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RESEARCH

Molecular mechanism of androgen receptor mutation in multigenerational mild androgen insensitivity syndrome

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Abstract

Objective: Androgen insensitivity syndrome (AIS) due to androgen receptor (AR) mutations creates a spectrum of clinical presentations based on residual AR function with the mildest impairment creating mild AIS (MAIS) whose undefined molecular mechanism and subtle clinical features leave it less understood and underdiagnosed.

Design: In silico modeling and *in vitro* androgen bioassay of the mutated AR are used to identify its structural and physiological mechanism. Clinical features and responses to high-dose testosterone treatment of three cases of MAIS across a six-generation family pedigree are described.

Methods: Structural and dynamic *in silico* molecular modeling and *in vitro* yeast-based androgen bioassays of the mutant AR are employed. Three cases of MAIS with consistent (gynecomastia and micropenis) and variable (infertility) clinical features across generations are reported, and the effects of high-dose testosterone treatment are studied.

Results: The missense AR exon 8 mutation (nucleotide **a**ga → **g**ga, p.R872G arginine to glycine), known to cause an increased ligand dissociation rate in mutant AR in binding assays, was analyzed. Modeling shows that the mutation weakens the closure energy of the 'lid' of the ligand-binding pocket, allowing easier ligand dissociation from the binding site but with unimpaired *in vitro* androgen bioactivity. High-dose testosterone treatment for 3 years in one young man caused increased virilization and height growth but was ineffective for treating micropenis. Genetic counseling allowed effective prediction of MAIS risks in progeny for carrier and noncarrier sisters.

Conclusions: The differential diagnosis and clinical management of MAIS is reviewed. The novel molecular mechanism of an AR ligand-binding domain mutation in MAIS may be present in other cases of MAIS.

Keywords: androgen insensitivity syndrome; androgen receptor; structural molecular modeling; clinical genetics

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Introduction

The androgen receptor (AR, MIM 313700) is a member of the steroid nuclear receptor family (NR3C4) (1), with its gene located on the X chromosome (Xq11–12) and comprising 8 exons that specify a 920 amino acid protein (2). The unliganded AR resides in a cytoplasmic multichaperone complex, which, upon binding a cognate native (e.g., testosterone and dihydrotestosterone) or a synthetic androgen, acts as a ligand-activated nuclear transcription factor moving into the nucleus to interact with DNA. Ligand-bound AR directs androgen action by binding to androgen response elements of target androgen-activated genes. AR-mediated androgen action is indispensable for species propagation, being required for producing and delivering sperm, although neither AR nor androgen action is essential for life.

Over 1000 AR mutations have been identified with over 600 germline mutations, around 30% arising *de novo* (3), causing androgen insensitivity syndrome (AIS, MIM 300068), the most frequent cause of XY disorders of sex development (DSD) (4, 5). AIS presents clinically in hemizygous males whose mothers are asymptomatic obligate heterozygous carriers. The clinical features of AIS depend on the functional severity of the germline inactivation of the AR mutation (6) presenting in three clinical phenotypes, complete (CAIS), partial (PAIS) and mild (MAIS). The clinical features of these variants reflect the spectrum of residual androgenic activity of the mutated AR, ranging from nil in CAIS, with complete AR inactivation and androgen resistance, to MAIS with near-full AR activation and the complete spectrum of functional AR bioactivity in between. CAIS inactivation results in a female somatic and external genitalia with usually no Mullerian duct derivatives (short vagina with no uterus or ovaries) but preserved Wolffian duct derivatives (undescended inguinal or abdominal testes). MAIS as the mildest form of AIS presents with normal male external genitalia but under-virilization due to defective androgen action, manifesting in reduced body hair, gynecomastia, micropenis and/or infertility (7). Between CAIS and MAIS, a spectrum of midrange AR bioactivity causes PAIS, characterized by variable severe hypospadias with reduced scrotal fusion, notably producing ambiguous genitalia in under-virilized males. Hypospadias is the most sensitive phenotypic feature of androgen insensitivity, reflecting the degree of residual AR activity in leading to masculine scrotal and urethral fusions with phallus formation.

MAIS is the least well-described form of AIS (3), with its relatively subtle phenotype rendering it underdiagnosed, so its true prevalence is unknown. In this study, we identify two generations of MAIS within an extended six-generation pedigree (Fig. 1), suggesting wider

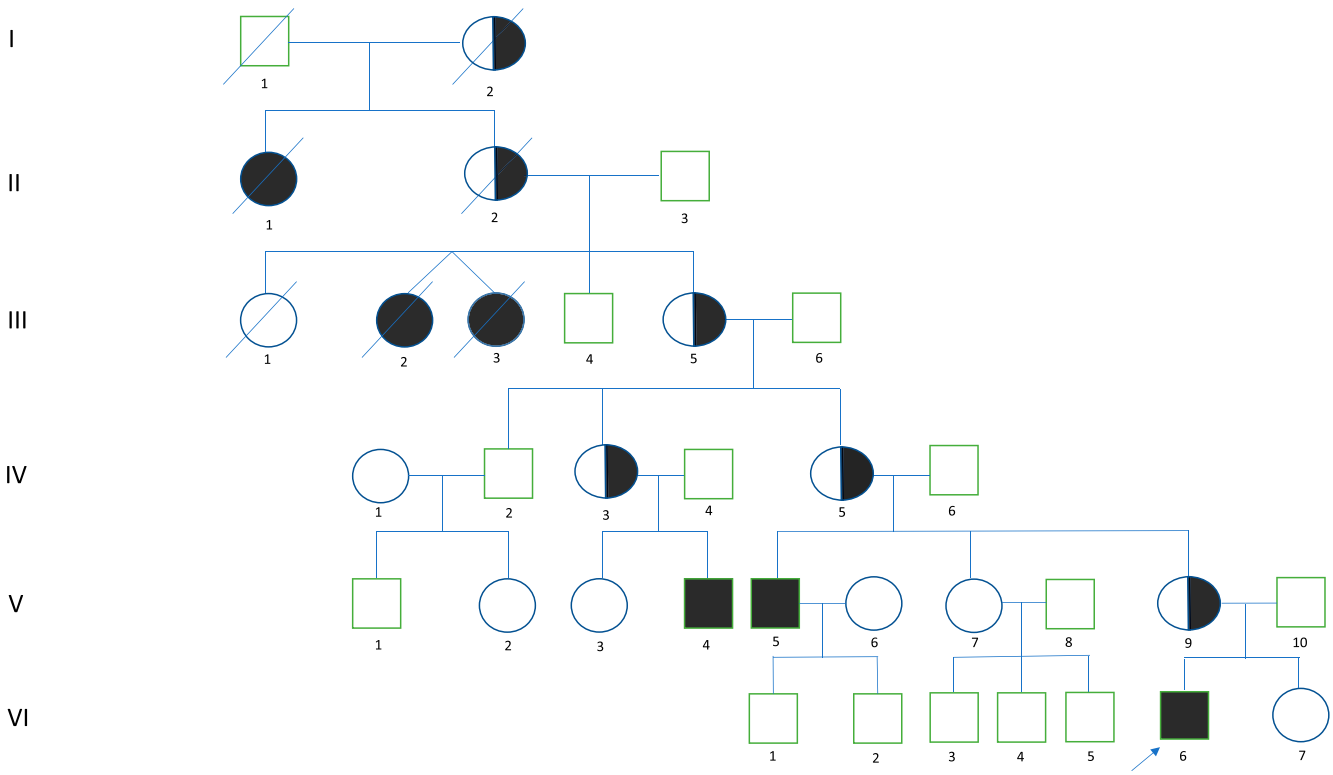

Figure 1

Chart showing the pedigree of the family. The index case (VI-6) is indicated by an arrow. The black-filled symbols indicate affected members. The half-filled symbols indicate carriers of the affected gene. III-2,3: monozygotic twins, both with features of 'hermaphroditism'. V-4: Gynecomastia and subfertility. V-5: propositus; delayed growth and puberty. VI-6: index case.

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involvement with phenotypic variation within the family. Family members are identified according to the notation in Fig. 1. Based on identifying the exon 8 point mutation in the AR ligand-binding domain (LBD) with functional AR-binding studies showing rapid dissociation of the ligand from the mutant compared with the WT AR (8), it is identified that the mutation loosens the binding strength of the 'lid' that closes the ligand-binding pocket. Nevertheless, the androgen bioactivity in a well-characterized *in vitro* androgen bioassay was not different from the WT AR.

Materials and methods

In silico molecular modeling

The protein–ligand complex of the WT human AR LBD (PDB ID: 2AM9) was prepared in the Maestro software (Maestro, Schrödinger LLC, USA, 2023) using the Protein Preparation Wizard (9). The structure of the R872G mutant complex was produced using the mutation function in Maestro.

For molecular dynamics studies, an orthorhombic solvent box filled with SPC water molecules, counterions and additional 0.15 M concentration of NaCl was constructed in the Desmond System Builder (10). Conventional molecular dynamics were performed at 200 ns, both on R872G and on WT after standard energy minimization and equilibration. Trajectories were clustered based on the root mean squared deviation (RMSD) matrix of backbone atoms.

To investigate the effect of the mutated site on secondary structure, a simulated annealing protocol was applied to both equilibrium conformations from conventional molecular dynamics (MD). Structures were gradually heated to 350 K and cooled down to 310 K for relaxation (11, 12). Furthermore, 2000-ns simulations were conducted to stabilize both models.

Metadynamics were also employed to study the conformation and energy difference on the mutated site and pocket lid, and the distance between the center of mass of lid helix (893–919) and mutated helix (851–882) was chosen as the collective variables to calculate ΔΔG (13).

In vitro androgen bioassay

A single colony of yeast cells cotransformed with pYEP-URA3-TEF1-hAR (NM_000044.6) or pYEP-URA3-TEF1-hAR (NM_000044.6 with a single mutation A to G) and pYEP-LEU2-AREminiHSV-TK-EGFP (site-directed mutagenesis of hAR cDNA with sequence confirmation was completed in plasmids constructed by Integrated DNA Technologies, Australia) were incubated overnight with orbital shaking (300 rpm) at 30 °C (sequences included in Fig. 3) using *in vitro* androgen bioassay methods similar to those described previously (14). The optical density of the culture was measured at A_{600} nm and diluted to 0.6. Aliquots of the diluted yeast culture were pipetted into a 24-well plate and treated with testosterone to a final concentration of 3.7×10^{-5} to 4.7×10^{-12} M and cultured overnight at 30 °C with orbital shaking (300 rpm). Cultures were then placed on ice for 20 min. Yeast cells were pelleted by centrifugation at 10,000 rpm for 5 min. The supernatant was discarded, and the pellet was resuspended in 1× PBS. The cell suspension was aliquoted in quadruplicate in 96-well black plates, and fluorescence was measured at ex488/em507. The relative androgenic activity was determined by measuring the EC_{50} value for the two AR strains. The EC_{50} values were determined from a sigmoidal curve fit using GraphPad Prism, version 10.2.3. All fluorescent values were corrected for vehicle-control (ethanol)-treated yeast.

Laboratory assays

Serum LH (Roche Cat# 11732234, RRID: AB_2800498), FSH (Roche Cat# 11775863, RRID: AB_2800499), testosterone (Roche Cat# 05200067, RRID: AB_2783736) and SHBG (Roche Cat# 03052001160, RRID: AB_2891165) were measured by routine automated immunoassays subject to external and internal quality control. Androgen sensitivity index (ASI) was calculated as the product of serum LH and serum testosterone. Semen analysis was performed according to the WHO manual 5th edition (15).

Cases

In 2023, the index case (VI-6 in Fig. 1), a 13-year-old boy, presented with bilateral gynecomastia and micropenis. He was the product of an uncomplicated pregnancy with normal childhood development and in good general health. He had no voice change and reported erections but no ejaculations. At the 50th centile for height and weight for age, he was in early puberty (Tanner stage 2) with no facial, trunk, axillary or pubic hair. He had 3 cm true bilateral gynecomastia with a stretched phallic length of 2–3 cm (<2.5th centile). He had no hypospadias or cryptorchidism and had bilateral testis volumes of 6–8 mL (orchidometry). Serum testosterone was 24.8 nmol/L (RR: 10–30 nmol/L), serum LH was 5.4 IU/L (RR: 1.7–8.6 IU/L), serum FSH was 8.5 IU/L (RR: 1.5–12.4 IU/L), serum SHBG was 91.6 nmol/L (RR: 15–80 nmol/L), and ASI was 134 IU/L nmol/L (Table 1). Genotyping confirmed the AR mutation, a missense nucleotide variation **a**ga → **g**ga in AR exon 8 resulting in a p.R872G (arginine to glycine) mutation. He underwent bilateral mastectomies and commenced a trial of topical dihydrotestosterone gel for micropenis.

In 1998, the index case's maternal uncle (V-11 in Fig. 1) was diagnosed as MAIS having presented at the age of 16 years with delayed puberty, bilateral gynecomastia, micropenis (stretched penile length (SPL) 7.5 cm, 3rd centile),

Table 1 Hormone levels before, during and after high-dose testosterone therapy for V-5 (upper panel) and VI-6 at diagnosis (lower panel).

Age (years and months)	Serum testosterone (nmol/L)	Serum LH (IU/L)	Serum FSH (IU/L)	Serum SHBG (nmol/L)	Estradiol (pmol/L)	PSA (ng/mL)	ASI (IU/L (nmol/L))
Reference range	11.0–35.0	1–10	1–8.5	10–50	55–165	0.2–0.5	
V-11 (1998+)							
17 and 11	**68.3**	8.4	1.7	**69.6**	105	<0.2	574
18 and 5	***76.3***	*3.6*	***0.7***	*46.9*			*275*
19 and 0	***102.0***	*7.7*	***0.8***	***60.3***		*<0.2*	*785*
20 and 1	***36.5***	*5.6*	***0.5***	***57.8***		*0.5*	*204*
21 and 2	***39.2***	*8.0*	*1.2*	***75.5***		*0.1*	*314*
23 and 10	**43.8**	**12.5**	1.8	**77.8**			548
26 and 2	**47.5**	**13.4**	2.1	**81.2**		0.2	637
VI-6 (2023)							
RR (adult)	10–30 nmol/L	1.7–8.6 IU/L	1.5–12.4 IU/L	15–80 nmol/L			
13 and 10	**24.8**	**5.4**	8.5	**91.6**			134

Boldface indicates abnormal value, including results for VI-6, when considering his early to mid-pubertal status. Italics indicate time of high dose testosterone treatment. RR indicates reference range for that analyte. Blood samples were taken at the time of the next testosterone dose, one week after the last testosterone injection (250 mg) and 4 months after the last testosterone implant (800 mg). Abbreviations: ASI, androgen sensitivity index; PSI, prostate specific antigen.

minimal truncal or facial hair and 10th centile for height and weight. In early to mid-puberty, he reported erections but no ejaculations. V-11's karyotype was 46 XY, so he was hemizygous. His mother and one of two sisters (mother of VI-6) were carriers, and the other sister was a noncarrier of the AR mutation. Subsequently, a case report of this mutation (8) was recognized by V-11 as his maternal first cousin in Canada (V-3), who presented with gynecomastia, micropenis and male infertility (Fig. 1). Functional AR-binding studies showed that this binding domain mutation increased androgen disassociation rate from the AR compared with the WT AR (8). V-11 underwent bilateral mastectomies at the age of 16 years and high-dose testosterone treatment for micropenis from the age of 18 years for 3 years with weekly 250 mg testosterone enanthate injections (double standard replacement dosage) for 12 months when he reported more ejaculatory fluid and increased hair growth (upper lip, abdomen and pubic). On treatment with subdermal testosterone implants (16, 17) at twice the testosterone replacement dose (8 × 200 mg per 4 months) for 2 years was associated with 7 cm height growth, voice deepening and increased body hair. Penis and testis size remained unchanged throughout the testosterone treatment. Sperm cryopreserved at the age of 22 years was discarded after two easily conceived children (at the ages of 32 and 34 years), and V-11 underwent vasectomy (Table 2).

The family gave permission to publish their medical details, including family tree, clinical results and test results. No ethics approval was required for an audit of completed tests and treatments in the course of medical management of all patients of our department.

Molecular modeling

Molecular dynamics simulated the dynamics of protein folding in the WT and R872G mutant of the AR LBD. The proteins heated to 350 K unfolded and were allowed to refold at 310 K. The R872G mutation (in helix residues 850–882) occurred earlier in the R872G mutant, indicating a relatively unstable structure. Two further extended simulations (2000 ns) were performed to study the refolding dynamics of the WT and R872G mutant at 310 K NPT (constant number of particles, temperature and pressure) ensemble. The interaction energy of lid helix (residue numbers 893–919) and LBD were calculated based on the equilibrium trajectory *via* the molecular mechanics with generalized born and surface area (MMGBSA) solvation method (18). At 2000 ns, the WT structure successfully forms almost the complete helix (res 850–882), whereas the R872G structure still shows a significant disorder (Fig. 2A). In the WT structure, the arginine at position 872 forms

Table 2 Semen analysis after cessation of high-dose testosterone treatment at the age of 21.

Age (years and months)	Semen volume (mL), RR > 2.0	Sperm concentration (M/mL), RR > 14	Total sperm count (M), RR > 39	Motility (%), RR > 50%	Morphology normal (%), RR > 30
22 and 2	0.9	2.9	2.6	7	11
23 and 10	1.8	17.5	31.5	34	11
26 and 2	1.4	23.0	32.2	30	4
28 and 4	1.2	37.0	44.4	41	8

Sperm was cryopreserved at the age of 22 but was discarded after he achieved two natural pregnancies when he was aged 32 and 34 years old after which he had a vasectomy.

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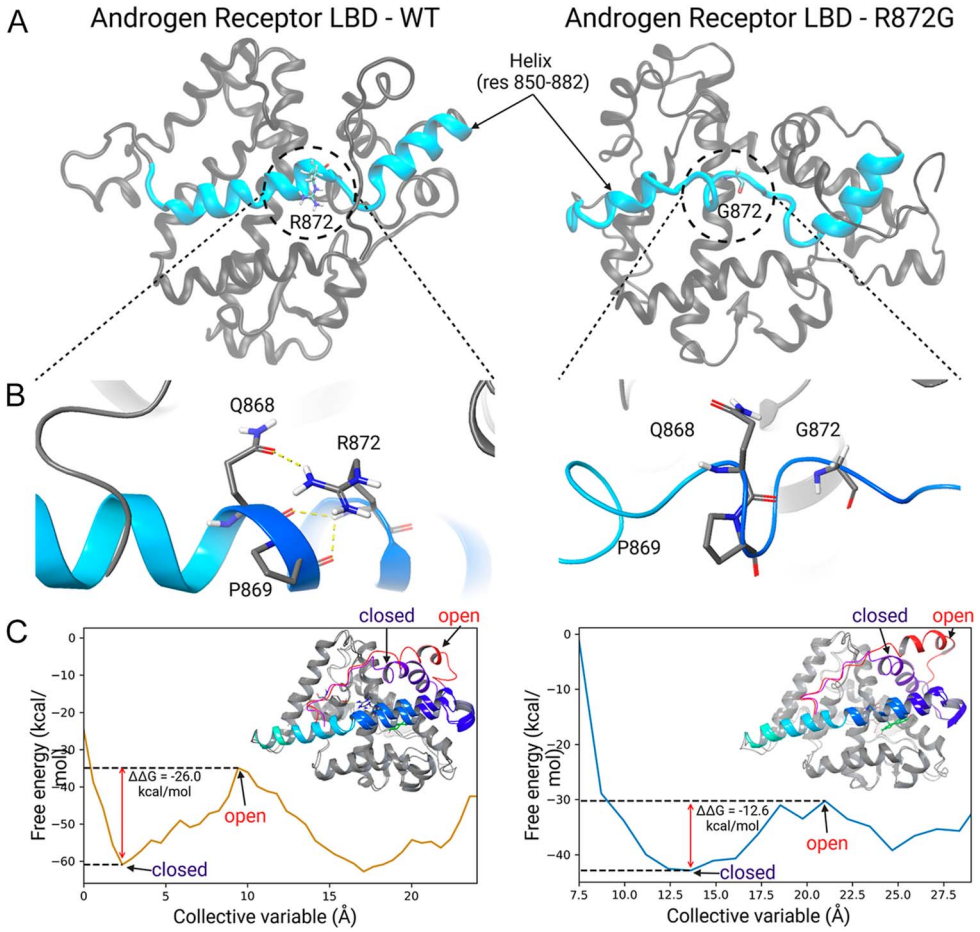

Figure 2

Molecular impact of the R872G mutation on the ligand-binding domain (LBD) of the human androgen receptor (AR). (A) Molecular dynamics simulation of wild-type (WT) and R872G mutants folding to form the protein at 2000 ns, after being heated to 350 K during simulated annealing procedures. (B) Molecular interactions present in the WT LBD between R872 and Q868 stabilize the helix (res 850–882). Hydrogen bonds are shown as dashed yellow lines. (C) Metadynamics analyses of the helix (res 893–919) that forms the lid of the binding pocket. In the R872G mutant, the open conformation of helix (res 893–919) is adopted more easily.

hydrogen bonds with GLN868 and PRO869, while these interactions are absent in the mutant structure (Fig. 2B). This suggests that arginine is essential for stabilizing the helix structure during protein folding.

Metadynamics analysis studied the structure of the helix (res 893–919), which forms the lid of the binding pocket within the LBD. The energy difference (ΔΔG) between the open and closed forms of the helix differs significantly between the WT and R872G mutant (−26 vs −12.6 kcal/mol) (Fig. 2C). This helix also interacts with ARG872 in the first helix *via* a hydrogen bond, whereas this interaction is absent in the R872G mutant. The proximity of the lid to the binding site may influence the binding kinetics of the ligand.

MMGBSA calculations based on the equilibrium trajectory of conventional MD for the interactions between the lid (res 893–919) and LBD also support the results of the metadynamics study. The ΔΔG between the WT and R872G mutant is −6.41 kcal/mol, indicating that the WT lid is more energetically stable as compared to the R872G mutant. Per residue energy decomposition analysis showed that ARG872 contributed more to stabilizing the helix compared to GLY872 in the mutant (−3.15 vs −0.16 kcal/mol).

Androgen bioassay

The testosterone dose–response curves for yeast strains containing the AR R872G variant compared to those with normal AR are shown in Fig. 3. The EC_{50} values in both strains are similar (2.2×10^{-9} vs 2.4×10^{-9}), indicating that the R872G mutation does not significantly affect AR's responsiveness to testosterone.

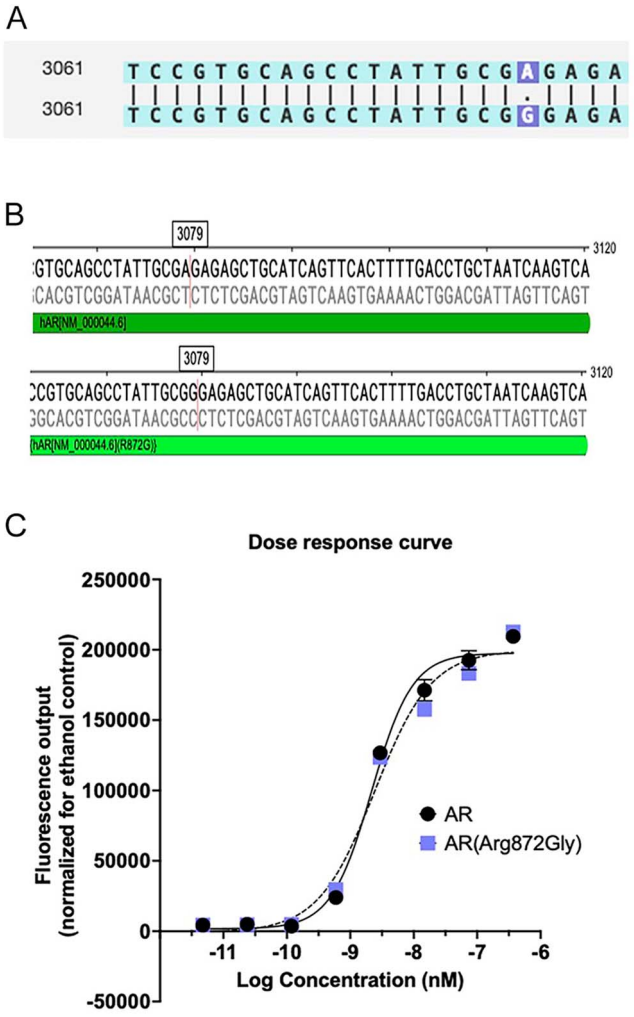

Figure 3

AR Arg872Gly shows no impairment in its capacity to respond to testosterone. (A) Sequence alignment between FASTA sequences of pYEP-URA3-TEF1-hAR (NM_000044.6) and pYEPURA3-TEF1-hAR (Arg872Gly). The figure shows the single change in base from adenosine to guanine at position 3080 of pYEP plasmid backbone. (B) Sequence alignment of pYEP-URA-TEF1-hAR (NM_000044.6) and pYEP-URA3-TEF1-hAR (Arg872Gly) plasmid backbones showing the mutation in the sequenced plasmids. (C) Dose–response curves for yeast strains harboring the normal hAR sequence or the Arg872Gly variant. Yeast cultures were treated for 24 h with serial dilutions of testosterone across the range 3.7×10^{-7} to 4.7×10^{-12} nM. Each point represents the mean of duplicate independent experiments (mean ± SEM).

Discussion

Molecular structural modeling

The present *in silico* molecular modeling experiments provided a plausible explanation for the older functional studies of this AR mutation, demonstrating a faster ligand dissociation rate compared with the WT AR (8). The present dynamic structural molecular modeling suggests that the arginine at position 872 in the LBD of the AR (mutated in this family) plays a major role in promoting the correct folding of the LBD, notably stabilizing the helix forming the 'lid' of the binding pocket, which effectively locks in the ligand to the binding site. The looser locking of the binding site 'lid' in the mutant AR provides a plausible explanation of its previously demonstrated faster ligand dissociation rate. Both testosterone and DHT had similar binding affinities to the mutant and WT ARs. The present finding suggests that a synthetic androgen with higher affinity to the ligand-binding site or even higher testosterone or DHT doses may be more effective in the affected men with this AR mutation if their dissociation rates are lower than for testosterone or DHT. Theoretical possibilities for more potent androgens include nandrolone derivatives (19, 20, 21) or some nonsteroidal synthetic androgens (SARMs) (22, 23), although none are yet marketed and their dissociation rates are not well characterized.

Diagnosis

As the most frequent cause of XY DSD, AIS arises from inactivating mutations in the X-linked AR gene causing end-organ resistance to androgen action evident in hemizygous males. AIS clinical features depend on the residual functionality of the mutated AR, with CAIS and PAIS having readily recognizable clinical features. By contrast, MAIS is represented by the mildest defects in virilization, typically produced by point mutations in the N-terminal (exon 1) or ligand-binding (exons 5–8) domains, but not by larger indels causing a greater disruption of AR function (3). The subtler clinical features of MAIS create underdiagnosis, rendering its prevalence still unknown. In the present cases, the AR mutation was originally shown to produce a faster dissociation rate from AR binding and is now shown to be due to the mutation modifying the 'lid' of the binding pocket to reduce its energetic closure of the bound ligand. We now further show that androgen bioactivity in an *in vitro* androgen-binding assay was unimpaired, consistent with the nearly fully normal adult male phenotype barring effects that were likely manifest during the fetal masculinization window of the first trimester of pregnancy, such as reduced body hair, gynecomastia and micropenis.

In the present family tree, we identify characteristic X-linked genetics but with significant phenotypic variation despite an identical genotype. The index case, his maternal uncle and his Canadian cousin all presented with gynecomastia and micropenis (8); however, the impact on male fertility was less consistent. In our cases, one older man was infertile (although details of his reproductive function and sperm output were not available) and the other was naturally fertile, whereas the adolescent index case had still undefined fertility. Although MAIS is often linked with male infertility,

often as the presenting feature (3, 7), other data indicate natural paternity with mutations in the LBD (3) and that testicular sperm from men with AIS may fertilize oocytes in IVF/ICSI procedures, indicating preservation of qualitative sperm fertilizing functionality (24, 25). These findings together with the discrepancies between fertility among our adult cases raise the possibility that a frequent association of MAIS with male infertility may reflect an ascertainment bias. This might reflect heightened awareness and surveillance for AIS with clinically minor features among male infertility populations (26), whereas among other non-infertile men, the diagnosis may not be considered and missed. Hence, whether infertility is an authentic feature of MAIS remains to be further determined.

Genotype–phenotype correlation in AIS is reportedly imperfect but not well understood. Postulated explanations include epigenetic variations, including in AR coactivators, in 5α-reductase type 2 activity and somatic mosaicism (3, 27). Our family tree includes a pair of monozygotic twins with female gender identity but who remained unmarried and childless with their medical records (from the 1950s) recording features of 'hermaphroditism', an obsolete term for intersex or DSD. Their descent is consistent with inheriting the same mutant AR as our index cases, but this genotype is inferred as no tissue was available for verification. Nevertheless, these features of phenotypic variability are consistent with previous reports indicating significant, and even striking, phenotypic variations within the same family with a single AR mutation (28, 29).

The most consistent clinical presentation of MAIS features of decreased body hair, gynecomastia and micropenis were present in all our cases. These likely reflect impaired prenatal androgen action during the masculinization programming window in the early fetal life (30, 31). It is unclear whether or to what degree impaired androgen action has further impact during minipuberty (32) when imprinting of androgen sensitivity of androgen-sensitive tissue may occur; however, androgen action during puberty is relatively normal or only minimally impaired. A young man with true gynecomastia and micropenis with reduced body hair should prompt the differential diagnosis of XY DSD (33) as causes of genetic, prenatal male under-virilization. For this differential diagnosis, hormonal screening can favor but not fully verify the diagnosis of AIS when the features of a high serum testosterone, LH and SHBG with a normal serum FSH are present. The product of serum LH and testosterone, the ASI, can serve as a screening test for AIS (34), although with limited specificity and sensitivity (26). An alternative combination of serum testosterone with sperm output is reportedly more effective for screening infertile men but is not applicable to younger boys (26). Nevertheless, these hormonal features cannot distinguish between clinical AIS phenotypes.

It is important to differentiate among other causes of XY DSD, such as 5α reductase type 2 deficiency (35), 17β-hydroxysteroid dehydrogenase type 3 deficiency (36), or gonadal dysgenesis. The latter is a miscellany of very rare genetic disorders, which remain genetically undefined (5, 37, 38). A high serum testosterone/dihydrotestosterone ratio (35) is indicative of 5α reductase type 2 deficiency, whereas a high serum androstenedione/testosterone ratio (36) is characteristic of 17β-hydroxysteroid dehydrogenase type 3 deficiency. These hormonal screening tests are most reliable when steroids are measured accurately by liquid chromatography-mass spectrometry rather than by less specific steroid immunoassays. The predominant use of steroid immunoassays has led to confusion about the thresholds and diagnostic reliability of steroid ratios, and further analysis of more accurate mass spectrometry-based steroid measurements may improve the diagnostic accuracy of circulating steroid measurements. Nevertheless, genetic diagnosis is essential to verify the diagnosis.

Interestingly, the clinical features of mild androgen insensitivity are also evident in Kennedy's syndrome, a genetic variant of motor neuron disease due to an excessive number of CAG triplet repeats (>37) in exon 1 of the AR but without mutations in other functional AR domains (39). Men with Kennedy's syndrome often display normal reproductive function at younger post-pubertal age before presenting with motor neuron disease symptoms in the fourth or fifth decades of life when associated with mild androgen insensitivity features, such as gynecomastia and testicular atrophy. The neurodegenerative features are most likely due to a toxic gain-of-function effect of the excessive CAG triplet repeat with protein accumulation in neurons rather than any functional defect in AR-mediated androgen action (39).

Management/treatment

Clinical management of MAIS is focused on cosmetic improvement of embarrassing clinical features such as gynecomastia, micropenis and psychosexual functions that can impair psychosocial adjustment. Specific options include plastic surgery, hormonal treatment, fertility management and psychological and genetic counseling.

Cosmetic surgery for gynecomastia is valuable for the mental well-being of affected males, especially adolescents, and it has superior and faster results than treatment with estrogen blockers or aromatase inhibitor drugs that require prolonged treatment with the risk of adverse effects. By contrast, urologic surgery is not required in the absence of hypospadias (3, 27). The risk of gonadoblastoma in impalpable gonads containing Y-chromosome elements is controversial and largely confined to more severe AIS variants (such as PAIS but

not CAIS) but not an issue for MAIS (40). Even with a significant risk of malignancy, prophylactic gonadectomy is no longer considered a routine but deferred till at least after the pubertal development is completed, at least for CAIS if not PAIS (41, 42).

Our family tree verifies that genetic counseling for MAIS with a known AR mutation is useful to screen female family members for the risk of MAIS in progeny. A heterozygous female carrier has a 50% risk to male offspring for MAIS, whereas an unaffected (noncarrier) female has no risk. An important caveat on the prediction of the affected male offspring is the risk of phenotypic variation. Psychological counseling in MAIS may be useful for males concerning their virilization, sexual function and fertility.

As an X-linked genetic disease, AIS is manifest in hemizygous males, whereas a single mutant inactive AR allele in heterozygous females renders them asymptomatic but obligate carriers with a 50% risk of mothering hemizygous sons (7, 27, 43). There are no reports of females homozygous for inactivating AR mutations and, owing to the sterility of potential fathers harboring strongly inactivating AR mutations, such homozygote females are unlikely to occur in nature; however, this limitation may not apply to mild AR mutations (e.g., MAIS) in men who have preserved fertility. Experimentally, homozygous inactivating AR knockout mutations have been produced in female mice using complex genetic engineering to circumvent the sterility of AIS males. These females display suboptimal female fertility, indicating a requirement for androgen function in optimal ovarian follicular recruitment and maturation (44), including protection against experimental polycystic ovarian syndrome (45). Hence, whether the reproductive performance of obligate heterozygous mother of children with AIS is reduced would be of interest but remains unknown.

The main hormonal treatment option for MAIS is high-dose testosterone treatment aiming to improve virilization, notably for micropenis, with the safety advantage of genetic protection against adverse pharmacological effects of any androgen (46). The minimally reduced androgen binding to the AR with preserved androgen bioactivity facilitates the use of high-dose testosterone for treating MAIS. Conventional testosterone replacement therapy for hypogonadism is not required for MAIS. There are no established guidelines to guide dosage, method or duration of androgen treatment for micropenis (46), but treatment in early puberty is thought to achieve best results. High-dose testosterone treatment prior to the completion of male puberty may increase circulating estradiol due to unimpeded aromatization of testosterone in AIS and limit statural growth due to premature epiphyseal closure. Nevertheless, MDM was treated with high-dose testosterone therapy for 3 years from the age of 18–21 for micropenis. Although he did experience increased statural growth and aspects of virilization, no effect on penile growth was achieved. This may reflect that defects in penile development due to impaired androgen action during the masculinization programming window of the early fetal life (30, 31) may not be repaired by androgen exposure later in development. The failure to suppress his elevated serum LH and SHBG levels is consistent with his underlying androgen resistance. Whether MDM experienced spermatogenic suppression during testosterone treatment or had a delayed completion of normal spermatogenesis cannot be deduced from these observational findings; nevertheless, he did subsequently display natural fertility.

In this context of androgen treatment, the nonaromatizable DHT topically has the advantage of reducing the theoretical risk of adverse estrogen-dependent effects limiting statural growth (46). A more specific treatment for micropenis is the topical application of androgens, either testosterone or its more potent pure androgenic (nonaromatizable) metabolite, DHT. Topical DHT gel was first reported for the treatment of micropenis in a 30-year-old man with PAIS due to an exon 2 (DNA binding domain (DBD)) AR mutation. After no clinical response to 1 year of treatment with high-dose injectable testosterone (weekly 250 mg testosterone enanthate), he had a modest increase in SPL during subsequent treatment with daily transdermal DHT treatment for 6 months applied topically to his microphallus (47). A subsequent case series of 3 related males (one adult and two adolescents aged 13 and 11 years) with PAIS due to a DBD (exon 4) mutation, treated with daily topical DHT gel (0.3 mg/kg) for 4 months, produced 40–63% growth of SPL in the adolescents but none in the 24-year-old adult previously treated with high-dose injectable testosterone (48). A further case series of 23 prepubertal children treated with daily topical DHT gel (0.1–0.3 mg/kg) for 6 months, which included two children with uncharacterized AR mutations who displayed similar doubling of SPL to children with 5α reductase type 2 deficiency or uncharacterized micropenis (49), was reported. In general, the effects of topical DHT gel treatment are most evident in younger boys. The index case is scheduled to undergo topical DHT gel treatment.

Natural or induced fertility in MAIS has been reported but only in individuals with AR mutations in the LBD (3), such as in our kindred. Semen analyses in MAIS are varied, with oligospermia or azoospermia reported in most cases. Patient MDM did not experience impaired fertility or spermatogenesis despite his MAIS phenotype, nor was his fertility apparently impaired by long-term high-dose testosterone therapy. In other individuals with MAIS, treatment with a relatively low dose of a synthetic androgen (mesterolone) was reported to temporarily induce spermatogenesis and fertility (3). The potential for long-term harm of high-dose testosterone treatment in MAIS, based on the hypothetical risks of accelerating atherosclerotic cardiovascular and late-life prostate

diseases, seems likely to be minimized by androgen resistance.

Declaration of interest

DJH has provided expert witness testimony to antidoping and professional standards tribunals, is supported by an NHMRC Investigator Grant. Other authors have no disclosures.

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