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SHORT COMMUNICATION

Novel *CYP11B2* mutation causing aldosterone synthase (P450c11AS) deficiency

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Abstract Aldosterone synthase (P450c11AS) deficiency is a rare autosomal recessive disorder, presenting with severe salt-losing in early infancy. It is caused by inactivating mutations of the *CYP11B2* gene. Here, we describe three unrelated Asian patients who have clinical and hormonal features compatible with aldosterone synthase deficiency and identify their *CYP11B2* mutations. Patient 1 was a Thai female infant. Patient 2 was an Indian boy, and patient 3 was a Thai male infant. All subjects presented at the age of 1–2 months with diarrhea, failure to thrive, and severe dehydration. Their plasma electrolytes showed hyponatremia, hyperkalemia, and acidosis. All patients had normal cortisol response and had elevated plasma renin activity with low aldosterone levels. The entire coding regions of the *CYP11B2* gene were amplified by polymerase chain reaction and sequenced. Patient 1 was homozygous for a previously described mutation, p.T318M. Patient 2 was homozygous for a novel c.666delC mutation inherited from both parents resulting in p.223F>Sfsx295. No *CYP11B2* mutation was detected in patient 3. *Conclusions:* We report the first *CYP11B2* defects in Southeast Asian families responsible for aldosterone synthase deficiency and identified a novel *CYP11B2* mutation. However, the affected gene(s) responsible for primary hypoaldosteronism other than *CYP11B2* remain to be determined.

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Introduction

Aldosterone is a hormone required for regulating intravascular volume and electrolyte balance. The genetic defects in aldosterone biosynthesis have been established [10]. In humans, the two 11β-hydroxylase iso-enzymes, 11β-hydroxylase enzyme (P450c11β) and aldosterone synthase enzyme (P450c11AS), which are 93 % identical in amino acid sequence, are encoded by two separate genes on the long arm of chromosome 8, *CYP11B1* and *CYP11B2*, respectively [2, 14]. P450c11AS is expressed exclusively in the zona glomerulosa, where it catalyzes 11β-hydroxylase, 18-hydroxylase, and 18-methyl oxidase activities. Disorders of P450c11AS cause aldosterone synthase deficiency that is formerly termed corticosterone methyl oxidase (CMO) deficiency, which was classified into two types according to the profiles of secreted steroids [9]. However, there are clinical, hormonal, and genotypic overlaps between CMOI and CMOII [16]. These disorders should now be considered as a continuous spectrum of the same disease.

Aldosterone synthase deficiency is an autosomal recessive disorder, which has been documented throughout Europe, North America, and part of Middle East [15], but there are very few reports from Asia [7]. Inadequate aldosterone production leads to decreased renal sodium resorption and potassium excretion. Affected infants usually develop poor weight gain, vomiting, diarrhea, and dehydration leading to hypovolemia at a few days to weeks of age. Some patients with milder phenotypes are diagnosed in childhood or adulthood [5, 13]. Hyponatremia, hyperkalemia, hyperreninemic hypoaldosteronism, and normal or elevated cortisol levels are characteristics of this disorder [15].

Interestingly, electrolytes usually normalize by 3–4 years of age, even if untreated [15]. Here, we describe three patients from different Asian families presenting with salt-losing in early infancy and identified their *CYP11B2* mutations in two of the three patients.

Case reports

Patient 1

A 2-month-old Thai female presented with vomiting and diarrhea with severe dehydration. She was born at term with a birth weight of 2,750 g. Parents denied consanguinity. At initial visit, her weight was 3,320 g (<the third centile). She had normal female genitalia without skin hyperpigmentation. Laboratory values showed serum Na 117 mmol/L, K 9.2 mmol/L, Cl 85 mmol/L, and CO_2 4 mmol/L. She received intravenous fluid to correct dehydration and electrolyte imbalance and was discharged, but the cause was undiagnosed. Two weeks later, she was sent back to the hospital due to diarrhea, vomiting, and clinical dehydration. Electrolytes showed Na 127 mmol/L, K 7.4 mmol/L, Cl 98 mmol/L, CO_2 15 mmol/L, BUN 11 mmol/L, and Cr 0.16 mg/dl. After fluid resuscitation, the adrenocorticotropic hormone (ACTH) test (cosyntropin 125 μg) was performed. Her baseline cortisol was 20 μg/dl and 60-min-stimulated cortisol was 50 μg/dl. Plasma renin activity (PRA) was 4,570 ng/dl/h [normal values for age (nl) 235–3,700], while plasma aldosterone was low at 4.03 ng/dl (nl, 5–90). Due to inappropriately low serum aldosterone level relatively to hyperkalemia and elevated PRA, she was diagnosed with primary hypoaldosteronism. She was treated with oral NaCl and fludrocortisone and responded well to the treatment. At her last visit (age 3.8 years), her height was 99.6 cm (+0.2 SD), and her weight was 14.5 kg (−0.3 SD).

Patient 2

An Indian male infant was born of term pregnancy with a birth weight of 3,300 g. Parental consanguinity was denied. He presented with diarrhea, poor feeding, and poor weight gain which led to the hospitalization at the age of 2 months. The patient had dark skin color but had no mucosal pigmentation. He had normal male genitalia with normally descended testes. Electrolytes showed serum Na 129 mmol/L, K 6.3 mmol/L, Cl 94 mmol/L, and CO_2 11 mmol/L. An ACTH test (cosyntropin 125 μg) revealed that cortisol rose from 6.4 to 19.7 μg/dl and post-stimulated 17OHP was 270 ng/dl (nl, 85–250). PRA was 6,309 ng/dl/h (nl, 235–3,700) and plasma aldosterone was 2.79 ng/dl (nl, 5–90), which suggested primary hypoaldosteronism. He had been treated with oral NaCl and fludrocortisone and remained well with catch-up growth within 3 months. When seen at age 7 years, his weight was 45.6 kg (+4.1 SD), and his height was 131.7 cm (+2.3 SD). Currently, he had poor compliance to medications, but has never had severe episode of salt wasting.

Patient 3

A Thai male infant was delivered at term with a birth weight of 3,110 g. His parents denied consanguinity. He presented at the age of 1 month due to episodic vomiting, diarrhea, and hypovolemic shock. Laboratory values showed serum Na 126 mmol/L, K 6.5 mmol/L, Cl 92 mmol/L, and CO_2 19 mmol/L. He received intravenous fluid resuscitation and his sepsis workup was negative. A month later, he came back to the hospital with diarrhea and severe dehydration. The examination revealed normal male genitalia and normally pigmented skin and mucosa. Serum electrolytes revealed Na 117 mmol/L, K 6.5 mmol/L, Cl 79 mmol/L, and CO_2 11 mmol/L. His plasma cortisol obtained while he was critically ill was >60 μg/dl. PRA was 3,020 ng/dl/h (nl, 235–3700) while plasma aldosterone was 7.35 ng/dl (nl, 5–90). He was diagnosed with primary hypoaldosteronism and had been treated with fludrocortisone. At age 3.5 years, he had been doing well with his weight of 13.2 kg (−1.2 SD) and height 96.5 cm (−0.4 SD).

Methods

PCR and DNA sequencing

With an informed consent, genomic DNA was extracted from peripheral leukocytes. All coding regions of *CYP11B2* and the exon–intron splicing junction boundaries were polymerase chain reaction (PCR)-amplified, using the primers and conditions as previously described [3] (Table 1), then sequenced. Sequence data analysis was done using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI).

Results

Direct sequencing revealed that patient 1 was homozygous for a previously described missense *CYP11B2* mutation located in exon 5, a cytosine-to-thymine substitution at nucleotide position 954 (c.954C>T) resulting in a threonine to methionine substitution at codon 318 (p.T318M) (NCBI cDNA reference sequence NM_000498.3 and CYP11B2 protein reference sequence NP_000489.3) (Fig. 1a). Patient 2 carried a homozygous novel frameshift mutation located in exon 3 (c.666delC), changing the downstream reading frame and causing premature translation stop at codon 295

Table 1 Oligonucleotides used for PCR amplification for *CYP11B2* mutation analysis

Exons amplified	Primer sequences for PCR 5′ to 3′	
	Forward	Reverse
1, 2	TATGTTTCCAGAGCAGGTTCCTGGGTGAGA	GCAGATGTGCTTTTGGGTCCTACCTC
3–5	AGGCAGCTTCTACCAGGGCCCCAGTCACTC	CCCCTCCCCTGCAAATCTCATCCCTTA
6–9	ATCAAGGTTTCAGATCCG	TGGCCTTGCTATTTGACA

(p.223F>Sfsx295) (Fig. 1b). Both parents were heterozygous carriers of the mutation and clinically asymptomatic. In patient 3, complete sequencing of all exons, all splice junctions, and 50 bp of the 5′ flanking region failed to reveal mutations in the *CYP11B2* gene.

Discussion

We described three unrelated Asian patients presenting with typical manifestations of aldosterone synthase deficiency and identified one previously described and one novel *CYP11B2* mutation. To our knowledge, this is the first report for CYP11B2 defects from Southeast Asia.

Patient 1 presented T318M mutation, which was first reported in a Slavic patient diagnosed with CMOII deficiency [16]. When transfected into MA-10 cells, the T318M mutant had severely impaired aldosterone synthase activity. T318 is a highly conserved residue that involves in cleavage of the dioxygen bond of O_2 which is essential for P450 catalysis [11]. The presence of this mutation in a Thai patient in our study suggests a possible recurrent mutation.

Patient 2 harbored a novel frameshift mutation, c.666delC (p.223F>Sfsx295). This mutant would result in truncated protein that lacks about half of the enzyme structure. The terminal half of CYP11B2 contains the important structure for proton transfer, accessory protein binding, heme binding, and substrate binding [12]. The p.223F>Sfsx295 protein is thus expected to be functionally inactive.

Aldosterone synthase deficiency is typically characterized by life-threatening salt loss in early infancy, but the clinical severity in this disorder gradually ameliorates with age. Abnormal steroid pattern may persist throughout life, but adolescent and adult patients are usually asymptomatic and do not require mineralocorticoid replacement [13]. However, the discontinuation of fludrocortisone in affected adults was associated with asymptomatic orthostatic hypotension and a rise in serum potassium levels [7]. Thus, patients should be informed that they are at risk if exposed to some stress such as dehydration and limited salt intake. The mechanism for reduced mineralocorticoid dependence with advancing age in these patients is not clearly understood. It is possibly from the increasing sensitivity to mineralocorticoid action and sodium intake with age. The recent study supports this hypothesis: the mineralocorticoid receptor expression in human kidney is very low in late gestational and at birth, but rising progressively afterward [8]. Another possible mechanism is an age-dependently impaired 11β-hydroxysteroid dehydrogenase type 2 activity, resulting in more cortisol availability for the mineralocorticoid receptor with age [4].

We could not identify *CYP11B2* mutation in patient 3. This is consistent with previous studies that also failed to detect mutations in *CYP11B2* in a number of

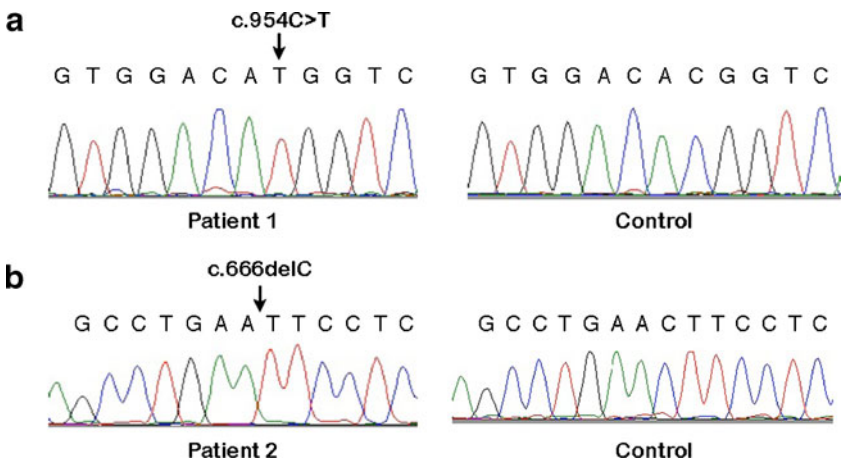

Fig. 1 Mutation analysis by direct DNA sequencing of the *CYP11B2* gene. **a** Patient 1 carrying homozygous p.T318M (c.954C>T). **b** Patient 2 carrying homozygous novel frameshift mutation (c.666delC), changing the downstream reading frame and causing premature translation stop at codon 295 (p.223F>Sfsx295). Each mutation is indicated by an *arrow*

kindreds with primary hypoaldosteronism [6]. It is possible that their pathologic mutations were in non-coding regions of *CYP11B2*. Additionally, these patients might have defects in transcriptional factors or other genes encoding components of the renin–angiotensin system (angiotensinogen, angiotensin-converting enzyme, and AT1-type angiotensin II receptor). Interestingly, the linkage analysis in some consanguineous families already excluded these genes, indicating that some other genes are etiologic [6].

Choi et al. recently discovered K^+ channel (*KCNJ5*) mutations in human adrenal aldosterone-producing adenomas and in a family with severe hyperaldosteronism and massive bilateral adrenal hyperplasia [1]. The mutated KCNJ5 channels caused chronic membrane depolarization, resulting in constitutive aldosterone production and cell proliferation. It is possible that defects in KCNJ5 channels could interfere the signal for aldosterone production leading to hypoaldosteronism as well. Yet, in this study, we sequenced the *KCNJ5* gene in patient 3, but failed to detect mutations. These results indicate that the affected gene(s) responsible for primary hypoaldosteronism other than *CYP11B2* remain to be determined.

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Conflict of interest The authors do not have a financial relationship with the organization that sponsored the research.

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