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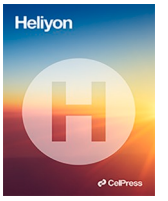

Case report

A *CYP11A1* homozygous exonic variant inducing an alternative splicing, frameshift and truncation in a family with congenital adrenal hyperplasia

Kheloud M. Alhamoudi [a,b], Meshael Alswailem [a], Balgees Alghamdi [a], Abdullah Alashwal [c], Ali S. Alzahrani [a,b,d,*]

[a] *Department of Molecular Oncology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia*
[b] *College of Medicine, Alfaisal University, Riyadh, Saudi Arabia*
[c] *Department of Paediatrics, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia*
[d] *Department of Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia*

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ABSTRACT

Background: Congenital adrenal hyperplasia (CAH) is a heterogeneous group of adrenal steroidogenesis disorders with variable degrees of glucocorticoid, mineralocorticoid and sex steroid deficiencies. *CYP11A1* gene encodes the mitochondrial cholesterol side-chain cleavage enzyme (P450scc), which initiates the first reaction in steroidogenesis by converting cholesterol to pregnenolone. Variants in this gene are extremely rare but associated with severe forms of CAH due to its early and critical function in various steroid biosynthesis pathways. Here, we report a *CYP11A1* exonic homozygous variant that, although exonic in location, affects splicing by creating an additional aberrant splicing site with frameshift and truncation of the gene.

Patients and methods: The proband is a 23-year old 46,XY patient raised as a girl. She was a product of normal pregnancy for first-degree relative parents. Soon after birth, she had vomiting, dehydration, hypotension, hyponatremia and hyperkalemia. She was started on glucocorticoids and mineralocorticoids with prompt recovery. Apart from a chronic need for these medications, her neonatal and childhood history was unremarkable. She sought medical advice at age 19 years for delayed puberty with primary amenorrhea and lack of breast development. On evaluation, she had normal external female genitalia, no breast development, undescended testes and absent uterus and ovaries. Her hormonal evaluation revealed very low estrogen, testosterone, cortisol, aldosterone, 17-hydroxyprogesterone, and androstenedione levels. ACTH, LH, FSH and renin were very high consistent with primary gonadal and adrenal failure. Her parents are healthy first-degree cousins. She has three sisters, all with 46,XX karyotype. One of them is clinically and biochemically normal while the other two sisters have normal female phenotype, normal uterus and ovaries, similar hormonal profile to the proband but different karyotype (46,XX) and absence of undescended testes. gDNA was used for whole exome sequencing (WES). Sanger sequencing was performed to confirm the detected variant and its segregation with the disease.

Results: WES identified a homozygous missense variant in *CYP11A1* changing the second nucleotide (GCG > GTG) at position 189 in exon 3 and resulting in a change of Alanine to Valine (p.

Abbreviations: Congenital adrenal hyperplasia, (CAH); whole-exome sequencing, (WES); variant of uncertain significance, (VUS).

* Corresponding author. Department of Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia, P.O Box 3354, Riyadh, 11211, Saudi Arabia.
E-mail address: aliz@kfshrc.edu.sa (A.S. Alzahrani).

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Ala189Val). This variant was confirmed by PCR and Sanger sequencing. It was found in a homozygous form in the proband and her two affected sisters and in a heterozygous form in the unaffected sister. *In-silico* analysis predicted this variant to create a new splicing site with frameshift and truncation of the gene transcript. This was confirmed by isolation of RNA, cDNA synthesis, gel electrophoresis and sequencing.
Conclusion: We describe a family with a very rare form of CAH due to a *CYP11A1* variant leading to creation of a new splice site, frameshift and premature truncation of the protein.

1. Introduction

Congenital adrenal hyperplasia (CAH) is a group of rare inherited autosomal recessive disorders that affect adrenal steroidogenesis. These conditions are characterized by variable degrees of impaired synthesis of some or all adrenal steroids including glucocorticoids, mineralocorticoids and adrenal androgens [1]. In some cases, gonadal steroidogenesis is also affected resulting in variable degrees of androgen or estrogen deficiencies [2].

Depending on the enzyme affected, patients may present with adrenal insufficiency, hypertension, hypokalemia, ambiguous genitalia, accelerated growth or precocious puberty. These symptoms, however, vary among patients depending on the gene affected and the severity of enzyme deficiency. To date, seven known human genes have been associated with CAH. These include *CYP21A2* [1], *CYP11B1* [3], *CYP11A1* [4], *HSD3B2* [5], *CYP17A1* [6], *StAR* [7] and *POR*. 21-hydroxylase *(CYP21A2)* and 11-Beta hydroxylase (*CYP11B1)* are the two most commonly mutated genes and are responsible for more than 90 % of cases [1,8]. Other types of CAH are rare. *CYP11A1* is the rarest form of CAH and is caused by variants in the *CYP11A1* gene [4].

CYP11A1 is a protein-coding gene located on chromosome 15 (NM_000781.3) chr15:74337762–74367646. It has nine exons and encodes 521 amino acids [9]. The protein is organized in a mitochondrial targeting sequence (MTS) and putative membrane-anchoring helices localized to the mitochondrial inner membrane [10]. *CYP11A1* gene encodes the mitochondrial cholesterol side-chain cleavage enzyme (P450scc), which is a member of the cytochrome P450 superfamily of enzymes [4]. These enzymes are monooxygenases that catalyse the first and rate-limiting biosynthesis step in steroidogenesis and also catalyse many reactions involved in drug metabolism [11,12]. The conversion of cholesterol to pregnenolone, which is the precursor of most steroid hormones, occurs through three sequential oxidation reactions (Koritz and Kumar, 1970). The 22-hydroxylation of cholesterol followed by the 20-hydroxylation of 22 (R)-hydroxycholesterol yields an oxidative scission of the C20–22 bond of 20(R), 22(R)-dihydroxycholesterol (the side-chain cleavage event) that is further cleaved between C20 and C22 to yield the C21-steroid pregnenolone and 4-methylpentanal (Shikita and Hall, 1973). In each enzymatic step, P450scc must receive a pair of electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) via the intermediary of electron donor partners, a flavoprotein termed "adrenodoxin reductase," (Adx) and an iron-sulfur protein termed "adrenodoxin reductase" (Kimura and Suzuki, 1965).

To date, 42 known human disease-causing *CYP11A1* variants have been described in cases with CAH (OMIM: 118485) (Supplementary Table 1). Among these variants, phenotype varies, presumably due to variant-specific effects on *CYP11A1* enzyme activity [13–21].

Here, we report a family with three siblings affected by CAH caused by a homozygous variant in exon 3 of the *CYP11A1* gene (c.566C>T, p.Ala189Val). This variant creates a new splicing site leading to frameshift and truncation of the *CYP11A1*.

2. Materials and methods

2.1. Study and ethical approval

The study was approved by the Institutional Review Board of the King Faisal Specialist Hospital and Research Centre (KFSHRC) (RAC number# 2130012), Riyadh, Saudi Arabia. The study was conducted in accordance with the Declaration of Helsinki. The probands' parents signed an informed consent form.

2.2. Patients

The proband (III -1) and her affected (III -2 and III -3) and unaffected (III -4) sisters underwent a full clinical and biochemical evaluation at King Faisal Specialist Hospital in Riyadh, Saudi Arabia.

2.3. Genomic DNA extraction

Fresh blood samples were collected from the proband (III-1), her two affected sisters (III-2 and III-3) and an unaffected sister (III-4) for DNA extraction. Genomic DNA (gDNA) was extracted using a commercial DNA extraction kit (QIAamp Blood Midi Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.4. Whole-exome sequencing and bioinformatics analysis

WES followed by data analysis and interpretation using an end-to-end-in-house bioinformatics pipeline was performed for the proband (III-1) as previously described [22,23]. *In-silico* computational prediction tools including Polyphen (available at http://genetics.bwh.harvard.edu/pph/) [24], SIFT (https://sift.bii.a-star.edu.sg/) [25], Varsome (https://varsome.com/) [26], MutationTaster (http://www.mutationtaster.org/) [27], PROVEAN (https://www.jcvi.org/research/provean) [28] and FATHMM (http://fathmm.biocompute.org.uk/index.html) [29] were used to predict the pathogenicity of the identified variant. The effect of the variant on RNA splicing was predicted using bioinformatics tools including BDGP splicing predictor program (http://www.fruitfly.org/seq_tools/splice.html), SKIPPY (http://research.nhgri.nih.gov/skippy) and Human Splice Finder (http://www.umd.be/HSF/).

2.5. Variant validation and segregation analysis

The identified *CYP11A1* homozygous variant (NM_000781.3, c.566C>T) was Sanger sequenced in three affected siblings and their unaffected sister. Sanger sequencing was performed as previously described [22] using the following primers: forward (5′-TGTTCCCCTTCACTCTCTTTTT -3′) and reverse (5- GGGTGGTCTTAAATTGCCTCT -3′).

2.6. RNA extraction and RT-PCR

Total RNA was extracted from fresh blood samples of the proband (III-1) and an independent unaffected control sample using TRIzol Blood RNA isolation protocol (Invitrogen) according to the manufacturer's instructions. The isolated RNA was quantified by spectrophotometer nanodrop (ND-1000). Total RNA was then reverse-transcribed using iScript reverse transcription supermix for RT-qPCR kit (Bio-Rad) as described previously [22]. PCR was performed using the following primers: forward 5′-TATGTCATCGACCCTGAAGA-3′ and reverse 5′-GGAACAGGTCTGGGGGAAG–3′ with an annealing temperature of 60 °C and 40 amplification cycles. The PCR products were then run on 2 % agarose gel electrophoresis supplemented with SYBR™ Safe Stain (Invitrogen). The effect of the splice variant identified in the proband was assessed by amplifying exons 3 and exon 4 in the *CYP11A1* gene and analysing the amplified PCR products by Sanger sequencing.

3. Results

3.1. Clinical description

The proband is currently 23 years old (III-1) raised as a female. She was born after an uneventful pregnancy to a first-degree relative couple. At birth, she looked like a normal girl and was named and raised as a girl. In the first few days of life, she developed severe vomiting, lethargy, poor feeding hypotension and severe dehydration. Serum sodium was low (126 mmol/l) and serum potassium was elevated >6 mmol/l. Cortisol was found undetectable and ACTH was extremely elevated (>2000 ng/dl). She was diagnosed with CAH and started on glucocorticoids and mineralocorticoids with prompt resolution of her symptoms. She was maintained on glucocorticoids and fludrocortisone but was not evaluated further. She had otherwise normal neonatal and childhood periods with normal milestones and growth. At age 19 years, she was referred to our hospital (KFSHRC) as she failed to achieve puberty with primary amenorrhea and

Table 1
Summary of biochemical and hormonal levels for all family members.

Characteristic/lab. Finding (normal ranges)	Proband (III-1)	Affected sister (III-2)	Affected sister (III-3)	Unaffected sister (III-4)
Current age (years)	23	14	11	8
Age at time of evaluation (years)	19	11	8	5
Weight/height (kg/cm)	55/171	24/126	28/124	21/104
Karyotype	46,XY	46,XX	46,XX	46,XX
Clinical presentation	Adrenal insufficiency, undescended testes, breasts Tanner 1, absent uterus and ovaries	Adrenal insufficiency	Adrenal insufficiency	healthy
External genitalia	Normal female	Normal female	Normal female	Normal female
Uterus and ovaries	Absent	Present	Present	present
S. Cortisol (nmol/l)	<1.5	<1.5	<1.5	246
ACTH (5–60 ng/l)	>2000	1017	623	32
17-OHP (0.5–3.0 nmol/l)	<0.4	<0.4	<0.4	ND
DHEAS (1.81–8.3 μmol/l)	0.04	ND	ND	ND
Andostenedione (1.0–12.2 nmol/l)	<1.0	<1.0	<1.0	ND
Testosterone (0.1–1.7 nmol/l)	0.7	<0.09	<0.09	ND
Estradiol (up to 99 pmol/l)	<18.4	ND	ND	ND
LH (2.4–12.6 u/l)	55.7	ND	ND	ND
FSH (3.5–12.5 u/l)	55.9	ND	ND	ND
Aldosterone (1.76–23.2 ng/dl)	1.55	ND	ND	ND
Renin (4.4–46.2 mU/l)	190.8	ND	ND	ND

lack of breast development. She was on oral dexamethasone 0.75 mg daily and fludrocortisone 0.1 mg daily. She looked well, weight was 55 kg, height 171 cm and her BMI 18.6. Blood pressure was104/66 mm Hg, heart rate 66 beats/minute, regular. No hirsutism or acne and skin was of normal color and texture. She had no dysmorphic features and her neurological, chest, cardiovascular and abdominal examinations were all normal. She had scanty pubic hair and no axillary hair. Genital examination revealed normal pre-pubertal female external genitalia without clitoromegaly but with two firm structures of about 3 × 1.5 cm size in the inguinal areas bilaterally. Breasts were Tanner I bilaterally. The rest of her physical examination was unremarkable. Biochemical and hormonal evaluation are summarized in Table 1. Cytogenetic evaluation showed a 46,XY karyotype. Pelvic magnetic resonance imaging and ultrasound of the pelvis showed undescended atrophied testes in the inguinal regions bilaterally, absent uterus, ovaries, fallopian tubes and upper third of the vagina. The family evaluation revealed two affected siblings (III-2 and III-3) who were products of normal pregnancy and spontaneous vaginal delivery but presented with severe adrenal insufficiency during the first few days after birth. They had normal female external genitalia and their biochemical and genetic evaluation was similar to the proband except that both of them have 46,XX karyotype (Fig. 1). Clinically, they appeared as normal females without ambiguous genitalia and normal development during their childhood. They have been treated with glucocorticoid and mineralocorticoid replacement therapy since the neonatal periods. The fourth sister had normal prenatal, postnatal and childhood periods and is healthy (III-4) (Table 1).

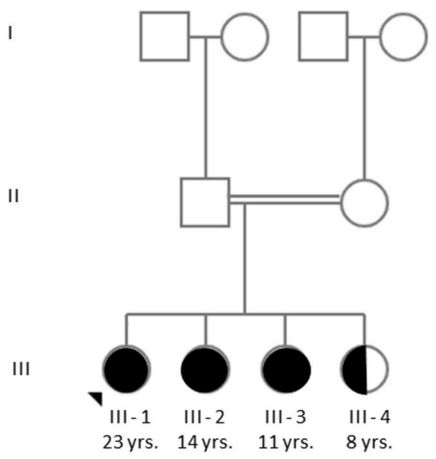
3.2. *Whole-exome sequencing and in-Silico analysis*

To identify the causative variant associated with the proband phenotype, WES and its bioinformatics analysis were performed for (III-1) as described previously [22]. We focused only on pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), and non-synonymous (NS) variants causing missense, nonsense, frame-shift, splice site variants (SS), coding insertions, or deletions (indel).

The bioinformatics analysis identified a single homozygous variant in exon 3 of the *CYP11A1* gene located on chr15(GRCh37): g.74637444, (c.566C>T, p.Ala189Val) that causes an amino acid change from Ala to Val at position 189, which was verified by Sanger sequencing (Fig. 2A).

This variant was listed in the ClinVar database as pathogenic or likely pathogenic (https://preview.ncbi.nlm.nih.gov/clinvar/variation/17518/). While it was reported in a mono-allelic form in a patient with a compound heterozygous *CYP11A1* variant, it has never been reported in a homozygous state nor was it observed in the homozygous state in the ExAC (http://exac.broadinstitute.

A)



B)

	III-1	III-2	III-3	III-4
Karyotype	46,XY	46,XX	46,XX	46,XX
Adrenal insufficiency	✓	✓	✓	_
Female genitalia	✓	✓	✓	✓
Uterus and fallopian tubes	_	✓	✓	✓
ovaries	_	✓	✓	✓
undescended atrophied testes	✓	_	_	_

Fig. 1. Figure 1. (A) Pedigree of the consanguineous family who had 3 affected daughters with severe adrenal insufficiency and complex sexual development. Pedigree generated using (https://www.progenygenetics.com); squares (males); circles (females); annotated symbols (affected individuals); open symbols (unaffected individuals); arrowheads (proband). **(B)** Summary of the clinical, cytogenetic and genital evaluation of the four siblings.

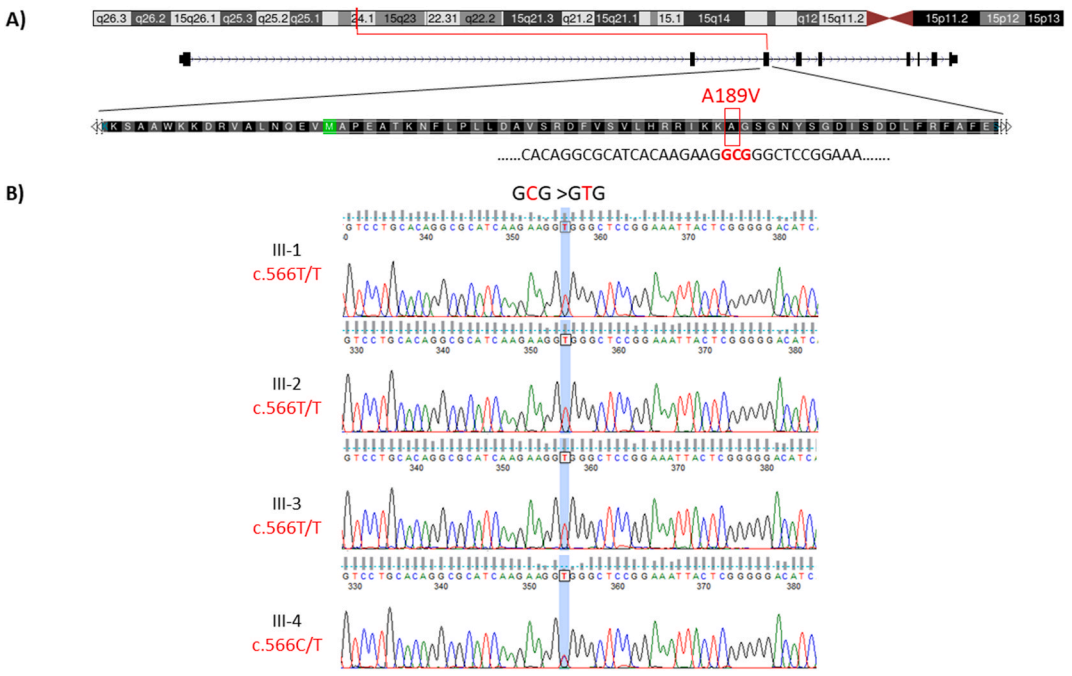


Fig. 2. Figure 2. Molecular characterization of the identified c.566C>T variant in *CYP11A1* gene. (A) Schematic diagram and annotation of the identified variant in the *CYP11A1* (c.566C>T, p.A189V is located on chromosome chromosome 15q24.1. The variant falls in the third exon. **(B)** Chromatogram Sanger sequencing segregation analysis of the proband (III-1), affected siblings (III-2 and III-3) and the healthy unaffected sister (III-4). Blue highlight represents the identified variant.

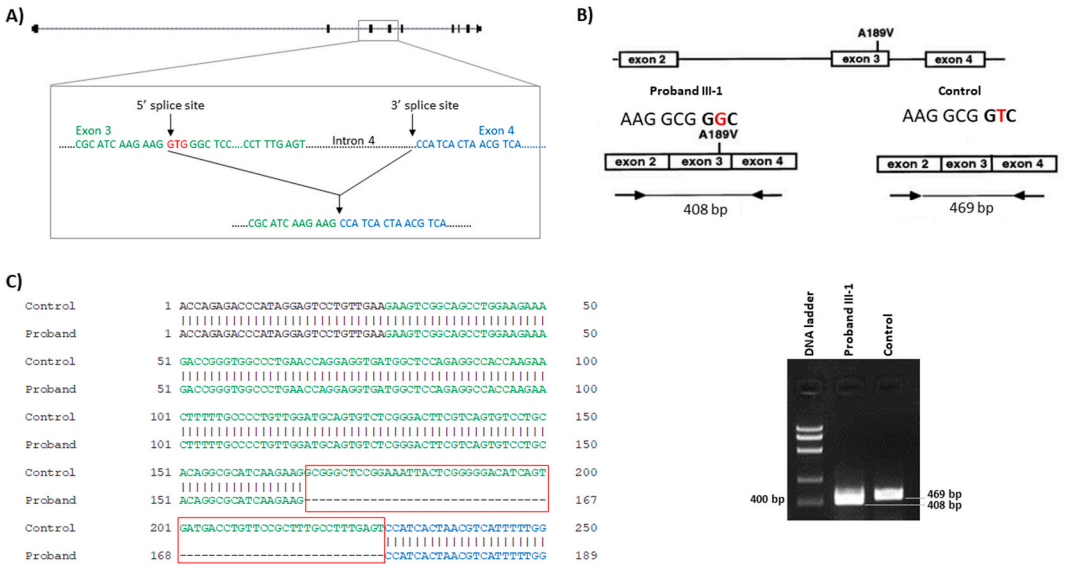


Fig. 3. Figure 3. Characterization of c.566C>T splicing variant in *CYP11A1* gene. (A) Schematic representation of the *CYP11A1* genomic region. Square represents the location of the identified variant. **(B)** Agarose gel (2%) demonstrating the results of the RT-PCR performed on the RNA extracted from the proband and an unrelated normal control, using primers located in exon 3 and exon 4. PCR amplification of the region around the variant showed a decrease in the size of the *CYP11A1* gene product in the proband (408 bp) compared the control sample (469 bp). **(C)** Alignment of the cDNA sequences of the proband and the control. The alignment file was generated using clustal Omega (EMBL-EBI) (https://www.ebi.ac.uk/Tools/msa/clustalo/).

org/), gnomAD (3.10e-6) (http://gnomad. broadinstitute.org/), 1000 Genomes Project (http://www.internationalgenome.org/), or in-house >18,000 exome database.

3.3. Sanger sequencing and characterization of the splice site variant

The *CYP11A1* variant c.566C>T was confirmed in the proband and her affected sisters by PCR and Sanger sequencing. The proband and her affected sisters were homozygous for the c.566C>T variant, whereas, it was heterozygous in the unaffected sister (Fig. 2B). This variant was reported in Ref. [13] (PMID: 12161514) and [30] (PMID: 34281122). It was predicted that the A189V variant creates a consensus splice-donor site sequence (Fig. 3A). To analyze the effect of the A189V variant in the *CYP11A1* gene on mRNA splicing, RT-PCR was performed. *CYP11A1* mRNA was inspected in both the affected proband (III -1), and unrelated control sample (Fig. 3B). The splice site for exons 2–4 was assessed from the cDNA sizes that correspond to the expressed mRNAs using RT-PCR. RT-PCR product revealed that the unaffected control sample yielded a 469-bp product; whereas the affected individual (III- 1) with the homozygous variant (c.566C>T) showed a 408-bp product (Fig. 3B). The cDNA sequencing analysis confirmed that A189V disrupted the canonical donor splice site resulting in an alternative splicing site that created a deletion of 61 nucleotides of exon 3 (Fig. 3C), which resulted in a reduction in the size of the CYP11A1 transcript in the proband's sample compared to the unrelated normal control. It was observed that codon 188 Lysine (K) was followed by Proline (P) in exon 4. Therefore, this variant is predicted to result in a frameshift in the reading frame, deleting 20 amino acids after Alanine (A) at position 189 leading to pre-mature termination codon in the mutant's transcript, and truncation at codon 205 as a result of aberrant RNA splicing (Fig. 4).

4. Discussion

In this report, we identified a missense variant in the *CYP11A1* gene associated with CAH. This was a homozygous missense variant c.566C>T, p.Ala189Val in exon 3 of *CYP11A1* in three affected siblings from a consanguinity family.

The identified c.566C>T variant is reported herein as a causal homozygous variant for the first time. All of the affected siblings presented during the early neonatal period with acute adrenal insufficiency and needed glucocorticoid and mineralocorticoid replacement. Although the oldest sister (III -1) had normal female external genitalia, she had a 46,XY karyotype, undescended testes and absent uterus and ovaries. The other affected sisters (III -2 and III -3) were 46,XX with normal external and internal genitalia. The variant found in the current family had been previously reported in a compound heterozygosity form with another variant in two unrelated patients; the first patient was diagnosed with CAH in late infancy with adrenal insufficiency, high ACTH levels, and hyperpigmentation [13]. In contrast to our patient, her cytogenetic evaluation revealed a 46,XX karyotype. The second reported patient presented with adrenal failure in the first year of life [30]. Her physical examination revealed normal external female genitalia, skin hyperpigmentation and extremely elevated levels of ACTH. The karyotype in this patient was 46,XY [30].

The mRNA analysis revealed that the biallelic homozygous p.Ala189Val variant detected in the proband (III -1) creates a new splice

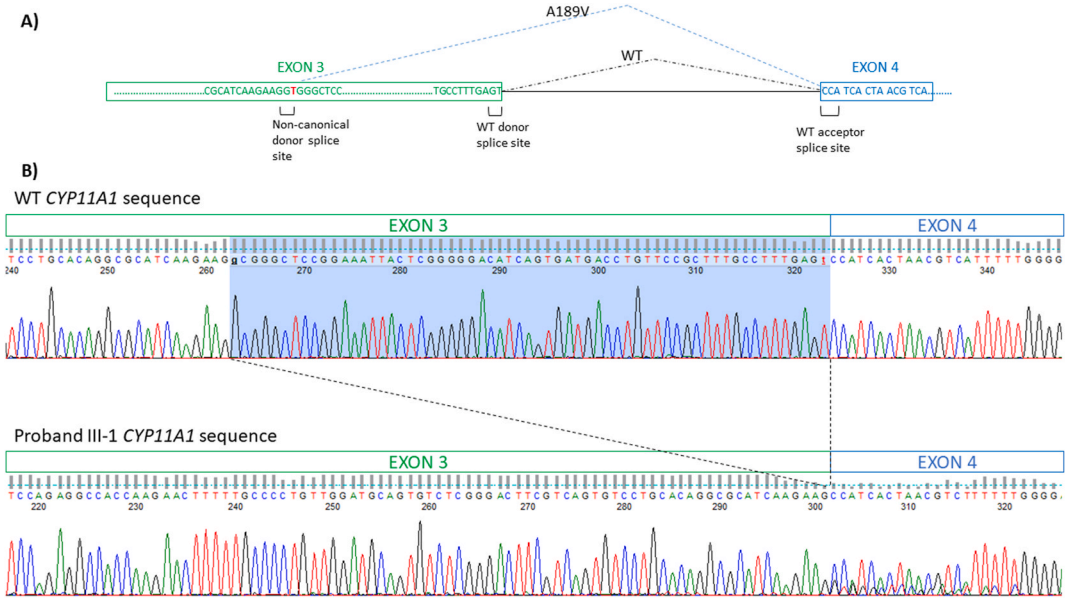


Fig. 4. Figure 4. Sanger sequencing of the wild-type control and proband's cDNA. (A) Schematic representation of the variant within exon 3 that creates a donor-splice site that leads to a defective transcript. Dashed lines above the gene structure represent different alternative splicing event. **(B)** Chromatograms sequence analysis showing a deletion of 61 bp on exon 3 (blue highlight) in the affected individual compared to the control.

site leading to a frameshift and an early premature termination codon downstream at codon 205, which would expectedly result in non-functional P450scc protein. The p.Ala189Val variant is located at exon 3; therefore, the truncated transcript lacks more than half of the wild-type transcript *CYP11A1* domains including the highly conserved heme-binding region of P450s. Similarly, Katsumata et al. identified this variant in the parental allele of *CYP11A1* as part of a compound heterozygous variant in trans with another point variant (p.R353W) that induces amino acid substitution in the maternal allele. The functional consequences and enzymatic activity of these variants were elucidated [13]. Katsumata et al. demonstrated that the mono-allelic p.Ala189Val heterozygous variant in *CYP11A1* gene had partially inactivated the P450scc activity by introducing a novel splice-donor site, which gave rise to two species of the transcripts; one transcript with a normal size and the other with a 61-nucleotide deletion of exon 3 downstream from codon 189 (Fig. 3). This deletion was predicted and validated to cause a shift in the open reading frame and create a premature termination at codon 205 [13]. Thus, the mono-allelic variant identified by Katsumata et al. did not completely abolish the enzymatic activity due to the expression of the other normal *CYP11A1* allele. Consequently, the patient was able to synthesize adrenal steroid hormones to some extent in the presence of elevated ACTH and plasma renin activity. Consistently, Matusik et al. identified the missense A189V variant, which was maternally inherited in one allele, as part of a compound heterozygous variant with another intronic splice site variant (c.1236+5G>A) in the second allele [30]. Although this study lacked functional work, it showed that the combination of defective splicing and p.Ala189Val variant would result in low levels of functional protein that partially abolishes the activity of the *CYP11A1* gene resulting in a minimum level of pregnenolone that is likely to be sufficient for gonadal steroidogenesis but insufficient for glucocorticoid production [30]. This likely explains the observed primary gonadal and adrenal failure in the proband of this study, who is homozygous for the exonic splice variant that doesn't completely abolish the enzymatic activity. Unlike the previous report in which this variant was mono-allelic, and splicing is likely to alternate between the canonical splice site and the new splice site, in our case, the variant is bi-allelic leading to complete or predominant replacement of the splicing from the canonical splice site to the new splice site created by the variant. This may explain the severity of the phenotype in our patient since the transcript is likely to be totally or mostly truncated.

Approximately 42 different genetic variants have been identified to date (Supplementary Table 1). A review of all variants identified in these previously reported cases shows that the majority of patients (47 out of 70) carried compound heterozygous variants. Whereas, 22 out of 70 patients had homozygous variants ([14][9,18,20,31–37]). Only one patient out of 70 had a missense heterozygous variant (c.809_814dup6) [15]. The most commonly detected variant was c.940G>A, p.E314K (45 %, n = 32), which has always been found in a compound heterozygosity form [33,38–42]. The c.835delA, p.Ile279TyrfsTer10 (17.1%, n = 12) has been found either as a bi-allelic homozygous or as compound heterozygous in trans with another variant [14,17,19,40,41]. Followed by the c.1351C > T, p. p.R451W (15.7 %, *n* = 11), which has always been reported as a homozygous variant [20,34].

Even though the correlation between the clinical findings and the enzymology of the P450scc mutations identified in these reported patients was imprecise, some of the mutations cause a complete loss of enzyme function, while others retain partial activity. The data from the reported patients with P450scc deficiency show how patients manifesting a heterozygous variant (missense or compound or frameshift) might partially lose CYP11A1 activity and hence would be predicted to have about 50 % of activity (from the normal allele that conferred some activity). The retained substantial P450scc activity has been reported in children with very late-onset adrenal insufficiency, hypospadias or glucocorticoid insufficiency [19,20]. Similarly, findings have shown that the partial dysfunction of the steroidogenic acute regulatory protein (STAR) has been associated with predominant glucocorticoid insufficiency (Baker et al., 2006). By contrast, patients with homozygous frameshift variants such as c835delA, have been predicted to cause the premature stop codon L288X; devoid of detectable P450scc enzymatic activity [14,17,19,42]. Patients with homozygous *CYP11A1* variant have been reported to have the most severe clinical course, which can be either female-typical external genitalia in 46,XY or a lack of puberty in 46, XX girls as they cannot produce any steroids [13–17,31,32,34,38]. This observation was consistent in the biallelic *CYP11A1* variant with the lack of detectable enzymatic activity using in vitro assay [18]. Consistently, it has been found that complete disruption of StAR causes congenital lipoid adrenal hyperplasia (CLAH) affecting both the adrenal and sex hormones (Metherell et al., 2009, Bakkar et al., 2023).

In summary, our analysis identifies a homozygous variant in *CYP11A1* in three siblings. This variant disrupts the function of *CYP11A1* by creating a new splicing site within exon 3 leading to frameshift and premature truncation of the gene.

Disclosure Summary

The authors declare that there is no conflict of interest related to the work included in this manuscript.

Funding statement

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Data availability statement

Data will be provided by the corresponding author upon potential requests.

CRediT authorship contribution statement

Kheloud M. Alhamoudi: Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Meshael Alswailem:**

Investigation, Methodology. **Balgees Alghamdi:** Project administration. **Abdullah Alashwal:** Data curation. **Ali S. Alzahrani:** Data curation, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35058.

References

[1] W.L. Miller, Molecular biology of steroid hormone synthesis, Endocr. Rev. 9 (1988) 295–318.
[2] W.L. Miller, R. Auchus, The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders, Endocr. Rev. 32 (2011).
[3] P.C. White, K.M. Curnow, L. Pascoe, Disorders of steroid 11 beta-hydroxylase isozymes, Endocr. Rev. 15 (1994) 421–438.
[4] B.C. Chung, K.J. Matteson, R. Voutilainen, T.K. Mohandas, W.L. Miller, Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta, Proc Natl Acad Sci U S A 83 (1986) 8962–8966.
[5] J. Simard, M.L. Ricketts, A.M. Moisan, V. Tardy, M. Peter, G. Van Vliet, Y. Morel, A new insight into the molecular basis of 3beta-hydroxysteroid dehydrogenase deficiency, Endocr. Res. 26 (2000) 761–770.
[6] T. Yanase, E.R. Simpson, M.R. Waterman, 17 alpha-hydroxylase/17,20-lyase deficiency: from clinical investigation to molecular definition, Endocr. Rev. 12 (1991) 91–108.
[7] D.M. Stocco, B.J. Clark, Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis, Biochem. Pharmacol. 51 (1996) 197–205.
[8] P.C. White, P.W. Speiser, Congenital adrenal hyperplasia due to 21-hydroxylase deficiency, Endocr. Rev. 21 (2000) 245–291.
[9] K. Morohashi, K. Sogawa, T. Omura, Y. Fujii-Kuriyama, Gene structure of human cytochrome P-450(SCC), cholesterol desmolase, J. Biochem. 101 (1987) 879–887.
[10] D. Lin, T. Sugawara, J.F.3R.D. Strauss, B.J. Clark, D.M. Stocco, P. Saenger, A. Rogol, W.L. Miller, Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis, Science 267 (1995) 1828–1831.
[11] D.M. Stocco, B.J. Clark, Regulation of the acute production of steroids in steroidogenic cells, Endocr. Rev. 17 (1996) 221–244.
[12] N. Strushkevich, F. Mackenzie, T. Cherkesova, I. Grabovec, S. Usanov, H.W. Park, Structural basis for pregnenolone biosynthesis by the mitochondrial monooxygenase system, Proc Natl Acad Sci U S A 108 (2011) 10139–10143.
[13] N. Katsumata, M. Ohtake, T. Hojo, E. Ogawa, T. Hara, N. Sato, T. Tanaka, Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans, J. Clin. Endocrinol. Metab. 87 (2002) 3808–3813.
[14] O. Hiort, P.M. Holterhus, R. Werner, C. Marschke, U. Hoppe, C.J. Partsch, F.G. Riepe, J.C. Achermann, D. Struve, Homozygous disruption of P450 side-chain cleavage (CYP11A1) is associated with prematurity, complete 46,XY sex reversal, and severe adrenal failure, J. Clin. Endocrinol. Metab. 90 (2005) 538–541.
[15] T. Tajima, K. Fujieda, N. Kouda, J. Nakae, W.L. Miller, Heterozygous mutation in the cholesterol side chain cleavage enzyme (p450scc) gene in a patient with 46, XY sex reversal and adrenal insufficiency, J. Clin. Endocrinol. Metab. 86 (2001) 3820–3825.
[16] H. Al Kandari, N. Katsumata, S. Alexander, M.A. Rasoul, Homozygous mutation of P450 side-chain cleavage enzyme gene (CYP11A1) in 46, XY patient with adrenal insufficiency, complete sex reversal, and agenesis of corpus callosum, J. Clin. Endocrinol. Metab. 91 (2006) 2821–2826.
[17] C.J. Kim, L. Lin, N. Huang, C.A. Quigley, T.W. Avruskin, J.C. Achermann, W.L. Miller, Severe combined adrenal and gonadal deficiency caused by novel mutations in the cholesterol side chain cleavage enzyme, P450scc, J. Clin. Endocrinol. Metab. 93 (2008) 696–702.
[18] P. Rubtsov, M. Karmanov, P. Sverdlova, P. Spirin, A. Tiulpakov, A novel homozygous mutation in CYP11A1 gene is associated with late-onset adrenal insufficiency and hypospadias in a 46,XY patient, J. Clin. Endocrinol. Metab. 94 (2009) 936–939.
[19] T. Sahakitrungruang, M.K. Tee, P.R. Blackett, W.L. Miller, Partial defect in the cholesterol side-chain cleavage enzyme P450scc (CYP11A1) resembling nonclassic congenital lipoid adrenal hyperplasia, J. Clin. Endocrinol. Metab. 96 (2011) 792–798.
[20] S. Parajes, C. Kamrath, I.T. Rose, A.E. Taylor, C.F. Mooij, V. Dhir, J. Grötzinger, W. Arlt, N. Krone, A novel entity of clinically isolated adrenal insufficiency caused by a partially inactivating mutation of the gene encoding for P450 side chain cleavage enzyme (CYP11A1), J. Clin. Endocrinol. Metab. 96 (2011) E1798–E1806.
[21] B.T. Le, C.M. Duong, T.Q. Nguyen, C.M. Nguyen, Two siblings with non-classic P450scc deficiency resulted from a novel mutation in CYP11A1 gene misdiagnosed as familial glucocorticoid deficiency, BMJ Case Rep. 15 (2022).
[22] K.M. Alhamoudi, B. Alghamdi, A. Aljomaiah, M. Alswailem, H. Al-Hindi, A.S. Alzahrani, Case report: severe gonadal dysgenesis causing 46,XY disorder of sex development due to a novel NR5A1 variant, Front. Genet. 13 (2022).
[23] M. Alswailem, A. Alsagheir, B.B. Abbas, O. Alzahrani, A.S. Alzahrani, Molecular genetics of disorders of sex development in a highly consanguineous population, J. Steroid Biochem. Mol. Biol. 208 (2021) 105736.
[24] I. Adzhubei, D.M. Jordan, S.R. Sunyaev, Predicting functional effect of human missense mutations using PolyPhen-2, Curr Protoc Hum Genet, Chapter 7 (2013), https://doi.org/10.1002/0471142905.hg0720s76. Unit7.20. 2013 Jan.
[25] N.L. Sim, P. Kumar, J. Hu, S. Henikoff, G. Schneider, P.C. Ng, SIFT web server: predicting effects of amino acid substitutions on proteins, Nucleic Acids Res. 40 (2012) W452–W457.
[26] C. Kopanos, V. Tsiolkas, A. Kouris, C.E. Chapple, M. Albarca Aguilera, R. Meyer, A. Massouras, VarSome: the human genomic variant search engine, Bioinformatics 35 (2019) 1978–1980.
[27] J.M. Schwarz, C. Rödelsperger, M. Schuelke, D. Seelow, MutationTaster evaluates disease-causing potential of sequence alterations, Nat. Methods 7 (2010) 575–576.
[28] Y. Choi, A.P. Chan, PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels, Bioinformatics 31 (2015) 2745–2747.
[29] M.F. Rogers, H.A. Shihab, M. Mort, D.N. Cooper, T.R. Gaunt, C. Campbell, FATHMM-XF: accurate prediction of pathogenic point mutations via extended features, Bioinformatics 34 (2018) 511–513.
[30] P. Matusik, A. Gach, O. Zajdel-Cwynar, I. Pinkier, G. Kudela, A. Gawlik, A novel intronic splice-site mutation of the CYP11A1 gene linked to adrenal insufficiency with 46,XY disorder of sex development, Int J Environ Res Public Health 18 (2021).

[31] S. Parajes, A.O. Chan, W.M. But, I.T. Rose, A.E. Taylor, V. Dhir, W. Arlt, N. Krone, Delayed diagnosis of adrenal insufficiency in a patient with severe penoscrotal hypospadias due to two novel P450 side-change cleavage enzyme (CYP11A1) mutations (p.R360W; p.R405X), Eur. J. Endocrinol. 167 (2012) 881–885.
[32] M.K. Tee, M. Abramsohn, N. Loewenthal, M. Harris, S. Siwach, A. Kaplinsky, B. Markus, O. Birk, V.C. Sheffield, R. Parvari, E. Hershkovitz, W.L. Miller, Varied clinical presentations of seven patients with mutations in CYP11A1 encoding the cholesterol side-chain cleavage enzyme, P450scc, J. Clin. Endocrinol. Metab. 98 (2013) 713–720.
[33] L.F. Chan, D.C. Campbell, T.V. Novoselova, A.J. Clark, L.A. Metherell, Whole-exome sequencing in the differential diagnosis of primary adrenal insufficiency in children, Front. Endocrinol. 6 (2015) 113.
[34] T. Guran, F. Buonocore, N. Saka, M.N. Ozbek, Z. Aycan, A. Bereket, F. Bas, S. Darcan, A. Bideci, A. Guven, K. Demir, A. Akinci, M. Buyukinan, B.K. Aydin, S. Turan, S.Y. Agladioglu, Z. Atay, Z.Y. Abali, O. Tarim, G. Catli, B. Yuksel, T. Akcay, M. Yildiz, S. Ozen, E. Doger, H. Demirbilek, A. Ucar, E. Isik, B. Ozhan, S. Bolu, I.T. Ozgen, J.P. Suntharalingham, J.C. Achermann, Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort, J. Clin. Endocrinol. Metab. 101 (2016) 284–292.
[35] J. Kaur, A.M. Rice, E. O'Connor, A. Piya, B. Buckler, H.S. Bose, Novel SCC mutation in a patient of Mexican descent with sex reversal, salt-losing crisis and adrenal failure, Endocrinol Diabetes Metab Case Rep 2016 (2016).
[36] A. Kim, M. Fujimoto, V. Hwa, P. Backeljauw, A. Dauber, Adrenal insufficiency, sex reversal, and angelman syndrome due to uniparental disomy unmasking a mutation in CYP11A1, Horm. Res. Paediatr. 89 (2018) 205–210.
[37] O. Kara, O. Gorukmez, A. Ekici, F. Celik, A novel homozygous mutation in CYP11A1 gene is associated with severe adrenal insufficiency in 46, XX patient, Fetal Pediatr. Pathol. 40 (2021) 518–522.
[38] M. Lara-Velazquez, A. Perdomo-Pantoja, P.R. Blackburn, J.M. Gass, T.R. Caulfield, P.S. Atwal, A novel splice site variant in CYP11A1 in trans with the p.E314K variant in a male patient with congenital adrenal insufficiency, Mol Genet Genomic Med 5 (2017) 781–787.
[39] C. Goursaud, D. Mallet, A. Janin, R. Menassa, V. Tardy-Guidollet, G. Russo, A. Lienhardt-Roussie, C. Lecointre, I. Plotton, Y. Morel, F. Roucher-Boulez, Aberrant splicing is the pathogenicity mechanism of the p.Glu314Lys variant in CYP11A1 gene, Front. Endocrinol. 9 (2018) 491.
[40] W. Kallali, E. Gray, M.Z. Mehdi, R. Lindsay, L.A. Metherell, F. Buonocore, J.P. Suntharalingham, J.C. Achermann, M. Donaldson, Long-term outcome of partial P450 side-chain cleavage enzyme deficiency in three brothers: the importance of early diagnosis, Eur. J. Endocrinol. 182 (2020) K15–k24.
[41] A. Maharaj, F. Buonocore, E. Meimaridou, G. Ruiz-Babot, L. Guasti, H.M. Peng, C.P. Capper, N. Burgos-Tirado, R. Prasad, C.R. Hughes, A. Maudhoo, E. Crowne, T.D. Cheetham, C.E. Brain, J.P. Suntharalingham, N. Striglioni, B. Yuksel, F. Gurbuz, S. Gupta, R. Lindsay, R. Couch, H.A. Spoudeas, T. Guran, S. Johnson, D. J. Fowler, L.S. Conwell, A.M. Mcinerney-Leo, D. Drui, B. Cariou, J.P. Lopez-Siguero, M. Harris, E.L. Duncan, P.C. Hindmarsh, R.J. Auchus, M.D. Donaldson, J. C. Achermann, L.A. Metherell, Predicted benign and synonymous variants in CYP11A1 cause primary adrenal insufficiency through missplicing, J Endocr Soc 3 (2019) 201–221.
[42] V. Kolli, H. Kim, A. Torky, Q. Lao, C. Tatsi, A. Mallappa, D.P. Merke, Characterization of the CYP11A1 nonsynonymous variant p.E314K in children presenting with adrenal insufficiency, J. Clin. Endocrinol. Metab. 104 (2019) 269–276.