Molecular Study of Nephronophthisis in 7 Unrelated Pakistani Families

Sofia Hussain,[1] Naureen Akhtar,[2] Reem Qamar,[1] Naima Khan,[1] Muhammad Naeem[1]

[1]Medical Genetics Research Laboratory, Department of Biotechnology, Quaid-I-Azam University, Islamabad, Pakistan
[2]Department of Pediatric Nephrology, The Children's Hospital and The Institute of Child Health, Lahore, Pakistan

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Nephronophthisis is an autosomal recessive cystic kidney disease characterized by tubular interstitial infiltration, periglomerular fibrosis, and cysts, and is the most frequent genetic cause of end-stage renal disease in children. Nephronophthisis is pleiotropic as almost all the causative genes are involved in primary cilium and centrosome function which are found in almost all human cells. Genetic heterogeneity in nephronophthisis makes the molecular and genetic diagnosis somewhat difficult. Homozygous deletions in the nephronophthisis 1 (*NPHP1*) gene are the major contributor of nephronophthisis cases, while other genes accounts for less than 3% each. Nephronophthisis-related ciliopathy is a term used for extrarenal symptoms in addition to nephronophthisis. Herein, we are reporting the molecular study of 7 children from independent families fulfilling the criteria of nephronophthisis. A deletion analysis of the *NPHP1* gene was performed in each case, and *NPHP5* mutation screening was performed in the absence of such deletion in patients with Senior Loken syndrome.

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Nephronophthisis is a genetically heterogeneous renal ciliopathy characterized by polyuria, polydipsia, mild proteinuria, and anemia. It follows an autosomal recessive pattern of inheritance, leading to end-stage renal disease (ESRD) in childhood and early adolescence. Clinically, 3 forms of nephronophthisis are distinguished as infantile, juvenile, and adolescent nephronophthisis with ESRD at the median ages of 1, 13, and 19 years, respectively. Ultrasonography is the most useful imaging technique for diagnosis of nephronophthisis. Unlike other kidney diseases, the kidneys are either normal in size or shrunken and are echogenic in nephronophthisis. There is no prophylaxis or treatment available for nephronophthisis, while dialysis and transplantation are the only treatment options at the stage of terminal kidney failure.[1]

Nephronophthisis and other cystic kidney diseases are referred to as *ciliopathies* based on the finding that all proteins mutated in the cystic kidney diseases of human or animal models are expressed in the primary cilia or centrosomes of renal epithelial cells. Nephronophthisis 1 (*NPHP1*) was the first gene identified in 1997 as the underlying cause of nephronophthisis. Homozygous deletion in *NPHP1* is the most frequent cause of nephronophthisis (about 21%) reported so far and mutations in other genes contribute less than 3% each.[2] A homozygous *NPHP1* deletion or compound heterozygosity for the *NPHP1* gene deletion combined with a single-point mutation in the *NPHP1* gene mostly cause isolated kidney disease. However, diverse extrarenal organ manifestations are reported in about 23% of these patients. Nephronophthisis associated with eye disease is known as Senior-Loken syndrome, which is genetically heterogenous and causative mutations are reported in several genes including *NPHP1*, *NPHP2/INVS*, *NPHP4*, *NPHP5/IQCB1*, *NPHP6/*

CEP290, NPHP10/SDCCAG8, AHI1, NPHP15/CEP164, TRAF3IP1, and *WDR19NPHP5*. However, *NPHP5* is considered as the classic Senior-Loken syndrome-causing gene.[3]

We investigated 7 Pakistani children from unrelated families affected with nephronophthisis designated here as N1, N3, N4, N7, N8, N10, and N13. The patients were diagnosed by a pediatric nephrologist at the Children's Hospital Lahore. Informed consent was obtained from all participating individuals and the study protocol was approved by the Institutional Review Board. All the patients presented with typical nephronophthisis symptoms of polyuria, polydipsia, nocturnal enuresis, and failure to thrive in the first 2 decades of life and demonstrated ESRD at a median age of 9.5 years, suggesting juvenile nephronophthisis. Concurrent occurrence of retinal degenerative changes was observed in 4 patients (N3, N4, N10, and N13), suggesting Senior-Loken syndrome. The parents were healthy in all families, indicating an autosomal recessive mode of inheritance. Gender distribution among patients showed a ratio of 1.3:1 (4 males and 3 females). Venous blood samples were obtained from seven affected and 20 unaffected individuals of the seven families for molecular analysis. Polymerase chain reaction screening of *NPHP1* deletion on chromosome 2q13 was performed on gDNA of all patients, based on polymerase chain reaction amplification of intronic region of 142 bp using primers: forward, GCTCCTTCCTGAGAAGACAG and reverse, CCACCTCTCATCCAGACACT.[4] This method allowed a noninvasive diagnosis of the disease, eliminating the need for kidney biopsy.[5] Polymerase chain reaction amplification was negative from the DNA of 6 patients (N1, N3, N4, N7, N8, and N13), indicating homozygous *NPHP1* deletion. The DNA of 20 unaffected individuals from all families and normal controls as well as of 1 patient from family (N10) did not demonstrate *NPHP1* deletion; a 142 bp polymerase chain reaction amplicon was seen on an ethidium bromide-stained 2% agarose gel indicating an intact *NPHP1* gene (Figure 1). Therefore, gDNA of the patient N10 was screened for mutation in *NPHP5* gene by direct DNA sequencing as *NPHP5* was the prime candidate gene for Senior-Loken syndrome.[3] DNA sequencing showed a nonsense mutation p.R455X (c.1363 C>T) in exon 13 predicting a truncated NPHP5 protein (Figure 2). This mutation was reported in 1 patient in homozygous and in another patient in compound heterozygous condition.[3,6] The NPHP5 is a centriolar protein involved in ciliogenesis. All *NPHP5* mutations identified so far lead to truncated products unable to bind Cep290 and therefore inhibit cilia formation.[7]

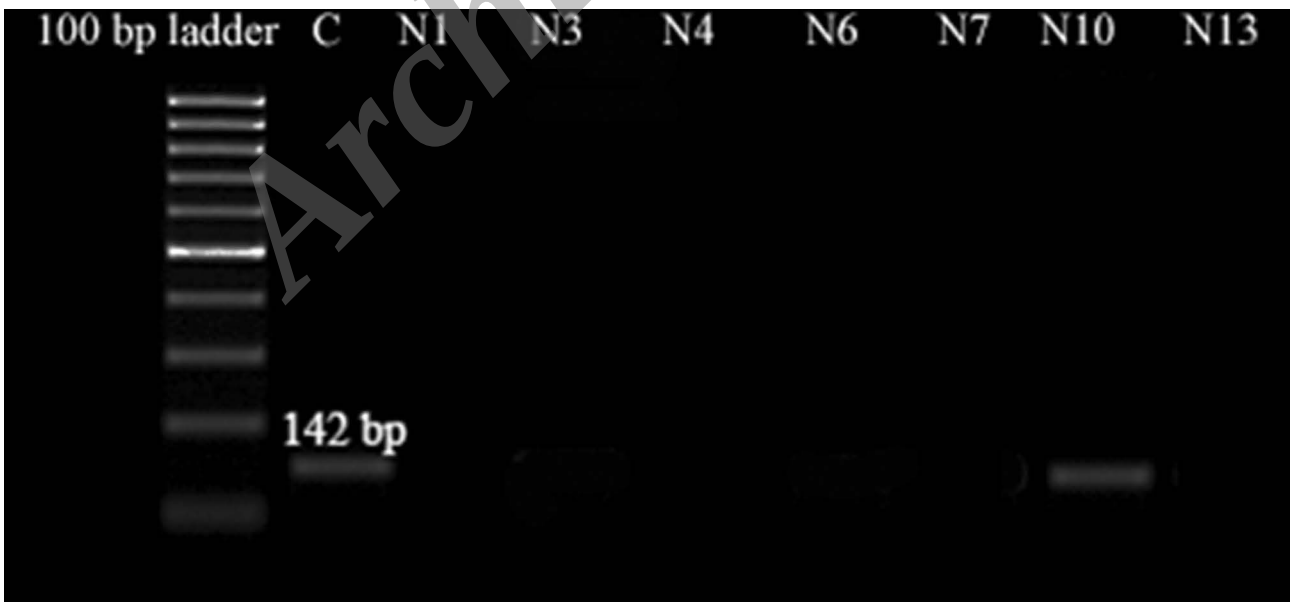

Figure 1. Molecular diagnosis of children affected with nephronophthisis. Two-percent agarose gel (stained with ethidium bromide) showing a 100 bp DNA molecular weight marker (lane 1), 142 bp polymerase chain reaction product in a healthy control individual and patient N10 (lanes 2 and 8, respectively), indicating an intact *NPHP1* gene and no amplification in patients N1, N3, N4, N7, N8, and N13 (lanes 3 to 7 and 9), indicating homozygous *NPHP1* deletion.

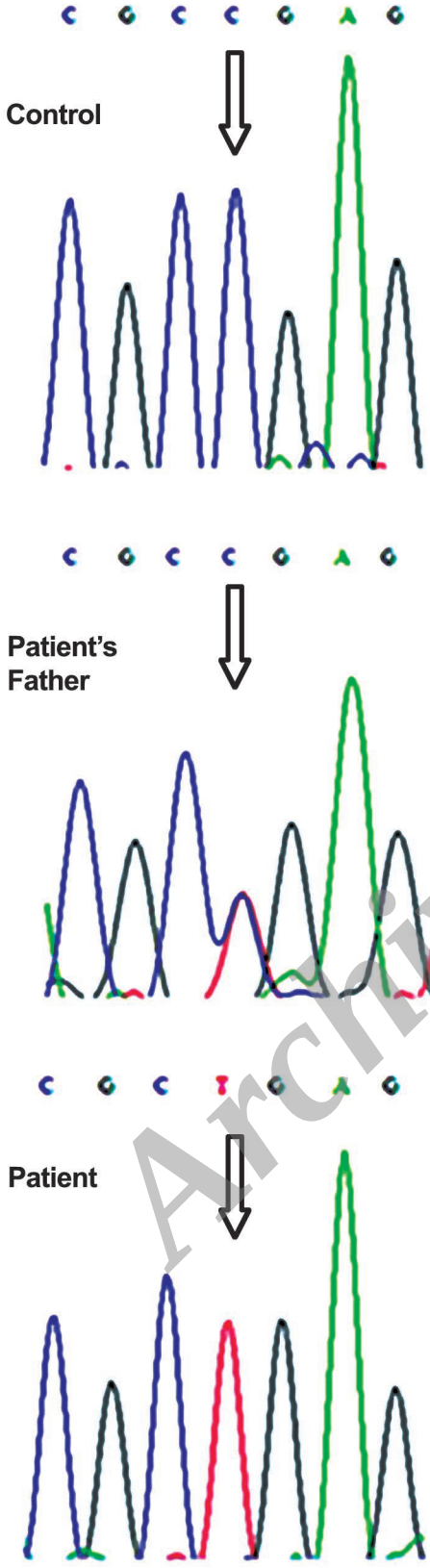

Figure 2. DNA sequence of part of exon 13 of *NPHP5* gene showing a nonsense mutation (c.1363 C>T / p.R455X) in the homozygous patient N10 (bottom), a heterozygous carrier (middle) and a normal control indicated by the arrows.

In summary, we report molecular study of 7 nephronophthisis patients from unrelated Pakistani families that identified *NPHP1* deletions in 6 patients (85.7%) and a nonsense *NPHP5* mutation in 1 patient (14.3%). The current study reinforces the importance of polymerase chain reaction detection of *NPHP1* deletions for the diagnosis of nephronophthisis and screening of *NPHP5* mutations in Senior-Loken syndrome patients with an intact *NPHP1* gene.

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CONFLICT OF INTEREST

None declared.

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Correspondence to:
Muhammad Naeem
Medical Genetics Research Laboratory, Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320, Pakistan
Tel: +92 51 9064 4122
E-mail: mnaeemqau@gmail.com

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