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Case report

Clinical Presentation and Genetic Analysis of Neonatal 11β-Hydroxylase Deficiency Induced by a Chimeric *CYP11B2/CYP11B1* Gene

Cai W et al. Chimeric Gene in Neonatal 11β-Hydroxylase Deficiency

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What is already known on this topic?

1. 11β-Hydroxylase deficiency (11β-OHD) is an autosomal recessive disorder caused by genetic variations in the *CYP11B1* gene.
2. Most cases of 11β-OHD are caused by single nucleotide variations or small insertions/deletions in the *CYP11B1* gene, but cases resulting from chimeric *CYP11B2/CYP11B1* genes are rare.

What this study adds?

1. This study presents a rare case of neonatal 11β-hydroxylase deficiency (11β-OHD) induced by a chimeric *CYP11B2/CYP11B1* gene.
2. It emphasizes the importance of considering gene fusion variants in the diagnosis of 11β-OHD, particularly in neonatal and early infantile cases.

Abstract

In terms of prevalence, 11β-hydroxylase deficiency (11β-OHD), a common form of congenital adrenal hyperplasia, closely follows 21-hydroxylase deficiency. 11β-OHD has been attributed to diminished enzymatic activity owing to *CYP11B1* gene variants, mainly encompassing single nucleotide variations and insertions-deletions. The involvement of chimeric *CYP11B2/CYP11B1* genes in 11β-OHD has been rarely reported. We conducted a genetic investigation on a male infant with generalized pigmentation and abnormal steroid hormone levels. Whole-exome sequencing revealed a heterozygous variant in *CYP11B1* inherited from the mother (NM_000497.4: c.1391_1393dup [p.Leu464dup]). Long-range polymerase chain reaction revealed an additional allele, a chimeric *CYP11B2/CYP11B1* gene, inherited from the father. The current case report emphasizes the need to consider the occurrence of gene fusion variants in the diagnosis of neonatal or early infantile 11β-OHD.

Keywords: 11β-hydroxylase deficiency, 11β-OHD, CYP11B1, chimeric gene

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Introduction

11β-Hydroxylase deficiency (11β-OHD) is an autosomal recessive hereditary disorder caused by genetic variations in the *CYP11B1* gene, accounting for approximately 5–8% of congenital adrenal hyperplasia (CAH) cases (1). After 21-hydroxylase deficiency, 11β-OHD is the second leading cause of CAH (2). The primary features of 11β-OHD include reduced synthesis of metabolic end products and accumulation of precursor substances. Patients commonly exhibit symptoms such as low-renin hypertension, hypokalemia, masculinized early puberty due to elevated androgen levels, and pseudohermaphroditism in female patients (3).

11β-Hydroxylase belongs to the cytochrome P450 enzyme family, with two isoforms encoded by *CYP11B1* and *CYP11B2* in humans. Given the high sequence homology between *CYP11B1* and *CYP11B2*, with approximately 95 and 97% identity in their coding and non-coding regions, respectively, there is a risk of recombination and fusion during mitosis (4,5). The chimeric *CYP11B2*/*CYP11B1* gene has been associated with familial hyperaldosteronism type I (HALD1, OMIM#103900), also known as glucocorticoid-remediable aldosteronism, predominantly characterized by clinical features of hypertension and hypokalemia (6). However, cases of 11β-OHD resulting from the chimeric *CYP11B2*/*CYP11B1* gene remain rare. Typically, patients with 11β-OHD exhibit single nucleotide variations (SNVs) and insertions-deletions (indels) in *CYP11B1*. The primary variant types are missense and loss-of-function variants, both of which can lead to reduced 11β-hydroxylase enzyme activity and disease onset (7,8).

Herein, we report an intriguing case of an infant who presented with widespread skin pigmentation shortly after birth, particularly prominent around the penis and nipples. Genetic testing revealed a frameshift variant in one allele of *CYP11B1*, with the other allele harboring a chimeric *CYP11B2*/*CYP11B1* gene. This case report suggests that the possibility of the chimeric *CYP11B2*/*CYP11B1* gene should be considered as the underlying cause of CAH when clinically suspected.

Materials and methods

Ethical compliance

The patient was from a non-consanguineous family. Informed consent was obtained from the infant's parents, and all research procedures were approved by the Medical Ethics Committee of the Anhui Children's Hospital (ID: EYLL-2018-020).

Whole-exome sequencing (WES) and Sanger sequencing

Genomic DNA was extracted from blood samples using a blood DNA extraction kit (TianGen, Beijing, China). DNA quality was assessed for optimal 260/280 ratios (1.6–2.0) and a total yield exceeding 1 μg. Targeted exonic sequences were captured using the xGen Exome Research Panel (Integrated DNA Technologies, Rockville, MD, USA), followed by high-throughput sequencing on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Raw sequencing data underwent quality control, alignment to the human genome reference build 19 via BWA software, and identification of SNVs and indels using the Genome Analysis Toolkit. Suspected variants were assessed for population frequency in gnomAD_ALL/EAS, with their pathogenicity predicted using various bioinformatics algorithms (SIFT, Polyphen2, MutationTaster). Variant pathogenicity was assessed following American College of Medical Genetics and Genomics (ACMG) guidelines. Suspected variants were validated by designing primers from Ensembl data (Table 1) and conducting Sanger sequencing on an ABI 3500XL Genetic Analyzer (Applied Biosystems, CA, USA).

Long-range polymerase chain reaction (L-PCR) and fusion variant verification

L-PCR was used to detect fusion genes in the patient and his parents, with normal samples serving as negative controls. The LA Taq Hot-Start Version kit (#RR042B, TaKaRa, Kusatsu, Japan) was used for PCR. Gel electrophoresis bands were examined to ascertain the formation of the chimeric *CYP11B2*/*CYP11B1* gene resulting from homologous recombination. Each reaction contained 100 ng of genomic DNA. Following PCR, the products were subjected to 1% agarose gel electrophoresis. Analysis was conducted on a gel imaging system (#1600; Tanon, Shanghai, China).

Validation was performed using a human *CYP11B1*/*CYP11B2* gene detection kit (WeHealth BioMedical, Shanghai, China).

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Initially, target libraries of *CYP11B1* and *CYP11B2* gene fragments were constructed from sample DNA. Following successful library quality assessment, we used the NovaSeq 6000 sequencing platform (Illumina). Subsequently, the relative depth of the differential sites on *CYP11B1* and *CYP11B2* was computed to discern *CYP11B1*/*CYP11B2* gene fusion outcomes.

Results

Case report

This case report involves a 2-month-old male infant (46, XY karyotype). He was the firstborn of his mother and was delivered vaginally at 37^{+2} weeks of gestation, with a birth weight of 2900 g and an unremarkable pregnancy history. Shortly after birth, the infant developed generalized skin pigmentation, prominent around the areola and penis. His blood pressure was 104/67 mmHg (reference values for individuals under 3 years of age not exceeding 100/60 mmHg). Auxiliary examinations revealed an elevated aldosterone level of 175.90 pg/mL (reference range: 12.00–170.80) and higher-than-normal levels of both supine angiotensin and renin activity. Measurement of steroid hormones revealed elevated levels of 11-deoxycortisol, dehydroepiandrosterone, and androstenedione, which exceeded reference ranges (Table 2). The patient's 24-year-old father presented with facial hemiparesis and a history of hypertension during adolescence, albeit without treatment. At the time of examination, his blood pressure was 165/110 mmHg, without additional auxiliary investigations. The patient's mother did not exhibit notable anomalies. The couple denied a consanguineous relationship.

WES results

A total of 54.9 million clean reads were obtained in the WES testing, with an average sequencing depth of 127.2X. The average coverage of target regions with a depth greater than 20X was 99.20%. Based on the WES analysis results, the patient had a heterozygous variation in *CYP11B1*; this variant was identified as NM_000497.4: c.1391_1393dup (p.Leu464dup). The presence of this variant in the patient's mother was confirmed using Sanger sequencing (Figure 1). The distribution frequency of this variant in gnomAD_ALL/EAS was 0.000007/0, indicating an extremely low occurrence rate. Moreover, the identified variant site has been previously reported to be deleterious to enzymatic activity (10,11). Following the ACMG guidelines, this variant was classified as likely pathogenic based on the following criteria: PM3 + PS3 + PM2 + PP4. Within the target sequence, we did not identify any SNV/Indel variations on the other allele of *CYP11B1*. Additionally, we did not identify any pathogenic variants in other genes associated with adrenal insufficiency.

***CYP11B1* and *CYP11B2* gene fusion variant**

The L-PCR results unequivocally confirmed the occurrence of a chimeric *CYP11B2*/*CYP11B1* gene through homologous recombination between *CYP11B1* and *CYP11B2*, constituting a paternally inherited pathogenic variation. Using the *CYP11B1*/*CYP11B2* gene detection kit, we performed a copy number analysis of the *CYP11B2*/*CYP11B1* genes within the sample DNA. Accordingly, we found that the proband exhibited a relative copy number of 1 for exons 1–6 of *CYP11B1*, indicative of a heterozygous deletion. Similarly, a relative copy number of 1 was observed for exons 7–9 of *CYP11B2*, indicating a heterozygous deletion. The proband's father exhibited the same copy number of deletion variants. Conversely, the mother's copy numbers of both *CYP11B1* and *CYP11B2* were within normal limits (Figure 2).

4 Discussion

Herein, we report the case of a neonate with 11β-OHD, primarily presenting with genital skin pigmentation and hypertension phenotypes. WES analysis revealed only one heterozygous variation in *CYP11B1* inherited from the mother, identified as c.1391_1393dup (p.Leu464dup). The other allele originated from the father and constituted a chimeric *CYP11B2*/*CYP11B1* gene, resulting in a compound heterozygous variation in *CYP11B1*. The diagnosis of 11β-OHD was established based on the patient's clinical presentation. Although routine next-generation sequencing can be effectively employed to identify SNVs/indels in the field of pediatric genetics, the detection of copy number variations or fusion variants in *CYP11B1* and *CYP11B2* presents a substantial challenge (12).

Distinct approaches have been employed to recognize chimeric *CYP11B2*/*CYP11B1* genes differently. Methods include customized probes for multiplex ligation-dependent probe amplification by Menabò et al., specific real-time PCR by MacKenzie et al., and optimized targeted sequencing algorithms by Xie et al., all aimed at enhancing the efficiency of chimeric

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CYP11B2/*CYP11B1* gene identification (11,13,14). However, these methods lack broad applicability. In the current case, the causative variant was identified using L-PCR and a dedicated kit. This could be partly attributed to the precise phenotypic assessment of the patient in a clinical setting. Given the strong clinical suspicion of CAH and recognition of a high sequence homology between *CYP11B1* and *CYP11B2*, additional verification of the chimeric *CYP11B2*/*CYP11B1* gene was performed, thereby establishing the patient's etiological diagnosis.

CYP11B1 variations are complex, with over 150 variants documented in the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/). These variants include missense, nonsense, splicing, and small indel variants. However, reports on chimeric *CYP11B2*/*CYP11B1* genes are relatively scarce. To date, only 11 cases of this variation have been reported (4,5,11,15–18), with the *CYP11B1* exon 7–9/*CYP11B2* exon 1–6 configuration commonly documented (4,5,11,15–18). Notably, Xie et al. have identified four unrelated Chinese patients who shared the same breakpoint (*CYP11B2* g.9559–9742) in their chimeric *CYP11B2*/*CYP11B1* genes, thereby suggesting a potential founder effect in the Chinese population, with a possible frequency of 1 in 10,000 (11). Accordingly, the distribution frequency of the chimeric *CYP11B2*/*CYP11B1* gene in the Chinese population might be higher than that predicted. Nevertheless, chimeric *CYP11B2*/*CYP11B1* genes remain underreported, possibly reflecting an oversight in their verification.

CYP11B1 is located in the chromosomal region 8q22 and comprises nine exons, spanning a length of 6.03 kb and encoding 503 amino acids. Within the zona fasciculata of the adrenal cortex, 11β-hydroxylase, encoded by *CYP11B1*, converts 11-deoxycortisol and 11-deoxycorticosterone into cortisol and corticosterone, respectively (1). In patients with 11β-OHD, reduced cortisol levels lead to feedback elevation of adrenocorticotropic hormone levels, resulting in increased adrenal androgen production; this can manifest as peripheral precocious puberty in males and virilization in females. Additionally, enhanced synthesis of corticosterone, a weak mineralocorticoid, can induce clinical manifestations such as hypokalemia and hypertension (19). Classical phenotypes frequently include virilization in females and enlarged genitalia in males. However, genital and hypertensive features might not be prominent in several infantile patients, potentially resulting in a misdiagnosis (20). The current case report revealed certain clinical indications, primarily suggesting a potential disease risk related to pigmentation surrounding the infant's genitalia. Notably, the patient's aldosterone and renin levels were slightly above the upper limits of the reference range, which may be related to varying degrees of elevated levels in newborns or during the early stages of infants (21,22). Therefore, for patients in the early stages of life, an increased awareness of molecular diagnostics is crucial, emphasizing the importance of selecting an appropriate molecular diagnostic approach.

Patients with 11β-OHD mainly require lifelong glucocorticoid replacement therapy. Importantly, early and accurate diagnosis, followed by glucocorticoid treatment, can prevent an Addisonian crisis and hypertension symptoms (23). Dexamethasone therapy should be avoided in the neonatal or pediatric period despite its stronger sodium-retaining properties than those of hydrocortisone. Dexamethasone potently suppresses the pituitary-adrenal axis, which can lead to growth retardation in neonates and children (24). In affected children, low-dose hydrocortisone could help achieve biochemical control within the normal range. During long-term treatment, combining low-dose hydrocortisone with gonadotropin-releasing hormone agonists and growth hormone may be considered to optimize final adult height recovery (25,26). However, the intricate nature of 11β-OHD demands a refinement of standard treatment protocols for pediatric patients to mitigate potential glucocorticoid-related morbidity.

In conclusion, we report the case of a male infant who exhibited generalized pigmentation centered on the genitals and nipples since birth. Using WES, we initially identified a heterozygous variation in *CYP11B1* inherited from the mother, subsequently verifying the presence of a chimeric *CYP11B2*/*CYP11B1* gene originating from the father. The current case report highlights the potential for atypical presentation and misdiagnosis of infantile 11β-OHD, underscoring the critical role of molecular diagnosis in such cases.

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Declaration of interest

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None.

Author contributions

Wenjuan Cai and Dan Yu: Conceptualization, Formal analysis, Methodology, Original draft; Jian Gao, Qian Deng, and Huihui Lin: Investigation, Data curation, Software, Visualization; Yuqing Chen: Funding acquisition, Supervision, Review.

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Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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TABLE 1. Primer sequences for Sanger sequencing

Variant*	Primers
Forward	5'-CTCTACTCTCTGGGTCGCAACC-3'
Reverse	5'-CAGGACCTACACAGCCTCAACC-3'

**CYP11B1* NM_000497.4: exon8:c.1391_1393dup

TABLE 2. Auxiliary examination data of the proband

Parameter	Measurement	Reference
Aldosterone	175.90 pg/mL	12.00–170.80 pg/mL
Angiotensin I	6.47 ng/mL	11.00–88.00 ng/L
Angiotensin II (recumbent)	72.40 pg/mL	25.00–60.00 pg/mL
Renin activity (supine)	5.03 ng/(mL*h)	0.15–2.33 ng/(mL*h)
11-Deoxycorticosterone	3.164 ng/mL	0.070–0.570 ng/mL
Dehydroepiandrosterone	6.020 ng/mL	<2.900 ng/mL
Androstenedione	1.937 ng/mL	0.060–0.780 ng/mL
Serum potassium	5.80 mmol/L	3.50–5.50 mmol/L
Serum sodium	137.6 mmol/L	135.0–145.0 mmol/L

Figure 1. Sanger sequencing results showing a heterozygous variation in the patient's *CYP11B1* (NM_000497.4: c.1391_1393dup [p.Leu464dup]), which was inherited from his mother

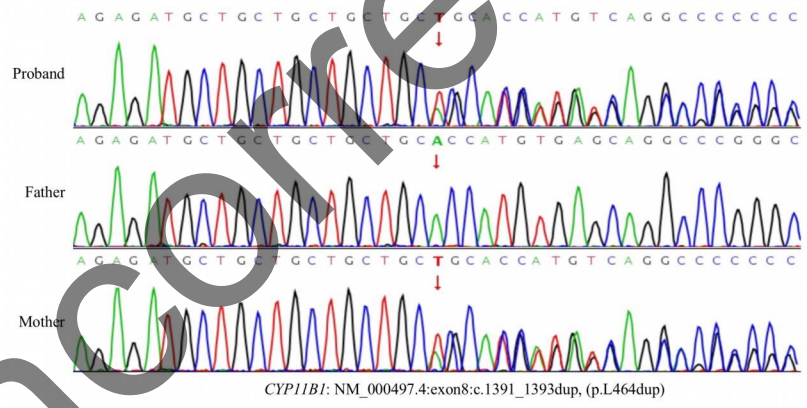

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Figure 2. Validation of the chimeric *CYP11B2*/*CYP11B1* gene. A. L-PCR results showing homologous recombination between *CYP11B1* and *CYP11B2* in the patient and his father, generating a chimeric *CYP11B2*/*CYP11B1* gene (indicated by red arrows). B. The patient exhibits heterozygous deletions in exons 1–6 of *CYP11B1* (blue dots) and in exons 7–9 of *CYP11B2* (blue dots). The patient's father carries the same copy number variation as the patient, while the mother shows no variations. Dashed lines represent the upper (1.3) and lower (0.7) limits of normal copy numbers. Copy numbers near 1 denote normal copy numbers (gray dots), while copy numbers near 0.5 indicate heterozygous deletion variations (blue dots). C. Schematic representation of the chimeric *CYP11B2*/*CYP11B1* gene occurrence in the patient

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