CASE REPORT

Mild androgen insensitivity syndrome (MAIS): the identification of c.1783C>T mutation in two unrelated infertile men

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SUMMARY

Two unrelated men complaining of primary male infertility presented to Orient Hospital in Damascus city. Physical examination showed moderate hypoandrogenic features. Both men were azoospermic. Hormone profiles revealed an elevation of follicle-stimulating hormone in one patient, but all the other hormones tested were within normal limits for both patients. Further genetic analyses, including karyotype and microdeletions in the AZF region of the Y chromosome, were normal in both patients. Mild androgen insensitivity syndrome was expected in the two patients. Sequencing analysis of the first exon in the androgen receptor (AR) gene have shown c.1783C>T mutation in the two patients with azoospermia. This paper sheds light on the need to screen for mutations in the AR gene, causing male infertility whenever mild hypoandrogenic features are present with unexplained male infertility.

BACKGROUND

The human androgen receptor (AR) is an essential steroid hormone receptor that plays a critical role in male sexual differentiation, development and preservation of the male phenotype, ignition and maintenance of spermatogenesis.¹ It is characterised by a modular structure consisting of four functional domains: an N-terminal domain, which serves transcriptional activation function; a central DNA-binding domain rich in cysteine residues; a hinge region containing the nuclear targeting signal and a C-terminal ligand-binding domain (LBD). LBD plays a critical role in nuclear localisation, receptor dimerisation and interaction with other proteins in addition to ligand binding.^{2,3} In humans, AR is a 110 kDa protein composed of 919 amino acids that are encoded by the AR gene located on the X chromosome at Xq11-12 and has eight exons.⁴

Mutations in the AR gene cause malfunctioning of the AR function, including loss of function, AR alterations and gain of function, many of which are associated with androgen insensitivity syndrome (AIS) (OMIM no 300068).⁵

AIS is a recessive genetic disorder composed of three different classes based on phenotype: complete androgen insensitivity syndrome (CAIS), partial androgen insensitivity syndrome (PAIS) and mild androgen insensitivity syndrome (MAIS).⁶ Individuals with CAIS syndrome have female external

genitalia with normal labia, clitoris and vaginal introitus, whereas those with PAIS syndrome have various degrees of ambiguous genitalia, including partial labial scrotal fusion, hypospadias, bifid scrotum and gynecomastia. By contrast, MAIS individuals have a moderate presentation with major complaint of disruption in the spermatogenesis process without any abnormalities in their secondary male sexual characteristics.⁷ Since 2004, the number of reported mutations in the AR gene increased from 605 to 1029; these mutations have been linked to prostate cancer, male infertility and breast cancer.⁸ Recent studies have suggested a connection between specific AR mutations and idiopathic male infertility.⁹ Identification of such mutations is essential for precise diagnosis and managements. In this study, we present a point mutation in the AR gene found in two unrelated infertile men.

CASE PRESENTATION

Two unrelated men aged 40 and 36 years were referred to Orient Hospital in Damascus Syria for reproductive counselling and treatment. Their main complaint was primary male infertility for 10 and 8 years, respectively. Their wives aged 34 and 32 years, respectively, were normal for all infertility investigations. Their onset of puberty was normal (by 14 and 15 years old). Both patients have no erection or ejaculate disturbance. Patients were questioned for any positive cases of infertility in their families and signed a written consent, according to the protocol approved by local Ethics Committee at Damascus University and health ministry.

A general physical examination for both patients has shown that they have moderate hypoandrogenic phenotype (low facial, axillary and pelvic hair), nearly normal male voice and normal external genitalia. Routine clinical and laboratory tests were performed for both patients. Semen analysis was done according to WHO criteria (WHO laboratory manual for the examination of human semen and semen-cervical interaction, 2010); both patients had azoospermia. Determination of testicular volume was performed by sonography using a 7.5 MHz probe (GE Voluson E800), which revealed a normal size for both patients (16 and 18 mL, respectively). Hormonal tests, including follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin (PRL) and testosterone, were done using ELISA by the

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Figure 1 Microscopic image of the testicular biopsy for patient 1.

Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Hormone profile results have shown an elevation of FSH hormone in patient 1 (24.6 U/L, normal values 1.5–15 U/L), but it was normal in patient 2 (11.7 U/L). Other hormones (LH, PRL and T) were within normal limits for both patients. Furthermore, previous medical reports of testicular biopsy and FNA for the two patients revealed that no mature sperms present in the testes, suggesting a Sertoli-cell-only syndrome ([figure 1](#)). In order to rule out any chromosomal aberration leading to male infertility, karyotype analysis was performed on peripheral blood lymphocytes according to standard G banding method and showed a normal karyotype: 46, XY ([figure 2](#)). In addition, genomic DNA was extracted from peripheral blood leukocytes for genetic studies, using QIAamp DNA blood mini kit (Qiagen, Germany) according to the manufacturer's instruction. Screening for microdeletions in the AZF region of Y chromosome was done by multiplex PCR. Twenty sequence-tagged site (STS) markers were analysed using Y chromosome deletion detection system, version 2 kit (Promega, Madison, Wisconsin, USA): SY81, SY86, SY84, SY182 (AZF a); SY121, SYPR3, SY124, SY127, SY128, SY130, SY133, SY134 (AZF b); SY242, SY208, SY254, SY255, SY157 (AZF c); SY145 and SY152 (proximal AZFc (AZFd)). SY14 (SRY) and SMCX locus ZFX/ZFY were also used as internal controls ([figure 3](#)). None of the patients with azoospermia had a Y chromosome microdeletion in the AZF region ([figure 2](#)). Based on some clinical findings

Figure 2 Karyotype and Y chromosome microdeletion results for both patients.

PCR conditions

initial denaturation	94° C for 2 min
35 cycles	94° C for 45 s
	57 °C for 30 s
	72 °C for 1 min
elongation cycle	72 °C for 5 min
Final hold	4 °C

Figure 3 PCR conditions for amplification of AZF region of Y chromosome.

(moderate hypoandrogenic features, accompanied with normal testosterone levels and no clear cause of male infertility), MAIS was expected in the two patients.

INVESTIGATIONS

Amplification of exon 1 in the AR gene was performed using a set of six primers in order to amplify three overlapping PCR fragments ([figure 4](#)).

In general, PCR reaction mixture (20 µL final volume) consisted of a total of 3 µL gDNA (20–200 ng), two primers (300 nM each), DMSO (5%), MgCl₂ (3.5 mM), dNTPs (200 µM), 1× buffer (Promega) and GoTaq 5 U/µL (Promega, USA). PCR cycling conditions are explained in [figure 5](#). PCR products were electrophoresed on 2% agarose gels (Promega) in 1× tris base 90 mM, boric acid 90 mM, EDTA 2 mM (TBE) buffer containing 0.5 µg/mL ethidium bromide, visualised and photographed using a UV transilluminator. PCR amplicons were analysed by terminator cycle sequencing using Big Dye v3.1 chemistry on an ABI PRISM 310 capillary DNA sequencer (Applied Biosystems, Foster City, California, USA) for the detection of mutations in the coding region of exon 1 in the AR gene.

OUTCOME AND FOLLOW-UP

Direct DNA sequencing of the exon 1 of AR gene revealed a missense mutation at codon 474 [c.1783C>T (p.Ala474Val)], in which a replacement of one nucleotide GCG=>GTG resulted in

Primer	Sequence (5'→3')	PCR fragment
AR-1F	TCCAGAATCTGTTCCAGAGCGTGC	288 bp
AR-2R	GCTGTGAAGGTTGCTGTTCTCAT	
AR-3F	TCCCAAGCCCATCGTAGAG	744 bp
AR-4R	GTAGACGGCAGTTCAGGTGTC	
AR-5F	ACCAAAGGGCTAGAAGGCGA	702 bp
AR-6R	CCGAAAGGCGACATTCTGGAAG	

Figure 4 Primers used to amplify exon 1 of the androgen receptor (AR) gene.

PCR conditions	PCR fragment 288 bp	PCR fragment 744 bp , 702 bp
initial denaturation	94° C for 7 min	94° C for 3 min
	94° C for 60 s	94° C for 45 s
35 cycles	62.5 °C for 60 s	53 °C for 45 s
	72 °C for 60 s	72 °C for 55 s
elongation cycle	72 °C for 7 min	72 °C for 5 min
Final hold	4 °C	4 °C

Figure 5 PCR conditions for amplification of exon 1 in the androgen receptor gene.

a change of the amino acid alanine to amino acid valine in both patients (figure 6).

DISCUSSION

Infertility affects approximately 15%–20% of couples¹⁰ of which male factor is responsible in about 40%–50% cases.^{11–12} One major reason for male infertility is the absence of total sperm in the semen (azoospermia), which can be due to many factors such as malfunction either in the pituitary gland or in the hypothalamus leading to hypogonadotropic hypogonadism and dual-side blockage in the seminal vesicles (obstructive azoospermia). However, non-obstructive azoospermia is mainly due to hypo-orchidism, which appears as hypospermatogenesis or maturation arrest or as a complete failure in the formation of sperm (Sertoli-cell-only syndrome).¹³ In non-obstructive azoospermia, routine clinical examination should be performed for anatomical integrity of genital system, alongside laboratory tests including FSH, LH, PRL and T, followed by karyotype and molecular analysis of the AZF region on chromosome Y due to the high prevalence of genetic abnormalities in Middle Eastern patients with idiopathic non-obstructive azoospermia.¹⁴ Certain mutations cause non-obstructive azoospermia as microdeletions in the AZF region of the Y chromosome or mutations in an essential gene

such as the AR gene.¹⁵ AR gene mutations cause a wide spectrum of abnormalities known collectively as AIS, which is an X-linked recessive genetic disorder. AIS presents in three different phenotypes: CAIS, PAIS and MAIS.¹⁶ The variety of phenotypes can be explained by the region of which the mutation alters or the linking strength of the modified receptor.¹⁷

MAIS is usually detected in normal phenotypic males and is not typically investigated except in cases of male infertility.^{18–20} It could explain some cases of idiopathic male infertility.²¹ MAIS has a mild presentation that is often unnoticeable. It can be difficult to distinguish between men with and without MAIS. A precise diagnosis of MAIS is usually done through confirmation of an AR gene mutation.^{9–22} Clinically, our two patients showed a normal phenotype with moderate hypoandrogenic features. Testicular biopsy may be part of the diagnostic process of infertile men, but usually it does not explain the real cause of infertility. It simply confirms a disruption of the spermatogenesis process in men with low/no sperm concentrations and elevated FSH.²³

Genetic analysis of our patients have revealed the c.1783C>T (p.Ala474Val) mutation in exon 1 of the AR gene, which is situated in the transactivation domain (TAD) of the AR. In particular, amino acid Ala474 is situated in the transcription activation unit 5. Therefore, alterations in it is expected to affect protein–protein interactions of the AR, leading to the deregulation of the expression of androgen-responsive genes.⁴ The mild phenotype of this mutation could be explained by the similar aliphatic nature of alanine and valine.

More than 1000 mutations in the AR gene have been described. Substitution mutations causing transformations of proteins are the most common. Variations like insertions or duplications are rare. Exon 1 is the longest coding region of the AR gene and have a substantial role in regulating AR activity.²⁴ Nearly 20.7% of the total mutations in the AR gene have been reported in exon 1, among which approximately 9.6% (mainly point mutations) result in the MAIS phenotype.^{8–25–26} The c.1783C>T mutation in the AR gene receptor was previously reported in different studies, which confirms the high frequency of this mutation in patients with infertility.^{9–15} Furthermore, all the phenotypes of the patients bearing the mutation were MAIS. These data come in agreement with our findings. Unfortunately, maternal blood samples of both patients were not available, and we could not confirm that these patients inherited the mutation from their respective mothers, or this mutation existed as a de novo mutation. Moreover, neither patients reported any family history of male infertility.

To date, few genetic studies have been conducted in Syria to identify common mutations and genetic abnormalities in infertile couples.¹⁴ In conclusion, this paper reports the existence of the c.1783C>T mutation in the AR gene receptor in unrelated men suffering from idiopathic male infertility. AR mutation screening should be expected when MAIS features are presented.

Figure 6 DNA sequence chromatograms of the c.1783C>T mutation in exon 1 of the androgen receptor gene found in two azoospermic men. Patients 1 and 2 showing a cytidine-to-thymidine substitution, compared with a normal fertile control. Arrows point to the mutated nucleotides.

Learning points

- Infertility may present as the primary or the sole symptom in mild androgen insensitivity syndrome (MAIS).
- Moderate hypoandrogenic features, normal testosterone levels and male infertility may be an indicative signs of MAIS.
- Androgen receptor mutation screening should be expected when MAIS features are presented.

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Contributors MA clinically approached the patient. AM performed genetic investigations. GAA performed the sequencing analysis. GAA and AM drafted the initial manuscript. MA revised and reviewed the manuscript. All authors have reviewed the final version of the manuscript and approved the submission process.

Competing interests None declared.

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