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LETTER TO THE EDITOR

Male Infertility

An infertile man with gynecomastia caused by a novel mutation of the androgen receptor gene

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Dear Editor,

We present here a rare case that an infertile man had gynecomastia for 8 years, which might be caused by a novel mutation (c. 728 T > G) found in the transactivation domain (TAD) of androgen receptor (AR) gene.

The AR, an intracellular ligand-activated transcription factor, is encoded by a single-copy gene located on Xq11-q12.[1] This gene comprises eight exons, of which exon 1 codes for the N-terminal TAD, exons 2–3 encode the DNA-binding domain, and exons 4–8 encode a C-terminal hormone-binding domain.[2] *AR* mutations cause androgen insensitivity syndrome (AIS), which is characterized by a complete or partial resistance to androgen. This can be subdivided into three phenotypes: complete AIS with typical female external genitalia, partial AIS with predominantly female, predominantly male, or ambiguous external genitalia, and mild AIS (MAIS) with typical male external genitalia. Until date, more than 1000 *AR* mutations have been annotated in the *AR* gene mutation database (http://www.androgendb.mcgill.ca), with more than 100 clustered in exon 1. This report describes a novel missense mutation in the AR TAD in an infertile man with gynecomastia.

A 30-year-old infertile man who had gynecomastia for 8 years was admitted to the reproductive medicine center of our hospital (**Figure 1a**). He was of medium height with a hairless face and no apparent Adam's apple. His penis was small, and the testes were palpable, but soft in texture. They were also decreased in size, with a testicular volume of 10 ml as measured by an orchidometer on each side (**Figure 1b**). The epididymis and vas deferens were observed bilaterally. Pelvic magnetic resonance imaging (MRI) showed that the prostate was decreased in size (**Figure 1c** and **1d**). Routine karyotyping revealed a 46, XY karyotype, 21pstk$^+$ with two trabants. Semen analysis could not be done due to an ejaculation, even with orgasm and hence we carried out a percutaneous testis biopsy. This revealed the degeneration of most seminiferous tubule basement membranes, resulting in a glassy appearance and the almost exclusive presence of Sertoli cells within the lumen of seminiferous tubules (**Figure 1g**).

Elevated serum levels of luteinizing hormone (LH, 12.7 IU l^{-1}) and follicle stimulating hormone (FSH, 33.66 IU l^{-1}) were found, although levels of testosterone (T), free T, estradiol, prolactin (PRL), adrenocorticotropic hormone (ACTH), and growth hormone (GH) were within normal ranges (11.0 nmol l^{-1}, 34.18 pg ml^{-1}, 95 pmol l^{-1}, 0.21 IU l^{-1}, 33.37 ng l^{-1}, and 0.332 ng ml^{-1} respectively). MRI revealed a 2-mm node in the patient's pituitary gland, which was indicative of pituitary microadenoma. Gadolinium-enhanced MRI was avoided because of the patient's allergic constitution. The patient had no response to the common or prolonged gonadotropin-releasing hormone (GnRH) provocative tests. The levels of testicular tumor markers α-fetoprotein and β-human chorionic gonadotropin were within normal ranges, and the thyroid functional test was normal.

Eight *AR* exons were sequenced from the patient, and a novel variation was identified in exon 1. This c. 728 T > G mutation was in the TAD (**Figure 1e** and **1f**) and resulted in a valine–glycine substitution at codon 243 (V243G). SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page = npsa_sopma.html) and Polyphen (http://genetics.bwh.harvard.edu/pph) prediction tools were used to determine how the mutation affects the secondary and tertiary structures of the protein. p.V243G was predicted to change the random coil structure of the protein into an extended strand, and to slightly affect protein stability. During sequence analysis, the patient was shown to have 22 CAG repeats and 14 GGC repeats, which are both within physiological ranges (10–35 and 4–24, respectively).[3] This, therefore, excluded Kennedy's disease in which the number of CAG repeats exceeds 40.

The AR TAD plays an important role in regulating AR activity by interacting with coregulators. Therefore, missense mutations in this region may down-regulate transcriptional activity. Goglia *et al.*[4] previously demonstrated that the A242S mutation was located in a region critical for correct AR folding, and that this substitution could lead to a moderate conformational change of the AR TAD associated with MAIS and azoospermia. The A242S mutation is highly conserved between different species, and is adjacent to the V243G mutation identified in the present case (**Figure 1h**).[4] Therefore, p.V243G may also affect the AR function, and this is supported by structural and functional analysis that showed the substitution changes a random coil into an extended strand,

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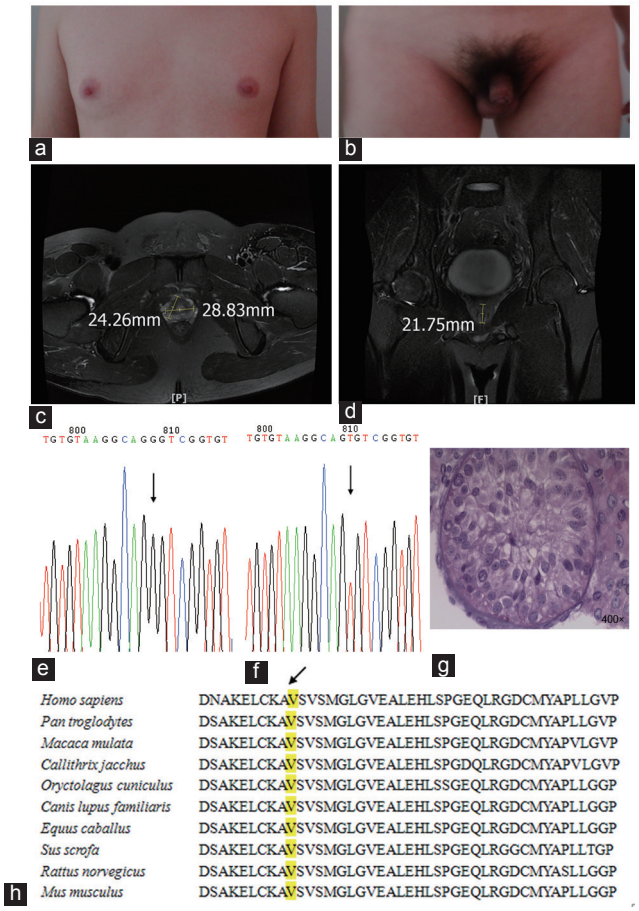

Figure 1: Characteristics of patient. (**a**) Enlargement of the right breast. (**b**) Small penis and testes. (**c** and **d**) Magnetic resonance imaging showing the decreased size of the prostate (22 mm × 24 mm × 29 mm). (**e**) Sequencing data of patient androgen receptor (AR) exon 1 showing homozygous mutation site (arrow) at c.728 T>G (p.243V>G). (**f**) Sequencing data of healthy control. (**g**) Microscopic analysis of testicular biopsies showing almost exclusive presence of Sertoli cells, although some germ cells were found within the lumen of seminiferous tubules. (**h**) Sequence comparison of the AR transactivation domain amino acid sequences between different species. Arrow marks codon 243.

altering the secondary structure of the protein and slightly impairing the AR interaction with androgen. It is, therefore, possible that this mutation is responsible for testicular hypoplasia as seen in a report by Hose *et al*.[5]

Because the levels of PRL, ACTH, GH, and TSH were all normal in the present case, it is likely that the pituitary microadenoma was nonfunctional. However, the lack of response to the GnRH test indicated that the mutation caused a dysfunction of androgen feedback in the hypothalamus and pituitary.[6] The observed elevated levels of LH are also likely to reflect the consequence of an impaired feedback regulation of T on LH secretion caused by AR dysfunction in the hypothalamic–pituitary axis.[6] Consistent with some reported studies, the concentration of T in our patient was within the normal range.[4,7] FSH levels in AIS patients can also be variable, as FSH secretion is additionally controlled by inhibin B, which is a marker of spermatogenesis, originating from the Sertoli cells.[8] In MAIS patients, low levels of inhibin B cause increased FSH secretion, correlating with the Sertoli cell-only phenotype.[9] Moreover, the chromosome karyotype 46, XY, 21pstk$^+$ with two trabants is a common polymorphism that might not be related to the aberrant expression of hormones.[10]

In summary, a novel mutation was identified in the AR TAD of an infertile male patient. This mutation could be responsible for various MAIS symptoms such as gynecomastia and impaired spermatogenesis. It will also enrich the *AR* database and lay the foundation for clinical diagnosis and therapy of affected patients.

AUTHOR CONTRIBUTIONS

HYX carried out the molecular genetic studies, participated in sequence alignment, and drafted the manuscript. CDL, XMG, and XY participated in the acquisition, analysis, and interpretation of data. LLT and LLW took part in data acquisition and sequence alignment. XYL and BCC conceived the study and helped to draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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