

A frameshift mutation in the *SCNN1B* gene in a family with Liddle syndrome: A case report and systematic review

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Received March 20, 2023; Accepted October 31, 2023

DOI: 10.3892/mmr.2023.13142

Abstract. Liddle syndrome is an autosomal dominant form of monogenic hypertension that is caused by mutations in *SCNN1A*, *SCNN1B* or *SCNN1G*, which respectively encode the α , β and γ subunits of the epithelial sodium channel. In the present study, DNA was extracted from leukocytes in peripheral blood obtained from all members of a family with Liddle syndrome. Whole-exome sequencing and Sanger sequencing were performed to assess the candidate variant and a co-segregation analysis was conducted. A frameshift mutation in *SCNN1B* (NM_000336: c.1806dupG, p.Pro603Alafs*5) in the family was identified, characterized by early-onset hypertension and hypokalemia. The mutation led to the truncation of the β subunit of the epithelial sodium channel and a lack of the conservative PY motif. Furthermore, a systematic review of follow-up data from patients with Liddle syndrome with *SCNN1B* mutations was performed. The follow-up data of 108 patients with pathogenic *SCNN1B* mutations from 47 families were summarized. Phenotypic heterogeneity was evident in patients with Liddle syndrome and early-onset hypertension was the most frequent symptom. Patients responded well to targeted amiloride therapy with significant improvements in blood pressure and serum potassium concentration. The

present study demonstrates that confirmatory genetic testing and targeted therapy can prevent premature onset of clinical endpoint events in patients with Liddle syndrome.

Introduction

Liddle syndrome is an autosomal dominant form of monogenic hypertension with early penetrance. It was first characterized by Liddle (1) in 1963, and its molecular basis lies in mutations in *SCNN1A*, *SCNN1B* and *SCNN1G*, which respectively encode the α , β and γ subunits of the epithelial sodium channel (ENaC). The ENaC serves an important role in the maintenance of blood pressure through the reabsorption of sodium and water. Each subunit of the ENaC has a highly conserved proline-rich sequence in the cytosolic C-terminus, known as the PY motif (2). Individuals with Liddle syndrome have gain-of-function mutations, which cause damage to the PY motif, resulting in excessive sodium absorption and volume expansion (3). Therefore, Liddle syndrome responds well to ENaC blockers, but not to spironolactone, which is a mineralocorticoid receptor antagonist (4).

Typical clinical characteristics of Liddle syndrome include early-onset hypertension, refractory hypokalemia, low plasma aldosterone and renin concentrations, and metabolic alkalosis (5). Previously, Fan *et al* (6) summarized the clinical characteristics of 54 children with Liddle syndrome, reporting that the median age of typical hypertension onset was 12.75 years and the median age at genetic diagnosis was 14 years. Although most patients with Liddle syndrome have distinctive clinical features, certain individuals do not and are thus at risk of misdiagnosis. Genetic analysis is the gold-standard method for diagnosing Liddle syndrome at the molecular level. However, the genotype-phenotype association in Liddle syndrome is still currently unknown because of the limited number of reported cases (7).

The present study described a family with Liddle syndrome, characterized by early-onset hypertension and hypokalemia. Whole-exome sequencing and Sanger sequencing were used to identify pathogenic mutations in the family. A systematic review of follow-up data from patients with Liddle syndrome with *SCNN1B* mutations was also conducted to assess the phenotypic heterogeneity and prognosis of Liddle syndrome after tailored therapy.

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Key words: Liddle syndrome, *SCNN1B*, genetic testing, phenotypic heterogeneity, hypertension

Materials and methods

Compliance with ethical standards. The present study was approved by the Ethics Committee of Fuwai Hospital, Peking Union Medical College (Beijing, China; approval no. 2020-1321) and was performed in accordance with the Declaration of Helsinki. All study participants provided written informed consent before study enrolment.

Subjects and clinical evaluation. The proband (Fig. 1; III-2) was a 28-year-old male patient who was admitted with severe hypertension to the Department of Hypertension, Fuwai Hospital (Beijing, China) in 2021. The hypertension of the patient had been incidentally diagnosed during a medical examination at the age of 16 years. During hospitalization, detailed screening tests, including imaging (echocardiography and computed tomography of the aortic and renal arteries), physical examination, biochemical assessments (including plasma serum, aldosterone, renin and urinary microalbumin concentration measurements) and hormone measurements (including growth hormone, cortisol and adrenocorticotrophic hormone concentration measurements), were performed to identify the etiology of hypertension. Moreover, the fundus lesions of hypertension were evaluated by mydriasis followed by fundoscopy. The patient had a family history of early-onset hypertension. Affected family members included the father (II-4) of the patient, two paternal aunts (II-1 and II-5) and a deceased paternal uncle (II-7). Therefore, seven biological relatives of the patient (five females and two males) with a median age of 42 years (interquartile range 35, 60 years) were enrolled in the present study. They underwent full clinical evaluation (including blood pressure measurement, medical history and symptom assessment), biochemical testing (including plasma serum, aldosterone and renin concentration measurements) and genetic analysis.

DNA sequencing. DNA was extracted from leukocytes in the peripheral blood of all enrolled family members using the QIAamp® DNA Blood Mini kit (cat. no. 51104; Qiagen GmbH) according to the manufacturer's protocol. The DNA concentration and quality was measured using a Qubit® 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.). Additionally, gel electrophoresis was carried out with a 1% agarose gel to visually inspect the DNA for intact, high molecular weight bands, to ensure that it was not degraded or fragmented. Whole-exome sequencing was performed on DNA from the blood of the proband using the SureSelectXT Human All ExonV6 kit (cat. no. 5190-8864; Agilent Technologies, Inc.) for exome capture and the Novaseq 6000 platform (Illumina, Inc.) was used for genomic DNA sequencing (paired end; 150 bp). The concentration of the library was measured using a Qubit® 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) and then the size distribution of the library was analyzed using a NGS 3K reagent kit (cat. no. CLS960013; PerkinElmer, Inc.) and the loading concentration of the final library was 17.56 nM. Except for the proband, Sanger sequencing was performed on the DNA of the family members to evaluate the candidate variant and to perform co-segregation analysis. The forward primer used was 5'-CCCACCCAAGAATCACCT CC-3' and the reverse primer used was 5'-TCAGGACAGGTA

GGGACGAG-3'. To ensure that the mutation was accurately identified, the exon was sequenced forward and backward, and all products were analyzed using Chromas 2.22 software (Technelysium Pty Ltd.).

Genetic analysis. The whole-exome sequenced data were first checked using in-house quality control software to remove low-quality reads. The sequenced data were then aligned to the reference human genome (GRCh37/hg19) using Burrows-Wheeler Aligner (version 0.7.12) (8). SAM tool (version 0.1.19; <https://sourceforge.net/projects/samtools/files/samtools/0.1.19/>) and Sambamba (version 0.8.2; <https://github.com/biod/sambamba/releases>) were used to sort bam files and perform duplicate marking, generating the final bam file (9,10). Single-nucleotide variants and insertion/deletion variants were identified using the SAM tool (version 0.1.19) (10) and copy number variants were detected using CoNIFER (version 0.3) (11). Annotation was performed using ANNOVAR software (version 322; <https://annovar.openbioinformatics.org/en/latest/>). Mutations with a frequency of $\geq 1\%$ in the 1000 Genomes Project (1000G_all; <https://www.internationalgenome.org/>), ESP6500 (ESP6500SIV2_ALL; <https://esp.gs.washington.edu/drupal/>) and gnomAD (gnomAD_ALL and gnomAD_EAS; <https://gnomad.broadinstitute.org/>) databases were removed. *In silico* analysis software, including MutationTaster (MutationTaster2021; <https://www.mutationtaster.org/>) and Phylogenetic Analysis with Space/Time Models (phast) Cons (version 1.3; compgen.bscb.cornell.edu/phast) were used to predict the pathogenicity of variants. The pathogenicity of the sequence variants was assessed using the method developed by the American College of Medical Genetics and Genomics (ACMG) (12).

Systematic review and genotype-phenotype association analysis. The follow-up data of patients with Liddle syndrome were analyzed to evaluate the prognosis of patients with Liddle syndrome after targeted therapy. 'Liddle syndrome', 'Liddle's syndrome', 'pseudoaldosteronism' and 'SCNN1B' were used as key words to search publications in English in the MEDLINE (<https://www.medline.com/>), Cochrane (<https://www.cochranelibrary.com/>) and Web of Science (<https://clarivate.com/products/scientific-and-academic-research/research-discovery-and-workflow-solutions/webofscience-platform/>) databases from December 1963 to July 2023. Patients that were genetically diagnosed with Liddle syndrome with available follow-up data were included in the analysis, while those without genetic diagnostic information or follow-up data were excluded. As the prevalence of Liddle syndrome is low, ranging from 0.91-1.52%, the genotype-phenotype association is still unclear (13,14). Therefore, a genotype-phenotype association analysis was performed by reviewing the clinical and biochemical characteristics of patients carrying the same mutation as the participants in the present study.

Statistical analysis. Statistical analysis was performed using SPSS software (version 16.0, SPSS, Inc.). The data are presented as mean \pm standard deviation or frequency (%), as applicable. To assess the differences in blood pressure and serum potassium concentration before and after treatment with an ENaC

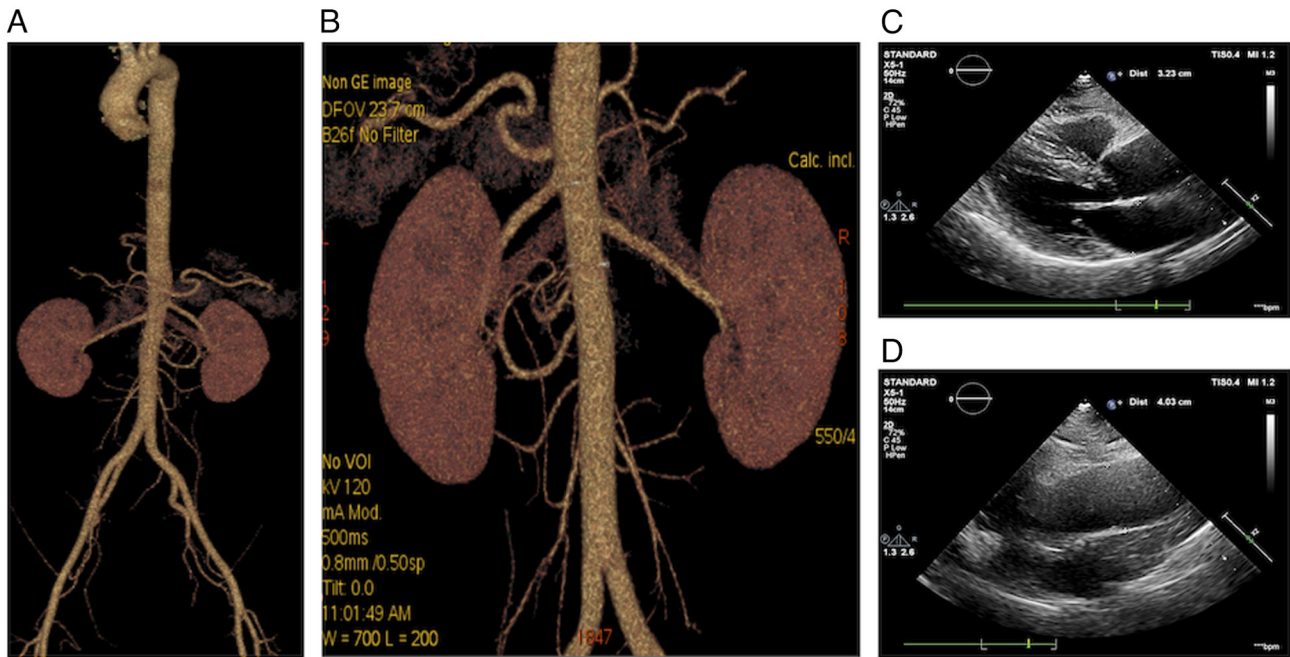


Figure 1. Imaging examination results of the proband. (A) Aortic artery computed tomography revealed normal findings. (B) Renal artery computed tomography also revealed a normal result. (C) Transthoracic echocardiography indicated thickening of the left ventricular posterior wall and interventricular septum. (D) Transthoracic echocardiography revealed the widening of the ascending aorta.

inhibitor, paired Student's two-tailed t-tests were used. To ensure the statistical robustness of the findings, a minimum of three replicates were conducted for each experiment. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinical and biochemical characteristics. The proband was 28 years old and had a history of hypertension, which was first diagnosed at the age of 16 years and had remained refractory to a 12-year combination therapy that included telmisartan, benidipine and bisoprolol. In December 2021, the proband was referred to Fuwai Hospital (Beijing, China) for a detailed evaluation of the hypertension of the patient.

Physical examination at the time of hospital admission demonstrated that the patient was overweight (body mass index, 26.2 kg/m^2) and had a high blood pressure ($200/140 \text{ mmHg}$; normal range, $<140/90 \text{ mmHg}$). Biochemical assessments demonstrated a serum potassium concentration of 3.18 mmol/l (normal, $3.50\text{--}5.30 \text{ mmol/l}$), a plasma aldosterone concentration of 1.0 ng/dl (normal, $3.0\text{--}35.3 \text{ ng/dl}$) in the standing position (Table I), a urinary microalbumin concentration of 56.7 mg/l (normal, $<30.0 \text{ mg/l}$) and a plasma chloride concentration of 108 mmol/l (normal, $98\text{--}106 \text{ mmol/l}$). The plasma renin concentration (5.4 mIU/ml ; normal range, $4.4\text{--}46.1 \text{ mIU/ml}$), aldosterone/direct renin concentration ratio [$0.19 \text{ (ng/dl)/(mIU/ml)}$; normal range, $<3.70 \text{ (ng/dl)/(mIU/ml)}$] and results of a low-dose dexamethasone inhibition test (cortisol, $3.00 \text{ } \mu\text{g/dl}$; normal range, $<22.45 \text{ } \mu\text{g/dl}$; and adrenocorticotropic hormone concentration, $<5 \text{ pg/ml}$; normal range $<46 \text{ pg/ml}$) were normal. In addition, the growth hormone concentration ($18.40 \text{ } \mu\text{g/dl}$; normal reference, $5.27\text{--}22.45 \text{ } \mu\text{g/dl}$), cortisol concentration ($<0.1 \text{ ng/ml}$; normal reference, $0.0\text{--}3.0 \text{ ng/ml}$) and adrenocorticotropic

hormone concentration (10.30 pg/ml ; normal reference, $0.00\text{--}46.00 \text{ pg/ml}$) were also within the normal range. The result of aortic and renal artery computed tomography was also normal (Fig. 1A and B). Transthoracic echocardiography demonstrated thickening of the left ventricular posterior wall and interventricular septum and widening of the ascending aorta (Fig. 1C and D). Evaluation of the retinal microvasculature suggested bilateral retinal artery stenosis and grade 1 hypertensive retinopathy.

The father (II-4) of the patient and two paternal aunts (II-1 and II-5) also had early-onset hypertension and chronic hypokalemia, for which they intermittently took potassium citrate supplementation. The paternal grandmother (I-1) of the patient was diagnosed with hypertension at the age of ~ 20 years and died of a stroke at 35 years of age. A paternal uncle (II-7) was reported to have had hypertension at the age of ~ 30 years, with little compliance to blood pressure control and died of cardiac arrest at the age of 50 years. The mother (II-3) of the proband was unaffected. Clinical and biochemical profiles of family members are presented in Table I. The frequency of early-onset refractory hypertension, hypokalemia and history of sudden mortality on the paternal side of the family of the proband was suggestive of an autosomal dominant disease (Fig. 2).

Identification of the *SCNN1B* gene mutation. The results of whole-exome sequencing in the proband demonstrated a frameshift variant in *SCNN1B* (NM_000336: c.1806dupG, p.Pro603Alafs*5). This mutation led to a heterozygous G insertion at base 1806 of the β subunit of ENaC. This resulted in the substitution of alanine in place of proline at codon 603 and a shortened open reading frame that created a premature stop codon at position 607. It also abrogated the highly conserved PY motif, which conformed to the criterion for protein-altering variant severity (PVS1) (12). This variant

Table I. Clinical and biochemical data of enrolled subjects in the present study.

A, Affected patients									
Family member	Hypertension diagnosis	Age at hypertension diagnosis, years	Blood pressure, mmHg	Heart rate, bpm	Symptoms/cardiovascular events/comorbidities	Anti-hypertensive treatment before genetic testing	Serum K ⁺ , mmol/l	Serum plasma renin ^a , mIU/ml	Serum plasma aldosterone ^a , ng/dl
II-1	Y	28	151/96	70	Cardiovascular disease and stroke	Nifedipine and Bisoprolol	3.21	3.4	2.8
II-4	Y	18	160/100	67	Hyperlipidemia, hyperuricemia and hyperthyroidism	Nifedipine, Bisoprolol and Telmisartan	2.80	4.6	3.3
II-5	Y	32	167/103	70	Syncope	Nifedipine, Losartan potassium hydrochlorothiazide compound tablet and Carvedilol	3.02	2.1	2.4
III-2	Y	16	200/140	75	-	Nifedipine, Bisoprolol and Telmisartan	3.18	5.4	1.0
III-3	N	-	114/85	69	Viral encephalitis and glioma	-	3.67	4.7	1.6
B, Unaffected patients									
Family member	Hypertension diagnosis	Age at hypertension diagnosis, years	Blood pressure, mmHg	Heart rate, bpm	Symptoms/cardiovascular events/comorbidities	Anti-hypertensive treatment before genetic testing	Serum K ⁺ , mmol/l	Serum plasma renin ^a , mIU/ml	Serum plasma aldosterone ^a , ng/dl
II-3	N	-	138/89	66	-	-	4.02	40.5	16.4
III-1	N	-	113/68	63	-	-	4.50	2.7	7.0
III-4	N	-	124/90	72	-	-	4.24	8.6	8.8

^aPatient took the test after remaining in a standing position for 2 h. Reference values for serum K⁺, 3.5-5.3 mmol/l; normal range of plasma renin concentration, 4.4-46.1 mIU/ml; normal range of plasma aldosterone concentration, 3.0-35.3 ng/dl. Y, yes; N, No.

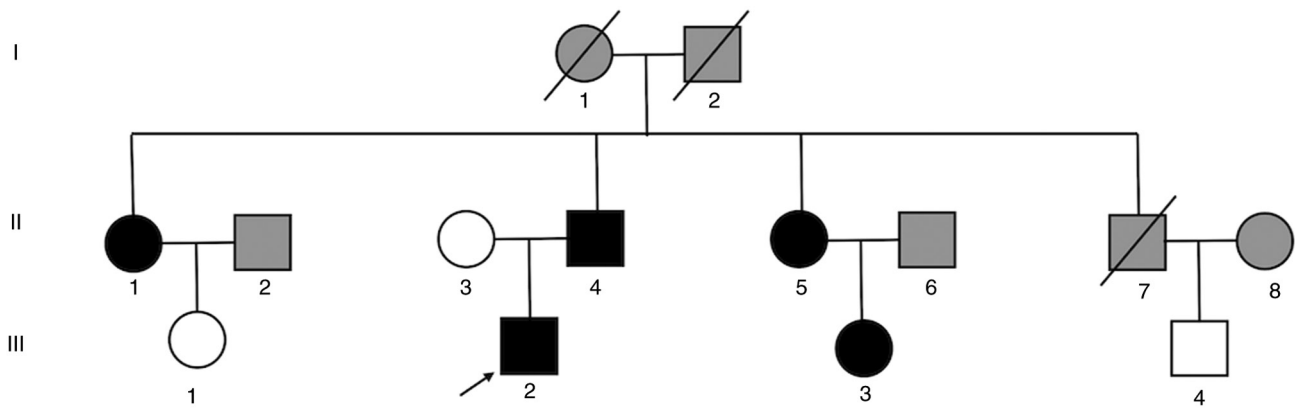


Figure 2. Pedigree diagram of the family with Liddle syndrome. An arrow indicates the proband; squares represent males; circles represent females; filled black symbols represent patients with Liddle syndrome identified using genetic testing; filled gray symbols indicate family members without genetic testing (and without Liddle syndrome); white symbols represent subjects without the identified mutation; and a line through the symbol represents the individual has died.

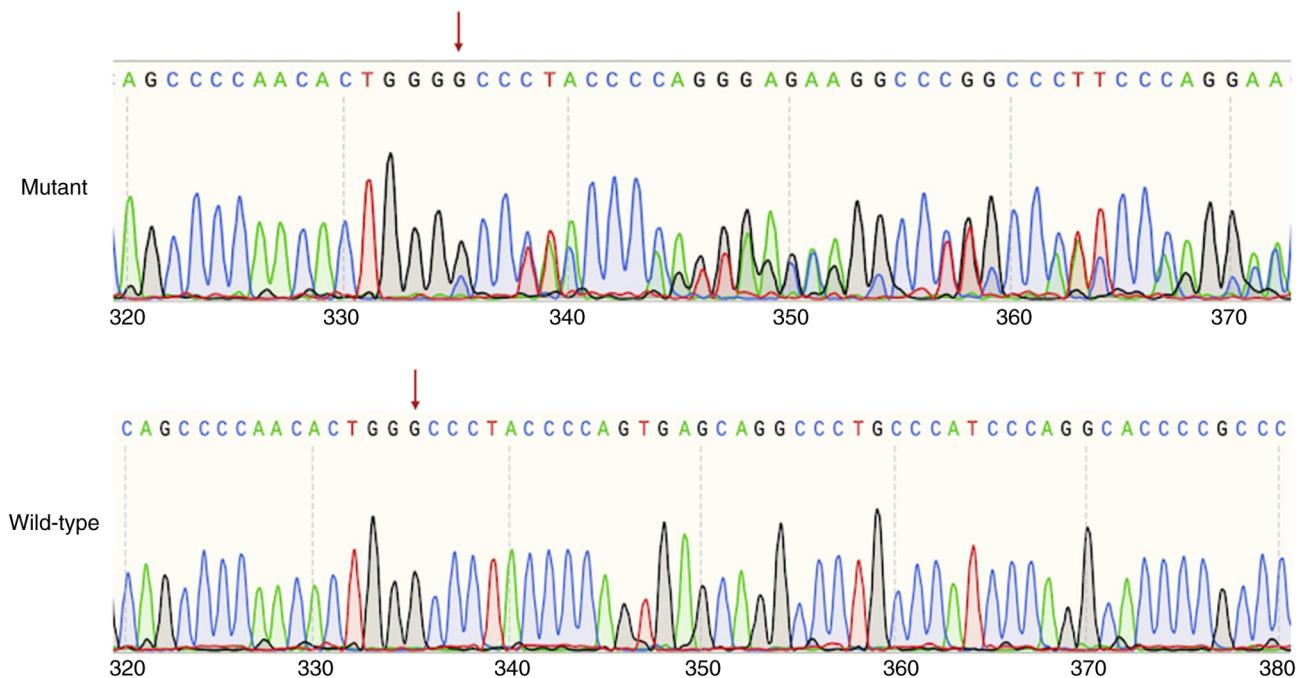


Figure 3. Sanger sequencing traces of exon 13 of *SCNN1B*. The frame-shift mutation (c.1806dupG) identified in affected patients with Liddle syndrome (upper) and the corresponding wild-type sequence in unaffected subjects (lower). The red arrows indicate the site of base insertion.

has not been reported in the 1000 Genomes Project or ExAC (<https://exac.broadinstitute.org/>) databases, which matches the criterion for pathogenic moderate 2 (PM2) (12). Sanger sequencing demonstrated that four affected family members (II-1, II-4, II-5 and III-3) had the same mutation as the proband (Fig. 3), indicating that they matched the criterion for pathogenic supporting 1 (PP1) (12). Therefore, it was assumed that the mutation identified in the proband was derived from the paternal lineage and probably from the grandmother who died at the age of 35 years as a result of early-onset severe hypertension. Moreover, the c.1806dupG mutation was predicted to be 'disease causing' and '1', determined according to the *in silico* analysis tools MutationTaster and phastCons, respectively, and aligning to the criterion for pathogenic supporting 3 (PP3) (12). Thus, according to the ACMG guidelines (12),

SCNN1B c.1806dupG was assessed as a pathogenic mutation based on the evidence of PVS1, PM2, PP1 and PP3.

Precise treatment and follow-up of patients with Liddle syndrome. All family members with the pathogenic mutation were prescribed amiloride (5 mg daily, oral administration), except for III-3, whose blood pressure was within a normal range. Lifestyle measures, including a low-sodium diet and exercise, were implemented. Combination antihypertensive therapy (50 mg losartan potassium/12.5 mg hydrochlorothiazide daily, oral administration) was prescribed to one family member (II-4), for whom amiloride alone was ineffective. Moreover, genetic counseling was provided to the affected individuals and DNA testing by amniocentesis was recommended. After 3 months of tailored treatment, blood pressure

Table II. Comparison of the phenotypes and treatment of patients carrying the p.P603Afs*607 mutation in the studies included in the systematic review.

First author, year	Family	Patient	Sex	Onset age of hypertension, years	Age at diagnosis, years	Maximum		Plasma aldosterone, ng/dl	Plasma renin activity/plasma renin concentration	Notes	Treatment with epithelial sodium channel inhibitors	
						blood pressure, mmHg	Serum K ⁺ , mmol/l				Blood pressure, mmHg	Serum K ⁺ , mmol/l (Refs.)
Cui <i>et al</i> , 2017	1	A	M	17	45	180/120	↓	↔	↓	Mother of the patient died of a stroke at the age of 32 years with unknown BP and serum potassium levels	120/80	↔
Fan <i>et al</i> , 2019	2	B1	M	23	31	230/150	↓	↔	↓	Mother of the patient died suddenly at the age of 28 years with unknown BP and serum potassium levels	150/85	↔
	3	B2	M	10	16	190/120	↓	↓	↓	-	125/85	↔
		II-2	M	48	73	220/120	↔	↔	↑	-	140/80	↔
Cui <i>et al</i> , 2017		II-4	F	25	70	200/130	↔	↓	↔	-	120/80	↔
		II-7	M	36	66	200/140	↓	↔	↔	Stroke at the age of 60 years	123/75	↔
		III-3	M	19	35	180/160	↔	↓	↔	-	140/100	↔
The present study		III-8	F	16	35	180/108	↔	↔	↑	-	120/90	↔
		III-15	F	16	37	180/110	↔	↓	↓	-	125/96	↔
		III-16 ^a	M	15	35	220/150	↓	↔	↔	-	126/88	↔
		IV-9	F	3	3	130/80	↔	↔	↑	-	N/A	N/A
		IV-10	M	3	10	150/130	↓	↓	↓	-	100/70	↔
		II-11	M	29	N/A	N/A	N/A	N/A	N/A	Died of stroke at the age of 39 years	N/A	N/A
The present study	4	II-1	F	28	62	151/96	↓	↓	↓	Stroke at the age of 58 years	125/95	↔
		II-4	M	18	53	160/100	↓	↔	↔	-	130/82	↔
The present study		II-5	F	32	59	167/103	↓	↓	↓	-	124/80	↔
The present study		III-2 ^a	M	16	28	200/140	↓	↓	↓	-	126/88	↔
The present study		III-3	F	-	40	114/85	↔	↓	↔	-	N/A	N/A

^aProband. M, male; F, female; BP, blood pressure; N/A, not applicable; ↓, lower compared with the normal range; ↑, higher compared with the normal range; ↔, within the normal range.

and electrolyte concentrations in the affected individuals were reassessed and these were demonstrated to be within normal ranges (Table II). Subsequently, all of the patients were followed up for ≥ 1 year and no abnormal blood pressure measurements, new cardiovascular events or serious adverse drug reactions were recorded.

Systematic review and genotype-phenotype association analysis. A total of 108 patients with Liddle syndrome from 47 families were identified through the literature search. Follow-up data of these individuals are detailed in Table SI. Median age at hypertension onset was ~ 18 years (interquartile range, 14, 23.5 years). Incidence of hypertension, hypokalemia, hypoaldosteronism and hyporeninemia was 97.2, 81.3, 65.1 and 80.2%, respectively. In patients without targeted therapy, the most common hypertensive damage was left ventricular hypertrophy (9.6%), followed by stroke (4.6%). A family history of stroke was reported in $\sim 12\%$ of the patients. After treatment with an ENaC inhibitor (amiloride or triamterene), blood pressure significantly decreased from $170.8 \pm 23.8/107.6 \pm 18.7$ to $124.2 \pm 9.9/79.4 \pm 9.9$ mmHg ($P < 0.001$) and serum potassium concentration significantly increased from 3.14 ± 0.5 to 4.3 ± 0.5 mmol/l ($P < 0.001$). During follow-up, only one mortality that was unexplained and no cardiovascular events were reported.

Moreover, 13 patients with Liddle syndrome were identified from three families, all with c.1806dupG in *SCNNIB* (15,16). The relevant clinical data for all individuals known to have the c.1806dupG mutation, to the best of our knowledge, are summarized in Table II. Individuals carrying this mutation had varying degrees of blood pressure elevation, except for III-3 in the present study (17/18, 94.4%). The median age at hypertension onset was 18 years (interquartile range, 16, 28 years) and the mean arterial blood pressure was 180/120 mmHg. Hypokalemia was identified in 10/17 patients (58.9%), hypoaldosteronism in 9/17 (52.9%) and hyporeninemia in 7/17 (41.1%).

Discussion

The present study demonstrated a frameshift base variant in *SCNNIB* (NM_000336: c.1806dupG, p.Pro603Alafs*5) causing Liddle syndrome that was characterized by early-onset refractory hypertension in a family. This mutation leads to the insertion of G at base 1806 and results in a truncated β subunit of the ENaC and deletion of the PY motif, with definite pathogenicity (16). Genotype-phenotype association analysis demonstrated wide phenotypic variability, including certain degrees of hypertension, hypokalemia, hyporeninemia and hypoaldosteronism. The results indicated that confirmatory genetic testing and targeted therapy could prevent early-onset clinical endpoints in patients with Liddle syndrome.

Liddle syndrome is a form of salt-sensitive hypertension that is caused by mutations in *SCNNIA*, *SCNNIB* and *SCNNIG*, which respectively encode the α , β and γ ENaC subunits. The ENaC serves a role in maintaining blood pressure homeostasis by controlling sodium and potassium handling in the kidneys (17). The functions of the ENaC include reabsorption of sodium ions from the lumen into epithelial cells and pumping these ions into the interstitial fluid with the assistance of the sodium/potassium ATPase on the basolateral membrane, as

well as potassium secretion (18). Each subunit of the ENaC contains a large extracellular domain, two transmembrane regions and relatively short cytoplasmic domains (N-terminus and C-terminus) (19). A highly conserved proline-rich sequence, known as the PY motif, has been identified in the C-terminus of the β and γ subunits, and is recognized by the domains of the ubiquitin ligase Nedd4-2. The binding of the ENaC to Nedd4-2 catalyzes the internalization of the ENaC and its degradation in proteasomes or lysosomes (18,20). Gain-of-function mutations in the subunits of ENaC result in deletion or alteration of the PY motif and subsequent blocking of ubiquitylation and internalization of ENaC (21). Dense accumulation of the ENaC at the cell surface increases sodium reabsorption and potassium secretion, thereby leading to the pathophysiological process of hypertension.

The prevalence of Liddle syndrome in patients with hypertension in the general population remains unknown. Previously, Tapolyai *et al* (22) studied 149 hypertensive US veterans and reported a similar biochemical phenotype to Liddle syndrome in 6% of patients. Wang *et al* (13) reported a Liddle syndrome prevalence of 1.52% in 330 hypertensive patients by sequencing exon 13 of *SCNNIB* and *SCNNIG*. Moreover, a study including 506 patients with early-onset hypertension reported a Liddle syndrome prevalence of 0.91% (14). Thus, Liddle syndrome may not be rare in specific populations with early-onset hypertension.

In the present study, a heterozygous G insertion at base 1806 of the β subunit of ENaC was identified. This leads to the substitution of alanine for proline at position 603 and premature emergence of a stop codon at position 607, resulting in the loss of 34 amino acids, including the PY motif (16). Further assessment (such as with biochemical assessments, hormone measurements and image examination) of the proband was performed due to difficulties in blood pressure control, although there were indications of Liddle syndrome based on several early signs, such as refractory hypertension, hypokalemia and a family history of hypertension. To the best of our knowledge, 14 frameshift mutations in β -ENaC have been identified in patients with Liddle syndrome (4,23,24). The mutation in *SCNNIB* identified in the present study is consistent with that in previous reports by Cui *et al* (15) and Fan *et al* (16). Furthermore, although there have been reports of a variety of frameshift mutations with certain base insertion or deletion locations in *SCNNIB*, including c.1781dupC (p.Ala595Argfs*13) (25), c.1789dupC (p.Arg597Profs*11) (26,27) and c.1800_1801insG (p.Thr601Aspfs*7) (28,29), all introduce a new stop codon at position 607 that is accompanied by deletion of the proline-rich PY motif. Missense and non-sense mutations have also been reported to alter the PY motif (15,30).

Liddle syndrome demonstrates wide phenotypic variability, including varying degrees of hypertension, hypokalemia, hyporeninemia and hypoaldosteronism (4,30). However, owing to its low prevalence, the genotype-phenotype relationship in Liddle syndrome is still unknown. In 2014, Gong *et al* (31) compared the characteristics of patients with p.Arg566* and p.Arg597Pfs*11 mutations in *SCNNIB* and reported marked phenotypic variability between families. The p.Arg563Gln mutation in the C-terminus of the β subunit of ENaC has also been associated with low-renin and low-aldosterone hypertension; however, only 2.8% of individuals with the p.Arg563Gln

variant present with the full Liddle syndrome phenotype (32,33). A previous study identified the p.Asn530Ser mutation in *SCNN1G* in the extracellular loop of the γ subunit, not only in a patient with the Liddle syndrome phenotype, but also in healthy controls, although available clinical and laboratory results were limited (29). A functional study of the p.Asn530Ser mutation in *Xenopus* oocytes reported that the open probability was two-fold higher in mutant ENaC compared with in wild-type ENaC, but with no marked change in the cell surface expression of ENaC (29). Moreover, the clinical features of patients may be similar even with certain mutation sites, as reported by Cui *et al* (15).

In the present study, a genotype-phenotype association analysis was performed to gain new insights into the phenotypic heterogeneity and clinical management of Liddle syndrome. A literature search identified 18 patients carrying the p.Pro603Alafs*5 mutation in four families (15,16). All of these patients were identified in China, suggesting that this mutation may be a hotspot variant in the Chinese population. Phenotypic heterogeneity was evident in patients with the same mutation, as demonstrated by the proportion of patients with hypertension (94.4%), hypokalemia (58.9%), hypoaldosteronism (52.9%) and hyporeninemia (41.1%). As demonstrated in the present study, although patient III-3 carried the pathogenic mutation, the blood pressure of the patient was within a normal range, which may be due to a combination of modifier genes and environmental factors, such as salt intake. Furthermore, stroke occurred in 3 patients and was fatal in one, and in the two families reported by Cui *et al* (15), the mothers of the two probands died before the age of 35 years and were strongly suspected to carry the same mutation. The patients with this mutation responded to targeted ENaC inhibitor therapy, with a return to normal blood pressure and potassium concentration.

Refractory early-onset hypertension, hypokalemia and the effective management of the symptoms of Liddle syndrome with amiloride are strong indicators of Liddle syndrome; however, molecular genetic testing is essential to diagnose the condition, especially in patients with isolated hypertension or hypokalemia (34). Genetic testing also has implications for the identification of first-degree relatives carrying the same pathogenic mutation, such as for guiding targeted drug use and eugenism. The present review demonstrated that 12% of patients with Liddle syndrome have a family history of stroke and are often associated with hypertensive target organ damage, such as left ventricular hypertrophy or stroke, similar to the findings of Qu *et al* (35). With the exception of one patient who died of an unknown cause, all patients with Liddle syndrome demonstrated significant improvements in blood pressure and serum potassium concentration after precise ENaC inhibitor therapy, and no cardiovascular disease-associated events were reported.

The present study has several limitations. First, a functional analysis to assess any increases in the amiloride-sensitive Na^+ current of *Xenopus* oocytes expressing p.Pro603Alafs*5 was not performed. Considering that the p.Pro603Alafs*5 variant results in the deletion of the final 34 amino acids in the β subunit of ENaC, including the PY motif, it is hypothesized that this mutation is pathogenic and leads to an increased channel activity and excessive sodium retention (2,7,16,36).

Second, the effects of amiloride in patients with Liddle syndrome were based on middle-term treatment only and so a limited duration of treatment was analyzed; long-term efficacy requires further follow-up studies. Third, the present study was a small-scale study. Therefore, large multicenter clinical studies should be conducted to screen genes for Liddle syndrome in patients with hypertension and to determine the prevalence of their involvement. Regular follow-up of patients should also be conducted to determine the long-term outcomes of patients with Liddle syndrome. In addition, the lack of available images of the eye was a limitation of the present study, which was due to the image storage capabilities of the fundus examination equipment not being fully implemented.

In the present study, results from whole-exome sequencing demonstrated a frameshift mutation (NM_000336: c.1806dupG, p.Pro603Alafs*5) in the β subunit of ENaC that was associated with Liddle syndrome in a family. The mutation in the extracellular domain leads to the substitution of alanine in place of proline at codon 603 and a shortened open reading frame which creates a premature stop codon at position 607 and results in a truncated β subunit of ENaC with deletion of the PY motif. Confirmatory genetic testing is essential and targeted therapy is necessary to prevent severe sequelae early in life (16). However, further studies are needed to characterize the long-term prognosis and reasons for the phenotypic heterogeneity in patients with Liddle syndrome.

Acknowledgements

Not applicable.

Funding

The present work was supported by CAMS Innovation Fund for Medical Sciences (grant nos. 2022-I2M-C&T-A-010 and 2022-I2M-C&T-A-011).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Sequence Read Archive repository, <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1020912/> and all other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YLu, XL, YLi and XZ designed the study and modified the manuscript. YLu, LS, DZ, KY, PF and LZ collected clinical information and performed data analysis. YLu and XL wrote the manuscript. YLu and XL confirm the authenticity of all the raw data. All authors read and approval the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Fuwai Hospital (approval no. 2020-1321). All of the participants provided written informed consent before study enrolment.

Patient consent for publication

Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin for the publication of any potentially identifiable patient information included in the present article.

Competing interests

The authors declare that they have no competing interests.

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