Coenzyme Q10 supplementation therapy for 2 children with proteinuria renal disease and ADCK4 mutation
Case reports and literature review
Chunyue Feng, MMa, Qiong Wang, MMa,b, Jingjing Wang, MMa, Fei Liu, MMa, Huijun Shen, MMa, Haidong Fu, MMa, Jianhua Mao, MDa,∗

Abstract
Rationale: Mitochondrial nephropathy has a poor prognosis and often progresses to the end-stage renal disease. Renal pathology often is focal segmental glomerulosclerosis (FSGS) and does not respond to steroid therapy or immunosuppressive therapy. Some patients are benefited from the therapy of coenzyme Q10, which affect the synthesis pathway of coenzyme Q10.

Patient concerns: Herein, we report 2 cases of children with proteinuria renal disease with ADCK4 mutation.

Diagnoses: Proteinuria renal disease with ADCK4 mutation.

Interventions: Compound heterozygous mutation in ADCK4 gene were detected with next-generation sequencing and confirmed by Sanger sequencing. Both of the patients were given coenzyme Q10 supplementation therapy.

Outcomes: The first patient showed a decreased proteinuria after coenzyme Q10 supplementation therapy, while the other was not improved.

Lessons: Based on the cases we reported and from the literature, recognition of ADCK4 mutation through early and accurate genetic screening could be helpful in avoiding unnecessary toxicities and in preventing complications arising in mitochondrial nephropathy.

Abbreviations: CKD = chronic kidney disease, FSGS = focal segmental glomerulosclerosis, NGS = next-generation sequencing.

Keywords: ADCK4 mutation, coenzyme Q10, mitochondrial nephropathy, proteinuria

1. Introduction
Most mitochondrial cytopathies are involved in multiple organ systems and often present with prominent neurologic and myopathic deficits in childhood.[1] Recent studies have found that mitochondrial dysfunction caused by genetic mutations in mitochondrial genes or nuclear genes can also lead to renal disease, for example, mitochondrial DNA A3243G mutation,[2] PDSS2 gene,[3] COQ2 gene,[4] and ADCK4 gene mutation.[5] Mitochondrial nephropathy has a poor prognosis and often leads to the end-stage renal disease. At present, only rare cases reported about the mitochondrial nephropathy and the correlated genetic mutation.

Next-generation sequencing (NGS) provides coverage of more than 95% of the exons, which contain 85% of disease-causing mutations in mendelian disorders and many disease-predisposing single nucleotide polymorphisms (SNPs) throughout the genome.[6] Here, we report 2 children with proteinuria in whom ADCK4 mutation were identified (c.748G>C, p.D250H and c.532C>T, p.R178W in first patient, and c.625C>G, D209H and c.614C>T, p.S205N in second patient). The results of NGS analysis was further confirmed by Sanger sequencing. One patient showed a reduced proteinuria after coenzyme Q10 supplementation therapy, although the other patient showed no improvement of proteinuria and renal injury.

2. Case presentation
2.1. Ethical approval
This study was approved by the Ethical Committee, Children’s Hospital, Zhejiang University School of Medicine. This case report was prepared in accordance with the Health Insurance Portability and Accountability Act (HIPAA) regulations. The
proteinuria was 413.4 mg/24 h (45.9 mg/kg/24 h), the serum proteinuria and microscopic hematuria; the result of 24-hours of abnormal urine for 10 days, and fever for 2 days. No edema or data collection and publication.

She was born by normal delivery after an uncomplicated pregnancy. Her growth history showed mental developmental retardation. The patient had no history of liver, heart, or neuromuscular diseases. Her parents are both healthy without consanguineous marriage or similar disease history in their family.

2.3. Case 2
A 11-year-old girl was admitted to the hospital because of abnormal urine for more than 2 years. Because of the diagnosis of primary nephrotic syndrome at another hospital, she received the treatment of prednisone and tacrolimus late, but there was no relief in the symptoms for her. On physical examination, no sign of edema and rash was found. Routine urine and blood tests relief in the symptoms for her. On physical examination, no sign of edema and rash was found. Routine urine and blood tests revealed proteinuria and micro-hematuria; the result of 24-hours of proteinuria was 2148.7 mg/24 h (55.5 mg/kg/24 h), the serum albumin was 40.3 g/L, the serum creatinine level was 30 μmol/L. Renal ultrasound showed that renal parenchyma echo enhancement. The pathology of renal biopsy revealed focal segmental glomerulosclerosis. Her medical history was unremarkable. Her parents are both healthy without consanguineous marriage and similar disease history in their family.

2.4. Next-generation sequencing
Genomic DNA was extracted from 5 mL peripheral blood of the patients and their parents, using a QIAamp Blood DNA mini Kit (Qiagen, Milano, Italy) according to the manufacturer’s instructions. Concentrations of DNA were determined by NanoDrop spectrophotometer (ThermoScientific, Waltham, MA). DNA samples were stored at −20°C until usage. Array capture was used to enrich the relevant human genes (SeqCap EZ Human Exome Library v2.0; Roche, Basel, Switzerland) and these genes were sequenced on the Illumina HiSeq 2000 platform (2016 Illumina, Inc., San Diego, CA).

The following principal steps were taken to prioritize the high-quality variants: variants within intergenic, in tronic, and untranslated region (UTR) regions and synonymous mutations were excluded from downstream analysis; variants with quality score less than 20 were excluded; only the conservation score (phyloP) from comparison of human and 43 vertebrates higher than 3 were considered; and after this prior selection, the remaining genes were filtered by the function. The software PolyPhen-2 and Provean software (http://provean.jcvi.org and http://genetics.bwh.harvard.edu/pph2/, respectively).

The NGS analysis was performed on the patients and their parents, Case 1 revealed with heterozygous ADCK4 mutation: c.748G>C (p.D250H) (MAF = 0) and c.532C>T (p.R178W) (rs398122978, MAF = 0) and confirmed by Sanger sequencing again. Family analysis showed other genetic genes about this patient, such as ARHGEF6 gene, ARID1A gene, and SETBP1 gene, which were related to mental retardation. But these mutations cannot contribute to proteinuria. Case 2 revealed heterozygous c.1802G>C (p.G601A) (rs114615449, MAF = 0.004) and c.1339C>T (p.E447K) (rs28939695, MAF = 0.007) mutations in NPHS1 gene and heterozygous c.625C>G (D209H) (MAF = 0) and c.614C>T (p.S205N) (MAF = 0) mutation in ADCK4 gene, which also confirmed by Sanger sequencing again. Family study showed that the patient had another mutation in NPHS1 gene mutation with his father (his father remains fine and no signs for nephropathy was found), and her ADCK4 gene genotype were inherited from her father and mother, respectively. Her father carried the mutation of c.625C>G (p.D209H), and her mother carried the mutation of c.614C>T (p.S205N) (Fig. 2) in ADCK4 gene. In addition to these 2 genes, we also identified other mutations in case 2, such as TMEM237 gene, C9 gene, SCN11A gene, KL gene, and so on, which could not be linked to the disease phenotype. Both of the patients denied the history of proteinuria.
The results of the analysis in the patients’ family members revealed an autosomal recessive model of inheritance in this disease (Fig. 3).

2.5. Treatment and follow-up

Case 1 and case 2 were both given the Coenzyme Q10 supplementation therapy in dose 15 to 30 mg/kg/d. After 12 months follow-up, the urine protein of case 1 turned to negative, and serum creatinine and urea nitrogen remains normal. However, case 2 still had gross proteinuria, serum creatinine, and urea nitrogen was increased gradually (Fig. 4).

3. Discussion

Mitochondrial disease is one of the most rare and complex genetic diseases, and its inheritance is related to maternal inheritance of mitochondrial genes and Mendel inheritance of nuclear genes. Studies have shown that mitochondrial dysfunction caused by either mitochondrial genes or nuclear genes mutations, may damage the kidney. Mitochondrial nephropathy can be characterized by glomerular lesion, renal tubular dysfunction, interstitial nephritis, and cystic renal disease. The mutation of Mitochondrial genes and nuclear genes encoding mitochondrial proteins can cause isolated...
glomerular lesions, manifesting proteinuria or nephrotic syndrome, though some patient accompanied by extra-renal symptoms.[12] The renal pathology often are FSGS, and do not respond to steroid therapy and immunosuppressive therapy, some patients are benefit from the therapy of coenzyme Q10, which affect the synthesis of coenzyme Q10 gene mutation.[5]

According to literature, ADCK4 localizes in mitochondria and foot processes in podocytes that interacts with COQ6 endogenously.[5] The ADCK4 gene (OMIM 615567) locates in chromosome 19p13.2 and contains 15 exons spanning approximately 12kb of DNA. ADCK4 disease typically manifests as an isolated nephropathy with occasional extrarenal symptomatology on contrast to the mutations in PDSS2, COQ2, and COQ6, those renal symptoms usually occur as part of a multisystemic disease complex encompassing progressive encephalopathy, seizures, and hypertrophic cardiomyopathy.[13] In patients with

**Figure 2.** Case 2 sequencing analysis demonstrating the detection of c.1802C>G (p.G601A) mutation in exon 14 and c.1339C>T (p.E447K) mutation in exon 11 of the NPHS1 gene, c.625C>G (D209H) mutation in exon 8 and c.614C>T (p.S205N) mutation in exon 8 of the ADCK4 gene.
ADCK4 mutant, advanced chronic kidney disease (CKD) at time of diagnosis was more prevalent than in patients with NPHS2 mutation. Of patients with ADCK4 disease, 38.5% presented with CKD stage 5, compared with 15.6% of patients with WT1 mutation and 2.9% of patients with NPHS2 mutation at time of diagnosis.\(^{14}\)

CoQ10 is an essential component of eukaryotic cells and is involved in crucial biochemical reactions such as the production of ATP in the mitochondrial respiratory chain, the biosynthesis of pyrimidines, and the modulation of apoptosis.\(^{15}\) The biosynthesis of CoQ10 requires at least 13 different genes. Mutations in these genes may cause primary CoQ10 deficiency, a clinically and genetically heterogeneous disorder. Primary CoQ10 deficiency was first described in 1989 but only in the last decade the molecular bases of this disorder have been elucidated.\(^{16}\) To date, mutations in 8 genes (PDSS1, PDSS2, COQ2, COQ4, COQ6, ADCK3, ADCK4, and COQ9) have been associated with CoQ10 deficiency presenting with a wide variety of clinical manifestations.\(^{17,18}\) It is of great importance that physicians should promptly recognize these disorders because most patients respond to oral administration of CoQ10.

We herein presented case 1 with onset at early infancy, and manifested by proteinuria and mental retardation. Genetic testing revealed mutation results fit with compound heterozygosity model: c.748G\(>\)C (p.D250H) and c.532C\(>\)T (p.R178W) mutation in the ADCK4 gene. The second patient’s renal pathology was FSGS, and showed no response to steroid therapy and immunosuppressive therapy. Genetic testing also revealed mutation results fit with compound heterozygosity model: c.625C\(>\)G (D209H) and c.614C\(>\)T (p.S205N) mutations in ADCK4 gene. From these mutations, c.614C\(>\)T (p.S205N) is a novel missense which was never reported before (Table 2).\(^{5,14,20,21}\) Further analysis finds that this mutation is harmful to protein structure related to this disease.

### Table 2

| References       | Nucleotide alteration | Amino acid change | Ethnic group |
|------------------|-----------------------|-------------------|--------------|
| Ashraf et al\(^{[5]}\) | c.101G\(>\)A          | p.W34\(^*\)       | European     |
|                  | c.954_956dup          |                   | European     |
|                  | c.532C\(>\)T          | p.R178W           | Arab         |
|                  | c.646delF             | p.F215Lfs*14      | Algerian     |
|                  | c.1430G\(>\)A         | p.R477Q           | Algerian     |
|                  | c.857A\(>\)G          | p.D286G           | ND (no data) |
|                  | c.1447G\(>\)T         | p.E483\(^*\)      | ND (no data) |
|                  | c.958C\(>\)T          | p.R320W           | Tunisian     |
|                  | c.1027C\(>\)T         | p.R343W           | Moroccan     |
|                  | c.1199-1200insA       | p.H400Nfs*11      | Turkish      |
|                  | c.1306-1362del        | p.D952Hfs         | Indian       |
| Korkmaz et al\(^{[14]}\) | c.203T\(>\)G          | p.L68R            | Turkish      |
|                  | c.929C\(>\)T          | p.P310L           | Turkish      |
|                  | c.1493-1494CC\(>\)AA  | p.A409E           | Turkish      |
|                  | c.1339dupG            | p.E4476fs*10      | Turkish      |
| Li et al\(^{[20]}\)   | c.625C\(>\)G          | p.D209H           | Chinese      |
| Wang et al\(^{[21]}\) | c.241G\(>\)T          | p.E81\(^*\)       | Chinese      |
|                  | c.1468C\(>\)T         | p.R400C           | Chinese      |
|                  | c.448C\(>\)T          | p.R150\(^*\)      | Chinese      |
|                  | c.748G\(>\)C          | p.D250H           | Chinese      |
|                  | c.737G\(>\)A          | p.S246N           | Chinese      |
|                  | c.1093C\(>\)G         | p.Q365E           | Chinese      |
| Current study 2017 | c.614C\(>\)T          | p.S205N           | Chinese      |
According to the clinical manifestation and genetic analysis, those 2 cases were diagnosed as mitochondrial nephropathy. Mitochondrial nephropathy caused by ADCK4 mutation is unresponsive to steroid and immunosuppressive therapy, and can rapidly progress to end-stage renal failure, and finally requires renal replacement. Clinical symptoms can be relieved in some patients with coenzyme Q10 administration. In this report, case 1 was not given steroid therapy, and only enough dose of coenzyme Q10 treatment was able to control the proteinuria and she revealed full response to CoQ10 therapy. Case 2 showed no response to steroid, immunosuppressive agents and CoQ10 administration together, and her glomerular filtration rate deteriorated progressively. Both patients we diagnosed with CKD, after 12 months follow-up, the glomerular filtration rate of case 1 is normal, while case 2 presented with CKD stage 2.

Ashraf et al[10] reported that 1 of 15 patients with SRNS and a homozygous ADCK4 frameshift mutation had partial remission following CoQ10 treatment. Korkmaz et al[14] reported 2 of 26 mutations. Furthermore, 1 patient reported by Park et al[19] showed complete remission of proteinuria with cyclosporine. One of our patients received CoQ10 supplementation when she was 11 years old and demonstrated no response. Combined with these and our data, it is implied that early recognition for ADCK4 mutation and early CoQ10 supplementation to patient would be necessary for patients with full or partial response to CoQ10 therapy and benign prognosis in future.

4. Conclusions

In conclusion, we reported 2 cases of mitochondrial nephropathy with ADCK4 gene mutation, and one respond to the CoQ10 therapy but the other did not. Although this is a rare disease, it is one of the important causes of the end-stage renal failure in childhood. Early detection and early intervention of this disease could contribute to the prevention of progress of CKD in children.

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References

[1] Chinnery PF. Mitochondrial disease in adults: what’s old and what’s new? EMBO Mol Med 2015;7:1503–12.
[2] Lowik MM, Hol FA, Steenbergen EJ, et al. Mitochondrial rRNALeu (UUR) mutation in a patient with steroid-resistant nephrotic syndrome and focal segmental glomerulosclerosis. Nephrol Dial Transplant 2004;20:336–41.
[3] López LC, Schuelke M, Quinzi CM, et al. Leigh syndrome with nephropathy and CoQ10 deficiency due to decapeptidyl dipeptide synthase subunit 2 (PDSS2) mutations. Am J Hum Genet 2006;79:1125–9.
[4] Diomed-Camassee F, Di Giandomenico S, Santorelli FM, et al. COQ2 nephropathy: a newly described inherited mitochondrialopathy with primary renal involvement. J Am Soc Nephrol 2007;18:2773–80.
[5] Ashraf S, Gee HY, Woerner S, et al. ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. J Clin Invest 2013;123:5179–89.
[6] Rabbah B, Mahdieh N, Hosomichi K, et al. Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. J Hum Genet 2012;57:621–32.
[7] Lightowler RN, Taylor RW, Turnbull DM. Mutations causing mitochondrial disease: what is new and what challenges remain? Science 2013;340:1494–9.
[8] Kloostwijk ED, Reichold M, Help-Woolley A, et al. Mitrargeting of peroxisomal EHHADH and inherited renal Fanconi’s syndrome. N Engl J Med 2014;370:129–38.
[9] Hildebrandt F. Genetic kidney diseases. Lancet 2010;375:1287–95.
[10] D’Aco KE, Manno M, Clarke C, et al. Mitochondrial rRNAphe mutation as a cause of end-stage renal disease in childhood. Pediatr Nephrol 2012;28:515–9.
[11] Au KM, Lau SC, Mak YF, et al. Mitochondrial DNA deletion in a girl with Fanconi’s syndrome. Pediatr Nephrol 2006;21:136–40.
[12] Gasser DL, Winkler CA, Peng M, et al. Focal segmental glomerulosclerosis is associated with a PDSS2 haplotype and, independently, with a decreased content of coenzyme Q10. Am J Physiol Renal Physiol 2013;305:1228–38.
[13] Malaga-Dieguez L, Susztak K. ADCK4 “reenergizes” nephrotic syndrome. J Clin Invest 2013;123:4996–9.
[14] Korkmaz E, Lipska-Zietkiewicz B, Boyer O, et al. PodoNet Consortium: ADCK4-associated glomerulopathy causes adolescence-onset FSGS. J Am Soc Nephrol 2015;27:63–8.
[15] Acosta MJ, Vazquez Fonseca L, Desbats MA, et al. Coenzyme Q biosynthesis in health and disease. Biochim Biophys Acta 2016;1857:1079–85.
[16] Doimo M, Desbats MA, Cerega C, et al. Genetics of coenzyme Q10 deficiency. Mol Syndromol 2014;5:156–62.
[17] Tasic V, Gucev Z, Polenakovic M. Steroid resistant nephrotic syndrome —genetic consideration. Pril (Makedon Akad Nauk Umet Odd Med Nauki) 2015;36:5–12.
[18] Desbats MA, Lunardi G, Doimo M, et al. Genetic bases and clinical manifestations of coenzyme Q10 (CoQ10) deficiency. J Inherit Metab Dis 2014;37:143–56.
[19] Park E, Kang HG, Choi YH, et al. Focal segmental glomerulosclerosis and medullary nephrocalcinosis in children with ADCK4 mutations. Pediatr Nephrol 2017;32:1547–54.
[20] Li G, Sun L, Shen Q, et al. Mitochondrial nephropathy in two children and literature review. Chin J Evid Based Pediatr 2015;10:426–33.
[21] Wang F, Zhang Y, Mao J, et al. Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. Pediatr Nephrol 2017;32:1181–92.