Case Report

Two Rare Copy Number Variants Involving Loss of *NPHP1*, *MALL*, and *MTLN* Genes Contribute to Nephronophthisis-Induced Nephropathy Progression in a Family: A Case Report

CH Liu[1,2], LJ Li[1], M Tian[1], GH Cao[1], SF Zhang[1], JT Li[1,2]

[1]Department of Nephrology and Rheumatology, Zhengzhou Key Laboratory of Pediatric Kidney Disease Research, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, [2]Henan Provincial Key Laboratory of Children's Genetics and Metabolic Diseases, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, China

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Abstract

Nephronophthisis (NPHP) is a common pediatric cystic kidney disease, accounting for approximately 10% of end-stage renal failure cases in children. NPHP is primarily diagnosed through the identification of indel mutations and copy number variants (CNVs), and patients carrying *NPHP1* mutations usually progress to renal failure at a mean age of 13 years old. However, the association between CNVs containing *NPHP1* variations and the progression of NPHP-induced disease remains unclear. Here, we report three NPHP patients in a family. The proband had developed stage 4 chronic kidney disease (CKD) at 9 years old, and her younger brother and older sister had developed renal failure at 8 and 10 years old, respectively. A genetic diagnosis showed that they carried two rare CNVs, including homozygous loss of *NPHP1*, *MALL*, *ACTR1AP1*, *MTLN, and LOC100507334*. Heterozygous deletions mainly consisted of non-coding RNA genes on both sides of the CNVs. The proband was in stage 4 of CKD while her brother had progressed to renal failure, probably due to more extensive heterozygous deletion of a 67.115 kbp fragment, which included *LIMS3-LOC440895*, *LOC440895*, *GPAA1P1*, *ZBTB45P1,* and *LINC0112* genes. This report demonstrates that larger CNV deletions, including homozygous *NPHP1*, *MALL,* and *MTLN* mutations and heterozygous deletions, presumably accelerate disease progression. Therefore, early genetic diagnosis plays a crucial role in the intervention and prognosis of these patients.

Keywords: *Copy number variant (CNV), nephronophthisis, NPHP1, renal failure*

Introduction

Nephronophthisis (NPHP) is a cystic kidney disease characterized by autosomal recessive genetics, which is the primary genetic factor that causes end-stage renal disease (ESRD) in children.[1] Although NPHP is considered a rare disease with an incidence of 0.1–0.2/10000 in live births, it is the most common genetic cause of ESRD in children with an incidence rate of 15%.[2] Therefore, early diagnosis of NPHP patients is vital for early intervention, treatment, and delay of disease progression.

NPHP is generally diagnosed by renal biopsy or genetic testing. More than twenty genes have been identified that are associated with NPHP, with NPHP1 (OMIM: 256100) being one of the most common types. Wolf *et al.* reported that NPHP patients usually develop ESRD at thirteen years old, which is consistent with our previous data.[3,4] Moreover, the complete homozygous loss of *NPHP1* accounts for the most significant proportion (20%-25%) of cases.[5] Adolescent NPHP is a heterogeneous disease, mainly caused by pathogenic mutations in *NPHP1* and *NPHP4*.[6] Recently, whole-exome sequencing (WES)

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Address for correspondence: Dr. JT Li, Department of Nephrology and Rheumatology, Children's Hospital Affiliated to Zhengzhou University, Zhengzhou - 450 018, China. E-mail: ljtsuc@yeah.net

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and copy number variant sequencing (CNV-seq) have been widely adopted to identify pathogenic factors in genetically heterogeneous diseases. However, there are few reports on NPHP being caused by large deletions in CNVs. Current reports reveal homozygous deletion of multiple genes involving *NPHP1* along with deletions in a few heterozygous genes. The impact of CNVs on NPHP-induced disease progression is not well elucidated.

This study used a genome-wide CNV-seq and WES to perform genetic testing in a family with renal failure. The results revealed that pathogenic factors of NPHP consist of microdeletions of chromosome 2, which included the homozygous loss of *NPHP1*, *MALL*, *ACTR1AP1*, *MTLN, and LOC100507334* genes. These deletions may be responsible for the pathogenesis of NPHP and the combination of these gene deletions likely contribute to a more severe phenotype compared to just a single *NPHP1* mutation. Moreover, our case presentation also demonstrated that heterozygous mutations of the genes on both sides of the CNVs could also be related to the severity of the disease.

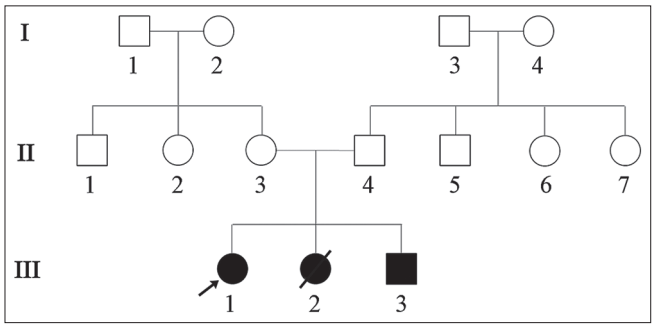

Figure 1: Pedigree of patients with renal failure. Square symbols represent males, and circles represent females. White symbols represent unaffected individuals, and black-filled symbols represent affected individuals

Case Report

One female child [Figure 1, III-1], aged nine years old, was admitted to our hospital with increased creatinine, no fever, no abdominal pain, no diarrhea, and no sallow complexion in a physical examination five days prior. After admission, renal function testing showed that urea was high at 14.5 mol/L, creatinine was at 220.4 mol/L, and uric acid was at 447.2 mol/L. Routine urine examination was regular and urine β2 microglobulin was high. Color Doppler ultrasound for the proband indicated a large liver and diffuse lesion in both renal parenchyma. Both kidneys were slightly smaller, with the right kidney around 81.7 × 29.5 mm and the left kidney around 85.2 × 48.4 mm. The result of voiding cystourethrography (VCU) was typical, and the vision and hearing examination was normal. The child was diagnosed with stage 4 CKD, but the cause was unclear.

The proband had a younger brother who was eight years old [Figure 1, III-3]. He was at stage 5 CKD and underwent kidney transplantation. Moreover, the

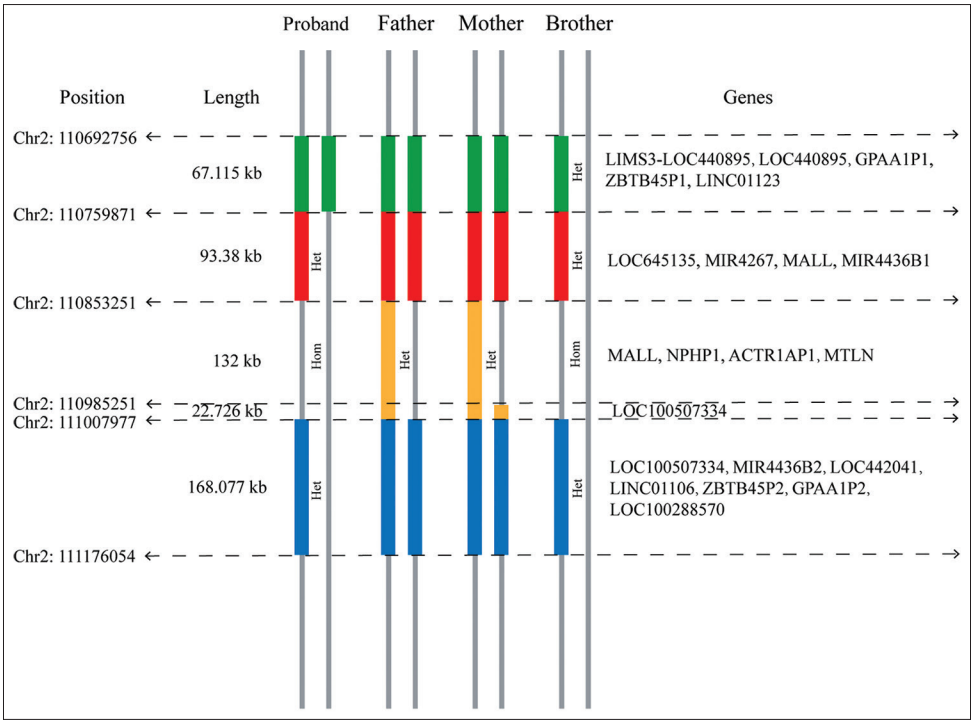

Figure 2: The chromosomal 2 microdeletions of patients obtained by copy number variant sequencing (CNV-seq). The green, red, yellow, and blue represent different regions of the chromosome 2. Het, heterozygous; Hom, homozygouss

proband had a ten-year-old sister [Figure 1, III-2], who had poor physical fitness and was prone to catching colds. She also had stage 5 CKD and eventually passed away. In contrast, their father [Figure 1, II-4] and mother [Figure 1, II-3] had normal phenotypes.

Molecular genetic analysis with CNV-seq and WES showed that the chromosome number of the pedigree was typical. However, the proband and her younger brother had homozygous deletions of CNV (Chr2:110853251–111007977), including *NPHP1*, *MALL*, *ACTR1AP1*, *MTLN, and LOC100507334* genes, and both of their parents had a heterozygous loss of CNV [Figure 2]. Surprisingly, the proband had two heterozygous deletion regions flank the homozygous loss of territory, located at Chr2:110759871–110853251 and Chr2:111007977–111176054 with lengths of 93.38 kb and 168.077 kb, respectively [Figure 2]. However, the younger brother had a larger heterozygous interval located at Chr2:110692756–110759871 with a length of 67.115 kb, which included multiple genes such as *LIMS3-LOC440895*, *LOC440895*, *GPAA1P1*, *ZBTB45P1,* and *LINC0112* [Figure 2]. Therefore, these variants confirmed the clinical diagnosis of the novel CNVs-induced renal impairments.

The proband reported in this paper was at stage 4 CKD, and symptomatic treatment was provided to correct metabolic acidosis, improve anemia, maintain internal environment stability, and lower blood pressure. At present, the child has no particular complaints upon last follow-up. According to the information provided by their parents, the proband's brother is currently awaiting a kidney transplant and her sister is deceased.

Discussion

Nephrocystins are encoded by the NPHP genes and are located in primary cilia. Nephrocystins are vital for ciliary function, usually in the kidney, liver, pancreas, central nervous system, and ocular tissues. Therefore, mutations of NPHP-related genes could induce cilia disease and present as different clinical syndromes.[7] Though *NPHP1* variants primarily present with NPHP1 disease, they also display other characteristics, such as Joubert syndrome, Senior-Løken syndrome, cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma, and hepatic fibrosis (COACH) syndrome.[8] This implies that some NPHP1 patients with further complications may have had other pathogenic genes that were not found. Moreover, CNV is an essential source of human genetic variation.[9] Various CNVs, including *NPHP1* and other suspected pathogenic genes, can cause multiple clinical symptoms.

In this report, both the proband's brother and her old sister had renal failure, and the proband progressed to stage 4 CKD. However, the exact etiology was unclear before being admitted to our hospital. Since multiple patients showed similar phenotypes in this family, we hypothesized that hereditary nephropathy was the possible pathogenesis. Subsequently, we performed the CNV-seq along with trio-WES to improve accurate diagnosis for the proband and her brother. The results indicated that the two patients carried various lengths of CNVs, including homozygous losses of *NPHP1*, *ACTR1AP1*, *MTLN, and LOC100507334* genes and partial loss of the *MALL* gene fragment.

Consistent with many other reports, our previous data also showed that the *NPHP1* mutation was a well-established causative factor of NPHP and can lead to renal failure.[3,6] Moreover, our recent data showed that patients carrying *NPHP1* mutations primarily presented with isolated NPHP and progressed to ESRD at a mean age of 12.9 ± 0.5 years in Chinese patients.[3] The male patient in this study presented with ESRD at eight years old, and the proband's sister was only ten years old when she died from renal failure. This is probably because the patients harbored multiple gene mutations. Although the *MTLN* and *MALL* genes have been reported to be associated with Joubert syndrome 4 and Nephronophthisis 1,[10] the direct evidence is scarce. Our research provides further evidence that these genes may be responsible for NPHP-induced nephropathy. On the other hand, the functions of *ACTR1AP1* and *LOC100507334* are vague and require further study.

Additionally, the patients had different lengths of heterozygous regions. Regrettably, the younger brother had a more severe condition and progressed to stage 5 CKD earlier than the proband, who had advanced to stage 4 CKD. This is probably because the brother carried a more extended heterozygous territory, which included *LIMS3-LOC440895*, *LOC440895*, *GPAA1P1*, *ZBTB45P1,* and *LINC0112*. Jaiswal *et al*.[11] reported that an evolutionarily conserved developmental gene *LIMS3-LOC440895* deletion might correlate with multiple congenital anomalies, but its exact function is unclear. Moreover, the *LINC0112* gene may be a cardiometabolic risk factor.[12] The role of the *LOC440895* has not been reported thus far. GPAA1P1 is a glycosylphosphatidylinositol (GPI), which plays an essential role in the process of more than 100 eukaryotic membrane proteins anchored to the cell surface[13]; thus, it may be related to signal transduction during development and disease. Additionally, *ZBTB45P1* is a pseudogene and is significantly associated with the prognosis of cancer patients.[14] Recent studies have shown that pseudogenes could regulate the expression of homologous or non-homologous genes and other

biological functions by interacting with RNA-binding proteins and miRNAs.[15] However, the relationship between *ZBTB45P1* and nephropathy has not been explored. Therefore, the functions of these genes in kidney disease may be a valuable topic for further study. Of course, we cannot rule out that other unknown pathogenic factors in this process remain to be explored.

NPHP is one of the most common genetic etiologies of ESRD in children, which mainly occurs in childhood and its typical clinical symptoms consist of polyuria, polydipsia, anemia, growth retardation and renal failure.[5] Unfortunately, there are no preventative or noninvasive treatments for NPHP. When NPHP patients progress to ESRD, dialysis, and transplantation are the only two options for managing the disease. In contrast, early intervention can be conducted for genetically screened positive patients, which can slow the progression of the disease. Early interventions can consist of supportive therapies, which includes the treatment of hypertension, anemia, growth retardation, and other symptoms associated with CKD. These treatments can prevent the accelerated decline of renal function and improve the quality of life for NPHP patients.

Conclusion

We presented a case of two patients who carried two rare CNVs, including the homozygous loss of *NPHP1*, *MALL*, *ACTR1AP1*, *MTLN, and LOC100507334* and heterozygous gene mutations on both sides of the CNVs, which contributed to NPHP-induced nephropathy progression. Moreover, the brother of the proband had a more extended heterozygous region, which included *LIMS3-LOC440895*, *LOC440895*, *GPAA1P1*, *ZBTB45P1,* and *LINC0112*. The larger extended heterozygous region was possibly related to the brother's more severe condition and earlier progression to renal failure compared to the proband. Our report expands on the type of CNVs, the NPHP genotypes and phenotypes spectrum, which can aid in genetic diagnoses and allow for early intervention in patients with NPHP.

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Declaration of patient consent

The authors certify that the parents of the patients have signed informed consent forms. In the form the parents have given their consent for the patient's clinical information to be reported in the journal.

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Conflicts of interest

There are no conflicts of interest.

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