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Case Report

Novel Mutation of NR5A1 in A Case of 46,XY Disorder of Sexual Development: A Case Report

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Abstract 46,XY disorder of sexual differentiation (DSD) is a type of sexual ambiguity which a patient with male chromosome does not completely developed male sex phenotype. Although the condition is known as androgen insensitivity syndrome, its pathophysiology is not always such unresponsiveness to androgen. Defective androgen production caused by molecular pathology on steroidogenic hormone regulating genes explain the phenotypes in a number of cases. Herein, we report a case of 46,XY DSD with gonadal dysgenesis who had a heterozygous germline mutation of NR5A1 at the position 9:124500710. The mutation is predicted to result in a substitution of Arginine with Cysteine at the codon 84 (p.R84C) of NR5A1, which encodes for a DNA binding domain of the transcription factor SF1. On familial study, the mutation was found derived from the maternal side who also carried a heterozygous p.R84C. With this novel mutation, our evidence was consistent with previous studies which have suggested that mutations within NR5A1 are associated with 46,XY DSD and primary ovarian insufficiency.

Keywords: Disorder of sexual development, NR5A1, SF1

Introduction

Gender development is a continuous process, beginning from chromosomal derived biological sex to sexual organs development and personality trait. Each step requires combination of signals from various genes in both sex chromosomes and autosomes. Human male sex is primarily determined by *SRY* gene on Y chromosome. With presence of *SRY*, the primordial gonad differentiates into testicular tissue that contains Leydig cells and Sertoli cells. Under influence of human chorionic gonadotropin (hCG) from the placenta, Leydig cells produce testosterone while Sertoli cells produce anti-Müllerian hormone (AMH)[1]. When T is the principal hormone that induces differentiation of Wolffian duct system (epididymis, vas deferens and seminal vesicles), AMH suppresses Müllerian structures (uterus and adnexa). Apart from SRY defects, germline mutations in various genes were reported to be involved in 46,XY disorder of sexual development (46,XY DSD) including Steroid 5-alpha reductase 2 (*SRD5A2*), androgen receptor (*AR*), Nuclear receptor subfamily 5 group A member 1 (*NR5A1*), Bone morphogenetic protein 4 (*BMP4*) and Wilms tumor 1 (*WT1*)[2,3].

NR5A1 (9q33.3, MIM #184757) provides an essential transcription factor for male sex development, the steroidogenic factor 1 (SF1).

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SF1 expresses in the urogenital ridge during early embryonic life and involves in the development of gonadal and adrenal tissue[4,5]. In male mice model, targeted deletion of *NR5A1* is associated with gonadal and adrenal agenesis together with persistent Müllerian structures. Animals with heterozygosity have a variety of subtle phenotypes such as testicular hypoplasia and abnormal steroid hormone production during stress. In humans, heterozygous mutations of *NR5A1* were reported in 46,XY DSD with or without adrenal insufficiency. In addition, some mutations in *NR5A1* were associated with ovarian insufficiency in 46,XX female[6].

With advancement in genome sequencing technique, rare mutations in human genome can be detected and the variant data is meaningful in understanding pathophysiology of various rare diseases. In this report, we demonstrate a case of 46,XY DSD with gonadal dysgenesis in whom a genetic study found a novel mutation of *NR5A1*.

Case Report

A female baby first presented to us on her 6-month age with a problem of ambiguous external genitalia. The girl was born term and was the first child of a mother with long history of infertility, irregular menstruation and early menopause. The second child was a normal male. On examination, the patient had female type external genitalia with deep labioscrotal fold, enlarged phallus that looked like a large clitoris and urogenital sinus (fusion of the urethra and the distal vaginal canal) (Figure 1). There was no palpable gonad within the inguinal canals. A chromosomal study by G-band karyotyping method showed 46,XY. Baseline testosterone was < 0.02 ng/ml and raised to 3.10 ng/ml after a single-dose hCG stimulation. A contrast genitogram showed urogenital sinus and grade 3 bilateral vesicoureteral refluxes. During voiding, the contrast retrograded into a posterior tract which was suspected to be a large vagina. Elongation of posterior urethra was noted.

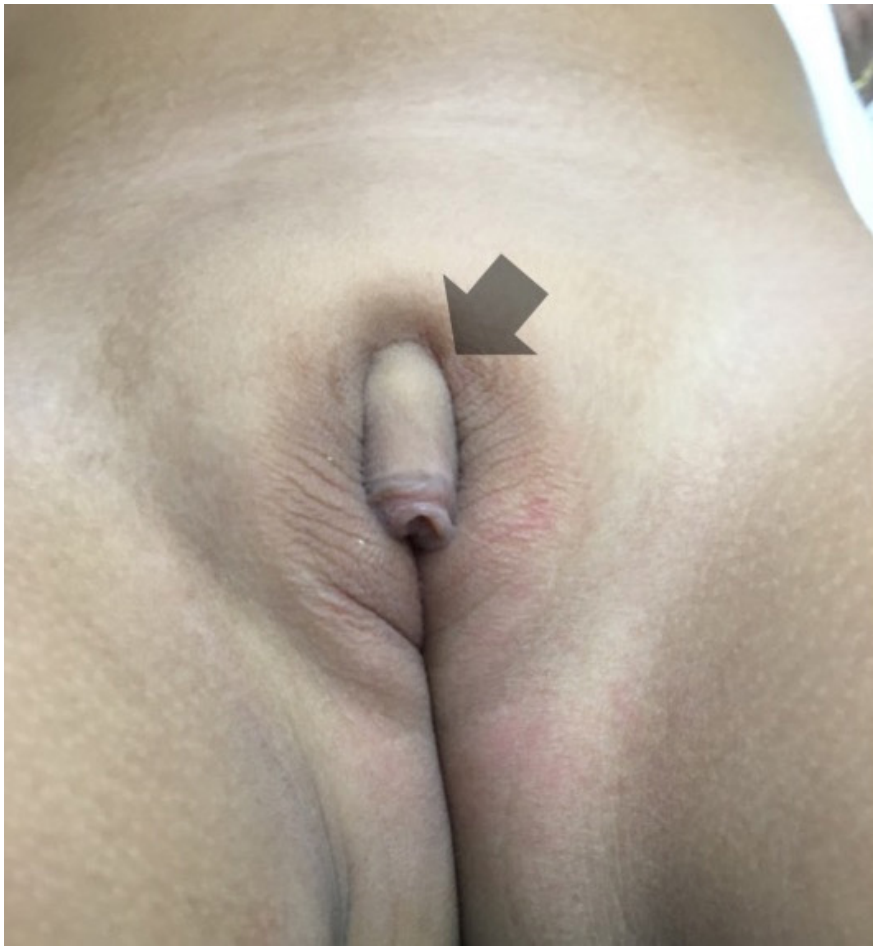


Figure 1 Appearance of the external genitalia in this 46,XY patient at 3 months old, showing cleft labioscortal fold without gonads and a small phallus (arrow)

The patient was assigned to be female and underwent a transabdominal gonadectomy and genital size reduction surgery when she was 4 years old. On laparotomy, there was no uterus and both gonads were found in the pelvis near the internal inguinal rings. Pathology of the resected gonads reported gonadal dysgenesis. The patient had regular follow-up visit with the endocrinologist and began to receive estrogen therapy when she was twelve. Her parents reported that the girl love to play outdoor sports and did well at the secondary school. Body weight steadily gain at the percentile 25 and the height began to spurt from the percentile 25 to the percentile 50 around the age 12-14. An augmentation vaginoplasty was performed when the patient aged 17 years, by using a U-flap ileal segment.

Genetic study was performed in 2017 under informed consent. Blood DNA was submitted for a Whole Exome Study (WES) using Illumina Hiseq-2000 (Illumina, San Diego, California, United States) platform with 100bp runs at an average mean target depth of 100x coverage. The raw sequence data in the FASTQ format were mapped to the reference genome (GRCh38/hg7) using the Lasergene 15.0 Bioinformatic suits (DNAstar, Wisconsin, United States). Annotation focused on the genes previously reported to be associated with 46,XY DSD according to a recent publication list[7]. The analysis showed that the most likely pathogenic variant was at the position 9:124500710 (DNA RefSeq NM 004959.3) which had C to T mutation at 10/19 reads. The position belonged to codon 84 of the gene NR5A1 and the variant led to substitution of Arginine (R) by Cysteine (C). At the time of this manuscript preparation (September 2019), the variant has never been annotated in the Single Nucleotide Polymorphism database (dbSNP) but a missense mutation in this same position causing different amino acid substitution (p.R84H) is reported in a study[8].

Validation by Sanger's dideoxynucleotide sequencing technique confirmed heterozygous mutation at the same point (Figure 2). Further study in both parents found identical heterozygous mutation in the mother and wildtype sequence in the phenotypically normal father and the brother.

Discussion

Modern high throughput genome study has allowed novel approach in various human diseases, especially rare diseases like endocrinological disorders. Previously, 46,XY DSD was categorized in a group of rare conditions known as 'androgen insensitivity' which meant that although testosterone can be produced, the embryonic tissue does not respond to the hormone and, as a consequence, the male genital organs do not develop well. With this pathophysiological paradigm, molecular pathology in focus was at the receptor gene, AR[6].

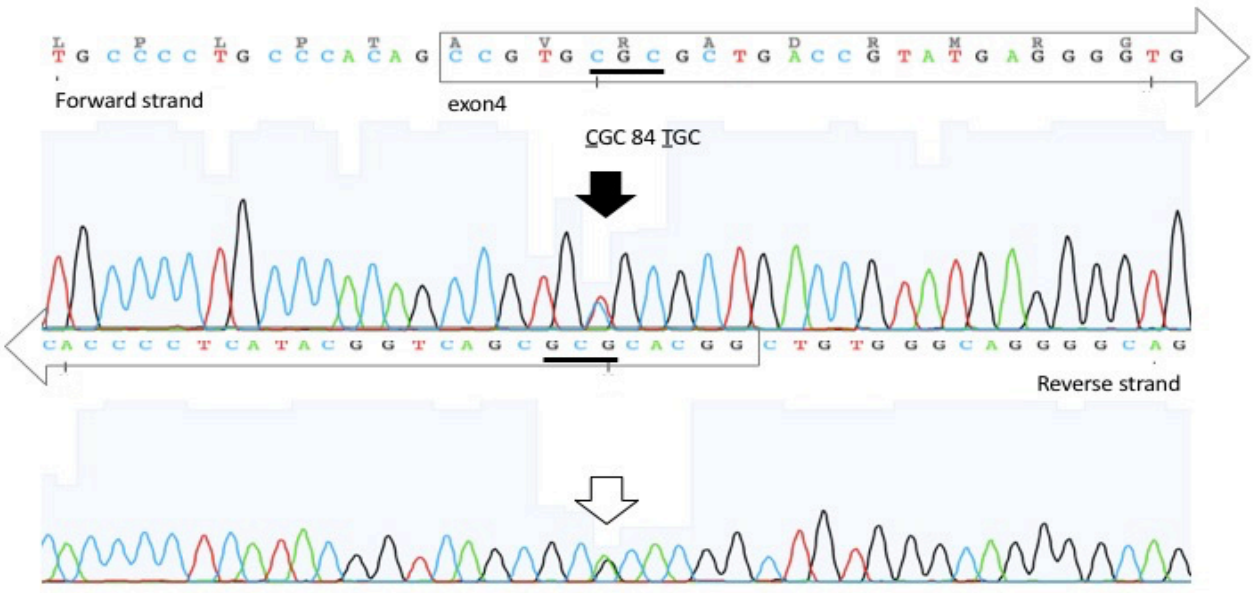
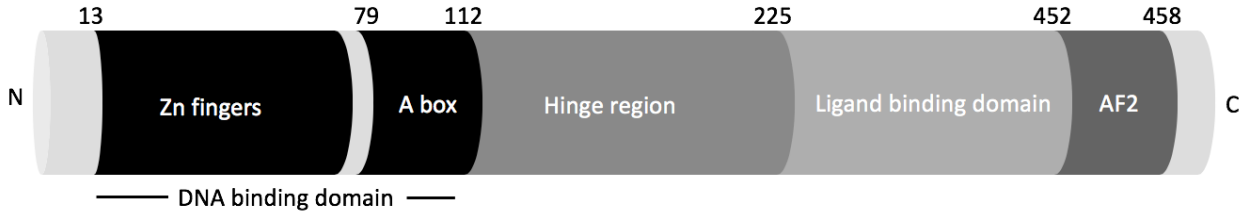


Figure 2 Electropherograms of the capillary electrophoresis, demonstrating heterozygous point mutation (C/T) at the position 9:124500710

Table 1 Structure of SF1 (NR5A1) protein (illustrated in the box below and positions of mutation reported in each category of disorders of sexual differentiation



Phenotypes	Genotypes
46,XY DSD female external genitalia, persistent Mullerian structures with adrenal insufficiency	p.G35E *(Achermann JC 1999)*, p.R92O *(Achermann JC 2002)*, p.R427W[a] *(Rocca MS 2018)*
6,XY DSD female external genitalia, without adrenal insufficiency	Del nt1058-1065 *(Correa RV 2004)*, D6fsX74 *(Hasegawa T 2004)*, p.V15M, p.M78I, p.G91S, p.L437Q *(Lin L 2007)*, p.C16X *(Mallet D 2004)*, p.C33S, p.R84H, p.Y138X *(Kohler B 2008)*. p.L376F, p.G328V *(Tantawy S 2014)*, p.K38*, p.S32N *(de Andrade, JGR 2014)*, p.C65Y *(Fabbri HC 2014)*, p.T03X, E07XQ299HfsX386 *(Hussain S 2015)*, p.G26A, p.C283R, p.L384Rfs*7, p.E455* *(Woo KH 2015)*, p.G90C, p.L298P, *(Rehkamper J 2017)*, p.T40R, p.T47C, p.G328W, p.A351E, p.Q460R *(Rocca MS 2018)*, p.G212S *(Wang H 2018)*, R39C, C247* *(Fabbri-Scallrt H 2017)* p.R84C *(This study)*
46,XY DSD micropenis and/or hypospadias and/or cryptorchidism	p.Q107X, c.103-2C>A, p.E11X *(Kohler B 2009)*, R281P *(Philibert P 2011)*, p.Y183X *(Warman DM 2011)*, p.G26A *(Woo KH 2015)*, S32N, K396Rfs*34 *(Fabbri-Scallrt H 2017)* p.N44X, p.C283*, p.T29K, p.E148fsX105, p.G35V, p.C370Y, p.E367G, S430I *(Wang H 2018)*
46,XY infertility	p.G165R, p.D257N, p.I323T *(Ropke A 2013)*
46,XX testicular/ovotesticular DSD	p.P125Rfs*171 *(Rehkamper J 2016)*, p.R92W *(Bashamboo A 2016, Takasawa K 2017),*

[a]R427W had borderline level of cortisol *(Rocca MS 2018)*, DSD: disorder of sex development

However, mutations of AR belong to minority group of 46,XY DSD reported by large cohorts that used a high-throughput genomic technique[7,9].

NR5A1, synonym *SF1* or adrenal 4-binding protein (*Ad4BP*), encodes a nuclear receptor protein SF1 which consists of 461 amino acids. The SF1 protein comprised of a DNA-binding domain (DBD), a ligand binding domain (LBD), 2 functional activation domains (named A box and AF2), and a hinge region (Illustrated in Table 1). As a transcription factor, *NR5A1* regulates expression of several enzymes essential for testosterone biosynthesis, *AMH*, *SOX9* and several genes involving in cholesterol mobilisation and steroid hormone biosynthesis[10,11]. Crucial roles of *NR5A1* is at the early stage of sexual organ diversification, consisting of differentiation of Wolffian structures and regression of Müllerian organs[3]. More than 80 mutations of *NR5A1* were reported in 46,XY DSD[11-21] and *NR5A1* mutations are estimated to contribute 8-15% of the cases[11]. Although clinical spectrum of 46,XY DSD individuals harboring *NR5A1* is wide, from isolated hypospadias to full female external genitalia, there is no genotype-phenotype correlation[22]. Most XY patients with NR5A1 reported in the literature had varying degree of poor development of external genitalia with or without undescended testicles[22,23]. In addition, missense mutations of *NR5A1* were also found in infertile males with normal genital organs[24]. In 46,XX, although phenotypes of *NR5A1* mutated are subtle, varying from asymptomatic to primary ovarian insufficiency[25], a recent evidence suggested that p.R92W mutation is specifically associated with 46,XX SRY-negative testicular DSD[26-28]. Genotypes of *NR5A1* reported in each category of DSD are reviewed from Pubmed database and are summarized in Table 1. Interestingly, the same mutation in NR5A1 may give different severity of phenotype in siblings which might be explained by uncovered genetic modifiers[29,30].

Our patient carried heterozygous mutation of *NR5A1* at the codon 84 (R84C) which was located at the DNA binding domain of the protein. Functional study of the position R84 has been studied by Köhler and colleagues since 2008. In their experiment, p.R84H showed reduced binding affinity with Cyp11a promotor when subcellular localization did not significantly change. The same study also showed reduced transcriptional activity of the cells transfected with p.R84H mutated *NR5A1*. Although our mutation p.R84C was not exactly identical to theirs, we assume that loss of arginine in this position impacts the transcription activity in the same way. The phenotypes in KÖhler's patient were similar to our case in that the patient had female type external genitalia with testicular dysgenesis and absence of the uterus. Although hormonal study had not been performed in the mother, irregular menstruation and early menopause suggested primary ovarian insufficiency that was related to a carrier state of the mutation.

In summary, we report a case of 46,XY DSD with gonadal dysgenesis without adrenal dysfunction. The case inherited a point mutation of *NR5A1* from a mother who was likely to have primary ovarian insufficiency. Our evidence supports the role of *NR5A1* in the development of 46,XY females and also supports the role of a whole exome study in an annotation of germline pathology in a patient with ambiguous genitalia.

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