

# Pediatric Nephrology

## Whole Exome Sequencing and variant spectrum in children with suspected inherited renal tubular disorder: East India Tubulopathy Gene Study --Manuscript Draft--

<b>Manuscript Number:</b>	PNEP-D-21-00548R1	
<b>Full Title:</b>	Whole Exome Sequencing and variant spectrum in children with suspected inherited renal tubular disorder: East India Tubulopathy Gene Study	
<b>Article Type:</b>	Original Article	
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<b>Funding Information:</b>	medgenome labs private limited (Partial funding)	Dr Rajiv Sinha
<b>Abstract:</b>	<p><b>Background:</b> Inherited tubulopathies are a heterogeneous group of genetic disorders making whole exome sequencing (WES) the preferred diagnostic methodology.</p> <p><b>Methods:</b> This was a multi-centric descriptive study wherein children (&lt;18 years) with clinically suspected tubular disorders were recruited for molecular testing through WES. Multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing were done when required. Variants were classified as per American College of Medical Genetics 2015 guidelines and pathogenic (P) / likely pathogenic (LP) variants were considered causative.</p> <p><b>Results:</b> There were 77 index cases (Male =73%; female). Median age of diagnosis</p>	

	<p>was 48 months (IQR 18.5 to 108 months). At recruitment, number of children in each clinical group were as follows: Distal Renal Tubular Acidosis (dRTA) =25, Bartter syndrome=18, Isolated Hypophosphatemic rickets (HP) =6, Proximal tubular dysfunction (pTD) = 12, Nephrogenic Diabetes Insipidus (NDI) =6, Kidney stone / Nephrocalcinosis (NC) =6 and Others =4.</p> <p>We detected 55 (24 novel) P/LP variants, providing genetic diagnoses in 54 children (70%). The diagnostic yield of WES was highest for NDI (100%), followed by HP (83%; all X-linked HP), Bartter syndrome (78%), pTD (75%), dRTA (64%), and NC (33%). Molecular testing had a definite impact on clinical management in 24 (31%) children. This included revising clinical diagnosis among 14 children (26% of those with a confirmed genetic diagnosis and 18% of the overall cohort), detection of previously unrecognized co-morbidities among 8 children (sensorineural deafness: n=5, hemolytic anemia: n=2, and dental changes: n=1) and facilitating specific medical treatment among 7 children (Primary Hyperoxaluria: n=1, Cystinosis: n=4, Tyrosinemia: n=2). Conclusion: WES is a powerful tool in the diagnosis and management of children with inherited tubulopathies in the Indian population.</p>
<p><b>Response to Reviewers:</b></p>	<p>Reviewers' comments:</p> <p>Reviewer #1:</p> <p>Major concerns</p> <p>Comment 1: The a priori inclusion criteria of this study remain unclear. Since the authors investigate multiple disease entities, they list a "phenotype description" for each disease entity in TI. However, it remains unclear if and to which extent this phenotype description needed to be fulfilled for the respective patient to be included in this supposedly prospective study in general and in the respective cohort in particular. Furthermore, the category "Others" seems to entirely consist of patients that did not fulfill any clear inclusion criteria beyond a presumptive diagnosis of tubulopathy by their treating physician. To avoid the impression of selective in- and exclusion of patients and post-hoc grouping of patient cohorts, the authors should clearly explain their inclusion process and, most importantly, add the actual phenotype of the respective patient in TII.</p> <p>Response: We would like to thank the reviewer for highlighting this concern. At the time of collecting blood sample for genetic analysis all centres were mandated to submit a detailed history. Criteria for classification into the different phenotypes were decided prior to starting the study in a PI meeting. It was agreed that those not falling into clear pattern but with unexplainable electrolyte, acid / base disorder or polyuria would be classified as "Others" (Added in Table 1). Classification into various groups was done after analysing the clinical data but before availability of genetic results. We have added this information in the method section (Page 4). In addition as per reviewer suggestion we have added details of the phenotype in Table 2.</p> <p>Comment 2: The patient NDI6 has been previously published by the authors, as mentioned in the manuscript (Das Indian Pediatr 56:325, 2019). The initial manuscript of this case report was received in February 2018 by Indian Pediatrics. This is four months before the start of enrollment ("June 2018") stated in the methods section of this manuscript and, together with point 1 of our major concerns, further corroborates the suspicion that this is a retrospective and not a prospective study as claimed in the manuscript.</p> <p>Response: We do appreciate this genuine concern and would like to clarify the sequence of events pertaining NDI6. The family was under follow up with the particular clinician during the period of project. In view of marriage in a close community (with hidden consanguinity), the family was apprehensive and wanted extended family screening, which was done using the project grant. This was done during the interim period wherein the case was under consideration for publication in the journal Indian Pediatrics and was undergoing revision. As most of our cohort were under follow up with various clinical teams and the genetic analysis was done with the current project grant we have included the child's data. If desired we can remove this patient from our series, and include as reference during discussion and would appreciate suggestion from reviewers / editorial board for the same.</p> <p>We, appreciate the concern that whether this was a prospective study or a</p>

retrospective data collection and would like to reaffirm that all the data were collected prospectively. As mentioned above clear inclusion criteria for different groups were decided prior to starting the study in a PI meeting and all centres had to submit a detailed clinical form without which no blood samples were analysed. Analysis of the clinical data submitted in the forms and actual allocation of the subjects into different sub groups was made at the end of recruitment but prior availability of genetic results. Despite this and keeping in mind that most of these children were under follow up of various clinical team and the fact that we included NDI6 who was diagnosed to have dRTA prior the start of the study we have removed the word prospective from the abstract and have refrained from terming this study as a prospective study in the main draft.

Comment 3: The discussion section of the manuscript needs to be shortened significantly. While very diligent, the discussion of heterozygous variants in recessive genes in the sense of carrier status is irrelevant for the results and expected in a WES study of this size.

Response: We do agree with the comments and have compiled with the suggestion (Page 10)

#### Minor concerns

1. p.6: A p-value for statistical significance testing between age of clinical presentation and age of genetic testing is irrelevant.

Response: We wanted to emphasise that in our region there was a significant gap between the clinical suspicion of an underlying tubulopathy and final genetic diagnosis but as per reviewers comment we have removed the "p" value (page 6).

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Response: Changes done in the Table 2

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Response: We meant to say that the genetic etiology was confirmed after we had put ACMG criteria for variant classification. This has been added in Table 2 (highlighted by yellow).

4. The entire manuscript would profit from thorough spellchecking.

Response: We have reviewed the manuscript for any missed spelling mistakes.

5. The authors should ensure that the formatting of the tables is uniform.

Response: Thanks for highlighting this and we have reformatted the tables.

#### Reviewer #2:

##### Major comments

Comment 1: The manuscript reports the usefulness of WES as a diagnostic tool of primary tubulopathies in Indian children. The manuscript does not provide any essential new finding unknown for the readers but it is interesting to describe the local experience in a large group of patients.

Response: We thank the reviewer for finding our study interesting. We also believe that keeping in mind the genetic heterogeneity seen across ethnic group it is essential to describe the genetic pattern seen in India which by itself has a huge population.

Comment 2: The manuscript must be substantially shortened because a lot of the information given in the Introduction and the Discussion is well known.

Response: Thanks for the comment and in accordance the Introduction and Discussion section have been shortened.

Comment 3: Table II should be removed from the manuscript and be moved to the Supplementary Material.

Response: We do agree that Table 2 is quite long but the Table is essential to the paper as it describes the phenotype and genotype of the cohort in details. If the editorial board deems necessary we are happy to move it to supplement.

Comment 4: The Objectives detailed in the Introduction could be summarized to indicate "To examine the utility of WES in children with a clinical diagnosis of tubulopathy" The study was not designed to provide reliable information on the epidemiology of tubulopathies in East India.

Response: We do agree with the reviewer that the study design was not appropriate to give us accurate information on epidemiology of tubulopathies in Eastern India and do apologise for adding this in the objective. In accordance we have removed this from the revised draft (page 4, introduction section)

Comment 5: Several paragraphs of the Discussion, should be much shorter, i.e. those on unsolved cases of DRTA.

Response: As said before we have tried to shorten the introduction and discussion part of the draft to make it crisper.

Minor comments:

1) - Abstract: Please, add "syndrome" to Bartter.

Response: Changes done in the abstract

2) Abstract and Results: Hypophosphatemic rickets must be replaced by X-linked hypophosphatemia (XLH) because there are several other forms of hypophosphatemic rickets and all mutations found were in the PHEX gene.

Response: Changes done in the abstract (Page 3) and results (Page 6).

3) Primary pRTA is a extremely rare condition. The term pRTA should be changed because the cases analyzed by the authors correspond to tubular disorders causing generalized proximal tubular dysfunction.

Response: As per suggestion we have changed pRTA to proximal tubular dysfunction (pTD)

4) Results (Table II and text): The interval between the ages at diagnosis and at genetic testing is of little interest for this manuscript and does not have to be compared statistically (First paragraph of the Results).

As mentioned in the response to Reviewer 1 we wanted to highlight the significant gap between the age of presentation and final genetic diagnosis but in accordance to the advice of the reviewers we have removed statistical comparison between the two groups.

Reviewer #3:

Comment 1: This is a well written paper describing a high diagnostic yield in a relatively under-described cohort. I don't think every paper that features WES needs to start with an explanation of NGS/MPS, cost dropping but this may just reflect the number of renal genetics papers I read.

Response: Thanks for your encouraging comments. We do believe it was important for us to underline the dropping cost of NGS which has made it cost effective even for emerging economies.

Comment 2: The methods seem sound though I don't understand why it says "Multiple databases (HGMD; Clinvar; MobiDetails; pubmed; google scholar) were searched prior to confirmation of novel variants" when all classification was conducted as per ACMG guidelines.

Response: Thanks for highlighting this. We do agree that mention of various databases were redundant and just mentioning that the classification was done in accordance to ACMG guideline would suffice. Hence we have deleted it in the updated draft (Page 5).

Comment 3: Overall I would be happy for this paper to be accepted as it is currently written though I would like to see some more information as to the clinical utility of the positive test results in terms of diagnostic certainty, management, surveillance and reproductive information.

Response: Thanks again for the encouraging comments. We did emphasize on the clinical utility in the previous draft (page 7 and Table 3). We have further expanded it by emphasizing the high incidence of consanguineous marriages and endogamy leading to hidden consanguinity in our population and need for extended family screening, genetic counselling and prenatal testing.

Editor-in-Chief comment:

We are especially concerned by the point raised by reviewer 1 regarding prior publication of one of these patients, and the implications regarding your study design. If the study was actually retrospective, then say so.

Response: We do appreciate the concerns and have discussed this in detail during our response to Reviewer 1 comments. We would like to re-emphasize that the inclusion criteria was determined prior the start of the study in a PI meeting and the clinical data were collected prospectively (Page 4). Analysis and sub-grouping of these children into various groups was done at the end of the study but prior availability of genetic results. At the same time it needs to be noted that many of the children were already being followed up at the respective centres (as obvious from our mention of the time at clinical suspicion and time to genetic testing) and were recruited for genetic testing once our study started. In light of the obvious concerns raised by both the reviewers and the Editorial board we have removed the term prospective from the abstract and have described the study as “descriptive” study. We have also elaborated on the sequence of events which made us include the data of NDI6 which was re analysed and family screening was undertaken as part of the grant received for the current study. We would abide by the decision of the editorial board regarding inclusion of NDI6.

Editorial Office comments:

1) Please change the author list to be consistent re order of given name or initials and surname, so given name or initials are listed first and surname is listed second for each author.

Response: This has been revised and we do apologise for the inconvenience.

2) Graphical abstract - Pediatric Nephrology expects Original Articles to be accompanied by a Graphical Abstract. This is a single slide which visually summarizes the main findings of the paper. The Graphical Abstract will be included with the online paper, and will be available as a PPT file for researchers to download and use in presentations, and also provides a convenient visual summary for use on social media. As such, including a Graphical Abstract may increase the penetrance and profile of your paper.

A Graphical Abstract template is attached, which includes instructions, a simple graphical library and some examples.

Response: This has been added.

3) Nomenclature - We have implemented the KDIGO nomenclature guidelines for all manuscripts. The full KDIGO nomenclature guideline is published in Kidney

International - [https://www.kidney-international.org/article/S0085-2538\(20\)30233-7/pdf](https://www.kidney-international.org/article/S0085-2538(20)30233-7/pdf)  
- followed by accompanying editorials in other kidney and pediatrics journals.

In brief:

- Where reasonable, please replace 'renal' with 'kidney' eg in 'renal tubular disorders', 'renal ammonium secretion' etc.

- In Table 3 and its caption, please replace 'end-stage kidney disease / ESKD' with 'kidney failure' or CKD stage 5 as appropriate

Please see the attached document for details and include any relevant changes in your revised manuscript.

Response: We have incorporated the changes as advised but have kept the term renal for distal renal tubular acidosis as it is a well recognised entity and is referred in OMIM also by this name. Will be happy to change further if editorial board desires.

#### 4) References

(a) In your reference list, while it is acceptable to abbreviate long author lists with 'et al', we encourage all authors to be listed where reasonable, and when the list is long please include 4 author names before 'et al'.

(b) Please abbreviate all journals names per PubMed guidelines and be consistent throughout the reference list. For example, ref. 1 and 2 should be 'Clin J Am Soc Nephrol' only; ref. 12 should be 'Nephrol Dial Transplant' only.

(c) Ref. 4 - please provide page numbers.

(d) Ref. 24, please provide journal volume and page numbers.

(e) Ref. 33, please provide page numbers.

Comment: Reference has been revised as advised

To,

The Editor,

Pediatric Nephrology

Dated: 29<sup>th</sup> September 2021

Subject: Revision of the article entitled “**Whole Exome Sequencing and variant spectrum in children with suspected inherited renal tubular disorder: *East India Tubulopathy Gene Study***”.

Dear Sir/ Madam,

We are thankful to you, for the comments received from the editorial board and the reviewers. We are hereby submitting the revised version as per the comments received.

We are looking forward for a positive response from your side.

Thanking you,

Yours sincerely,

Dr Kausik Mandal (Corresponding author)

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Reviewers' comments:

Reviewer #1:

Major concerns

Comment 1: The a priori inclusion criteria of this study remain unclear. Since the authors investigate multiple disease entities, they list a "phenotype description" for each disease entity in TI. However, it remains unclear if and to which extent this phenotype description needed to be fulfilled for the respective patient to be included in this supposedly prospective study in general and in the respective cohort in particular. Furthermore, the category "Others" seems to entirely consist of patients that did not fulfill any clear inclusion criteria beyond a presumptive diagnosis of tubulopathy by their treating physician. To avoid the impression of selective in- and exclusion of patients and post-hoc grouping of patient cohorts, the authors should clearly explain their inclusion process and, most importantly, add the actual phenotype of the respective patient in TII.

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*Comment: Reference has been revised as advised*

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**Title: Whole Exome Sequencing and variant spectrum in children with suspected inherited renal tubular disorder: *East India Tubulopathy Gene Study***

**Short title: *India tubulopathy gene study***

**Authors:**

Rajiv Sinha<sup>1,5</sup>, Subal Pradhan<sup>2</sup>, Sushmita Banerjee<sup>1,3</sup>, Afsana Jahan<sup>4</sup>, Shakil Akhtar<sup>1</sup>, Amitava Pahari<sup>5</sup>, Sumantra Raut<sup>6</sup>, Prince Parakh<sup>7</sup>, Surupa Basu<sup>1</sup>, Priyanka Srivastava<sup>8</sup>, Snehamayee Nayak<sup>2</sup>, SG Thenral<sup>9</sup>, V Ramprasad<sup>9</sup>, Emma Ashton<sup>10</sup>, Detlef Bockenhauer<sup>11</sup>, Kausik Mandal<sup>12</sup>

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35  
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41  
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44 for submission of manuscript.

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49 **Consent to participate:** Informed consent obtained taken from the parents / guardians as well as from the children  
50 (aged  $\geq$  13 years)

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4 **Abstract**

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7 **Background:** Inherited tubulopathies are a heterogeneous group of genetic disorders making whole exome  
8 sequencing (WES) the preferred diagnostic methodology.  
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11 **Methods:** This was a multi-centric descriptive study wherein children (<18 years) with clinically suspected tubular  
12 disorders were recruited for molecular testing through WES. Multiplex ligation-dependent probe amplification  
13 (MLPA) and Sanger sequencing were done when required. Variants were classified as per American College of  
14 Medical Genetics 2015 guidelines and pathogenic (P) / likely pathogenic (LP) variants were considered causative.  
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20 **Results:** There were 77 index cases (Male =73%; female). Median age of diagnosis was 48 months (IQR 18.5 to  
21 108 months). At recruitment, number of children in each clinical group were as follows: Distal Renal Tubular  
22 Acidosis (dRTA) =25, Bartter syndrome=18, Isolated Hypophosphatemic rickets (HP) =6, Proximal tubular  
23 dysfunction (pTD) = 12, Nephrogenic Diabetes Insipidus (NDI) =6, Kidney stone / Nephrocalcinosis (NC) =6 and  
24 Others =4.  
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30 We detected 55 (24 novel) P/LP variants, providing genetic diagnoses in 54 children (70%). The diagnostic yield of  
31 WES was highest for NDI (100%), followed by HP (83%; all X-linked HP), Bartter syndrome (78%), pTD (75%),  
32 dRTA (64%), and NC (33%). Molecular testing had a definite impact on clinical management in 24 (31%) children.  
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34 This included revising clinical diagnosis among 14 children (26% of those with a confirmed genetic diagnosis and  
35 18% of the overall cohort), detection of previously unrecognized co-morbidities among 8 children (sensorineural  
36 deafness: n=5, hemolytic anemia: n=2, and dental changes: n=1) and facilitating specific medical treatment among 7  
37 children (Primary Hyperoxaluria: n=1, Cystinosis: n=4, Tyrosinemia: n=2).  
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45 **Conclusion:** WES is a powerful tool in the diagnosis and management of children with inherited tubulopathies in  
46 the Indian population.  
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## Introduction

The development of kidney tubules is an important evolutionary advance that facilitated the move from the ocean onto land. Kidney tubules reabsorb the majority of the glomerular filtrate and maintain volume and electrolyte balance, thereby preserving the “milieu interieur” critical for normal physiology [1]. Multiple transporters and channels perform this important task and their dysfunction can lead to various disorders, collectively called “tubulopathies”, which in children are usually due to variants in genes involved in tubular transport [2]. Establishing the underlying genetic etiology of these inherited tubulopathies are important as it not only provides a clear diagnosis for the patient and family, it also impacts on management, genetic counseling, and screening of relatives at risk [3]. Classically, individual candidate genes for a known disorder were sequenced one at a time. This approach is both cumbersome and expensive, since tubulopathies comprise a heterogeneous group of disorders [4]. Next Generation Sequencing (NGS) / Massively Parallel Sequencing enables sequencing of multiple genes simultaneously saving time and money [3, 5] Over the last decade its cost has dropped considerably making it affordable also for emerging economies. Based on various studies, use of NGS has been endorsed in international guidelines on nephrotic syndromes [6–8]. NGS based diagnostic algorithms have also been published in some other kidney disorders including atypical HUS and Alport syndrome [9, 10]. Unfortunately, NGS based studies on tubulopathies are limited and mainly restricted to developed countries with scanty representation from emerging economies like India [11–20]. The decreasing cost of NGS based molecular testing and superior yield of whole exome sequencing (WES) has resulted in a shifting trend towards WES for variant testing; especially for disorders with genetic heterogeneity [3]. As with any powerful tool, widespread use of NGS has also thrown up various challenges particularly that of variant classification, which can be more difficult in populations/ disease groups with scant genomic data [3, 4, 21]. With this perspective, we initiated the *East India Tubulopathy Gene Study* to examine the utility of WES among children with clinically suspected tubular disorders.

## Methods

### *Study details*

We performed a multi-centric, descriptive cross-sectional study with enrolment from June 2018 to March 2020. Children (age  $\leq 18$  years) under follow up for clinically suspected tubulopathy, defined as per pre-decided criteria (Table I) were eligible for inclusion. Inclusion criteria, case definition (Table I), details of data collection, sample collection and details of genetic tests to be conducted were agreed upon by the investigators before the start of the study. At the time of collecting blood sample for genetic analysis all centres were mandated to submit a detailed data sheet which included patient demographics, presence of consanguinity and a detailed clinical history of the proband. Clinical data sheet was shared with the genetic lab but detailed analyses of the clinical data and classification of the subjects into different phenotypes was made at end of recruitment but prior availability of genetic results.

Consent was taken from the parents/ guardians as well as from the children (aged  $\geq 13$  years) after pre-test counseling by the site Primary Investigator (PI). Post-test genetic counseling was offered to all families.

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4 Ethical approval was obtained from the Institute of Child Health Ethics Committee.  
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#### 6 *Whole Exome sequencing and data analysis*

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8 Sample collection, DNA extraction, WES, tools for annotation and the list of genes included in the initial  
9 tubulopathy panel are described in the supplemental appendix.  
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11 After a detailed literature search and considering the relative frequency of different tubular disorders in different  
12 populations we generated a pre-designed virtual panel of 38 tubulopathy genes (Supplemental Table I in  
13 supplemental appendix). During initial data analysis, putative variants in only these 38 tubulopathy genes were  
14 analyzed. In the absence of detection of any causative variant(s), the search was expanded to any other relevant  
15 gene, considering the human ontology terms that were consistent with the clinical phenotype.  
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20 It was agreed that in case 100% coverage was not achieved and there was a high clinical likelihood of a particular  
21 gene with incomplete coverage being causative, gaps would be sequenced by Sanger sequencing. Multiplex ligation-  
22 dependent probe amplification (MLPA) was used for confirmation of any suspected copy number variation (CNV).  
23 When indicated, Sanger sequencing for variants detected in the proband was also done in parents for segregation  
24 analysis.  
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28 Classification of the variants as pathogenic (P) or likely pathogenic (LP) was done based on 2015 American College  
29 of Medical Genetics and Genomics (ACMG) guidelines [22]. For calculation of the diagnostic yield, pathogenic (P)  
30 or likely pathogenic (LP) variants in line with the mode of inheritance were considered significant.  
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#### 33 *Statistical Analysis*

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35 Continuous variables were expressed as median with Inter-quartile Range (IQR) and dichotomous variable as  
36 percentages.  
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## 41 **Results**

### 42 *Patients*

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44 During the study period, 77 index cases (73% male, n=56) with clinical phenotypes corresponding to various tubular  
45 disorders underwent WES (Table I & II). The median age of the cohort at clinical presentation was 24 (IQR 8.5 to  
46 50) months and at genetic testing was 48 (18.5 to 108) months.  
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### 50 *Coverage in WES*

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52 Most of the tubulopathy genes had 100% coverage, except for *SLC34A3* (96.56%), *WNK4* (99.16%) and *OCRL*  
53 (99.98%).  
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### 56 *Distal RTA (dRTA) phenotype*

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58 Twenty-five children with a clinical phenotype of dRTA (60% male) were assessed and a definitive genetic  
59 diagnosis was established in 16 (64%). In total, 20 different variants were seen, of which 14 were P/LP. Seven of  
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4 these were novel (Table I). Median age at clinical presentation and at testing were 24 (IQR: 4.8 to 36) months and  
5 50 (IQR: 35 to 107.5) months, respectively (Table II). P/LP variants were commonly detected in *SLC4A1* (n=6) and  
6 *ATP6V0A4* (n=5), followed by *ATP6V1B1* (n=4) and *WDR72* (n=1). Sensorineural hearing loss (SNHL) was  
7 observed in 7 children; *ATP6V0A4* (n=3), *ATP6V1B1* (n=2) and no P/LP variant identified (n=2) (Table II).  
8 Hemolytic anemia was found among 2 children with variants in *SLC4A1* (Table II).  
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11 Although genotyping did not result in any change of the clinical diagnosis; reverse phenotyping did lead to the  
12 diagnosis of previously missed clinical co-morbidities in 7 children (SNHL=4, hemolytic anemia =2 and dental  
13 anomalies =1) (Table II and Table III).  
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#### 15 *Bartter Syndrome (BS) phenotype*

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17 BS phenotype was the second most common clinical diagnosis (n=18, 89% male) with a median age at presentation  
18 of 18 (IQR 7 to 44) months and at testing of 25 (IQR 11 to 60) months (Table II). The diagnostic yield was 78%  
19 (n=14, Table I). The total numbers of variants detected were 21 of which 18 were P/LP and of these, 9 were novel.  
20 Interestingly, only 8 children with BS phenotype were found to harbor causative variants in BS disease genes,  
21 namely *KCNJ1* (n=5), *SLC12A1* (n=1), and *CLCNKB* (N=2). Six new diagnoses were made; one child was found to  
22 have Gitelman syndrome and 5 children had a primary non-tubular diagnosis: cystic fibrosis (CF) = 4 and congenital  
23 secretory chloride diarrhea (CCD) = 1 (Table II).  
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#### 30 *Isolated Hypophosphatemic rickets phenotype (HP)*

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32 6 children with HP phenotype (50% male) with median age at clinical presentation of 27 (20 to 30) months  
33 underwent NGS at 47 (IQR 26 to 132) months of age (Table II). Genetic diagnosis was established in 5 (83%)  
34 children (Table 1). All were X-Linked HP, 4 P/LP variants were detected in *PHEX* gene, one was novel with a large  
35 intra-genic deletion involving Exon 16 to 20 (Table I and Table II). The deletion was confirmed by MLPA.  
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#### 39 *Proximal tubular dysfunction (pTD) phenotype*

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41 12 children (75% male) with a clinical diagnosis of *pTD* were investigated with a median age at diagnosis and  
42 testing of 21 (IQR: 12 to 41) months and 72 (IQR: 18.5 to 127.5) months, respectively (Table II). A genetic  
43 diagnosis was established in 9 (75%, Table I). The total numbers of variants found were 11 of which 10 were P/LP  
44 and of these, 2 were novel (Table I). The most common genetic diagnosis was cystinosis (n=4) and for three of them  
45 this was previously clinically not suspected. Apart from this we had 2 diagnoses each of tyrosinemia and Fanconi-  
46 Bickel syndrome and one of Lowe syndrome.  
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#### 50 *Nephrogenic Diabetes Insipidus (NDI)*

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52 Six children (83% male) with a phenotype of NDI with a median age at clinical presentation of 34 (17 to 71) months  
53 underwent genetic testing at 192 (IQR 24 to 144) months (Table II). A genetic diagnosis was established in all of  
54 them (100%, Table I). Six variants (all P/LP) were reported, of which 3 were novel. X-linked recessive NDI was the  
55 most common genetic diagnosis (N=4). Two children were found to have secondary inherited NDI (Table II and  
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4 Table III) with the new primary diagnoses identified as nephronophthisis (NDI5) and dRTA (NDI6). Details of the  
5 child with dRTA (NDI6) have been published previously[23]. Of note, the variant identified in NDI6 was identical  
6 to the variant in dRTA8.  
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#### 8 9 *Nephrocalcinosis / Stones*

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11 6 children (all male) with a clinical phenotype of nephrocalcinosis / kidney stones (NC) were investigated. The  
12 median age at presentation was 81 (IQR: 12 to 156) months and at time of testing 96 (IQR: 17 to 168) months  
13 (Table II). NGS revealed four variants of which three were P/LP and a genetic diagnosis could be established in two  
14 children (33%) (Table I). NC1 was found to have a novel homozygous variant in *HOGA1* establishing a genetic  
15 diagnosis of Primary Hyperoxaluria type 3 (Table II). Genotyping led to a new diagnosis for NC2. Despite  
16 presenting with only nephrocalcinosis and hypercalciuria, NC2 was found to have a likely pathogenic homozygous  
17 variant identified in *KCNJ1* (Exon 2: c.146G>A), establishing a genetic diagnosis of Bartter syndrome type 2. The  
18 variant identified in NC2 was identical to that in BS4. Although a novel LP variant in *SLC12A1* was identified in  
19 NC4 this was only present in heterozygous form and hence a genetic diagnosis could not be confirmed.  
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#### 25 26 *Other disorders with kidney tubulopathy*

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28 4 children (75% male) underwent NGS for suspected tubulopathies whose phenotypes did not match any of the  
29 above categories (Table II). Median age at presentation and clinical diagnosis were 48.5 (IQR: 1 to 121) months and  
30 57 (IQR: 1.5 to 130.5) months respectively. Relevant variants were detected in 3 children and genetic diagnosis was  
31 thereafter established in two children (50%). In none of them the genetic diagnosis established was previously  
32 suspected based on reported clinical phenotype (Table I). Others1 had a causative variant in *GATA3*, establishing a  
33 diagnosis of Barakat syndrome and Others2 had a causative variant identified in *HNF1B*, establishing a diagnosis of  
34 Renal Cyst and Diabetes (RCAD) syndrome (Table II). Others3 also had a phenotype consistent with RCAD, but  
35 there was insufficient evidence to establish the identified variant in *HNF1B* as causative in accordance with ACMG  
36 criteria.  
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#### 41 42 *Overall diagnostic yield and revision of clinical diagnosis*

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44 In summary among 77 children with clinically suspected tubulopathy, WES established a genetic diagnosis in 54  
45 (70%). As detailed in Table II and summarized in Table III; WES resulted in a revision of the clinical diagnosis in  
46 14 children (26% of those with a genetic diagnosis and 18% of the overall cohort) and had direct impact on clinical  
47 management in 24 children (44% of those with a genetic diagnosis and 31% of the overall cohort).  
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#### 50 51 **Discussion**

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53 Analyzing our data from this large cohort of children with tubulopathies from India, we confirm WES to be a useful  
54 diagnostic tool with an overall diagnostic yield of 70%. Of particular relevance is the fact that it changed the clinical  
55 diagnosis in 18%, highlighting the diagnostic challenges in a low-resource environment. Notably, for some of these  
56 newly established diagnoses, such as cystinosis and tyrosinemia specific treatment is available and could be offered  
57 post WES results. Genotyping also helped reveal other associated pathologies, such as SNHL, hemolytic anemia and  
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4 dental anomalies facilitating appropriate treatment. Thus, in addition to the usual benefits of a genetic diagnosis,  
5 such as allowing genetic counseling and cascade screening of relatives at risk, WES directly impacted on the clinical  
6 management in 31% of our cohort (Table III), further emphasizing its potential benefits. .  
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9 Although few in numbers, studies on use of NGS for clinically suspected tubulopathy has usually shown an  
10 impressive genetic yield. For example, among a large, mostly European cohort of children (n=384) Ashton et al  
11 confirmed an underlying genetic diagnosis in 64% (n=245) [11]. This striking yield is also confirmed in our study  
12 (70%) and provides strong support for the utility of WES, even in the Indian population. There are some notable  
13 differences with regards to the clinical diagnoses in our cohort compared to that from Ashton *et al* [11]. Ours  
14 included a larger proportion of patients with a clinical diagnosis of dRTA and only one child with Gitelman  
15 syndrome, who, in fact, had a clinical diagnosis of BS. Indeed, our tubulopathy cohort was quite similar to a  
16 previous Indian publication that included 67 children with clinically proven tubulopathy. Among that cohort dRTA  
17 (44%), BS (22%) and pTD (12%) were the most common diagnoses, with only 2 children having a clinical diagnosis  
18 of Gitelman syndrome and none PHA (24).  
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25 Distal RTA was the most common phenotype in our cohort and our diagnostic yield of WES (64%) is in  
26 concordance with the data presented by Ashton *et al.*(58%) and Palazzo *et al.* (72%) [11, 14]. Worldwide, *SLC4A1*,  
27 *ATP6V0A4* and *ATP6V1B1* are the most commonly reported underlying genes, explaining up to 80% of cases of  
28 primary dRTA [24, 25]. In contrast to both Ashton *et al.* and Palazzo *et al.*, *SLC4A1* variants were more common in  
29 our cohort [11, 14]. This likely reflects population-specific differences, as 5 of the 6 children with *SLC4A1*-  
30 associated dRTA in our cohort were homozygous for the p.(Ala858Asp) variant, which is more frequently found in  
31 tropical Asia and associated with protection from malaria [26]. We also identified a child with dRTA secondary to a  
32 homozygous variant in *WDR72* (dRTA17), which is a relatively newly recognized disease gene [27] that had not  
33 been included by either Ashton *et al.* or Palazzo *et al.*[11, 14]. Of note, genotyping led us to identify SNHL in four  
34 of our children with dRTA and hemolytic anemia in two.  
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41 BS was our next most common clinical diagnosis (n=18) and in contrast to previous reports from Europe, where  
42 *CLCNKB* was the most common disease gene [11, 13], we found a higher incidence of *KCNJI* variants in our small  
43 cohort. Interestingly, genotyping of our BS phenotype revealed a significant number of children with cystic fibrosis  
44 (CF) and congenital secretory chloride diarrhea (CCD). Such findings have been reported previously and termed as  
45 “Pseudo Bartter syndrome” [28–30] highlights the importance of assessing urinary electrolytes, which is often not  
46 done in facilities with resource constraints.  
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50 Genotyping our cohort of children with pTD phenotype resulted in a diagnostic yield of 75%. Cystinosis was the  
51 most common diagnosis. Similar to a recently published Indian cohort and in contrast to Western cohorts, the  
52 p.(Thr7PhefsTer7) protein change was most commonly identified [31]. Even though cystinosis is the most common  
53 cause of renal Fanconi syndrome, we identified three new cases in which it had not been clinically confirmed. This  
54 again highlights the diagnostic challenges in emerging economies, where the assay for leukocyte cysteine content is  
55 not easily available and clinical expertise for suspecting cystinosis or for detection of corneal cysteine crystals may  
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4 vary across regions. With easier availability of genetic tests, NGS may be a cheaper and more practical mode of  
5 diagnosis [31].  
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8 Both of our cohorts of HP (X-linked HP) and NDI had a high diagnostic yield (greater than 80%) and as expected,  
9 variants in *PHEX* and *AVPR2*, respectively, were most common. Interestingly, genotyping of our NDI cohort  
10 revealed a couple of new primary diagnoses that had not been considered clinically. Although rare, secondary  
11 inherited NDI associated with nephronophthisis or dRTA has been described before and can be difficult to  
12 distinguish just based on clinical phenotype [32–34].  
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16 We had a very small cohort of children with the clinical phenotype of stone / nephrocalcinosis (n=6), among whom  
17 two children had a causative variant identified (NGS yield = 33%). Whereas NC1 was confirmed to have  
18 nephrocalcinosis secondary to Primary Hyperoxaluria type 3, NC2 was found to have a diagnosis of BS.  
19 Interestingly NC2 presented with only nephrocalcinosis and hypercalciuria and the diagnosis was purely based on  
20 genetic testing. The LP homozygous variant identified in NC2 (c.146G>A) had been previously reported in a similar  
21 case report of isolated nephrocalcinosis and hypercalciuria from India albeit in a compound heterozygous form  
22 (c.146G>A and c.657C>G) [35]. Although our numbers were small, larger international cohorts of paediatric stone  
23 disease / nephrocalcinosis have also shown similar diagnostic yield from genetic testing, ranging between 7 to 30 %  
24 [36, 37].  
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31 Our cohort also included a group of children with suspected tubular disorders who could not be classified into any of  
32 the classical phenotypes. These children primarily had persistent unexplained electrolyte problems and hence were  
33 included in the study as per pre decided criteria. *HNF1B* merits special mention in this group as the heterogeneity of  
34 the associated phenotype is well recognized and includes features of tubulopathy [38, 39]. Similar to Ashton *et*  
35 *al.*[11], we also included *HNF1B* in our tubulopathy panel and identified variant in two of our children of which one  
36 was significant (Table II).  
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41 DRTA19 was found to carry a heterozygous variant in *ATP6V1B1*: c.1181G>T, p.(Arg394Gln). This has been  
42 reported repeatedly in heterozygous form with a distal RTA phenotype [12, 40]. If tested, the variant is usually *de*  
43 *novo*, as it was in our study, consistent with the absence of a family history. While we classified the variant as LP we  
44 did not include it in our diagnostic yield, as no causative variant was identified on the other allele. Yet, by  
45 identifying an additional subject with dRTA associated with this heterozygous variant clearly raises questions about  
46 a potential dominant disease mechanism and therefore this variant is a strong candidate for future comprehensive  
47 study [3]. Another potentially interesting variant was found in patient dRTA22; a heterozygous variant in *RHCG*  
48 (c.837G>A p.(Met279Ile). Although the gene is not a recognized kidney tubulopathy gene, it has been associated  
49 with reduced kidney ammonium secretion and worsening of metabolic acidosis in acid-challenged mice [41]. We  
50 could not assess whether the variant was inherited from one of the parents and if it segregated with a dRTA  
51 phenotype.  
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58 Our study has limitations. Some of the known tubular disorders were surprisingly missing from our cohort (like  
59 PHA) or were reported in unexpectedly low numbers (like Gitelman syndrome, stone/nephrocalcinosis). Although  
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4 this might represent an altered prevalence of these disorders in our population, some bias in recruitment could also  
5 be responsible. Though clinicians from all regions of Eastern India contributed, most of the patients were from two  
6 large cities (Kolkata and Bhubaneswar). This may indicate a lack of awareness or interest regarding tubulopathies  
7 among pediatricians outside the bigger cities. We did carry segregation analyses on majority of the parents as and  
8 when indicated; but were unable to do it in a few. For example, parents of dRTA22 refused further testing even  
9 though it was deemed necessary. Additionally, due to unavoidable limitations involving time and funding we were  
10 unable to undertake further functional studies of our suspected novel variants. Despite these limitations our results  
11 strongly support our hypothesis that NGS is a useful diagnostic tool for clinically suspected tubulopathies in the  
12 Indian population. Indeed, post WES, new clinical diagnoses were established in 18% of our cohort, compared to  
13 6.5% reported by Ashton *et al.*[11]. Notably, among the European cohort, most cases of discrepancy between  
14 clinical and genetic diagnosis concerned BS type 3 and Gitelman Syndrome, which can be clinically  
15 indistinguishable and the precise diagnosis has little impact on the direct clinical management. In contrast, in our  
16 cohort, NGS results had a direct and substantial impact on clinical management in 24 (31 %) children; including  
17 those where genetic confirmation enabled us to offer specific treatment as well as helped in identifying important  
18 associated co-morbidities (Table III).

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27 In conclusion, we demonstrate a high diagnostic yield of NGS among Indian children with clinically suspected  
28 tubulopathy and highlight its utility in establishing correct diagnoses. Most of the tubulopathies are autosomal  
29 recessive and as consanguineous marriages and endogamy leading to hidden consanguinity are not uncommon in our  
30 region extended family screening, genetic counseling and prenatal testing in at-risk population are likely to be  
31 useful.  
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8 **Legends to tables**  
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10 **Table I: Clinical phenotypes, involved genes and yield of Next Generation Sequencing**  
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12 Initial clinical phenotypes with respective number of patients, the phenotype definition, and the number of genetic  
13 diagnoses with details of the identified causative variants.  
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15 N.B. (\*) “Others” included suspected tubulopathies with a phenotypic pattern not matching the other categories  
16 (phenotypic details of these children are provided in Table III). (\*\*)The dRTA variant identified in the child NDI 6  
17 (Table II) was similar to the variant reported in the child dRTA 8 (Table II); hence the total number of P/LP variant  
18 is 55.  
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21 Ddavn: desmopresin, dRTA: distal renal tubular acidosis, FTT failure to thrive, Mx: management, NAGMA normal  
22 anion gap metabolic acidosis, OMIM: Online Mendelian Inheritance in Man, P/LP: pathogenic / likely pathogenic.  
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26 **Table II: Genotype pattern among distal Renal Tubular Acidosis (dRTA) phenotype, Bartter Syndrome**  
27 **phenotype, hypophosphatemic rickets phenotype, proximal renal tubular acidosis (pRTA) phenotype,**  
28 **Nephrogenic Diabetes Insipidus (NDI) phenotype Nephrocalcinosis / stone phenotype and “Others”**  
29 **phenotype i.e. those whose phenotypic pattern could not be classified in any of the set patterns**  
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32 (+ve): positive, (-ve): negative, BS: Bartter Syndrome phenotype, CNV: copy number variation, dRTA: distal renal  
33 tubular acidosis phenotype, F: female, HP: Hypophosphatemic rickets phenotype, M: male. MLPA-Multiplex  
34 ligation-dependent probe amplification, NC: nephrocalcinosis / stone phenotype, NA: not available, NDI:  
35 nephrogenic diabetes insipidus phenotype, pRTA - proximal renal tubular acidosis phenotype, SNHL: sensorineural  
36 hearing loss, WES: whole exome sequencing  
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39 **Table III: Impact of Whole Exome Sequencing results on clinical management**  
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41 Cases grouped according to initially suspected phenotype and the impact of WES on management, such as revision  
42 of diagnosis or change in treatment  
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44 CCD: Chloride Secretory Chloride Diarrhea, CKD: Chronic kidney disease, CTNS: cystinosis, CF: Cystic fibrosis,  
45 ESKD: End Stage Kidney Disease, Mx: management, PH3 Primary Hyperoxaluria 3, RCAD: Renal Cyst and  
46 Diabetes syndrome, SNHL: sensorineural hearing loss, WES: whole exome sequencing  
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50 **Supplemental Appendix:**  
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52 a) Supplemental methods  
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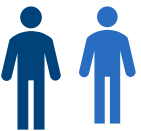
54 b) Supplemental Table I with list of genes included in the initial tubulopathy panel  
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# Whole Exome Sequencing and variant spectrum in children with suspected inherited renal tubular disorder: *East India Tubulopathy Gene Study*



**HYPOTHESIS: Whole exome sequencing is useful for management of children with tubulopathy in India**

Children with clinically suspected tubulopathy underwent Whole Exome Sequencing (WES)



Suspected phenotype (Total 77)	Clinical implications of genetic testing	
	Revision of diagnosis: 14 (18%)	Impact on clinical Management: 24 (31%)
Distal Renal Tubular Acidosis (dRTA) n=25	Revision of diagnosis: None	Identification of co-morbidities: SNHL: 4, Hemolytic anemia: 2 and Dental problems: 1 (Total 7)
Bartter Syndrome n=18	Non tubulopathy: 5 Diagnosed to another type of tubulopathy: 1	Identification of Congenital Chloride Diarrhea and Cystic Fibrosis facilitated specific treatment : 5
Hypophosphatemic rickets n=6	None	None
Proximal Tubular Dysfunction (pTD) n=12	Total 3	Facilitated specific treatment : cystinosis: 4 and tyrosinemia: 2
Nephrogenic Diabetes Insipidus (NDI) n=6	Non tubulopathy diagnosis: 1 Another type of tubulopathy: 1	Identification of dRTA allowed treatment with alkali: 1 Diagnosis of nephronophthisis changed the management plan: 1
Isolated kidney stone or nephrocalcinosis n=6	Diagnosed as Bartter syndrome: 1	Diagnosis of Bartter syndrome helped in appropriate management planning: 1 Identification of HOGA variant helped in prognostication: 1
Others n=4	Non-tubulopathy diagnosis: 1 Diagnosed to another type of tubulopathy: 1	Identifying HNF1B phenotype helped in prognostication: 1 Audiological assessment conducted post availability of genetic result diagnosed SNHL: 1

## Summary

Total number of children: 77

Total number of variants: 68

Total number of Pathogenic or Likely pathogenic variants: 55

Novel Pathogenic / Likely pathogenic variants: 24

Positive yield of WES: 70% (n=54)

**CONCLUSION: WES had a definite impact on clinical management in nearly one third (31%) children with clinically suspected tubulopathy from Eastern India**

Sinha et al. 2021



**Pediatric Nephrology**

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International Pediatric Nephrology Association

**Table I: Clinical phenotypes, involved genes and yield of Next Generation Sequencing**

Suspected phenotype Total (n=77)	Phenotype description	Confirmed Genetic diagnoses (n=54; 70 %)	Variants detected: Total (n= 68) / P or LP (n=55) / Novel among P/LP (n=24)	Identified causative genes with # OMIM ID and respective number of patients with variants
Distal Renal Tubular Acidosis (dRTA) n=25	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	16 (64 %)	20/14/7	a. <i>ATP6V0A4</i> # 602722 DISTAL RENAL TUBULAR ACIDOSIS 3: n=5 b. <i>ATP6V1B1</i> # 267300; DISTAL RENAL TUBULAR ACIDOSIS 2: n=4 c. <i>SLC4A1</i> # 179800 DISTAL RENAL TUBULAR ACIDOSIS 1: n=6 d. <i>WDR72</i> # 613211 AMELOGENESIS IMPERFECTA Type II A 3: n =1
Barter syndrome (n=18)	Polyuria/ Polydipsia/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	14 (78%)	21/18/9	a. <i>CLCNKB</i> # 607364 BARTTER SYNDROME TYPE 3: n=2 b. <i>KCNJ1</i> # 241200 BARTTER SYNDROME TYPE 2: n =5 c. <i>SLC12A1</i> # 601678 BARTTER SYNDROME TYPE 1:n=1 d. <i>SLC12A3</i> # 263800 GITELMAN SYNDROME: n=1 e. <i>SLC26A3</i> # 214700 CONGENITAL SECRETORY CHLORIDE DIARRHEA: n=1 f. <i>CFTR</i> # 602421 CYSTIC FIBROSIS: n= 4
Isolated Hypophosphatemic rickets (n=6)	Rickets with low serum phosphate, posphaturia but no glycosuria or proteinuria	5 (83%)	4/4/1	<i>PHEX</i> # 307800 X LINKED DOMINANT HYPOPHOSPHATEMIC RICKETS: n =5 Hemizygous male = 3; Heterozygous female= 2
Proximal Tubular Dysfunction (pTD) (n=12)	Rickets/ Poyuria/ Polydipsia/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria/ proteinuria	9 (75%)	11/10/2	a. <i>CTNS</i> # 219800 NEPHROPATHIC CYSTINOSIS: n=4 b. <i>FAH</i> # 276700 TYROSINEMIA TYPE 1: n=2 c. <i>OCRL</i> # 309000 LOWE OCULO CEREBRO RENAL SYNDROME: n =1 d. <i>SLC2A2</i> # 227810 FANCONI BICKEL SYNDROME: n=2

Gitelman (n=0)	Polyuria/ Polydipsia/ Metabolic alkalosis/ Hypokalemia/ Hypomagnesemia	-----	-----	
Nephrogenic Diabetes Insipidus (N=6)	Hypernatremia, polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	6 (100 %)	6/6 <sup>(**)</sup> /3	a. <i>AVPR2</i> # 304800 DIABETES INSIPIDUS, NEPHROGENIC, X-LINKED: n=4 b. <i>NPHP4</i> # 606966; NEPHRONOPHTHISIS 4: n =1 c. <i>ATP6V1B1</i> # 267300; DISTAL RENAL TUBULAR ACIDOSIS TYPE 2: n =1*
Isolated kidney stone or nephro- calcinosis (n=6)	Nephrocalcinosis or kidney stone in absence of Bartter or distal Renal Tubular Acidosis phenotype	2 (33%)	4/3 <sup>(***)</sup> /2	a. <i>HOGA1(+)</i> # 613616; PRIMARY HYPEROXALURIA TYPE 3: n=1 b. <i>KCNJ1</i> # 241200 BARTTER SYNDROME TYPE 2: n =1
Others <sup>(*)</sup> (n=4)	Not falling in any of the above criteria but with unexplainable electrolyte, acid / base disorders or polyuria Individual phenotypes detailed in Table	2 (50%)	3/2/0	a. <i>GATA3</i> # 146255 BARAKAT SYNDROME: n=1 b. <i>HNF1B</i> # 137920 <i>RENAL CYST AND DIABETES SYNDROME</i> : n =1

N.B. (\*) “Others” included suspected tubulopathies with a phenotypic pattern not matching the other categories (phenotypic details of these children are provided in Table III). (\*\*)The dRTA variant identified in the child NDI 6 (Table II) was similar to the variant reported in the child dRTA 8 (Table II); hence the total number of P/LP variant is 55.

Ddvp: desmopresin, dRTA: distal renal tubular acidosis, FTT failure to thrive, Mx: management, NAGMA normal anion gap metabolic acidosis, OMIM: Online Mendelian Inheritance in Man, P/LP: pathogenic / likely pathogenic

Table II: Genotype pattern among distal Renal Tubular Acidosis (dRTA) phenotype, Bartter Syndrome phenotype, hypophosphatemic rickets phenotype, proximal tubular dysfunction (pTD) phenotype, Nephrogenic Diabetes Insipidus (NDI) phenotype Nephrocalcinosis / stone phenotype and “Others” phenotype i.e. those whose phenotypic pattern could not be classified in any of the set patterns

Identifier Sex / Age at presentation / Age at genetic test in months	Clinical indication for inclusion criteria in the group	Gene in which causative variant(s) were detected / genetic diagnosis / inheritance pattern	Type of mutation	Variant description and ENST ID	Pathogenic/ Likely Pathogenic/ Variant of Unknown significance (VUS)	Novel or reported	Evidence of pathogenicity as per ACMG criteria	
dRTA1 F / 8 / 12	Polyuria/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V0A4/ dRTA Type 3 with or without SNHL / Autosomal recessive	Compound	Exon 3: c.106C>T p.(Gln36Ter) Exon 6: c.355C>T p.(Gln119Ter) ENST00000310018.2	Pathogenic  Pathogenic	Novel  Novel	PVS1, PM2, PM3  PVS1, PM2, PM3	Genetic confirmation:(+ve) Genotype / Phenotype: (+ve) SNHL: (-ve)
dRTA2 M / 4.5 / 108	NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V0A4/ dRTA Type 3 with or without SNHL / Autosomal recessive	Homozygous	Exon 13: c.1185delA p.(Tyr396ThrfsTer12)  ENST00000310018.2	Pathogenic	Reported	PVS1, PS4_M,PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) SNHL: (-ve)
dRTA3 M / 34 / 46	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V0A4 / dRTA Type 3 with or without SNHL / Autosomal recessive	Homozygous	Exon 15: c.1571C>T p.(Pro524Leu) ENST00000310018.2	Likely Pathogenic	Reported	PM2, PP3, PP4, PS4_M, PM3_supp	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) SNHL: (+ve)
dRTA4 F / 4 / 4	Polyuria/ NAGMA/ FTT/ Hypokalemia	ATP6V0A4 / dRTA Type 3 with or without SNHL / Autosomal recessive	Homozygous	Exon 15: c.1571C>T p.(Pro524Leu) ENST00000310018.2	Likely Pathogenic	Reported	PM2, PP3, PP4, PS4_M, PM3_supp	Genetic confirmation:(+ve) Genotype / Phenotype:(+ve) SNHL: (+ve); detected on reverse phenotyping
dRTA5 M / 3 / 72	Polyuria/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V0A4/ dRTA Type 3 with or without SNHL / Autosomal recessive	Homozygous	Exon 18: c.1955C>G p.(Pro652Arg) ENST00000310018	VUS	Novel	PM2, PP3, PP4	Genetic confirmation: (- ve) Genotype / Phenotype: (- ve) SNHL: (+ve)
dRTA6 M /26 / 36	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V0A4 / dRTA Type 3 with or without SNHL / Autosomal recessive	Homozygous	Exon 8: c.596T>A p.(Leu199Ter) ENST00000310018	Likely Pathogenic	Novel	PVS1, PM2, PM3	Genetic confirmation +ve Genotype / Phenotype: +ve SNHL –ve



dRTA7 M / 22/48	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V0A4 / dRTA Type 3 with or without SNHL / Autosomal recessive	Compound	Intron 16: c.1691+1G>A (5' splice site) Exon 12: c.1170G>A (p.Glu390(=))  ENST00000310018	Pathogenic  VUS	Reported Novel	PVS1, PM2, PS4_S  PM2, PM3	Genetic confirmation: (- ve) Genotype / Phenotype: (- ve) SNHL -ve N.B. The synonymous second variant is just proximal to the splice site. However, splicing prediction tools did not show deleterious splicing effect.
dRTA8 M/19/161	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V1B1 / dRTA Type 2 with progressive SNHL/ Autosomal recessive	Homozygous	Exon 1: c.91C>T p.(Arg31Ter) ENST00000234396	Pathogenic	Reported	PVS1, PS4, PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype:(+ve) SNHL: (-ve)
dRTA9 M / 9 / 36	Polyuria/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V1B1 / dRTA Type 2 with progressive SNHL/ Autosomal recessive	Compound	Intron 3: c.273+2T>G (5' splice site)  Exon 7: c.611C>A p.(Ala204Glu) ENST00000234396	Pathogenic  Likely Pathogenic	Novel  Novel	PVS1, PM2, PM3, PP4  PM2, PM3_M, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) SNHL: (+ve); detected on reverse phenotyping
dRTA10 F / 60 / 80	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V1B1 / dRTA Type 2 with progressive SNHL/ Autosomal recessive	Homozygous	Exon 5: c.403A>T p.(Lys135Ter) ENST00000234396.4	Pathogenic	Novel	PVS1, PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) SNHL: (+ve); detected on reverse phenotyping
dRTA11 M/ 52 / 107	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	SLC4A1 / dRTA Type 4 with hemolytic anemia / Autosomal recessive	Homozygous	Exon 19: c.2573C>A p.(Ala858Asp) ENST00000262418.6	Likely Pathogenic	Reported	PM2, PP3, PP4. PS3, PP4	Genetic confirmation:(+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series Haemolytic Anemia: (+ve); detected on reverse phenotyping
dRTA12 F/ 28/ 36	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia	SLC4A1 / dRTA Type 4 with hemolytic anemia/ Autosomal recessive	Homozygous	Exon 19: c.2573C>A p.(Ala858Asp) ENST00000262418.6	Likely Pathogenic	Reported	PS3_M, PS4_M, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series Hemolytic anemia: (-ve)

dRTA13 M / 36 / 62	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	SLC4A1 / dRTA Type 4 with hemolytic anemia/ Autosomal dominant	Heterozygous	Exon: 14: c.1766G>G/A p.(Arg589His) ENST00000262418	Pathogenic	Reported	PS2_strong, PS3_strong, PS4_moderate, PM3, PM5, PP1_strong, PP3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Haemolytic anaemia: (- ve)
dRTA14 M / 34 / 34	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	SLC4A1 / dRTA Type 4 with hemolytic anemia/ Autosomal recessive	Homozygous	Exon 19: c.2573C>A p.(Ala858Asp) ENST00000262418.6	Pathogenic	Reported	PS3_M, PS4_M, PM3_M, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series Hemolytic anemia: (+ve); detected on reverse phenotyping
dRTA15 F / 2 / 6	Polyuria/ NAGMA/ FTT/ Hypokalemia	SLC4A1 / dRTATyoe 4 with hemolytic anemia/ Autosomal recessive	Homozygous	Exon 19: c.2573C>A p.(Ala858Asp) ENST00000262418.6	Pathogenic	Reported	PS3_M, PS4_M, PM3_M, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series Hemolytic anemia: (-ve)
dRTA16 F/66/72	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	SLC4A1 / dRTA Type 4 with hemolytic anemia/ Autosomal recessive	Homozygous	Exon 19: c.2573C>A p.(Ala858Asp) ENST00000262418.6	Pathogenic	Reported	PS3_M, PS4_M, PM3_M, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series Hemolytic anemia: (-ve)
dRTA17 M / 52 / 108	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia	WRD 72/ Hypomaturation/ Amelogenesis Imperfecta/ dRTA Autosomal Recessive	Homozygous	Exon 13: c.1715T>A p.(Leu572Ter) ENST00000396328.1	Pathogenic	Novel	PVS1, PM2, PM3_supp	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Characteristic dental changes detected on reverse phenotype
dRTA18 M / 4 / 8	NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V1B1/ dRTA Type 2 with progressive SNHL/ AR	Homozygous	Exon 7: c.611C>A p.(Ala204Glu)  ENST00000234396.10	Likely Pathogenic	Novel	PM2, PM3_M, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) SNHL: (+ve); detected on reverse phenotyping

dKTA19 F / 18 / 156	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia/ Nephrocalcinosis	ATP6V1B1 / dRTA with progressive SNHL/ Autosomal recessive	Heterozygous	Exon 12: c.1181G>A p.(Arg394Gln)  ENST00000234396	Likely pathogenic	Reported	PM2, PP4, PP3, PM6_M, PS4_M	Genetic confirmation: (- ve) Genotype / Phenotype: (- ve) N.B. Second mutation was not detected. This variant has been found in heterozygous condition in affected individuals (unpublished data)
dRTA20 F / 36 / 78	Polyuria/ Polydipsia/ NAGMA	NPHP3 / Nephronophthisis type 3/ Autosomal recessive	Compound	Exon 2: c.449C>C/T p.(Ala150Val) Exon 13: c.1975C>C/T p.(Pro659Ser) ENST00000337331	VUS  VUS	Novel  Reported	BS1, BS2, BP6  BP4, PM2	Genetic confirmation: (- ve) Genotype / Phenotype: (- ve) SNHL +ve N.B. Despite classical phenotype of dRTA no causative variants in dKTA disease genes were found and the NPHP3 variants were classified as VUS.
dRTA21 M / 36 / 52	Polyuria/ Polydipsia/ NAGMA/ FTT/ Hypokalemia	NPHP4 / Nephronophthisis 4/ Autosomal recessive	Compound	Exon 2: c.122C>T p.(Pro41Leu) Exon 22: c.3145C>T p.(Pro1049Ser) ENST00000378156.4	VUS  VUS	Reported Reported	BP4  PP3	Genetic confirmation: (- ve) Genotype / Phenotype:(- ve) N.B. Despite classical phenotype of dRTA no causative variants in dKTA disease genes were found and the NPHP4 variants were classified as VUS.
BS1 F/ 58/ 108	Polyuria/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	CLCNKB / Barter 3/ Autosomal recessive	Homozygous	Exon 9: c.849_851delTCT p.(Phe285del)  ENST00000375679.4	Likely Pathogenic	    Novel	PM2,PM4, PP5, PS4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)

BS2 M / 3 / 2	Polyuria/ Metabolic Alkalosis/ FTT/ Hypokalemia	CLCNKB / Barter 3/ Autosomal recessive  CASR / Hypocalcemia 1 with Barter/ Autosomal dominant	Homozygous  Heterozygous	del 19 (Exons 1-19 del)  ENST00000375679  Exon 4: c.970G>A p.(Ala324Thr)  ENST00000498619	Pathogenic  VUS	Novel  Novel	PVS1, PM2  PM1, PM2, PP3, BP1	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) The large homozygous deletion was initially detected by CNV analysis of WES data and later on confirmed by MLPA N.B. The heterozygous variant in CasR was re- confirmed by Sanger due to presence of pseudogene.
BS3 F / NA / 72	Polyuria/ Polydipsia/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	KCNJ1 / Antenatal Bartter Syndrome Type 2/ Autosomal recessive	Homozygous	Exon 2: c.212C>T p.(Thr 71Met) ENST00000392664	Likely Pathogenic	Reported	PS1, PM2, PM3, PP2, PP3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
BS4 M / 34 / 43	Polyuria/ Polydipsia/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	KCNJ1 / Antenatal Bartter Syndrome Type 2/ Autosomal recessive	Homozygous	Exon 2: c.146G>A p.(Cys49Tyr) ENST00000392664.2	Likely Pathogenic	Reported (same as BS4)	PM2, PM3, PM5, PP2, PP3, PS3, PS4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series
BS5 M / 28 / 46	Polyuria/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	KCNJ1 / Antenatal Bartter Syndrome Type 2/ Autosomal recessive	Homozygous	Exon 2: c.124C>A p.(Leu42Ile) ENST00000392664.2	Likely Pathogenic	Novel	PM2,PP2, PM3, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent mutation in our series
BS6 M / 60 / 114	Polyuria/ Polydipsia/ Metabolic Alkalosis/ FTT/ Hypokalemia	KCNJ1 / Antenatal Bartter Syndrome Type 2/ Autosomal recessive	Homozygous	Exon 2: c.124C>A p.(Leu42Ile) ENST00000392664.2	Likely Pathogenic	Novel (same as BS 6)	PM2, PP2, PM3, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent variant in our series

BS7 M / 52 / 156	Polyuria/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	KCNJ1 / Antenatal Bartter Syndrome Type 2/ Autosomal recessive	Homozygous	Exon 2: c.716delG p.(Gly239GlufsTer14) ENST00000392664.2	Pathogenic	Novel	PVS1, PM2, PM3,PP3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
BS8 M / 19 / 24	Polyuria/ Polydipsia/ Metabolic Alkalosis/ FTT/ Hypokalemia	SLC12A1/ Bartter Syndrome Type 1/ Autosomal recessive	Homozygous	Intron 18: c.2295+1G>C NM_000338	Pathogenic	Novel	PVS1, PM2, PM3, PP3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
BS9 M / NA / 60	Polyuria/ Metabolic Alkalosis/ FTT/ Hypokalemia/	SLC12A3 / Gitelman Syndrome/ Autosomal recessive	Compound	Exon 3: c.472C>T p.(Arg158Trp)  Exon 7: c.911C>T p.(Thr304Met)  ENST00000438926.2	Likely Pathogenic  Likely Pathogenic	Novel  Reported	PM1, PM2, PM3, PM5, PP2, PP3, PP4, PM5, PM2, PM3, PP3, PS4, PM1, PM5	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype:(- ve)
BS10 M / 1 / 5	Metabolic Alkalosis/ FTT/ Hypokalemia	SLC26A3 / Congenital secretory chloride diarrhea/ Autosomal recessive	Homozygous	Exon 10: c.1169G>A p.(Gly390Glu) ENST0000034010.5	Likely Pathogenic	Novel	PM1, PM2, PM3, PP2, PP3, PP4	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) N.B. Clinical features were reviewed post availability of genetic result and was found to match with Congenital Secretory Chloride Diarrhea
BS11 M / 10 / 13	Metabolic Alkalosis/ FTT/ Hypokalemia	CFTR / Cystic Fibrosis/ Autosomal recessive	Compound	Intron 10: c.1393-1G>A  Exon 10: c.1367T>C p.(Val456Ala) ENST00000003084.6	Pathogenic  Pathogenic	Reported  Reported	PVS1, PS4, PM2, PM3,PP4  PS4, PM2, PM3, PP3, PM5	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) N.B. Clinical features were reviewed post availability of genetic result and was found to match with Cystic Fibrosis

BS12 M / 9 / 11	Metabolic Alkalosis/ FTT	CFTR / Cystic Fibrosis/ Autosomal recessive	Compound	Exon 11: c.1521_1523del p.(Phe508del) Exon 19: c.3119T>C p.(Leu1040Pro) ENST0000003084.6	Pathogenic  Likely pathogenic	Reported  Novel	PVS1, PS3, PS4, PM2, PP4  PM1, PM2, PM3, PP2, PP3	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype:(+ ve) N.B. Clinical features were reviewed post availability of genetic result and was found to match with Cystic Fibrosis
BS13 M / 7 / 26	Metabolic Alkalosis/ FTT/ Hypokalemia/	CFTR / Cystic Fibrosis/ Autosomal recessive	Compound	Exon 14: c.2125C>T p.(Arg709Ter) Exon 5: c.563T>C p.(Leu188Pro) ENST0000003084.6	Pathogenic  Likely Pathogenic	Reported  Reported	PVS1, PS4, PP5, PM2, PM3  PM2, PM3, PP3, PP4	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) N.B. Clinical features were reviewed post availability of genetic result and was found to match with Cystic Fibrosis
BS14 M / 6 / 8	Metabolic Alkalosis/ FTT/ Hypokalemia/	CFTR / Cystic Fibrosis/ Autosomal recessive	Compound	Exon 6: c.719T>G p.(Leu240Arg) Exon 22: c.3472C>T p.(Arg1158Ter) ENST0000003084.6	Likely Pathogenic Pathogenic	Novel  Reported	PM1, PM2, PM3, PP3  PVS1, PS4, PM2, PM3	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) N.B. Clinical features were reviewed post availability of genetic result and was found to match with Cystic Fibrosis
BS15 F / 18 / 18	Polyuria/ Metabolic Alkalosis/ FTT/ Hypokalemia/ nephrocalcinosis	SLC12A1/ Bartter Syndrome Type 1/ Autosomal recessive	Compound	Exon 7: c.904C>T p.(Arg302Trp)  Exon 15: c.1898T>A p.(Val.633Asp)	VUS  VUS	Reported in DbSNP  Novel	PM1, PP3, PP4  PM1, PP4, PM2,PP3	Genetic confirmation: (- ve)  Genotype / Phenotype: (- ve)
BS16 M / 44 / 44	Polyuria/ Polydipsia/ Metabolic Alkalosis/ FTT	CLCNKB / Bartter syndrome Type 3/ Autosomal recessive	Heterozygous	Exon 7: c.584G>T p.(Ser195Ile) ENST000003756794	VUS	Novel	PP4	Genetic confirmation: (- ve) Genotype / Phenotype: (- ve)

HP1 M / 30 / 46	Rickets with low serum phosphate, phosphaturia but no glycosuria or proteinuria	PHEX / Hypophosphatemic rickets / X linked dominant	Hemizygous	Exon 21: c.2078G>A p.(Cys693Tyr)  ENST00000379374.4	Likely Pathogenic	Reported	PM1, PM2, PP3, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent mutation in our series
HP2 M / 30 / 96	Rickets with low serum phosphate, phosphaturia but no glycosuria or proteinuria	PHEX / Hypophosphatemic rickets / X linked dominant	Hemizygous	Exon 21: c.2078G>A p.(Cys693Tyr) ENST00000379374.4	Likely Pathogenic	Reported	PM1, PM2, PP3, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Recurrent mutation in our series  N.B. Younger brother underwent genetic study at 20 months and had same mutation.
HP3 M / 18 / 24	Rickets with low serum phosphate, phosphaturia but no glycosuria or proteinuria	PHEX / Hypophosphatemic rickets / X linked dominant	Hemizygous	Intron 15: c.1645+4A>T (5' splice site) ENST00000379374.4	Likely Pathogenic	Reported	PS1_S, PM2, PP3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
HP4 F / 24 / 47	Rickets with low serum phosphate, phosphaturia but no glycosuria or proteinuria	PHEX / Hypophosphatemic rickets / X linked dominant	Hemizygous	Intron 15: c.1645+1G>A (5' Splice variant) ENST00000379374.4	Pathogenic	Reported	PVS1, PM2, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
HP5 F / 30 / 46	Rickets with low serum phosphate, phosphaturia but no glycosuria or proteinuria	PHEX / Hypophosphatemic rickets / X linked dominant	Hemizygous	Exon 16 to 20 c.(1645+1_1646-1)_(2250_?)del (Deletion) ENST00000379374.4	Likely Pathogenic	Novel	PVS1, PM2	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) The deletion was confirmed by MLPA. Mother also had similar variant detected.
pTD1 F / 22 / 87	Rickets/ Polyuria/ Polydipsia/ NAGMA + FTT + Hypokalemia/ Hypophosphatemia/ glycosuria/ proteinuria	CTNS / Nephropathic cystinosis / Autosomal recessive	Compound	Exon 3: c.18_21del p.(Thr7PhefsTer7)  Exon 11: c.944A>G p.(Gln315Arg)  ENST00000381870.3	Pathogenic  Likely Pathogenic	Reported  Reported	PVS1, PS4_M, PM2, PM3  PM2, PM3, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) N.B. Younger brother underwent genetic study at 65 months of age and had same mutation.

pTD2 M / 86 / 87	Rickets/ Polyuria/ Polydipsia/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria/ proteinuria	CTNS/ Nephropathic cystinosis / Autosomal recessive	Homozygous	Exon 7: c.422C>T p.(Ser141Phe) ENST00000381870.3	Likely Pathogenic	Reported	PM2, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
pTD3 M / 34 / 111	Rickets/ Polyuria/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria/ proteinuria	CTNS / Nephropathic cystinosis / Autosomal recessive	Homozygous	Exon 3: c.18_21del p.(Thr7PhefsTer7) ENST00000381870.3	Pathogenic	Reported	PVS1, PS4_M, PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
pTD4 F / 18 / 18	Rickets/ Polyuria/ Polydipsia/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria	CTNS / Nephropathic cystinosis / Autosomal recessive	Homozygous	Exon 3: c.61_61+1delinsCT p.(Glu21LeufsTer39) ENST00000381870.3	Pathogenic	Novel	PVS1, PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
pTD5 M / 20 / 21	Rickets/ Polyuria/ NAGMA + FTT + Hypokalemia/ glycosuria	FAH / Tyrosinemia Type 1/ Autosomal recessive	Homozygous	Exon 10: c.835C>T p.(Gln279Ter) ENST00000407106.1	Pathogenic	Novel	PVS1, PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
pTD6 M / 34 / 72	Polyuria/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria	FAH / Tyrosinemia Type 1/ Autosomal recessive	Compound	Exon12:c.928C>T p.(Gln310Ter )  Exon 3: c.192G>T p.(Gln64His) ENST00000407106.1	Pathogenic  Pathogenic	Novel  Reported	PVS1, PM2, PM3  PM1, PM2, PM3, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
pTD7 M / NA / 156	Rickets/ Polyuria/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria/ proteinuria	OCRL / Dents's disease 2 ; Lowe's Oculocerebrorenal syndrome/ X linked recessive	Hemizygous	Exon 16: c.1621C>T p.(Arg541Ter) ENST00000371113.4	Pathogenic	Reported	PVS1, PS4, PM2	Genetic confirmation: (+ve) Genotype / Phenotype:(+ve)
pTD8 M / 5 / 6	Rickets/ NAGMA + FTT + Hypokalemia/ Hpophosphatemia/ glycosuria/ proteinuria	SLC2A2/ Fanconi Bickel Syndrome / Autosomal recessive	Homozygous	Exon 10: c.1246G>A p.(Gly416Ser) ENST00000314251	Likely Pathogenic	Reported	PM1, PM2, PM3, PP3, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)



pTD9 F / 48 / 204	Rickets/ Polyuria/ NAGMA + FTT + Hypokalemia/ Hypophosphatemia/ glycosuria/ proteinuria	SLC2A2/ Fanconi Bickel Syndrome / Autosomal recessive	Homozygous	Intron 1: c.16-1G>A (3' splice site) ENST00000314251	Pathogenic	Reported	PVS1, PM2, PM3	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
pTD10 M / 122 / 144	NAGMA + FTT + Hypokalemia/ Hypophosphatemia/ glycosuria/ proteinuria	OCRL / Dents's disease 2 ; Lowe's Oculocerebrorenal syndrome / X linked recessive	Hemizygous	Exon 11: c.973_975del p.(Leu325del) ENST00000371113.4	VUS	Reported	PM2, PP4, PM4_sup	Genetic confirmation: (- ve) Genotype / Phenotype:(- ve) Despite the phenotype matching Dent's disease the variant was classified as VUS.
NDI1 M / 5 / 7	Hypnatremia, polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	AVPR2 /Nephrogenic diabetes insipidus / X linked recessive	Hemizygous	Exon 3: c.262G>A p.(Val88Met) ENST00000358927.2	Pathogenic	Reported	PS3, PS4, PM1, PM2, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype:(+ve)
NDI2 M / 17 / 192	Hypnatremia, polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	AVPR2 /Nephrogenic diabetes insipidus / X linked recessive	Hemizygous	Exon 3: c.650C>T p.(Pro217Leu) ENST00000358927.2	Likely Pathogenic	Novel	PM1, PM2, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
NDI3 M / 48 / 84	Hypnatremia, polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	AVPR2 / Nephrogenic diabetes insipidus / X linked recessive	Hemizygous	Exon 3: c.541C>T p.(Arg181Cys) ENST00000358927	Pathogenic	Reported	PM1, PM2, PP4, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)

NDI4 M/71 / 144	Polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	AVPR2 / Nephrogenic diabetes insipidus / X linked recessive	Hemizygous	Exon 3: c.815T>C p.(Met272Thr) ENST00000358927.2	Likely Pathogenic	Novel	PM1, PM2, PP3, PP4	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
NDI5 M / 90 / 96	Hypertremia, polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	NPHP 4/ Nephronophthisis 4 / Autosomal recessive	Homozygous	Exon 2: c.12G>A p.(Trp4Ter) ENST00000378156	Pathogenic	Novel	PVS1, PM2, PP4	New diagnosis Genetic confirmation:(+ve) Genotype / Phenotype: (+ve) Genetic result revealed NDI to be secondary to Nephronophthisis
NDI6 F / 20 / 24	Hypertremia, polyuria, urinary concentration defect (analysed by paired serum and urine osmolality) and failed ddAVP challenge test	ATP6V1B1 / distal RTA Type 2 with progressive SNHL / Autosomal recessive	Homozygous	Exon 1: c.91C>T p.(Arg31Ter) ENST00000234396	Pathogenic	Reported	PVS1, PS4, PM2, PM3, PP5	New diagnosis Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Genetic result revealed NDI to be secondary to dRTA This has been reported previously by one of the authors (Ref: 20).
NC1 M / 6 / 17	Bilateral nephrolithiasis	HOGA1 / Primary Hyperoxaluria 3 / Autosomal recessive	Homozygous	Intron 6: c.834+2T>C NM_138413	Pathogenic	Novel	PVS1, PS2, PM2	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve)
NC2 M / 72 / 72	Bilateral nephrocalcinosis , hypercalciuria	KCNJ1 (-)/ Antenatal Bartter Syndrome Type 2/ Autosomal recessive	Homozygous	Exon 2: c.146G>A p.(Cys49Tyr) ENST00000392664.2	Likely Pathogenic	Reported	PM2, PM3, PM5, PP2, PP3, PS3, PS4	Genetic confirmation: (+ve) Genotype / Phenotype: +ve Variant similar to BS 4
NC3 M / 168 / 168	Bilateral nephrocalcinosis, hypercalciuria	CLDN19 / Renal hypomagnesemia 5 with ocular involvement/ Autosomal recessive	Heterozygous	Exon 1: c.115G>A (p.Ala39Thr) ENST00000296387.6	VUS	Reported in GenomAD	PP3	Genetic confirmation: (-ve) Genotype / Phenotype:(-ve)

NC4 M / 12 / 12	Bilateral nephrocalcinosis	SLC12A1 / Bartter Syndrome Type 1 / Autosomal recessive	Heterozygous	Intron 25: c.3096+2T>A (5' splice site)	Likely pathogenic (Likely to be an incidental carrier finding rather than disease causing)	Novel	PVS1, PM2	Genetic confirmation: (-ve) Genotype / Phenotype: (-ve) N.B. Apart from nephrocalcinosis no other features of Bartter such as metabolic alkalosis or hypokalemia were present. We were also unable to find mutation in any other tubulopathy related genes.
Others1 F / 146 / 149	Polyuria / Polydipsia/ Hypocalcemia/ Hypomagnesemia	GATA3 / Hypoparathyroidism, sensori-neural deafness and renal dysplasia/ Autosomal Dominant	Heterozygous	Exon 4: c.829C>T (p.Arg277Ter)  ENST00000379328.3	Pathogenic	Reported	PVS1, PS2, PM2, PP3, PP5	Genetic confirmation: (+ve) Genotype / Phenotype: (+ve) Presented with polyuria convulsion, hypocalcemia and hypo-magnesemia. Reverse phenotyping revealed SNHL
Others2 M / 1 / 2	Polyuria / FTT/ Hypomagnesemia	HNF1B / Renal Cysts and Diabetes Syndrome / Autosomal dominant	Heterozygous	Encompassing deletion in Exon 5: c.(1045+1_10461_1206+1_1207-1) del ENST00000617811.5 MLPA was not done	Likely pathogenic	Reported	PVS1, PM2	New diagnosis Genetic confirmation : (+ve) Genotype / Phenotype: (+ve) Presented with polyuria, hypocalcemia, hypomagnesemia, failure to thrive and bilateral echogenic kidneys. On re evaluation child was found to have bilateral kidney cysts. Father / Grand Father were found to have bilateral kidney cysts and diabetes which they were unaware of.

Others3 M / 96 / 112		HNF1B / Renal Cysts and Diabetes Syndrome / Autosomal dominant	Heterozygous	Exon 4: c.1006C>G (p.His336Asp)  NM_000458	VUS	Reported in genomAD and clinvar	PP3, PM6	Genetic confirmation: (- ve) Genotype / Phenotype: (- ve) Presented with polyuria, hypocalcemia, hypo- magnesemia, failure to thrive and bilateral echogenic kidneys. On re evaluation child was found to have bilateral kidney cysts. Parents were normal.
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No relevant mutations in known tubulopathy related genes were detected for **dRTA22** (F / 5 / 48), **dRTA23** (F / 7 / 42 month), **dRTA24** (M, 74 / 180), **dRTA25** (F / 36 / 122), **BS17** (M / 10 / 11), **BS18** (M / ? / 21), **IHP6** (F / 18 / 144), **pTD11** (M / 19 / 19), **pTD12** (M / 0.05 / 0.1), **NC5** (M / 90 / 120) and **NC6** (M / 156 / 168), **Others4** (M, presented at 1 month with hypertension, hyperkalemia, normal creatinine and normal ultrasound scan of kidneys).

N.B. BS: Bartter Syndrome phenotype, CNV: copy number variation, dRTA: distal renal tubular acidosis phenotype, F: female, Genetic confirmation +ve / -ve: Confirmation / non-confirmation of genetic aetiology post variant classification, HP: Isolated Hypophosphatemic rickets phenotype, M: male. MLPA-Multiplex ligation-dependent probe amplification, NC: nephrocalcinosis / stone phenotype, NA: not available, NDI: nephrogenic diabetes insipidus phenotype, pTD - proximal tubular dysfunction phenotype, SNHL: sensorineural hearing loss, WES: whole exome sequencing

**Table III: Impact of Whole Exome Sequencing results on clinical management**

Suspected phenotype (n=77)	Clinical implications of genetic testing	
	Revision of diagnosis:  N=14; 18%	<i>Impact on clinical Management</i> (excluding impact on genetic counselling, but including identification of associated features):  N =24; 31%
<b>Distal Renal Tubular Acidosis</b> (dRTA) n=25	Revision of diagnosis: None	<i>n=7; 28%</i> Post availability of genetic reports additional co-morbidities were identified in 7 children (SNHL = 4, hemolytic anemia = 2 and dental changes = 1).
<b>Bartter Syndrome</b> n=18	<i>n = 6; 33%</i> I. Non tubulopathy (n=5): CCD = 1 and CF = 4 II. Another type of tubulopathy: (n=1) One patient with Bartter phenotype was found to have a genetic diagnosis of Gitelman syndrome	<i>n=5; 26%</i> Identification of CCD and CF facilitated specific treatment.
<b>Hypophosphatemic rickets</b> n=6	None	None
<b>Proximal Tubular Dysfunction</b> (pTD) n=12	<i>n = 3; 25%</i> N.B. Prior to availability of genetic report cystinosis was not suspected in 3 out of 4 children with causative CTNS variants.	<i>n=6; 50%</i> Confirmation of cystinosis (n=4) and tyrosinemia (n=2) facilitated specific treatment.  N.B. Tyrosinemia was clinically suspected in both children but WES confirmed the diagnosis and supported offer of treatment with nitisonine
<b>Nephrogenic Diabetes Insipidus</b> (NDI) n=6	<i>n = 2; 33%</i> I. Non tubulopathy diagnosis(n=1): Nephronophthisis II. Diagnosed to another type of tubulopathy: (n=1) dRTA	<i>n=2; 33%</i> Identification of dRTA allowed treatment with alkali supplementation. Diagnosis of nephronophthisis changed the management plan.

<p><b>Isolated kidney stone or nephrocalcinosis</b> n=6</p>	<p><i>n =1(17%)</i></p> <p>I. Diagnosed as Bartter syndrome (n=1) N.B. Prior to availability of genetic report Bartter Syndrome was not being suspected. In NC2</p>	<p><i>n=2, 33%</i></p> <p>Diagnosis of Bartter syndrome in NC2 which initially presented with isolated nephrocalcinosis and hypercalciuria helped in appropriate management planning such as avoiding the use of thiazide</p> <p>Identification of HOGA variant in the child helped in specifying treatment (such as avoiding unnecessary use of Vitamin B6) as well as in prognostication (risk of CKD Stage 5 is low in PH3).</p>
<p><b>Others</b> n=4</p>	<p><i>n=2; 50%</i></p> <p>I. Non-tubulopathy diagnosis (n=1): Hypo-parathyroidism, SNHL and renal dysplasia (Barakat syndrome). II. Diagnosed previously clinically unsuspected variant of tubulopathy: (n=1) RCAD</p>	<p><i>n=2; 50%</i></p> <p>Audiological assessment conducted post availability of genetic result diagnosed SNHL in Others1. Among Others 2 identifying HNF1B phenotype informed prognosis, including the risk of diabetes and gout.</p>

Cases grouped according to initially suspected phenotype and the impact of WES on management, such as revision of diagnosis or change in treatment

CCD: Chloride Secretory Chloride Diarrhea, CKD: Chronic kidney disease, CTNS: cystinosis, CF: Cystic fibrosis, Mx: management, PH3 Primary Hyperoxaluria 3, RCAD: Renal Cyst and Diabetes syndrome, SNHL: sensorineural hearing loss, WES: whole exome sequencing



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