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Original Article

Use of whole-exome sequencing to identify a novel ADCY10 mutation in a patient with nephrolithiasis

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Abstract: Nephrolithiasis is a prevalent condition with high morbidity, and the incidence and prevalence of nephrolithiasis have been increasing worldwide. Although dozens of monogenic reason of nephrolithiasis have been identified, the fraction of the disease caused by single genes has not been determined. In this study, employing total exon sequencing technology, we investigated two patients in south-central China with primary nephrolithiasis and identified a novel *ADCY10* mutation c.2186G > A (p.G729E) and a known *ADCY10* mutation c.2182G > A (p.E728K). The results of our study suggest that *ADCY10* plays an important role in nephrolithiasis.

Keywords: ADCY10, nephrolithiasis, kidney stone, molecular diagnosis

Introduction

Nephrolithiasis (NL) is a disease caused by the aggregation of crystals, leading to the formation of stones in the kidney [1]. NL is a common disease worldwide, and the incidence and prevalence of nephrolithiasis have been increasing globally [2]. NL presents a significant problem for individuals and society because the costs for treating and preventing NL are substantial. Almost 9% of the population will have a kidney stone at any point in life, generally during adulthood [3]. In addition, stone recurrence is a prevalent problem with an estimated recurrence rate of 14% after 1 year and 35% after 5 years [4]. NL comprises renal colic, hematuria, flank pain, urinary tract infections, and blockage of urine flow [5]. NL is associated with significant morbidity because of colicky pain, the necessity of surgical procedures, and progression to CKD [6]. NL and related conditions, such as nephrocalcinosis (NC), emphasize that two-thirds of hypercalciuric stone formers have relatives with NL [7]. Hypercalciuria is the most common metabolic abnormality associated with NL and NC; however, approximately 30% of individuals with kidney stones have been reported to have no obvious underlying metabolic defect (idiopathic NL) [8]. NL, NC, and hypercalciuria are likely to have a genetic basis, as up to 65% of kidney stone patients have been reported to have an affected family member [9]. Currently, at least 30 genes have been shown to cause single-gene forms of NL/NC by autosomal-dominant, autosomal-recessive, or X-linked transmission [10].

ADCY10 (adenylate cyclase 10) is a protein coding gene. *ADCY10* encodes soluble adenylyl cyclase, which is associated with sperm-specific enzymes; therefore, *ADCY10* is usually considered to be associated with asthenozoospermia [11]. Some studies listed ADCY10 as a disease gene for familial idiopathic hypercalciuria, which is a common cause of kidney stones (MIM#143870) [12]. *ADCY10* was shown to consist of 33 exons, and the open reading frame extended to nucleotide 5053. The ADCY10 protein was predicted to consist of 1518 amino acids [13]. The molecular function of ADCY10 is to catalyze the formation of the signaling molecule cAMP, and it may function as a sensor that mediates responses to changes in cellular bicarbonate and CO_2 levels [14]. Some

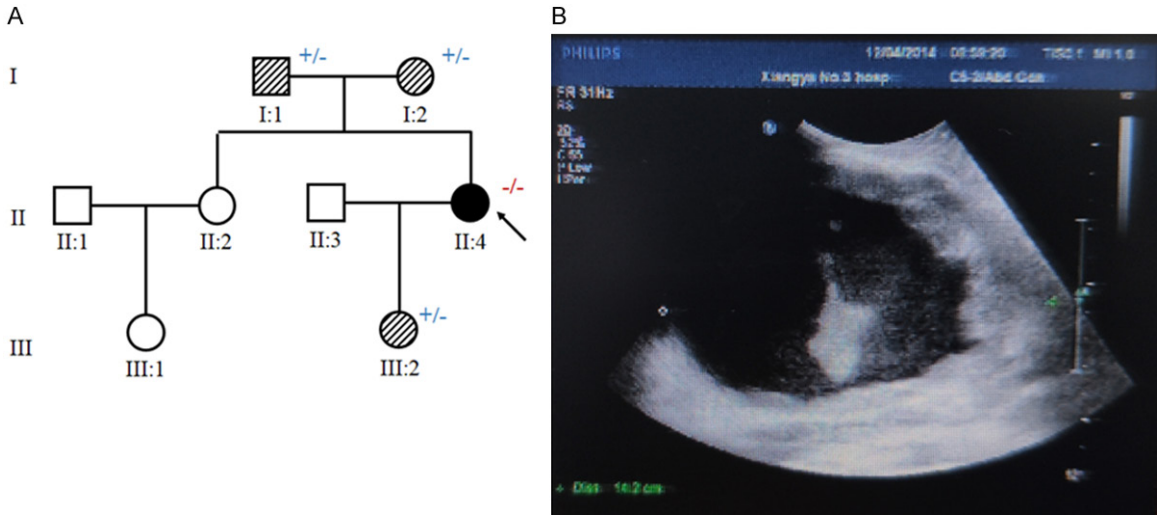

Figure 1. A. Pedigree of the patient with nephrolithiasis. Family members are identified by generation and number. Squares indicate male family members; circles indicate female member; symbol with bias indicate carrier; arrow indicate proband, "+" means normal sequence; "-"means mutation; II:4 is propositus. B. Ultrasound report of the patient with nephrolithiasis shows that the patient suffers from parenchymal lesions of both kidneys, atrophy of both kidneys, multiple stones in the right kidney, hydrocephalus in the right kidney, left kidney cyst. Left kidney size 50 × 27 mm, right kidney size 59 × 30 mm, morphological rules, contour please, substantive echo enhancement, left kidney upper section can be explored and a size of about 15 × 16 mm no echo area, boundary clear, with envelope, right kidney collection system separation 30 mm, there is a kidney can probe a number of strong light group accompaniment, the larger 10 × 7 mm.

studies have suggested that a genetic factor is the primary cause of hypercalciuria, and elevated urinary calcium excretion may result in calcium loss, decreased bone mineral density (BMD), osteoporosis, and progression toward nephrolithiasis [15]. Mutations or polymorphisms in the soluble ADCY10 have been linked to bone loss in nephrolithiasis patients with absorptive hypercalciuria. In this study, we identified a mutation in *ADCY10* in a patient with nephrolithiasis [16].

Materials and methods

Clinical summary

The study protocol was approved by our institutional ethics committee, and the parents of the patient provided informed consent.

The proband was 55 years old and presented with chronic nephritis, right kidney stones, and kidney cysts (**Figure 1A**). Ultrasound diagnostic reports show that the patient suffers from double kidney material lesions and atrophy, and the right kidney shows multiple stones and hydronephrosis, a left kidney cyst, and a lower abdomen mixed echo pack after screening the biochemical criteria of the patients. The report shows that the larger stones is 10 × 7 mm in right kidney, left kidney have no obvious stone (**Figure 1B**). At the same time, the proband suffered from long-term chronic nephritis and CKD, and indicators, such as creatinine, carbamide, and hemoglobin, showed a significant increase compared to common indicators (**Table 1**). Notably, the patient had 50 years of proteinuria.

Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes of the proband and his healthy parents using a QIAamp DNA Blood Mini Kit (250) (QIAGEN, Valencia, CA). Briefly, DNA of the proband was captured with the Agilent SureSelect Human All Exon V5 Kit (Agilent, California, USA) and sequenced on an Illumina HiSeq 4000 (Illumina Inc, San Diego, USA). The sequencing reads were aligned to the NCBI human reference genome (gh19/NCBI37.1) by Burrows-Wheeler Aligner software. ANNOVAR is performed to annotate the Variant Call Format file. The American College of Medical Genomics (ACMG) guideline was used to classify the variants. Pathogenic, likely pathogenic and uncertain significance single-nucleotide variants (SNVs) and short insertions and deletions (InDels) were filtered as follows:

Table 1. Long term follow-up of the patient with renal calculus

Time/phenotype	20040820	20050515	20071220	20091202	20100309	20110124	20120122	20130112
Proterinuria	+	+	+	+	+	+	+	+
chronic nephritis	√	√	√	√	√	√	√	√
Stage	CKD5	CKD5	CKD5	CKD5	CKD5	CKD5	CKD5	CKD5
renal calculus								
renal cyst								
renal hypertension	√	√	√	√	√		√	√
renal anemia					√		√	√
secondary hyperparathyroidism			√				√	
osteoarthritis								
Time/phenotype	20140526	20150624	20151203	20160503	20161028	20170418	20181207	20190531
Proterinuria	+	+	+	+	+	+	+	+
chronic nephritis	√	√	√	√	√	√	√	√
Stage	CKD5	CKD5	CKD5	CKD5	CKD5	CKD5	CKD5	CKD5
renal calculus		√	√	√	√	√	√	√
renal cyst		√	√	√	√	√	√	√
renal hypertension	√	√		√	√	√	√	√
renal anemia	√	√		√	√	√	√	√
secondary hyperparathyroidism	√		√	√	√	√	√	
osteoarthritis			√	√	√	√	√	√

(i) Variants within intergenic, intronic, and untranslated regions and synonymous mutations were excluded from subsequent analyses. (ii) High-frequency (minor allele frequency > 0.01) polymorphisms found in the Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), 1000 Genomes Project, and Exome Sequencing Project (ESP), were excluded. (iii) Nonsynonymous SNVs were further analyzed. Bioinformatics programs SIFT, Polyphen2, MutationTaster, and CADD were used to predict the possible impacts of variants. Sanger sequencing was used to validate the candidate variants. Genomic array screening was performed to identify the potential microdeletion or uniparental disomy with the Infinium OmniZhongHua-8 Kit v1.4 (Illumina, California, USA). We also used the short tandem repeats (STR) typing method to study paternity. Two-tailed Student's t-tests based on ANOVA were used for two-group comparisons. For multiple comparisons, we conducted one-way ANOVAs with Dunnett's correction to analyze differences among the control group and one or more independent treatment groups. Differences were considered to be significant at $P < 0.05$ with significance indicated in figures as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NS represents no significant difference.

Results

For the DNA sample of the proband, whole-exome sequencing (WES) generated an average of 6 Gb data with an appropriately 99% coverage and a depth of > 50 ×. Unique SNPs were identified after the exclusion of common variants. Finally, only one mutation located in ADCY10 exon 17 induces amino acid changes (**Figure 2B**), *ADCY10* mutation c.2186G > A (p.G729E) (**Figure 2A**), passed the filtration, which MutationTaster showed to be disease-causing NL. This variant was validated via Sanger sequencing (**Figure 2B**).

The genomic array screening did not show structural variants at the *ADCY10* mutation c.2186G > A (p.G729E) or nearby. In addition, paternity was confirmed by the STR typing method. The mutation was consistent with the coseparation and was not found in our 200 control cohorts, dbSNP (https://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi) or the Exome Variant Server database (**Figure 2A**). Three programs analyzing the pathogenicity of genetic mutations, MutationTaster, polyphen2 and SIFT, predicted that the mutation is probably deleterious.

Combining our sequencing results and the analyses from bioinformatics programs, we conclude that the region plays an important role in causing NL.

Discussion

As the incidence of kidney stone disease continues to rise and because current therapies do

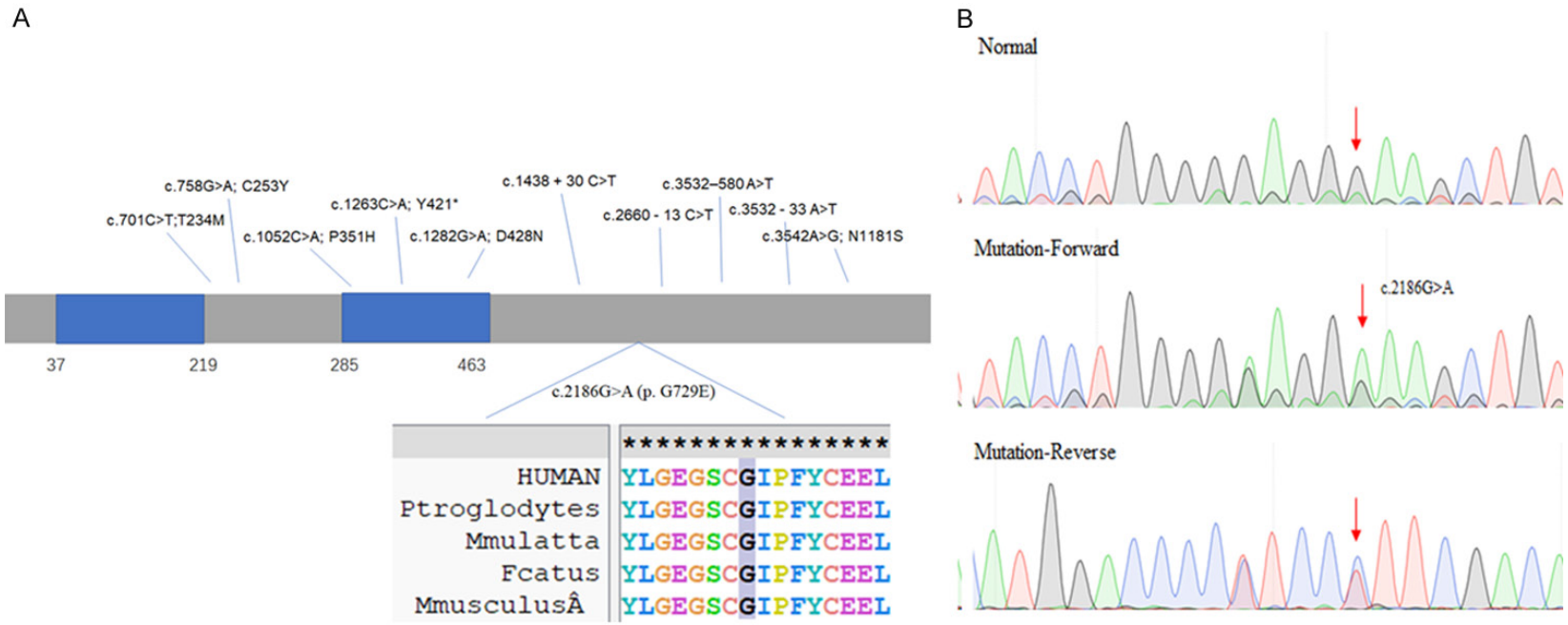

Figure 2. A. The domain of ADCY10, at the mutation position c.2186G > A (p.G729E), Conservative analysis and Comparison of the functional domains: Compared sequence of amino acid, mutation sites (gray) show a high degree of conservatism in different species. Known ADCY10 mutation causing NL. B. Sanger Sequencing confirmed the variation in patients; Sequence analysis of new mutations of c.2186G > A (p.G729E).

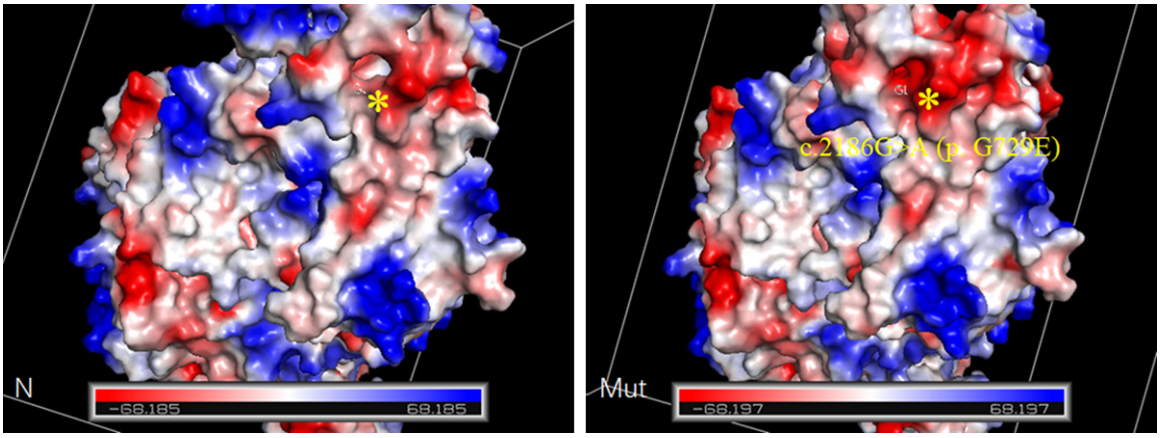

Figure 3. The mutation is c.2186G > A, and the protein change happen in position 729, glycine to glutamic acid, and the protein charge changes, the sign * shows: the mutation cause amino acid glycine to glutamic.

not prevent recurrence, it is essential to consider new and targeted strategies to prevent kidney stones [1, 2]. During the past decade, genetic studies have associated an increasing number of genes with kidney stone formation and recurrence, demonstrating the polygenetic nature of the disease [3-5]. The exact role of these genes, particularly *ADCY10*, in which we found the putatively deleterious allele, and the evidence for single-gene causation is controversial. The OMIM phenotype for *ADCY10* implies susceptibility to absorptive hypercalciuria [6, 7].

ADCY10 belongs to the adenylyl cyclase class-4/guanylyl cyclase family, encoding adenylate cyclase typ10, which has adenylate cyclase activity and interacts selectively and noncovalently with magnesium (Mg) ions activating the enzyme [8, 9]. *ADCY10* is associated with spermatogenesis and participates in the process of cAMP biosynthesis. The longest transcript, corresponding to NM 018417, has 33 exons and translates into a 1610 amino acid-long polypeptide with a mass of ~187 kDa [15, 17]. The N-terminal guanylate cyclase domains are required for enzyme activity. Fragments of isoforms containing the first 470 amino acid residues are fully active [9, 10]. While the mutation is c.2186G > A, and the protein change occurs at position 729, converting glycine to glutamic acid, which causes protein charge changes (**Figure 3**), the position has not been included in the known domain. Thus, the role of ADCY10 in the pathophysiology of kidney stone disease, as well as the clinical relevance of common variants, warrants further investigation.

Some studies have suggested that a genetic factor is the primary cause of hypercalciuria, and elevated urinary calcium excretion may result in calcium loss, decreased bone mineral density (BMD), osteoporosis, and progression toward nephrolithiasis [11, 12]. We diagnosed bone density changes in the patient information. Mutations or polymorphisms in soluble *ADCY10* have been linked to bone loss in kidney stone patients with absorptive hypercalciuria.

Advances in whole-genome sequencing technologies may result in the identification of low-frequency variants, which may explain aspects of nephrolithiasis genetics that have not been characterized to date [13, 14].

In conclusion, our findings expand the spectrum of *ADCY10* mutations, and more importantly, our study further indicates that *ADCY10* plays an important role in nephrolithiasis.

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Disclosure of conflict of interest

None.

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References

[1] Siraj MA, Mundil D, Beca S, Momen A, Shikatani EA, Afroze T, Sun X, Liu Y, Ghaffari S, Lee W, Wheeler MB, Keller G, Backx P and Husain M. Cardioprotective GLP-1 metabolite prevents ischemic cardiac injury by inhibiting mitochondrial trifunctional protein-alpha. J Clin Invest 2020; 130: 1392-1404.

[2] Zhang W, Li D, Wei S, Guo T, Wang J, Luo H, Yang Y and Tan Z. Whole-exome sequencing identifies a novel CCDC151 mutation, c.325G > T (p.E109X), in a patient with primary ciliary dyskinesia and situs inversus. J Hum Genet 2019; 64: 249-252.

[3] Zhang J, Jia H, Wang J, Xiong Y, Li J, Li X, Zhao J, Zhang X, You Q, Zhu G, Tsai FF, Espina M and Wan X. A novel deletion mutation, c.1296delT in the BCOR gene, is associated with oculo-facio-cardio-dental syndrome. Sci China Life Sci 2019; 62: 119-125.

[4] van der Wijst J, van Goor MK, Schreuder MF and Hoenderop JG. TRPV5 in renal tubular calcium handling and its potential relevance for nephrolithiasis. Kidney Int 2019; 96: 1283-1291.

[5] Mehdi WA, Mehde AA, Yusof F, Raus RA, Resen AK and Ghazali H. The endothelial nitric oxide synthase gene G894T, glutathione S-transferase (GSTM1 and GSTT1) polymorphisms as a risk factor in the patient with nephrolithiasis. Int J Biol Macromol 2019; 140: 719-726.

[6] Li Z, Zhu P, Huang H, Pan Y, Han P, Cui H, Kang Z, Xun M, Zhang Y, Liu S, Wang J and Wu J. Identification of a novel COL4A5 mutation in the proband initially diagnosed as IgAN from a Chinese family with X-linked Alport syndrome. Sci China Life Sci 2019; 62: 1572-1579.

[7] Jirackova J, Hyspler R, Alkanderi S, Pavlikova L, Palicka V and Sayer JA. Novel CYP24A1 mutation in a young male patient with nephrolithiasis: case report. Kidney Blood Press Res 2019; 44: 870-877.

[8] Akbari A, Pipitone GB, Anvar Z, Jaafarinia M, Ferrari M, Carrera P and Totonchi M. ADCY10 frameshift variant leading to severe recessive asthenozoospermia and segregating with absorptive hypercalciuria. Hum Reprod 2019; 34: 1155-1164.

[9] Pozdniakova S and Ladilov Y. Functional significance of the adcy10-dependent intracellular cAMP compartments. J Cardiovasc Dev Dis 2018; 5.

[10] Plain A and Alexander RT. Claudins and nephrolithiasis. Curr Opin Nephrol Hypertens 2018; 27: 268-276.

[11] Daga A, Majmundar AJ, Braun DA, Gee HY, Lawson JA, Shril S, Jobst-Schwan T, Vivante A, Schapiro D, Tan W, Warejko JK, Widmeier E, Nelson CP, Fathy HM, Gucev Z, Soliman NA, Hashmi S, Halbritter J, Halty M, Kari JA, El-Desoky S, Ferguson MA, Somers MJG, Traum AZ, Stein DR, Daouk GH, Rodig NM, Katz A, Hanna C, Schwaderer AL, Sayer JA, Wassner AJ, Mane S, Lifton RP, Milosevic D, Tasic V, Baum MA and Hildebrandt F. Whole exome sequencing frequently detects a monogenic cause in early onset nephrolithiasis and nephrocalcinosis. Kidney Int 2018; 93: 204-213.

[12] Sayer JA. Progress in understanding the genetics of calcium-containing nephrolithiasis. J Am Soc Nephrol 2017; 28: 748-759.

[13] Halbritter J, Baum M, Hynes AM, Rice SJ, Thwaites DT, Gucev ZS, Fisher B, Spaneas L, Porath JD, Braun DA, Wassner AJ, Nelson CP, Tasic V, Sayer JA and Hildebrandt F. Fourteen monogenic genes account for 15% of nephrolithiasis/nephrocalcinosis. J Am Soc Nephrol 2015; 26: 543-551.

[14] Chou PS, Kuo CN, Hung KS, Chang WC, Liao YC, Chi YC, Chou WP, Tsai SJ, Liu ME, Lai CL, Chou YH and Chang WP. Osteoporosis and the risk of symptomatic nephrolithiasis: a population-based 5-year follow-up study in Taiwan. Calcif Tissue Int 2014; 95: 317-322.

[15] Sakhaee K, Maalouf NM, Kumar R, Pasch A and Moe OW. Nephrolithiasis-associated bone disease: pathogenesis and treatment options. Kidney Int 2011; 79: 393-403.

[16] Ichikawa S, Koller DL, Curry LR, Lai D, Xuei X, Edenberg HJ, Hui SL, Peacock M, Foroud T and Econs MJ. Association of adenylate cyclase 10 (ADCY10) polymorphisms and bone mineral density in healthy adults. Calcif Tissue Int 2009; 84: 97-102.

[17] Visser L, Westerveld GH, Xie F, van Daalen SK, van der Veen F, Lombardi MP and Repping S. A comprehensive gene mutation screen in men with asthenozoospermia. Fertil Steril 2011; 95: 1020-4, e1-9.