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ARTICLE

Steroid metabolism gene variants and their genotype-phenotype correlations in Chinese early-onset hypertension patients

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Abstract

The genetic factors related to early-onset hypertension are largely unknown. This study aimed to determine the spectrum of steroid metabolism gene variants and the clinical relationships of these variants to phenotypes in Chinese patients with early-onset hypertension. A total of 306 consecutive early-onset hypertensive patients were recruited. All coding exons and flanking intronic regions of *KCNJ5*, *CYP11B1*, and *CYP17A1* were sequenced. Long-distance polymerase chain reaction was used to search for a *CYP11B1/CYP11B2* chimeric gene. Pedigree investigations and genotype–phenotype analyses were performed for patients with rare variants. Nine rare variants were detected in eight patients (2.6%), but no *CYP11B1/CYP11B2* chimeric gene was identified. One patient and two of her siblings were found to carry compound heterozygous mutations (C183Y and T390R) in *CYP17A1* and were eventually diagnosed with atypical congenital adrenal hyperplasia. Patients with rare variants had younger ages of onset [17 (16, 20) vs. 30 (23, 35) years old, $p = 0.010$] and higher systolic blood pressure (148.5 ± 9.6 vs. 137.9 ± 17.8 mmHg, $p = 0.021$) than those without rare variants. Additionally, the patients and their relatives carrying rare variants exhibited increased serum free corticosterone [230.4 (7.4, 533.0) vs. 1.9 (0.9, 6.7) ng/ml, $p = 0.001$] and 11-deoxycorticosterone [16.16 (0.59, 33.23) vs. 0.77 (0.41, 0.96)ng/ml, $p = 0.038$] levels. Genetic testing is useful for the etiologic diagnosis of early-onset hypertension. Rare variants in steroid metabolism genes were associated with more severe clinical expression and abnormal circulating steroid metabolites in patients with early-onset hypertension.

Keywords genotype–phenotype correlation · steroid metabolism · hypertension · congenital adrenal hyperplasia · rare variant

Introduction

Hypertension, which increases the risk of stroke, heart attack, and kidney failure, is a global cause of morbidity and mortality. Many cases of early-onset hypertension are associated with an inherited disturbance or secondary forms of hypertension [1]. Adrenal steroid metabolism has a close relationship with blood pressure, and three types of familial hyperaldosteronism (FH) and two types of congenital adrenal hyperplasia (CAH) are Mendelian forms of

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hypertension that are related to steroid metabolism [2, 3]. These conditions cause increased levels of aldosterone and its precursors, including corticosterone, deoxycorticosterone, and other hybrid steroids, affecting salt and water balance through renal cortical mineralocorticoid receptors and leading to hypokalemia and elevated blood pressure [4, 5]. FH is autosomal dominant. FH type I (FH-I, glucocorticoid-remediable aldosteronism, GRA) is a consequence of abnormalities in the chimeric *CYP11B1/CYP11B2* gene. In these patients, the aldosterone synthase gene (*CYP11B2*) is regulated by the promoter of *CYP11B1*; thus, aldosterone biosynthesis is regulated by adrenocorticotropic hormone (ACTH) instead of by AngII, resulting in the dysregulated production of aldosterone. An abnormality in the *KCNJ5* gene is responsible for FH type III (FH-III). In contrast, the pathogenic gene causing FH type II remains unknown [6]. CAH is a recessively inherited disorder caused by deficiencies in 11β-hydroxylase (*CYP11B1* mutation) and 17 α-hydroxylase/17,20-lyase (*CYP17A1* mutation) [7, 8]. Despite knowledge of links between these genes and inherited hypertension, the association of steroid metabolism gene variants and early-onset hypertension is unclear. In the current study, we sequenced these genes in an early-onset hypertension cohort and analyzed clinical and genetic data to determine the variant profile and associations of these variants with clinical expression.

Subjects and methods

Enrollment of subjects

Between 2012 and 2014, we consecutively enrolled 306 early-onset hypertensive subjects from the Hypertension Center of Fuwai Hospital, Chinese Academy of Medical Sciences. Early-onset hypertension was defined as diagnosis before age 40 years. Subjects with secondary causes of hypertension, including renal hypertension, renovascular hypertension, aortic coarctation, pheochromocytoma, hyperthyroidism, and Cushing syndrome but not primary aldosteronism were excluded. Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the Ethics Committee of Fuwai Hospital. All researchers were trained to participate in the study. Blood pressure was measured using a mercury sphygmomanometer, with the subject placed in the sitting position for at least 15 min before beginning the measurements. The average of three values measured every 5 min was used. For accurate measurement, a large cuff size (16 cm × 42 cm) was used for patients who had arm circumferences greater than 50 cm. Hypertension was defined as blood pressure >140/90 mmHg on three separate occasions or the use of antihypertensive drugs. Clinical data were collected, including target organ damage and complication details. The left ventricular mass index was calculated using the Mosteller formula. The development of secondary sex characteristics was evaluated by Tanner stage [9, 10].

Sequencing of *KCNJ5*, *CYP11B1*, and *CYP17A1*

Targeted sequencing of three steroid metabolism-related genes was performed at Bestnovo Medical Technology Co. Ltd. (Beijing, China). DNA was extracted from peripheral blood leukocytes and purified. All coding exons and flanking regions of the *KCNJ5*, *CYP11B1*, and *CYP17A1* genes were enriched using a custom-designed library (Agilent Technologies, Santa Clara, CA, USA) and subsequently sequenced using a Genome Analyzer HiSeq 2500 (Illumina Inc., San Diego, CA, USA). Sequencing reads were mapped to the human reference genome GRCh37/hg19 with Burrows-Wheeler Aligner (BWA) (Version 0.7.12-r1039). After the removal of polymerase chain reaction (PCR) duplications with Picard (Version 1.112), variants were determined using Varscan (Version 2.2.5).

Variants were described according to the guidelines for mutation nomenclature of the Human Genome Variation Society (http://www.hgvs.org/). A variant was defined as novel if it was absent in the Human Gene Mutation Database. To exclude common polymorphisms and likely neutral variants, the following were excluded in the genotype-phenotype relationship analysis: (i) synonymous variants; (ii) variants with a minor allele frequency of ≥ 1% in the 1000 Genomes Project [11] and Exome Variant Server (ESP; http://evs.gs.washington.edu/EVS/); (iii) missense variants predicted as benign by both PolyPhen-2 and SIFT; [12, 13] and (iv) intronic variants not predicted by Human Splicing Finder as affecting the splice site [14]. All rare variants included in the study were validated by Sanger sequencing using an ABI 3500xl or ABI 3100 autosequencer (Life Technologies, Carlsbad, CA, USA).

Genotyping of the *CYP11B1/CYP11B2* chimeric gene

For GRA detection, long-distance PCR (LD-PCR) of the *CYP11B1/CYP11B2* chimeric gene was performed using Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA). The PCR primers used for chimeric gene detection were 1998B1F (5′-TCT ACG CTC ATG CAC CCC CAA TGA GTC CCT G-3′) and 1998B2R (5′-AGT GGA GTC CTC CAG CTG CCT CTC AAC C-3′). Primers for the wild-type *CYP11B1* gene, 5′-TCT ACG CTC ATG CAC CCC CAA TGA GTC CCT G-3′ and 5′-AGT GGA GTC CTC CAG CTG CCT CTC AAC C C-3′, were used as a control [15, 16]. A PCR fragment approximately 4 kb in

Table 1 Rare *KCNJ5, CYP11B1*, and *CYP17A1* gene variants detected in patients with early-onset hypertension

Gene	Variants type	NA Change	AA Change	Affected patients	known pathogenic mutation	SIFT/ Polyphen	1000 G allele frequency	ESP allele frequency
KCNJ5								
exon2	missense	c.T834A	p.H278Q	1/306	No	++/++	0	0
exon3	missense	c.C1123T	p.R375W	1/306	No	++/++	0.0005	0.000077
CYP11B1								
exon3	nonsense	c.C421T	p.R141X	1/306	Yes	N/A	0	0
exon4	missense	c.C610G	p.L204V	1/306	No	-/++	0.0005	0
exon6	missense	c.G1099T	p.A367S	1/306	No	++/++	0	0
CYP17A1								
exon3	missense	c.G548A	p.C183Y	1/306	No	++/++	0	0
exon4	missense	c.C683T	p.T228I	1/306	No	-/+	0	0
exon6	nonsense	c.987delC	p.Y329X	2/306	No	N/A	0	0
exon7	missense	c.C1169G	p.T390R	1/306	Yes	N/A	0	0

NA nucleotide acid, *AA* amino acid, ++ probably damaging or deleterious, + possibly damaging or deleterious, − benign or tolerated, *ESP* exome variant server

size was considered to indicate the presence of the *CYP11B1/CYP11B2* chimeric gene.

Statistical analysis

The distribution of quantitative variables was tested for normality by a one-sample Kolmogorov–Smirnov test. Values are shown as the mean ± SD, *n* (%), or median (P25, P75). The mean levels of variables were compared using one-way ANOVA or a Mann–Whitney U test, and a chi-squared test was applied for analyzing the distribution of categorical variables. A two-sided *p*-value of <0.05 was considered statistically significant. All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA) for Windows statistical package.

Results

Variant profiles of steroid metabolism-related genes

A total of 45 *KCNJ5*, *CYP11B1*, and *CYP17A1* gene variants were detected in the 306 subjects (Supplementary Table 1), including nine rare variants found in eight (2.6%) of the patients. Two of the identified rare variants were in *KCNJ5*, three in *CYP11B1*, and four in *CYP17A1* (Table 1). Multiple rare variants were found in two patients: a trans-compound heterozygous variant in *CYP17A1* (p. C183Y and p. T390R) and a cis-compound heterozygous variant in *CYP11B1* (p. A367S and p. L204V, both inherited from the father). None of the 306 patients was found to carry a chimeric *CYP11B1/CYP11B2* gene (representatives of the LD-PCR products are shown in Supplementary Fig. 1).

Diagnosis of CAH by genetic testing

The patient carrying the trans-compound heterozygous rare variant in *CYP17A1* (proband, II-1 in Fig. 1 and Table 2) was a 19-year-old female with irregular menstruation who was diagnosed with essential hypertension by a routine hypertension diagnosis procedure. Her blood pressure remained at 150/110 mmHg when she was treated daily with 40 mg of valsartan. The T390R variant is a known pathogenic mutation in CAH [17], whereas the C183Y variant is novel. Both p. T390R and p. C183Y are located in a highly conserved region (Fig. 1): P183 in the helical region and P390 adjacent to a beta sheet near the membrane attachment site. A pedigree investigation revealed these two variants to be independently inherited from her parents, consistent with the recessive inheritance of CAH caused by mutations in the *CYP17A1* gene. Further physical and laboratory examinations showed that the patient lacked pubic hair and had decreased levels of plasma dehydroepiandrosterone (DHEA) and increased levels of ACTH, progesterone, and 17-hydroxyprogesterone (17OHP) (Table 2). Ultrasound examination showed that the patient also had an infantile uterus and multiple ovarian cysts (Fig. 2). A computed tomography scan of II-1 showed bilateral adrenal hyperplasia (Fig. 3). After treatment with 0.75 mg/day of dexamethasone and 30 mg/day of nifedipine, her blood pressure was controlled (120/70 mmHg), and her menstruation became regular.

In addition, pedigree analysis revealed that both of the proband's siblings (II-2 and II-3) were also carriers of p. C183Y and p. T390R in *CYP17A1* and were masked hypertension patients. The karyotypes of II-1, II-2, and II-3 were 46XX, 46XY, and 46XX, respectively

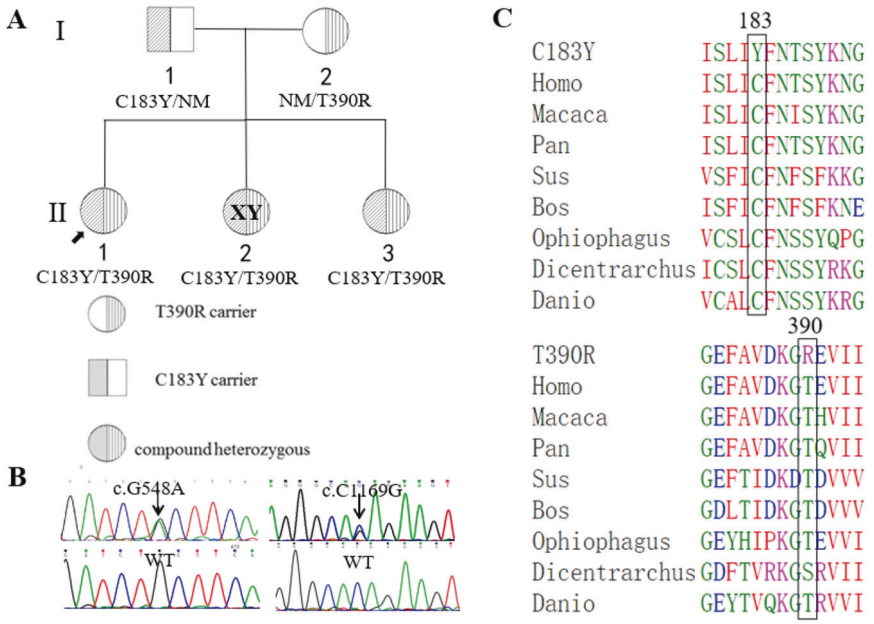

Fig. 1 Family tree of the CAH family, genetic work-up of the two *CYP17A1* rare variants, and analyses of conservation during species evolution. **a** Pedigree of the CAH family studied in this work. Males are indicated by squares, and females are indicated by circles. Filled squares/circles indicate variant carriers. The proband is indicated by an arrow. The *CYP17A1* genotype is shown for family members. (NM, nonmutated; WT, wild-type). The 46,XY patient was raised as a female. **b** The original sequence (forward) of the *CYP17A1* gene with the two rare variants. **c** Protein sequence homology of mutation-affected regions among species, as determined using Clustal Omega. The C183Y and T390R substitutions are highly conserved among species

(Supplementary Fig. 2). II-2 was a 15-year-old female who had pseudohermaphrodism with a male chromosomal complement. Ultrasound examination of this patient showed no uterus or ovaries, and bilateral undescended testes were detected by pelvic computed tomography (Fig. 2). II-3 was an 8-year-old female with normal secondary sex characteristics for her age, and a normal uterus and ovaries were detected by ultrasound (Fig. 2). Steroid analysis indicated increased levels of ACTH, 17OHP, and progesterone in both of these subjects, with II-2 having a more severe clinical and steroid hormone disorder (Table 2). Based on the combination of clinical manifestations and genetic testing, both were also diagnosed with CAH. II-2 was treated by orchiectomy to prevent the potential development of testicular neoplasms.

Clinical manifestation of *KCNJ5* carriers

Two patients carried rare variants in *KCNJ5*. The individual carrying p. R375W was hypertensive at 40 years old without a family history of hypertension and was diagnosed with aldosterone-producing adenoma (APA). The patient harboring p. H278Q displayed resistant hypertension with normal serum K^+ levels, plasma renin activity and aldosterone levels (Supplemental Table 2). His father, who carried the same variant, had primary hypertension without increased aldosterone and adrenal hyperplasia or adenomas. Neither subject had a phenotype similar to that of several previously reported FH-III patients, who developed hypertension at ages younger than 20 years old and had severe resistant arterial hypertension and hypokalemia [18, 19].

Clinical manifestations of multiple *CYP11B1* carriers

One patient carried a compound heterozygous rare variant of *CYP11B1* (p. A367S and p. L204V, both inherited from his father). He was diagnosed with essential hypertension and had normal male genitalia; normal adrenal glands were observed via computed tomography. His steroid analysis showed a mildly increased progesterone level (1.38 ng/ml) but a normal aldosterone to renin ratio (14.1) and normal cortisol (21.88 μg/dl), DHEA (557 μg/dl), testosterone (4.1 ng/ml), ACTH (31.7 pg/ml), follicle-stimulating hormone (1.32 IU/L), luteinizing hormone (4.5 IU/L), and 17OHP (1.68 ng/ml) levels. Overall, his clinical manifestation was inconsistent with previously reported 11β-hydroxylase-deficiency patients.

Genotype-phenotype analysis

A total of eight patients carried rare variants of *KCNJ5*, *CYP11B1*, or *CYP17A1*, whereas 298 did not carry any rare variants of these genes. Compared with these 298 patients,

Table 2 Clinical characteristics of familial individuals with trans-compound heterozygous mutations in *CYP17A1*

Parameter (reference range)	II-1	II-2	II-3
Chromosome karyotype	46, XX	46, XY	46, XX
Age at diagnosis (years)	19	15	8
Age of admitted (years)	19	15	8
Height (cm)	165	168	135
Weight (kg)	65	95	28.5
Blood pressure (mm Hg)	160/100	156/86	110/70
MBP (mm Hg)	152/103	145/76	129/83
Tanner stages	B2, P1	G1, P1	B1, P1
ACTH (0–46 pg/mL)	108↑	87↑	86.5↑
Cortisol (4.0–22.3 μg/dL)	7.18	3.23↓	5.03
24hUFC (12.3–103.5 μg/dL)	30.45	32.68	16.24
17OHP (0.1–0.8 ng/mL)	1.93↑	2.16↑	1.29↑
PRA (0.93–6.56 ng/mL/h)	0.15↓	0.76↓	0.01↓
ALD (6.5–29.6 ng/dL)	13.29	10.49	9.48
K (3.5–5.5 mmol/L)	4.1	3.9	4.1
Na (135–145 mmol/L)	140	140	139
P (0.38–2.28 ng/mL)	14.46↑	9.58↑	6.78↑
E2 (27–122 ng/L in the early follicular phase; <47 ng/L in males; <60 ng/L in prepubescent girls)	57	9	11
DHEA (51–321 μg/dL in females; 44–332 μg/dL in males; <96 μg/dL in prepubescent girls)	32.9↓	34↓	18.8
FSH (<10 IU/L in the early follicular phase and prepubescent girls; 1.27–19.26 IU/L in males)	5.52	21.87↑	7.56
LH (2.12–10.89 IU/L in the early follicular phase; 1.24–8.62 IU/L in males; <6 IU/L in prepubescent girls)	10.09	22.4↑	0.62
PRL (<30 ng/mL in the early follicular phase and prepubescent girls; 2.64–13.13 ng/mL in males)	9.17	11.03	9.37
T (0.1–0.75 ng/mL in the early follicular phase; 1.75–7.81 ng/mL in males; <0.2 ng/mL in prepubescent girls)	0.24	0.23↓	<0.1

MBP mean blood pressure during 24-h ambulatory blood pressure monitoring, *UFC* urinary free cortisol, *Na* serum sodium, *K* serum potassium; *PRA* plasma renin activity; *ALD* aldosterone; *ACTH* adrenocorticotropic hormone; *DHEA* dehydroepiandrosterone, *E2* Estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *P* progesterone, *PRL* prolactin, *17OHP* 17 hydroxyprogesterone, *T* testosterone, *G* genitals, *B* breasts, *P* pubic hair, ↑ values above the upper limit of the normal range, ↓ values below the lower limit of the normal range

the subjects with rare variants exhibited a significantly younger age of hypertension onset [17 (16, 20) vs. 30 (23, 35) years old, $p = 0.010$] and a higher systolic blood pressure recorded during 24-h ambulatory blood pressure monitoring (148.5 ± 9.6 vs. 137.9 ± 17.8 mmHg, $p = 0.021$) (Table 3).

A total of eight patients and four relatives carried rare variants of *KCNJ5*, *CYP11B1*, or *CYP17A1*. Compared with 12 other patients who were selected as controls (matched age, sex, and blood pressure), these rare variant carriers had higher concentrations of serum free corticosterone [230.4 (7.4, 533.0) vs. 1.9 (0.9, 6.7)ng/ml, $p = 0.001$] and 11-deoxycorticosterone [16.16 (0.59, 33.23) vs. 0.77 (0.41, 0.96)ng/ml, $p = 0.038$] (Table 4).

Discussion

Abnormal biosynthesis, metabolism, or activity of steroid hormones can result in elevated blood pressure. The present study is the first to detect variant profiles of genes influencing steroid metabolism in early-onset hypertensive patients.

By using next-generation sequencing, we diagnosed one patient with 17 alpha-hydroxylase/17,20-lyase deficiency in a cohort of 306 patients. CAH and FH are rare, even among those with early-onset hypertension. Screening of these diseases in young hypertensive patients has been suggested [1], especially in those with a family history. Nonetheless, diagnosing these diseases based solely on clinical manifestations [6, 20], especially in patients with an atypical phenotype [21, 22], is rather difficult. Genetic diagnosis provides another means of screening such patients.

Diagnosis of CAH is usually established by abnormal secondary sexual characteristics, glucocorticoid deficiency, and hypokalemic hypertension. Among the proband and her two siblings who carried the *CYP17A1* compound heterozygous variants, two presented with atypical sexual and steroid profile disorders. All three of these patients had mildly elevated blood pressure, though their serum potassium and cortisol levels were in the normal ranges. Neither the proband nor her two siblings could have been diagnosed precisely without genetic testing, and appropriate treatment was administered once the diagnosis was clear. Indeed, all three patients showed normal blood pressure following treatment with dexamethasone combined with a low dose antihypertension drug. Routine hormone treatments were given to prevent infertility, and an operation was performed on the pseudohermaphrodism patient to prevent potential testicular neoplasms. As genetic detection of their conditions notably changed the medical strategies and clinical events, conducting genetic screening is worthwhile, especially in early-onset hypertensive patients.

In our early-onset hypertension cohort, no patients were found to carry the chimeric *CYP11B1/CYP11B2* gene. Gates et al. randomly chose 300 hypertensive patients attending a hypertensive clinic and failed to identify any GRA mutation-positive individuals by Southern blotting [23]. Our results illustrate that random screening for GRA

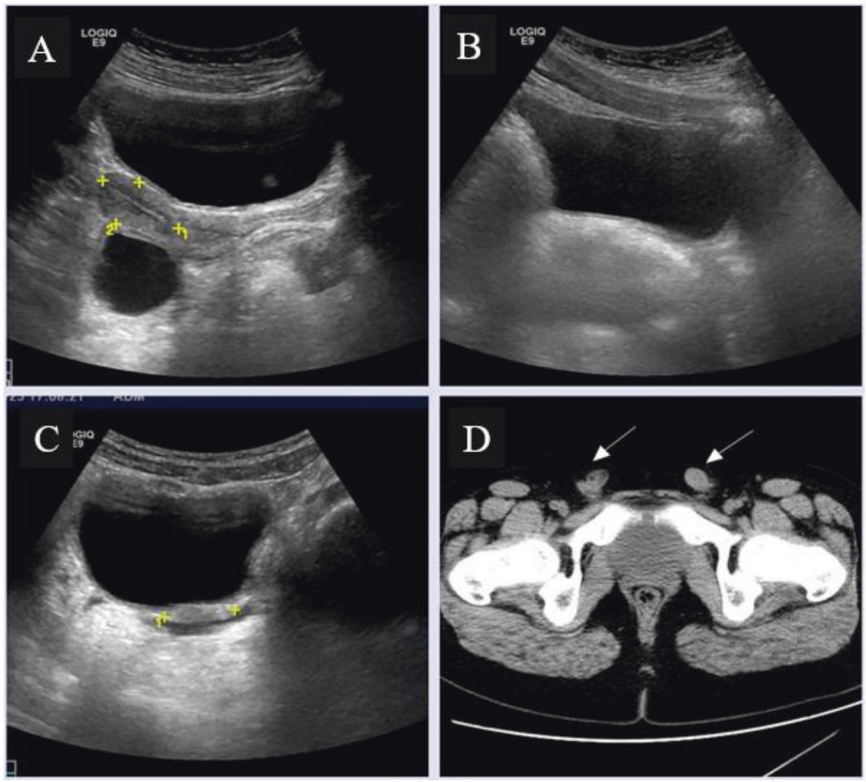

Fig. 2 Reproductive organ development of CAH patients. **a** Ultrasound examination of II-1 shows an infantile uterus and multiple ovarian cysts. The uterus was 3.5 cm × 3.4 cm × 2.0 cm, and the ovarian cysts ranged in size from 2.5 cm × 2.0 cm × 2.1 cm to 4.8 cm × 2.9 cm × 4.2 cm. The thickness of the uterine endometrium was 0.25 cm (second day of menstruation). **b** Ultrasound examination of II-2 showed no uterus or ovaries. **c** Ultrasound examination of the uterus and ovaries for II-3 revealed normal characteristics for her age. **d** Computed tomography of II-2 shows an undescended testicle

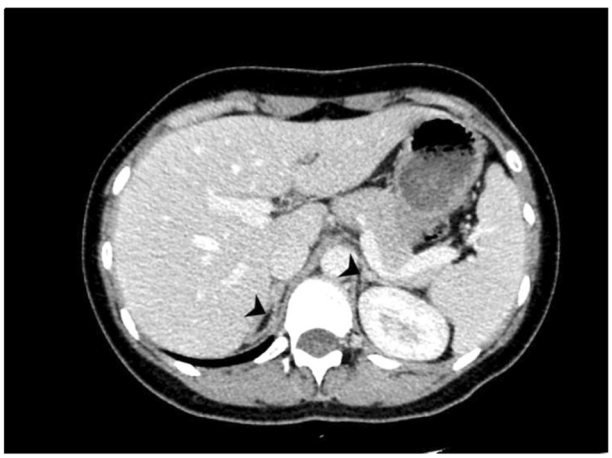

Fig. 3 Images of adrenal glands in CAH patients. **a** computed tomography scan of II-1 shows bilateral adrenal hyperplasia

in hypertensive patients might be nonsignificant in clinical practice.

However, FH patients may display a phenotype that is indistinguishable from sporadic primary aldosteronism. Mulatero et al. did not diagnose FH-III in any individual in a cohort of 300 consecutive primary aldosteronism (PA) patients, though the diagnosis of FH-III was based only on clinical manifestation and urinary steroid metabolites [20]. By searching for mutations in *KCNJ5*, researchers in 2011 found one FH-III family comprising two patients among 46 patients from 21 FH families [18]. They searched for FH patients only among PA patients with a family history of PA. The present study is the first to use genetic screening for FH in hypertension patients without a family history of PA. We screened non-PA and non-APA patients to determine the existence of other phenotypes. Interestingly, neither of the two *KCNJ5* rare variant carriers had been previously diagnosed with FH-III because they lacked a typical clinical manifestation. One of these subjects was diagnosed with APA at 40 years of age, and the other presented with resistant hypertension without adrenal abnormalities. Whether these rare *KCNJ5* variants play roles in the onset of APA and resistant hypertension is unknown, but functional studies might be useful for demonstrating the significance of rare variants. Although rare variants without clear functional information may have limited significance for the diagnosis of these diseases, they are useful for the exploration of disease mechanisms.

With regard to steroid metabolism-related hypertension (CAH, GRA, and FH-III), potassium excretion and water and sodium retention caused by overactivation of mineralocorticoid receptors are common. Furthermore, overactivation of mineralocorticoid receptors can promote inflammation, fibrosis, arteriosclerosis, left ventricular hypertrophy, retinopathy, and chronic kidney disease progression [24, 25]. Thus, the addition of a mineralocorticoid receptor antagonist (eplerenone, spironolactone) is indicated if blood pressure control is unsatisfactory [4]. Although CAH is an autosomal recessive condition, carriers of

Table 3 Comparison of the clinical features between the patients with and without rare variants in *KCNJ5*, *CYP11B1*, or *CYP17A1*

Parameter (reference range)	All	With rare variant ($n = 8$)	Without rare variant ($n = 298$)	p value
Age (years)	35.0 (28.0, 42.3)	20.5 (17.8, 29.5)	35.5 (28.0, 43.0)	0.009*
Female (n (%))	89 (29.1)	4 (50)	85 (28.5)	0.237
Age of on-set (years)	29 (23, 35)	17 (16, 20)	30 (23, 35)	0.010*
HT duration (years)	5 (1, 10)	4.5 (0.3, 7.8)	5 (1, 10)	0.487
Hyperlipemia (n(%))	49 (16.0)	0 (0)	49 (16.4)	0.363
CHD (n (%))	28 (9.2)	0 (0)	28 (9.4)	1.000
Stroke (n (%))	25 (8.2)	1 (4.0)	24 (8.1)	0.498
Diabetes (n (%))	28 (9.2)	0 (0)	28 (9.4)	1.000
Vascular disease (n (%))	11 (3.6)	1 (9.1)	10 (3.4)	0.256
BMI (kg/m^2)	26.8 ± 4.4	23.8 ± 2.7	26.9 ± 4.4	0.021*
MSBP (mmHg)	138.2 ± 17.7	148.5 ± 9.6	137.9 ± 17.8	0.021*
MDBP (mmHg)	89.5 ± 12.8	95.6 ± 6.6	89.3 ± 12.9	0.051
Serum cortisol (4.0–22.3 μg/dL)	14.75 (11.05, 19.58)	11.4 (4.1, 21.3)	14.9 (11.6, 19.6)	0.279
Serum K (3.5–5.5 mmol/L)	3.8 ± 0.5	3.6 ± 0.3	3.8 ± 0.5	0.123
PRA (0.93–6.56 ng/ml/h)	0.45 (0.16, 1.07)	0.76 (0.23, 1.94)	0.45 (0.16, 1.06)	0.459
ALD (6.5–29.6 ng/dL)	0.17 ± 0.05	1.38 ± 1.86	0.93 ± 0.45	0.369
MAlb/Cr (30–300 mg/g)	21.0 (8.0, 69.8)	72.3 (19.9, 81.6)	20.9 (7.75, 67.21)	0.220
BaPWV (cm/s)	1434 (1259, 1684)	1486 (1220, 1832)	1433 (1259, 1683)	0.639
LVMI (g/m^2)	95.7 (82.5, 112.5)	118.6 (83.4, 168.4)	95.5 (82.4, 111.8)	0.221

HT hypertension, *CHD* coronary heart disease, *BMI* body mass index, *MSBP* mean systolic blood pressure during 24-h ambulatory blood pressure monitoring, *MDBP* mean diastolic blood pressure during 24-h ambulatory blood pressure monitoring, *UK* urine potassium, *PRA* plasma renin activity, *ALD* aldosterone, *BaPWV* brachial ankle pulse wave velocity, *MAlb/Cr* urinary microalbumin/creatine ratio, *LVMI* left ventricular mass index, * $p < 0.05$

Table 4 Plasma mineralocorticoid precursor in patients with and without a rare variant in *KCNJ5*, *CYP11B1*, or *CYP17A1*

	Carriers ($n = 12$)	non-carrier ($n = 12$)	p value
Corticosterone - ng/ml	230.4 (7.4, 533.0)	1.9 (0.9, 6.7)	0.001
11-deoxycorticosterone – ng/ml	16.2 (0.6, 33.2)	0.8 (0.4, 1.0)	0.038

heterozygous *CYP17A1* mutations reportedly have lower adrenal 17α-hydroxylase activity and altered adrenal gland reserve for steroid biosynthesis when compared with carriers of the wild-type gene [26]. Similarly, individuals with heterozygous *CYP11B1* mutations have reduced 11β-hydroxylase enzyme activity [27]. In practice, some heterozygotes are rather difficult to distinguish from atypical CAH patients [28, 29]. In our study, we found that the levels of some mineralocorticoid precursors, such as those for corticosterone and deoxycorticosterone, were higher in patients with *CYP11B1* or *CYP17A1* heterozygous rare variants than in controls. It is possible that heterozygous rare variants may cause reduced activity of 11β-hydroxylase and 17α-hydroxylase, which is associated with reduced cortisol and accumulation of mineralocorticoid precursors. Therefore, rare variants of *KCNJ5*, *CYP11B1*, and *CYP17A1* might play a role in mineralocorticoid receptor overactivation. Mineralocorticoid receptor antagonists may be useful in these patients if blood pressure is not well controlled. In our study, carriers of rare variants in *KCNJ5*, *CYP11B1*, or *CYP17A1* generally had an earlier age of hypertension onset and higher blood pressure. Nonetheless, the significance of these rare variants needs to be verified in a larger population.

In conclusion, our results show that genetic testing is useful in the etiologic diagnosis of early-onset hypertension. Rare variants in steroid metabolism genes are associated with more severe clinical expression and abnormal circulating steroid metabolite levels in patients with early-onset hypertension.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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