# Two siblings with non-classic P450scc deficiency resulted from a novel mutation in *CYP11A1* gene misdiagnosed as familial glucocorticoid deficiency

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## SUMMARY

P450scc deficiency due to *CYP11A1* gene mutations is a rare cause of primary adrenal insufficiency (PAI) in children. We reported two young siblings with PAI presented with hyperpigmentation. They were referred to our clinic with a diagnosis of familial glucocorticoid deficiency (FGD), another rare cause of PAI. However, further hormonal evaluation revealed increased plasma renin activity, which was not congruent with the diagnosis of FGD. Genetic analysis showed the compound heterozygous mutations in exon 8 of the *CYP11A1* gene, including a missense mutation, R466W (c1396C>T), and a nonsense mutation, R439X (c1315C>T). A case responded well to hydrocortisone, while another case received prednisolone due to her unresponsiveness to hydrocortisone. To correctly diagnose P450scc deficiency, physicians should be alerted with the similarity between this disease and FGD because of their predominant glucocorticoid deficiency. Long-acting glucocorticoids may be used with caution to reach treatment goals.

## BACKGROUND

Primary adrenal insufficiency (PAI) in children is a rare, life-threatening condition characterised by an impaired production of glucocorticoids, with or without mineralocorticoid and androgen deficiency, due to a primary pathology in the adrenal gland.[1] The most common cause of PAI in children is congenital adrenal hyperplasia (CAH).[2] CAH is a group of autosomal recessive enzymatic defects in steroidogenesis.[2–4] Cholesterol is transported into the mitochondrial cytochrome of the adrenal gland by the steroidogenic acute regulatory (StAR) protein and is initially converted to pregnenolone by the cholesterol side-chain cleavage enzyme (P450scc) encoded by the *CYP11A1* gene.[3–5] The disruption of either StAR or P450scc activity is a rare cause of CAH and will result in PAI, in which the clinical presentations are due to the insufficient steroidogenesis of the adrenal and the gonads including glucocorticoid, mineralocorticoid, progesterone and sex steroids.[2–5] Signs and symptoms of CAH can occur early in the neonatal period or later during infancy and include vomiting, volume depletion, hyponatraemia, hyperkalaemia, hypoglycaemia, hyperpigmentation and failure to thrive.[6] Since P450scc also plays an essential role in the progesterone synthesis of the placenta to assure term gestation, mutations of the *CYP11A1* gene may lead to miscarriage or premature birth.[7]

Patients with P450scc deficiency show a variety of clinical presentations ranging from a severe form considered as classic P450scc deficiency to a mild form which is non-classic P450scc deficiency.[3 8] The clinical presentation may vary from being incompatible with term gestation to late-onset adrenal insufficiency after the neonatal period (9 months–9.7 years of age).[3] The lack of P450scc enzyme activities leads to a decrease in the production of adrenal steroids and an increase in the production of adrenocorticotropic hormone (ACTH) and plasma renin activity.[3 6] Patients with a 46,XX karyotype with either StAR or P450scc deficiency have normal female external genitalia.[3] However, those with a 46,XY karyotype may have normal female or male genitalia, female genitalia with clitoromegaly, or male genitalia with hypospadias or cryptorchidism.[3]

There are a few case reports about the disorders in the initial steps of steroid hormone synthesis due to mutations in *CYP11A1* gene.[3] All reported cases presented with clinical manifestations of adrenal insufficiency and with varying degrees of severity at different ages depending on P450scc enzyme residual activities.[3] Here, we reported a novel mutation of *CYP11A1* gene in two siblings with PAI. In addition, these siblings presented with only clinical presentations of glucocorticoid deficiency, which is usually misdiagnosed as familial glucocorticoid deficiency (FGD) (ACTH resistance), another rare cause of PAI.[2] We have also reported our use of the long-acting glucocorticoids to treat PAI in one of our patients as she did not respond to the recommended hydrocortisone therapy.

## CASE PRESENTATION

The two cases were the second and the third child of a three-child family. They were referred to our outpatient clinic with a diagnosis of PAI due to FGD. They were born to non-consanguineous marriage partners. There was no family history of tuberculosis, HIV infection, or diseases related to the adrenal gland and autoimmune disorders. Their parents and the oldest child did not have hyperpigmentation. There was no sign or symptom suggesting HIV infection in these two siblings.

### Case 1

Case 1 was the youngest child in her family. She was a full-term baby, born by caesarean delivery with a birth weight of 3000 g and with otherwise uneventful antenatal and perinatal periods.

**Table 1** Laboratory test results of cases 1 and 2

| Laboratory tests | Case 1 | Case 2 | Reference ranges |
|---|---|---|---|
| Morning plasma ACTH (pg/mL) | >2000 | >2000 | 2.7–63.3 |
| Morning serum cortisol (µg/dL) | 1.44 | 0.62 | 6.20–19.40 |
| 17OH-progesterone (ng/mL) | 0.22 | 0.24 | 0.07–0.69 |
| Direct renin concentration (µIU/mL) | 382.1 | 203.0 | 4.4–46.1 |
| Aldosterone (ng/dL) | 3.69 | 5.28 | 2.21–35.3 |
| Serum sodium (mmol/L) | 142.0 | 139.0 | 130–145 |
| Serum potassium (mmol/L) | 3.93 | 4.31 | 3.4–5.1 |
| Total serum calcium (mmol/L) | 2.55 | 2.42 | 2.1–2.8 |
| Dehydroepiandrosterone sulfate (µg/mL) | <0.002 | 0.006 | 0.5–3.5 |

ACTH, adrenocorticotropic hormone.

Reviewing her medical history found that she was hospitalised with a diagnosis of severe septic shock and FGD when she was 2 years old. There was no detailed medical record of this hospital admission, but she has undertaken an oral hydrocortisone therapy (20 mg/m$^2$/day in two divided doses) since then. At our first examination, she was 5 years and 6 months old. She was generally well with a normal physical evaluation, and no signs of dehydration or salt craving, except diffuse hyperpigmentation. Her height (108.5 cm), weight (18.5 kg), blood pressure (85/55 mm Hg) and pulse rate (88/min) were within the corresponding normal ranges for age and sex. The hyperpigmentation was accentuated on her face, extremities and skin folds. She had a normal external female genitalia.

Morning plasma ACTH and serum cortisol examinations that were performed on this patient confirmed PAI. To identify underlining conditions, she also undertook other laboratory and imaging tests including serum electrolyte, 17OH-progesterone, aldosterone, plasma renin activity, dehydroepiandrosterone sulfate, abdominal ultrasonography and karyotype. Her blood test results are summarised in table 1. Abdominal ultrasound showed undetectable adrenal glands, and normal uterus and ovaries. Karyotype analysis confirmed 46,XX karyotype, matching with her normal female phenotype.

### Case 2

Case 2 was the second child in the family. She was a premature baby because of premature rupture of membranes at 34 weeks of gestation with a birth weight of 2100 g. She had a normal growth and development without any noticeable health problem. After the diagnosis of FGD of her younger sibling (case 1), she was brought to the same hospital for evaluation because of her hyperpigmentation when she was 5 years old. She was also diagnosed with an FGD and has been treated with oral hydrocortisone (20 mg/m$^2$/day in two divided doses) since then. Our first physical examination, which was performed when she was 8 years and 10 months old, found no abnormality, except diffuse hyperpigmentation, significantly in sun-exposed areas like case 1. There was no sign of dehydration or salt craving. Her height (131.5 cm), weight (40 kg), blood pressure (90/50 mm Hg) and pulse rate (80/min) were within the corresponding normal ranges for age and sex. She was prepubertal with a normal external female genitalia. She was asked to undertake the same laboratory and imaging tests as case 1. Her laboratory test results are summarised in table 1. Abdominal ultrasound showed undetectable adrenal glands, and normal ovaries and prepubertal uterus. She had a 46,XX karyotype, matching with her normal female phenotype. At 11 years of age, examination revealed Tanner stage 2 of breast development, growth acceleration but no axillary or pubic hair. Evaluation of her hypothalamic–pituitary–gonadal axis at this time found that there was an increase in levels of luteinising hormone (LH) (9.78 IU/L), follicle-stimulating hormone (5.09 IU/L) and oestradiol (30.4 pg/mL) .[9]

## INVESTIGATIONS

Genetic testing using DNA samples extracted from peripheral blood leucocytes was performed on two cases and their family members, including parents and the oldest child. The results showed compound heterozygous mutations in exon 8 of the *CYP11A1* gene, including a missense mutation R466W, (c1396C>T), and a nonsense mutation, R439X (c1315C>T) that were found in both cases. Based on the Clinvar database, these mutations were considered as novel mutations.[10] However, the nonsense mutation R439X has been reported elsewhere.[11] Hence, only R466W variant was considered as a novel mutation. These two variants were analysed using Intervar—bioinformatics software recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology 2015 Guidelines.[12]

Genetic analysis showed that these variants were classified as variants of uncertain significance. Given that nonsense mutations are highly likely to be non-functioning,[11] the novel missense mutation resulted in mild enzymatic defects in our cases presented with the non-classic form of P450scc deficiency. The missense mutation was inherited from the mother, while the nonsense mutation was inherited from the father. The oldest child (ie, older sister of cases 1 and 2) was clinically normal and had the nonsense mutation only.

## DIFFERENTIAL DIAGNOSIS

The two cases presented with hyperpigmentation and blood tests showing extremely low morning cortisol and high ACTH concentration. Therefore, as suggested elsewhere, a diagnosis of PAI was made without the need to perform corticotropin stimulation test.[2 6] However, regarding the identification of the aetiology of PAI, the increased plasma renin activity in these cases did not match well with their previous diagnosis of FGD, in which the mineralocorticoid production is usually intact.[2] This made the previous diagnosis of FGD questionable. Some causes of PAI including infections, injury and malignancies of the adrenal can possibly be ruled out based on the clinical features, laboratory tests and imaging.[2] However, other causes of PAI such as autoimmune diseases and genetic defects (CAH, adrenal hypoplasia, adrenoleucodystrophy and ACTH insensitivity syndromes) need specific diagnosis tests, including 21-hydroxylase antibodies and genetic analysis, respectively.[2 6] Gene analysis can be performed in Viet Nam, but 21-hydroxylase antibodies are not available. In addition, CAH, along with other genetic defects, is the most common cause of PAI in children.[2] Hence, genetic analysis had been performed to detect genetic mutations before autoimmune diseases were considered. Genetic analysis also helped differentiate between genetic causes of PAI, including FGD.

## TREATMENT

At our centre, the recommended oral hydrocortisone therapy (8–15 mg/m$^2$/day), divided into three doses and administered every 8 hours, was used for two cases to optimise their height growth.[6] Case 1 responded to the dose of 12 mg/m$^2$/day demonstrated by her decreased hyperpigmentation. In contrast, case 2 was treated using a dose of 15 mg/m$^2$/day, but the severity of her hyperpigmentation was not improved after 3 months. Hence, case 2's treatment therapy was replaced by prednisolone with

equivalent dose. Case 2's hyperpigmentation improved after 6 months with prednisolone treatment.

Mineralocorticoid replacement is not recommended for non-classic CAH type due to steroid 21-hydroxylase deficiency.[13] Although the two cases had mineralocorticoid insufficiency demonstrated by an increased plasma renin activity, they did not have serum electrolyte imbalance. There were also no clinical signs of aldosterone deficiency including poor weight gain, salt craving or dehydration. Therefore, they had been carefully followed up without any mineralocorticoid treatment. Mineralocorticoid replacement will be considered when the patients grow up and require more physical activities.

## OUTCOME AND FOLLOW-UP

Both patients were stable with hydrocortisone or prednisolone treatment in the subsequent follow-ups until 26 months since their admission to our clinic. Their parents were consulted about the need of these medications in their children's whole life, especially when the patients are stressful or sick. The two patients have been followed up at our clinic every 6 months for a re-evaluation and drug dosage adjustment. Genetic counselling was also conducted for their parents with a focus on the risk of disease in their future children.

## DISCUSSION

CAH is a group of autosomal recessive disorders. An increasing incidence of CAH due to consanguineous marriage has been reported.[14] Classic P450scc deficiency is a rare, severe form of CAH due to the complete insufficiency of all adrenal and gonadal steroids.[3 15] In Viet Nam, to the best of our knowledge, consanguineous marriage is rare. Given that newborn screening for CAH has just been available at some private hospitals, information on the magnitude of CAH gene mutation and associated clinical manifestations is scarce due to limited laboratory resource. A published study examining the clinical manifestation of 21-hydroxylase (CYP21A2) deficiency—the most common type of CAH in 209 Vietnamese patients—found that salt-wasting was the most common manifestation (77%), followed by simple virilising (21%) and non-classic type (2%).[16] However, gene analysis was not performed in this study. Our patients had no clinical signs and symptoms of adrenal insufficiency until the age of 2 and 5 years, except progressive hyperpigmentation. Although our patients had high levels of plasma renin activity, their levels of mineralocorticoids were partially preserved, demonstrated by normal serum sodium and potassium levels as well as no clinical signs of poor weight gain, salt craving and dehydration. Both patients had a 46,XX karyotype and normal female external genitalia. Hence, our patients' condition was also considered as the non-classic P450scc deficiency.

The partially inactivating mutations of *CYP11A1* gene presented as non-classic P450scc deficiency affect glucocorticoid production more predominantly than other adrenal gland steroids.[2–5] Consequently, the clinical presentation of non-classic P450scc deficiency is similar to that of FGD, which is an isolated glucocorticoid deficiency because of the hereditary unresponsiveness to ACTH.[2] Our cases were previously misdiagnosed as FGD because of the predominant hyperpigmentation manifestation and high-plasma ACTH. However, patients with non-classic P450scc deficiency also have asymptomatic mineralocorticoid and gonadal steroid deficiency, which will become symptomatic and more severe when they grow up and reach their puberty.[2] In light of this, paediatric endocrinologists should be alerted to this presentation of the non-classic P450scc deficiency to correctly diagnose its aetiology. To differentiate between the non-classic P450scc deficiency and FGD, it is important to perform plasma renin measurement in patients presenting with only glucocorticoid insufficiency and hyperpigmentation.

Regarding treatment, hydrocortisone is the only suggested glucocorticoid replacement therapy.[6] Other synthetic, long-acting glucocorticoids including prednisolone and dexamethasone are not recommended because of the risk of their adverse

## Patient's perspective

### Mother's perspective

I did not realize the dark skin of my kids until the hospital admission of the youngest one a few years ago. I was very upset at when I knew about the diagnosis of familial glucocorticoid deficiency - an inherited disease. This was because my husband and I are totally healthy, and my oldest kid is healthy too. No genetic test was done to confirm our kids' disease at that time. The treatment had been going on without any improvement of my kids' skin condition since the last 2 years. The middle kid was bored at school because she had no close friend. Honestly, none of her classmates wanted to play with her because of her skin condition. My youngest kid was still naïve and happy at her age which helps me so much. Nevertheless, I really wanted them to have a normal life as others. This urged me to seek help from a paediatric endocrinologist. Now, I am truly happy to know about the cause of the disease and how the treatment works to help maintain my kids' normal life. It was so amazing that my youngest kid's dark skin improved after 3 months and that of the middle one improved after she used the new medication. The lack of medication and follow-ups with their doctor due to the COVID-19 lockdown caused me too much trouble. Hopefully, everything is good in the end, especially, when the doctor confirmed that my middle kid experiences her puberty at a proper age. I greatly thank the doctor for everything and am more optimistic with the future of my kids.

### Case 2's perspective

I loved your clinic. It was so beautiful with a lot of cartoon pictures. My sister (ie, Case 1) also liked these pictures, and she loved to run around to look at them. I was ok with the pain caused by blood draw, but she hated it. I liked to play with her at home because I had no close friend. My classmates sometime made fun of my skin colour and did not really want me to join them. I really wanted to join my classmates and have close friends. Could you help me with this?

## Learning points

- P450scc deficiency due to *CYP11A1* gene mutations is a rare cause of primary adrenal insufficiency and is diagnosed using gene analysis.
- Given that patients with non-classic P450scc may be misdiagnosed as having familial glucocorticoid deficiency, to differentiate these conditions, it is important to perform plasma renin measurement in patients presenting with only glucocorticoid insufficiency and hyperpigmentation.
- The levels of adrenal and sex steroids deficiency are dependent on the residual P450scc enzyme activity. Hence, a long-term follow-up is required to decide if hormone replacement therapy is needed.

effects.[6] However, no data are available for the comparison of the long-term adverse effects of various glucocorticoids in children. In addition, some long-acting glucocorticoids that were used as a substitution for hydrocortisone in children with CAH such as dexamethasone and prednisolone showed no long-term adverse effects, especially on growth velocity.[17 18] Our case 2's hyperpigmentation was not improved even with the highest recommended dose of hydrocortisone. Given that the mother of two cases was their direct caregiver, she confirmed the patients' high compliance with medication. Hence, case 2's non-response to hydrocortisone due to drug non-compliance was ruled out. Her treatment therapy was replaced by using an equivalent dose of prednisolone with a comparable anti-inflammatory potency after discussing with her parents. She has been on prednisolone for 23 months and shows no adverse effects of medication. However, the long-term adverse effects of prednisolone on this patient, especially growth retardation, have been continuously monitored.

The production of sex steroids is also partially impaired in patients with non-classic P450scc deficiency due to the retained P450scc activity. Hence, it is pivotal to closely follow up the patients to examine if their pubertal development is appropriate.[2 15] The average age for girls to begin puberty is 10–11 years.[9] Patient follow-up found that case 2 had the first signs of puberty at the age of 11 years with height acceleration, breast development and pubertal levels of hypothalamus–pituitary–gonadal axis hormones. The LH level was higher than that of clinical Tanner stage 2 proving an inappropriate oestrogen production. She has been continuously followed up to ensure her adequate oestrogen biosynthesis through puberty and in adulthood.[8] Similarly, case 1 has also been followed up.

In conclusion, PAI caused by non-classic P450scc deficiency is a very rare disease in children and is due to mutations of *CYP11A1* gene. By describing two clinical cases with a novel mutation, this case report adds to the body of knowledge of molecular pathogenesis of non-classic P450scc deficiency leading to PAI. The report also shows an unusual presentation of non-classic P450scc deficiency, which may cause misdiagnosis. If the recommended hydrocortisone therapy is ineffective, long-acting glucocorticoids may be used with a close patient follow-up.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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