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LETTER TO THE EDITOR

Male Endocrinology

# Genetic sequencing of a patient with Kallmann syndrome plus 5α-reductase type 2 deficiency

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Dear Editor,

Kallmann syndrome (KS) is a phenotypically and genetically heterogeneous disorder featured by hypogonadotropic hypogonadism and congenital hyposmia or anosmia, accompanined with renal dysplasia, hearing loss, and craniofacial defects sometimes. More than 20 genes have been identified causing KS either alone or in combination.

5α-reductase type 2 deficiency (5α-RD) caused by *SRD5A2* gene mutation is a rare disease characterized by elevated ratio of testosterone (T) to dihydroteststorone (DHT). The clinical spectrum of 5α-RD is heterogeneous, ranging from the classic phenotype to males with hypospadias and even isolated micropenis. Here, we report a male KS patient accompanied with mild 5α-RD diagnosed from genetic sequencing and clinical analysis.

A 28-year-old male (46, XY) was referred for no secondary sexual characteristics and anosmia. At birth, he had bilateral cryptorchidism, anosmia, and deafness in the left ear. **Figure 1a** shows the patient's olfactory bulbs and tracts while **Figure 1b** shows the normal individual. **Figure 1c** shows his flat pituitary. Bilateral orchidopexy was performed at age five, and there is no prior treatment. The distance between the top of the head to the superior margin of the symphysis pubis was 87.2 cm and from the inferior margin of the symphysis pubis to the sole of the foot was 93.0 cm, and distance between outstretched arms was 187.0 cm. He had bilateral cubitus valgus, and his bone age was 16 years old (**Figure 1e**). No remarkable frontotemporal hairline recession or acne was noted. Each testicle was in the scrotum without hypospadias. Genital development was Tanner genital stage 1. His pelvic MRI showed prostate dysplasia, mullerian duct cyst (**Figure 1f**), and seminal vesicles dysplasia (**Figure 1g**). The MRI of normal seminal vesicles is given in **Figure 1h**.

Normal respond of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) was seen after the subcutaneous injection of triptorelin 100 mg. The results of the triptorelin stimulation test at baseline and 15, 30, 60, 90, and 120 min after injection were LH 0.08, 1.30, 1.48, 1.67, 1.81, and 2.26 (normal: 2–12) IU $l^{-1}$; FSH 1.02, 2.03, 2.47, 3.03, 3.13, and 4.20 (normal: 1–8) IU $l^{-1}$, respectively. T, DHT, and T/DHT ratio were 0.32 (normal: 1.58–8.77) μg $l^{-1}$, 47.58 (normal 57.5–355.0) ug $l^{-1}$, and 6.78 at baseline, and 4.41 ug $l^{-1}$, 168.5 ug $l^{-1}$, and 26.40 after human chorionic gonadotropin (HCG) stimulation, respectively. Other hypothalamic-pituitary-axes' functions were normal.

The patient was treated with HCG 2000 U i.m. 3 times every week for 9 months, and then human menopausal gonadotropin (HMG) 75 U i.m. was added 3 times a week for another 3 months. Mild

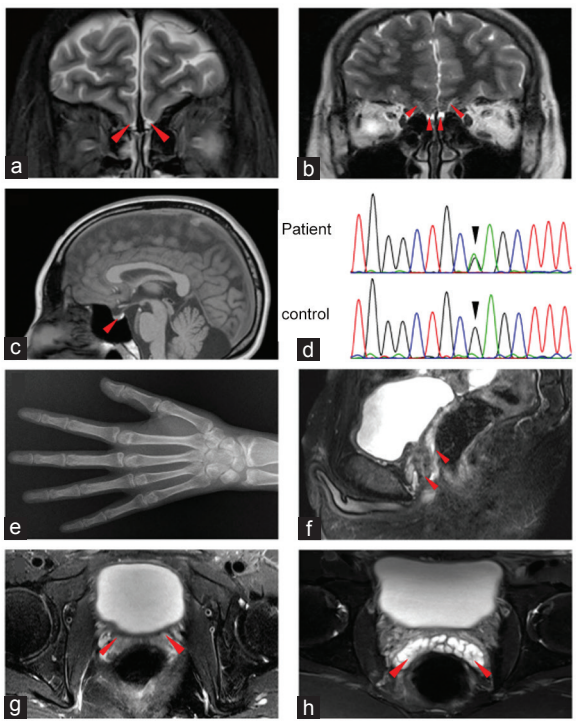


**Figure 1:** Image characters (**a**) Fat-saturated coronal T2-weighted MR image shows bilateral agenesis of the olfactory bulbs and tracts (arrows). (**b**) Coronal T2-weighted MR image shows normal olfactory tracts and bulbs (arrows). (**c**) Sagittal postcontrast T1-weighted image shows a flat pituitary but no lesion (4 mm height, arrow). (**d**) Direct sequencing of the *SRD5A2* gene shows c. 680G> A (p.R227Q) substitution at exon 4 in the patient (top arrow) and the sequence of a normal control subject (bottom arrow). (**e**) X-ray picture of the patient's left wrist shows a 16-year-old bone age. (**f**) Sagittal T2-weighted MR image shows a Mullerian duct cyst (the upper arrow) and a dysplastic prostate (the lower arrow). (**g**) T2-weighted axial MR image shows the patient's bilateral agenesis of seminal vesicles. (**h**) Axial T2-weighted MR image shows normal seminal vesicles. MR: magnetic resonance.

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improvements in secondary sexual characters are summarized in **Table 1**.

All exons and splice junctions of 23 related genes were screened using Panel or Sanger sequencing. The results were validated by Sanger sequencing. The screened genes were *KAL1*, *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, *GNRHR*, *GNRH1*, *KISS1R*, *KISS1*, *TACR3*, *TAC3*, *CHD7*, *WDR11*, *HS6ST1*, *SEMA3A*, *NR0B1*, *LHB*, *LEP*, *LEPR*, *FSHβ*, *PCSK1*, *SRD5A2*, and *NSMF*. *SRD5A2* gene variant (c. 680G>A) (**Figure 1d**) was identified in addition to two nonpathogenic variants in *KAL1* and *GnRH1* (rs808119, rs6185). **Supplementary Table 1** shows genes information and sequencing results. The failure to find pathogenic genes involved in KS could be due to the mutations mapping outside the coding regions or other candidate genes.

Both 5α-RD and severe congenital KS could result in low level of DHT in fetus and prostatic dysplasia. When KS co-existed, it is hard to diagnose 5α-RD according to DHT-dependent signs such as facial or body hair, prostate enlargement, and scalp hairline recession. Hence, identification of mild enzyme defect should be based on genetic diagnosis and T/DHT ratio.

Mild 5α-RD as stated above was due to the heterozygous c. 680G>A in exon 4. Makridakis *et al*.[1] reported that the mutant retained 3.2% of normal enzyme activity and slowed the rate of enzyme-catalyzed reaction to 0.06 nmol·l$^{-1}$·mg$^{-1}$ (normal, 1.7–2.2 nmol l$^{-1}$ mg$^{-1}$). Interestingly, the heterozygous state was found in 5% of their normal Chinese controls. Thus, a remote hypothesis of a dominant effect of some mutations, in particular, cellular environments cannot be completely excluded.[2] Since people with the same mutation may have divergent phenotypes even in one family, genetic and functional heterogeneity (due to the presence of *SRD5A1* and some interacting factors) should be taken into account. The elevated T/DHT ratio post-HCG stimulation is more sensitive and reliable in diagnosis of 5α-RD[3] and helpful to distinguish between androgen insensitive syndrome and other conditions arising from T synthesis defects. The diagnostic cut-off point of T/DHT ratio has not been precisely defined, varied from 3.6 to 18 in diverse ethnic groups pre or after HCG stimulation.[4–6] Obviously, high T/DHT ratio of 26.4 after HCG stimulation is another reason for the diagnosis of mild 5α-RD.

Some authors reported that DHT regulated the expression of genes relevant for normosmia idiopathic hypogonadotropic hypogonadism (nIHH) such as *GnRH1*, *KISS1*, and *KISS1R* and significantly stimulate the migration of FNC-B4 placode *in vivo*.[7,8] KS might be similar with nIHH in some way. The interaction between KS and 5α-RD need further investigation.

Feyles *et al*.[9] reported that the earlier the orchiopexy performed the better the restoration of spermatogenesis. In 5α-RD, the differentiation from spermatogonium to spermatocyte was defective at an age of 4–6 years, which resulted in a lack of spermatocytes but normal spermatogonium.[10] Dysplasia prostate and seminal vesicles epididymis are the primary causes for azoospermia and few semen. Testicular biopsy is an approach to confirm the testicular spermatozoa.

In conclusion, we first reported a male with KS and 5α-RD diagnosed with gene sequencing that will boost the knowledge about geneotype-phenotype connection. If a KS patient had prostate dysplasia, cryptorchidism, unremarkable frontotemporal hairline recession, or a minor response to gonadotropin therapy, *SRD5A2* sequencing might be considered.

**Table 1: Clinical features and laboratory data**

| | *Before therapy* | *After therapy* |
|---|---|---|
| Voice change | –[a] | – |
| Bear hair/pubic hair/armpit hair | –/–/– | Viliform (1-2 mm)/ Tanner P3/– |
| Testosterone level (ng ml$^{-1}$) | 0.46±0.15 | 3.65±1.93 |
| Left testicle/right testicle (length × width × thickness) (cm) | 1.0×0.4×0.6/ 1.2×0.3×0.6 | 1.6×0.8×1.1/ 1.3×0.7×0.9 |
| Head of epididymis (left/right) (cm) | Smaller/less clear | 0.7×0.4/less clear |
| Stretched length of penis (cm) | 1.4 | 2.0 |
| Semen | – | Few, no sperm |

[a]Not detected

## AUTHOR CONTRIBUTIONS

WJ collected the clinical data, performed the literature search, and drafted the manuscript. LYZ and FCL carried out the molecular genetic studies. ZHL conceived of the study and revised the manuscript. YW, WH, and QQY participated in the follow-up of treatment. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declared no competing financial interests.

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Supplementary information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

## REFERENCES

1 Makridakis NM, di Salle E, Reichardt JK. Biochemical and pharmacogenetic dissection of human steroid 5 alpha-reductase type II. *Pharmacogenetics* 2000; 10: 407–13.
2 Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, *et al*. Molecular genetics of steroid 5 alpha-reductase 2 deficiency. *J Clin Invest* 1992; 90: 799–809.
3 Hiort O, Willenbring H, Albers N, Hecker W, Engert J, *et al*. Molecular genetic analysis and human chorionic gonadotropin stimulation tests in the diagnosis of prepubertal patients with partial 5 alpha-reductase deficiency. *Eur J Pediatr* 1996; 155: 445–51.
4 Nicoletti A, Baldazzi L, Balsamo A, Barp L, Pirazzoli P, *et al*. SRD5A2 gene analysis in an Italian population of under-masculinized 46, XY subjects. *Clin Endocrinol (Oxf)* 2005; 63: 375–80.
5 Ko JM, Cheon CK, Kim GH, Kim SH, Yoo WH, *et al*. Clinical characterization and analysis of the SRD5A2 gene in six Korean patients with 5alpha-reductase type 2 deficiency. *Horm Res Paediatr* 2010; 73: 41–8.
6 Zhang M, Yang J, Zhang H, Ning G, Li X, *et al*. A novel SRD5A2 mutation with loss of function identified in Chinese patients with hypospadias. *Horm Res Paediatr* 2011; 76: 44–9.
7 Morelli A, Fibbi B, Marini M, Silvestrini E, De Vita G, *et al*. Dihydrotestosterone and leptin regulate gonadotropin-releasing hormone (GnRH) expression and secretion in human GnRH-secreting neuroblasts. *J Sex Med* 2009; 6: 397–407.
8 Morelli A, Marini M, Mancina R, Luconi M, Vignozzi L, *et al*. Sex steroids and leptin regulate the "first Kiss" (KiSS 1/G-protein-coupled receptor 54 system) in human gonadotropin-releasing-hormone-secreting neuroblasts. *J Sex Med* 2008; 5: 1097–113.
9 Feyles F, Peiretti V, Mussa A, Manenti M, Canavese F, *et al*. Improved sperm count and motility in young men surgically treated for cryptorchidism in the first year of life. *Eur J Pediatr Surg* 2014; 24: 376–80.
10 Hadziselimovic F, Dessouky N. Differences in testicular development between 5alpha-reductase 2 deficiency and isolated bilateral cryptorchidism. *J Urol* 2008; 180: 1116–20.

**Supplementary Table 1: Screened genes and sequencing results**

| *OMIM*[a] | *Symbol* | *Hereditary mode* | *Nonsynonymous variants* | *RefSeq* |
|---|---|---|---|---|
| *[b]300836 | *KAL1* | XR | c. 1600G>A | NM_000216.2 |
| *136350 | *FGFR1* | AD | -[d] | NM_023110.2 |
| *607123 | *PROKR2* | AR | - | NM_144773.2 |
| *607002 | *PROK2* | AR? | - | NM_001126128.1 |
| *600483 | *FGF8* | AD/AR | - | NM_033163.3 |
| *138850 | *GNRHR* | AR | - | NM_000406.2 |
| *152760 | *GNRH1* | AR | c. 47G>C | NM_000825.3 |
| *604161 | *KISS1R* | AR | - | NM_032551.4 |
| *603286 | *KISS1* | AR | - | NM_002256.3 |
| *608137 | *NSMF* | AD | - | NM_001130969.1 |
| *162330 | *TAC3* | AR | - | NM_013996.2 |
| *162332 | *TACR3* | AR | - | NM_001059.2 |
| *608892 | *CHD7* | AD | - | NM_017780.3 |
| *606417 | *WDR11* | AD | - | NM_018117.11 |
| *604846 | *HS6ST1* | AD? | - | NM_004807.2 |
| *603961 | *SEMA3A* | AD? | - | NM_006080.2 |
| *300473 | *NR0B1* | XD? | - | NM_000475.4 |
| *152780 | *LHB* | AD | - | NM_000894.2 |
| *164160 | *LEP* | AR? | - | NM_000230.2 |
| *601007 | *LEPR* | AR? | - | NM_001003679.3 |
| *136530 | *FSHβ* | AR? | - | NM_000510.2 |
| *162150 | *PCSK1* | AR? | - | NM_000439.4 |
| +[c]607306 | *SRD5A2* | AR | c. 680G>A | NM_000348.3 |

[a]: Each MIM entry in the column is designated by the MIM numbering system. [b]:An asterisk (*) before an entry number indicates a gene. [c]:A plus sign (+) before an entry number indicates that the entry contains the description of a gene of known sequence and a phenotype. [d]: No nonsynonymous variant was identified