Original Article

Next-generation sequencing in children of West India with suspected inherited tubulopathies

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ABSTRACT

Background: The renal tubules maintain homeostasis through an array of proteins coded by multiple genes, which directly or indirectly transport water and solutes in the tubules. Malfunction of these transport proteins leads to inherited tubulopathies. **Objective:** The objective of the study was to analyze the clinical utility of next-generation sequencing (NGS) in the diagnosis and management of children with tubulopathies. **Materials and Methods:** This retrospective study was conducted in a tertiary care nephrology center in West India between September 2016 and September 2020. All children ≤18 years with suspected inherited tubulopathy, where an NGS was sent, were included. Clinical exome sequencing (CES) covering 6670 genes was done in 14 children. CES included 73 tubulopathy genes. The test result was interpreted as per American College of Medical Genetics classification: No pathogenic variant, variant of unknown significance (VUS), and likely pathogenic and pathogenic variant. **Results:** The median age (IQR) of the cohort at the onset of disease was 18 months (4–65). History of consanguinity was present in 2 children (14.2%). Five children (35.7%) had Fanconi syndrome, two had distal renal tubular acidosis (d-RTA), two had Bartter/Gitelman syndrome, two had rickets, two had nephrocalcinosis**,** and one had low-molecular-weight proteinuria. Nine children (64%) had pathogenic variants detected in eight genes: ATP6V0A4, CTNS1, FAH1, PHEX, SLC12A1, SLC4A1, SLCA2, and CLDN 16. Five (35.7%) were novel variants. Two children had three VUS in FAT1, EYA1, and KCNJ1 gene. Three children (21.4%) had no genetic variant. Bartter syndrome type 1 and d-RTA were the most common genetic diagnoses with two patients each. Other diagnoses were tyrosinemia type 1, nephropathic cystinosis, Fanconi Bickel syndrome, X-linked hypophosphatemic rickets, and familial hypomagnesemia, hypercalciuria, and nephrocalcinosis in one each. **Conclusion:** The yield of NGS in children with tubulopathy was remarkably high. NGS was useful in the diagnosis and management in these children.

Key words: Next-generation sequencing, Inherited tubulopathy, Indian children

The renal tubules maintain homeostasis or "milieu interieur" for normal cellular function through an array of proteins coded by multiple genes [1,2]. The proteins directly or indirectly transport water and solutes in the tubules. Malfunction of these transport proteins leads to a set of diseases under inherited tubulopathies. They usually manifest from an early age and are associated with symptoms of polyuria, polydipsia, failure to thrive, acid-base abnormality, refractory rickets, tetany, seizures, and rarely hypertension with preserved kidney function [3]. Clinicians usually group them by analyzing the clinical picture into various syndromes such as Fanconi syndrome, proximal renal tubular acidosis (p-RTA), distal RTA (d-RTA), Bartter and Gitelman syndrome, various magnesium disorders, monogenic hypertension, and nephrogenic diabetes insipidus. The diseases and the underlying proteins and genes are enumerated in a recent review article [2]. Although making an accurate clinical diagnosis and management is possible in most cases, genetic testing may lead to a new diagnosis, revision of the clinical diagnosis, confirmation of the clinical diagnosis, genotype-phenotype correlation, reverse phenotyping, and adequate genetic counseling.

Sequencing of a particular suspected gene by Sanger sequencing used to be done earlier but it is time consuming and might miss an unsuspected disease [4]. Next-generation sequencing (NGS) is increasingly being used to overcome these shortcomings as it allows the sequencing of a large number of genes in a short time [5]. More than 50 genes causing inherited tubulopathies have been identified using the techniques of targeted gene panel, whole-exome sequencing, and whole-genome sequencing [2]. We aimed to retrospectively analyze the clinical utility of NGS in the diagnosis and management of children with renal tubulopathies at a tertiary care referral nephrology center in West India.

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MATERIALS OF METHODS

The medical records of all children under the age of 18 years with suspected inherited tubulopathy, where a request for NGS was sent between September 2016 and September 2020, were screened. Five milliliters of blood in EDTA were sent from the patient after obtaining written informed consent to Medgenome Labs Pvt. Ltd., a private commercial laboratory in Bengaluru, India. Demographic details, history of consanguinity, similar illness in the family, early onset of kidney failure, the involvement of multiple systems, and syndromic features were noted. Children with suspected inherited tubulopathies were included. Children with tubular dysfunction secondary to chronic kidney disease (CKD), drugs, cystic kidney diseases, hypodysplasia, and other congenital anomalies of the kidney and urinary tract (CAKUT) were excluded from the study.

Clinical presentation of tubulopathies was further classified into Fanconi syndrome, Bartter/ Gitelman syndrome, d-RTA, monogenic hypertension, nephrogenic diabetes insipidus, hypophosphatemic rickets, and magnesium disorders. Clinical exome sequencing (CES) covering 6670 disease-causing genes encompassing both nuclear and mitochondrial genes was requested in the index patient. It covered disease-associated genes including coding variants, splice variants, reported deep intronic variants, and copy number variation. The CES included 73 genes involved in tubulopathies. The list of the genes tested and the corresponding disease is given in Table 1.

CES was done by NGS by Illumina platform at a depth of 80–100x. Sanger sequencing was done when a sibling or any other family member was to be tested for the same gene or to confirm the variant in NGS. The test result was interpreted as per American College of Medical Genetics classification: No pathogenic variant, variant of unknown significance (VUS), and likely pathogenic and pathogenic variant [6]. Utility of genetic testing was assessed by finding out how many new diagnoses were made, changed the diagnosis in how many children, how it helped in reverse phenotyping, and genetic counseling.

RESULTS

From September 2016 to September 2020, 82 samples were sent for NGS from 72 children, eight parents, one sibling, and one fetus. Out of 72 children, 14 (19.4%) had suspected inherited tubulopathy. CES was sent in all 14 children. The median age (IQR) of the cohort at the onset of disease was 18 months (4–65). The median age (IQR) at diagnosis was 78 (45–120). History of parental consanguinity was present in 2 children (14.2%). Family history of similar illness in a sibling was present in one child.

The most common clinical presentation in the cohort was Fanconi syndrome in 5 children (35.7%). Two had dRTA, two had Barter/Gitelman syndrome, two had rickets, two had nephrocalcinosis, and two had low-molecular-weight proteinuria. The clinical characteristics of the patients are depicted in Table 2.

Nine children (64%) had a pathogenic variant, two had VUS, and three had no genetic variant. Inheritance was autosomal recessive in 8 (88.8%) and X-linked dominant in one child. Nine pathogenic

Table 1: Genes causing tubulopathy covered in clinical exome sequencing

Name of the gene	Disease
GATM	Fanconi renotubular syndrome 1
SLC34A1	Fanconi renotubular syndrome 2
EHHADH	Fanconi renotubular syndrome 3
HNF4a	Fanconi renotubular syndrome 4
SLC2A2	Fanconi Bickel syndrome
CTNS	Cystinosis
GALE	Galactose epimerase deficiency
GALK1	Galactokinase deficiency with cataract
GALT	Galactosemia
ALDOB	Hereditary fructose intolerance
G6PC	Glycogen storage disease type 1
SLC37A4	Glycogen storage disease 1B
ATP7B	Wilson disease
CLCN5	Dent disease type 1
OCRL	Lowe syndrome
CA2	Renal tubular acidosis type 3
SLC34A3	Hereditary hypophosphatemic rickets with hypercalciuria
PHEX	X-linked hypophosphatemic rickets
SLC3A1	Cystinuria A
SLC7A9	Cystinuria B
SLC7A7	Lysinuric protein intolerance
SLC6A19	Hartnup disorder
SLC36A2	Iminoglycinuria
SLC1A1	Dicarboxylic aminoaciduria
SLC12A1	Bartter type 1
KCNJ1	Bartter type 2
CLCNKB	Bartter type 3
BSND	Bartter type 4a
MAGED2	Bartter type 5
CLDN 16	Hypomagnesemia type 3
CLDN 19	Hypomagnesemia type 5
CaSR	Autosomal dominant hypocalcemia
FAM111A	Kenny-Caffey syndrome type 2
SLC12A3	Gitelman syndrome
KCNJ 10	EAST/SeSAME syndrome
WNK4	Pseudohypoaldosteronism type 2b
WNK 1	Pseudohypoaldosteronism type 2c
KLHL3	Pseudohypoaldosteronism type 2d
CUL3	Pseudohypoaldosteronism type 2e
TRPM6	Hypomagnesemia type 1
FXYD2	Hypomagnesemia type 2
KCNA1	Autosomal dominant hypomagnesemia
HNF1B	HNF1B related kidney disease
PCBD1	Hyperphenylalaninemia BH4 deficient
EGF	Hypomagnesemia type 4
EGFR	Neonatal inflammatory skin and bowel disease type 2
CNNM2	Hypomagnesemia, seizure, and mental retardation type 1

(*Contd...*)

Table 1: (*Continued*)

Name of the gene	Disease
ATP1A1	Hypomagnesemia, seizure, and mental retardation type 2
SCNN1A, SCNN1B, SCNN1G	Pseudohypoaldosteronism type 1
NR3C2	Pseudohypoaldosteronism type 1A
SCNN1B, SCNN1G	Liddle syndrome
HSD11B2	Apparent mineralocorticoid excess
CYP11B1	Glucocorticoid remediable aldosteronism
CYP17A1	Congenital adrenal hyperplasia type 5
CYP21A2	Congenital adrenal hyperplasia type 1
HSD3B2	Congenital adrenal hyperplasia type 2
CYP11B1	Congenital adrenal hyperplasia type 4
AVPR2, AQP2	Nephrogenic diabetes insipidus
GNAS	SIADH
SLC4A1, ATP6V1B1, ATP6V0A4, FOXI1, WDR 72	D RTA
ENPP1	Hypophosphatemic rickets with calcification
DMP1	Hypophosphatemic rickets, AR

Table 2: Clinical characteristics of patients with suspected tubulopathies

Characteristics	Total (n=14) (%)
Age in months at onset, median (IQR)	18 (4–65)
Sex F: M	8:6
Parental consanguinity	2 (14.2)
Family history	1 (7%)
Clinical presentation	
Fanconi syndrome	5 (35.7)
Bartter phenotype	2 (14.2)
Rickets	2 (14.2)
Nephrocalcinosis	2 (14.2)
Low-molecular-weight proteinuria	1 (7.1)

variants were detected in eight genes: ATP6V0A4, CTNS1, FAH1, PHEX, SLC12A1, SLC4A1, SLCA2, and CLDN 16. Five (35.7%) were novel variants not described earlier. Four were missense, three nonsense, and two frameshift mutations. Three VUS were detected in two children in FAT1, EYA1, and KCNJ1 gene. Three children (21.4%) had no genetic variant. Bartter syndrome type 1 and distal RTA were the most common genetic diagnoses with two patients each. Other diagnoses were tyrosinemia type 1, nephropathic cystinosis, Fanconi Bickel syndrome, X-linked hypophosphatemic rickets, and familial hypomagnesemia, hypercalciuria, and nephrocalcinosis (FHHNC) in one each. The spectrum of genetic diagnosis is depicted in Table 3. Clinical and genetic details of patients with genetic disease are given in Table 4.

Out of five children with Fanconi syndrome, three were diagnosed with a genetic disease, namely, nephropathic cystinosis, tyrosinemia type 1, and Fanconi Bickel syndrome and two did not have a genetic variant. Two children each with suspected Bartter syndrome and d-RTA were confirmed to have the disease.

Table 3: Spectrum of genetic diagnosis

ACMG classification	n, %
Pathogenic	9 (64%)
Likely pathogenic	0
VUS	2 (14%)
No variant	3 (22%)
Inheritance	
Autosomal recessive	8 (88.8%)
X-linked dominant	1 (11.2%)
Diagnosis	
Bartter syndrome Type 1	2
Distal RTA	2
Tyrosinemia Type 1	1
Nephropathic cystinosis	1
Fanconi Bickel syndrome	1
d RTA	3 (21.4%)
X-linked hypophosphatemic rickets	1
FHHNC	1

ACMG: American College of Medical Genetics, VUS: Variant of unknown significance, RTA: Renal tubular acidosis, FHHNC: Familial hypomagnesemia, hypercalciuria, and nephrocalcinosis

The child with nephropathic cystinosis (S No 2) had onset of Fanconi syndrome with polyuria, polydipsia, failure to thrive, glycosuria, aminoaciduria, and rickets from 5 months of age. He progressed to CKD Stage 4 by 5 years. A slit-lamp examination at this stage demonstrated cystine crystals and CES confirmed nephropathic cystinosis. He had a novel mutation in the CTNS1 gene. He is currently on oral cysteamine and cysteamine eye drops. The other child (S No 3) presented with genu valgum and on evaluation was found to have Fanconi syndrome. She was diagnosed to be tyrosinemia type 1 by CES 2 years after the onset of disease. Subsequent evaluation revealed an enlarged liver with normal liver enzymes. She is being evaluated for possible liver transplantation. Another child also had Fanconi syndrome for 4 months of age along with transaminitis, hypouricemia, and enlarged liver and kidney in ultrasound. She was suspected to have glycogen storage disease but got diagnosed to have Fanconi Bickel syndrome by CES. Two children got diagnosed with d RTA but none of them had sensorineural hearing loss or ovalocytosis.

The child with FHHNC (S No 9) presented with a recurrent history of nephrolithiasis and failure to thrive. On investigation, she had CKD Stage 4, medullary nephrocalcinosis, hypercalciuria, and hypomagnesemia. On further evaluation, her younger sibling also was found to be similarly affected. CES confirmed pathogenic mutation in Claudin 16 (CLDN 16). The other girl with nephrocalcinosis was brought to attention because of UTI and ultrasound revealing dense medullary nephrocalcinosis. She was suspected to have primary hyperoxaluria but there was a VUS in the KCNJ1 gene which caused Bartter syndrome type 2. On further evaluation, she has persistent metabolic alkalosis and hyperchloriduria. The Sanger sequencing of the same gene and parental screening has been planned to establish pathogenicity.

Table 4: Details of patients with a genetic etiology of tubulopathy

S. No.	Age mo	Clinical presentation	Clinical diagnosis	Gene	Location	Codon change	Protein change	Zygosity	Inheritance	Final diagnosis
1	4	RTA	dRTA	ATP6V0A4	Exon 13	c. 1185del	p.Tyr396ThrfsTer12	Homozygous	AR	d RTA with preserved hearing
2	60	Fanconi syndrome, cystine crystals in cornea	Cystinosis	CTNS1	Exon 7	c. 461G>T	p. Ser154Ile	Homozygous	AR	Nephropathic cystinosis
3	48	Fanconi syndrome, rickets	Proximal RTA	FAH	Exon 3	c. 192G>T	p.Gln64His	Homozygous	AR	Tyrosinemia type 1
4	180	Rickets	Hypophosphatemic rickets	PHEX	Exon 15	c. 1645C>T	p.Arg549Ter	Heterozygous	XLD	X Linked Hypophosphatemic Rickets
5	36	Bartter syndrome	Bartter syndrome	SLC12A1	Exon14	c. 1685C>T	p.Ala562Val	Homozygous	AR	Bartter Syndrome Type 1
6	72	Bartter syndrome	Bartter syndrome	SLC12A1	Exon 22	c. 2716C>T	p.Gln906Ter	Homozygous	AR	Bartter Syndrome Type 1
7	120	RTA	d RTA	SLC4A1	Exon 19	c. 2573C>A	p.Ala858Asp	Homozygous	AR	d RTA
8	8	Fanconi syndrome, transaminitis, hypouricemia, enlarged liver, kidney	Glycogen storage disease	SLCA2	Exon 3	c. 339del	p.Phe114LeufsTer16	Homozygous	AR	Fanconi Bickel Syndrome
9	180	Nephrocalcinosis, CKD	FHHNC	CLDN16	Exon 2	c. 374T>C	p.Phe125Ser	Homozygous	AR	FHHNC

RTA: Renal tubular acidosis, AR: Autosomal recessive, XLD: X-linked dominant, FHHNC: Familial hypomagnesemia, hypercalciuria, and nephrocalcinosis, CKD: Chronic kidney disease

DISCUSSION

We discuss the utility of NGS in the diagnosis and management of inherited tubulopathies in children from a West Indian population. There are no published data yet on NGS in Indian children with tubulopathy. More than 60 genes have been discovered to cause renal tubulopathies.[2] Seventy-three disease-causing genes were tested by NGS in our cohort (Table 1). Remarkably, a high proportion (64%) of children had a pathogenic variant; similar to 66% reported by Ashton *et al*. [7] This was also higher than reported in other diseases such as steroid-resistant nephrotic syndrome (SRNS) (26%), CAKUT (17%), nephrolithiasis/ nephrocalcinosis (25%), and ciliopathies (50%) [8]. This suggests that in a large majority of tubulopathies, a genetic cause can be established.

CES in our cohort included a much higher number of genes (73 compared to 37) including many genes causing Fanconi syndrome, PHEX, and FGF 23, which were not included in the study by Ashton *et al*. This is probably the reason we found pathogenic variants in three out of five children with Fanconi syndrome. Fanconi syndrome is the most common inherited renal tubulopathy in children caused by many diseases, may be primary with no underlying disease [3]. NGS was diagnostic in three out of five children in our cohort. Two of them were clinically being treated non-specifically with supplements in absence of a specific diagnosis. After NGS, they were diagnosed with tyrosinemia type 1 and glycogen storage disease which have specific treatments.

Tyrosinemia type 1 is caused by a deficiency of fumarylacetoacetase (FAH), the last enzyme in tyrosine degradation [9,10]. The chronic form of tyrosinemia type 1 leads to progressive cirrhosis of the liver and hepatocellular carcinoma. The child with tyrosinemia (S No 3) presented with Fanconi syndrome, refractory rickets, and very minimal liver involvement. The presence of succinylacetone in plasma and urine is diagnostic of tyrosinemia type I [11]. However, the test is not routinely available in commercial laboratories in India. Hence, the diagnosis of tyrosinemia remains elusive in most children.

Commercial availability of NGS now makes the diagnosis of these diseases possible within a short time as illustrated in our case. NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) which prevents the formation of pathogenic fumarylacetoacetate improves prognosis and quality of life of patients with tyrosinemia [12]. Therefore, definitive diagnosis should be made in case of suspected tyrosinemia and started with NTBC. Some children might benefit from liver transplantation [13].

Fanconi Bickel syndrome is an autosomal recessive disorder characterized by failure to thrive, "doll-like" face, hepatomegaly, nephromegaly, and severe rickets [11]. It is caused by the mutations in glucose transporter gene SLC2A2, also referred to as GLUT2, expressed in the liver, kidney, intestine, and pancreatic islet cells [14]. The child with Fanconi Bickel syndrome (S No 8) was suspected to have glycogen storage disease. In the absence of genetic testing, diagnosis is usually established by an invasive liver biopsy [15]. We could establish the diagnosis by NGS, avoiding an invasive liver biopsy with its attendant complications.

Nephropathic cystinosis is another disease which presents with Fanconi syndrome, has a definite treatment in the form of cysteamine [16]. Renal function is generally normal at presentation. However, most patients progress to kidney failure by 10 years of age if not treated with cysteamine [17]. The presence of cystine crystals in the cornea is usually diagnostic [11]. Other tests such as blood cysteine level and lysosomal cysteine level are extremely difficult to get done in India. However, definitive diagnosis can also be made by NGS in absence of these tests and the absence of cystine crystals. The diagnosis of cystinosis was made clinically in our patient and was confirmed by NGS.

Bartter/Gitelman syndrome was the most common tubulopathy in a previously published cohort of children with tubulopathies by detection of monogenic mutations [8]. Genetic diagnosis could be established in 74% of the children. It was also the most common diagnosis in our cohort affecting two children. Both had clinical Bartter syndrome and were confirmed to have Bartter syndrome type 1. One interesting child presented with recurrent urinary tract infection and dense medullary nephrocalcinosis who was suspected to have primary hyperoxaluria was found to have a VUS in the KCNJ1 gene to our surprise. The variant causes antenatal Bartter syndrome [18] but has been reported to cause a very mild Bartter phenotype in adults [19].

Distal RTA is another common tubulopathy, which accounted for the second most common tubulopathy in the earlier cohort. NGS could yield 58% diagnosis in that cohort [8]. One child (S No1) was found to have a pathogenic variant in ATP6V0A4, which causes d-RTA with preserved hearing [20]. Hearing evaluation in this child revealed normal hearing. The other child (S No 7) had a pathogenic variant in SLC4A1 which involves band three proteins in red blood cell (RBC) membrane leading to ovalocytosis [21]. However, our child did not have ovalocytosis or increased osmotic fragility of RBC.

We could confirm our clinical diagnosis of hypophosphatemic rickets in a child who had a pathogenic variant in the *PHEX* gene. The advent of new promising monoclonal antibody burosumab in the treatment of hypophosphatemic rickets necessitates making of correct molecular diagnosis [22]. The presence of hypomagnesemia and hypercalciuria in a child with nephrocalcinosis and CKD leads us to suspect FHHNC, which was confirmed by detection of a pathogenic variant in claudin 16. This also led to the same diagnosis in her sibling who had a similar presentation. FHHNC is an autosomal recessive disease due to pathogenic mutation in claudin 16 and claudin 19 genes [23]. The claudins are paracellular proteins involved in the paracellular transport of magnesium in the thick ascending limb of loop of Henle. Involvement of claudin 19 also leads to ocular involvement causing myopia [23].

NGS established new diagnosis of tyrosinemia 1 and Fanconi Bickel syndrome in two children guiding specific treatment. We were able to avoid an invasive liver biopsy in the child with Fanconi Bickel syndrome. In seven other children, it confirmed our clinical diagnosis, reverse phenotyping, and genetic counseling.

NGS is not without challenges for clinicians. One is cost; Rs. 16,000 is not affordable for all. Second, VUS is a challenge

for clinicians as a demonstration of pathogenicity will require testing in parents and functional analysis which is cumbersome, time consuming, and expensive in the clinical setting [24]. The possibility of VUS and not able to detect any variant should be explained to parents while sending the sample for NGS and counseled properly. The detection of VUS in KCNJ1 highlights the phenotypical variability of autosomal recessively inherited diseases and argues for broad testing with clinical exome or whole exome which can give a completely different diagnosis than a clinically suspected disease. Three children did not have any genetic variant in CES. As shown in earlier studies too, in up to one-third of children with inherited tubulopathies, no genetic disease could be established. Whole-exome sequencing or whole-genome sequencing could help in detecting genetic variants in this situation [25].

The limitation of our study is being a retrospective study with less number of patients in each group. A future prospective multicenter study including all children with suspected genetic kidney diseases should provide more evidence in this area.

CONCLUSION

Our study reiterates the emerging use of sequencing of exomes by NGS in the management of children with suspected kidney diseases. NGS has a high yield in inherited tubulopathies and can be a useful clinical tool in making an accurate diagnosis, genotype-phenotype correlation, and adequate genetic counseling. The genetic cause may not be found in one-third of children with tubulopathy after sequencing of exomes of all known disease-causing genes. Whole-exome sequencing or whole-genome sequencing might be useful in these children; however, these need confirmation with a large multicenter prospective study.

AUTHORS' CONTRIBUTIONS

AS conceptualized the study, collected data, and wrote the manuscript. KV contributed in collecting data and writing the manuscript. HP contributed in giving critical inputs and writing the manuscript. All the authors approved the final version of the manuscript. AS will act as guarantor of the article.

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