A Family with Liddle Syndrome Caused by a Novel Missense Mutation in the PY Motif of the Beta-Subunit of the Epithelial Sodium Channel

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Objective To identify the gene mutation in β and γ subunits of the epithelial sodium channel (ENaC) in an adolescent and family members with Liddle syndrome, an autosomal dominant form of secondary hypertension.

Study design We screened an adolescent with severe hypertension who was clinically diagnosed with Liddle syndrome for mutations in the C-terminus of the *SCNN1B* and *SCNN1G* genes. We also screened for these mutations in his family members, in 100 hypertensive patients, and in 100 controls.

Results The index case, a 14-year-old boy, was diagnosed with Liddle syndrome by the identification of a novel missense mutation, P614L, in the PY motif of the β subunit of the ENaC. Testing of relatives considered at risk revealed 6 subjects heterozygous for the mutation. All genetically affected subjects had a history of severe hypertension as well as hypokalemia. No other variants in the β or γ subunits of the ENaC were detected.

Conclusion Based on direct DNA sequencing, we have detected a novel mutation that causes Liddle syndrome. This confirms the diagnosis and helps guide effective therapy for this adolescent and his affected relatives. These findings provide further evidence that the conserved PY motif is critical to regulation of ENaC activity. *(J Pediatr 2013;162:166-70)*.

Hypertension in children is often secondary to a group of disorders with Mendelian inheritance. Recent advances in molecular biology have uncovered the pathogenesis of hypertension in many of these conditions. Remarkably, in each case the mechanism has been up-regulation of sodium reabsorption in the distal nephron, with accompanying expansion of extracellular volume. Liddle syndrome is an autosomal dominant form of salt-sensitive hypertension caused by mutations in an epithelial sodium channel (ENaC). Basic features of Liddle syndrome include low levels of plasma renin activity (PRA) and aldosterone and increased potassium excretion, resulting in low levels of serum potassium and metabolic alkalosis.[1] The disorder responds to inhibitors of epithelial sodium transport (eg, amiloride or triamterene), but not to spironolactone therapy.[2]

Composed of 3 subunits, α, β, and γ, the ENaC is considered to mediate the rate-limiting step for sodium absorption in the distal nephron.[3,4] All mutations reported in Liddle syndrome delete or alter a conserved proline-rich amino acid sequence, PPPXY, referred to as the PY motif, resulting in increased channel activity.[2,5-24]

A genetic analysis of the amiloride-sensitive ENaC is recommended when assessing patients with low-renin, salt-sensitive hypertension not responsive to spironolactone treatment. We report an adolescent with Liddle syndrome caused by a novel missense mutation, P614L, in the PY motif of the ENaC β subunit. This missense mutation was found in 6 members of the adolescent's family as well.

Methods

Clinical examination of the 14-year-old boy at the hypertension ward of the Fuwai Hospital raised the suspicion of Liddle syndrome because of early-onset, treatment-resistant hypertension, spontaneous hypokalemia, and suppressed PRA. A total of 19 at-risk subjects (excluding the index case) were available for genetic testing, and 14 of these subjects were also tested for endocrine function. To evaluate whether the variations were common genetic polymorphisms, we also recruited 100 unrelated hypertensive patients and 100 normotensive subjects, all of whom provided informed consent.

PRA and plasma aldosterone concentration were measured in samples drawn with the patient in the upright position. Concentrations of aldosterone in both plasma and urine were measured by radioimmunoassay using the DSL-8600

ENaC	Epithelial sodium channel
PRA	Plasma renin activity

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kit (Diagnostic Systems Laboratories, Webster, Texas). PRA was measured at pH 5.7 by radioimmunoassay.

Genomic DNA was extracted from blood leukocytes. The final exon of the β and γ subunits of the ENaC were amplified by polymerase chain reaction using β subunit primers 5′-GACAGTCCCAAGTTATTCCC-3′ and 5′-GCATCCTGT ACCAGCACC-3′ and a pair of primers for the γ subunit according to Hiltunen et al.[12] All samples were sequenced using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, California). The exons were sequenced in both forward and reverse directions to confirm the presence or absence of mutations.

The study was approved by the Ethics Committee of Fuwai Hospital, Peking Union Medical College, Beijing, China. All participants provided written informed consent.

Results

Clinical and Biochemical Features

The index case was admitted to our hospital for evaluation of hypertension (blood pressure, 186/114 to 226/130 mmHg) and hypokalemia (2.31 mmol/L). Laboratory studies showed a serum sodium level of 142.7 mmol/L and a serum chloride level of 101.7 mmol/L. There was suppression of PRA (0.27 ng/mL/hour; reference value, 0.93-6.56 ng/mL/hour) and decreased urinary aldosterone concentration (1.31 nmol/day; reference value, 2.8-22.2 nmol/day). The serum aldosterone level was 0.15 ng/mL (reference value, 0.065-0.296 ng/mL). Special serial clinical studies revealed no adrenal or kidney abnormalities.

Suppressed PRA, low serum potassium, and elevated blood pressure were found in cases II-3, III-3, III-4, III-6, III-7, and III-8 (Table I). II-3 was a 58-year-old man diagnosed with hypertension 37 years earlier with nonoptimal blood pressure control thereafter. Examination detected resting tachycardia of 106 beats/min and blood pressure of 200/100 mmHg. Transthoracic echocardiography revealed mild concentric hypertrophy of the left ventricle and left atrial dilation. Complications of hypertension were not found in any other affected family members.

Genetic Analysis

Figure 1 shows the extended kindred of the index case. As shown in Figure 2, a novel point mutation in exon 13 of the β-ENaC gene, *SCNN1B*, was detected in the proband (III-9), in which 614Pro (CCG) changed to 614Leu (CTG), P614L. This mutation was also found in II-3, III-3, III-4, III-6, III-7, and III-8. II-10, II-5, and II-8 were also hypertensive and died of stroke, at age 31, 20, and 32 years, respectively. This P614L variant was not detected in any other family members, and no other variants of β-ENaC or γ-ENaC were detected. None of the randomly selected 100 patients with hypertension or the 100 controls had the mutation, indicating that this is not a common genetic polymorphism.

Tailored Therapy for Mutation Carriers

All patients with hypertension in this family were encouraged to make lifestyle modifications, such as eating a healthier diet, quitting smoking, and getting more exercise. The 7 mutation carriers diagnosed with Liddle syndrome were treated with amiloride 10 mg/day in conjunction with a low-sodium diet (2 g NaCl/day). In addition, II-3 was also treated with a beta-blocker, a diuretic, and an angiotensin-converting enzyme inhibitor. At 1 month after initiation of treatment, blood pressure and plasma potassium were controlled at normal levels (Table I).

Discussion

This study reports a novel missense mutation changing 614Pro (CCG) to 614Leu (CTG) in the PY motif of the

Table I. Clinical and biochemical characteristics of the first-degree relatives of the kindred

Subject	Age, years	Age at onset of hypertension	BP before amiloride treatment, mm Hg*	Pk^+ before amiloride treatment, mmol/L	PRA, ng/mL/hour†	Aldosterone, ng/mL‡	Pk^+ after amiloride treatment, mmol/L	BP after amiloride treatment, mmHg*
Affected								
II-3	58	21	200/100	2.58	0.83	0.13	4.01	126/76
III-3	36	19	186/100	2.41	0.46	0.17	4.52	130/86
III-4	30	15	180/110	2.64	0.82	0.30	4.71	128/80
III-6	30	18	166/106	2.32	0.38	0.42	3.96	136/76
III-7	28	20	172/94	2.78	0.88	0.32	4.04	126/80
III-8	24	18	196/120	2.78	0.03	0.14	4.45	120/80
III-9	14	14	192/120	2.46	0.27	0.15	4.01	134/78
Unaffected§								
II-1	67	52	174/90	3.55	1.48	0.15	-	118/72
II-6	54	48	166/94	3.53	1.15	0.13	-	120/78
II-13	43	-	130/86	3.79	1.37	0.39	-	-
III-1	42	-	126/84	3.62	1.46	0.19	-	-
III-2	39	-	102/78	3.89	7.29	0.14	-	-
III-5	26	-	112/68	4.68	8.35	0.16	-	-
III-10	19	-	116/70	4.41	3.53	0.16	-	-

BP, blood pressure; *Pk*, plasma potassium.
*Mean of 3 measurements.
†PRA: reference value, 0.93-6.56 ng/mL/hour.
‡Aldosterone reference value 0.065-0.296 ng/mL.
§Unaffected: The unaffected subjects were not treated with amiloride.