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 hypercalciuric 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 CO2 levels [14]. Some
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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 (hg19/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:
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Table 1. Long term follow-up of the patient with renal calculus

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(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
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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).
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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 polygenic 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


