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A 46,XX ovotesticular disorder of sex development likely caused by a SF-1 (*NR5A1*) variant

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Abstract

Background—A variant in steroidogenic factor-1 (SF-1, encoded by the gene *NR5A1*), p.Arg92Trp, has recently been reported in multiple families with 46,XX ovotesticular or testicular DSD. This amino-acid change impacts the DNA-binding domain and perturbs gonadal differentiation pathways.

Methods—Whole-exome sequencing was performed on a 46,XX subject with ovotesticular DSD.

Results—Exome results identified a heterozygous *NR5A1* variant, p.Arg92Gln, in the 46,XX ovotesticular DSD proband. This arginine-to-glutamine change has been previously reported in the homozygous state in a 46,XY patient with gonadal and adrenal dysgenesis, though 46,XY and 46,XX heterozygous carriers of this variant have not been previously reported to have any clinical phenotype.

Conclusions—The *NR5A1* p.Arg92Gln variant, which has thus far only been seen in a family with 46,XY DSD, most likely contributes to the ovotesticular DSD in this case. In light of the recent reports of unrelated 46,XX subjects with testicular or ovotesticular DSD with the *NR5A1* variant, p.Arg92Trp, it appears that other mutations in the DNA binding domain have the potential to impact the factors determining testicular and ovarian differentiation. This case demonstrates the variability of phenotypes with the same genotype and broadens our understanding of the role of SF-1 in gonadal differentiation.

Key terms

SF-1; disorder of sex development; ovotestes; exome sequencing

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Introduction

Steroidogenic factor-1 (SF-1), encoded by the *NR5A1* gene, was identified in the early 1990's as a nuclear receptor with an essential role in adrenal and gonadal development [1,2]. Homozygous pathogenic *NR5A1* variants cause complete gonadal dysgenesis and adrenal insufficiency[3]. Heterozygous *NR5A1* variants can also cause gonadal dysfunction in 46,XY individuals and have been shown to be associated with a wide spectrum of phenotypes ranging from infertility without genital anomalies to significant undervirilization with hypospadias, microphallus, and/or undescended testes to overt genital ambiguity[4]. In 46,XX individuals, many of the same heterozygous *NR5A1* variants have also been associated with gonadal dysfunction, presenting as primary ovarian insufficiency[5].

Recently, Bashamboo et al. presented four families with a specific variant in *NR5A1*, p.Arg92Trp, with ovotesticular or testicular disorders of sex development (DSD) in 46,XX individuals[6]. This was followed by three additional cases with the same variant reported by Baetens et al. [7]. Individuals with ovotesticular DSD have both testicular and ovarian tissue and are most often seen in the setting of a 46,XX karyotype. The amino acid altered by this variant is located in the accessory DNA-binding domain and is highly conserved. While the role of *NR5A1* in early gonadal development had been well established, these reports demonstrated a previously undescribed role for *NR5A1* in gonadal fate determination, with the missense variant p.Arg92Trp leading to disruption of ovarian-specific developmental pathways, namely, the Wnt/ β -catenin pathway, which normally suppresses the expression of testis genes[6,7]. To date, only this single *NR5A1* variant has been described in patients with 46,XX ovotesticular or testicular DSD.

Methods and Subjects

Clinical History

This study was approved by the Boston Children's Hospital Institutional Review Board. Written informed consent was obtained for all participants.

The patient is a 46,XX individual of European ancestry (England, Scotland, Ireland, France) born with ambiguous genitalia and raised as a girl. She had not had any prior surgeries. She was initially seen at our institution at age 3 and was noted to have significant clitoromegaly (2.5 cm length, 1.1 cm width) without palpable gonads. She had an anogenital ratio of 0.5 and rugated labia majora. Her evaluation for congenital adrenal hyperplasia (CAH) revealed normal baseline and stimulated adrenal hormones and precursors. Ultrasound and MRI imaging identified a uterus measuring $2.0 \times 0.7 \times 0.4$ cm and possible abdominal gonads, with oval-shaped gonadal structures located at the junction of the pelvis and inguinal regions, measuring $0.8 \times 0.7 \times 0.6$ cm on the left and $1.0 \times 0.7 \times 0.6$ cm on the right. The structure on the left contained at least two cysts, potentially consistent with ovarian follicles.

To assess for the presence of testicular tissue, additional evaluation included an AMH measurement as well as an hCG stimulation test. AMH was 6.47 ng/ml (reference range 0.256–6.34 ng/ml, conversion factor to pmol/L: 7.1429) and baseline serum total testosterone was 2 ng/dL (conversion factor to nmol/L: 0.0347). After 3 days of stimulation

with intramuscular hCG 1500 units daily, testosterone rose to 39 ng/dL. Based on this evidence of testosterone-producing tissue, the subject underwent laparoscopy, which identified bilateral gonadal structures that appeared to be ovotestes. The testicular-appearing material was resected from both gonads, and pathology showed dysgenetic testicular tissue with absent germ cells (Figure 1). Cytogenetics on the testicular tissue confirmed a 46,XX karyotype.

Genetic Analyses

Whole-exome sequencing of blood- and testis-derived genomic DNA was performed at the Broad Institute (Cambridge, Massachusetts, USA) on the proband and her parents, as previously described[8]. For hybrid selection, we used the custom Illumina Content Exome capture kit (Illumina, San Diego, California, USA). Sequencing reads were aligned to the hg19 reference genome[9]. We applied the Genome Analysis Toolkit (GATK) for base quality score recalibration and indel (insertion-deletion) realignment[10]. Variant quality score recalibration was simultaneously performed for SNP and indel discovery according to GATK Best Practices recommendations [11,12]. We used SnpEff (<http://snpeff.sourceforge.net/>) for functional annotation. We filtered for variants that were found in less than 1% of the reference population based on allele frequencies from the Exome Aggregation Consortium (<http://exac.broadinstitute.org/>)[13]. Based on the quality standards at the Broad Institute, at least 80% of the exome had 20X coverage. Results were reviewed for variants in known DSD-related genes [14]. Sanger sequencing was performed to confirm the variant of interest.

Results

Exome sequencing on the 46,XX subject with bilateral ovotestes identified an extremely rare missense variant in *NR5A1*, a gene recently implicated in ovotesticular DSD. The variant, c. 275G>A, p.Arg92Gln (Figure 2), was inherited from the proband's father [5,15], a phenotypically normal adult male who has fathered two children. The variant was not present in the ExAC database but had previously been reported in an unrelated family with a homozygous 46,XY proband with gonadal dysgenesis and adrenal insufficiency and unaffected 46,XX heterozygotes (mother and sister of affected proband) [16]. *In silico* prediction of the missense variant using Polyphen-2 yielded a score of 0.932, considered "probably damaging" by the software[17]. We compared whole-exome sequencing results from both blood- and testis-derived DNA from the proband to identify somatic mutations that may be contributing to the phenotype; this identified no differences to suggest somatic mutations.

Discussion

We have identified a paternally inherited, heterozygous variant in *NR5A1*, p.Arg92Gln, in a patient with 46,XX ovotesticular DSD. The p.Arg92Gln change is at the same position as variants in recently reported 46,XX ovotesticular and testicular DSD cases but involves a different amino-acid change[6,7]. Based on other reported families, this change is not always pathogenic in the heterozygous state [16,18], nor does it impact synergy of SF-1 with β -catenin based on *in vitro* studies[6]. Early studies looking at DNA binding in the

p.Arg92Gln variant showed an impact on DNA-binding specificity when present with other reported pathogenic variants such as p.Gly35Glu[19]. Other studies have confirmed partial loss of function of the p.Arg92Gln variant with respect to activation of SF-1 target gene promoters[20]. A recent report looking at Turkish families with primary adrenal insufficiency identified another family carrying the same p.Arg92Gln variant with unaffected heterozygous parents and a homozygous 46,XX phenotypically female daughter with adrenal insufficiency [18]. This report does not describe the gonadal function of the mother or the affected daughter.

The fact that the p.Arg92Gln variant is absent in large databases but has now been seen in three different families with abnormalities in gonadal and/or adrenal function provides evidence that multiple missense mutations at this amino-acid residue can impact the function of SF-1. Reviewing this heterozygous variant in the context of the American College of Medical Genetics and Genomics (ACMG) criteria[21], we find that it is “likely pathogenic” for the 46,XX ovotesticular DSD phenotype given that it is absent from control databases, alters an amino-acid residue previously associated with the same phenotype, and has been seen as clearly pathogenic when homozygous in a patient with adrenal and gonadal insufficiency. Additional evidence includes *in silico* prediction, with a Polyphen-2 score in the “probably damaging” range, as well as a disease phenotype consistent with a variant in this gene.

The recent report of four families with 46,XX testicular or ovotesticular DSD by Bashamboo et. al. is the first to describe a missense mutation in *NR5A1* leading to these 46,XX ovotesticular or testicular phenotypes[6]. This was quickly followed by Baetens et. al. with three additional unrelated (ovo)testicular DSD subjects with the identical p.Arg92Trp variant[7]. The reports provide evidence of the p.Arg92Trp variant leading to decreased synergy of SF-1 with β -catenin. The model proposed for p.Arg92Trp in the setting of 46,XX DSD involves decreased expression of *NROB1* and other anti-testis genes, secondary to the decreased synergy with β -catenin. This subsequently leads to increased expression of *SOX9* and other pro-testis genes, resulting in testicular or ovotesticular DSD[6]. Notably, some of these additional families include unaffected 46,XX carriers, demonstrating incomplete penetrance of the p.Arg92Trp variant.

Although we have not directly tested the molecular function of the p.Arg92Gln variant, we presume that the p.Arg92Gln variant, previously reported in 46,XX carriers without identified phenotypes, can sometimes lead to similar perturbations in signaling as the p.Arg92Trp variant and can thereby lead to ovotesticular DSD in some individuals. It is possible that other genetic variants, whether in *NR5A1* or other genes, or nongenetic factors could explain this incomplete penetrance, although we did not find any other *NR5A1* variants in our subject. There is also strong evidence of variable penetrance of other *NR5A1* variants, particularly in 46,XY DSD cases[22], and it is likely that additional examples demonstrating variable penetrance will be observed with the p.Arg92Gln variant as more individuals carrying this variant are identified.

Our work provides further support for the unfolding story showing *NR5A1* as a cause of 46,XX ovotesticular DSD and suggests that the p.Arg92Gln variant may do so separately

from effects on Wnt signaling [6]. Instead, the altered amino-acid apparently influences another aspect of SF-1 action leading to the ovotesticular DSD phenotype through a mechanism that has yet to be identified. Further study of the functional effects of this variant may reveal how *NR5A1* influences gonadal fate specification. In particular, functional defects common to both p.Arg92Gln and p.Arg92Trp (as opposed to being associated with only one of the variants) are more likely to be relevant to the phenotype.

Our case, along with other recently published reports, highlights the contribution of *NR5A1* to multiple steps in the development of ovarian or testicular tissue from the undifferentiated gonad, with a previously unappreciated role in gonadal fate specification in addition to the well-established involvement of *NR5A1* in gonadal development. These recent ovotesticular DSD cases have been crucial to providing insights for future research.

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Established Facts

- *NR5A1* has an essential role in adrenal and gonadal development, and individuals with homozygous mutations in *NR5A1* have adrenal and gonadal dysgenesis.
- Heterozygous pathogenic *NR5A1* variants in 46,XY individuals have been shown to be associated with varying degrees of undervirilization, ranging from infertility to significant undervirilization.
- Heterozygous pathogenic variants in 46,XX individuals have been associated with premature ovarian insufficiency.
- Recent reports have associated testicular and ovotesticular DSD in 46,XX individuals with a specific *NR5A1* variant, p.Arg92Trp.
- Another report identified homozygosity of a very rare variant at the same amino acid, p.Arg92Gln, in a patient with gonadal dysgenesis and adrenal insufficiency.

Novel Insights

- Heterozygosity of the deleterious *NR5A1* variant p.Arg92Gln appears to be associated with ovotesticular DSD in a 46,XX subject

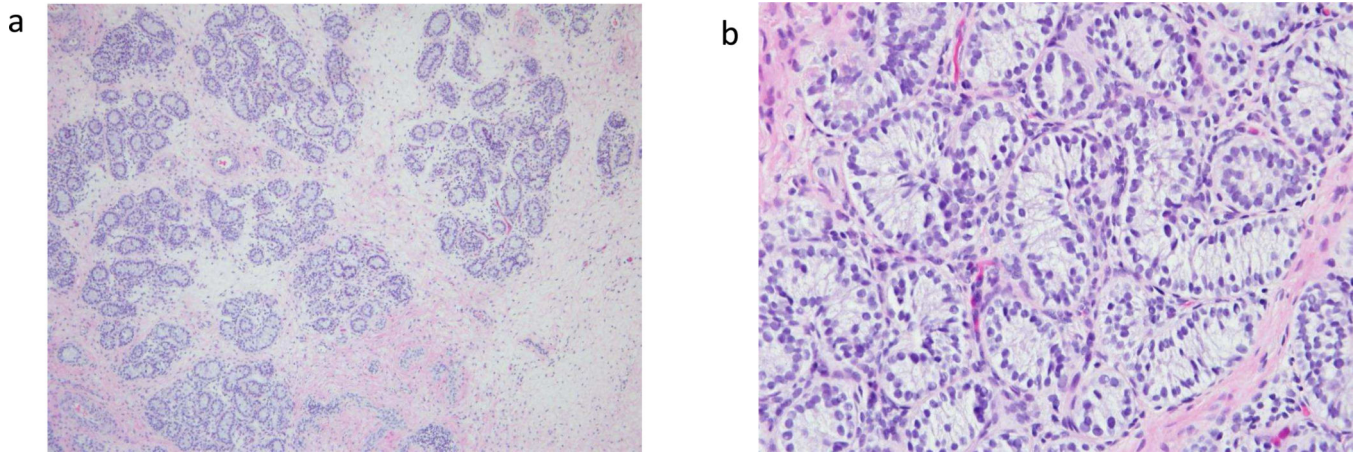


Figure 1.

a) Left gonad at intermediate magnification - testicular tissue demonstrating seminiferous tubules

b) Right gonad at high magnification – seminiferous tubules without visible germ cells

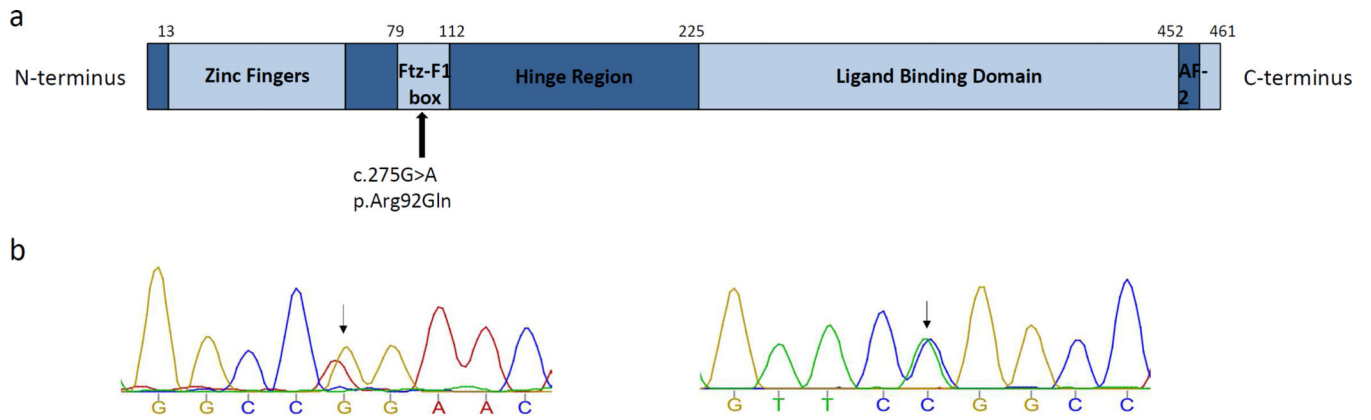


Figure 2.

a) SF-1 protein structure, with the location of the heterozygous mutation in the 46,XX ovotesticular DSD subject indicated. = Activation function domain 2; Ftz- F1 = fushi tarazu factor 1 box. (adapted from Wong, J Mol Endocrinol, 1996 and Camats, JCEM, 2012) b) Chromatograms obtained by Sanger sequencing indicating a G>A transition in exon 4 of *NR5A1* (Chr 9:127262964) the subject. On the left is the forward read, and on the right is the reverse read.

*Figure not to scale.