Mutational Analysis of a Novel Mutation (p.H232R) in the *SRD5A2* Causing 46,XY Disorder of Sex Development

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Research

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Abstract

Background: Over 100 mutations in *SRD5A2* gene have been identified in subjects with 46,XY DSD. Exploring *SRD5A2* mutation and elucidating its molecular mechanism will find the domains function of 5 α -reductase 2 enzyme and identify the cause of 46,XY DSD. Previously, we reported a novel compound heterozygous p.H232R/p.Q6X mutation of *SRD5A2* gene in a case with 46,XY DSD. Whether the compound heterozygous p.Q6X/p.H232R mutation in *SRD5A2* gene causes 46,XY DSD occurrence is needed to be further explored.

Results: In order to clarify the cause of 46,XY DSD in the case's family, *SRD5A2* sequencing were performed. The heterozygous p.H232R mutation were identified in the case's father, so we concluded that the heterozygous p.H232R mutation originated from paternal family and didn't cause 46,XY DSD occurrence. The heterozygous p.Q6X mutation were identified in the case's mother, maternal uncle and maternal grandfather, indicating that the heterozygous p.Q6X mutation descended from maternal family and didn't cause 46,XY DSD occurrence. In order to clarify p.H232R mutation in *SRD5A2* on DHT production, p.H232R mutant *SRD5A2* plasmids were transfected with HEK293 cells and LC-MS detected that DHT production decreased compared with wild-type *SRD5A2* infected ones.

Conclusions: Our findings confirmed that the compound heterozygous p.Q6X/p.H232R mutation in *SRD5A2* gene is the cause of 46,XY DSD. p.H232R mutation reduced DHT production while attenuated 5 α -reductase 2 enzymatic catalytic efficiency.

Background

46,XY disorder of sex development (DSD) characterize incomplete gonadal development and female phenotype in an individual with a normal 46,XY karyotype(1). The clinical phenotype of 46,XY DSD mainly include bilateral undescended testes and female external genitalia, but without uteruses or ovaries(2–4). The predicted morbidity among objects with 46, XY DSD is up to 1:20,000. Among them, the morbidity of hypospadias and cryptorchidism can reach 1:200 to 1:300(5, 6). Many etiologies can give rise to 46,XY DSD, the common causes including androgen insensitivity syndrome (AIS)(7–9) and steroid 5 α reductase type 2 deficiency(10, 11). AIS is an Xlinked recessive genetic disorder that is caused by androgen receptor dysfunction(3). 5 α reductase type 2 deficiency is an autosomal recessive disorder caused by decreased or loss of 5 α reductase type 2 function(12).

5 α reductase type 2, which is coded by *SRD5A2* gene, mainly expresses in epididymides, seminal vesicles, prostate and genital skin(13). The function of 5 α reductase type 2 is the conversion of testosterone (T) to dihydrotestosterone (DHT), which is responsible for male sexual development(14, 15). The loss of 5 α reductase type 2 function fail to differentiate normally into the external genitalia, urethra and prostate and causes 5 α reductase type 2 deficiency. To date, over 100 mutations of the *SRD5A2* gene have been identified(16).

Recently, we identified the compound heterozygous mutation (p.H232R/p.Q6X) of *SRD5A2* gene in two cases who were diagnosed as 46, XY DSD from one family(17). Through DNA sequencing, the heterozygous p.H232R mutation in *SRD5A2* gene originated from paternal family and the heterozygous p.Q6X mutation in *SRD5A2* gene descended from maternal family. In order to elucidate the effect of p.H232R mutant on 5αreductase type 2 enzymatic activity, HEK293 cells were transfected with p.H232R mutant and LC-MS found that the mutation obvious decrease DHT production, indicating that p.H232R mutation reduced) as c catalytic efficiency. Hence, our results confirmed compound heterozygous mutation (p.Q6X/ p.H232R) as cause of 46, XY DSD and p.H232R mutation decreased 5αreductase type 2 enzymatic catalytic efficiency.

Results

Clinical phenotype of the 46,XY DSD patients.

Case-1 III-1: We previously reported a 2yearold female case who was diagnosed as 46,XY DSD. The clinical examination showed the proband has normal female external genitalia, but with blindending vagina. An ultrasound test revealed that the proband have testis in the left labium majus pudendi and right groin, but did not have a uterus or ovaries (Fig. 1a). The karyotype analysis displayed that the proband had a 46, XY karyotype with no apparent anomalies in the chromosome number or chromosomal structure (Fig. 1b). Compared to normal males, folliclestimulating hormone (FSH), luteinizing hormone (LH), and T (< 0.1 ng/ml) levels of the proband were lower (Fig. 1c,Table 1).

Table 1 Characteristics of sex hormones of the the proband		
Sex Hormone	Mean	Normal Male Range
FSH (mIU/ml)	0.54	1.27–19.26
LH (mIU/ml)	0.12	1.24–8.62
E2 (pg/ml)	27	20–47
PROG (ng/ml)_	0.35	0.1–0.84
T (ng/mL)	< 0.1	1.75–7.81
PRL(ng/ml)	10.74 ng/mL	2.64–13.3

Case-2 III-2: The case-1’s mother at 25 weeks’ gestation came to our hospital for the type-B ultrasonic examination. The type-B ultrasonic examination results showed that the child did not have male external genitalia (Fig. 1d). Cytogenetic analysis revealed that the child had a 46, XY karyotype with no apparent anomalies in the chromosome number or chromosomal structure (Fig. 1e)). The results of FISH assay to test chromosome 13/16/18/21/22/X/Y uncovered that the child have normal X, Y chromosome (Fig. 1f).

These results indicated that the fetus is a 46, XY DSD patient. So, the mother decided to terminate the pregnancy. Family members of the proband were examined and pedigrees were generated. (Fig. 1g).

Genetic analysis identified the hereditary sources of SRD5A2 mutation

To find the hereditary sources of *SRD5A2* gene mutation, Sanger sequencing was performed in 8 members of three generations in the case-1's family. The sequencing results showed that the case-1 of 46,XY DSD was compound heterozygous for the previously reported nonsense mutation (c.16C > T/p. Q6X) (Fig. 2a, III-1) ⁽¹⁷⁾ and the novel missense *SRD5A2* mutation (c. 695A > G/p. H232R) (Fig. 2b), III-1). The case-2 III-2 was also the nonsense *SRD5A2* mutation p. Q6X (Fig. 2a, III-2) and the novel missense *SRD5A2* mutation p. H232R (Fig. 2b, III-2). Amazingly, the case-1's mother had the mutation p. Q6X (Fig. 2a, II-2). On the contrary, the case-1's father and younger sister III-3 had the *SRD5A2* mutation p. H232R (Fig. 2b, II-1 and III-3). These results demonstrated that the missense mutation p. Q6X of 46, XY DSD cases rooted in the maternal family and the novel *SRD5A2* mutation p. H232R came from the paternal family.

To further search the hereditary sources of the mutation p.Q6X /p.H232R, Sanger sequencing was done in the case-1's maternal uncle, grandfather and grandmother. As expected, the maternal uncle and grandfather had the nonsense mutation p. Q6X (Fig. 2a, II-3 and I -1), but not the novel mutation p. H232R (Fig. 2b, II-3 and I -1). The case-1's grandmother neither had the mutation p. Q6X (Fig. <link rid="fig2">2</link>a, I-2) nor the mutation p. H232R (Fig. <link rid="fig2">2</link>b, I-2). These results confirmed that the nonsense *SRD5A2* mutation p. Q6X originated from the case-1's grandfather.

Pedigree analysis demonstrated that the *SRD5A2* mutation presented an evidently autosomal recessive inheritance. Neither the novel *SRD5A2* mutation p. H232R nor the nonsense mutation p. Q6X alone cause 46,XY DSD occurrence. However, the *SRD5A2* mutation p. H232R and p. Q6X occurring simultaneously will give rise to 46,XY DSD occurrence.

p.H232R mutation reduced the 5 α -reductase type 2 enzymatic catalytic efficiency

To investigate the effect of p.H232R mutation on the enzymatic activity of *SRD5A2* gene, we transfected wild-type, p.H232R mutant *SRD5A2* or control pcDNA3.1-GFP plasmids into HEK293 cells. qPCR and western blot confirmed that wild-type or p.H232R mutant *SRD5A2* transfection upregulated the *SRD5A2* expression in HEK-293 cells (Fig. 3a b). DNA sequencing confirmed that p.H232R mutation existed in the cells transfected with p.H232R mutant *SRD5A2* (Fig. 3c). LC-MS analysis showed that p.H232R mutation in *SRD5A2* gene obviously decreased DHT production (Fig. 3d). Using GraphPad.Prism.8.0, we found the apparent Michaelis constant (K_m) of 0.6 μ M, maximum velocity of enzyme-catalyzed reaction (V_m) of 431.0 nmol DHT /mg protein/h and catalytic efficiency V_{max}/K_m of 718.3 nmol DHT /mg protein/h/(μ mol/L). In contrast, the cells transfected with plasmid vectors expressing WT *SRD5A2* had a K_m of 0.9 μ M and V_{max} of 659.8 nmol DHT /mg protein/h and catalytic efficiency V_{max}/K_m of 733.1 nmol DHT /mg protein/h/(μ mol/L). Our mutagenesis studies showed that the replacement of H232R with a less acidic glutamine reduced the 5 α -reductase 2 catalytic efficiency.

Conservative analysis of SRD5A2 in humans and other species

We used MEGA software to perform conservation analysis of the residue 6 and residue 232 (marked by a red box) of the *SRD5A2* gene (Fig. 4a b), The H232 amino acid was found to be highly homologous in different species including human, mouse, rat, cow, nematode, zebrafish, chicken, macaque, chimpanzee and clawed frog, indicating a high degree of conservation across species. These data suggested that the H232 amino acid was important in organisms and mutation at this site has a major effect on gene expression.

In silico prediction of pathogenicity for human SRD5A2 p.H232R mutation

Because p.H232R was the novel mutation identified and its pathogenicity is unknown, we first predicted the pathogenicity of this mutation by software. Three widely used software tools for in silico were used to predicted human *SRD5A2* p.H232R variant. The software tools Polyphen2 (18) score was 1, the software tools SIFT (19) score was 0.007 and the software tools PROVEAN (20) score was - 7.330. These scores illustrated that human *SRD5A2* p.H232R mutation is highly likely to be a pathogenic mutation. Details are shown in Table 2.

Table 2
The prediction of pathogenicity for SRD5A2 p.H232R mutation

Gene	Nucleotide substitution	Amino acid substitution	software		
			Polyphen2	SIFT	PROVEAN
SRD5A2	c.695A > G	p.H232R	1	0.007	-7.330
The pathogenicity score of each software is described below. PolyPhen2 benign with a score of 0.0. Score was 0.447 ~ 0.909 indicating possibly damaging. Score was 0.910 ~ 1.000 indicating probably damaging. SIFT score < 0.05 was considered as affecting protein function and ≥ 0.05 was considered as tolerated. The PROVEAN score threshold is -2.5, deleterious effect with a score ≤ -2.5 and neutral effect with a score > -2.5.					

Discussion

In the present research, we first found that the two 46,XY DSD patients in one family had the compound *SRD5A2* mutation (p.H232R /p. Q6X)(17). Next, the further sequencing demonstrated that the novel *SRD5A2* p.H232R mutation or the nonsense p.Q6X mutation alone will not result in 46,XY DSD occurrence. LC-MS found that the novel mutation (p.H232R) obvious decrease DHT production and reduced *SRD5A2* catalytic efficiency.

Mutations in the *SRD5A2* gene are the common genetic defects of 46,XY DSD, which include point mutations, deletions, and insertions(21). The function of *SRD5A2* is conversion of T (testosterone) into DHT (dihydrotestosterone)(22). During early development, DHT is responsible for the formation of the male external genitalia, urethra and prostate (23, 24). Some mutation in *SRD5A2* gene will cause 5α-reductase-2 deficiency syndrome(12). From one Thai patient with male pseudohermaphroditism, the

sequencing results found the compound mutation (p.Q6X/p.G203S) in *SRD5A2* gene(25). In one Chinese patient with Hypospadias, the sequencing revealed the compound mutation (p.G203S/p.Q6X) in *SRD5A2* gene(26). In our research, the two 46,XY DSD patients had the compound heterogeneous *SRD5A2* mutation (p.H232R/ p.Q6X). The heterogeneous *SRD5A2* mutation (p. Q6X) or the heterogeneous *SRD5A2* mutation (p. Q6X) alone will not give rise to 46,XY DSD occurrence. Our and others research support the notion that the compound heterogeneous *SRD5A2* mutation (p.H232R/ p.Q6X) results in 46,XY DSD occurrence.

SRD5A2 gene, located on chromosome 2p23.1, encodes a 254-amino acid protein that contains five exons and four introns(24). The p.Q6X mutation in *SRD5A2* gene, reported only in Asian people, created a drastically truncated protein and the enzymatic activity was completely impaired in truncated proteins due to the lack of either T or the NADPH-binding domain(26, 27). Histidine 232 is located in a stretch of three histidine (residues 230–232) in the *SRD5A2* enzyme. H230P mutation in *SRD5A2* protein inactivate *SRD5A2* enzyme activity. H231R mutation in *SRD5A2* protein impaired the *SRD5A2* isozyme activity since the mutation primarily affected the ability of the enzyme to bind testosterone(28, 29). Therefore, we speculate that H232R mutation in *SRD5A2* protein could inactivate or impair the *SRD5A2* enzyme activity. Together with our findings, the compound heterogeneous *SRD5A2* mutation (p.H232R/ p.Q6X) could inhibit DHT formation and cause 46,XY DSD occurrence.

Most *SRD5A2* mutations identified can reduce DHT levels and cause 5 α -Reductase deficiency, which is an autosomal recessive disorder (30, 31). The p.H232R mutation, located in exon 4 of *SRD5A2*, is in the T-binding domain and most likely affects the binding of 5 α - reductase 2 to T, which rendering 5 α - reductase 2 less active. It was reported that p.H231R mutations in exon 4 of the *SRD5A2* gene cause 46,XY DSD and the mechanism associated with impaired binding T (28). Because both amino acids 231 and 232 are located in exon 4 and are the same mutation of histidine to arginine, the mutational impact is likely to be similar, both preventing binding of the enzyme to substrate or coenzyme. Our enzymatic activity analysis of the *SRD5A2* p.H232R mutation showed the Vmax/Km value was lower than the WT *SRD5A2* and reduced the *SRD5A2* catalytic efficiency, suggesting that p.H232R mutation contributed to the development of genital ambiguity.

Conclusions

our research revealed that the compound heterogeneous *SRD5A2* mutation (p.H232R /p.Q6X) may very likely lead to 46,XY DSD occurrence and elucidated the effect of the *SRD5A2* p. H232R mutation on *SRD5A2* catalytic efficiency. Future research is needed to construct a mouse model of 46, XY DSD with the *SRD5A2* p. H232R mutation, replicate the 46, XY DSD disease phenotype, and further investigate the regulatory mechanism of p. H232R on steroid 5 α -reductase 2 activity in vivo.

Methods

Patients

Case-1, a 2-year-old girl with 46, XY DSD came to our hospital. Physical examinations of female external genitalia were done. B-ultrasound was done to detect male or female internal genital organs, including testis, uterus, and ovaries. Hormone assay and karyotype analysis were performed. 219 DSD-related genes were sequenced.

Case-2, Case-1's younger sibling, who is at 25 weeks' gestation, is suspected with 46, XY DSD. Type-B ultrasonic test, chromosome karyotype analysis and *SRD5A2* gene sequencing were done for 46, XY DSD. When she is aborted, physical examinations were performed for female external genitalia and HE staining for testis tissue and epididymis tissue.

An informed written consent for the genetic studies were obtained from the family, and all researches were approved by the Medical Ethical Committee of the People's Hospital of Xingtai City.

Hormone assays

Serum levels of FSH, LH, estradiol (E2), progesterone (PROG), T, and prolactin (PRL) for the proband were measured by radioimmunoassay.

Karyotype analysis

Peripheral blood cells from the proband were cultured in RPMI1640 medium supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum (FBS) for 72 hours. Colchicine (20 mg/ml) was added for two hours to arrest cells in metaphase and inhibit spindle body formation. Samples were incubated for 20 minutes with 75 mM potassium chloride to spread out the spindle body and fixed in Carnoy's solution. Fixed cells were dropped onto glass slides and placed in an incubator at 75 °C for three hours to air-dry. Giemsa solution was used to stain the G-bands of the chromosomes.

FISH analysis

The uncultured amniotic fluid cells from the unbirthed sibling of the proband were obtained for the FISH test. The probes were used to test chromosome 13, 21, 16, 22, 18, X and Y.

Genetic analysis

The peripheral blood samples of the case-1, her unbirthed sibling, her birthed sibling, her father, her mother, her maternal uncle, her grandfather, her grandmother were obtained for DNA sequencing. The genomic DNA was extracted according to the manufacturer's instructions using the TIANamp Blood DNA Kit (TIANGEN, China). The sequencing of the proband's 219 DSD-related genes was performed by BGI using the Illumina Genome Analyzer IIx for (supplementary Table 1). The mutation genes in the case-1, her unbirthed sibling, her birthed sibling, her father, her mother, her maternal uncle, her grandfather, her grandmother were sequenced by sanger sequencing. The gene mutations were identified by comparing the sequencing results of the case to the UCSC reference genome using the BWA tool.

Construction of human *SRD5A2* site-directed mutagenesis

Human *SRD5A2* cDNA was kindly provided from professor Jiahuai Han (Xiamen University) and was subcloned into vector pcDNA3.1 (with 3Flag tag, purchased from Life Technologies). To generate 695A>G mutation in *SRD5A2* gene, the pcDNA3.1-*SRD5A2* plasmid was used as template and the QuikChange II Site-Directed Mutagenesis Kit (Catalog #200523, Agilent) was used to produce the mutataion site according to manufacturer's instruction. The primers for cloning the 695A>G mutation in *SRD5A2* gene as following:

SRD5A2 mF-695: gcgagcttttcaccaccGtaggttctacctaagatgtttg

SRD5A2 mR-695: catcttgaggtagaacctaCggtggtgaaaagctcgag

Transfection assay

HEK293 cells (purchased from the Chinese Academy of Sciences Cell Bank in Shanghai) were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10 % fetal bovine serum (Gibco, USA) and 1 % streptomycin/penicillin (Gibco, USA). The cells were transiently transfected with 2.0 µg wild-type or p.H232R mutant *SRD5A2* plasmids in each well of 6 well plate using the TurboFect™ Transfection Reagent (Thermo Scientific) accoding to the manufacturer's protocol. The transfected HEK293 cells were cultured in 5% CO₂ at 37 °C for 48 h before the assays.

DNA sequencing of transfection plasmids

HEK293 cells were transfected with wild-type or p.H232R mutant *SRD5A2* plasmids for 48 hours and Sanger sequencing were used to assess the DNA sequence. Briefly, the DNA samples were isolated by TIANamp Genomic DNA kit (Cat# DP304-02, TIANGEN, China). The concentration and purity of the DNA were determined by NanoDrop (at 260/280 nm, ND2000C, Thermo). The DNA samples were amplified by polymerase chain reaction (PCR) and sequenced by Genewiz corporation (Suzhou, China).

Primer sequences for PCR are listed below.

SRD5A2-Fseq: AGCCCGTTAAGCAGTTGAGG

SRD5A2-Rseq: CGGCTTCTTCCGCTTCTTGA

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) were performed to assess the mRNA expression after wild-type or p.H232R mutant *SRD5A2* plasmids transfection for 48 hours. The total RNA was isolated by RNAiso Plus (Cat# 9109, TaKaRa) and reverse transcription was done using the PrimeScript™ RT reagent kit (RR047A, Takara) according to the manufacturer's protocol. qPCR were performed using PrimeScript™ RT reagent Kit (RR037A, Takara) by ViiA7 Real-time PCR System (ABI). The PCR cycle included: 95°C for 30s, followed by 40 cycles of 95°C for 5 s and 60°C for 34s. The relative quantification for SRD5A2 mRNA was assessed by 2-ΔΔCt method.

Primer sequences for qPCR are listed below.

SRD5A2F: GCCACTTTGGTCGCCCTT

SRD5A2R: CTCCGTGTGCTTCCCGTAG

β -actinF: AGAGCTACGAGCTGCCTGAC

β -actinR: AGCACTGTGTTGGCGTACAG

Western Blot

Western Blot were performed as previously described Briefly(32), after HEK293 cells transfected with wild-type or p.H232R mutant *SRD5A2* plasmids 48 hours, the cells were collected from 6-well plates and lysed by RIPA buffer (P0013, Beyotime Biotechnology). The whole-protein lysates were separated by 12% SDS-PAGE and then transferred to nitrocellulose membranes. After blocked by 5% milk, the membranes were incubated with primary antibodies monoclonal anti-flag (F9291, sigma) and β -Actin (sc-69879, santa) at 4°C overnight. After rinsed by Tris-buffered saline containing 1% Tween-20 (TBST), the membrane was then incubated with appropriate HRP-conjugated secondary antibodies (sc516102, Santa Cruz) and detected by an ECL Plus kit (P1050, Applygen).

Kinetic assays

Kinetic assays were performed to assess the steroid 5 α -reductase 2 activity after transfected p.H232R mutant *SRD5A2* plasmid into HEK293 cells. The cells were collected from 6-well plates and resuspended in 200 μ l of Tris-Citrate buffer (pH 5.5). The resuspended cells were sonicated 2 min (HD3100, BANDELIN) and subsequently incubated at 37°C for 15 min with 500 μ M of NADPH (CAS: 100929-71-3, [N302057](#), aladdin) and various concentrations of T (0.25–8.0 μ mol/L, CAS: 58-18-4, M163044, aladdin). The steroids were extracted with chloroform, condensed by a freeze-drying apparatus and re-dissolved in chromatographic methanol for Liquid Chromatography–Mass Spectrometry (LC–MS) analysis, using buffer A containing 1 mM Formate (F112034, aladdin) and buffer B containing 100% methanol. DHT were quantified according the concentration and peak area of the standard DHT (CDCT-C10255010, ANPEL Laboratory Technologies (Shanghai) Inc.). The protein concentration was determined using BCA assays (P0012S, Beyotime). The rate of enzyme production (nmol/mg prot/h) were calculated as indicators of enzyme activity. The data were processed by Prism8 (GraphPad). The experiments on each groups were repeated three times. The data were represented as mean $\pm$ SEM.

Sequence alignment of *SRD5A2* in humans and other species

FASTA format *SRD5A2* sequences of the homologous species were downloaded from the NCBI database. Protein sequences of *SRD5A2* between human and the other homologous species were aligned by MEGA software.

Pathogenicity analysis for human *SRD5A2* p.H232R mutation

The pathogenicity of human SRD5A2 p.H232R mutation were analyzed using the following bioinformatics programs:

Polyphen-2: <http://genetics.bwh.harvard.edu/pph2/>

SIFT: <http://sift.jcvi.org>

PROVEAN: http://provean.jcvi.org/seq_submit.php

Declarations

Ethics approval and consent to participate:

Experimentation was approved by the Medical Ethical Committee of the People's Hospital of Xingtai City. Parental/guardian consent obtained.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Competing interests

The authors declare no competing financial interests.

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Authors' contributions

LL, JZ, QL, QL, PL, YC, SL, SH, QW, LL, JY and PH carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. SL and XD carried out the immunoassay. HY participated in the sequence alignment. XD and HY designed the study. All authors read and approved the final manuscript.

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References

1. Berglund A, Johannsen TH, Stochholm K, Viuff MH, Fedder J, Main KM, et al. Morbidity, Mortality, and Socioeconomics in Females With 46,XY Disorders of Sex Development: A Nationwide Study. *J Clin Endocrinol Metab.* 2018; 103(4):1418-28.
2. Gomez-Lobo V, Amies Oelschlager AM, North American Society for P, Adolescent G. Disorders of Sexual Development in Adult Women. *Obstet Gynecol.* 2016; 128(5):1162-73.
3. Mongan NP, Tadokoro-Cuccaro R, Bunch T, Hughes IA. Androgen insensitivity syndrome. *Best Pract Res Clin Endocrinol Metab.* 2015; 29(4):569-80.
4. Byers HM, Mohnach LH, Fechner PY, Chen M, Thomas IH, Ramsdell LA, et al. Unexpected ethical dilemmas in sex assignment in 46,XY DSD due to 5-alpha reductase type 2 deficiency. *Am J Med Genet C Semin Med Genet.* 2017; 175(2):260-7.
5. Fabbri-Scallet H, de Mello MP, Guerra-Junior G, Maciel-Guerra AT, de Andrade JGR, de Queiroz CMC, et al. Functional characterization of five NR5A1 gene mutations found in patients with 46,XY disorders of sex development. *Hum Mutat.* 2018; 39(1):114-23.
6. Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al. Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care. *Horm Res Paediatr.* 2016; 85(3):158-80.
7. Bashamboo A, Donohoue PA, Vilain E, Rojo S, Calvel P, Seneviratne SN, et al. A recurrent p.Arg92Trp variant in steroidogenic factor-1 (NR5A1) can act as a molecular switch in human sex development. *Hum Mol Genet.* 2016; 25(16):3446-53.
8. Petroli RJ, Hiort O, Struve D, Gesing JK, Soardi FC, Spinola-Castro AM, et al. Functional Impact of Novel Androgen Receptor Mutations on the Clinical Manifestation of Androgen Insensitivity Syndrome. *Sex Dev.* 2017; 11(5-6):238-47.
9. Jung EJ, Im DH, Park YH, Byun JM, Kim YN, Jeong DH, et al. Female with 46, XY karyotype. *Obstet Gynecol Sci.* 2017; 60(4):378-82.
10. Andonova S, Robeva R, Vazharova R, Ledig S, Grozdanova L, Stefanova E, et al. New Territory for an Old Disease: 5-Alpha-Reductase Type 2 Deficiency in Bulgaria. *Sex Dev.* 2017; 11(1):21-8.
11. Bertelloni S, Baldinotti F, Russo G, Ghirri P, Dati E, Michelucci A, et al. 5alpha-Reductase-2 Deficiency: Clinical Findings, Endocrine Pitfalls, and Genetic Features in a Large Italian Cohort. *Sex Dev.* 2016; 10(1):28-36.
12. Kang HJ, Imperato-McGinley J, Zhu YS, Rosenwaks Z. The effect of 5alpha-reductase-2 deficiency on human fertility. *Fertil Steril.* 2014; 101(2):310-6.
13. Zhang K, Li Y, Mao Y, Ma M. Steroid 5-alpha-reductase type 2 (SRD5A2) gene V89L polymorphism and hypospadias risk: A meta-analysis. *J Pediatr Urol.* 2017; 13(6):630 e1- e9.
14. Sata F, Kurahashi N, Ban S, Moriya K, Tanaka KD, Ishizuka M, et al. Genetic polymorphisms of 17 beta-hydroxysteroid dehydrogenase 3 and the risk of hypospadias. *J Sex Med.* 2010; 7(8):2729-38.

15. Chan AO, But WM, Lee CY, Lam YY, Ng KL, Loung PY, et al. Aetiological bases of 46,XY disorders of sex development in the Hong Kong Chinese population. *Hong Kong Med J*. 2015; 21(6):499-510.
16. Kim JH, Kang E, Heo SH, Kim GH, Jang JH, Cho EH, et al. Diagnostic yield of targeted gene panel sequencing to identify the genetic etiology of disorders of sex development. *Mol Cell Endocrinol*. 2017; 444:19-25.
17. Li SP, Li LW, Sun MX, Chen XX, Wang XF, Li ZK, et al. Identification of a novel mutation in the SRD5A2 gene of one patient with 46,XY disorder of sex development. *Asian J Androl*. 2018; 20(5):518-9.
18. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet*. 2013; Chapter 7:Unit7 20.
19. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003; 31(13):3812-4.
20. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015; 31(16):2745-7.
21. Fu XH, Zhang WQ, Qu XS. Correlation of androgen receptor and SRD5A2 gene mutations with pediatric hypospadias in 46, XY DSD children. *Genet Mol Res*. 2016; 15(1):15018232.
22. Audet-Walsh É, Yee T, Tam I, Giguère V. Inverse Regulation of DHT Synthesis Enzymes 5 α -Reductase Types 1 and 2 by the Androgen Receptor in Prostate Cancer. *Endocrinology*. 2017; 158(4):1015-21.
23. Kang H-J, Imperato-McGinley J, Zhu Y-S, Rosenwaks Z. The effect of 5 α -reductase-2 deficiency on human fertility. *Fertility and sterility*. 2014; 101(2):310-6.
24. Okeigwe I, Kuohung W. 5-Alpha reductase deficiency: a 40-year retrospective review. *Current opinion in endocrinology, diabetes, and obesity*. 2014; 21(6):483-7.
25. Sahakitrungruang T, Wacharasindhu S, Yeetong P, Snabboon T, Suphapeetiporn K, Shotelersuk V. Identification of mutations in the SRD5A2 gene in Thai patients with male pseudohermaphroditism. *Fertil Steril*. 2008; 90(5):2015 e11-5.
26. Zhang M, Yang J, Zhang H, Ning G, Li X, Sun S. A novel SRD5A2 mutation with loss of function identified in Chinese patients with hypospadias. *Horm Res Paediatr*. 2011; 76(1):44-9.
27. Wang Y, Li Q, Xu J, Liu Q, Wang W, Lin Y, et al. Mutation analysis of five candidate genes in Chinese patients with hypospadias. *Eur J Hum Genet*. 2004; 12(9):706-12.
28. Nordenskjöld A, Ivarsson SA. Molecular characterization of 5 alpha-reductase type 2 deficiency and fertility in a Swedish family. *J Clin Endocrinol Metab*. 1998; 83(9):3236-8.
29. Wigley WC, Prihoda JS, Mowszowicz I, Mendonca BB, New MI, Wilson JD, et al. Natural mutagenesis study of the human steroid 5 alpha-reductase 2 isozyme. *Biochemistry*. 1994; 33(5):1265-70.
30. Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, Griffin JE, et al. Molecular genetics of steroid 5 alpha-reductase 2 deficiency. *J Clin Invest*. 1992; 90(3):799-809.
31. Mendonca BB, Batista RL, Domenice S, Costa EM, Arnhold IJ, Russell DW, et al. Steroid 5alpha-reductase 2 deficiency. *J Steroid Biochem Mol Biol*. 2016; 163:206-11.

32. Dou XW, Liang YK, Lin HY, Wei XL, Zhang YQ, Bai JW, et al. Notch3 Maintains Luminal Phenotype and Suppresses Tumorigenesis and Metastasis of Breast Cancer via Trans-Activating Estrogen Receptor- α . *Theranostics*. 2017; 7(16):4041-56.

Figures

Figure 1

Clinical features of the proband (III-1) and her unbirtherd sibling (III-2). a. B ultrasound detection of the proband III-1 internal reproductive organs showed that the proband had no uterus and ovaries, but testes on the left labia and right groin. b. karyotype analysis revealed that the proband III-1 was 46,XY type. c. Hormonal investigations of proband III-1' serum. d. Prenatal system B ultrasound testing at 25 weeks of the proband's sister III-2 revealed no abnormalities. e. Karyotype analysis of the proband's sister III-2 was 46,XY. f. Fluorescence in situ hybridization (FISH) prenatally diagnosed 13 / 16 / 18 / 21 / 22 / X / Y chromosomes in the proband's sister III-2. g. Pedigree diagrams of the probands' families, the proband III-

1 and his sister III-2 were 46, XY DSD patients with mutations in SRD5A2, and other relatives did not suffer from 46, XY DSD.

a c.16C>T (p.Q6X)

b c.695A>G (p.H232R)

Figure 2

Sequence analysis of SRD5A2 in the proband and his relatives and. a. SRD5A2 p.Q6X (c.16C > T) mutation site analysis in proband III-1 and her relatives. The proband III-1, younger sister III-2, mother II-2, maternal uncle II-3 and her granduncle I-1 carried the p.Q6X (c.16C >T) mutation, while the others did not.

b. SRD5A2 p.H232R (c.695A > G) mutation site analysis in proband III-1 and her relatives. The proband III-1, younger sister III-2, younger sister III-3 and her father II-1 carried the p.H232R (c.695A> G) mutation, but the others did not.

Figure 3

Validation of SRD5A2 wild-type and p. H232R mutant HEK293 cell models. a. The transcription of SRD5A2 gene in HEK-293 cells transfected with WT and p.H232R mutant plasmids by using the qRT-PCR. The internal control was β-actin gene. The amounts of SRD5A2 transcripts were calculated by the standard 2-ΔΔCt method and were made as a histogram. b. Western blot results of SRD5A2 protein in HEK-293 cells transfected with WT and p.H232R mutant plasmids. The internal control was β-actin. c. Sequencing analysis showed mutation c.695A>G in SRD5A2 were found in HEK-293 cells transfected with p.H232R mutant plasmids, causing amino acid 232 to change from histidine to arginine. d. Enzyme activity analysis of SRD5A2 WT and mutants H232R catalyzing testosterone (T) to dihydrotestosterone (DHT) by LC-MS.

Figure 4

Conservation of the mutation site. a. Conservation analysis of the SRD5A2 p.Q6X (c.16C > T). b. Conservation analysis of the SRD5A2 p.H232R (c.695A > G).

Supplementary Files

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