Case Report

Dual Heterozygous Mutations in CYP21A2 and CYP11B1 in a Case of Nonclassic Congenital Adrenal Hyperplasia

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Background/Objective: Nonclassic congenital adrenal hyperplasia (NCCAH) may be overlooked or mistaken for polycystic ovarian syndrome. Unlike congenital adrenal hyperplasia (CAH), the enzymatic activities of 21-hydroxylase or 11β-hydroxylase in NCCAH are not completely lost. In this case, NCCAH presented in a patient with CYP21A2 and CYP11B1 heterozygous mutations, one of which is a variant of unknown significance in CYP11B1.

Case Report: A 30-year-old woman presented with a chief complaint of irregular menses and hirsutism. Previous medical history was significant for a prolactin level of 34.7 ng/mL (reference range, 2.0-23.0 ng/mL), a total serum testosterone level of 77 ng/dL (reference range, 25-125 ng/dL, not sex-specific), and a 2-mm x 3-mm pituitary lesion. An adrenocorticotropic hormone stimulation test increased the 17-hydroxyprogesterone level from 444 ng/dL at baseline to 837 ng/dL at 60 minutes (baseline female reference range and stimulated reference ranges are 10-300 ng/dL and <1000 ng/dL, respectively). Gene sequencing revealed a heterozygous pathogenic CYP21A2 variant and a heterozygous, previously undescribed variant of unknown significance in CYP11B1.

Discussion: Unlike CAH, NCCAH presents more subtly and later in life, and salt wasting and hypertension are not typically seen. Although mutations in CYP11B1 that cause steroid 11β-hydroxylase deficiency more commonly lead to the CAH phenotype, cases have been reported of CYP11B1 mutations leading to NCCAH, depending on the location of the mutations.

Conclusion: This patient’s case demonstrates physical examination and laboratory findings suggestive of NCCAH. Our case adds to the database of described mutations in CYP11B1 and suggests that heterozygous mutations in 2 different genes may present phenotypically as NCCAH.

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Introduction

Congenital adrenal hyperplasia (CAH) is a condition caused by various autosomal recessive mutations that lead to enzymatic dysfunction in steroidogenesis. It is classically caused by a defective steroid 21-hydroxylase enzyme from a mutation that produces a classic constellation of symptoms in the neonatal period, including genital virilization, hyponatremia, hyperkalemia, and adrenal insufficiency or crisis.1 A more common but often overlooked constellation of symptoms can occur, known as nonclassic congenital adrenal hyperplasia (NCCAH), which often presents later in childhood, adolescence, or adulthood. It is characterized by precocious puberty, menstrual irregularity, hirsutism, and acne and can be mistaken for polycystic ovarian syndrome (PCOS).2

A less common cause of CAH is a defect in steroid 11β-hydroxylase from a mutation in CYP11B1, resulting in hypertension, hypokalemia (in contrast to the salt-wasting symptoms of classic steroid 21-hydroxylase deficiency), and androgen excess symptoms.3 The severity of clinical symptoms is determined by the

Abbreviations: CAH, congenital adrenal hyperplasia; COVID-19, Coronavirus disease 2019; CYP, cytochrome; Gly, glycine; HSD, 3-beta-hydroxysteroid dehydrogenase; Leu, Leucine; NCCAH, nonclassic congenital adrenal hyperplasia; PCOS, polycystic ovarian syndrome; Phenylalanine, phenylanine; POR, P450 oxioreductase; STAR, steroidogenic acute regulatory protein gene; Val, valine; VUS, variant of unknown significance.

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degree to which the mutation impairs the function of the affected enzyme. In rare cases, it is possible for an individual to have 2 separate mutations in both 21-hydroxylase and 11β-hydroxylase. There is a paucity of reports of such dual mutations in the literature, and the clinical manifestations of such a case are not thoroughly described. Here we report a case wherein a pair of heterozygous mutations in both CYP21A2 and CYP11B1 was detected in a new diagnosis of NCAH. The mutation detected in CYP11B1 is a previously undescribed mutation.

Case Report

The patient is a 30-year-old woman who presented with a chief complaint of irregular menses and hirsutism. Since adolescence, she menstruated every 2 to 3 months, accompanied by mild cramping. Her menstrual flow typically lasted for 6 to 7 days, and she occasionally had spotting between periods. Her facial hair was bothersome enough to require regular shaving. She never had acne, breast tenderness, or breast discharge. She experienced headaches once or twice a month but denied experiencing blurry vision and diplopia. She had never had children or tried to become pregnant but wanted to start a family with her husband in the coming years. She denied experiencing changes in thirst or urination, muscle aches, skin changes, bruising, rashes, frequent infections, anxiety, or depression. She denied the use of tobacco, alcohol, or recreational substances. She had a family history of irregular menses (her mother and sister) and type 1 diabetes (her mother and grandmother). Physical examination revealed no visual field defects, facial plethora, or telangiectasia. There was hirsutism at the chin. There was no goiter. Her skin was warm and dry, with no obvious rashes or bruises, and her abdomen was soft, with normal hair distribution and typical external genitalia for her age. Her body mass index was 23, her heart rate was 86 beats per minute, and her blood pressure was 124/80. Her previous medical history included a prolactin level of 34.7 ng/mL (reference range, 2.0-23.0 ng/mL) and an AM total serum testosterone level of 77 ng/dL (reference range, 25-125 ng/dL, not sex-specific). She also had a 2-mm × 3-mm posterior pituitary lesion on magnetic resonance imaging. A pelvic ultrasound 3 years before presentation was reported to be normal. Other laboratory parameters before the initial evaluation can be found in Table 1. As part of this medical team’s initial evaluation, laboratory findings revealed the following: the AM 17-hydroxyprogesterone level was 554 ng/dL (female reference range, 10-300 ng/dL), the total testosterone measured by Liquid Chromatography-Tandem Mass Spectrometry level was 83 ng/dL (reference range for females and children, 9-55 ng/dL), and the prolactin level was 29.6 ng/mL (reference range, 2.0-23.0 ng/mL). The findings of the further laboratory testing performed at that time can be found in Table 2. The patient was started on cabergoline (0.25 mg) biweekly, which decreased her prolactin level to 4.4 ng/dL in 4 weeks. At the lowered prolactin level, the patient continued to report menstrual irregularities and hirsutism. A 250-mcg cosynotropin test was performed. The AM 17-hydroxyprogesterone level increased from 44 ng/dL at time 0 to 837 ng/dL at 60 minutes (Table 3). The adrenocorticotropic hormone level was 178 pg/mL (reference range, 7.2-63 pg/mL) and the baseline AM cortisol level was 24.1 mcg/dL (reference range, 8-25 mcg/dL). After a 1-mg dexamethasone suppression test, her serum AM cortisol level was 197 ng/dL (140-295 ng/dL for 1-mg dexamethasone ingested the previous evening) and her AM serum cortisol level was 0.5 mcg/dL (8-24 mcg/dL) (Table 3).

Genetic testing was performed with CAH gene sequencing and a copy number variation detection panel via Next Gen Sequencing with PGxome exome capture probes to analyze CYP11A1, CYP11B1, CYP17A1, CYP21A2, HSD3B2, POR, and STAR, and Sanger sequencing for the genotype.

Table 1

| Test                  | Result | Reference range |
|-----------------------|--------|-----------------|
| Na (mmol/L)           | 138    | 135-145         |
| K (mmol/L)            | 4.4    | 3.5-5.0         |
| Cl (mmol/L)           | 107    | 98-108          |
| HCO3 (mmol/L)         | 21     | 22-29           |
| BUN (mg/dL)           | 10     | 5-20            |
| Creatinine (mg/dL)    | 0.74   | 0.60-1.20       |
| Glucose (mg/dL)       | 102    | 70-99           |
| Ca (mg/dL)            | 9      | 8.5-10.5        |
| Alkaline phosphatase (units/L) | 51    | 5-15            |
| AST (units/L)         | 31     | 13-39           |
| ALT (units/L)         | 10     | 7-52            |
| Total bilirubin (mg/dL) | 0.5 | 0.0-1.0         |
| Total protein (g/dL)  | 6.8    | 6.4-8.0         |
| Albumin (g/dL)        | 4.2    | 2.5-5.0         |
| TSH (mU/mL)           | 2.086  | 0.40-4.200      |
| Prolactin (ng/mL)     | 34.7   | 2.0-23.0        |
| Total testosterone serum (ng/dL) | 77  | 25-125         |

Table 2

| Test                  | Result | Reference range |
|-----------------------|--------|-----------------|
| 17-OH progesterone (ng/dL) | 554 | 10-300 ng/dL |
| Total testosterone F/C LC-MS/MS (ng/dL) | 83 | 9-55 |
| Serum prolactin (ng/mL)       | 29.6  | 2.0-23.0        |
| DHEA sulfate (mcg/dL)         | 223   | 35-430          |
| Estradiol (pg/mL)             | 261   | N/A             |
| IGFl (ng/mL)                  | 202   | 87-368          |
| Serum sex hormone-binding globulin (nmol/L) | 106.61 | 30-135 |
| Free testosterone F/C LC-MS/MS (pg/mL) | 6.8 | 0.8-7.4 |
| LH (mU/mL)                    | 11.5  | N/A             |
| LH (mU/mL)                    | 35.5  | N/A             |

Table 2 Endocrine Work-up for Hyperandrogenism

Table 1 Initial Laboratory Work-up

| Test                  | Result | Reference range |
|-----------------------|--------|-----------------|
| Na (mmol/L)           | 138    | 135-145         |
| K (mmol/L)            | 4.4    | 3.5-5.0         |
| Cl (mmol/L)           | 107    | 98-108          |
| HCO3 (mmol/L)         | 21     | 22-29           |
| BUN (mg/dL)           | 10     | 5-20            |
| Creatinine (mg/dL)    | 0.74   | 0.60-1.20       |
| Glucose (mg/dL)       | 102    | 70-99           |
| Ca (mg/dL)            | 9      | 8.5-10.5        |
| Alkaline phosphatase (units/L) | 51    | 5-15            |
| AST (units/L)         | 31     | 13-39           |
| ALT (units/L)         | 10     | 7-52            |
| Total bilirubin (mg/dL) | 0.5 | 0.0-1.0         |
| Total protein (g/dL)  | 6.8    | 6.4-8.0         |
| Albumin (g/dL)        | 4.2    | 2.5-5.0         |
| TSH (mU/mL)           | 2.086  | 0.40-4.200      |
| Prolactin (ng/mL)     | 34.7   | 2.0-23.0        |
| Total testosterone serum (ng/dL) | 77  | 25-125         |

Abbreviations: Na – sodium; K – potassium; Cl – chloride; HCO3 – Bicarbonate; BUN – Blood Urea Nitrogen; Ca – Calcium; AST – Aspartate Transaminase; ALT – Alanine Transaminase; TSH – Thyroid Stimulating Hormone

Nonclassic congenital adrenal hyperplasia (NCAH) may go undiagnosed in women.

The number of known mutations in CYP11B1 causing CAH and nonclassic CAH is growing.

A digenic cause with heterozygous variants may be a genetic makeup of the condition.

The genetic diversity of nonclassic congenital adrenal hyperplasia ought to remind clinicians to consider it as a diagnosis, particularly in young women whose hyperandrogenism or menstrual irregularities cannot be explained by polycystic ovarian syndrome or another presumed endocrine abnormality when the patient is not responding to standard medical treatment.

Highlights

- Nonclassic congenital adrenal hyperplasia (NCAH) may go undiagnosed in women.
- The number of known mutations in CYP11B1 causing CAH and nonclassic CAH is growing.
- A digenic cause with heterozygous variants may be a genetic makeup of the condition.

Clinical Relevance

The genetic diversity of nonclassic congenital adrenal hyperplasia ought to remind clinicians to consider it as a diagnosis, particularly in young women whose hyperandrogenism or menstrual irregularities cannot be explained by polycystic ovarian syndrome or another presumed endocrine abnormality when the patient is not responding to standard medical treatment.
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In evaluating for NCCAH, she was found to have an inconclusive metabolic panel.12 Given the continued diagnostic uncertainty, genetic testing was performed, and our patient was found to be heterozygous for a known pathogenic CYP21A2 variant, a G110Efs mutation, c.332_339del, which is predicted to cause a frameshift mutation resulting in premature protein termination per the genetic sequencing laboratory report provided by Prevention Genetics of Marshfield, Wisconsin. Additionally, she was found to be heterozygous in the CYP11B1 gene for a sequence variant designated c.1123C>T, which is predicted to result in the amino acid substitution p.Leu375Phe; this was reported as a VUS. The majority of cases of CAH due to 11β-hydroxylase deficiency present with the previously described classic CAH constellation of findings.13 Less commonly, this mutation can lead to NCCAH, primarily when the pathogenic mutation is found outside of exon 8. In another 2018 case study and literature review, Wang et al.14 described that most mutations in exon 8 result in classic CAH symptoms due to severe disruption of enzymatic structure and activity. In contrast, they reported that most mutations in exon 3 cause more minimal alterations in enzyme structure and, thus, lead to the milder nonclassical presentation. In our case, the mutation was located in exon 7, a location associated with both nonclassic and classic symptoms.13,14 Furthermore, our team used Robetta protein structure prediction software to simulate the mutant CYP11B1 protein on the basis of our described VUS. The predicted protein structure for wild type and mutant protein is shown in Figure 1. The mutated Phe residue was simulated to be situated near the “roof” of the enzymatic active site and could potentially affect residue binding at this location. In addition, in vitro functional assessment to recreate the mutation and to observe enzymatic changes would be a further method of characterizing her VUS.15

Phenotypes of CYP11B1 deficiencies can be milder and can be similar to nonclassic 21-hydroxylase deficiency.16 These milder forms are differentiated, in part, by less virilization and more normotension, as seen in our patient. At least 100 mutations of CYP11B1 have been found to cause CAH because of 11β-hydroxylase deficiency.17 Moreover, a growing number of case reports of novel mutations of CYP11B1 are adding to the understanding of the genetic variations of this condition.14,16-19

With respect to combined 21- and 11β-hydroxylase deficiency, a 1985 report described this phenomenon in 5 people among 3 different families.18 Two of the individuals in that case report were females whose symptoms included acne and, similar to our patient, hirsutism and menstrual abnormalities. As for the other 3 individuals, 2 were asymptomatic and 1 was a virilized XX female raised as a male. All 5 patients had elevated androgen levels. Our patient’s case differs because she clinically presented with NCCAH. Our patient seems to show that mutations in 2 different genes can potentially present phenotypically as NCCAH. This could enrich beyond CYP21A2 deletions is needed to identify the genotypes of those with CAH due to the diversity of genetic mutations.20 Had our patient not had a supportive physical examination and biochemical findings suggestive of NCCAH, her genetic results could have been interpreted to suggest that she is simply a carrier for the 2 genes

**Table 3**

| Test                                      | Result | Reference Range |
|-------------------------------------------|--------|-----------------|
| ACTH (pg/mL)                              | 178.0  | 7.2-63.0        |
| Baseline 17-OH progesterone (ng/dL)       | 444    | 10-300          |
| 60-min 17-OH progesterone (ng/dL)         | 837    | <1000           |
| Baseline cortisol (mcg/dL)                | 24.1   | 8-25            |
| Cortisol after 1mg (mcg/dL)               | 0.5    | 8-24            |
| dexamethasone suppression test (mcg/dL)   | 837    | 1-10            |
| FSH (mIU/mL)                              | 4.1    | N/A             |
| LH (mIU/mL)                               | 10.0   | N/A             |

*Abbreviations: OH = hydroxy; ACTH = adrenocorticotropic hormone; FSH = Follicle Stimulating Hormone; LH = Leutinizing Hormone; N/A = Not Available (due to unknown phase of ovulation) ACTH stimulation labs bolded.*

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Fig. 1. CYP11B1 Wild type and mutant. Arrows indicate the implicated amino acid. Robetta protein structure prediction software demonstrating CYP11B1 wild type (left) and mutant with VUS (p.Leu375Phe) (right).

polymerase chain reaction to amplify targeted regions. This revealed a heterogeneous pathogenic CYP21A2 variant (c.332_339del; p.Gly111Valfs*21) and a heterogeneous, previously undescribed variant of unknown significance (VUS) in CYP11B1 (c.1123C>T; p.Leu375Phe).

The patient was referred to genetic counseling for further discussion of risks to future children of CAH. She was offered oral contraceptives in an attempt to lower her testosterone level until the patient decided to attempt pregnancy. She was maintained on cabergoline 0.25 mg biweekly but lost to follow-up during the COVID-19 pandemic.

**Discussion**

Our patient presented with symptoms including irregular periods and shaving, both of which were bothersome for the patient. She was treated for hyperprolactinemia without resolution of her symptoms. Additionally, PCOS was considered as an explanation for her presentation; however, she did not fully meet the diagnosis criteria.11 In evaluating for NCCAH, she was found to have an elevated 17-hydroxyprogesterone level at baseline prior to adrenocorticotropic hormone stimulation, although the stimulated 17-hydroxyprogesterone level fell slightly below 1000 ng/dL. Between 1000 ng/dL and 1500 ng/dL is the range considered to be inconclusive.12 Given the continued diagnostic uncertainty, genetic testing was performed, and our patient was found to be heterozygous for a known pathogenic CYP21A2 variant, a G110Efs mutation, c.332_339del, which is predicted to cause a frameshift

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discussed given the heterozygosity. However, given our findings, it is both intriguing and reasonable to at least consider a digenic cause with heterozygous variants in CYP21A2 and CYP11B1 causing her NCAH. In vitro testing of the VUS and further biochemical testing including 11-deoxycorticosterone, 11-deoxycortisol, or progesterone levels may further indicate whether either the mutation in 21-hydroxylase or 11β-hydroxylase is causing an increase in upstream substrates. This can help indicate whether there is a biochemical significance that we could extrapolate as possibly contributing to the clinical presentation. These tests could reveal a less disease—causing mutation in CYP11B1 given that our patient was normotensive and without hyponatremia or hyperkalemia. In addition, the VUS in CYP11B1 should be cataloged and hopefully examined in the context of other patients if found elsewhere, and our patient’s genetic variation, in a way not previously described in case reports or other literature, perhaps contributes to the relative commonness of NCAH.

Conclusion

This case demonstrates a patient with physical examination, biochemical, and genetic findings suggestive of NCAH. Her case suggests that dual heterozygous mutations in separate genes can be seen in NCAH, which may further help to identify the genotypes of those with NCAH. We further conclude that our patient’s case ought to remind clinicians to consider NCAH as a diagnosis, particularly in young women whose hyperandrogenism or menstrual irregularities cannot be explained by PCOS or hyperprolactinemia when the patient is not responding to standard medical treatment.

Disclosure

The authors have no multiplicity of interest to disclose.

References

1. Merke DP, Auchus RJ. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. N Engl J Med. 2020;383(13):1248–1261.
2. New MI. Extensive clinical experience: nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2006;91(11):4205–4214.
3. White PC, Speiser PW. Steroid 11 beta-hydroxylase deficiency and related disorders. Endocrinol Metab Clin North Am. 1994;23(2):325–339.
4. Auchus RJ. The classic and nonclassic congenital adrenal hyperplasias. Endocr Pract. 2015;21(4):383–389.
5. Tonetto-Fernandes V, Lemos-Marini SH, Kuperman H, Ribeiro-Neto LM, Verreschi IF, Kater CE. Serum 21-deoxycorticisol, 17-hydroxyprogesterone, and 11-deoxycortisol in classic congenital adrenal hyperplasia: clinical and hormonal correlations and identification of patients with 11beta-hydroxylase deficiency among a large group with alleged 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2006;91(6):2179–2184.
6. Gillis D, Speiser P, Zhou Z, Rosler A. Combined 21-hydroxylase and 11beta-hydroxylase deficiency: patient report and molecular basis. J Pediatr Endocrinol Metab. 2000;13(7):945–949.
7. Penny R, Vecsei P. Congenital adrenal hyperplasia due to combined 21- and 11 beta-hydroxylase deficiency. J Endocrinol Invest. 1989;12(10):723–728.
8. Hurwitz A, Brautbar C, Milwidsky A, et al. Combined 21- and 11 beta-hydroxylase deficiency in familial congenital adrenal hyperplasia. J Clin Endocrinol Metab. 1985;60(4):631–638.
9. Dall’Asta C, Barbetta L, Libè R, Passini E, Ambrosi B. Coexistence of 21-hydroxylase and 11 beta-hydroxylase deficiency in adrenal incidentalomas and in subclinical Cushing’s syndrome. Horm Res. 2002;57(5-6):192–196.
10. Tonetto-Fernandes V, Lemos-Marini SH, De Mello MF, Ribeiro-Neto LM, Kater CE. 21-Hydroxylase deficiency transiently mimicking combined 21- and 11beta-hydroxylase deficiency. J Pediatr Endocrinol Metab. 2008;21(5):487–494.
11. Azziz R, Carmina E, Dewailly D, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91(2):456–488.
12. New MI, Lorenzen F, Lerner AJ, et al. Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab. 1983;57(2):320–326.
13. Kandemir N, Yılmaz DY, Gonç EN, et al. Novel and prevalent CYP11B1 gene mutations in Turkish patients with 11-β-hydroxylase deficiency. J Steroid Biochem Mol Biol. 2017;165(Pt A):57–63.
14. Wang D, Wang J, Tong T, Yang Q. Non-classical 11β-hydroxylase deficiency caused by compound heterozygous mutations: a case study and literature review. J Ovarian Res. 2018;11(1):82.
15. Mesman RLS, Calléja FMGR, Hendriks G, et al. The functional impact of variants of uncertain significance in BRCA2. Eur J Hum Genet. 2019;27(1):303–308.
16. Menabò S, Polat S, Baldazzi L, et al. Congenital adrenal hyperplasia due to 11beta-hydroxylase deficiency: functional consequences of four CYP11B1 mutations. Eur J Hum Genet. 2014;22(5):610–616. Published correction appears in Eur J Hum Genet. 2020;28(5):692.
17. Ben Charfeddine L, Riepe FG, Kablou N, et al. Two novel CYP11B1 mutations in congenital adrenal hyperplasia due to steroid 11β-hydroxylase deficiency in a Tunisian family. Gen Comp Endocrinol. 2012;175(3):514–518.
18. Korne N, Riepe FG, Götze D, et al. Congenital adrenal hyperplasia due to 11β-hydroxylase deficiency: functional characterization of two novel point mutations and a three-base pair deletion in the CYP11B1 gene. J Clin Endocrinol Metab. 2005;90(6):3724–3730.
19. Long Y, Han S, Zhang X, et al. The combination of a novel 2 bp deletion mutation and p.D63H in CYP11B1 cause congenital adrenal hyperplasia due to steroid 11β-hydroxylase deficiency. Endocr J. 2016;63(3):301–310.
20. Finkelstein GP, Chen W, Mehta SP, et al. Comprehensive genetic analysis of 182 unrelated families with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab. 2011;96(1):E161–E172.