ORIGINAL ARTICLE

A Novel Frameshift Mutation of *SCNN1G* Causing Liddle Syndrome with Normokalemia

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BACKGROUND
Liddle syndrome (LS) is an autosomal dominant disorder caused by single-gene mutations of the epithelial sodium channel (ENaC). It is characterized by early-onset hypertension, spontaneous hypokalemia and low plasma renin and aldosterone concentrations. In this study, we reported an LS pedigree with normokalemia resulting from a novel *SCNN1G* frameshift mutation.

METHODS
Peripheral blood samples were collected from the proband and eight family members for DNA extraction. Next-generation sequencing and Sanger sequencing were performed to identify the *SCNN1G* mutation. Clinical examinations were used to comprehensively evaluate the phenotypes of two patients.

RESULTS
Genetic analysis identified a novel *SCNN1G* frameshift mutation, p.Arg586Valfs*598, in the proband with LS. This heterozygous frameshift mutation generated a premature stop codon and deleted the vital PY motif of ENaC. The same mutation was present in his elder brother with LS, and his mother without any LS symptoms. Biochemical examination showed normokalemia in the three mutation carriers. The mutation identified was not found in any other family members, 100 hypertensives, or 100 healthy controls.

CONCLUSIONS
Our study identified a novel *SCNN1G* frameshift mutation in a Chinese family with LS, expanding the genetic spectrum of *SCNN1G*. Genetic testing helped us identify LS with a pathogenic mutation when the genotypes and phenotype were not completely consistent because of the hypokalemia. This case emphasizes that once a proband is diagnosed with LS by genetic testing, family genetic sequencing is necessary for early diagnosis and intervention for other family members, to protect against severe cardiovascular complications.

Keywords: blood pressure; hypertension; Liddle syndrome; *SCNN1G* gene; Frameshift mutation; Normokalemia.

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Over the past several decades, with the development of numerous genetic studies on hypertension, the heritability of hypertension has been widely recognized and mainly separated into three forms, monogenic, polygenic, and epigenetic hypertension.[1–3] Genetic factors are thought to contribute to estimated 30% of blood pressure (BP) variance.[4] As an important part of secondary hypertension, monogenic hypertension disorders follow Mendelian inheritance patterns because of single-gene mutations, most of which are associated with volume expansion, electrolyte abnormalities, and low serum renin levels.[5,6]

Liddle syndrome (LS, OMIM #177200), an autosomal dominant form of monogenic hypertension, is caused by a gain-of-function mutation in the epithelial sodium channel (ENaC) genes, *SCNN1A*, *SCNN1B*, and *SCNN1G*, which encode three homologous subunits, α-ENaC, β-ENaC, and γ-ENaC, respectively. These mutations can increase the membrane density of the ENaC or the open probability of

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the ion channel being open, leading to extensive sodium reabsorption and subsequent hypertension.[7] Therefore, LS patients typically present with early-onset hypertension, hypokalemia, low renin and aldosterone levels, and metabolic alkalosis.[8] However, phenotypic diversity allows severe phenotypes and mild forms to coexist among LS patients, even those carrying the same causative mutation.[9]

In this study, we describe the clinical, biochemical, and genetic features of a *de novo* LS family. A novel *SCNN1G* frameshift mutation was identified in three family members and confirmed by genetic screening.

METHODS

Subjects

The proband (Figure 1), a 17-year-old male, suffered from uncontrolled hypertension for 3 years. From the age of 14, he was found high BP, with a maximum BP as high as 190/120 mm Hg. He received medication of bisoprolol and a calcium antagonist, but this failed to control his BP (140/90 mm Hg). Biochemical examination showed low plasma renin concentration (PRC) and plasma aldosterone concentration (PAC) in the upright position (Table 1). There were no other significant symptoms. On investigation of his family members, we found that his elder brother (subject III-3) had similar abnormalities as the proband. He was also diagnosed as hypertension (maximum BP: 180/110 mm Hg) for 3 years with symptoms of dizziness. Although he was treated with irbesartan and nifedipine, his BP was still uncontrolled (160/100 mm Hg) with unrelieved symptoms. Low PRC and PAC were detected for him (Table 1). While his parents showed no abnormal symptoms, this situation aroused a suspicion of secondary hypertension, particularly monogenic hypertension. Most of the family members were enrolled in this study. Every participants underwent clinical evaluation, biochemical examination, and genetic testing (Table 1).

The specific treatment of LS relies on ENaC blockers, such as amiloride and triamterene. In our study, only a compound amiloride containing 2.5 mg amiloride and 25 mg hydrochlorothiazide in each tablet were available for LS patients. Hydrochlorothiazide may diminish the efficacy of amiloride by increasing the sodium delivery to the collecting duct where it competes with amiloride for ENaC binding.[10] Both of LS patients (subjects III-3 and III-4) took two tablets of compound amiloride every day. During therapy, it is worthwhile monitoring serum electrolytes, especially to prevent hyperkalemia.[11]

This study was approved by the Ethics Committee of Fuwai Hospital and has been performed in accordance with the Declaration of Helsinki. All participants in this family offered Informed consent.

Imaging and laboratory examination

Imaging examinations were performed including echocardiography, abdomen ultrasound, and computed tomography (CT) of the kidneys, adrenal glands, and renal arteries. Laboratory testing covered general biochemical measurements associated with hypertension, such as serum electrolytes, urine protein, microalbuminuria, and blood gas analysis. PRC and PAC were measured at Fuwai Hospital by means of a chemiluminescence immunoassay using the LIAISON Direct Renin kit (DiaSorin S.p.A, Vercelli, Italy) and the LIAISON Aldosterone kit (DiaSorin Inc., Stillwater).

Genetic analysis

All participants provided venous blood samples for genetic testing. Genomic DNA was extracted from peripheral blood

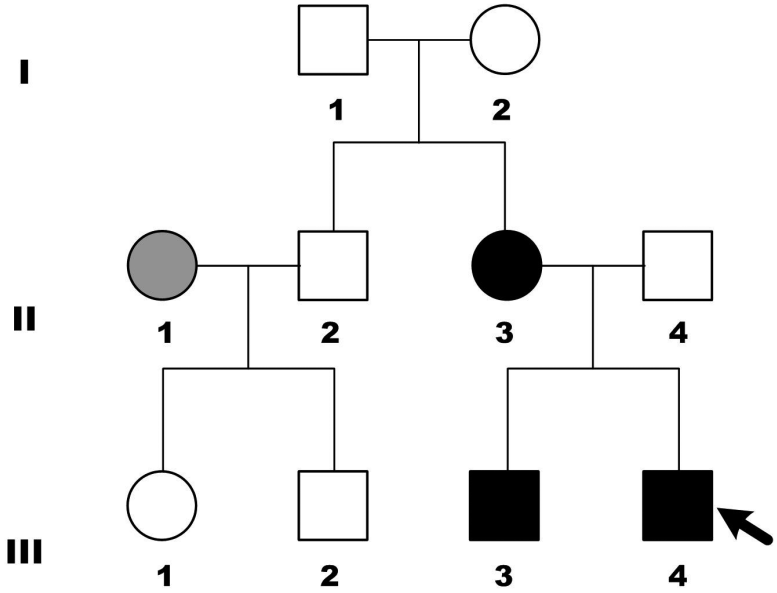

Figure 1. Family pedigree. Black filled symbols, subjects carrying the identified *SCNN1G* mutation (c.1756delC, p.Arg586Valfs*598); empty symbols, subjects without identified mutation; grey filled symbols, not sequenced; black arrow indicates the proband.

Table 1. Clinical and laboratory features of all participants

Subjects	Gender	Age, years	Onset age of hypertension, years	BP, mm Hg[a]	Serum K^+, mmol/l	PRC, μIU/ml	PAC, ng/dl	Follow-up[c] BP, mm Hg[a]	Follow-up[c] Serum K^+, mmol/l	Genotype[b]
II-3	F	46	-	120/86	5.25	14.8	3.0	-	-	1756delC/-
III-3	**M**	**23**	**19**	**180/110**	**4.38**	**3.0**	**2.9**	**124/78**	**4.67**	**1756delC-**
III-4	**M**	**17**	**14**	**190/120**	**3.68**	**2.0**	**2.2**	**130/83**	**4.05**	**1756delC/-**
I-1	M	73	66	165/78	4.72	8.0	1.5	-	-	-/-
I-2	F	74	63	178/86	4.93	7.7	4.0	-	-	-/-
II-2	M	50	48	143/102	4.37	8.0	1.5	-	-	-/-
II-4	M	46	-	130/90	4.57	3.0	8.0	-	-	-/-
III-1	F	17	-	124/80	4.15	11.3	9.5	-	-	-/-
III-2	M	6	-	114/74	4.43	8.5	10.3	-	-	-/-

The features of two patients with Liddle syndrome are shown in bold.

BP, blood pressure; F, female; M, male; serum K^+: reference value, 3.5–5.3 mmol/l; PRC, plasma renin concentration (upright): reference value, 4.4–46.1 μIU/ml; PAC, plasma aldosterone concentration (upright): reference value, 3.0–35.3 ng/dl.

[a]Mean of three measurements.

[b]1756delC/-: subjects had identified heterozygous mutation; -/-: subjects had no identified mutation.

[c]Follow-up results were obtained one month after treatment with compound amiloride.

leucocytes using the QIAamp DNA Blood Mini kit (QIAGEN, Hilden, Germany) by standard protocols. Next-generation sequencing of 41 monogenic hypertension-related genes was performed for the proband (Supplementary Table 1). All coding exons were enriched using custom-made SureSelect Target Enrichment System (Agilent Technologies, Inc., Santa Clara, CA). Captured DNA library were sequenced on Illumina Hiseq X Ten according to standard protocol for paired-end 150bp reads. Paired-end reads were aligned to the reference genome (hg19) using the Burrows–Wheeler Aligner and duplicated reads were marked by Picard.[12] SNVs and indels were detected by SAMtools, and Annovar[13] was used for annotation. A mutation in γ-ENaC was identified by polymerase chain reaction (PCR) using gene-specific primer pairs: *SCNN1G*: (GenBank accession number NM_001039): forward primer: 5′-CTTGGGAATCAGGGTTCCTGTG-3′, reverse primer: 5′-AAGCAGGCTTTTTGGTCAGAGT-3′.[14] The PCR products were sequenced bidirectionally using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA).

RESULTS

Clinical features

The proband and his elder brother were admitted to the hypertension center of Fuwai Hospital for further evaluation. On admission, the proband did not complain of any symptoms. Biochemical measurements (Table 2) showed low PAC (supine position: 2.8 ng/dl; upright position: 2.2 ng/dl) and PRC (supine position: 1.8 μIU/ml; upright position: 2.0 μIU/ml), elevated microalbuminuria (42 mg/l) and uric acid (459 μmol/l), and decreased aldosterone concentration (0.74 μg/24 h) and sodium (103.03 mmol/24 h) in urine. Fundus examination indicated mild retinal arteriostenosis. Serum potassium was measured several times within the normal range. Echocardiogram and x-ray showed a normally sized heart, and a CT scan excluded renal and adrenal abnormalities. Laboratory results of the proband's elder brother were similar to the proband (Table 2) in terms of low PAC (supine position: 2.6 ng/dl; upright position: 2.9 ng/dl) and PRC (supine position: 0.5 μIU/ml; upright position: 3.0 μIU/ml), elevated microalbuminuria (33.1 mg/l) and uric acid (459.36 μmol/l), decreased urine sodium (129.87 mmol/24 h), and mild retinal arteriostenosis. Echocardiogram detected left atrial enlargement (left atrial diameter, 41 mm.), and no abnormalities were found in serum potassium or CT scan of the kidney and adrenal glands. After a month of medication, their BP was successfully controlled, with a normal serum potassium level (Table 1). Both of their parents were healthy without any symptoms or signs. Another three hypertensives (subjects I-1, I-2, and II-2) were diagnosed with primary hypertension.

Genetic findings

A heterozygous frameshift mutation in exon 13 of *SCNN1G,* p.Arg586Valfs*598, was found in the proband (Figure 2), resulting from the deletion of a single cytosine residue from a sequence of four consecutive cytosines in codons 585–586. This not only generates a premature stop codon at position 598, but also directly deletes the PY motif. This new stop codon results in a truncated γ-ENaC protein lacking the remaining 52 amino acids. This mutation was only detected in three family members (subjects II-3, III-3, and III-4). The identified mutation was also did not found in 100 hypertensives or 100 healthy controls. This variant was not found in the Exome Aggregation Consortium (http://exac.broadinstitute.org/), the 1000 Genomes Project database (http://browser.1000genomes.org), or the Human Gene Mutation Database (http://www.hgmd.org). *In silico*

Table 2. Laboratory examinations compared between the proband and his elder brother in hospitalization

	Normal reference value	Proband	Elder brother
Renal function			
Blood urea nitrogen	2.86–7.90 mmol/l	4.95	4.92
Creatinine	44–133 μmol/l	78	97.17
Uric acid	148.8–416.5 μmol/l	459[a]	459.36[a]
Serum electrolytes			
Sodium	137–147 mmol/l	139.88	141.3
Potassium	3.5–5.3 mmol/l	3.68	4.38
Chloride	99–110 mmol/l	103.57	103.7
Calcium	2.2–2.75 mmol/l	2.27	2.43
Phosphorus	0.97–1.60 mmol/l	1.33	1.09
Blood gas analysis			
pH	7.350–7.450	7.419	7.401
HCO_3^-	21.0–27.0	25.7	26.2
Actual base excess	−3.0–3.0	1.7	1.6
Standard base excess	−3.0–3.0	1.7	1.9
Urine electrolytes			
Sodium	130–260 mmol/24 h	103.03[a]	129.87[a]
Potassium	25–125 mmol/24 h	31.55	51.74
Urinalysis			
Red blood counts	0–25/μl	0.1	1.3
Proteinuria	0.03–0.14 g/24 h	0.04	0.09
Microalbuminuria	<30 mg/l	42[a]	33.1[a]
Aldosterone	1.19–28.1 μg/24 h	0.74[a]	-
Plasma hormone levels			
Renin, supine	2.8–39.9 μIU/ml	1.8[a]	0.5[a]
Renin, upright	4.4–46.1 μIU/ml	2.0[a]	3.0[a]
Aldosterone, supine	3.0–23.6 ng/dl	2.8[a]	2.6[a]
Aldosterone, upright	3.0–35.3 ng/dl	2.2[a]	2.9[a]

[a]Abnormal laboratory examination values.

analysis predicted that it would be a probably deleterious mutation using MutationTaster2. Furthermore, there were no mutations in *SCNN1A* or *SCNN1B* in this family.

DISCUSSION

In this study, we report a novel frameshift mutation (p.Arg586Valfs*598) in γ-ENaC with LS. Family screening showed that the identified mutation arose *de novo* and originated from the proband's mother. Genetic analysis demonstrated that this pathogenic mutation generates a premature stop codon and deletes the PY motif.

Amiloride-sensitive ENaCs are expressed in various tissues, such as the apical portion of the epithelial cells of the distal nephron, distal colon, lung, and ducts of exocrine glands.[15] Under physiological conditions, ENaC expression and activity in the distal nephron is essential to maintain electrolytes homeostasis, together with the renal outer medullary K^+ channels and Na^+ /K^+ ATPase.[16] The ENaC is composed of three subunits, α-ENaC, β-ENaC, and γ-ENaC, which share 30–40% homologous identity.[17] Each subunit passes through the membrane twice, creating two transmembrane regions separated by a large glycosylated extracellular loop with short intracellular N- and C-terminus.[18] A proline-rich sequence called the PY motif is completely conserved in all three subunits, located at the C-terminus.[19] The E3 ubiquitin-protein ligase Nedd4-2 catalyzes the ligation of ubiquitin to the ENaC subunit, leading to ENaC internalization and eventual degradation in a proteasome or lysosome.[20,21] Mutation or deletion of the PY motif reduces the rate of ENaC ubiquitylation and consequent internalization, leading to an increase in the number of ENaCs in the membrane, and consequently enhancing Na^+ reabsorption and increasing water and sodium retention and potassium loss.[22,23] Therefore, this suggests that the PY motif plays a crucial role in the activity of the ENaC.

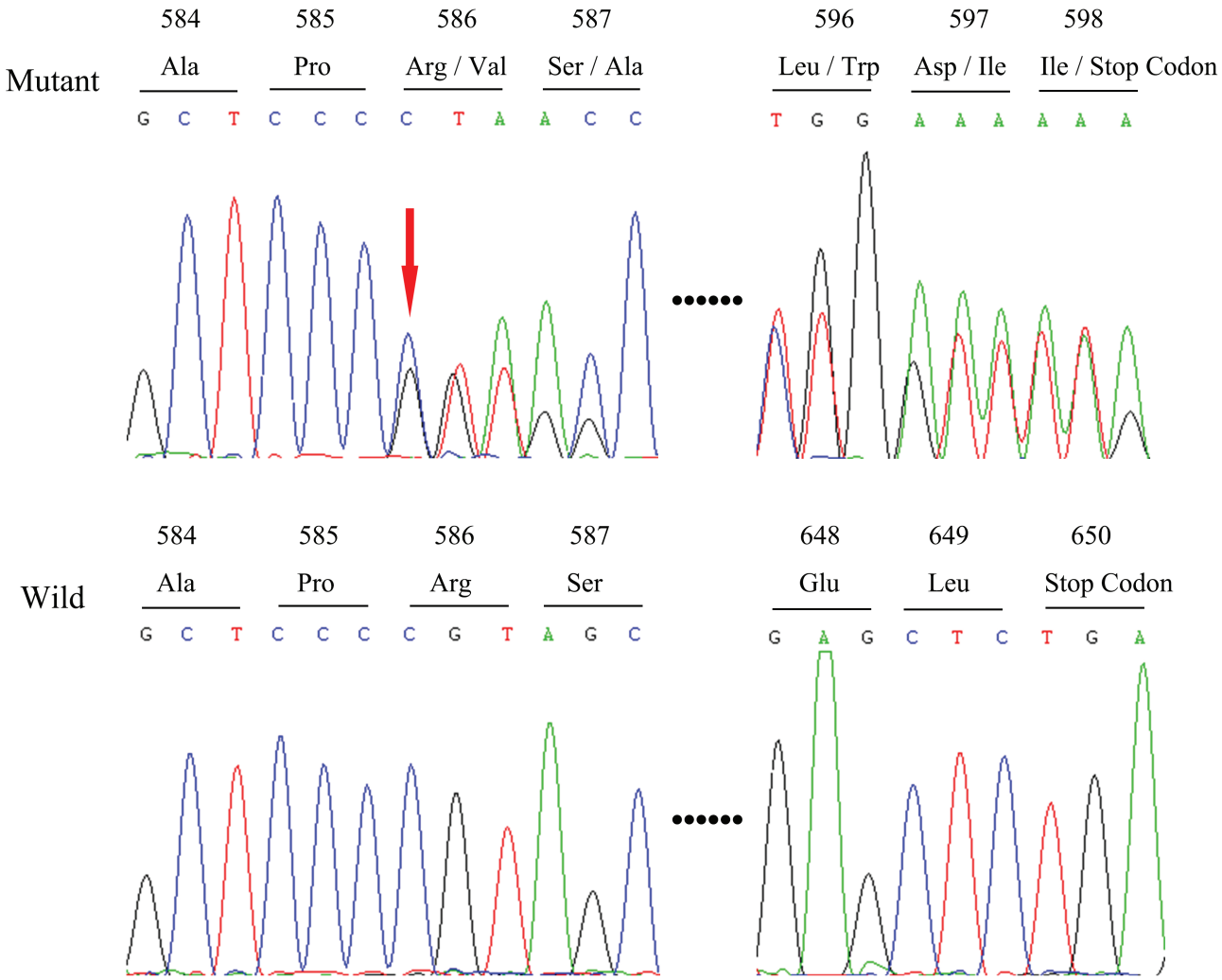

Figure 2. Sanger sequencing identifying the heterozygous *de novo* mutation in *SCNN1G*. The arrow indicates the mutation site (c.1756delC, p.Arg586Valfs*598), which generated a premature stop codon at position 598.

SCNN1G mutations are malignant and rare in LS. To date, only six different mutations in *SCNN1G* have been identified from different countries. Compared with the genetic spectrum of *SCNN1B*, most of *SCNN1G* mutations are nonsense mutations. In 1995, Hansson *et al.* firstly identified *SCNN1G*, in which the nonsense mutation c.1718G>A caused LS.[24] In the following decades, another three nonsense mutations in *SCNN1G* were reported.[25–27] All these nonsense mutations in *SCNN1G* truncated the carboxy terminus of γ-ENaC and directly deleted the PY motif. Wang *et al.* found a frameshift mutation caused by deletion of AGCTC at codon 583 in *SCNN1G*, resulting in a new termination site at codon 585 of the γ-ENaC and the deletion of the PY motif.[28] In this study, a novel frameshift mutation in *SCNN1G*, c.1756delC, was regarded as arising *de novo*, because the mutation came from the proband's mother and was not detected in her parents. This mutation, with a single cytosine nucleotide deletion, generated a new stop codon at position 598 and caused loss of the PY motif. It was predicted that a dysfunctional truncated γ-ENaC protein would be translated.

The families with LS show marked phenotypic variation in BP, serum potassium, and other clinical manifestations.[9] A total of 54 genetically confirmed LS probands with 26 different mutations were summarized in terms of 100% hypertension, 94% hypokalemia, 84% suppressed PAC, and 94% suppressed plasma renin activity or PRC.[29] Systematic review of the reported LS cases revealed that 92.4% hypertension, 71.8% hypokalemia, and 58.2% hypoaldosteronemia were present in a total of 200 individuals affected by LS with genetic diagnosis, belonging to 72 different families.[7] The LS symptoms in the proband presented more typical than in all LS cases. Hypertension and hypokalemia are common phenotypes in LS subjects. Usually, hypertensives seek medical treatment for uncontrolled elevated BP, and then recurrent spontaneous hypokalemia will lead to a suspicion of LS after excluding common hypokalemic reasons,[30] such as primary aldosteronism, congenital adrenal hyperplasia, low potassium intake, gastrointestinal potassium loss, and licorice ingestion.[31] In our research, the proband and his elder brother manifested with early-onset hypertension and low PAC and PRC. Their mother, with an identified

mutation, did not present any abnormalities. Their serum potassium levels were within the normal range. It has previously been reported that two LS patients with a *de novo* *SCNN1G* mutation suffered from hypertension[27,28] and hypokalemia.[28] Therefore, LS should be considered even in patients without spontaneous hypokalemia. As a result of the genetic sequencing, subject II-3 was identified as an asymptomatic *SCNN1G* mutation carrier. It will be necessary for her to be followed up for the rest of her life, as well as early interventions can be made to achieve a better prognosis, such as keeping a healthy lifestyle and reducing salt intake.[8]

The mechanisms leading to variable LS phenotypes remain unclear, although there are several hypotheses that might explain this. Dietary habits favoring a low salt intake could influence the phenotypic consequences.[32] Genetic factors such as gene polymorphisms could affect the phenotypic manifestation of the disease.[33] Epigenetic factors, including methylation, post-translational histone modification, and noncoding microRNAs, have been indicated to be significantly associated with hypertension.[34,35] Gene environment interactions also play an important part in modulating BP.[6] In the future, exploring these mechanisms may allow alteration of the progress and prognosis of LS by interventions for these factors.

In summary, we identified a novel pathogenic mutation of *SCNN1G* in a LS pedigree. Our study has expanded the genetic spectrum of *SCNN1G* and enriched the association between genotype and phenotype in LS. Genetic sequencing helps to identify causative mutations for LS cases, especially for those mutation carriers who manifest atypical clinical features. Combined with genetic and clinical characteristics, intervention as early as possible is beneficial to improve prognosis.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *American Journal of Hypertension* online.

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DISCLOSURE

The authors declared no conflict of interest.

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