A Novel Mutation of *KCNJ1* Identified in an Affected Child with Nephrolithiasis

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Abstract

Nephrolithiasis is not common in children, but the incidence is gradually increased in these years. Urinary tract malformations, urinary infection, dietary habits, geographic region and genetic factor are involved in the etiology of nephrolithiasis. For the affected child, it is especially important to elucidate the etiology, which may provide an accurate diagnosis, a personalized therapy and effective follow-up strategy. Here to seek the etiology of a ten-year old boy incidentally found with nephrolithiasis, next generation sequencing (NGS) including a panel with 248 genes involved in hereditary kidney diseases was performed for the boy and identified two mutations of *KCNJ1*, c.89G>A (p.C30Y) and c.65G>T (p.R22M), and the later was a novel missense mutation originated from his father. The child was confirmed with type II Bartter syndrome (BS) caused by *KCNJ1* mutations. Our study suggests that BS may be difficult to get diagnosed at early stage based on clinical manifestations or biochemical laboratory tests, and NGS is an efficient way to determine the etiology and provide further treatment and guide fertility counselling for the affected family.

1 Introduction

Nephrolithiasis is not common in children compared to adults, but the incidence of pediatric nephrolithiasis is gradually increased in these years [1]. Urinary tract malformations, urinary infection, dietary habits, geographic region and genetic factor are involved in the etiology of nephrolithiasis [2,3]. There are at least 30 genes shown to cause monogenic forms of nephrocalcinosis or nephrolithiasis in autosomal-dominant, autosomal-recessive, or X-linked transmission pattern [4]. For the affected child, it is especially important to detect the exact causative mutation of monogenic disease and know the etiology, which may provide an accurate diagnosis, a personalized therapy and effective follow-up strategy.

In the present study, we described the clinic features of one 10-year old child who was incidentally found with nephrolithiasis and detected with the genetic mutation, which suggests that NGS is very important and efficient to identify the etiology of nephrolithiasis, which may not easily diagnosed by manifestation or chemical laboratory tests.

2 Materials And Methods

2.1 Enrollment of human subjects

The study was approved by the Ethical Committee of Zhengzhou University (Study NO. KS-2018-KY-36) and was conducted with the written consent of all the participants. The proband was a 10-year old boy who was incidentally found with nephrolithiasis when he was admitted to the First Affiliated Hospital of Zhengzhou University, China because of the fraction of right clavicle and firstly diagnosed as medullary sponge kidney. To seek the etiology of nephrolithiasis, the proband and his parents were referred to the Center of Genetics and Prenatal Diagnosis for genetic counselling, and the peripheral blood samples were

obtained from this family and genomic DNA was extracted from peripheral blood lymphocytes for further genetic test.

2.2 Next generation sequencing (NGS) and Sanger sequencing

Targeted-NGS using inherited kidney disease panel including 248 disease-causing genes was performed by a commercial company (MyGenostics, Inc., Beijing, China). To validate the variants screened by NGS, the related fragments were performed PCR amplification for the proband and his parents, and the primers of the fragments were listed in Table 1. PCR products were bi-directionally sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA) in the Center of Genetics and Prenatal Diagnosis of the First Affiliated Hospital of Zhengzhou University.

2.3 Bioinformatics analysis

The harmful prediction was analyzed according to the scoring conditions using three kinds of software including SIFT, PolyPhen-2 and MutationTaster. The pathogenicity of the mutation site was annotated according to the American College of Medical Genetics and Genomics standards and guidelines.

3 Results

3.1 Clinical examinations

The proband was 10-year old, and the weight was 28kg (-1standard deviation [SD] for age), with the height 130cm (-2 SD). The other general physical examinations of the proband were normal, with normal blood pressure of 123/65mmHg. Biochemical laboratory tests showed blood routine, electrolyte, liver function, renal function, urinary routine were normal. The urinary ultrasound showed enhanced echo of bilateral renal collecting system, several echogenic foci with posterior shadow, the largest one on the left kidney 8mm × 6mm, on the right side 9mm × 6mm (Figure 1), the blood perfusion was normal, and multiple high echo spots in the prostate with posterior shadow, the largest one 6.4mm × 4.6mm. Abdominal computed tomography (CT) showed multiple high density shadows in bilateral renal medulla and left high density nodules of urethra prostate. Glomerular filtration rate (GFR) in the left kidney was 34.07ml/min shown by SPECT-CT indicating mild impairment of left renal function (normal range: 40-50 ml/min), and GFR in the right kidney was 51.11ml/min. Traced back to the previous history of the proband, he was born prematurely at 31 weeks of gestation with low birth weights about 1.7kg following polyhydramnios. No obvious abnormality was seen until 4 years ago, and he presented intermittent cramps, fatigue and muscle weakness and these symptoms released after taking oral potassium chloride.

3.2 Mutation analysis

Two heterozygous mutation sites of *KCNJ1* gene (NM_153767) were found in the proband by NGS and convinced by PCR and Sanger sequencing, which were originated from his father and mother (Figure 2).

Both mutations were located at exon 4. One site c.89G>A (p.C30Y) was a known pathogenic mutation of *KCNJ1* [5]. The other one c.65G>T (p.R22M) was a novel missense mutation, leading to the arginine being substituted by methionine at codon 22, predicted to be deleterious by SIFT, Polyphen-2 and MutationTaster (table 1), which might be responsible for this family.

4 Discussion

In the present study, the proband, a ten-year old boy primarily incidentally detected with bilateral nephrolithiasis was found with compound heterozygous mutations of *KCNJ1* gene by NGS. A novel heterozygous missense mutation c.65G>T (p.R22M) identified by Sanger sequencing was inherited from the phenotypically healthy father, which could be discerned from a polymorphism since the harmful predictions by all three bioinformatics softwares were pathogenic and this mutation was not found as SNP in the global SNP database. Through the symptoms and genetic test, the proband was confirmed with type II Bartter syndrome (BS II).

KCNJ1 gene is one of the five types of genes involved in the etiology of BS which is a group of rare tubulopathies. Patients with different type of BS present with overlapping clinical phenotypes as polyuria, polydipsia, volume contraction, muscle weakness and growth retardation induced from hypokalaemia, hyperreninism and hyperaldosteronism. According to the onset and severity of BS, it can be grouped into three types: the hypocalciuric-hypomagnesemic variant described by Gitelman *et al*., the classic syndrome originally described by Bartter *et al*., and the antenatal hypercalciuric variant associated with severe systemic manifestations classical type [6]. *KCNJ1* gene encodes the apical potassium inwardly-rectifying channel (ROMK) in the thick ascending limb of the Henle's loop (TALH) in the distal nephron to ensure adequate luminal potassium available for the efficient functioning of the Na-K-2Cl cotransporter which function in salt reabsorption [7]. Effective chloride reabsorption in the TALH prevents renal salt wasting and is an essential mechanism to maintain tubular concentrating capability. Loss-of-functional mutations in the *KCNJ1* gene cause antenatal/neonatal Bartter syndrome type II (aBS II) in autosomal recessive pattern [8].

The Phenotype in most of patients with BS II can begin in utero with marked fetal polyuria presenting polyhydramnios from 24 weeks of gestation and premature delivery. During neonatal period, patients may have life-threatening volume depletion caused by severe renal salt wasting or failure to thrive. During childhood, other secondary symptoms including developmental retardation, fever, vomitting, occasional diarrhea may present resulted from metabolic alkalosis, hyposthenuria, hyperreninaemic, hyperaldosteronism which was stimulated by elevated plasma concentration of prostaglandin E2 (PGE2). The basic deficiency of antenatal BS is the malfunction of mTAL chloride transport, which involves an interaction among the apical Na-K-2Cl cotransporter (*NKCC2*), the luminal ATP-sensitive potassium channel ROMK (*KCNJ1*), the basolateral chloride channel (ClC), a basolateral K-CL cotransporter and the Na-K-ATPase. Therefore, any gene encoding or involving in these channels or transporters will result in defective chloride transport. *NKCC2*, *KCNJ1*, *CLCNKB* for chloride channel and *BSND* gene encoding

barttin, a subunit for ClC-Ka and ClC-Kb have been confirmed with antenatal BS [9,10]. Rare disease shall also be differentiated such as Rabson-Medndenhall syndrome caused by *INSR* [11].

The other equally important feature in antenatal BS is hypercalciuria. Continuous loss of calcium results in nephrocalcinosis, nephrolithiasis and osteopenia [12,13], usually medullary nephrocalcinosis is seen [14,15]. Hypercalciuria and associated nephrocalcinosis are present in approximately 85% of infants with this neonatal BS [16]. The prevalence of nephrolithiasis is high, but the prevalence secondary to BS is not known very well, and may be lower than the prevalence of nephrocalcinosis. Both nephrocalcinosis and nephrolithiasis share a well-recognized heritability [17,18], and around 15% of the patients were detected with causative genes [4]. Although low plasma potassium concentration, secondary low urinary citrate, tubulointerstitial damage, chloride deficiency, and increased intracellular chloride activity were also suggested to contribute to the hypercalciuria, the exact pathogenesis of nephrocalcinosis or nephrolithiasis in BS remains unclear [19]. Renal function is generally well preserved. In the present study, GFR of the proband was lower than the normal population. According to the ten-year outcome study by Puricelli E et al. [20], 25% of the patients with type I or type II BS had GFR lower than the normal range, which may be resulted from nephrocalcinosis. More than 30 genes have been reported to be with the etiology of nephrolithiasis [4]. Two-thirds of the genes currently known to be associated with nephrolithiasis coding for membrane proteins or enzymes involved in renal tubular transport[21]. The TALH and connecting tubules (CNT) have a central role in maintenance of fluid, electrolytes and acid-base homeostasis. Therefore, mutations of genes involved in TALH and CNT function can result in phenotypically severe disease. 14 of all genes are of paramount importance accounting for 15% of nephrolithiasis or nephrocalcinosis [22]. Recessive causes were more frequent among children, whereas dominant disease occurred more abundantly in adults. Therefore, NGS panel including genes involved in functions of TALH, connecting tubules, systemic disorders such as chromic hypercalcemia from vitamin D, primary hyperoxaluria, ARPT deficiency, distal renal tubular acidosis, Dent's disease, cystinuria and family hypomagnesemia with hypercalciuria shall be applied [23,24]. In this study, 248 genes associated with hereditary kidney diseases were all included in the panel, and no other suspicious gene mutations were found except *KCNJ1* gene.

KCNJ1 gene mutation associated antenatal BS is phenotypically distinct from the other disease because of prominent polyhydramnios with preterm delivery together with discontinuous fatigue, still phenotypic variability presents in patients with *KCNJ1* mutation and absence of enough recognition for this type of disease may exist. The patient in the present study was not gotten accurate diagnosis until he was ten-years old and incidentally found bilateral nephrolithiasis, although he had the previous infant history with polyhydramnios and preterm delivery, and the intermittent cramps, fatigue and muscle weakness during childhood.

There are other causes which could also induce either of these symptoms. The clinicians or parents may ignore the real etiology beneath the manifestations and the clinical misdiagnosis of BS was nearly 25%, especially in developing countries [25]. Also the onset of BS type may be late. One adult male patient

initially presented with an incidental finding of nephrocalcinosis was diagnosed as a late-onset BS due to detection of a homozygous *KCNJ1* missense mutation (c.658C > T, p.L220F) [26].

Our case showed that the presentations in patients with BS may not be unusual, and specific disorders within the spectrum of BS or nephrolithiasis may not easily be diagnosed or differentiated by rigorous clinical manifestations. Genetic test, especially NGS is a very efficient tool to distinguish specific disorder from multiple confusing spectrums.

Declarations

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Author's contributions

Qinghua Wu and Saisai Yang conceived the ideas and wrote the manuscript; Huirong Shi, Cong Wang analyzed the data; Chihhong Lou, Shumin Ren, Zhihui Jiao and Xiangdong Kong revised the draft. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials

The original data of WES are not available according to the Chinese policies of Human Genetic Resource, but the VCF file is available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethics approval and consent to participate.The study was approved by the First Affiliated Hospital of Zhengzhou University(approval number KS-2018-KY-36). The guardians of the patient pro-vided written informed consent.

Consent for publication

Written informed consent was obtained from the guardians of the patient for publication of this Case Report and any accompanying images. The copy of the written consent is available for review by the Editor of this journal.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

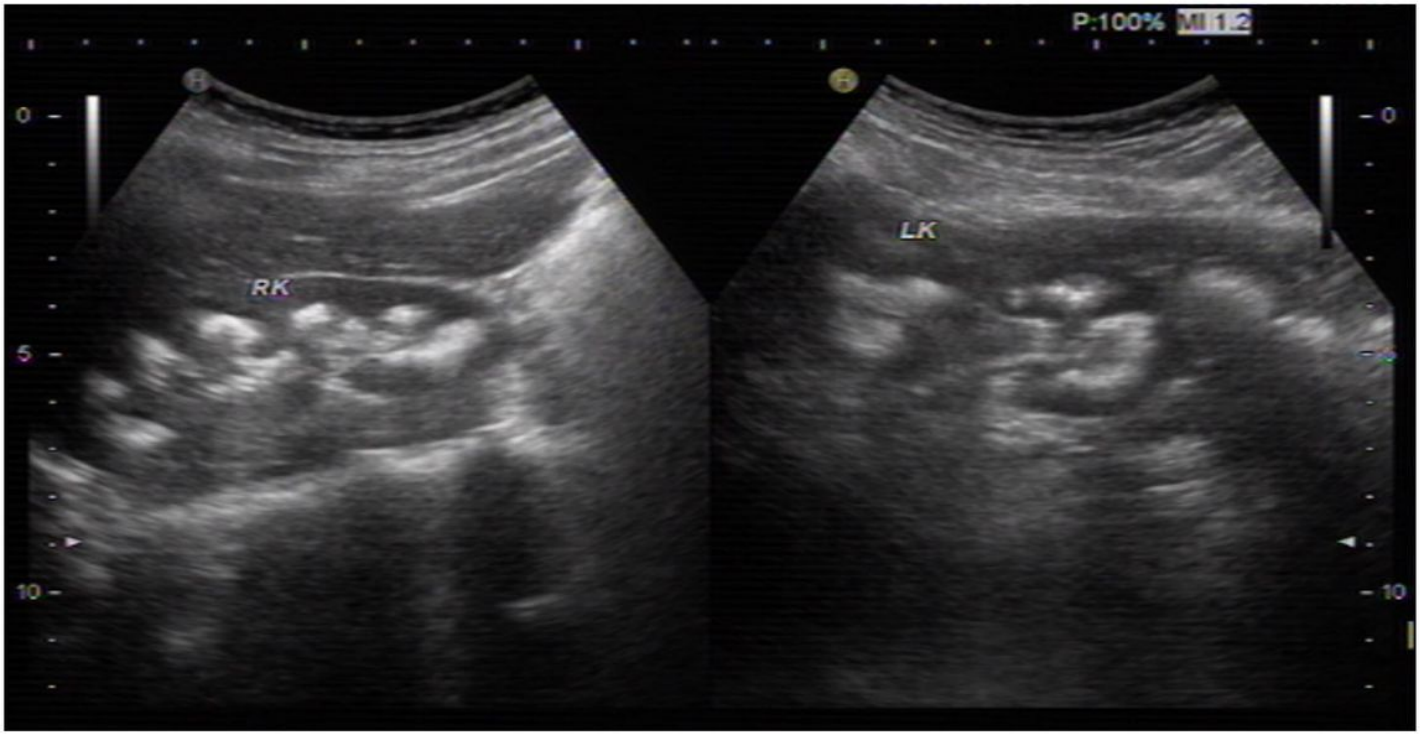

Figure 1

The urinary ultrasound showed enhanced echo of bilateral renal collecting system.

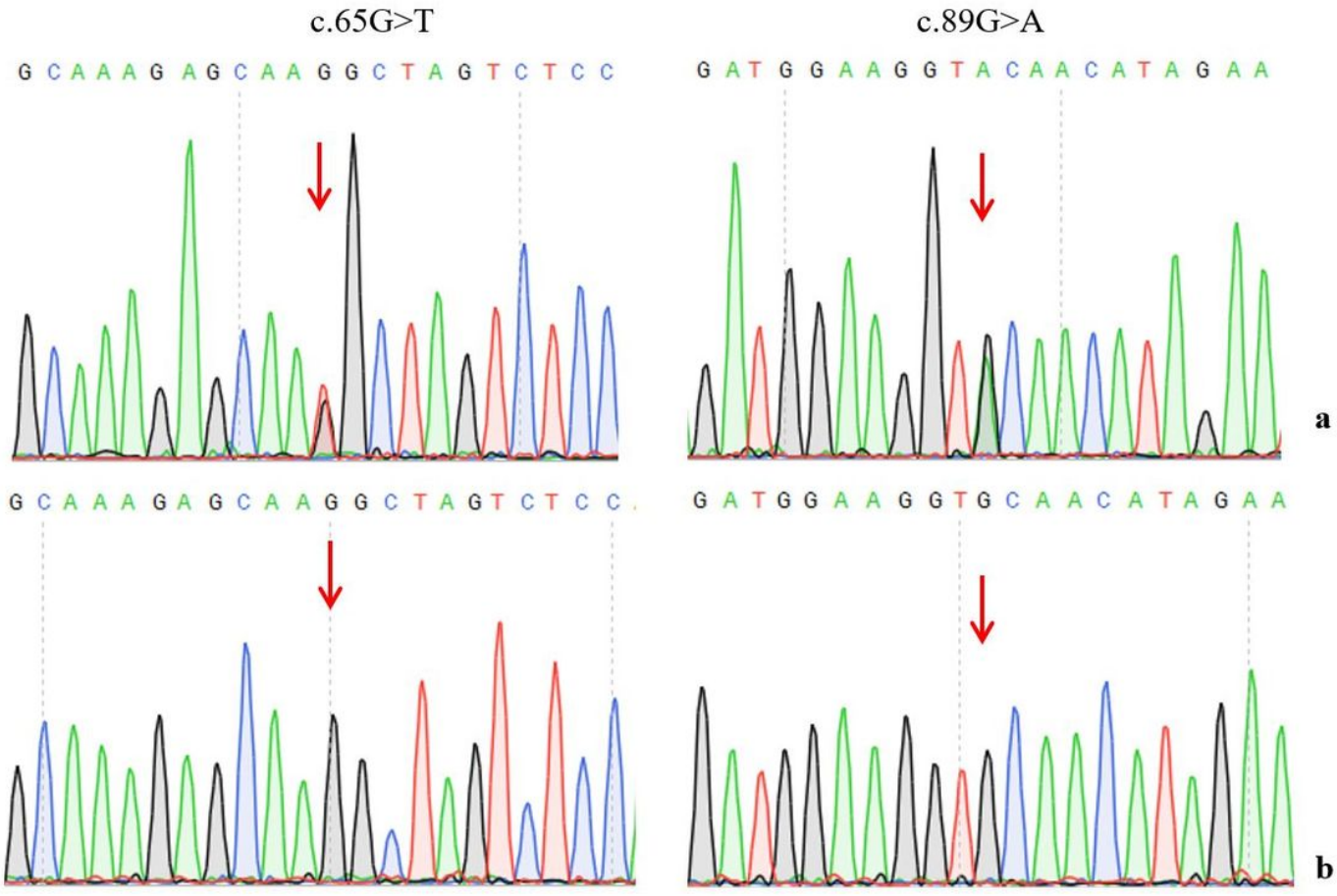

Figure 2

Direct sequencing results of *KCNJ1*. Compound mutations c.65G>T and c.89G>A in *KCNJ1* gene (NM_153767) in the proband (a), originated form his father and mother. The sites as normal control were shown in (b).

Supplementary Files

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- table1.pptx