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A Novel Case of Somatic *KCNJ5* Mutation in Pediatric-Onset Aldosterone-Producing Adenoma

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Aldosterone-producing adenoma (APA), a subtype of primary aldosteronism, is a common cause of secondary hypertension in adults. Somatic *KCNJ5* mutations have been identified in about 12%–80% of adult-onset APA. In contrast, there has been no previous reported case of pediatric-onset APA in whom a somatic *KCNJ5* mutation was confirmed.

We report an 11-year-old Japanese girl who had experienced recurrent headaches and nausea for more than 2 years before hypertension was observed (blood pressure, 150/82 mm Hg). Plasma renin activity was <0.1 ng/mL per hour even after a captopril-challenge or upright furosemide-loading test. Plasma aldosterone concentrations (PACs) before and after saline-infusion test were 28.0 and 40.6 ng/dL, respectively. Plasma adrenocorticotropic hormone (ACTH) and serum cortisol levels were 16.5 pg/mL and 16.7 μg/dL, respectively. The patient was diagnosed with APA in the left adrenal gland on the basis of selective adrenal venous sampling after ACTH stimulation (PAC in the left adrenal vein, 3630 ng/dL) and histopathologic findings of the tumor obtained by laparoscopic left adrenalectomy. Sanger sequencing of *KCNJ5* using genomic DNA from peripheral lymphocytes and laser-captured microdissected APA tissues demonstrated the presence of a somatic *KCNJ5* mutation p.L168R, previously reported only in adult-onset APA. Immunohistochemistry detected strong immunoreactivity for CYP11B2, but not for CYP11B1 in the APA, consistent with the endocrinologic findings in this patient.

Somatic *KCNJ5* mutations are also identified in pediatric-onset APA. Further cases are needed to elucidate functional characteristics of pediatric-onset APA with a somatic *KCNJ5* mutation.

Freeform/Key Words: primary aldosteronism, aldosterone-producing adenoma, somatic mutation, *KCNJ5*, pediatric

Primary aldosteronism (PA) is caused by autonomous secretion of aldosterone from the adrenal cortex. PA accounts for ~10% of secondary hypertension in adults, and 30% to 80% of the adult-onset

Abbreviations: ACTH, adrenocorticotropic hormone; APA, aldosterone-producing adenoma; FH, familial hyperaldosteronism; PA, primary aldosteronism; PAC, plasma aldosterone concentration.

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PA is due to aldosterone-producing adenoma (APA) [1]. The Japan Endocrine Society guidelines for the diagnosis and treatment of PA recommend measurement of plasma renin activity and plasma or serum aldosterone concentration (PAC) in all patients initially diagnosed with hypertension [2]. However, PA is not a major cause of secondary hypertension in children [3]. Only 23 children younger than age 15 years with APA have been previously reported. The clinical characteristics of pediatric-onset APA remain elusive.

In 2011, somatic *KCNJ5* mutations were identified in adult-onset APA [4]. The somatic *KCNJ5* mutations of adult-onset APA was detected in 43% (range, 12% to 80%) with ethnic variation [5]. However, pediatric-onset APA in patients in whom a somatic *KCNJ5* mutation was confirmed has not been reported.

We report an 11-year-old Japanese girl with APA with a somatic *KCNJ5* mutation and review the clinical presentations of previously reported cases of pediatric-onset APA and familial hyperaldosteronism (FH) type III carrying germline *KCNJ5* mutations.

1. Case Report

An 11-year-old girl was referred to us for hypertension and hypokalemia. She was diagnosed with "cyclic vomiting" on the basis of recurrent headaches and nausea for more than 2 years. There was no family history of hypertension or any other symptoms associated with hypokalemia. Her height and weight were 154.4 cm (75th to 90th percentile) and 41.5 kg (25th to 50th percentile), respectively. Her blood pressure was 149/105 mm Hg (95th percentile for age, 126/82 mm Hg), and her heart rate was 92 beats/min. Her serum potassium was 2.7 mEq/L. Venous blood pH and HCO_3^- were 7.437 and 29.5 mEq/L, respectively, indicating metabolic alkalosis. Her plasma renin activity was <0.1 ng/mL per hour (reference range, 0.6 to 7.5 ng/mL per hour), and her PAC was 28.6 ng/dL (reference range, 0.2 to 20 ng/dL). Plasma ACTH and serum cortisol levels were 16.5 pg/mL and 16.7 μg/dL, respectively. These findings were consistent with PA, and further tests, including captopril-challenge, upright furosemide-loading,

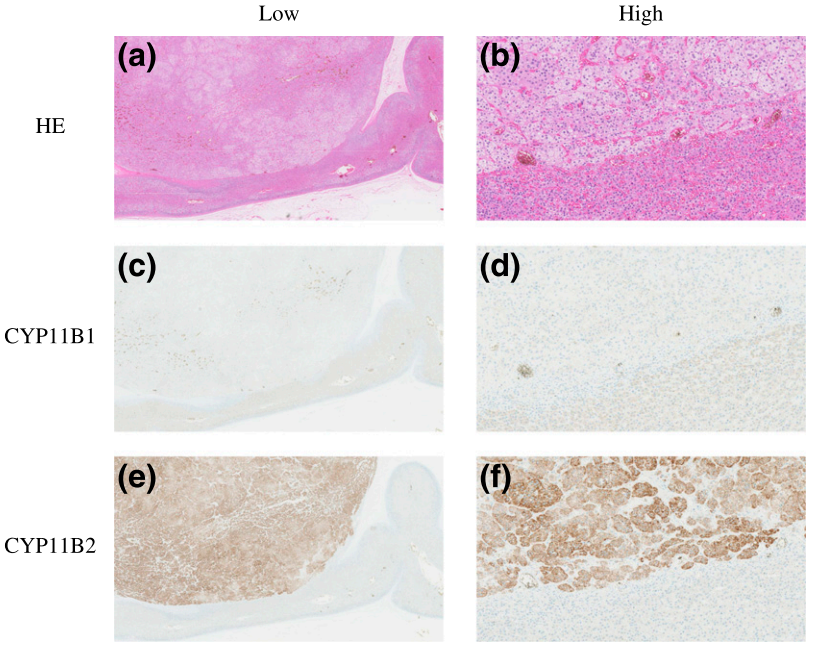

Figure 1. Histopathology of APA in lower-magnification (×2.5) and higher-magnification (×20) views. (a, b) Hematoxylin-eosin (HE) staining indicates a well-defined nodule with bright clear cells in the left adrenal gland. (c-f) Immunohistochemical staining detected strong immunoreactivity for CYP11B2, but not for CYP11B1, in the APA. Positive immunoreactivities are shown in brown.

and saline-loading tests, indicated autonomous aldosterone secretion (Supplemental Table 1), confirming initial diagnosis.

Because the patient and her family preferred tumorectomy to medication, we attempted to localize the putative adenoma by imaging evaluation and selective adrenal venous sampling. We could not detect adenoma by computed tomography and magnetic resonance imaging of the adrenal glands. Selective adrenal venous sampling showed that PAC of the left vein after ACTH stimulation (3630 ng/dL) was significantly higher than the cutoff level for APA (PAC > 1400 ng/dL), whereas serum cortisol level of the right vein after ACTH stimulation was not elevated enough (Supplemental Table 2) [2]. We then proceeded with laparoscopic left adrenalectomy. Macroscopically, the tumor was well circumscribed, and the size was 20 × 15 × 8 mm (Supplemental Fig. 1). Histopathologic examination showed that the tumor was a well-defined adrenocortical adenoma consisting of bright clear cells that were not typical for fasciculata or glomerulosa cells (Fig. 1). After adrenalectomy, her blood pressure and serum potassium levels were 110/76 mm Hg and 4.4 mEq/L, respectively.

We performed immunohistochemical staining using rabbit polyclonal antihuman CYP11B1 antibodies (5000-fold dilution) [6] and mouse monoclonal antihuman CYP11B2 antibody (10-fold dilution of hybridoma supernatant) that was produced by using a peptide corresponding to the amino acid residues from 41 to 52 as an antigen [7]. Immunohistochemistry detected strong immunoreactivity for CYP11B2, but not for CYP11B1 in the APA, compared with the residual adrenal cortex (Fig. 1).

We obtained written informed consent from the patient's parents to perform molecular studies, which were approved by the Ethics Committee of the Keio University School of Medicine. We extracted genomic DNA from peripheral lymphocytes and APA cells isolated from paraffin sections using laser-capture microdissection (PALM MicroBeam; Carl Zeiss MicroImaging GmBH, Jena, Germany). We analyzed *KCNJ5* in these two genomic DNA samples by polymerase chain reaction–based sequencing. The primers are shown in Supplemental Table 3. We detected a previously reported mutation [4], c.503T>G, p.L168R,

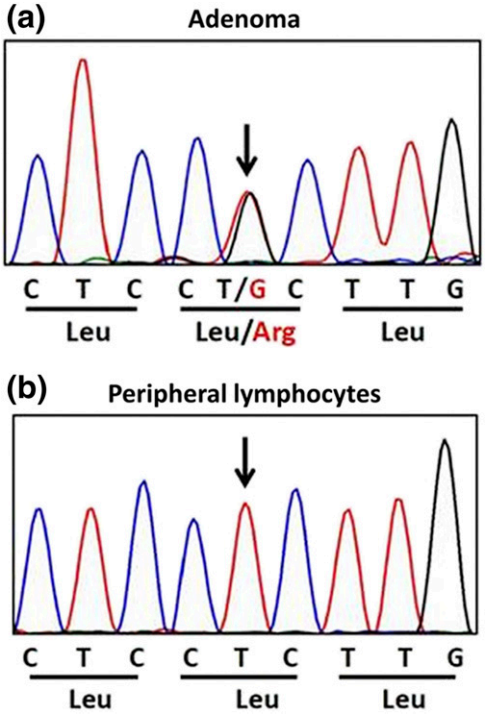

Figure 2. Chromatographs of Sanger sequencing of *KCNJ5*. (a) p.L168R was identified in genomic DNA extracted from aldosterone-producing adenoma. The heterozygous substitution of leucine by arginine is indicated by an arrow. (b) The genotype of genomic DNA from the patient's peripheral lymphocytes was wild-type.

in APA cells but not in peripheral lymphocytes (Fig. 2), indicating the presence of a somatic *KCNJ5* mutation in APA cells.

2. Discussion

We report an 11-year-old girl with APA who presented with recurrent headaches and nausea. She was not diagnosed with APA until severe hypertension and hypokalemia were evident. We identified a somatic *KCNJ5* mutation in the APA cells.

To our knowledge, there has been no previously reported case of pediatric-onset APA with a somatic *KCNJ5* mutation detected in APA cells using laser-capture microdissection. Somatic *KCNJ5* mutations were previously identified in 65% to 69% of Japanese patients with adult-onset APA, most frequently in the fifth decade of life [5]. Whether somatic *KCNJ5* mutations are sufficient for development of APA is still not clear. Taking into account that the patient already had symptoms that suggested the presence of APA at 9 years of age, we believe that the somatic *KCNJ5* mutation could be associated with pediatric-onset APA. Further studies are required to understand whether somatic *KCNJ5* mutations are a major genetic contributing factor in pediatric-onset APA.

Functional and morphologic characteristics of APA with a somatic *KCNJ5* mutation are not fully understood. Monticone *et al.* [8] reported that *KCNJ5*-mutated APAs in Italy were composed mainly of fasciculata-like cells with higher CYP11B1 and lower CYP11B2 expressions than those in APAs of other causes. Okamura *et al.* [9] found that *KCNJ5*-mutated APAs in Japan had higher *CYP11B2* and lower *CYP11B1* mRNA levels than those without mutation. The APA in our patient expressed abundant CYP11B2 and scarce CYP11B1. We speculate that the combination of abundant CYP11B2 and scarce CYP11B1 can be characteristic for pediatric-onset or Japanese APA with a somatic *KCNJ5* mutation.

Table 1. Clinical Features of Pediatric-Onset APA

Patient	Age at Onset, y	Age at Diagnosis, y	Blood Pressure, mm Hg	PRA , ng/mL per h	PAC, pmol/L	Potassium, mEq/L	Initial Symptoms	Supplemental Reference
1	1	3	160/110	NA	NA	2.4	P	1
2	6	7	150/106	<0.1	NA	4.1	E, behavioral change, back pain	2
3	7	10	200/100	0.07	28,530	1.4	E, P	3
4	NA	8	150/120	<0.2	1108	2.2	Asymptomatic	4
5	7	9	130/90	NA	NA	2.0	P	5
6	7	7	140/100	<0.2	693	3.3	H	6
7	7	10	125/100	0.04	1222	2.5	E, P	7
8	NA	10	170/130	0.02	604	3.5	Asymptomatic	8
9	3	5	160/104	<0.15	1235	1.4	M, P	9
10	9	9	160/110	0.08	1665	1.2	E, M, P	10
11	7	10	180/120	<0.2	1711	2.3	H	11
12	9	11	170/110	0.18	1040	2.2	H	12
13	10	11	180/120	0.3	1320	1.9	D, H, tinnitus	13
14	1	10	130/100	0.6	NA	2.8	H, M, P	14
15	10	11	230/126	<0.3	1260	1.9	D, H, tinnitus	15
16	4	5	150/80	0.1	3047	1.5	D, M, P	16
17	0	0	137/97	0.5	>4100	3.1	Asymptomatic	17
18	11	11	140/96	NA	2216	2.4	M, palpitation, paresthesia	18
19	6	8	130/90	3.59	1025	2.9	H, paresthesia	19
20	12	12	200/115	<0.2	475	4.2	D	20
21	13	13	140/100	<0.2	1385	2.0	M	21
22	9	10	170/100	0.1	1160	<1.5	H, P, palpitation	22
23	13	15	150/100	3.9	2789	2.3	H	23
This case	9	11	149/105	<0.1	792	2.7	H, nausea	—

Abbreviations: D, dizziness; E, enuresis; H, headache; M, muscular weakness; NA, not available; P, polyuria and polydipsia; PRA, plasma renin activity.

Table 2. Clinical Features of FH Type III

Patient	*KCNJ5* Genotype	Age at Onset, y	Age at Diagnosis, y	Blood Pressure, mm Hg	PRA , ng/mL per h	PAC, pmol/L	Potassium, mEq/L	Initial Symptoms	Supplemental Reference
24	p.T158A	3	5	230/140	NA	NA	2.8	H, P	24, 25
25	p.T158A	NA	7	188/140	0.3	3805	1.8	NA	25
26	p.T158A	NA	4	148/114	0.2	5127	1.9	NA	25
27	p.G151R	NA	1	174/85	<0.2	NA	2.1	Muscular weakness	26
28	p.G151R	NA	1	120/70	0.1	NA	1.4	NA	26
29	p.G151R	NA	1	130/90	<0.2	2440	3.2	P, failure to thrive	26
30	p.G151R	NA	4	127/80	<0.1	638	1.7	NA	26
31	p.G151E	NA	11	160/120	NA	NA	3.0	P	26, 27
32	p.G151E	NA	2	135/85	<0.1	NA	3.5	Asymptomatic	26, 28
33	p.G151E	NA	1	153/94	<0.1	2520	2.6	NA	26
34	p.G151E	NA	0	130/NA	<0.1	8230	4.4	NA	26
35	p.G151E	NA	4	NA	<0.1	NA	NA	NA	26
36	p.G151E	NA	<6	NA	NA	NA	NA	NA	26
37	p.I157S	NA	2	150/100	0.22	3269	2.7	NA	29
38	p.I157S	NA	7	170/100	<0.1	NA	NA	NA	29
39	p.G151E	2	2	130/80	0.1	3130	2.8	P	30, 31
40	p.G151E	<10	24	190/115	0.5	1479	2.9	P	30, 31
41	p.Y152C	48	62	180/110	<0.6	665	2.1	H, nausea	32
42	p.G151R	1	4	130/90	0.1	641	3.1	P	33
43	p.E145Q	0	2	115/65	0.2	>5000	2.3	P, failure to thrive	34

Abbreviations: H, headache; NA, not available; P, polyuria and polydipsia; PRA, plasma renin activity.

Our patient was diagnosed with PA more than 2 years after her first visit for headache and nausea. We reviewed previously reported cases of pediatric-onset APA and cases of FH type III due to germline *KCNJ5* mutations in patients younger than age 15 years (Tables 1 and 2). Blood pressure at the first visit for medical attention was not reported in eight patients with APA and five with FH type III (patients 1, 3, 7, 9, 11, 12, 19, 23, 24, 31, and 41 to 43) (Supplemental references 1, 3, 7, 9, 11, 12, 19, 23–27, 32–34). One patient with APA (patient 5) showed mild hypertension but was not evaluated in the next 2 years (Supplemental reference 5). It is important to stress the importance of blood pressure measurement in children who experience headaches or symptoms associated with hypokalemia. Wyszyńska *et al.* [10] reported that hypertension in children is secondary to a known disease in 98% of cases. Therefore, children with hypertension should be further evaluated to reveal possible underlying causes.

According to the clinical features of pediatric-onset APA, the initial symptoms of pediatric-onset APA were not specific and were similar to those of FH type III. In addition, onset ages between pediatric-onset APA (range, 0 to 13 years) and FH type III (range, 0 to 48 years) overlapped, even though patients with FH type III were diagnosed at a younger age than those with APA. Molecular analysis would help distinguish between APA and FH type III in pediatric-onset PA because imaging studies, including computed tomography and magnetic resonance imaging, are not always informative [2], and selective adrenal venous sampling is difficult for young children.

In conclusion, somatic *KCNJ5* mutations are associated with pediatric-onset APA as well as adult-onset APA. Further cases are needed to elucidate molecular, functional, and morphologic characteristics of pediatric-onset APA with a somatic *KCNJ5* mutation.

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