln)	Hetero	
H049	Great Britain	5			Hetero	
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 1	
H059	Netherlands	5	NM_000479.3:c.1016T>C	NM_000479.3:p.(Leu339Pro)	Hetero	
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	<a href="#">Knebelmann et al 1990</a>
H080	Australia	5	NM_000479.3:c.1165G>T	NM_000479.3:p.(Glu389*)	Homo	
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	
H045	Great Britain	5	NM_000479.3:c.1396G>T	NM_000479.3:p.(Glu466*)	Hemi	
H047	Kosovo	5	NM_000479.3:c.1425_1426ins1397_1419	NM_000479.3:p.(Val477Serfs*3)	Homo	<a href="#">Lang-Muritano et al 2001</a>
H056	Greece				Hetero	
H052	Italy	5	NM_000479.3:c.1430T>C	NM_000479.3:p.(Val477Ala)	Homo	
H053	Japan	5	NM_000479.3:c.1457A>C	NM_000479.3:p.(Asn486Thr)	Hetero	<a href="#">Morikawa et al 2014</a>
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	<a href="#">Nishi et al 2012</a>
H031	Great Britain	5	NM_000479.3:c.1518C>G	NM_000479.3:p.(His506Gln)	Hetero	
H068	Great Britain				Hetero	
H069	Pakistan	5	NM_000479.3:c.1574G>A	NM_000479.3:p.(Cys525Tyr)	Homo	<a href="#">Carré-Eusèbe et al 1992</a>
H053	Japan	5	NM_000479.3:c.1579G>T	NM_000479.3:p.(Val527Leu)	Hetero	<a href="#">Morikawa et al 2014</a>
H072	Turkey	5	NM_000479.3:c.1591T>C	NM_000479.3:p.(Tyr531His)	Homo	<a href="#">Nalbantoglu et al 2015</a>
H075	Morocco	5	NM_000479.3:c.1598G>T	NM_000479.3:p.(Gly533Val)	Homo 2	
H073	Great Britain	5	NM_000479.3:c.1606C>T	NM_000479.3:p.(Leu536Phe)	Hemi	
H070	Pakistan	5	NM_000479.3:c.1637C>A	NM_000479.3:p.(Ala546Glu)	Hetero	
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	

Supplemental table 2

Patient	Origin	Exon-intron	Mutation	Effect	Alleles	Publication
R001	Maghreb	1	NM_020547.2:c.2T>C	NM_020547.2:p.(Met1?)	Homo	
R002	Belgium	1	NM_020547.2:c.3G>A	NM_020547.2:p.(Met1?)	Homo	
R003	Guadeloupe	1	NM_020547.2:c.6_9del	NM_020547.2:p.(Gly3Leufs*40)	Hémi	
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	<a href="#">Korkmaz et al 2017</a>
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	
R010	Great Britain	2	NM_020547.2:c.160C>T	NM_020547.2:p.(Arg54Cys)	Hetero	
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:g.53424471G>A	NM_020547.2:r.232_233ins232+1_232+12 NM_020547.2:p.(Gly78Asp_Cys79insGluValGlnArg)	Homo	<a href="#">Imbeaud et al 1995</a>
R015	France N	3	NM_020547.2:c.238C>T	NM_020547.2:p.(Arg80*)	Hetero	<a href="#">Guerrier et al 1989</a>
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	
R019	Argentina	3	NM_020547.2:c.352G>A	NM_020547.2:p.(Ala118Thr)	Hetero	
R020	Denmark	4	NM_020547.2:c.425G>T	NM_020547.2:p.(Gly142Val)	Hetero	
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*)	Hemi	
R025	Great Britain	5	NM_020547.2:c.596del	NM_020547.2:p.(Glu199Glyfs*10)	Hetero	<a href="#">Messika-Zeitoun et al 2001</a>
R026	Switzerland				Hetero	
R027	USA	5th intr	NC_000012.12:g.53425638C>T	NM_020547.2:r.621_622ins621+1_622-1 NM_020547.2:p.(Ile209Valfs*73)	Hetero	<a href="#">Hoshiya et al M 2003</a>
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	<a href="#">Abduljabbar et al 2012</a>
R030	Egypt	6	NM_020547.2:c.767A>C	NM_020547.2:p.(His256Pro)	Homo	<a href="#">Mazen et al 2016 abstr. ESPE</a>
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	
R035	Netherlands	6	NM_020547.2:c.846T>G	NM_020547.2:p.(His282Gln)	Hetero	<a href="#">Imbeaud et al 1995</a>
R036	Netherlands				Homo	
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	
R039	Israel	7	NM_020547.2:c.928C>T	NM_020547.2:p.(Gln310*)	Homo	<a href="#">Elias-Assad et al 2016</a>
R040	Brazil	7	NM_020547.2:c.967G>A	NM_020547.2:p.(Gly323Ser)	Homo	<a href="#">Nishi 2012</a>
R041	Argentina-Sweden	7th intr	NC_000012.12:g.53429012T>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:g.53429626G>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	
R025	Great Britain	9	NM_020547.2:c.1217G>A	NM_020547.2:p.(Arg406Gln)	Hetero	
R052	Morocco	9	NM_020547.2:c.1219C>T	NM_020547.2:p.(Arg407*)	Homo	
R053	Saudi Arabia				Homo	<a href="#">Abduljabbar et al 2012</a>
R054	France S				Hetero	
R009	Italy				Hetero	
R017	Italy				Hetero	
R057	Brazil				Homo	<a href="#">Nishi 2012</a>



Supplemental table 2

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	
R061	Iran	10	NM_020547.2:c.1317_1325del	NM_020547.2:p.(Tyr440_Ala442del)	Homo	
R062	USA	10	NM_020547.2:c.1332_1358del	NM_020547.2:p.(Gly445_Leu453del)	Hetero	<a href="#">Imbeaud et al 1996</a>
R063	Sweden				Homo	
R054	France S				Hetero	
R065	France S				Hémi	
R066	Netherlands				Hetero	
R067	France N				Hemi	
R068	France N				Homo	
R069	Germany				Homo	
R019	Argentina				Hetero	
R071	Russia				Hemi	
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	
R046	France N				Hetero	
R059	Spain				Hetero	
R012	Argentina				Hetero	
R055	Switzerland				Homo	
R010	Great Britain				Hetero	
R035	Netherlands				Hetero	<a href="#">Imbeaud et al 1995</a>
R056	Netherlands				Homo	
R041	Argentina-Sweden				Hetero	
R016	France N				Hetero	
R027	USA				Hetero	<a href="#">Hoshiya et al 2003</a>
R064	Portugal				Homo	<a href="#">Rosal-Goncalves et al 2010</a>
R062	USA	10	NM_020547.2:c.1373T>C	NM_020547.2:p.(Val458Ala)	Hetero	<a href="#">Imbeaud et al 1996</a>
R070	Italy	10	NM_020547.2:c.1412G>A	NM_020547.2:p.(Arg471His)	Hetero	<a href="#">Avolio et al 2003</a>
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	<a href="#">Avolio et al 2003</a>
R066	Netherlands	11	NM_020547.2:c.1511G>A	NM_020547.2:p.(Arg504His)	Hetero	