Screening for homozygous deletion of the NPHP1 gene in Pakistani Nephronophthisis patients

Sajid Amin, Hameed Ur Rehman, Rukhsana Gul, Muhammad Imran, Wasif Ullah, TajUd Din, Safia Gul, Maleeha Azam, Sajid Awaiz and Afzal Ahmad

Abstract

The present study was conducted from August 2014 to August 2015 to screen the Pakistani NPHP patients for NPHP1 gene deletions. In order to evaluate the homozygous deletion of the NPHP1 gene in Pakistani NPHP patients, we analyzed NPHP1 gene in a cohort of eleven families diagnosed with NPHP for the detection of NPHP1 gene deletion and genotyping was done via conventional Polymerase chain reaction by using three pairs of primers for exons 5, 7 and 20 of NPHP1 gene and two pairs of primers for exon 4 and 6 of LHX9 gene (control) from chromosome 1. The results were checked on 2% Agarose gel. The present study results demonstrate that 45% of patients (5/11) were positive for homozygous NPHP1 gene deletion and 6/11 patients were found not linked to NPHP1 gene. The six patients were excluded from NPHP1 gene deletion, suggesting further heterogeneity in the present NPHP cohort. Finding of further heterogeneity in Pakistan NPHP patients has significant importance for genetic counseling, early prognosis and disease management.

Keywords: Renal ciliopathy, NPHP1 gene, homozygous deletion, kidney failure

1. Introduction

Nephronophthisis (NPHP) is considered as a renal ciliopathy which means “disappearance of nephrons,” it is a congenital, progressive and genetically heterogeneous cystic kidney disease leading to kidney failure in the first three decades of life [1-3]. It is considered as the third (incidence rate of 10 to 15%) among all hereditary pediatric renal anomalies leading to end-stage renal disease (ESRD). The incidence rate of NPHP has been reported quite low in the Western Countries as 1/50,000 live births have been reported in a Canadian population [4], whereas in the USA the estimates are 9/8.3 million. In European population the prevalence of NPHP is reported as 1 in 61800 live births [5]. A study in Finland reported the prevalence of juvenile NPHP to be 0.13 for 10,000 live births [6]. The incidence rate of NPHP could be higher in communities where consanguineous marriages are traditional e.g., Middle East, West Asia and North Africa, where cousin unions are accounting for more than 20 to 50% of all marriages [7-8]. Pakistan has the highest rate of consanguinity which is about 60% or more [9], however, for Pakistani population there is very limited prevalence data available for NPHP and the disease are considered to be very rare. Clinically NPHP is characterized by polyuria, polydipsia, nocturia, anemia and lethargy (secondary to anemia) [10]. However, right after the onset of primary symptoms, the dysfunction of the cortical collecting duct of nephron occur [11] and result in the loss of salt (sodium) conservation and disability of the patient’s kidneys to concentrate urine. Decreased urine concentration diagnosed by a low urinary osmolarity (<400 mosm/kg) in the first urine sample in the morning. Kidney size is observed either normal or reduced through renal ultrasound, while echogenicity and corticomedullary cysts are increased [12]. A diagnostic renal biopsy of NPHP, revealed that there is a characteristic triad of tubular basement membrane disruption, tubulointerstitial fibrosis, and cysts are restricted to the corticomedullary junction [13].

NPHP is a monogenic disorder that is mostly inherited in an autosomal recessive pattern and thirteen causative genes of NPHP have been reported till date [14]. The first causative gene to be discovered for NPHP was the NPHP1 gene on chromosome 2q13. It code for a protein product named nephrocystin-1. Nephrocystin-1 is considered to be confined to the primary renal cilium [15] to epithelial cell adherence junctions and to the focal adhesions [16-17], and is associated with various proteins which are critical for the maintenance of the cellular scaffolding or cytoskeleton.
These proteins include; jouberin, filamin A and B, ack1 [18], tensin, protein tyrosine kinase 2B (PTK2B) [19], and β-tubulin. Among the NPHP patient the most frequent genetic mutations are homozygous deletions of approximately 250 kb DNA in the region 2q13 on chromosome number 2. Compound heterozygous mutations for the NPHP1 gene deletion are also identified, along with a single point mutation in the NPHP1 gene [20]. The present study was conducted to screen the Pakistani NPHP patients for homozygous deletions of NPHP1 gene.

2. Materials and Methods
A total of 11 patients were recruited from august 2014 to august 2015 in the pediatric Nephrology Centre, Children Hospital Lahore, Pakistan. A complete family history of renal disease and consanguinity was taken. Blood samples were collected along with their family members. Written informed consent was obtained. The study was approved by the Ethics Review board, CIIT Islamabad. Genomic leukocyte DNA was extracted from the peripheral EDTA- blood by using standard Phenol-Chloroform method [21].

The patient’s medical record was investigated for the symptoms like polyuria, polydypsia, enuresis, growth retardation, blood pressure, renal insufficiency/ renal failure and other extrarenal manifestation (night blindness). Kidney size, corticomedullary cysts and corticomedullary differentiation for each patient were examined by ultrasound. Kidney function was examined by blood tests (urea, creatinine) [22].

All the patients diagnosed with initial symptoms of NPHP (polyuria, polydypsia, growth retardation and renal insufficiency) were included in the present study. Patient with extrarenal the manifestation (night blindness) were also included. All other with renal anomalies were excluded. The PCR primers were designed by using online software Primer3 (primer3.ut.ee). The reference sequences for the primers were obtained and compared from the three different online databases; UCSC Genome Browser, Ensembl and Gene [23]. The amplifying specificity of primers was tested by using UCSC InSilico PCR (ge.ucsc.edu/cgi-bin/hgPcr) and self-pairing of primers was checked by using Oligo Analysis Tool (www.operon.com/tools/oligo-analysis-tool.aspx). Primers constitute more than four back to back self-pairing within inter-primers or intra-primers were discarded. At the site of annealing, the possibility of SNPs was tested by using SNP masker (bioinfo.egr.bcei/snpmasker/). Three pairs of primers for exons 5, 7 and 20 of NPHP1 gene and two pairs of primers for exon 4 and 6 of LHX9 gene were used for the analysis of NPHP1 gene deletions. The two pairs of primer against two exons of LHX9 gene were used as internal control. The primer sequence from (5’ > 3’) are as follow; NPHP1-E5F, CACTCATAGCTGGTTGTCTTTG; NPHP1-E5R, CAGGTGTACAGGCAGAGTTC; NPHP1-E7F, TGGAGGGTTAGGTGGCAGTA; NPHP1-E7R, TTTGATATCCTTTCCCACTTTG; NPHP1-E20F, AATTGGCACCTCTCCACACTAC; NPHP1-E20R, AATCTGGAGGATTGCTACTCG; LHX9-E4F, ATATGGCCTCCTGGTTGTCTTT; LHX9-E4R, TGGGCAAAACACACTCTTCT; LHX9-E6F, ACCCCCTAAAGGCAAAGTGTGC; LHX9-E6R, CCTAATAGTGTCTTGTCTCTCACTGC.

PCR for was performed on a Thermo Electron Corporation System (PXE 0.2 Thermal Cycler) or Applied BiosystemsGeneAmp® PCR System 2700 (ABI, Foster City, CA). Each PCR reaction contained 40-50ng of DNA, 0.2mM dNTP’s, 2.5mM MgCl₂, 1X Taq buffer, 0.1µM forward primer and reverse primer each, 1U TaqPolymerase and Gibco® DNase, RNase free water. PCR conditions were as follows; 1 cycle of Initial denaturation at 95 ºC for 5min, followed by 35 cycles comprising three steps each, denaturation at 95 ºC for 30sec, primer annealing at 59 ºC, for 30sec and primer extension at 72 ºC for 30sec. Lastly, 1 cycle of a final extension at 72 ºC for 7minutes [21].

4. Results

By pedigree analysis, the present study found that 7/11 families (NPH1, 2, 3, 4, 7, 8 and 11) had only one child affected with NPHP, in others four families (NPH5, 6, 9 and 10) 2 individuals were affected. Among total NPHP patients more males were found to be affected than females (7 male and 4 female). The consanguineous marriages of all the parents and the death of siblings in some families strongly recommend the autosomal recessive mode of inheritance of the disease (Fig. 1).

Clinical evaluation of all the patients was carried out by pediatric nephrologists. The age of onset of symptoms in all patients was found in the range of 6 to 15 year. Growth retardation along with typical NPHP symptoms including polydipsia, polyuria and secondary enuresis were found in all patients. Renal cysts were seen in the seven patients (NPH2-II:1, NPH4-II:1, NPH5-II:1, NPH6-II:1, NPH8-II:1, NPH9-II:1, NPH11-II:1). Reduced kidney size was observed in nine patients. However, two patients (NPH3-II:3 and NPH10-II:3) were found with whom normal kidney size.7/11 patients (NPH1-II:3, NPH2-II:3, NPH3-II:3, NPH4-II:3, NPH5-II:3, NPH8-II:3, NPH9-II:3) were found with the extra-renal manifestation of night blindness (table. 1). We categorized the seven NPHP patients with retinitis pigmentosa, as syndromic juvenile NPHP (NPHP-associated with retinitis pigmentosa), and the other four patients as isolated juvenile NPHP.
Table 1: Clinical and genetic details of all the eleven enrolled families (NPH1-11).

<table>
<thead>
<tr>
<th>Family IDs (NPH1-11)</th>
<th>Symptom/ Phenotypes</th>
<th>NPH1</th>
<th>NPH2</th>
<th>NPH3</th>
<th>NPH4</th>
<th>NPH5</th>
<th>NPH6</th>
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<tr>
<td></td>
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<td>10y</td>
<td>6y</td>
<td>14y</td>
<td>9y</td>
<td>14y</td>
<td>15y</td>
<td>7y</td>
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<td>8y</td>
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All the 11 families (NPH1-11) were tested for NPHP1 gene deletion mutations. Four patients (NPH4, 5, 8, and 9) were found with the deletion of NPHP1 gene as a whole or part of it, and one patient (NPH3) was found with mutation/deletion in only exon5 of NPHP1 gene. The amplified PCR products were analyzed on 2% agarose gel. The deleted or mutated exons were found failed to amplify concluding the deletion/mutation of targeted exons of NPHP1 gene.

5. Discussion

Nephronophthisis is a pediatric renal anomaly and remains an important cause of the end stage renal disease among the developed and under developing countries. The initial symptoms, including polyuria, nocturia, anemia, lethargy and polydipsia, are mild and they experienced no edema, hypertension or urinary tract infection that lead to a delay in the diagnosis of NPHP [22]. Ultimately, the primary symptoms resulted into secondary symptoms which leads to cyst formation and reduced of normal kidney size [12]. In the present study, we found a reduced kidney size in 9/11 patients and cyst formation was observed in 7/11 patients.

To date 13 genes (NPHP1-13) are known to be associated with isolated or syndromicNPHP.However NPHP1 gene deletion is one of the most frequently occurring mutations in NPHP patients. TheNPHP1 deletion is mainly caused by the deletion of about 250kb DNA on chromosome 2 that harbors NPHP1 gene flanking by two large inverted repeats of approximately 300- 330kb. The distal inverted repeat contain a 45kb report, which is inversely repeated to the upstream of the first exon of the NPHP1 gene [23] and the deletion breakpoint of NPHP1 to be present in this chromosomal region. The deletion spectrum of NPHP1 gene is about 40 to 60% of the total NPHP patients worldwide. In the present study 11 families suffered from autosomal recessive, congenital, juvenile NPHP were subjected to the mutational analysis of NPHP1 gene, the frequency of homozygous deletion of the NPHP1 gene in NPHP patients from Pakistani population was found to be 45%, which shows similar percentage of homozygous deletion of the NPHP1 gene in NPHP patients worldwide (25 to 50%). Among the five patients that had NPHP due to NPHP1 gene mutation, four patients showed homozygous deletion of the complete NPHP1 gene, while in one patient exon-5 was found not amplified, which was considered to be a partial deletion of only exon-5 on NPHP1 gene. Otto and co-worker [23] observed 21% out of 470 patients in a cohort of 28 families from France and North Africa with homozygous deletions of NPHP1 gene. Similarly, 46% of total 28 unrelated NPHP patients were found with homozygous or heterozygous deletion of the NPHP1 gene by Friedelheim and coworkers [24]. In our study the data showed 36.4% patients (4/11) were affected with isolated NPHP and 63.6% (7/11) had NPHP associated with retinitis pigmentosa, the condition is known as Senior-Loken Syndrome (SLS syndrome). All the NPHP-associated retinitis pigmentosa patients were observed with homozygous deletion of NPHP1 gene. O’toole and co-workers [25] demonstrate that all NPHP proteins are localized to the primary renal cilia; however NPHP1 and NPHP4 are interacting partners of a protein RPGR (retinitis pigmentosa GTPase regulator) present in the photoreceptor cells of the retina. Therefore NPHP1 and NPHP4 can express in photoreceptor cells of retina. Involvement of NPHP1 with retinitis pigmentosa is highly backed up by the fact that the primary cilia of renal epithelial cells are known to produce NPHP1 gene products. However, there is a definite possibility of other autonomous mutations causing renal damage in NPHP patients as these mutations alone do not manifest RP as the incidence of retinitis pigmentosa varies from family to family with NPHP5 mutations being a common factor in all such cases [26]. This complex nature of NPHP related diseases also leads to believe that the ultimate phenotype relies on the interaction between multiple cilia-associated gene mutations [20]. This study was conducted only for the spectrum of homozygous deletion of the NPHP1 gene in NPHP patients from Pakistani population, therefore for the patients with no homozygous deletion in NPHP1gene further investigations are needed for the detection of mutation either in NPHP1 gene or other known NPHP genes. Further investigation of NPHP heterogeneity might be helpful in genetic counselling, early molecular diagnosis, and disease management.

6. Conclusion

From the above study, it may be concluded that study results demonstrate that 45% of patients (5/11) were positive for homozygous NPHP1 gene deletion and 6/11 patients were found not linked to NPHP1 gene. The six patients were excluded from NPHP1 gene deletion, suggesting further heterogeneity in the present NPHP cohort. Finding of further heterogeneity in Pakistani NPHP patients has significant importance for genetic counseling, early prognosis and disease management.

7. Acknowledgement

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8. References


