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Original Article

Isolated micropenis reveals partial androgen insensitivity syndrome confirmed by molecular analysis

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Abstract

Partial androgen insensitivity syndrome (PAIS) is the milder variant of androgen receptor (AR) defects. The subtle effects of AR mutations present in a patient with micropenis, peno-scrotal hypospadias, infertility, clitoromegaly and posterior labial fusion. We studied the association of isolated micropenis with the genetic defects resulting in androgen resistance, that is, *AR* gene defects and 5-α reductase type 2 (*SRD5A2*) deficiency. We describe two cases of isolated micropenis: one in a 14-year-old boy and the other in a 3-year-old boy who was followed until he was 10 years old. There were no findings of hypospadias, cryptorchidism or gynecomastia in either of these patients. Serum gonadotrophin and androgen levels were obtained and karyotyping was done. Human chorionic gonadotropin (hCG) stimulation testing assessed the functional capacity of the testes. DNA was extracted from peripheral leukocytes, and all exons of the *SRD5A2* and *AR* genes were amplified by polymerase chain reaction and sequenced. In both patients, baseline testosterone (T) level was low and the values were elevated after hCG testing. The sequence of the *SRD5A2* gene was normal in patient 1, and a heterozygous polymorphism, V89L, was found in patient 2. Two known mutations, P390S and A870V, were identified in patients 1 and 2, respectively. Mutations in the *AR* gene can be associated with isolated micropenis without other features of PAIS, such as hypospadias or gynecomastia. This underlines the importance of including *AR* gene analysis in the evaluation of isolated micropenis with normal plasma T to ensure proper management of the patient and appropriate genetic counseling for the family.

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1 Introduction

Micropenis can be a manifestation of underlying growth hormone deficiency, hypogonadotrophic hypogonadism, or defects in androgen biosynthesis and action. It has been associated with abnormalities in the androgen receptor (AR) leading to partial androgen insensitivity syndrome (PAIS) [1–4]. However, in addition to micropenis, these patients had other features of PAIS, such as scant pubic hair development, gynecomastia during adolescence and penile chordae during infancy [1–4].

In a study from the UK, the association of gonadotrophin deficiency, testosterone biosynthesis and AR

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binding was studied in 18 boys with isolated micropenis. No evidence of abnormal AR binding was found in these patients [5]. The length of the CAG trinucleotide on exon 1 that encodes for the polyglutamine tract (with a polyglutamine length of 28 or more) has been associated with male infertility [6], but no association between the CAG trinucleotide repeat and micropenis has been observed [7]. Severe micropenis has also been described in association with a polymorphism in the steroidogenic factor-1 gene [8]. The Gly146Ala polymorphism was found to reduce the function by approximately 20% [8]. In this study, a higher percentage of patients with severe micropenis carried this polymorphism compared with control subjects and patients with mild micropenis [8]. A polymorphism causing androgen production defects might also explain severe isolated micropenis, but the number of cases showing this association is unknown.

PAIS with evidence of *AR* gene defects has not been reported in the context of isolated micropenis [4]. In this study, we sought a link between isolated micropenis and an AR defect. We describe two patients with isolated micropenis and *AR* gene mutations, P390S and A870V. The P390S mutation has been described in relation to infertility and the A870V mutant has been related to hypospadias and cryptorchidism [9, 10]. This is the first report of these mutations in association with isolated micropenis.

2 Materials and methods

2.1 Hormonal testing

Human chorionic gonadotropin (hCG) stimulation tests were performed to assess the functional capacity of the testes, as previously described [11]. Steroids were analyzed by radioimmunoassay after selective solvent extraction, column chromatography, hydrolysis and high-performance liquid chromatography tandem mass spectrometry.

2.2 DNA isolation and sequencing

DNA was extracted from peripheral leukocytes and all exons of the *AR* genes were amplified by polymerase chain reaction using primer pairs and standard conditions. The *SRD5A2* gene was also analyzed in both patients. DNAs from the mothers of both patients were also obtained and extracted and the *AR* gene was analyzed using standard conditions. Written informed consent and assent to perform the mutational analysis was obtained from the parents and subjects, respectively. The study was approved by the Institutional Review Board of Maimonides Medical Center, Brooklyn, and Hôpital Lapeyronie, Centre Hospitalier Universitaire, Montpellier.

2.3 AR gene mutation prediction model

Amino-acid substitutions were studied *in silico* to predict the effects using PolyPhen (Polymorphism Phenotyping, http://genetics.bwh.harvard.edu/pph/index.html) [12] and SIFT [13] software (http://sift.jcvi.org/). The PolyPhen algorithm predicts the functional effects of amino-acid changes by considering evolutionary conservation, physicochemical differences, and the proximity of the substitution to the predicted functional domains and/or structural features. The SIFT algorithm predicts the functional importance of the amino-acid substitutions based on the alignment of orthologous and/or paralogous protein sequences. Original sequences of proteins were obtained from the Ensembl and UniProt/Swiss, Prot databases.

2.4 Patient description

Patient 1: A 14-year-old boy was seen in a pediatric endocrinology clinic for evaluation of micropenis. He had been born full term and the micropenis had been noted at the time of birth; however, the exact measurement was not available for our review. Both parents were healthy and the family had no history of delayed puberty or infertility. Physical examination revealed axillary hair at Tanner stage 2 and pubic hair at Tanner 3. The testes were descended bilaterally and 8 cm^3 in volume, the scrotum was rugated normally, and the stretched penile length was 2.0 cm (below –2 SD). No gynecomastia or hypospadias was noted. The patient's height was 163 cm (50%, +0.05 SD). His karyotype was a normal 46,XY complement. The anti-Mullerian hormone and inhibin-B levels were in the normal range (Table 1). The ultrasensitive luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were in the range of early puberty (Table 1). The baseline testosterone (T) concentration was low and rose to 15.29 nmol L^{-1} (442 ng dL^{-1}) after the hCG stimulation test, the T:dihydrotestosterone (DHT) ratio was elevated to 31.85 and the T:Δ4 (androstenedione) ratio was normal (Table 1). The penis was carefully measured during the examination by a pediatric endocrinologist. After assessment and diagnosis, the patient was treated with T replacement. Within 6

Table 1. Hormonal data and hCG stimulation test in patients 1 and 2.

Test	Patient 1 (14 years old)		Patient 2 (3 years old)	
	Pre-stimulation	Post-stimulation	Pre-stimulation	Post-stimulation
Δ4, nmol L^{-1} (ng dL^{-1})	1.19 (34)	2.84 (81)		
T, nmol L^{-1} (ng dL^{-1})	0.52 (15)	15.29 (442)	0.35 (10)	22.84 (660)
DHT, nmol L^{-1} (ng dL^{-1})	0.08 (2.2)	0.48 (14)		
T: DHT ratio	6.50	31.85		
T: Δ4 ratio	0.44	5.38		
LH, IU L^{-1}	0.72		0.10	
FSH, IU L^{-1}	3.9		0.6	
Inhibin-B, pg mL^{-1}	86			
AMH, ng dL^{-1}	20			

Abbreviations: AMH, anti-Mullerian hormone; Δ4, androstenedione; DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; hCG, Human chorionic gonadotropin; LH, luteinizing hormone; T, testosterone.

months, his penile length increased from 2 to 4 cm (–2 SD to –1 SD); T replacement was continued until 16 years of age.

Patient 2: At 3 years of age, this boy was referred to a pediatric endocrinology clinic because of isolated micropenis. He had been born to non-consanguineous parents at 39 weeks of gestation, after an uncomplicated pregnancy and delivery (birth weight: 3.6 kg, birth length: 51 cm). His height was 101 cm (+2 SD) and his weight was 15 kg (+1 SD). His stretched penile length was 2.5 cm (–3.3 SD), and the testes were descended bilaterally and 2 cm^3 in volume with pubic hair development at Tanner stage 1. The urethral meatus was located at the top of the glans. Bone age was 2.6 years. Basal LH and FSH were in the normal prepubertal range. T was 0.35 nmol L^{-1} (10 ng dL^{-1}) and increased to 22.84 nmol L^{-1} (660 ng dL^{-1}) after hCG stimulation testing. The stretched penile length after hCG testing was 4 cm (–1.7 SD), which indicated a good clinical response. This penile length was conserved 6 months later. Nevertheless, a decrease in penile length was observed at 10 years, to 3.5 cm (–2.5 SD). We thus decided to treat the patient with T enanthate (Androtardyl®, Bayer Santé, Puteaux cdx, France) at 100 mg m^{-2} (bodily area), 100 mg every month, for 3 months. This yielded a very good clinical response, with penile length increasing to 5 cm (+0.9 SD) after T injections. No history of micropenis, cryptorchidism, hypospadias or infertility was found in the mother's family.

3 Results

Patient 1: The *SRD5A2* gene was analyzed and was found to be normal. However, a Pro390Ser mutation on exon 1 of the *AR* gene was identified. The mother carried the same mutation, which is consistent with an X-linked pattern of PAIS (Figure 1A).

Patient 2: An A870V mutation on exon 8 of the *AR* gene was found. The mother was found to be a carrier for the A→V substitution identified in the propositus (Figure 1B). The *SRD5A2* gene was analyzed and a V89L polymorphism on exon 1 was found in a heterozygous state. The same polymorphism has previously been described [14].

3.1 Prediction of mutation effects

To assess the potential deleterious effect of the amino-acid change, we used two types of software to predict the functional effects. First, we performed testing with PolyPhen, a tool that predicts the possible impact of an amino-acid substitution on the structure and function of a human protein using straightforward physical and comparative analysis. The mutations were evaluated as 'Probably damaging'.

The same assessment was performed with SIFT software. SIFT uses protein sequence conservation data to calculate the probability of a mutation being deleterious. Scores less than 0.05 suggest potential pathogenicity. Similar to the PolyPhen method, SIFT placed the mutations in the 'Affects protein function' class, with a score of 0.03, and confirmed the first evaluation with the PolyPhen algorithm.

4 Discussion

Mutations in the *AR* gene are associated with a

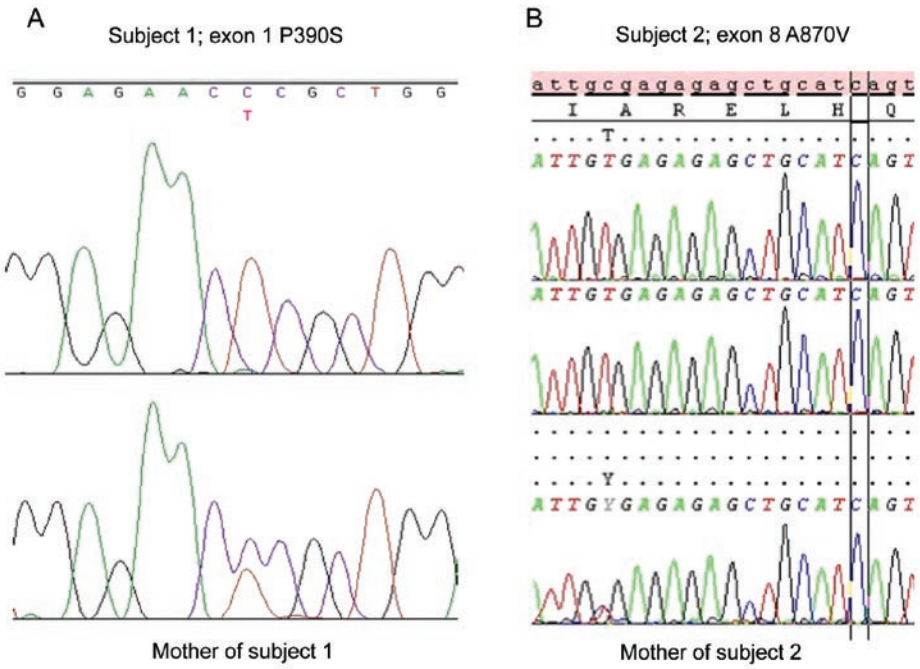

Figure 1. (A): The sequence of the patient with P390S exon 1 mutation and, below, the sequence of the mother of subject 1 carrying the same mutation. (B): The sequence of the patient with A870V exon 8 mutation and, below, the sequence of the mother of subject 2.

wide spectrum of phenotypes. To date, hundreds of mutations have been described, with clinical phenotypes from externally normal females with no androgenic effects to partial androgen insensitivity characterized by genital ambiguity and males with undervirilization at one end of the spectrum. Moreover, the same *AR* mutation can result in different clinical presentations within the same family and even among siblings [3, 15]. In one report, for example, a sibling with clitoromegaly and labial fusion was raised as a girl while the other sibling with micropenis and peno-scrotal hypospadias was raised as a boy [15]. In another report of an extended Chinese family with an *AR* mutation, some of the family members were infertile with gynecomastia and/or hypospadias, whereas other members were fertile [3].

Patient 1 was evaluated for isolated micropenis. The patient's normal stature and the detection of pubertal levels of LH and FSH ruled out growth hormone deficiency and hypogonadotrophic hypogonadism as the cause of isolated micropenis. The patient's T levels were low at baseline and increased normally after the hCG stimulation test. The T:DHT ratio was high (> 20:1) and consistent with a 5-α reductase type 2 enzyme deficiency [16]. However, it was shown in previous studies that the T:DHT ratio is not sensitive for 5-α reductase type 2 deficiency [17]. The T:Δ4 ratio was normal (> 0.8) and is a very sensitive marker for 17 βHSD-3 enzyme deficiency [18]. Upon hCG stimulation, the T:DHT ratio was high and initially the diagnosis of 5-α reductase deficiency was proposed. The entire coding region of the *SRD5A2* gene was analyzed and found to be normal. We further investigated whether *AR* defects could have led to micropenis. We found a mutation on exon 1 of the *AR* gene, which led to the Pro390Ser change. This proline to serine change at position 390 was previously reported to be associated with decreased spermatogenesis and infertility in men [9]. At the time patient 1 was evaluated, he was at Tanner 3 and only had micropenis; gynecomastia was not noted on examination. The testicular size was also compatible with Tanner 3 development. At this point, we cannot exclude the possibility of abnormal breast development later on in puberty.

Patient 2 was evaluated at 3 years of age for micropenis. The patient had a good response to hCG stimulation testing as the T levels were high, and we observed a subsequent increase in the penile length from 2.5 cm (–3.3 SD) to 4 cm (–1.7 SD). A similar response to T

Table 2. Androgen receptor gene mutations leading to micropenis.

Phenotype	Mutation	Exon	Functional affect	References
Isolated micropenis	P390S	1	Transactivation defect	This report
Isolated micropenis	A870V	8	Hormone binding	This report
Reported slightly diminished penile size	A840C (presumed mutation)	7	Hormone binding	[3]
Reported slightly diminished penile size, gynecomastia	G824L	7	Hormone binding	[2]
Micropenis, penile chordae	L712F	4	Transactivation defect	[4]

replacement was obtained later on. It has been reported that androgen resistance can be overcome by exogenous androgen therapy. Therefore, despite the good response to exogenous androgens, we decided to analyze the *AR* gene and found that patient 2 carried an A870V mutation on exon 8, which is situated in the hormone-binding domain of the receptor.

There have been some reports of micropenis with *AR* mutations, but a direct association has not been reported [3, 4]. In a large Chinese family with the Arg840Cys mutation, some of the family members were infertile with gynecomastia and/or hypospadias, whereas others were fertile but had a 'small penis', according to their wives [3]. These patients, reportedly with micropenis, were not examined by clinicians, nor was their DNA analyzed for *AR* mutations. There was no report of gynecomastia or hypospadias in these patients. As shown in table 2, another family member was found to have a 'slightly diminished penile length' and gynecomastia, but the patient's DNA was not studied for *AR* gene mutation [3]. In another report, a 16-year-old boy was described with a 'slightly diminished penile length', normal scrotal size and Tanner stage 3–4 gynecomastia [2]. Unfortunately, the measurements and SD of penile length were not provided. This patient had elevated T and LH levels with normal levels of inhibin-B. This patient was fertile but had a low seminal volume, low sperm concentration and highly immotile sperms [2]. Another report was on infants with micropenis and penile chordae. This patient was the brother of a newborn boy with PAIS and the L712F mutation of the *AR* gene [4]. Of note, these patients were examined in infancy and may or may not develop gynecomastia in puberty.

The Pro390Ser mutation on exon 1 of the *AR* gene in patient 1 has also been reported to be associated with infertility and seminoma [9, 19, 20]. The variation lies within the first exon encoding for the transactivation domain of the receptor [9]. Interestingly, this mutation is located within a region of the *AR* gene that is important for transcriptional activity of the receptor [21]. In the *in vitro* model, the Ser390 AR mutant did not lead to gross alterations in transcriptional activity in the presence of the combination of T and DHT, compared with the wild-type AR [9]. The authors suggested that the *in vitro* model was oversimplified and would not reflect the changes induced *in vivo* leading to defective spermatogenesis [9]. This might explain our patient's mild presentation of isolated micropenis without other features of PAIS. The A870V mutation in patient 2 has already been reported in association with peno-scrotal hypospadias and bilateral cryptorchidism [10]. Although the phenotype of the patient in this earlier study was more severe than that of our patient, he was assigned male gender and responded very well to T treatment, presenting normal prepubertal male genitalia after the treatment and surgical correction. Other mutations affecting this amino acid in the hormone-binding domain have been reported and lead to a wide range of phenotypes, from isolated gynecomastia to ambiguous genitalia to complete sex reversal [22–24].

We conclude that *AR* gene mutations may be linked with the phenotype of isolated micropenis without other features of PAIS, such as hypospadias or gynecomastia. This underlines the importance of evaluating the *AR* gene sequence in cases of isolated micropenis, as the identification of a mutation will have an impact on patient management and will orient appropriate genetic counseling for the family.

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