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RAPID COMMUNICATION

A novel heterozygous *SF1/NR5A1* gene variant causes 46,XY DSD-gonadal dysgenesis with hypergonadotropic hypogonadism without adrenal insufficiency

Steroidogenic factor 1 (SF1/NR5A1; nuclear receptor subfamily 5 group A member 1) is an essential orphan protein involved in gonadal embryogenesis, sex determination, and reproductive endocrinology.[1] Furthermore, NR5A1 is a transcription factor that regulates a number of target genes [*e.g.*, *SRY* (sex determining Region Y), *SOX9* (SRY-box transcription factor 9), *GATA4* (GATA binding protein 4), *AMH* (anti-Mullerian hormone), *STAR* (steroidogenic acute regulatory protein), and *CYP11A1* (cytochrome P450 family 11 subfamily A member 1)] crucial to reproductive biology. Several human *NR5A1* gene variants have been associated with 46,XY disorders of sex development (DSD), a congenital condition in which the gonadal or anatomical sex is atypical.[2] While NR5A1's role as a transcription factor essential to adrenal development is supported by several *in vitro* and *in vivo* experiments in knockout mouse models, to date, adrenal insufficiency in humans due to *NR5A1* gene mutations is extremely rare.[3] In this regard, next-generation sequencing approaches are a powerful tool in defining population genetics of rare diseases and allow more focused clinical genetic screening programs to be established. Therefore, the molecular etiology of this genetic steroid disorder is still unknown. Likewise, genomic analyses performed in our laboratory have revealed several non-synonymous mutations in *NR3C4/AR* (androgen receptor) and *SRD5A2* (steroid 5 alpha-reductase 2) genes in patients with 46,XY DSD.[4,5] We also showed that mutations of the NR3C4/AR protein reduced or nulled responses to androgens, testosterone, and dihydrotestosterone. Moreover, mutations in the SRD5A2 protein affect its enzymatic activity due to erroneous interactions between amino acid residues, the substrate testosterone, or nicotinamide adenine dinucleotide phosphate. Here, a novel *NR5A1* gene variant is described in a Mexican patient raised as a female due to her phenotype. This female patient has a 46,XY karyotype, undescended atrophied testes, and hypergonadotropic hypogonadism. This study identified a novel heterozygous missense *NR5A1* variant (c.89G > A, p.Cys30Tyr) in the first zinc finger of the DNA-binding domain that explains the 46,XY-female phenotype to a genetic condition, and adds to the variety of 46,XY DSD-causing mutations that impair the development of gonadal and phenotypic sex.

The purpose of this study was to identify nucleotide variants in potentially essential genes for the determination and differentiation of sex from a 16-year-old female of Mexican ancestry with primary amenorrhea, mammary glands (Fig. S1A), and pubic hair at Tanner stage III. Her external genitalia were bilateral labia minora and majora with vaginal introitus and clitoris 1.0 cm in length (Fig. S1B). The patient was reared as a female, though psychosocial evaluation now displays a male sex identity. A pelvic ultrasound (Fig. S1C) revealed undescended atrophied testes with the presence of right inguinal testis (cryptorchidism) measuring 1.0 cm (Fig. S1D) without Müllerian structures; no uterus, ovaries, seminal vesicles, or prostate were visualized. Furthermore, testicular histology showed atrophic seminiferous tubules and azoospermia (Fig. S1E). At admission, the patient hormone profiles revealed high levels of follicle-stimulating hormone and luteinizing hormone; low levels of androstenedione, testosterone, and dihydrotestosterone; normal levels of 17-hydroxyprogesterone and dehydroepiandrosterone sulfate (Table S1). The cytogenetics analysis on peripheral blood lymphocytes revealed that the patient showed a 46,XY karyotype with apparently normal adrenal function.

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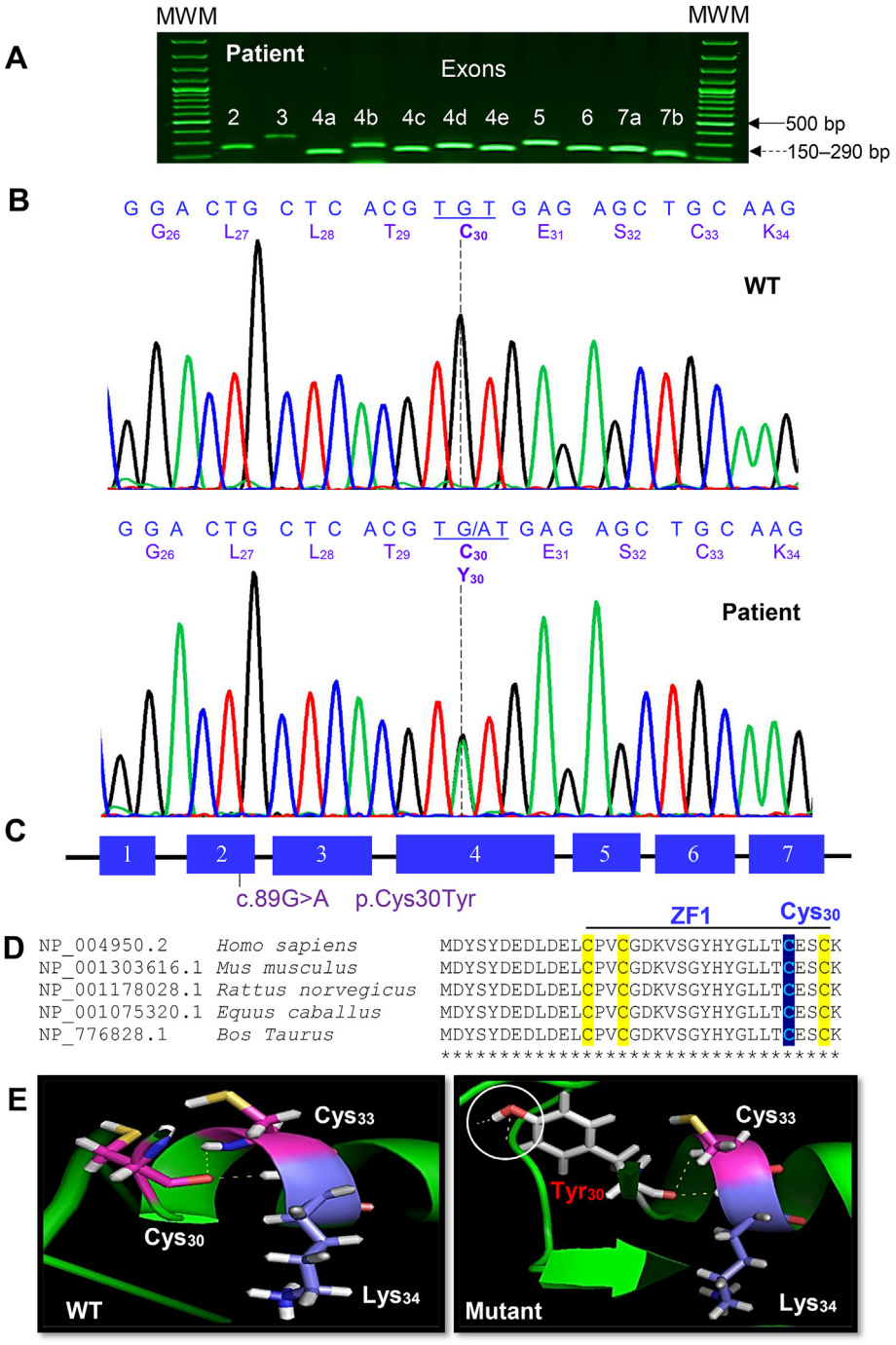

Figure 1 Genomic assays, *in silico* prediction analysis, and three-dimensional structure of the mutant NR5A1 from the 46,XY DSD patient and WT-NR5A1. **(A)** PCR amplification of transcribed exons (2—7) of the *NR5A1* gene from the 46,XY DSD patient. Agarose gel shows a specific amplicon (no additional PCR product) of the expected size between 150 and 290 base pairs (dotted arrow). The size of the molecular weight marker is 100 base pairs. The arrow indicates 500 base pairs. **(B)** Partial electropherogram of *NR5A1* gene from 46,XY DSD patient (P) and healthy control subject or WT. *NR5A1* gene sequencing revealed a heterozygous single nucleotide variant in the 89 position. **(C)** Sanger sequencing indicated a G > A transition at position 89 (c.89G > A) that encodes a missense substitution Cys for a Tyr at codon 30 (p.Cys30Tyr) of exon 2. **(D)** Comparative sequence analysis with human (NP_004950.2), mouse (NP_001303616.1), rat (NP_001178028.1), horse (NP_001075320.1), and cow (NP_776828.1) revealed that missense substitution was located in a highly conserved region of the first zinc finger of the DNA-binding domain. **(E)** A three-dimensional model of WT and mutant human NR5A1. The WT and p.Cys30Tyr missense mutations are indicated. The white circle indicates the tyrosine side chain, which exhibits different interactions.

The first genomic evaluations of a 46,XY DSD patient were carried out by sequencing the *SRY*, *LHCGR* (luteinizing hormone/choriogonadotropin receptor), *HSD17B3* (hydroxysteroid 17-beta dehydrogenase 3), *SRD5A2*, and *NR3C4*/*AR* genes; molecular genetic analysis excluded mutations in these genes associated with gonadogenesis, which are specifically involved in sex determination and gonadal function in humans. The results indicated that the full-length gene sequences were similar to the controls. Subsequently, genomic analysis of *NR5A1* was performed by individually amplifying each exon (Table S2). PCR analysis of all coding regions of the *NR5A1* gene revealed a normal electrophoretic migration profile. The PCR products displayed only one specific amplicon (expected molecular size of 150—290 base pairs); all exonic regions (2—7; for detailed sequencing analysis, 4 and 7 exons were divided into several fragments) analyzed by PCR excluded extensive insertions, deletions, and duplications (Fig. 1A). Bidirectional Sanger sequencing assays (Fig. 1B) identified in the first transcribed exon of the *NR5A1* gene a heterozygous single nucleotide variant in the 89 position (c.89G > A), which resulted in a missense substitution of the codon TGT (cysteine) with TAT (tyrosine) in the 30 position (p.Cys30Tyr) of the NR5A1 protein (Fig. 1C). The missense substitution was localized by alignment of sequences in the third cysteine of the first zinc finger of the DNA-binding domain, a structural region with a protein identity of 100 % (Fig. 1D). Using nuclear magnetic resonance data from Protein Data Bank no. 2FF0-NR5A1 WT-protein, its mutant (p.Cys30Tyr), and PyMOL software, the 3D structure was obtained for structural analysis. In the identified NR5A1 mutation, the interactions observed were via the backbone of the amino acids Cys33 and Lys34, which maintain standard-type interactions. However, the Tyr30 residue of mutant NR5A1 structure has other interactions via the aromatic R group of the side chain (Fig. 1E). All bioinformatic tools for pathogenic prediction classified the p.Cys30Tyr variant as potentially damaging, affecting protein function, likely deleterious, and thermodynamically destabilizing at the protein level (Table S3).

In summary, this study reveals a novel missense variant in the *NR5A1* gene from a 46,XY DSD female with hypergonadotropic hypogonadism and normal adrenal function. Sex disorders, including 46,XY, rely on molecular diagnoses according to genetically-regulated endocrinopathies, and this information is used to inform clinical treatment. The data from this study support and further develop the genotype-structure—function-phenotype correlation framework established by previous studies in this complex field.

Ethics declaration

This study was conducted according to the Declaration of Helsinki and the standard investigation protocols of the Institutional Ethical Committee for Investigation in Humans of the INCMNSZ (No. BRE-2614-18-21-1).

Declaration of competing interest

The author declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.101160.

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