Case Report

Liddle's Syndrome: A Case Report

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*A thirty-eight years old female presented with frequent proximal weakness, severe hypertension, and persistent kaliuresis despite hypokalemia. After normalized serum potassium level, hyporeninemic hypoaldosteronism was detected. Pedigree study supported an autosomal dominant inherited disease. A causative mutation for Liddle's syndrome (LS) in this patient was identified to be a novel frameshift mutation. DNA sequencing resulted in exon 13 of SCNN1B gene: SCNN1B NM_000336.2:c.1724_1730dupGGCCCAC [p.Pro575Argfs*17]. Since LS is a rare existing clinical syndrome in Thailand, correct diagnosis should be confirmed by genetic studies. Therefore, proper management could be given.*

Keywords: *Liddle's syndrome, Hypokalemia and hypertension*

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Fourteen years prior to investigation, a 24-year-old Thai woman from Prapradang, Samutprakarn, presenting with chronic hypertrophic tonsillitis subsequently received tonsillectomy at Siriraj Hospital. On admission, systolic blood pressure/diastolic blood pressure (SBP/DBP) levels with averages of 125.00±5.77/87.50±5.00 mmHg were recorded. Recovery from surgery without complication was noted. Nine years prior to the investigation, high SBP/DBP levels with averages of 156.67±11.55/106.67±5.77 mmHg were noted by a general practitioner. She was initially given nifedipine 30 mg/day and subsequently switched to hydrochlorothiazide 25 mg/day plus atenolol 25 mg/day. However, she did not come for follow-up. Afterward, she was treated by a local health care provider in a community hospital. Averaged SBP/DBP levels of 145.27±14.61/105.82±6.15 mmHg, were observed from her appointment/treatment logbook during June 2002 to January 2010.

Case Report

Clinical management at Siriraj Hospital

On the first visit at hypertension clinic, Siriraj Hospital, she was 38 years old. Her SBP/DBP was 170.67±14.47/118.50±10.61 mmHg, body weight was 76 kg, height was 167 cm, body mass index (BMI) was 27.25 kg/m^2, and abdominal circumference was 32 inches. She worked as a security officer at an airport in Bangkok. At that visit, her chief complaints were lightheadedness and frequent episodes of proximal muscle weakness, which happened spontaneously during her daily activity. She was treated with atenolol 25 mg/day, amlodipine 5 mg/day, and losartan 50 mg/day, initially. Laboratory findings revealed serum sodium of 141 mmol/L, potassium of 2.9 mmol/L, chloride of 104 mmol/L, bicarbonate of 26 mmol/L, and creatinine of 0.50 mg/dL. Subsequently, her treatment was changed to amlodipine 5 mg/day, diltiazem 200 mg/day, and doxazosin 1 mg/day. Her averaged SBP/DBP levels were brought down to 142.67±7.57/93.33±4.16 mmHg. She denied of taking other medication, i.e., licorice, carbenoxolone, herbal medication, aldosterone antagonist, etc. Physical examinations were all unremarkable, despite mild hypokalemia was detected. In addition, proximal muscle weakness could not be detected. Serum and urine electrolytes were determined from time to time. Episodes of spontaneous hypokalemia with kaliuria were demonstrated (Table 1).

Laboratory tests

Taking high potassium (K) diet was encouraged and K replacement was provided at a rate of 60 mEq daily. When serum potassium had attained normokalemic level, endocrine tests were performed.

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Basal plasma renin level of 0.54 ng/mL/hour, serum aldosterone level of 1.60 ng/dL, and the aldosterone renin ratio (ARR) of 0.52 were demonstrated on March 26, 2010. A state of hyporeninemic hypoaldosteronism was detected.

After holding back potassium replacement and encouraging high K diet intake, spontaneous hypokalemia was still detected. Repeated serum and urine electrolytes examination were carried out (Table 1). Adequate potassium replacement had to be given to achieve normokalemic level. Determinations of aldosterone and renin levels were repeated again. Basal plasma renin level of 1.07 ng/mL/hour, serum aldosterone level of 0.56 ng/dL, and the ARR of 2.96 were found on May 9, 2011. Hyporeninemic hypoaldosteronism was, again, confirmed.

Clinical diagnosis

Differential diagnosis of Liddle's syndrome (LS) and the syndrome of apparent mineralocorticoid excess (SAME) were considered. Family history was thoroughly interviewed (Fig. 1). Her mother had suffered from liver cancer and passed away at the age of 56 years old. Her father passed away from lung infection at the age of 81. Both of her parents had hypertension without known history of proximal muscle weakness or concurrent electrolytes abnormalities. Her eldest brother, aged 52, was a known hypertensive patient with coexisting hypokalemia and intermittent proximal muscle weakness. However, he did not receive any proper investigation or treatment. He had two sons, his elder son, aged 25, was a healthy normotensive individual, his younger was a documented case of congenital muscular dystrophy and passed away at the age of 13 from respiratory failure and concurrent infection. Both her younger sister and elder brother aged 49 and 38 were healthy normotensives. The studied patient (proband) also had two sons. Her elder son, aged 21,

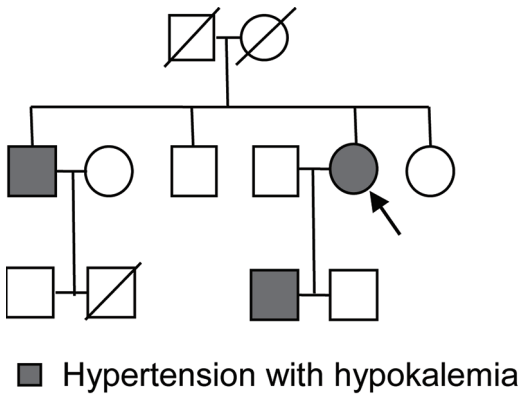

Fig. 1 Examination of the family pedigree.

Table 1. Serum & urine electrolytes study

Parameters		Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	HCO_3 (mmol/L)	Osmolality (mOsmol/kg)	UKER (mmol/day)	TTKG	UV (mL)
First set of serum & urine electrolytes studies									
4-2-10	Serum	143	3.4	104	29	300.0	18.40	9.29	860
	Urine	104	21.4	82	-	443.0			
9-2-10	Serum	142	3.6	100	32	304.0	23.54	7.49	1,100
	Urine	76	21.2	61	-	385.0			
19-2-10	Serum	144	3.4	105	26	277.0	37.44	5.19	1,300
	Urine	88	28.8	79	-	452.0			
2-3-10	Serum	141	3.1	104	31	288.0	19.82	5.55	560
	Urine	98	35.4	85	-	580.0			
Repeated serum & urine electrolytes studies									
14-12-10	Serum	143	3.4	105	31	295.0	26.66	4.41	1,240
	Urine	133	21.5	121	-	423.0			
20-12-10	Serum	143	3.1	102	27	293.0	35.09	7.39	1,020
	Urine	81	34.4	75	-	440.0			
22-12-10	Serum	144	3.4	105	27	293.0	34.88	3.24	1,020
	Urine	116	34.2	223	-	643.0			

Na = sodium; K = potassium; Cl = chloride; HCO_3 = bicarbonate; UKER = urine potassium excretion rate; TTKG = trans-tubular potassium gradient; UV = urine volume

had high blood pressure and hypokalemia, his serum sodium of 142 mmol/L, potassium of 3.2 mmol/L, chloride of 101 mmol/L, bicarbonate of 29 mmol/L, and creatinine of 0.99 mg/dL were observed. Her younger son, aged 15, was normotensive and normokalemia, he suffered from the occulo-auriculo-vertebral congenital defect, Goldenhar syndrome.

Therefore, presumptive diagnosis of LS, an autosomal dominant inherited hypokalemic hypertensive disease was made and the patient was subsequently referred for further evaluation to a medical geneticist. Meanwhile, associated features of SAME were looked up. Withholding potassium replacement was carried out. Spironolactone was initiated and titrated to control the blood pressure levels, whereas other antihypertensive drugs, e.g., amlodipine, diltiazem, and doxazosin were adjusted or discontinued. Finally, her SBP/DBP levels could be maintained at 123.0±7.4/85.0±2.0 mmHg with 2.5 mg of amlodipine, 120 mg of diltiazem SR, and 200 mg of spironolactone. The mean serum potassium level could not be normalized. There was no significant difference between the serum potassium levels before and after receiving of spironolactone (3.34±0.18 vs. 3.43±0.19 mmol/L, p = 0.50). Concurrently, morning fasting serum adrenocorticotropic hormone (ACTH) level of 17.61 pg/mL and serum cortisol level of 4.94 mcg/dL were observed. Repeated morning fasting serum cortisol was 9.38 mcg/dL. Therefore, SAME could be excluded.

Genetic studies

Subsequently, ethylenediamine tetraacetic acid (EDTA) blood was submitted to the Molecular Genetics Laboratory. Mutation analysis of the SCNN1B and SCNN1G causing LS in the patient with hypertension was conducted. Polymerase chain reaction (PCR) amplification of exon 13 of both SCNN1B and SCNN1G genes was performed using previously published primer sequences and followed by direct DNA sequencing[1]. Duplication (insertion) of a GGCCCAC at nucleotide position 1724 to 1730 (c.1724_1730dupGGCCCAC) in exon 13 of SCNN1B was identified. This novel mutation resulted in frameshift translation starting from codon 575, then premature termination at the 17th residue, codon 591 (SCNN1B NM_000336.2:c.1724_1730dupGGCCCAC [p.Pro575Argfs*17]) (Fig. 2). Her eldest son had also submitted EDTA blood for the mutation analyses of the SCNNIB causing LS and duplication (insertion) of a GGCCCAC at nucleotide position 1724 to 1730

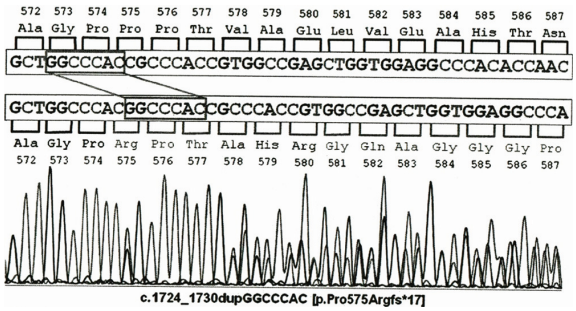

Fig. 2 Mutation analysis of the SCNNIB and SCNN1G causing Liddle's syndrome in the patients.

(c.1724_1730dupGGCCCAC) in exon 13 of SCNN1B could also be documented.

Discussion

Personal approach with verbal consent was obtained from the patient and her family members prior to report of her medical findings. LS is a rare autosomal dominant monogenic form of hypertensive disease with somewhat variable clinical expression[1]. Hypertension, hypokalemia, persistent urinary potassium excretion, suppressed plasma renin activity (PRA), and blunted aldosterone secretion rate are useful clinical clues in identifying the existing clinical condition[2]. To demonstrate persistent kaliuresis in hypokalemic hypertensive patients, daily urinary potassium excretion rate, and the trans-tubular potassium gradient (TTKG) in the present of hypokalemia and adequate sodium intake are ones of the most useful tools[3]. In the present scenario, the mean serum potassium level was 3.34±0.18 mmol/L, whereas the mean amount of daily urinary potassium excretion rate was inappropriately high at 27.95±7.86 mmol. Moreover, the TTKG as an index reflecting the conservation of potassium in the cortical collecting ducts (CCD) of the kidneys is useful in finding out the causes of hypokalemia. The TTKG estimates the ratio of potassium in the lumen of the CCD to that in the peritubular capillaries. During potassium depletion or hypokalemia, the TTKG should fall to less than 3, indicating appropriately reduced urinary excretion of potassium[4]. The averaged TTKG in this patient was inappropriately high at 6.08±2.08. Hyporeninemic hypoaldosteronism could be demonstrated with blunted basal aldosterone secretion related to the low renin secretion at 1.08±0.74 ng/dL and 0.81±0.38 ng/mL/hour, respectively. Lastly, the relatively low in the averaged basal ARR of 1.74±1.72 could be confirmed. All of the findings are compatible with a diagnosis of LS[5].

Since, LS is a rare disease and the defective product of the gene is a structural protein; the amiloride sensitive epithelial sodium channel (ENaC). Male and female siblings in the present family seem to have equal chance of inheriting the allele and being affected. Genetically setting in the present patient was, therefore, suggested of an autosomal dominant heritance supported by the examination of the pedigree[6].

Since, hypertension in the LS is attributable to increasing of sodium reabsorption in the distal nephron, spironolactone has no role in treatment. Her serum potassium level could not be normalized with spironolactone 200 mg/day and there was no statistical difference in serum potassium levels between pre/post treatment with spironolactone ($p = 0.50$). Furthermore, her blood pressure could not be normalized with aldosterone antagonist either. Low salt diet and treatments with antagonist of the amiloride-sensitive ENaC, amiloride or triamterene, can correct hypertension and biochemical abnormalities in LS by closing the sodium channels[7]. However, in Thailand, only combinations of hydrochlorothiazide and amiloride or triamterene are available in the market. Therefore, hydrochlorothiazide 25 mg plus triamterene 50 mg per tablet were given instead. However, worsening of her serum potassium level was detected and probably aggravated by hydrochlorothiazide.

Works from Lifton's group and others had identified several deletions/mutations that cause LS, all of which map to beta and gamma ENaC and lead to elevated channel numbers and activity at plasma membrane[8]. LS caused by mutations in the amiloride-sensitive ENaC gene led to excessive salt and water resorption from the distal nephron, volume expansion, and suppression of PRA and aldosterone secretion. Previous reports on LS were usually associated with missense and truncate mutations at the C-terminus of the amiloride-sensitive ENaC gene[9-12]. Alternatively, insertion of SCNN1B, which led to frameshift translations and premature termination of the encoded protein, caused LS to be reported[1,13,14]. However, mutational analysis of the patient's genomic DNA in the present study disclosed a duplication (insertion) of a GGCCCAC at nucleotide position 1724 to 1730 (c.1724_1730dupGGCCCAC) in exon 13 of SCNN1B and this novel mutation could result in frameshift translation starting from codon 575, then premature termination at the 17th residue, codon 591 (SCNN1B NM_000336.2:c.1724_1730dupGGCCCAC [p.Pro575Argfs*17]). Hiltunen et al[1] had supported the study by revealing a single nucleotide insertion in the exon 13 of beta ENaC resulted in frameshift at codon 601 coding for threonine localized in the intracellular domain of the beta ENaC subunit. The mutation caused a translational frameshift and was predicted to create a premature stop codon at position 607, thus deleting the PY motif of the beta ENaC[1]. Nagano et al had stated a frameshift mutation of the beta subunit caused by a single cytosine insertion at the codon 595, introducing a new stop codon at 605 and deleting the last 34 amino acids from the normally encoded the protein[13]. Ma et al[14] had also found a 1bp, INS, 600G causing a frameshift mutation. The mutation introduced a new stop codon at position 605 and deleted the last 34 normal amino acids from the C-terminus of beta ENaC[14].

LS is rarely found in Thailand. Only a few cases had been described. A young child presenting with severe hypertension had arisen a suspicious case of LS in the Department of Pediatric, Faculty of Medicine Siriraj Hospital. LS caused by a novel P615H missense mutation in the proline-rich domain of the SCNN1B gene coding for the beta-subunit of amiloride-sensitive ENaC was reported[15]. The other two cases had been reviewed by Jameekornrak[16], the novel nonsense mutation, W572X, and a missense mutation, L609F in exon 13 of SCNN1G had been found in heterozygous forms[16]. The novel frameshift mutation of SCNN1B found in this patient is another genetic abnormality led to LS. Awareness of clinical features of LS, appropriate laboratory evaluation including genetic studies and proper management can be pursued.

Conclusion

A thirty-eight years old female presented with frequent proximal muscle weakness, severe hypertension, and persistent kaliuresis despite hypokalemia was found. After normalized serum potassium level, hyporeninemic hypoaldosteronism was detected. Pedigree study had supported the autosomal dominant inherited disease. Affected with LS in the present patient was due to a novel frameshift mutation. DNA sequencing resulted in exon 13 of SCNN1B gene: SCNN1B NM_000336.2:c.1724_1730dupGGCCCAC [p.Pro575Argfs*17]. Definite diagnosis led to proper management in this patient.

What is already known on this topic?

LS is extremely rare, even so, it could be found in Thailand. At present, only six cases were reported. Sawathiparnich et al[15] had examined a

proband with three other members in her family (mother, aunt, and elder sister). The genetic abnormality was P615H missense mutation in the proline-rich domain of the SCNN1B gene coding for the beta-subunit of amiloride-sensitive ENaC in all of them[15]. The other two had been reviewed by Jameekornrak[16] in 2010, the novel nonsense mutation, W572X, and a missense mutation, L609F in exon 13 of SCNN1G had been found in heterozygous forms during surveillance of 101 hypertensive patients presenting with low PRA and suppressed serum aldosterone at Siriraj Hospital[16]. Only one case had been proven after clinical and laboratory suggestion, whereas, the others were documented after surveillance or pedigree study.

What this study adds?

Since, clinical and laboratory findings of the LS are somewhat variable penetrance, and clinical expression[1] which could delayed and be difficult to make a clinical diagnosis. This report described findings which can be exercised as clinical clues to identify possible case of LS, simple and feasible clinical and laboratory examinations, i.e., high BP, low potassium, TTKG >3, UKER >20 mmol/L, etc., which have been already available, can be utilized prior to demonstration of low PRA, and suppressed serum aldosterone in a suspected case. Moreover, this novel mutation, duplication (insertion) of a GGCCCAC at nucleotide position 1724 to 1730 (c.1724_1730dupGGCCCAC) in exon 13 of SCNN1B, resulted in frameshift translation starting from codon 575, then premature termination at the 17th residue, codon 591 (SCNN1B NM_000336.2: c.1724_1730dupGGCCCAC [p.Pro575Argfs*17]) which was obtained in the author's report had raised another genetic abnormality of LS in Thailand. Since, genetic study is important for case documentation, the study done in this patient including her son revealed a new genetic mutation of the LS.

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Potential conflicts of interest

None.

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กลุ่มอาการลิดเดิล: รายงานผู้ป่วย 1 ราย

เมธา ผู้เจริญชนะชัย, พีระ บูรณะกิจเจริญ, ชนินทร์ ลิ่มวงศ์

*ผู้ป่วยหญิงอายุ 38 ปี มาด้วยอาการแสดงของกล้ามเนื้อส่วนต้นอ่อนแรงบ่อย ความดันโลหิตสูง และตรวจพบว่ามีการขับโปแทสเซียมออกทางปัสสาวะตลอดเวลาที่ร่างกายยังอยู่ในภาวะโปแทสเซียมในเลือดต่ำ ภาวะระดับเรนินและอัลโดสเตอโรนต่ำในเลือดได้ถูกตรวจพบหลังจากระดับโปแทสเซียมในเลือดได้รับการแก้ไขจนได้ระดับปกติ การศึกษาพงศาวลีได้สนับสนุนโรคที่มีการถ่ายทอดทางพันธุกรรมโดยจีนออโตโซมแบบลักษณะเด่น ผู้ป่วยกลุ่มอาการลิดเดิลรายนี้เกิดจากการกลายพันธุ์ชนิดใหม่ซึ่งทำให้เกิดการขยับลำดับการอ่านและแปลรหัสพันธุกรรม การเรียงลำดับพันธุกรรมนี้ส่งผลในแอกซอน 13 ของจีน SCCN1 เบต้า เริ่มที่ตำแหน่ง (codon) 575 โปรลีน และทำให้หยุดการอ่านรหัสก่อนกำหนดที่ตำแหน่ง (codon) 591 อาร์จินีน (SCNN1B NM_000336.2:c.1724_1730dupGGCCCAC [p.Pro575Argfs*17]) เนื่องจากกลุ่มอาการลิดเดิลเป็นกลุ่มอาการทางคลินิกที่พบได้น้อยมากในประเทศไทย การวินิจฉัยให้ถูกต้องควรยืนยันโดยการตรวจทางพันธุกรรม เพื่อให้การรักษาที่เหมาะสม*
