BSMMUJ-66671

Mutation of NPHS1, NPHS2, WT1, LAMB2, COL4A5 and other genes in children with idiopathic steroid resistant nephrotic syndrome

Sharmim et al.

Type of the review: Anonymous Reviewer/Disclosed Author

MECHANICAL EDITING

30 September 2023

Comment 1

Please reduce the tables/figures up to 6 as per journal's rules.

Response

Reduced table and figure number up to 6.

Comment 2

Please provide ORCID iDs.

Response

Attached are the ORCID numbers which are available.

Comment 3

Provide DOI (PMID if DOI not available)/ URL numbers for all references.

Response

Given DOI numbers for all references.

TECHNICAL EDITING

Round 1

18 December 2023

We want to thank the learned reviewer for his nice review. Our point-by-point response has been attached below:

Reviewer's comments

Screening Points

Comment 1

How would you rate the originality and depth of the manuscript? (average 4.5 out of 10)

Response

Revised the manuscript to improve the depth of the manuscript.

Comment 2

Is the manuscript written in a scholarly manner? (average 4 out of 10)

Response

Revised the methods section.

Comment 3

Does the manuscript have the potential to make a valuable contribution to the world of knowledge? (average 4.5 out of 10)

Response

Revised the conclusion and major findings.

Comment 4

Does the manuscript meet ethical standards? (average 6.5 out of 10)

Response

Described the ethical issues so that it is understandable.

Reviewer C

Major Points

Comment 1

The number of patients included in this study was too small to derive any conclusions.

Response

We included all the patients of Idiopathic Steroid Resistant Nephrotic Syndrome (SRNS) who got treatment from the department of Pediatric Nephrology during the study period.

Comment 2

The number of genes tested was very few.

Response

We did Next Generation Sequencing (NGS) of whole exome. It included Nephrotic Syndrome gene Panel as well as other gene. Nephrotic Syndrome gene Panel included *ADCK4*, *ARHGDIA*, *CD2AP*, *CFH*, *COQ2*, *COQ6*, *CUBN*, *DGKE*, *ITGA3*, *ITGB4*, *LAMB2*, *MEFV*, *MYO1E*, *NPHS1*, *NPHS2*, *PDSS2*, *PLCE1*, *PTPRO*, *SCARB2*, *SMARCAL1*, *TTC21B*, *ACTN4*, *ARHGAP24*, *INF2*, *LMX1B*, *PAX2*, *TRPC6*, and *WT1*). NGS also could find the other de-novo gentic mution. As above 1 year NPHS1, NPHS2, WT1, AND LAMB2 genes are more common genetic mutation and our research proposal was approved with the title-" Analysis of 4 Genes Mutation (NPHS1, NPHS2, WT1, AND LAMB2) in Children with Steroid Resistant Nephrotic Syndrome". So we mentioned only these 4 genes. Moreover as we did NGS, we also found COL4A5 gene mutation which was not mentioned in our title.

Comment 3

From the introduction to the conclusion of the manuscript there are multiple shortcomings and mistakes. The frequency of Nephrotic Syndrome quoted in the first sentence is from New Zealand. It is well-established that the prevalence is much higher in Asians.

Response

Changed data has been given. Change data- The incidence of childhood NS under 15 years is 2–7 in 100,000 annually in children with a prevalence of 16 in 100,000 and the Asian population have a higher prevalence.

Comment 4

The rationale of this research is not clear. Mutations are now well reported in not only the 4 genes but in many other genes.

Response

Actually, we searched for known and unknown genes that's why we did next generation sequencing (NGS) which included huge genes.

Comment 5

The definitions of Nephrotic Syndrome and SRNS are used from ISKDC and APN which are at least 30 to 40 years old. There are IPNA and KDIGO Guidelines for NS especially SRNS published recently.

Response

During the study period we used this definition (ISKDC and APN). That time recent IPNA and KDIGO guidelines was not available.

Comment 6

There is no reference to data of mutated genes found in other South Asian cohorts, especially India and Pakistan.

Response

Given. One Indian study included 25 children of 4months to 18 years which showed NPSH2 gene mutation in 3 patients, and PLCe1, NPHS1mutation one in each variety. All were presented above 1 year of age.

Comment 7

The Objectives of the research are not fulfilled by the results and the conclusion drawn is not supported by the results.

Response

Frequency of mutation-8%, type of mutation- NPHS2 and COL4A5 gene mutation, their histology- diffuse MesPGN and FSGS.

Minor Points

Comment 1

There are too many tables that could be merged into fewer.

Response

Tables are merged and made it fewer.

Comment 2

If there were limited resources, perhaps only patients with a family history of NS, Infantile, and Congenital NS should have been included.

Response

As the aim of our study was to see the frequency of genetic origin of idiopathic SRNS in children, we excluded infantile and congenital nephrotic syndrome. Infantile and Congenital NS are separate entity of nephrotic syndrome. They are mostly genetic in origin which is well known. But as there are few studies in idiopathic SRNS in children worldwide as well as no published data in our country, we included all the cases of idiopathic SRNS cases in between 1-18 years. We did not include only familial SRNS because we have only limited number of cases in the study period as well. Recent IPNA guideline recommend that, if available, that genetic testing be performed in all children diagnosed with primary (Idiopathic) SRNS

Comment 3

Biopsy was done only on 17 patients. Why was it not done in 1/3rd of patients? The inclusion of Proliferative GN as SRNS makes data further murky.

Response

The biopsy was done only 17 patients because renal biopsy was done only those patients whose parents gave the consent and whose physical condition were permitted renal biopsy. Proliferative GN was included other than secondary cause.

Comment 4

In this cohort 88% of children had non-consanguineous parents. Is that a usual trend in the country?

Response

As now a days people have built awareness on disease related to consanguineous marriage like thalassemia might be the cause of it. Moreover we found this finding in our study.

Reviewer H

Comment 1

American and British English were mixed up. Tables are repeated in the text, which should be avoided, only highlighting points will be mentioned.

Response

Corrected as much as possible. Tables are curtailed.

Comment 2

Figure of NGS data showing the mutations comparing the wild sequence can be presented. Discussion on the selection of the 1 to 18 age group can be justified or highlighted as in most of the cases the results were compared with the patients aged up to 25 years.

Response

We showed the final result of genetic mutation and the comparisons can be done on the relevant issues.

Comment 3

A comparison of mutations should be made (with type, position etc.). During comparisons, it will be preferable to mention the population of the comparing data

Response

Given in table-2

Comment 4

Line 66- percentage may give a false impression, one subject is enough. Line 68- percentage may give a false impression, one subject is enough

Response

Removed the percentages.

Comment 5

Line 72- from this result, it cannot be concluded as not uncommon.

Response

Conclusion has changed. New conclusion- Genetic mutation of SRNS patients 1-18 year of age showed NPHS1 and COL4A5 gene mutation. Histopathologically they showed diffuse mesangial proliferative glomerulonephritis and focal segmental glomerulosclerosis.

Comment 6

Line 78- ? as the study did not include below 1 year aged patient. Line 79- from only one mutation in each of the two genes among the four studied, it cannot be highlighted as not uncommon. Line 105- Statistics should be corrected, the incidence in which of the population needs to be mentioned

Response

Corrected accordingly. Statistics has corrected as follows –
The incidence of childhood NS under 15 years is 2–7 in 100,000 annually in children with a prevalence of 16 in 100,000 of children and the Asian population have a higher prevalence. Approximately10- 20% will be steroid resistant nephrotic syndrome (SRNS) of all nephrotic syndrome.

Comment 7

Line 167- the name of the device with the company and country of origin for the DNA quantity and quality analysis should be mentioned

Response

DNA quantification and quality was assessed by Thermoscientific Nanodrop2000 Spectrophotometer (USA origin, company name- Thermoscientific)

Comment 8

Line 167- the name with the company and country of origin of the NGS machine should be mentioned

Response

Name of NGS machine- HiSeq™ 4000 Illumina sequencer machine, Company Illumina, Country of origin-USA

Comment 9

Line 169- The algorithm of BWA software should be mentioned

Response

Picard and GATK version 3.6. Clinically relevant mutations were annoted using published variants in literature and a set of disease databases- Clin Var, OMIM, GWAS, HGMD,swiss Var.

Comment 10

Line 175- It did not mention whether normality tests were done or not.

Response

Normality testing had been done among all the data first.

Comment 11

Line 52- spelling correction of nephrotic syndrome. Line 64- majority

Response

Corrected spelling- nephrotic syndrome

Executive Editor's comments

Comment 1

The title should run continuously without a parenthesis.

Response

Corrected title- Analysis of Genetic Mutation of NPHS1, NPHS2, WT1, AND LAMB2 gene in Children with Idiopathic Steroid Resistant Nephrotic Syndrome

Comment 2

1. Abstract: The line on consent is confusing and misleading. The children under 18 years cannot give consent. Probably the authors intended to mention the assent of children below 12 and asent and parental consent for those aged 12 or above.
2. The conclusion's first clause has no link to the study's objective. Kindly revise it. Moreover, what is bad renal histopathology variety?

Response

1. As abstract has to be written only 200 words, here we mentioned it in a short but in methodology it was written in detail. Correction in abstract- . Histopathological study of renal tissue was performed among 17 patients whose parents agreed to renal biopsy.
2. Corrected conclusion: Genetic mutation of idiopathic SRNS patients of 1-18 year was showed NPHS1 and COL4A5 gene mutation. Histopathologically they showed diffuse mesangial proliferative glomerulonephritis and focal segmental glomerulosclerosis.

Comment 3

Highlight is a repetition of the Conclusion of the Abstract! Kindly replace these with bullets that provide an overview of the article.

Response

- Genetic mutation of Idiopathic SRNS patients of 1-18 year could be occurred.
- Genetic mutation patient of above 1 year children may have non-minimal change disease(non-MCD) on renal histopathologigy.

Comment 4

Introduction: Appears lengthy. The last paragraph is exceedingly long to read at a stretch. Please divide it into two thematic paragraphs.

Response: Length is reduced

Comment 5

1. The whole of the Methods section has been written in one paragraph! These should be given in two to three paragraphs.
2. Consent-related problems are also visible here (line 158). Line 175 is unnecessary. I do not see any method for the patients' history (as the results are given in Table 2) and the analytical methods for the biochemical variables.

Response

1. Consent: After taking assent of below 12 years children, assent and parental consent above 12 years of children who was fulfilled the inclusion criteria initially enrolled in this study.
2. History: Thorough history was taking regading age, sex, age of onset of disease,consangunity, family history of renal disease, hematuria etc, Method divided into 3 paragraphs

Comment 6

1. Results: The first line of the Results section is a Method. It is unnecessary.

2. Tables 1-3 could be given in one table. Age categories have not been done judiciously. Three out of four groups have small numbers to analyze. These could be removed. Having the mean (SD) could be enough. Remove the male-female ratio, and total residence category (Table 1). From Table 2, only positive categories could be given in the table, rest is implied and understandable, e.g. hematuria in 16 (44%) means rest did not have hematuria. Table 3 will automatically match the previous two tables' contents given in one new table.
3. The biochemical data given in a single subject is meaningless. Remove them. Do the other data given in this table have any implications for clinical practice or research?

Response

1. Removed from the result
2. Data of table 1-3 are given in a single table,age catagories and male-female ratio are removed,only positve catagories are given(table2,3)
3. Biochemical parameters are removed

Comment 7

Fig 1: Make it a bar chart and label the horizontal axis as "Number of subjects".

Response

Fig 1 have changed according to comment(bar chart with horizontal axis as "Number of subjects")

Comment 8

Kindly revise it to fit into a **Brief article** (1500-word mani text, 200-word abstract, 3 tables/graphs, 20 references).

Response

I am agree with “Brief Article”. I have tried to reduce the number of word and number of references. Abstract- 223, text-1557, tables/graphs-4 ref-21

Round 2

19 December 2023

Executive Editor’s comments

We want to thank the learned reviewer for his nice review. Our point-by-point response has been attached below:

Comment 1

The abstract's word count is 223, which should be 200 or less.

Response

Reduced to 200 words

Comment 2

The Highlights do not encompass the overview of the study. Kindly revise it.

Response

- Eight percent children of idiopathic SRNS above 1 year had genetic mutation in this study.
- Genetic mutation patients had non-minimal change disease(non-MCD) on renal histopathology.

Comment 3

A brief article should not have more than three data visuals (tables or graphs). I have hand-written suggestions to revise the tables in the attached files. This will reduce the number of tables from 3 to 2.

Response

Reduced to 2 tables and 1 figure.