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A Novel Intronic Pathogenic Variant in *STAR* With a Dominant Negative Mechanism Causes Attenuated Lipoid Congenital Adrenal Hyperplasia

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Abstract

Lipoid congenital adrenal hyperplasia (LCAH) is typically inherited as an autosomal recessive condition. There are 3 reports of individuals with a dominantly acting heterozygous variant leading to a clinically significant phenotype. We report a 46,XY child with a novel heterozygous intronic variant in *STAR* resulting in LCAH with an attenuated genital phenotype. The patient presented with neonatal hypoglycemia and had descended testes with a fused scrotum and small phallus. Evaluation revealed primary adrenal insufficiency with deficiencies of cortisol, aldosterone, and androgens. He was found to have a de novo heterozygous novel variant in *STAR*: c.65-2A>C. We report a case of a novel variant and review of other dominant mutations at the same position in the literature. Clinicians should be aware of the possibility of attenuated genital phenotypes of LCAH and the contribution of de novo variants in *STAR* at c.65-2 to the pathogenesis of that phenotype.

Keywords

endocrinology, genetics and molecular medicine, pediatrics, STAR, lipoid congenital adrenal hyperplasia

Introduction

The steroidogenic acute regulatory (STAR) protein (OMIM #600617) is part of a transduceosome complex on the mitochondrial membrane and is responsible for the rate-limiting step of steroid synthesis, translocation of cholesterol from the outer to the inner mitochondrial membranes where the side chain cleavage enzyme (P450scc) converts cholesterol to pregnenolone.[1-3] Deficiencies in the STAR protein lead to the most severe form of congenital adrenal hyperplasia, lipoid congenital adrenal hyperplasia (LCAH), which manifests as severe adrenal insufficiency, salt wasting, and phenotypically female external genitalia in most XY individuals.[1,4] Clinical manifestations of glucocorticoid and mineralocorticoid deficiencies can be present at birth or be delayed by months to years due to residual STAR-independent mechanisms of steroid synthesis, which are eventually destroyed by buildup of cholesterol esters in the adrenal grands and gonads.[1,5] In contrast, testicular hormonogenesis is affected in utero as evidenced by development of external female genitalia in XY individuals, with genital development typically complete by about 12 weeks gestation.[6] There have been a number of nonclassic LCAH cases reported with a variety of attenuated phenotypes including late-onset adrenal insufficiency and XY individuals with virilized genitalia.[7-11] LCAH is typically a recessive condition and heterozygous carriers of pathologic variants are usually asymptomatic.[1] Only 3 prior reports in the literature have described individuals with a dominantly acting heterozygous variant leading to a clinically significant phenotype.[9,12,13] We present here a fourth individual with a novel heterozygous variant in *STAR* resulting in adrenal insufficiency and salt wasting in an undervirilized, but phenotypically male child. Additionally, we review the 3 other previously reported cases of LCAH due to a dominant heterozygous variant at the c.65-2 location.

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Case Presentation

History, Clinical Examination, and Hormonal Laboratory Findings

The proband was born full-term to nonconsanguineous parents by spontaneous vaginal delivery following an uncomplicated gestation. On the day of delivery, he was found to have persistent hypoglycemia with blood glucoses of 20 to 30s mg/dL. Initial physical examination was notable for bilateral descended testes and a fused scrotum with a small phallus (1.5 cm × 0.08 cm). Laboratory evaluation showed low cortisol at <0.2 µg/dL (both at time of hypoglycemia and following 1 µg adrenocorticotropic hormone [ACTH] stimulation test, with normal for these being ≥18 µg/dL), and he was started on hydrocortisone for adrenal insufficiency. At 10 days of life, he developed hyponatremia (sodium 130 mmol/L) and hyperkalemia (potassium 7.2 mmol/L). Fludrocortisone was added with resultant normalization of electrolytes. Additional neonatal evaluation included normal newborn screen (although undetectable 17-hydroxyprogesterone), normal pituitary on brain magnetic resonance imaging, normal chromosomal microarray, and 46,XY karyotype. He had an elevated ACTH >1250 pg/mL (normal 0-46), elevated plasma renin activity 193 ng/mL/h (normal 2-37), and low aldosterone 3 ng/dL (normal 6-179) indicative of primary adrenal insufficiency. Serum 17-hydroxyprogesterone was also undetectable at <10 ng/dL. At 2 months of age, during the minipuberty of infancy, he had an elevated luteinizing hormone 17.5 mIU/mL (normal 0.02-7.0) and follicle-stimulating hormone 7.9 mIU/mL (normal 0.16-4.1), and low total testosterone 5.6 ng/dL (normal 60-400) consistent with hypergonadotropic hypogonadism. He received testosterone 25 mg intramuscularly every 28 days for 4 doses, to replace the absent testosterone surge during the minipuberty of infancy.

Genetic Analysis

The patient underwent genetic testing at a commercial laboratory. The testing included sequencing of exonic regions and at least 10 bases of flanking intronic regions for 69 genes associated with differences in sex development. Initial sequencing and copy number variant detection was done using standard methods for next-generation (short read) sequencing. Read depth was at least 20× for all regions included on the panel. Potential pathogenic variants were confirmed using Sanger sequencing, also using standard methods.

Results revealed a heterozygous novel variant in *STAR* at c.65-2A>C, confirming a diagnosis of LCAH. Targeted variant testing was negative for the variant in both parents, confirming a de novo pathogenic variant in the proband. No other potential pathogenic variants were detected in genes associated with a difference of sex development.

Discussion and Conclusion

LCAH typically results from biallelic pathogenic variants in *STAR*. There are 3 previously reported cases of a heterozygous pathogenic variants with a dominant negative mechanism resulting in an attenuated phenotype.[9,12,13] All 3 resulted from the same c.65-2A>G pathogenic variant. Details of the clinical presentations and laboratory evaluations for the previously reported and currently reported cases are summarized in Table 1. All 3 previously reported cases presented in the first week of life with primary adrenal insufficiency manifesting as hypoglycemia or adrenal crisis. Two of the 3 cases had undervirilization of the external genitalia, while the third had typical phenotypically male genitalia and only demonstrated hypogonadism during puberty. Laboratory evaluation demonstrated XY karyotype, elevated ACTH, and low cortisol in all individuals. All patients required glucocorticoid replacement. One of the patients was able to stop mineralocorticoid replacement at the time of puberty induction. Two of the 3 individuals were assigned a male gender, while the third was assigned a female gender and underwent gonadectomy at 3 years of age. Baquedano et al[12] performed mRNA sequencing and western blotting for STAR in testicular tissue from patient 2. They demonstrated abnormal splicing resulting in an in-frame loss of exon 2 (p.Gly22_Leu59del), causing loss of most of the mitochondrial targeting sequence (MTS). In vitro STAR function was assessed with measurement of pregnenolone production in cell culture. COS-1 cells were transfected with either mutant or wild-type STAR cDNA. Production of pregnenolone was reduced both in cells transfected with only the mutant STAR vector and in cells cotransfected with mutant and wild-type STAR vectors, proving a dominant negative effect of the mutant protein. Immunofluorescence studies using anti-STAR and mitotracker red demonstrated that the mutant STAR protein lacking the MTS remained external to the mitochondria. The specific mechanism of the dominant negative action of this pathogenic variant is not known, but STAR is a component of the transduceosome complex, which locates to the mitochondrial membrane and regulates importation of cholesterol into the mitochondria. Presumably, the stable mutant STAR protein lacking the amino acids corresponding to exon 2 disrupts the transduceosome complex and decreases hormonal synthesis sufficiently to result in the phenotype of attenuated LCAH.

The term dominant negative signifies that the phenotype resulting from a mutation with a dominant negative mechanism is more severe than the phenotype resulting from haploinsufficiency (full loss of function of one copy) for the gene. As illustrated in Figure 1, the STAR protein lacking the MTS is itself nonfunctional because of not being trafficked into the mitochondria and additionally interferes with the function of the STAR protein encoded by the other (nonmutated) allele. As a result, individuals with mutations at the c.65-2A>G position have manifestations of lipoid

Table 1. Clinical Characteristics, Laboratory Values, Treatment, and *STAR* Mutations of Reported Heterozygous Cases[a].

	1 (our patient)	2 (Argentina)	3 (Russia)	4 (Japan)
Age at presentation	DOL 1	DOL 7	DOL 2	DOL 1
Clinical presentation	Hypoglycemia Adrenal crisis	DSD Adrenal crisis	DSD Hypoglycemia Adrenal crisis	Hypoglycemia
Genital examination	Small phallus (1.5 cm), fused scrotum, bilateral descended testes	Small phallus (0.5 cm) with penoscrotal hypospadias and poorly developed corporal tissue, complete labial fusion, and bilateral inguinal gonads	Small phallus (<1 cm), hypoplastic scrotum, urogenital sinus, and bilateral abdominal testes	Typical phallus and bilateral descended testes
Cortisol	<0.2 μg/dL	<1 μg/mL	0.8 μg/dL	0.3 μg/dL
ACTH	>1250 pg/mL (0-46)	143 pg/mL	238 pg/mL (10-185) 654 pg/mL (0-46)	4858 pg/mL
PRA/renin	193 ng/mL/h (2-37)	28 ng/mL/h	>500 uIU/mL (4.4-46.1)	>80 ng/mL/h
LH	2 months: 17.5 mIU/mL (0.02-7.0)	3 months: 0.57 mIU/mL		20 years: 18.8 IU/L
FSH	2 months: 7.9 mIU/mL (0.16-4.1)	3 months: 3.8 mIU/mL		20 years: 34.2 IU/L
Testosterone	2 months: Total testosterone 5.6 ng/dL (60-400)	3 months: <0.05 ng/mL	Negative hCG stimulation test	20 years: Total testosterone 5.02 ng/mL
Treatment	2 years old: Hydrocortisone 12 mg/m^2/day Fludrocortisone 0.1 mg/day Testosterone 25 mg IM Q28 days × 4 doses in the minipuberty	10 years old: Hydrocortisone 11 mg/m^2/day Fludrocortisone 0.1 mg/day 14 years old: Hydrocortisone 11.4 mg/m2/day Conjugated estrogens 0.625 mg/day	3 years old: Hydrocortisone 3.75 mg/m^2/day Fludrocortisone 0.05 mg/day	20 years old: Hydrocortisone 21.2 mg/m^2/day Fludrocortisone 0.05 mg/day
Pathogenic variant	c.65-2A>C	c.65-2A>G	c.65-2A>G	c.65-2A>G
Reference		Baquedano et al[12]	Kalinchenko et al[13]	Ishii et al[9]

Abbreviations: DOL, day of life; DSD, disorder/difference of sex development; ACTH, adrenocorticotropic hormone; PRA, plasma renin activity; LH, luteinizing hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin.
[a]Normal value in parentheses where available.

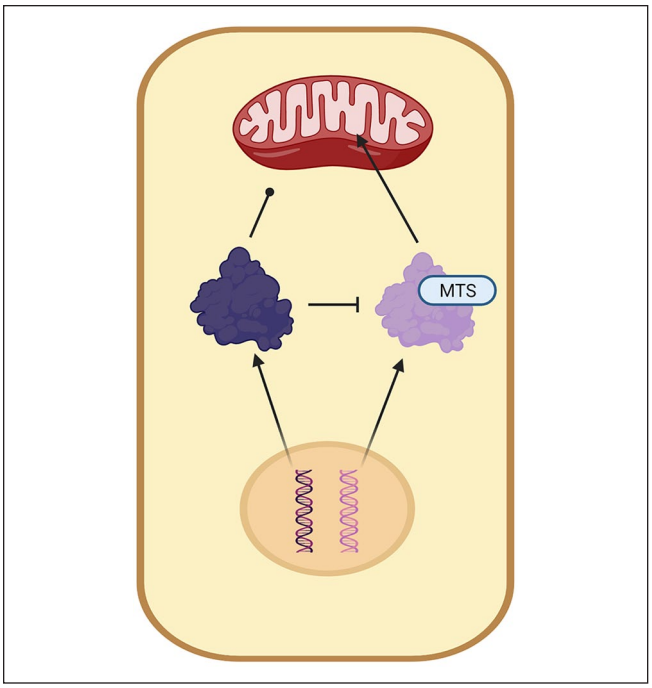

Figure 1. Dominant negative mechanism. The allele of the *STAR* gene with a c.65-2 A>G or A>C mutation is in the dark color on the left. The wild-type allele is light and on the right. Both alleles result in translation of a stable protein but the allele with one of these specific mutations does not have a full MTS and is not trafficked into the mitochondria. The protein is not functional outside of the mitochondria and also inhibits the function of the STAR protein expressed from the wild-type allele, accounting for the dominant negative action and the occurrence of a clinical phenotype in individuals with only a heterozygous mutation (and not biallelic mutations). Created using BioRender.com.

congenital adrenal hypoplasia, whereas those who are carriers for typical recessive mutations do not have any manifestations. The functional protein translated from one wild-type allele of the gene has sufficient function for normal hormonogenesis as long as it is not interfered with by the protein expressed from the allele with the mutation.

While we have not performed functional analysis of the mRNA and protein products resulting from c.65-2A>C, given the phenotypic similarity of our patient to those previously described with dominant negative *STAR* pathogenic variants, and given that the single nucleotide variant occurs at the same position, we infer that the c.65-2A>C variant results in the same transcript lacking exon 2 as c.65-2A>G.

For those clinicians evaluating patients with a difference of sex development, ambiguous genitalia, or primary adrenal insufficiency, it is important to be aware of attenuated phenotypes of LCAH and the contribution of de novo variants in *STAR* at c.65-2 to the pathogenesis of that phenotype. Laboratories and clinicians should recognize that heterozygous variants in *STAR* may be diagnostic if they result in an intact protein that lacks a functional MTS due to the dominant negative effect that can result.

Authors' Note

This case was previously submitted as an abstract to the Pediatric Endocrine Society meeting in Fort Worth, TX (April 2020, but cancelled due to COVID-19), and the meeting abstract was published in *Hormone Research in Paediatrics*. 2020;93:1-185.

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Declaration of Conflicting Interests

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Ethics Approval

Our institution does not require ethical approval for reporting individual cases.

Informed Consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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