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Two novel mutations of the CYP11B2 gene in a Japanese patient with aldosterone deficiency type 1

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Abstract. Isolated hypoaldosteronism is a rare and occasionally life-threatening cause of salt wasting in infancy. A 2-month-old Japanese boy of unrelated parents was examined for failure to thrive and poor weight gain. Laboratory findings were hyponatremia, hyperkalemia, high plasma renin and low aldosterone levels. Spot urine analysis by gas chromatography-mass spectrometry (GC-MS) showed that urinary excretion of corticosterone metabolites was elevated. Whereas excretion of 18-hydroxycorticosterone metabolites was within the normal range, excretion of aldosterone metabolites was undetectable. The patient was therefore suspected to have aldosterone synthase deficiency type 1. Sequence analysis of *CYP11B2*, the gene encoding aldosterone synthase (CYP11B2), showed that the patient was a compound heterozygote for c.168G>A, p.W56X in exon 1 and c.1149C>T, p.R384X in exon 7. p.W56X was inherited from his mother and p.R384X was from his father. Since both alleles contain nonsense mutations, a lack of CYP11B2 activity was speculated to cause his condition. To our knowledge, this is the first Japanese patient in which the molecular basis of aldosterone synthase deficiency type 1 has been clarified. This case also indicates that spot urinary steroid analysis is useful for diagnosis.

Key words: Aldosterone, CYP11B2, Mutations, Urinary steroid profile

ALDOSTERONE is the principal mineralocorticoid hormone in humans and is synthesized by aldosterone synthase (CYP11B2) in the adrenal zona glomerulosa. Conversion of deoxycorticosterone (DOC) to aldosterone requires hydroxylation at position 11 β to form corticosterone (B), hydroxylation at position 18 to form 18-hydroxycorticosterone (18OHB), and finally oxidation at position 18. All three reactions require the same enzyme, CYP11B2 [1, 2].

Aldosterone synthase deficiency usually presents in infancy as a life-threatening electrolyte imbalance. Affected children typically display failure to thrive, vomiting and severe dehydration [1, 2]. The biochemical features are hyperkalemia, hyponatremia and metabolic acidosis, with elevated plasma renin activity and low or undetectable aldosterone [1, 2]. Patients

with aldosterone synthase deficiency type 1 have undetectable aldosterone levels, increased levels of 18-hydroxy-11-deoxycorticosterone (18OHDOC), reduced levels of 18OHB and an increased B/18OHB ratio. Aldosterone synthase deficiency type 1 is caused by mutations in *CYP11B2* that completely abolish enzymatic activity [3-6]. In contrast, aldosterone synthase deficiency type 2 is characterized by low aldosterone levels, increased 18OHB and 18OHDOC levels, and an increased 18OHB/aldosterone ratio. This form of aldosterone synthase deficiency is caused by mutations that decrease both 18-hydroxylase and 18-oxidase activities, but do not affect 11 β -hydroxylase activity [2, 7, 8].

To our knowledge, there is no report of Japanese patient in whom the molecular basis of aldosterone synthase deficiency type 1 has been clarified. Herein, we describe a Japanese patient with aldosterone synthase deficiency type 1 in whom a spot urinary steroid profile and *CYP11B2* were analyzed.

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Table 1 Laboratory findings in the patient

Blood	Value	Normal range for age	Urine steroid profiles (mg/g creatinine)	Value at 3 months	Reference values (2-4 months of age, 18 males, 2.5-97.5 percentile)
Na (mEq/L)	130	136-145	THDOC	0.44	0.01-0.07
K (mEq/L)	6.4	3.5-5.0	THB 5 β THB	1.09	<0.01-0.11
			5 α THB	6.92	0.18-2.10
PRA (ng/mL/hr)	156	3.66-12.05	THA 5 β THA	2.37	0.05-0.70
			5 α THA	4.22	0.29-1.55
Aldosterone (ng/dL)	10	26.9-75.8	18OHTHA	0.45	0.15-0.77
Cortisol (μ g/dL)	10.2	7.1-17.1	THAld	undetectable	0.03-0.20

THDOC, tetrahydrodeoxycorticosterone; THB tetrahydrocorticosterone; THA, tetrahydro-11-dehydrocorticosterone; 18OHTHA, 18-hydroxy-tetrahydro-11-dehydrocorticosterone; THAld, tetrahydroaldosterone

Methods

Genetic study

The ethical committee of Hokkaido University School of Medicine approved this study. Written informed consent to participate in the study was obtained from the patient's parents.

Blood samples were obtained from the patient and both parents, from which genomic DNA was extracted. All nine exons of *CYP11B2* of the patient, his father and mother were specifically amplified by PCR in two fragments (from exon 1 to exon 6 and from exon 3 to exon 9) as previously described [4]. After amplification, the PCR products were purified and sequenced directly using an ABI PRISM Dye Terminator Cycle Sequencing Kit and an ABI 373A automated fluorescent sequencer (PE Applied Biosystems, Foster City, CA).

Case report

The patient was apparently normal at birth (40 wk gestation). Birth weight was 3262 g (+0.22 SD), and length was 50 cm. The parents are Japanese and non-consanguineous. At 3 months of age he was admitted to hospital for failure to thrive and poor weight gain. Upon medical examination, his body weight was 4196g (-2.9 SD), and his skin was not pigmented. Initial laboratory examinations showed hyponatremia (130 mEq/L) and hyperkalemia (6.4 mEq/L), metabolic acidosis (pH, 7.148, vein) and elevated blood urea nitrogen (16.6 mg/dL, normal range for this age 3-12 mg/dL). Endocrinological evaluation showed low plasma aldosterone concentration (10 ng/dL, nor-

mal range for this age 26.9-75.8 ng/dL) and markedly elevated plasma renin activity (PRA) 156 ng/mL/hr, normal range for this age, 3.66-12.05 ng/mL/hr) (Table 1). Serum cortisol level was 10 μ g/dL and plasma adrenocorticotropin (ACTH) was 75 pg/mL. His blood 17-hydroxyprogesterone was within the normal range. His urinary Na was inappropriately elevated (44.2 mEq/L) despite of his hyponatremia. There were no signs of salt-wasting nephropathy or enteropathy, urinary tract infection or obstruction. An abdominal computed scan detected no hypertrophy of the adrenal gland. He was suspected to have an adrenal disease and hydrocortisone (5 mg/day), fludrocortisone (0.05mg/day), and NaCl (1g/day) were initiated. After treatment, he showed weight gain.

A spot urine sample was obtained before treatment and its steroid profile was analyzed by gas chromatography-mass spectrometry (GC-MS) as described previously [9, 10]. The results are summarized in Table 1. Urinary excretion B metabolites [tetrahydrocorticosterone (THB) and tetrahydro-11-dehydrocorticosterone (THA)] were elevated. Whereas excretion of metabolites of 18OHB was within the normal range, aldosterone and its metabolites were undetectable (Table 1, Fig. 1). These findings indicated aldosterone synthase deficiency type 1.

After confirmation of the diagnosis of aldosterone synthase deficiency type 1, hydrocortisone was gradually discontinued while fludrocortisone and NaCl supplementation were continued. The patient is currently seven months old, and in good health. The dose of fludrocortisone has been increased to 0.1 mg/day.

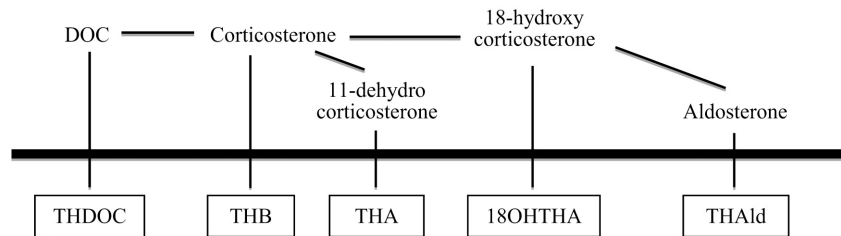


Fig. 1 Schematic representation of steroids and their urine metabolites

THDOC, tetrahydrodeoxycorticosterone; THB, tetrahydrocorticosterone; THA, tetrahydro-11-dehydrocorticosterone; 18OHTHA, 18-hydroxy-tetrahydro-11-dehydrocorticosterone; THAld, tetrahydroaldosterone

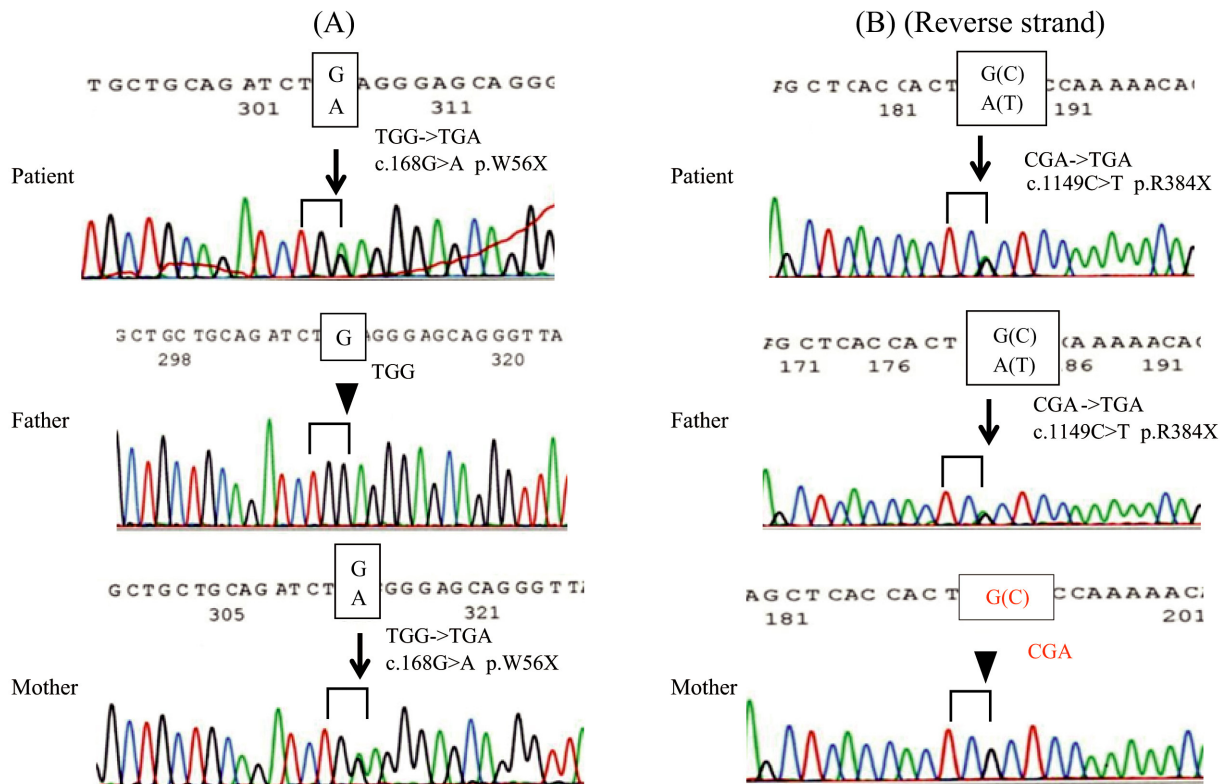


Fig. 2 Results of direct sequencing

Heterozygous mutations are marked by arrow and only wild type nucleotide is marked by arrow head.

(A) Sequence of exon 1. The heterozygous base change from G to A point mutation at c.168 in exon 1 leads to a change of tryptophan 56 to a termination codon (p.W56X) in the patient and his mother. His father had only wild type nucleotide. (B) Sequence of exon 7. Note that the sequence shown in the figure B is reverse strand. The heterozygous base change from G to A point mutation at c.1149 in exon 7 leads to a change of arginine 384 to a termination codon (p.R384X) in the patient and his father. His mother had only wild type nucleotide.

Results

Direct sequencing of all nine exons of *CYP11B2* of the patient identified a heterozygous G to A change at codon 56 in exon 1, resulting in a stop codon (c.168G>A, p.W56X Fig. 2A). Sequencing of *CYP11B2* in the patient also identified a heterozygous C to A change at codon

384 in exon 7 resulting in a stop codon (c.1149C>T, p.R384X) (Fig. 2B). Sequencing of *CYP11B2* in the parents demonstrated that the mother was heterozygous for c.168G>A in exon 1 and the father was heterozygous for c.1149C>T in exon 7 (Fig. 2A, B). These two mutations have been previously undescribed.

Discussion

Children with aldosterone synthase deficiency typically display failure to thrive, vomiting and severe dehydration; the biochemical features are hyperkalemia, hyponatremia and metabolic acidosis with elevated PRA, and low or undetectable aldosterone. Aldosterone synthase deficiency type 1 is caused by mutations that abolish all CYP11B2 activity *in vitro*. Therefore, B and 18OHDOC produced by CYP11B1 become the terminal steroids. Consequently, increased angiotensin II stimulates *CYP11B1* expression and enhanced CYP11B1 activity overproduces B [1, 2]. CYP11B1 can catalyze the 18-hydroxylation of B to form 18OHB only to a minimal extent however [11], and cannot further oxidase 18OHB to aldosterone. The patient's urine steroid profile was typical of aldosterone synthase deficiency type 1 in that aldosterone metabolites were undetectable and levels of the B metabolites of THA and THB were elevated. Nguyen *et al.* [4] reported an aldosterone synthase deficiency type 1 patient, in whom a GC-MS steroid profile analysis of a spot urinary sample, which also showed no 18-oxygenated steroid metabolites, was useful for diagnosis. Since it is sometimes difficult in infants to collect urine samples over a 24 hour period, spot urinary steroid profiling is a useful tool to diagnose aldosterone synthase deficiency and other congenital adrenal diseases [10].

Nonsense mutations and frame shift mutations leading to premature stop codon in *CYP11B2* have been previously reported in patients with aldosterone syn-

thase deficiency type 1 [2, 3-6, 12]. Furthermore, several missense mutations (S315R, L324Q, R374W, R384P, L451F, and L461P) have been identified in type 1 patients. In some cases, *in vitro* expression analysis demonstrated a complete loss of enzymatic activity in these mutants [2-5, 13]. We have identified here two novel nonsense mutations of *CYP11B2*, and using familial analysis, confirmed that the patient was a compound heterozygote. While the functional consequences of the mutant alleles were not determined *in vitro*, since both mutations were nonsense, mRNA transcribed from the two alleles would presumably be subject to nonsense-mediated decay [14]. CYP11B2 activity in the patient would therefore be completely impaired, and the genotype is consistent with aldosterone synthase deficiency type 1.

While Mitsuuchi *et al.* [12] and Nomoto *et al.* [13] reported aldosterone synthase deficiency type 1, their patients were of North America and Turkish origin, respectively. To our knowledge, therefore, there is no previous report of a Japanese patient with aldosterone synthase deficiency type 1 in whom the molecular basis of the disease has been characterized.

In conclusion, this is the first report of a Japanese patient with aldosterone synthase deficiency type 1, in whom the molecular basis has been clarified and in which a spot urine profile has been used in diagnosis of this disease. Aldosterone synthase deficiency is very rare; however this disease is one of a number of diseases associated with hyponatremia in infancy.

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