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## LETTER TO THE EDITOR

Male Infertility

# An insertion mutation in the androgen receptor gene in a patient with azoospermia

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Dear Editor,

We present here a case of an infertile man with azoospermia, from whom an insertion mutation (g. 1283\_1284insAGTTTGCTG) near the beginning of the CAG repeat in the exon 1 of androgen receptor (*AR*) gene is demonstrated.

The human *AR* is a ligand-activated transcription factor. Encoded by a single-copy gene located on Xq11–12, it is composed of 8 exons.<sup>1</sup> There are four domains in the protein structure, including the N-terminal transactivation domain (TAD) encoded by exon 1, DNA-binding domain encoded by exons 2 and 3, hinge region encoded by part of exon 4, and C-terminal ligand-binding domain encoded by exons 4–8.<sup>2</sup> The TAD consists of two polymorphic sites, characterized by different numbers of CAG and GGN repeats in exon 1, resulting in variable lengths of polyglutamine and polyglycine stretches.<sup>3</sup> Mutations in the *AR* gene may result in the androgen insensitivity syndrome (AIS) with various phenotypes in XY individuals. Males with Complete AIS (CAIS) show the female phenotype with female external genitalia. In partial AIS, the patients have genital ambiguity while males with mild AIS (MAIS) are phenotypically normal with or without normal spermatogenesis.<sup>4</sup>

More than 1000 mutations in the *AR* gene have been reported, most of which pertains to substitutions leading to transformations of proteins. Variations of insertions or duplications are rare, and the overwhelming majority of them are in regard to CAIS.

A 25-year-old infertile man presented to our department with a history of hypoplasia of testis. The scrotum was small and testes were palpable but decreased in size, with a one-sided testicular volume of 4 ml measured by an orchidometer. Routine examination of semen was conducted with the consequence indicating the volume and pH of semen was normal (2.2 ml and 7.2 ml, respectively), however, the total amount and concentration of spermatozoa were down to zero. In addition, no sperm could be detected even after centrifugation. Scrotum biopsy failed by the rejection of patient (**Table 1**). Hormonal evaluation revealed that total testosterone (T), free T and estradiol were in the physiological range (16.62 nmol l<sup>-1</sup>, 43.06 pg l<sup>-1</sup> and 111.0 pmol l<sup>-1</sup>, respectively), whereas luteinizing hormone (LH, 17.00 IU l<sup>-1</sup>) and follicle stimulating hormone (FSH, 49.70 IU l<sup>-1</sup>) were in high levels (**Table 1**).

Eight *AR* exons were sequenced from the patient, and molecular genetic testing of the *AR* gene revealed a mutation in exon 1, which resulted in a glutamine to leucine substitution at codon 58, caused by a CAG to CTG transversion. Besides, a more remarkable variation (g. 1283\_1284insAGTTTGCTG) was found between codon 56 and codon 57, which meant three proteins (serine-leucine-leucine) were added in **Figure 1a**. The point mutation was at the beginning of CAG repeat, and the insertion was close to it in the meanwhile. To predict how the mutation affected protein function and stability, SOPMA (<http://www.ibcp.fr/predict.html>) prediction tool was performed. The results, to be specific, the ratio of four main spatial structures in normal and variant sequences were as follows: alpha helix 27.56%–25.10%, extended strand 9.06%–8.37%, beta-turn 1.57%–3.19%, and random coil 61.81%–63.35% (**Figure 1b**).

The number of CAG and GGC triplets was determined during analysis of exon 1. The triplet number (21 CAG and 17 GGC) was within the normal range (10–35 and 4–24, respectively),<sup>3</sup> therefore, the possibility that the length of the CAG repeat contributed to infertility in this patient could be excluded, especially Kennedy's disease in which the number of CAG repeats exceeds 40.<sup>5</sup>

Routine examination of semen delivered signs that spermatogenesis may be impaired with no sperm being detected, which could be considered as azoospermia. It was reported that an elevated level of serum FSH, together with either normal or low serum T levels, was associated with primary testicular failure. In contrast, elevated serum LH levels with normal or elevated T levels indicated androgen resistance at the hypothalamic-pituitary level.<sup>6</sup> In this case, the elevation of LH was the consequence of an impaired negative feedback control of T on LH secretion due to insensitivity of the *AR*. Then the deficiency of spermatozoa in patient might be responsible for the inverse feedback toward FSH. Consistent with the previous study, the T was within the normal level.

Exon 1, as the longest coding region of the *AR* gene, plays a significant role in regulating *AR* activity and encodes roughly 58% of the protein.<sup>7</sup> According to the *AR* gene mutations database (<http://www.androgendb.mcgill.ca>, last updated 2013), approximately 20.7% of entire mutations have been reported in exon 1, among which only 9.6% (mainly point mutations) result in the MAIS phenotype. Of the total, just 24 insertion mutations (about 2.2%) are covered, the majority of which occur in CAIS patients. Our finding differed from them with the insertion of nine nucleotides, which avoided causing a frame shift and stopping in advance. It meant the length and structure of proteins did not change violently, without contributing to CAIS.

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**Table 1: Hormonal features and examination of semen in the patient**

Characteristic	Measure	Normal range
Age (year)	25	
Karyotype	46, XY	
LH (IU l <sup>-1</sup> )	17.00	1.24–8.62
FSH (IU l <sup>-1</sup> )	49.70	1.27–19.26
T (nmol l <sup>-1</sup> )	16.62	6.07–27.10
Free T (pg ml <sup>-1</sup> )	43.06	15.00–50.00
Estradiol (pmol l <sup>-1</sup> )	111.0	73.4–275.25
Color of semen	Ivory white	
Volume of semen (ml)	2.2	2.0–6.0
pH of semen	2	2–7
Total amount of sperm	0	More than 40×10 <sup>6</sup> ml <sup>-1</sup>
Concentration of sperm	0	More than 20×10 <sup>6</sup> ml <sup>-1</sup>
Remarks	No sperm after centrifugation	

LH: luteinizing hormone; FSH: follicle stimulating hormone; T: testosterone

But obviously, it varied a lot from alterations caused merely by point mutations.

The p.Gln58Leu substitution presented in this study has been previously reported.<sup>8</sup> In accordance with the study, an extra leucine is located immediately upstream of the polyglutamine tract and is likely to slightly change the conformation of this domain, thereby perhaps altering interaction with other co-activators and/or repressors. Furthermore, together with notable changes that three proteins, Ser-Leu-Leu, inserted near the point mutation, the final result was that a chain of proteins involving five ones altered around the beginning of polyglutamine tract, from “LQ” to “SLLL” (Figure 1a). With the help of SOPMA, we identified that the specific gravity of the alpha helix and extended strand reduced while beta turn and random coil structure increased. It demonstrated the variation induced a moderate change of the predicted protein structure in the TAD of the AR, where was known to interact with co-factors.<sup>9,10</sup>

To testify the prevalence of insertion mutations associated with MAIS and damaged spermatogenesis, we analyzed relevant studies in exon 1 by AR database. Ferlin *et al.*<sup>4</sup> covered infertility in a subject with a mutation at codon 57 of the AR gene, with one leucine duplicated, the position of which was definitely identical to our finding. Our present case showed two more amino acids (serine and leucine) compared to them. Moreover, as the polyglutamine tract coded by the CAG repeat is involved in regulating the AR transactivation function, it is crucial to spermatogenesis.<sup>8</sup> Therefore, we believed that the protein function may be affected, for the variation was located near the beginning of polyglutamine, where was conserved region for the correct folding of AR protein and resemble outcomes could be referred.

However, for our expose of variation includes a substitution and an insertion, further studies are needed to elucidate the detailed roles of these mutations in male infertility separately.

In summary, an insertion mutation together with a known point variation was described in the TAD of AR gene. They could be responsible for the impaired spermatogenesis, and this research would enrich the AR database and extend the current understanding of AR pathology.

#### AUTHOR CONTRIBUTIONS

YHC carried out the genetic studies, conducted the analysis of mutations and drafted the manuscript. HYX and CDL participated in the acquisition, analysis, and interpretation of data. ZYW and ZHZ took part in data acquisition and sequence alignment. ZGW and BCC conceived the study and assisted to modify the manuscript. All authors read and approved the final manuscript.

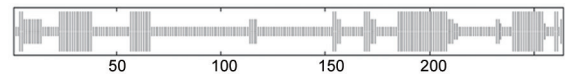
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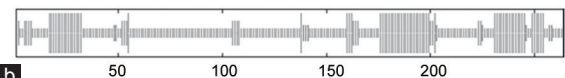
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**a**

Sequence of normal



Sequence of the patient



**b**

**Figure 1:** Results of sequencing and structure prediction. (a) “>0” and “>1” stand for sequences from the normal and the patient, respectively. Alignments of them revealed the insertion of three proteins including serine-leucine-leucine at codon 57 in yellow block, along with the point mutation at codon 58 in green block. (b) The ratio of four main spatial structures in normal and variant sequences was described with various colors: blue, red, green, and purple line represents alpha helix, extended strand, beta-turn, and random coil, respectively.

#### COMPETING INTERESTS

The authors declared no competing interests.

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