Design and method: DNA was extracted from leucocytes in peripheral blood obtained from all family members. Whole-exome sequencing and Sanger sequencing were performed to verify the candidate variant and conduct co-segregation analysis. We also reviewed the clinical and biochemical characteristics of patients with the same mutation described in the literature and performed a genotype–phenotype correlation analysis.

Results: Whole-exome sequencing identified a c.1806dupG frameshift variant in SCNN1B leading to substitution of alanine for proline at position 603 and premature emergence of a stop codon at position 607. Phenotypic heterogeneity is evident in patients with the mutation reported in the literature and is reflected in the proportions with hypertension (94.4%), hypokalemia (58.9%), hypoaldosteronemia (52.9%), and hyporeninemia (41.1%). Patients with the mutation have a good response to targeted ENaC inhibitor therapy.

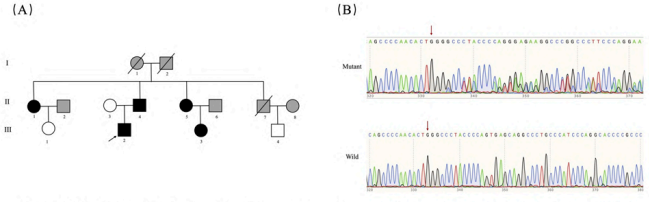

Figure. (A) Pedigree of the family with Liddle syndrome. Squares indicate males; circles indicate females. Filled symbols represent individuals with Liddle syndrome. Unfilled symbols represent subjects that did not carry the frame-shift mutation. Gray symbols mean individuals without genetic testing. Deceased members are indicated with diagonal lines. The arrow indicates the index case. (B) Sanger sequencing identified the heterozygous frame-shift mutation in *SCNN1B*. The arrow indicates the mutation site (c.1806dupG).

Conclusions: We have identified a frameshift mutation (c.1806dupG) in SCNN1B in a Chinese family characterized by early-onset hypertension and hypokalemia. Confirmatory genetic testing is necessary and targeted therapy should be used to prevent premature onset of clinical endpoints events in patients suspected to have mutation. Genotype-phenotype correlation analysis involving the P603 residue variant provides further evidence of phenotypic heterogeneity and guidance for clinical management of Liddle syndrome.

FUNCTIONAL ANALYSIS OF AN NF1 SPLICING MUTATION THAT CAUSED EXON SKIPPING AND LED TO A TRUNCATED PROTEIN IN NEUROFIBROMATOSIS TYPE 1

Yiting Lu, Di Zhang, Tao Tian, Kunqi Yang, Xianliang Zhou, Peng Fan. Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, CHINA

Objective: Neurofibromatosis type 1 (NF-1) is caused by mutations in the NF1 gene that encodes neurofibromin, a negative regulator of RAS proto-oncogene. Approximately one-third of the reported pathogenic mutations in NF1 are splicing mutations, suggesting that splicing mutations are not rare. The objective of this study was to identify the pathogenicity of splicing mutation in a Chinese family with NF-1 and determine the effects of the pre-mRNA splicing mutation by in vitro functional analysis.

Design and method: Next-generation sequencing was used to screen candidate mutations and Sanger sequencing was performed for validation. We performed a minigene splicing assay to determine the effect of the splicing mutation on NF1 expression and three-dimensional structure models of wild-type and mutant-type neurofibromin were generated using SWISS-MODEL and PROCHECK method, respectively.

Results: Next-generation sequencing analysis identified a pathogenic splicing mutation c.479 + 1G>C in NF1 in the proband who had typical pigmental lesions and severe renal artery hypertension. We speculated that the causative mutation originated from the proband's mother who died of glioma. The minigene splicing assay showed that the c.479 + 1G>C mutation caused the skipping of exon 4, leading to a Glutamine to Valine substitution at position 97 in neurofibromin and an open reading frame shift terminating at codon 108. Three-dimensional modelling of neurofibromin showed that several major domains were missing in the truncated protein, which would have affected its function.

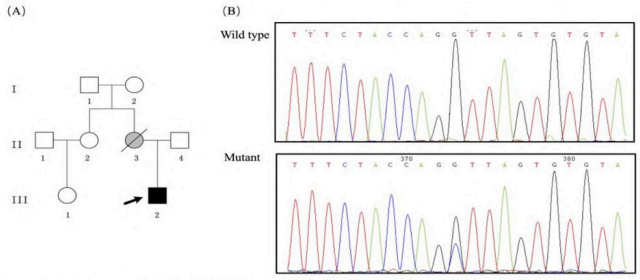

Figure 1. A splicing mutation c.479+1 G>C in *NF1* identified in a Chinese family. (A) Pedigree of the family. (B) Sanger sequencing results of *NF1* gene in the patient with neurofibromatosis type 1 and normal subject. The black arrow indicates the proband.

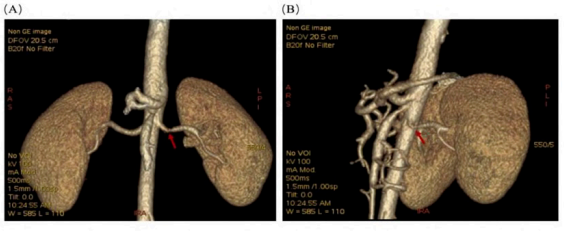

Figure 2. Left renal artery stenosis detected in the proband. The red arrow indicates the vascular lesion. (A) The Sagittal view. (B) The coronal view.

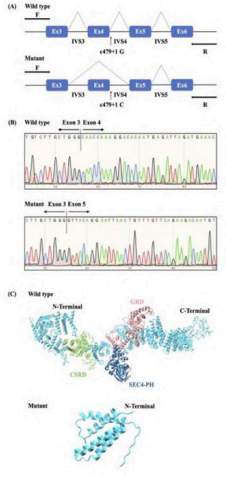

Figure 3. (A) Schematic diagram of the splicing process (c.479+1G>C). (B) The skipping of exon 4 in the mutant type demonstrated by Sanger sequencing. (C) The predicted three-dimensional models of wild-type and mutant-type neurofibromin. CSRD, cysteine-serine-rich domain; SEC14-PH, Sec14-homologous domain and pleckstrin homology domain; GRD, GAP-related domain.

Conclusions: The splicing mutation c.479 + 1G>C identified in a Chinese pedigree with NF-1 caused the skipping of exon 4 and resulted in a truncated protein. Our findings would be of great significance for the molecular diagnosis and management of NF-1 and showed that the minigene splicing assay is a powerful tool for analyzing mRNA expression outcomes caused by donor splicing site variation.

COMPARISON OF GENE EXPRESSION OF EPICARDIAL ADIPOSE TISSUE AND LEFT VENTRICULAR MYOCARDIUM IN END-STAGE HEART FAILURE

Jan Kyselovic[1], *Jana Mlynarova*[1], *Peter Musil*[1], *Michal Hulman*[2], *Ivo Gasparovic*[2], *Andrea Gazova*[3]. [1]Clinical Research Unit, 5th Department of Internal Medicine, Faculty of Medcine,Comenius University, Bratislava, SLOVAK REPUBLIC, [2]Clinic of Cardiac Surgery, National Institute for Cardiovascular Diseases, Faculty of Medicine, Comenius University, Bratislava, SLOVAK REPUBLIC, [3]Institute of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Comenius University, Bratislava, SLOVAK REPUBLIC