Association of ACE and MDR1 Gene Polymorphisms with Steroid Resistance in Children with Idiopathic Nephrotic Syndrome

Mohana Priya Chinambedu Dhandapani, Vetrivel Venkatesan, Namburav Bolam Pongaswamy, Karpina Gowrishanker, Frazad Nageswara, and Venkatachalam Perumal

Aim: The purpose of the study was to investigate the distribution of insertion/deletion (I/D) polymorphisms of the angiotensin-converting enzyme (ACE) gene and three exonic polymorphisms of the multidrug resistance 1 (MDR1) gene (C3435T, C1236T, and G2677T) in children diagnosed with idiopathic nephrotic syndrome (INS). Materials and Methods: The study group consisted of 100 healthy controls and 150 INS patients, of which 50 were steroid resistant. Genomic DNA from blood samples was isolated from both of these groups and genotyping of the ACE and MDR1 genes was performed by polymerase chain reaction (PCR) using specific primers. Results: There was no significant difference observed in the genotypic distribution and D allele frequency of the ACE gene. The two single nucleotide polymorphisms (SNPs), C1236T and C3435T, of the MDR1 gene showed no significance, whereas the SNP G2677T/A was significantly associated with the genotypes GT and GA of the MDR1 gene, indicating it may be a potential marker to detect drug resistance. Conclusion: Screening these polymorphisms will pave the way to better understand the molecular mechanisms of the disease, which may be useful in developing targeted therapies for INS patients.

Introduction

Idiopathic nephrotic syndrome (INS) is a primary glomerular disease in children with clinical manifestation of histological variants, such as minimal change disease (MCD), diffuse mesangial proliferation, and focal segmental glomerulosclerosis (FSGS) (Barisoni et al., 2007). MCD accounts for 52-78% and the FSGS for 10% of nephrotic syndrome (NS) (Swierczewska et al., 2013). Childhood INS patients are categorized into either steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS) patients. Steroid responsiveness is the most important prognostic indicator of INS (Bagga and Savasta, 2005). Various genetic markers studied to predict susceptibility and course of INS, polymorphisms in the angiotensin-converting enzyme (ACE) gene and multidrug resistance 1 (MDR1) gene have been shown as some of the probable genetic risk factors in the development and progression of disease (Rigat et al., 1990; Jafar et al., 2011).

The ACE gene consists of either an insertion (I) allele or a deletion (D) allele, forming three possible genotypes: II, ID, and DD (Hohenfellner et al., 2001). Children with a history of INS were found to have an increased prevalence of homozygosity for the deletion allele (D) of the ACE gene (Lee et al., 1997; Sardaroglu et al., 2000). DD homozygous or D allele is associated with elevated circulating and tissue ACE activity compared with I allele (Rigat et al., 1990). Therefore, it has been thought that the D/D genotype may link to the ACE-related pathophysiology of renal diseases, in particular FSGS, or may predispose a person to INS. The MDR1 gene plays a vital role in drug-drug interaction (Gottsmann et al., 2002). Single-nucleotide polymorphisms (SNPs) of the MDR1 gene may change the protective role of P-glycoprotein (Pgp) and thus influence disease risk and patient response to drug treatment (Konar, 2007). Two additional SNPs, C2677T/A (rs2032582) in exon 21 and C1236T (rs128513) in exon 12, were found to be in linkage disequilibrium with MDR1. C3435T also seems to affect P-gp function (Jafar et al., 2011). The putative genetic regulation of MDR1 gene expression has not been clearly delineated in patients with INS yet.

Considering the importance of the ACE gene in renal pathophysiology, it was decided to investigate the distribution of insertion/deletion (I/D) polymorphisms of ACE in...
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children with SSNS and SRNS. Furthermore, studies were undertaken to examine the distribution of the most frequent three exonic polymorphisms of the MDR1 gene (C3435T, C1236T, and G2677T/A) in INS patients with age and gender matched healthy children to determine whether they will be useful markers to detect drug resistance and responsiveness to steroids.

Materials and Methods

The study group consisted of 250 children, of which 150 children were with INS with the mean age of onset as 5.39 years and 100 healthy children without a familial history of renal disease were with the mean age of 5.86 years. All cases fulfilled the criteria of the International Standard of Kidney Disease in Children (1984) for the diagnosis of the NS. Of the 150 samples, 50 children were steroid resistant and biopsy confirmed FSGS. On ethical approval, 3 mL of blood samples was collected from the study subjects with the written informed consent from the parents.

Genotyping of ACE and MDR1

Genomic DNA was isolated from whole blood (Genetix Biotech) and genotyping of ACE and MDR1 genes was performed by polymerase chain reaction (PCR) using specific primers and annealing temperatures described by Rigat et al. (1990) for the ACE gene and by Iafar et al. (2011) for the MDR1 gene. The ACE gene II genotype was detected as a 490 bp fragment and DD genotype was identified as a 190 bp fragment, while ID genotype was identified by the presence of both 490 and 190 bp fragments. For the MDR1 gene, C1236T; G2677T/A, and C3435T genotypes, PCR products were sequenced (ABI PRISM Genetic Analyzer 3730) and analyzed using SeqScape analysis software V2.5.

Statistical analysis

The allele frequency and distribution of genotypes in the cases and the controls were calculated and their relationship with drug resistance was statistically evaluated using SPSS Statistical software (Version 16.0). The odds ratio at 95% confidence interval facilitated the comparison of genotype frequency distribution between the controls and cases. A p-value of <0.05 was accepted as significant.

Results

ACE and MDR1 polymorphisms in children with INS

The distribution of ACE and MDR1 genotype frequency among the cases and the controls follows Hardy–Weinberg equilibrium (Tables 1 and 2). The frequency of D allele of the ACE gene was 0.37 in patients and 0.42 in controls. There was no significant difference in the frequency of either I or D allele of the ACE gene between the cases and the controls.

The distribution of genotypes of the MDR1 gene for C1236T and C3435T in patients and healthy controls did not show any significant difference. However, the SNP G2677T/A showed a significant association with the genotypes, GT and GA (p < 0.05). The calculation of haplotype frequency of C1236T, G2677T/A, and C3435T genetic variations in the MDR1 gene showed the (i) CT-GT-CT in INS (17.3%) and in controls (12%), (ii) CT-CT-CT in INS (8.6%) and in controls (8%).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control n=150 (%)</th>
<th>Cases n=150 (%)</th>
<th>OR</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>12 (12)</td>
<td>27 (18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>44 (44)</td>
<td>58 (38.7)</td>
<td>0.58</td>
<td>0.26–1.28</td>
<td>NS</td>
</tr>
<tr>
<td>TT</td>
<td>34 (34)</td>
<td>65 (43.3)</td>
<td>0.33</td>
<td>0.16–0.62</td>
<td>NS</td>
</tr>
<tr>
<td>CC</td>
<td>14 (14)</td>
<td>16 (10.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>71 (71)</td>
<td>101 (67.3)</td>
<td>1.24</td>
<td>0.57–2.71</td>
<td>NS</td>
</tr>
<tr>
<td>TT</td>
<td>15 (15)</td>
<td>33 (22)</td>
<td>1.92</td>
<td>0.75–4.94</td>
<td>NS</td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme; CI, confidence interval; I/D, insertion/deletion; INS, idiopathic nephrotic syndrome; NS, nonsignificant.

Discussion

The ACE I/D gene polymorphism can alter the enzyme activity and better predict the outcome of renal diseases (Lee et al., 1997; Serdaroglu et al., 2005). In the present study, there was no significant relationship between D allele frequency of ACE and INS. Further analysis of genotypes in FSGS and MCD patients also revealed lack of association in the genotypic distribution or allelic frequency between control subjects or SRNS/SSNS patients. This observation is supported by the earlier findings such as that of Lee et al. (1997), which showed no difference in the ACE genotype between children with FSGS or MCD and normal controls. In contrast, Pasun et al. (2011) have observed that DD genotype was significantly high in SRNS patients compared with SSNS patients. Similarly, the frequency of the DD genotype was shown to be higher in FSGS patients than in controls in Indonesian (Yong et al., 2006), Korean (Lee et al., 1997), and Japanese (Hori et al., 2001) populations. In the present study, the lack of association of D allele frequency or DD genotype with the INS might be due to the sparseness of data and
therefore a large sample size is required to further clarify the association of DD genotype or D allele with the onset of INS in a South Indian population.

Second, three known SNPs of the \textit{MDR1} gene (C1236T, C3435T, and G2677T/A) were studied to analyze the correlation between the genotypes and the INS. In the present study, there was no significant association in genotype and allele frequencies of either C1236T or C3435T polymorphism between control subjects and INS patients, which was similarly reported by Youssif \textit{et al.} (2013). These polymorphisms also did not have any significant impact on steroid resistance in our patient population.

G2677T/A, a missense mutation, results in an amino acid change from alanine to serine or threonine, which may lead to increased efficiency of the mutated protein by efflux of glucocorticoid or its active metabolites resulting in steroid resistance (Kim \textit{et al.}, 2001). In the present study, the frequency distribution of the heterozygous mutant genotype of the G2677T/A gene is much higher in patients with INS compared with controls. In contrast, Jafar \textit{et al.} (2011) have observed that the frequency distribution of the homozygous mutant genotype of the G2677T/A gene is much higher in patients with INS compared with controls and also higher in patients with SRNS compared with SSNS and suggested that NS patients who carry the T/A would be more resistant to steroids and susceptible for developing SRNS. However, in our study, further analysis of genotypes in the G2677T/A gene mutation did not show any significant correlation between SSNS/RNS patients and healthy controls. Nevertheless, the prevalence of heterozygous mutant genotype of the G2677T/A gene in INS patients probably may contribute to the P-mediated drug-resistant phenotype by either increased expression or function of P-gp. The higher frequency of G2677T/A gene polymorphism in patients with INS also indicates that it may be a marker for susceptibility to developing NS. These results also suggest that the evaluation of \textit{MDR1} G2677T/A polymorphism is of greater importance for individualized pharmacotherapy. However, when this polymorphism was compared between FSGS and MCD cases, no significant difference in either genotypes or alleles was found; thus, it is concluded that there is no association between pathological type of SRNS and \textit{MDR1} gene polymorphism in the three tested sites (C1236T, G2677T/A, and C3435T).

Screening for the common genetic causes of NS will prevent unnecessary steroid therapy of these children. For better understanding of the correlation between these gene polymorphisms, allele frequency, and diseases conditions, large cohort studies in different areas need to be conducted. A better understanding of the molecular mechanisms of the disease may also yield new information about etiology and will be helpful in developing targeted therapies against the disease.

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\textbf{Author Disclosure Statement}

No competing financial interests exist.

References


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Report of novel genetic variation in *NPHS2* gene associated with idiopathic nephrotic syndrome in South Indian children

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**Abstract**

**Background** Steroid-resistant nephrotic syndrome (SRNS) is found in 10–20% of children with idiopathic nephrotic syndrome (INS). In SRNS patients, common histopathological subtypes are Focal segmental glomerulosclerosis (FSGS) (53%) and minimal change disease (MCD) (27%). Familial forms of FSGS constitute podocyte diseases with varying severity and age of onset. Podocin gene (*NPHS2*) mutations cause childhood-onset steroid-resistant FSGS and MCD to adult-onset FSGS. In view of genetic variations and susceptibility to the disease, the present investigation was undertaken to study the pattern of genetic mutation in children from South India.

**Methods** Mutation analysis was carried out by direct sequencing of the entire *NPHS2* gene (eight exons) using specific primers in 200 INS (100 SRNS and 100 steroid sensitive) children and 100 healthy controls. The allele and genotype frequencies of *NPHS2* gene were calculated for both cases and controls as per Hardy–Weinberg equilibrium.

**Results** Among the SRNS patients, 18% revealed both heterozygous and homozygous mutations. Out of 12 mutations, 8 were homozygous and 4 were heterozygous. Interestingly, we found two novel SNPs in exon 4 of *NPHS2* gene, which are documented and submitted to dbSNP database (Ref rs12401711 and rs12401708).

**Conclusion** Mutational analysis of *NPHS2* would be advisable at the start of treatment. The genetic variations detected in the study would serve as the important molecular marker in treating the children’s at early stage, which also enables to detect carriers, prenatal diagnosis and provide genetic counseling to couples at risk.

**Keywords** Steroid-resistant nephrotic syndrome · *NPHS2* mutations · Polymorphism

**Introduction**

Idiopathic Nephrotic syndrome (INS) is a glomerular disorder occurring mainly in children [1, 2]. The annual incidence of INS is projected around 2–7/100,000 children and a prevalence rate of 16/100,000 children below 16 years of age [3]. Based on the response to steroid therapy, INS is classified into steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS) [4]. In most patients, the severity of the disease was controlled after steroid treatment. Nevertheless, 10% of children with INS did not respond to either steroids or to any other immunosuppressive therapy, and progressed to end-stage renal disease (ESRD) [5]. In SRNS, 75% of patients revealed renal histologic features of FSGS, while 25% of the cases exhibited MCNS.

FSGS and MCNS diseases are due to aberrations of genes that govern podocyte structure and function [6]. One
of the causative genes for this disease, NPHS2, encodes a novel protein named podocin, which is expressed only in the podocytes at the cytoplasmic part of the slit diaphragm in both fetal and mature kidney [7]. Mutations in the NPHS2 gene are a main cause of autosomal-recessive SRNS in childhood with approximately 6.4–30 % of sporadic and 20–40 % of familial cases [8–10]. Previous studies have shown that SRNS patients with an NPHS2 mutation had more severe clinical manifestations compared to SRNS patients without the NPHS2 mutation [10–12].

Podocin performs a mechanotransduction function by interacting with the cytoskeleton and slit-diaphragm nephrin to stabilize the permeability unit of podocytes [8, 13]. There are different NPHS2 mutations (missense, nonsense and frameshift mutations), which play a key role in the glomerular filtration barrier function. Huber et al. [12] demonstrated that mutations in the NPHS2 cause disruption of nephrin targeting to lipid raft microdomains. The prevalence of NPHS2 mutations in Indian children with SRNS is limited. The aim of the study was to evaluate the frequency of NPHS2 gene mutations in correlation with the pathogenesis of INS in South Indian children.

Materials and methods

Subjects and blood samples

The study group consisted of 200 children with INS and 100 healthy controls without a familial history of renal disease were included in the study. The mean age of onset for INS is 5.39 and for controls 5.86. All cases fulfilled the criteria of the International Standard of Kidney Disease in Children (1981) for the diagnosis of the NS. Of the 200 children included, 100 children were steroid resistant and biopsy confirmed FSGS and rest were SSNS. All these children had massive proteinuria >40 mg/m²/day or >50 mg/kg body weight/day or a random sample of urinary protein-to-creatinine ratio exceeding 2 mg/mg which effectively resulted in severe hypoalbuninaemia of serum albumin less than 2.5 g/dl. On ethical approval, 3 ml of blood samples was collected from the study subjects with the written informed consent from the parents.

All these children had initially a course of prednisolone when found resistant had a course of either IV cyclophosphamide or oral cyclophosphamide, followed either oral cyclosporine or oral tacrolimus and then mycophenolate mofetil along with steroids. None of the children had progressed into chronic kidney disease stage 2 or more during the study period. The study was approved by the Institutional Medical Ethics Committee (Ref No: IEC-N/1/10/June/17915). Written informed consent was obtained from the parents of both cases and healthy controls before collecting 3 ml of peripheral blood from the study subjects.

Mutation and genotyping analysis

Genomic DNA from blood sample was isolated and purified using the QIAamp blood kit (QIAGEN GmbH, Germany). All eight exons of the NPHS2 gene were amplified using exon-flanking primers [8] and the primers are listed in Table 1. The PCR reaction mixture was made up in dH2O water containing 100 ng genomic DNA, 0.2 μM of each primer, and PCR Taq master mix (Amplicon). The cycling conditions had a range of annealing temperatures from 50 to 60 °C (50 °C-exon 6, 55 °C-exons 2, 3, 4 and 5 and 60 °C exons 1, 7 and 8) [8]. Yield and purity were determined by electrophoresis on 0.8 % agarose gel. PCR was carried out. The amplified PCR products were separated on 2 % agarose gel electrophoresis and visualized under UV transilluminator after staining with ethidium bromide. The amplified products were subjected to mutation analysis using Big Dye Terminator V.1.1. (Applied Biosystems, Foster City, California) by direct sequencing of all exons in ABI Prism™ 3730 Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Sequences were evaluated with Seqscape analysis software V2.5.

Statistical analysis

Mutational analysis was carried out using Seqscape analysis software. The allele frequency and distribution of genotypes for the SNPs in cases and controls were calculated, and their relationship with drug resistance was statistically evaluated using SPSS Statistical Software (Version 16.0). Hardy–Weinberg equilibrium performed for both cases and controls. The odds ratio at 95 % confidence interval (CI) facilitated the comparison of genotype frequency distribution between the controls and cases. A p value of less than 0.05 was accepted as significant. Pair wise linkage disequilibrium was calculated for the SNPs and the D' and r² statistic measures showed a strong association coefficient of 0.9995 for both SRNS and SSNS against control group.

Results

Patient characteristics

NPHS2 gene mutation screening was performed in 200 INS patients and 100 healthy children without a familial history of renal disease as healthy controls; among the patients 100 patients were SRNS and 100 patients were SSNS. Age of onset between 1 and 16 years with the mean age of
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Table 2 Summary of NPHS2 gene variations in cases (INS) and controls detected in the study

<table>
<thead>
<tr>
<th>Sample details</th>
<th>Gene location</th>
<th>Nucleotide change</th>
<th>Aminosacid change</th>
<th>Mutation status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 100)</td>
<td>All exons</td>
<td>No change</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>SSNS (N = 100)</td>
<td>All exons</td>
<td>No change</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>SRNS-number of patients (P) = 100</td>
<td>Exon 4</td>
<td>n21237 (T&gt;C)</td>
<td>L167P (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P44</td>
<td>Exon 4</td>
<td>n21240 (G&gt;A)</td>
<td>R168H (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P7, P73 and P96</td>
<td>Exon 4</td>
<td>n21306 (A&gt;G)</td>
<td>H52R (novel)</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>P23</td>
<td>Exon 5</td>
<td>n21371 (C&gt;T)</td>
<td>R196G (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P24</td>
<td>Exon 8</td>
<td>n29515 (C&gt;T)</td>
<td>A297V (reported)</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>P47, P3 and P96</td>
<td>Exon 8</td>
<td>n23795 (C&gt;A)</td>
<td>L204L (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P46 and P4</td>
<td>Exon 8</td>
<td>n23975 (C&gt;A)</td>
<td>L204L (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P43 and P48</td>
<td>Exon 4</td>
<td>n21253 (G&gt;A)</td>
<td>No change</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>P51</td>
<td>Exon 1</td>
<td>n5221 (T&gt;C)</td>
<td>S46P (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P62</td>
<td>Exon 1</td>
<td>n5250 (G&gt;A)</td>
<td>No change</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P78</td>
<td>Exon 5</td>
<td>n23841 (A&gt;G)</td>
<td>Q291L (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P93</td>
<td>Exon 8</td>
<td>n29680 (C&gt;T)</td>
<td>S192F (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P98</td>
<td>Exon 4</td>
<td>n21260 (C&gt;T)</td>
<td>P175S (novel)</td>
<td>Homozygous</td>
</tr>
</tbody>
</table>

SSNS: steroid sensitive nephrotic syndrome, SRNS: steroid resistant nephrotic syndrome, NT: nucleotide

Table 3 NPHS2, Exon 4 G/A polymorphism frequency distribution of patients with INS and healthy subjects for SNPs (rs1130236897) and (rs1130236897)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Genotypes</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 100)</td>
<td>GG 66 (66 %)</td>
<td>GA 32 (32 %)</td>
<td>AA 2 (2 %)</td>
</tr>
<tr>
<td>SSNS (N = 100)</td>
<td>78 (78 %)</td>
<td>20 (20 %)</td>
<td>2 (2 %)</td>
</tr>
<tr>
<td>SRNS (N = 100)</td>
<td>71 (71 %)</td>
<td>25 (25 %)</td>
<td>4 (4 %)</td>
</tr>
</tbody>
</table>

CI: confidence interval, INS: idiopathic nephrotic syndrome, NS: Non significant

Table 4 Distribution of genotype and allele frequency of NPHS2 exon 4, polymorphism in study subjects and control group

<table>
<thead>
<tr>
<th>SNPs (rs1240711 and rs1240708)</th>
<th>Genotypes</th>
<th>Allele frequency</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (N = 100)</td>
<td>GG 66</td>
<td>GA 32</td>
<td>AA 2</td>
</tr>
<tr>
<td>Expected</td>
<td>67.2</td>
<td>29.5</td>
<td>3.2</td>
</tr>
<tr>
<td>SSNS (N = 100)</td>
<td>Observed 78</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Expected</td>
<td>78.2</td>
<td>19.6</td>
<td>1.2</td>
</tr>
<tr>
<td>SRNS (N = 100)</td>
<td>Observed 71</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Expected</td>
<td>69.2</td>
<td>27.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

SNP: single nucleotide polymorphism, NS: non significant

Synonymous and 4 were missense type. Of the four missense mutations, one R168H at n21240 (G>A) in exon 4 and the other A297V at n29515 (C>T) in exon 8 were already published [10, 15, 16]. Nevertheless, clinical symptoms were not different in patients showing homozygous or heterozygous mutations.

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The mutation R168H nt21240 (G>A) observed in exon 4 was widely investigated in various populations in relation to kidney disorders [15, 17]. Yu et al. [18] were the one who identified this homozygous NPHS2 mutation for the first time in Chinese children with FSGS, which showed a substitution of histidine for arginine at 168th position (podocin R168H). This change has been shown to induce apoptosis, a marked loss and aggregation of actin filaments and activation of extracellular signal-regulated kinase (ERK) pathway in podocytes [19]. The abnormal retention of podocin R168H in endoplasmic reticulum (ER) significantly up-regulated ER stress markers and also led to the mis-localizations of other crucial slit diaphragm molecules like nephrin, CD2-associated protein (CD2AP) and the transient receptor potential-C channel-6 (TRPC6), [19, 20]. These studies implicate that podocin R168H induce different degrees of podocyte injury, which may disrupt glomerular filter function, ultimately leading to nephrotic syndrome.

The missense mutation A297V is located in an alanine- and glutamate-rich region of stomatin protein family. Tory et al. [21] reported that this mutation was found to be pathogenic, with a milder phenotype and a disease onset in late childhood or adulthood. Caridi et al. [10] reported the presence of podocin in the glomeruli of a patient with composite heterozygous mutation of NPHS2 (R229Q and A297V). It has been reported that more than 116 pathogenic mutations have been identified with the disease. These mutations may change the expression of the gene and presumably, the structure of the protein. Among missense variants, severity of the disease appears to be determined by the amino acid substitution on specific functional domains and on the intracellular trafficking of podocin.

The existence of NPHS2 gene mutations only in SRNS group and their absence in SSNS and healthy controls observed in the present study indicates a strong relationship between the occurrence of SRNS and these NPHS2 gene mutations. Among the 18 % of children, who showed NPHS2 mutations, 12 % were male and 6 % were females. These children were treated with various immunosuppressive medications, 3 % children were lost for follow up, 4 % children were under remission for the past 3 years, 6 % children were under partial response and continued to be under small doses of prednisolone, 4 % children have shown no response and continued to be under active treatment. One child expired due to renal failure and its complications. According to the guidelines of International Study for Kidney Disease in children [1981], initial steroid resistance is defined as NS that does not respond to 1 month’s treatment with oral prednisolone at a dosage of 60 mg/m^2/day or 2 mg/kg/day. Children with late resistance is defined as initial steroid responders who developed resistance later than ≥6 months of treatment. In the present study, 24 % children showed initial steroid resistance while 76 % children were later non-responsive.

The detection of these NPHS2 mutations is of clinical importance as the preponderance of these mutations only in SRNS not only confirms genetic heterogeneity in SRNS but also underscores molecular defect leading to the non-responsiveness of these patients to steroid therapy. Failure to respond to steroid treatment has an important ramification for the risk of developing progressive renal failure later in life leading to ESRD. The higher frequency of these mutations (18 %) observed in SRNS is of noteworthy, though not significant, confirming the previous findings that mutations in the NPHS2 is known to cause SRNS, occurring in both sporadic and familial cases of SRNS [10, 11, 22, 23]. Lipska et al. [24] also reported that NPHS2 mutations were to be found in the highest prevalence in patients with SRNS in Polish population.

In the present study, the detection of NPHS2 mutations is of clinical importance as the identification of the underlying gene defect in SRNS has underscored the understanding of the pathogenesis of nephrotic syndrome. Earlier studies have shown that in addition to the structural injury to podocyte, identification of genetic mutations in numerous podocyte and podocyte-related proteins have been shown to play pivotal roles in the development of podocyte injury and proteinuria and are associated with SRNS and/or FSGS [2, 7, 9, 12]. The most implicated mutation involves the genes encoding the proteins such as nephrin, podocin, CD2-associated protein and alpha-actinin-4 that are important in maintaining its structure and function of podocytes. NPHS1 gene encodes nephrin, a key component of the podocyte slit diaphragm and is responsible for the Finnish-type congenital nephrotic syndrome [9, 13]. NPHS2 gene encodes, an integral membrane protein, podocin which interacts with nephrin and is responsible for FSGS. In case of alterations in some of the genes encoding these proteins, the podocyte foot process along with the glomerular basement membrane and slit diaphragm can lose its normal structure and alter its function leading to protein leakage or proteinuria causing NS and often the renal biopsy tissue will show a picture of FSGS [12, 28].

Further, mutational analysis in SRNS would help in preventing unnecessary exposure to immuno-suppressants and their adverse effects, besides helping in prognostication. Although 12 mutations were observed in SRNS, the significance of these mutations is not much known. Moreover, there was no subtle phenotypic difference between patients who bear or do not harbor these mutations. Further analyses are required to study the protein structure modifications and its implications to the diseases. Caridi et al. [10] have reported homozygous or compound
heterozygous mutations in NPHS2 for 14 of 120 patients with SRNS (12%). In contrast to those data, MANYAMA et al. [25] did not detect any mutation in NPHS2 in a study conducted in 36 Japanese children with SRNS. Vasudevan et al. [26] reported that the prevalence of NPHS2 mutations observed in a group of Indian children with sporadic SRNS is low (4%) and is similar to the Chinese (3%), Korean (0%), and the Japanese (0%) populations [16, 25, 27]. In contrast, NPHS2 gene mutations are more prevalent in Europe and North America affecting 10.5–28% of the sporadic SRNS children [28, 29]. Diversity in the NPHS2 gene mutation pattern is thought to be due to variations in ethnicity and environment [10, 11, 29]. The small number of NPHS2 exons and the detection of a frequent mutation will allow rapid screening of these individuals for mutation of NPHS2. This gives the advantage of avoiding unnecessary immunosuppressive therapeutic trials and renal biopsy.

In conclusion, our results demonstrate that mutational analysis of NPHS2 would be advisable at the start of treatment. The genetic variations detected in the study would serve as the important molecular marker in treating the children’s at early stage, which also enables to detect carriers, prenatal diagnosis and provide genetic counseling to couples at risk. Still, furthermore studies required to rule out the protein modifications and its implications to the diseases to know the functional significance of the protein.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest and they are responsible for the content and writing of this article.

References

Identification of novel gene biomarkers associated with steroid responsiveness in children with Nephrotic syndrome


Erratum to: Report of novel genetic variation in NPWS2 gene associated with idiopathic nephrotic syndrome in South Indian children

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Erratum to: Clin Exp Nephrol
DOI 10.1007/s10157-016-1237-0

The original version of this article unfortunately contained errors.

In the “Results” section of the main text, under the heading "NPWS2 gene mutation analysis", the sentence that begins with “The heterozygous mutations should read:

The heterozygous mutations were observed one in exon 4 [nt21253 (G>A) in 2 %], one in exon 5 [nt23795 (C>T) in 2 %], one in exon 8 [nt20515 (C>T) in 3 %] and one in intronic region [nt21306 (A>G) in 1 %].

In Table 2, the values of “Amino acid change” for P23 and P24, and “Nucleotide change” for P46 and P4 were shown incorrectly. The corrected Table 2 is shown here.

The online version of the original article can be found under doi:10.1007/s10157-016-1237-0.

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### Table 2: Summary of *NPHS2* gene variations in cases (NS) and controls detected in the study

<table>
<thead>
<tr>
<th>Sample details</th>
<th>Gene location</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Mutation status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 100)</td>
<td>All exons</td>
<td>No change</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>SSNS (n = 100)</td>
<td>All exons</td>
<td>No change</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>SSNS-number of patients (P) = 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P44</td>
<td>Exon 4</td>
<td>nt21237 (T&gt;C)</td>
<td>L167P (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P7, P73 and P96</td>
<td>Exon 4</td>
<td>nt21340 (G&gt;A)</td>
<td>R168H (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P23</td>
<td>Intron</td>
<td>nt21306 (A&gt;G)</td>
<td>No change (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P24</td>
<td>Exon 5</td>
<td>nt23771 (C&gt;T)</td>
<td>R196* (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P47, P3 and P76</td>
<td>Exon 8</td>
<td>nt29515 (C&gt;T)</td>
<td>A297V (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P46 and P4</td>
<td>Exon 5</td>
<td>nt23795 (C&gt;T)</td>
<td>L204L (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P43 and P48</td>
<td>Exon 4</td>
<td>nt21253 (G&gt;A)</td>
<td>No change</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P51</td>
<td>Exon 1</td>
<td>nt5221 (T&gt;C)</td>
<td>S46P (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P62</td>
<td>Exon 1</td>
<td>nt5250 (G&gt;A)</td>
<td>No change</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P78</td>
<td>Exon 5</td>
<td>nt23411 (A&gt;T)</td>
<td>Q216L (reported)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P93</td>
<td>Exon 8</td>
<td>nt29860 (C&gt;T)</td>
<td>S192F (novel)</td>
<td>Homozygous</td>
</tr>
<tr>
<td>P98</td>
<td>Exon 4</td>
<td>nt21260 (C&gt;T)</td>
<td>P175S (novel)</td>
<td>Homozygous</td>
</tr>
</tbody>
</table>

SSNS steroid sensitive nephrotic syndrome, SRNS steroid resistant nephrotic syndrome, NT nucleotide
Representative picture of NPHS1 ss# 1966531516 (novel) published in dbSNP database

<table>
<thead>
<tr>
<th>RefSNP</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism:</strong> human (<em>Homo sapiens</em>)</td>
<td><strong>Variation Class:</strong> SNV: single nucleotide variation</td>
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<tr>
<td><strong>Molecule Type:</strong> Genomic</td>
<td><strong>RefSNP Alleles:</strong> A/G (FWD)</td>
</tr>
<tr>
<td><strong>Created/Updated in build:</strong> 147/147</td>
<td><strong>Allele Origin:</strong></td>
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<td><strong>Map to Genome Build:</strong> 107/Wight 1</td>
<td><strong>Ancestral Allele:</strong> Not available</td>
</tr>
<tr>
<td><strong>Validation Status:</strong></td>
<td><strong>Variation Viewer:</strong> VarView</td>
</tr>
<tr>
<td><strong>Clinical Significance:</strong> NA</td>
<td><strong>Clinical Significance:</strong> NA</td>
</tr>
</tbody>
</table>

Fasta sequence (Legend)


CTCAGTGGACC CTATGCAAGC CCCCC
R
CCCCGGGCCC AGGAGACCT TCACT
Role of ACE gene I/D polymorphism in Idiopathic Nephrotic Syndrome

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Abstract: Idiopathic nephrotic syndrome (INS) is a common chronic renal disorder in children characterized by shared pathologic solubility of the glomerular filter. It is difficult to predict at onset the clinical course in terms of steroid responses. Various genetic markers have been studied to predict the cause and treatment response in nephrotic syndrome. Angiotensin converting enzyme (ACE) insertion/deletion (ID) polymorphism has been used as a marker and predictor of clinical course in various multifactorial disorders, including focal segmental glomerulosclerosis. Hence the present study was aimed to identify the role of ACE ID polymorphism in nephrotic syndrome. Genomic DNA was isolated from 50 children’s with nephrotic syndrome and 50 healthy control subjects without nephrotic syndrome. Genotyping of the ACE gene was done by polymerase chain reaction using specific primers for exon 16 of the ACE gene. The allele and genotype frequencies were calculated for cases and controls. The relative risk of the various genotypes was determined by calculating the odds ratio (OR) at 95% confidence interval (CI). The results revealed a increased frequency of DD in nephrotic syndrome compared to the controls. The distribution of genotypes in patients and controls was in Hardy Weinberg equilibrium.

Introduction: Nephrotic syndrome (INS) is among the most common type of kidney diseases seen in children. It is characterized by massive proteinuria, hypoaalbuminemia and edema. One of the kidney’s most important functions is filtration of the blood by glomeruli, allowing excretion of fluid and waste product, while retaining all blood cells and the majority of blood proteins within the blood stream. Under normal conditions, molecules greater than 200 kDa are unable to cross the filtration barrier.

Hypovolemia or hypotension stimulates Renin release from the kidney which cleaves Angiotensinogen to Angiotensin I. ACE converts angiotensin I to angiotensin II which binds with AT1 / AT2 receptors this activation leads to vasoconstriction, vascular growth, hypertension and aldosterone synthesis.

Inhibition of ACE results in the decreased formation of angiotensin II.

- leading to systematic dilatation of the arteries and veins and a diuresis
- Diminishes aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extracellular volume.

Aim: The aim of this study is to investigate the distribution of ACE gene I/D polymorphism among children with and without INS

Materials and Methods: The study included 50 children’s with idiopathic nephrotic syndrome who were sterile sensitive with the age of onset more than 1 year and less than 16 years. 50 healthy children of similar age without familial history of renal disease were selected as controls. The study was approved by the institutional medical ethics committee.

Results: The allele and genotype frequency of ACE gene in cases and controls were determined (Table 1). Of the 50 cases analyzed, 38% were homozygous ID, 42% were heterozygous I/D and 20% were DD. Among the 50 controls analyzed 35% were ID, 38% were ID and 27% were DD. The I/D allele frequency was 0.6 in cases and 0.57 in controls and that of D allele was 0.4 and 0.43 in cases and controls respectively. The distribution of genotypes in cases and controls was in Hardy-Weinberg equilibrium. No significant difference was observed in the ACE genotypes between cases and controls, suggesting lack of association of ACE genotypes and INS.

Table 1:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>ID</td>
<td>30</td>
<td>46</td>
</tr>
<tr>
<td>ID</td>
<td>30</td>
<td>46</td>
</tr>
</tbody>
</table>

Discussion: The study revealed no significant association between D allele frequency and INS. The reports from Japan (1) and Korea (2) stated that the D allele frequency was significantly higher in FSGS patients than in controls. The Korean report also indicated significant relationship between D allele and clinical severity. Several other studies did not observe any relationship between D allele and INS.

In conclusion our results correlate and deviate with other studies. More detailed analysis with large sample size are required to establish the role of ACE I/D polymorphism in INS.

References: I.

II.

Presented at the 5th International Conference on Genetic and Molecular Diagnosis in Modern Medicine, Chennai. 26th to 29th Jan 2012 at SRM University.
Identification of mutations in NPHS2 gene-potential causative factor for steroid resistant nephrotic syndrome in Indian children

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Abstract
Idiopathic Nephrotic Syndrome (INS) is one of the most common primary glomerular diseases in children. On the basis of the patients’ response to steroid treatment, INS is classified as steroid-sensitive NS (SSNS) or steroid-resistant NS (SRNS). Mutations in the NPHS2 gene encoding the protein podocin has recently been found to be associated with the recessive form of SRNS. Failure to respond to steroid treatment (SRNS) has an important ramification as the risk of developing progressive renal failure is higher, which will consequently lead to end-stage renal disease (ESRD). Aim: To evaluate the frequency of NPHS2 gene mutations in Indian children in correlation with the pathogenesis of SRNS. Methods: The study group consisted of 100 children-50 SSNS and 50 SRNS (biopsy confirmed FSGS). Genomic DNA was isolated from both the groups and then subjected to polymerase chain reaction using specific primers for NPHS2 gene, followed by sequencing. Results and Discussion: The NPHS2 gene analysis revealed two mutations, one in Exon 4 and other in Exon 5 leading to amino acid changes. Thus we propose that NPHS2 gene mutations is a causative factor for steroid resistance in SRNS patients. These molecular defects in NPHS2 will result in the translation of defective podocin protein which presumably cause glomerular barrier dysfunction, leading to nephrotic syndrome.

Introduction:
Nephrotic syndrome is a disease characterized by disruption of the glomerular filtration barrier of the kidney, abnormalities in podocytes, causing them to leak large amounts of proteins into the urinary system (Proteinuria and Hypoalbuminuria). Idiopathic Nephrotic Syndrome (INS) as the prefix ‘Idiopathic’ implies, is a disease of unknown etiology commonly reported in children. INS is broadly defined as the association of NS with nonspecific glomerular abnormalities, including Minimal Change Disease (MCD), Focal segmental Glomerulosclerosis (FSGS) and mesangial proliferative glomerulonephritis (MPGN). On the basis of the patient’s response to steroid treatment, INS is descriptively classified as steroid-sensitive NS (SSNS) or steroid-resistant NS (SRNS), both of which show highly similar clinical manifestations. Clinicians rely heavily on the histology patterns such as MCD that has been shown to be associated with SSNS (50-80%) and FSGS for SRNS patients (10-20%). Thus, researchers are adapting molecular approaches to identify novel and accurate biomarkers that could aid in the diagnosis and prognosis of NS worldwide. In India, recent studies suggest an association of single nucleotide variations in genes expressed (e.g. NPHS1, NPHS2 and C2CAD) within podocytes to that of drug resistance in INS patients. Thus, identification and validation of genetic biomarkers such as mutations in genes NPHS1, NPHS2 and C2CAD would help the physician in appropriate planning and better treatment strategies for the patients. Hence, this study was aimed at looking at the genetic spectra of NPHS2 gene in children diagnosed with INS to identify and validate novel genetic markers that would reflect on the patient’s response to steroid treatment.

Aim:
To evaluate the frequency of NPHS2 gene mutations in Indian children in correlation with the pathogenesis of SRNS.

Methods:
Blood sample
DNA Isolation
Qualitative and quantitative analysis
PCR to amplify specific sequence
Sequencing

Results:
The NPHS2 gene analysis revealed mutations only among the SRNS (14%) subjects and were absent in the SSNS, suggesting their disease-causing nature. Two mutations were noticed one in Exon 4 (Fig1) with amino acid change from arginine to histidine at position 168 and other in Exon 5 (Fig2) were at position 165 the arginine amino acid sequence as been converted to a stop codon resulting in a truncated gene expression.

Discussion and Conclusion:
Our data suggest a significant risk (4%) of two genetic mutation in NPHS2 gene with steroid resistant in INS patients, which is concordance with the published data. In this study, we report two genetic mutations in NPHS2 gene that were unique to samples isolated from SRNS subjects. However, this data needs to be further validated and characterized to determine the exact functional role of these mutations with the development of drug resistance such as steroids.

References:

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Identification of novel gene biomarkers associated with steroid responsiveness in children with Nephrotic syndrome 130