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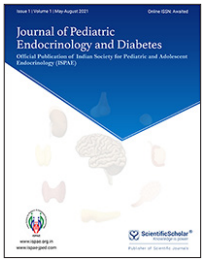

*Case Report*

# A novel p.Leu197Pro homozygous variant in *HSD3B2* as a cause of 46,XY DSD with hyperpigmentation in an infant

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## ABSTRACT

We report a novel missense variant of the *HSD3B2* gene in a 46,XY child born to third degree consanguineous parents presenting with undervirilization and progressive hyperpigmentation. The steroid profile showed elevated concentrations of 17-hydroxyprogesterone, but normal androstenedione and testosterone. The adrenocorticotropic hormone was elevated. The direct DNA sequencing of the child revealed a new homozygous missense variant in the *HSD3B2* gene, resulting in the amino acid substitution of proline for leucine at codon 197. We have described a hitherto novel *HSD3B2* gene variant in an undervirilized male infant causing 3β-hydroxysteroid dehydrogenase 2 deficiency.

**Keywords:** Congenital adrenal hyperplasia, Disorders of sexual differentiation, *HSD3B2*, Novel mutation

## INTRODUCTION

Disorders of sex development (DSD) are often medical and social emergencies to the patient, the family, and the treating physicians. Advances in genetic evaluation have facilitated prompt and accurate diagnoses of these disorders. Congenital adrenal hyperplasia (CAH) is the most common cause of DSD in pediatric endocrinology practice, with a wide spectrum of presentations. We report here a case of 3β-hydroxysteroid dehydrogenase 2 deficiency (3βHSD2), in a 46,XY infant with hyperpigmentation and undervirilization due to a novel genetic mutation in the *HSD3B2* gene.

## CASE REPORT

The infant presented at 24 days of life and was the first child of third-degree consanguineous parents after a spontaneous conception. The infant was born at term by emergency cesarean section because of fetal distress after an uneventful pregnancy with a birth weight of 3.54 kg. The infant's APGAR scores were 8 and 9 at 1 and 5 min after birth, respectively. There were no other significant events in the perinatal period. However, the attending pediatrician noted atypical genitalia. A karyotype was ordered before referring him to the endocrine unit, where he was first seen at the age of 24 days. There were no features of salt wasting by then, and genital examination revealed perineal hypospadias, micropenis, and palpable gonads within the bifid scrotum with significant genital hyperpigmentation. The parents reported that the infant's

maternal uncle had similar phenotypic features and died in infancy. Plasma biochemical and hormone concentrations on day 25 of life were as follows: plasma glucose 91 mg/dL, sodium 134 mmol/L, and potassium of 5.1 mmol/L. The basal 17-hydroxyprogesterone (17OHP), was 2868 ng/dL (49–410 ng/dL); the basal cortisol, was 22.6 μg/dL (3–22.4 μg/dL); adrenocorticotropic hormone (ACTH), 477 pg/mL (<46 pg/mL) and testosterone, 212 ng/dL (75–400 ng/dL). Post-Synacthen (125 μg IM) hormonal profile was as follows: cortisol, 28.2 μg/dL (3–22.4 μg/dL); 17OHP, >5400 ng/dL (49–410 ng/dL); and androstenedione, >1000 ng/dL (70–360 ng/dL). Of note, the steroid profile was assayed using chemiluminescence immunoassays (CLIA) and the apparently normal cortisol levels in the unextracted sample might be due to the cross-reacting analytes of the sample. However, the markedly elevated levels of 17OHP suggested that this is a case of CAH with hyperpigmentation. The child was in mini-puberty (luteinizing hormone level 1.91 U/L) and the testosterone and androstenedione were appropriately normal. Ultrasonography (USG) showed mildly prominent adrenals bilaterally and both testes in the labioscrotal folds. The karyotype was 46,XY. The typical clinical picture of 46,XY DSD in this infant with genital hyperpigmentation and elevated 17OHP and ACTH with normal testosterone and androstenedione levels suggests 3-beta-hydroxysteroid dehydrogenase 2 deficiency. Pending molecular genetic confirmation, he was given hydrocortisone (10 mg/m$^2$/day) in three divided doses [Figure 1]. The hyperpigmentation improved and the child was growing well. Subsequently, mineralocorticoid replacement was added as the plasma renin concentration was high on follow-up (plasma renin, direct (CLIA) 141.30 μIU/mL [2.80–39.90 μIU/mL]). To identify the enzyme deficiency, a clinical exome sequencing was requested which showed a homozygous missense variation in exon 4 of the *HSD3B2* gene [Figure 1].

DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The sequences obtained were aligned to human reference genome (GRCh38.p13) using Sentieon aligner.[1,2] In addition to single nucleotide variants (SNVs) and small indels, copy number variants were detected from targeted sequence data using the ExomeDepth (v1.1.10) method.[3] Clinically, relevant mutations were annotated using published variants in the literature and a set of diseases databases – ClinVar, OMIM (updated on May 11, 2020), GWAS, HGMD (v2020.2), and SwissVar.[4,5] Exon 4 of the *HSD3B2* gene was polymerase chain reaction-amplified and the products were sequenced using Sanger sequencing. Sequencing of the *HSD3B2* gene showed that the patient carried a novel homozygous missense variation located in exon 4 (chr1:g.119422091T>C; Depth: ×169) that results in the amino acid substitution of proline for leucine at codon 197 (p.Leu197Pro; ENST00000543831.5). The allelic frequency was reported to be close to 100%.

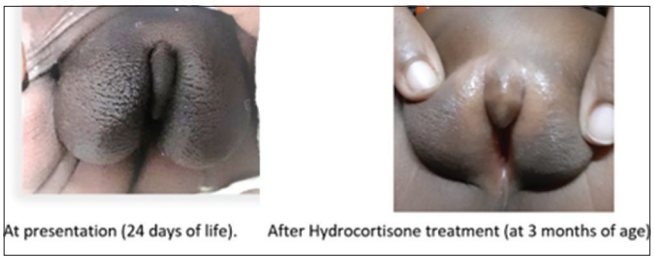


**Figure 1:** Pigmentation of genitalia before and after treatment initiation.

A Sanger sequencing validation confirmed the presence of the homozygous variation [Figures 2 and 3]. The Sanger sequencing of parents is pending.

## DISCUSSION

Our proband presented with atypical genitalia and progressive hyperpigmentation suggesting an underlying adrenocortical biosynthetic defect with excess production of ACTH. Notably, he was born to third-degree consanguineous parents and had a maternal uncle who had a similar presentation and died in infancy pointing to an autosomal recessive inheritance pattern. Although the 21-hydroxylase deficiency is the most common form of CAH, other rarer forms may present with a significant clinical disorder. Our patient is a 46,XY DSD presenting with undervirilization and progressive hyperpigmentation. Such a phenotype is consistent with 3βHSD2 deficiency. The differential diagnoses include lipoid CAH (LCAH), combined 17-hydroxylase/17,20-lyase deficiency, P450 oxidoreductase deficiency, and side chain cleavage enzyme deficiency.

LCAH (due to defect in the steroidogenic acute regulatory protein) and rarely side chain cleavage enzyme (practically its phenocopy) are characterized by generalized steroid underproduction and may present with undervirilization in the setting of 46,XY DSD.[6] However, 17OHP, androstenedione, and testosterone are low in these cases. Combined 17-hydroxylase/17,20-lyase deficiency (another less common form of CAH) characterized by hypertension and hypokalemia in undervirilized males is not compatible with normal androgen levels.[7] P450 oxidoreductase deficiency is another differential but is usually associated with skeletal malformations that resemble those observed in Antley-Bixler syndrome.[8]

Elevated ratios of delta-5 (17-hydroxy-pregnenolone [17OHPreg] and dehydroepiandrosterone [DHEA]) to delta-4 steroids (17OHP and androstenedione) are used to diagnose 3βHSD2 deficiency.[9] Very high DHEA sulfate (DHEAS) levels are a more readily available measure that points toward this diagnosis.[10] In our case regrettably, delta-5 steroids were not measured due to resource constraints. However, often an accurate diagnosis by

| Gene (Transcript) # | Location | Variant | Zygosity | Disease (OMIM) | Inheritance | Classification |
|---|---|---|---|---|---|---|
| ***HSD3B2* (+)** (ENST00000543831.5) | Exon 4 | c.590T>C **(p.Leu197Pro)** | Homozygous | congenital Adrenal hyperplasia due to 3-beta-hydroxysteroid dehydrogenase 2 deficiency | Autosomal recessive | Uncertain Significance |

| Analysis for: **Homozygous variation in *HSD3B2* gene** | **Gene Name: *HSD3B2* (Exon 4)** | |
|---|---|---|
| # | **Variation detected in NGS** | **Sanger validation result*** |
| 1. | **chr1:g.119422091T>C; c.590T>C; p.Leu197Pro** | **Present (Homozygous)** |

* The variant analysis in Sanger sequencing is based on the *HSD3B2* reference sequence ENST00000543831.5 [1]. The exon number and nucleotide numbers will differ based on the reference file chosen and the database used.

**Figure 2:** Variant analysis.

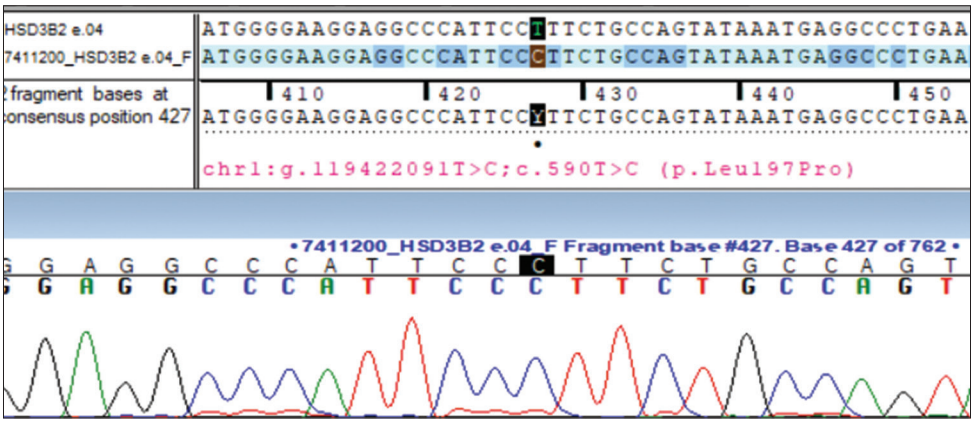


**Figure 3:** Sequence chromatogram.

hormonal criteria is precluded in these rarer forms of CAH due to the unpredictability of functional consequences of the variations in the mutations.

The fetal adrenal cortex along with the placenta, testes, and fetal liver plays a major role in the regulation of the steroid hormonal milieu needed for the maintenance of fetal and postnatal survival through the secretion of cortisol, aldosterone, DHEAS, androstenedione, and testosterone. A recent review of the various mutations leading to the clinical presentation of 3βHSD2 deficiency enumerated over 40 different variants across various geographical regions.[11] Several case reports till date have discussed the spectrum of phenotypic expression in 3βHSD2 deficiency. Variability of genetic expression resulted in significant heterogeneity in the clinical expression in these cases. Most of the reported cases of 3βHSD2 deficiency in 46,XY infants presented with severe hypospadias and varying degrees of incomplete labioscrotal fusion. Notably, testicular descent was normal in all these cases.[12] The degree of salt wasting though was variable reflecting the ontogenetic differences in the temporal and spatial regulation of HPA axis and androgen production in the fetal adrenal and the testes.

3βHSD2 deficiency affects cortisol production by blocking the conversion of 17OHPreg to 17OHP and our case had generalized hyperpigmentation due to ACTH elevation. With the deficiency of 3βHSD2, very high amounts of delte-5 steroids (17OHPreg and DHEA) are released from the adrenals. A significant proportion of these steroids are peripherally converted by the 3-beta-hydroxysteroid dehydrogenase type 1 (3βHSD1) to 17OHP and androstenedione. However, the circulating androstenedione may not be effectively taken up by the adrenals and testes to maintain sufficient androgen exposure for differentiation of external genitalia during the critical period in-utero.

Since testosterone and subsequently dihydrotestosterone (DHT) production in the first trimester is under the stimulation of human chorionic gonadotropin, there is no effective feedback loop to raise the testosterone levels to adequate levels in 3βHSD2 deficiency during this period. The inadequate masculinization of the external genitalia

in our case points to inadequate DHT exposure during the masculinization programming window *in utero*, as the levels of testosterone are not sufficient.[13]

*HSD3B2* gene encodes a 372 amino acid-containing enzyme with at least two reported sites with catalytic activity (Catalytic activity has been reported for amino acids 154–158 and 269–273).[11] Molecular analysis studies of the enzyme have flagged several functionally important regions in the *HSD3B2* gene.[14] The missense variant in our case caused a substitution of proline for leucine at the 197$^{th}$ position which is about 12 amino acids away from the putative substrate binding domain. It was classified as a variant of uncertain significance. However, the *in silico* predictions of the variant were deemed probably damaging by PolyPhen-2 (HumDiv) and damaging by SIFT and Mutation Taster2. The reference codon is conserved across species. However, the structure-function relationship is far more complex and without targeted transfection studies, remain speculative at best.

## CONCLUSION

We report a novel missense variant from India in the *HSD3B2* gene, leading to a classical presentation of CAH due to 3βHSD2 deficiency in a 46,XY infant.

### Declaration of patient consent

Patient's consent not required as the patients' identity is not disclosed or compromised.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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