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Research Article

GENE-GENE INTERACTIONS BETWEEN PODOCYTE GENES AND MULTI-DRUG RESISTANCE GENE IN CHILDHOOD NEPHROTIC SYNDROME

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ABSTRACT:

Aim: Nephrotic Syndrome is a kidney disorder that causes the body to excrete too much protein in the urine affecting both adults and children, in which steroid responsiveness is an importation manifestation. The present study is aimed at understanding the steroid responsiveness in NS patients by performing an extended analysis on the interactions of podocyte encoding genes (*NPHS1* and *NPHS2*) in combination with polymorphisms of *ACE* and *MDR1* genes. **Methods:** The comparative analysis between the 3 *MDR1* polymorphisms with *NPHS1/NPHS2* mutations among the patients was performed by Chi-square analysis. The gene-gene and functional profiling of the genes was assessed using bioinformatics tools such as BioGRID and GeneMania databases. All the statistical analysis was performed using SPSS v16.0 software. **Results:** The findings of the study revealed that 9% of the SRNS patients showed *NPHS1* mutation and 18% of the patients had *NPHS2* mutations (18%). Functional profiling revealed important functions such as glomerular development and slit diaphragm which are key players in nephrotic syndrome.
Conclusion: This study suggests that the *MDR1* SNP both independently and in combination with mutations in the *NPHS1/NPHS2* genes could be a potential genetic marker to detect drug resistance and responsiveness to steroids.
KEY WORDS: Gene-gene interactions, nephrotic syndrome, steroid responsiveness, podocyte

INTRODUCTION

Nephrotic syndrome (NS) is a predominant manifestation of glomerular disease, which is characterized by heavy proteinuria and hypoalbuminemia or hypoproteinemia. The progressive forms of the disease can cause Chronic Kidney Disease (CKD) and/or End-Stage Renal Disease (ESRD).[1]Theetiology of NS is increased permeability of serum protein through the damaged basement membrane in the renal glomerulus and has been reported to occur in both children and adults. However, it is said to occur 15 times more common in children than in adults.[1] NS can be classified into two types, based on the patient's response to steroid therapy, namely-steroid sensitive NS (SSNS) and steroid resistant NS (SRNS). This is because steroid responsiveness is the most important prognostic indicator of NS and appears to be the single most important clinical parameter in differentiating patients of primary NS.[2] Predominantly patients with primary NS respond to glucocorticoid therapy and are considered as SSNS. Patients belonging to the SRNS group are those who do not respond to glucocorticoid therapy. In addition, substantial inter-individual differences among NS patients have also been encountered in terms of responsiveness to steroid therapy such as steroid resistance and pattern of disease relapse.[3] Podocytes are highly differentiated polarized epithelial cells. NS are characterized by abnormalities in the podocyte and injury of this cell type typically leads to marked proteinuria.[4] Genetic mutations and polymorphisms in podocyte specific genes have been reported to alter the structure and cellular functions resulting in NS. Studies have reported the association of mutation and single nucleotide polymorphisms in several various genes including *NPHS1*, *NPHS2, MDR1, CD2AP* and *PLCE1* with NS in children. These genes have been reported to be involved in major functions such as establishment of podocyte slit diaphragm, cell growth and differentiation. It is understood that presence of mutations and/or SNPs in candidate genes may influence the response to treatment in NS patients, which is mainly due to inter-individual variation. There are only limited studies on mutations in candidate genes reported in Indian population, which includes our previous study on the influence of *NPHS1, NPHS2, ACE* and *MDR1* in children with NS.[5-7] However, the interactions between these candidate genes were unexplored. Hence, the present study is aimed at understanding the steroid responsiveness in NS patients by performing an extended analysis on the interactions of podocyte encoding genes (*NPHS1* and *NPHS2*) in combination with polymorphisms of *ACE* and *MDR1* genes.

MATERIALS AND METHODOLOGY

The analysis for the gene-gene interactions between *NPHS1, NPHS2* and *MDR1* was performed using the genotyping data in our previous study.[5-7]The study group comprised of 200 children with steroid resistant (SRNS) and steroid□sensitive nephrotic syndrome (SSNS) and 100 age-matched healthy children after obtaining appropriate informed consent. The study was approved by the Institutional Ethics committee of Sri Ramachandra University, Chennai, India.

The relationship between *MDR1, NPHS1* and *NPHS2* genes were determined by comparing the genotypes of the *MDR1* polymorphism with the mutations in both *NPHS1* and *NPHS2* genes among the SRNS patients using SPSS v16.0 software. The comparative analysis for the three genes was not performed in SSNS group as both *NPHS1* and *NPHS2* mutations were not observed. The gene-gene interactions between the 3 genes studied were performed using the BioGRID database.[8]BioGRID is an open access

interaction repository which comprises vast information on protein-protein, protein-chemical and genetic interactions curated from biological literature for all organisms. Each interaction is assigned a unique identifier that tracks the source, date of entry and name of the curator. The interactions obtained from the database, were curated based on strong experimental evidence. The information obtained from such curations provides biological processes as gene annotations or ontology terms (GO), the number of genes involved for various disease conditions. The outcome of the BioGRID dataset was confirmed using GeneMANIA database, [9] which provides extended information about gene functions using the Enriched Gene Ontology category. This database can predict the function of either a single gene query or multiple sets of genes. In addition to providing information on gene functions, GeneMANIA also provides network layout of the gene-gene interactions among the query genes as well as the non-query/predicted genes.

RESULTS

Comparative analysis of *MDR1* polymorphisms with *NPHS1* mutation

The distribution of genotypes for MDR1 polymorphism (C3435T) among the SRNS patients was found to be 8%, 74% and 18% for CC, CT and TT genotypes respectively as shown in Figure 1a. The frequency of *NPHS1* mutation was found to be higher in individuals with CT and TT genotypes. However, statistical significance was observed only in TT genotype (χ^2 =5.13, p=0.04), indicating that presence of both the risk allele of MDR1 polymorphism and *NPHS1* mutation could increase the severity of NS. In case of the tri-allelic G2677T polymorphism, the genotype distribution among SRNS group was observed as 9%, 41%, 34%, 11% and 5% for GG, GT, TT, GA and TA respectively (Figure 1b). *NPHS1* mutation was found to be higher in individuals with GT and GA genotypes, however, the difference was not statistically significant. The genotypes of C1236T polymorphism showed a distribution of 20%, 44% and 36% for CC, CT and TT genotypes respectively (Figure 1c). The frequency of *NPHS1* mutation was found to be 4%, 4% and 1% in the individuals with CC, CT and TT genotypes, but there was no significant difference.

Comparative analysis of MDR1 polymorphisms with NPHS2 mutation

The comparative analysis between the 3 *MDR1* polymorphisms and *NPHS2* mutation revealed the following results. The analysis of C3435T polymorphism and *NPHS2* mutation showed that the mutation was observed in 1%, 13% and 3% of the SRNS patients with CC, CT and TT genotypes respectively. The distribution of the genotypes of C3435T polymorphism and presence/absence of *NPHS2* mutation are illustrated in Figure 1d. Meanwhile in case of the tri-allelic polymorphism G2677T, the frequency of the genotypes were found to be 9%, 33%, 28%, 10% and 3% for GG, GT, TT, GA and TA genotypes respectively. Among these 5 genotypes of *MDR1* polymorphism, *NPHS2* mutation was observed in GT, TT, GA and TA genotypes (Figure 1e). The distribution of genotypes for C1236T polymorphism was observed as 17%, 35% and 31% for CC, CT and TT, in that, the frequency of *NPHS2* mutation was found to be 3%, 9% and 5% respectively (Figure 1f).

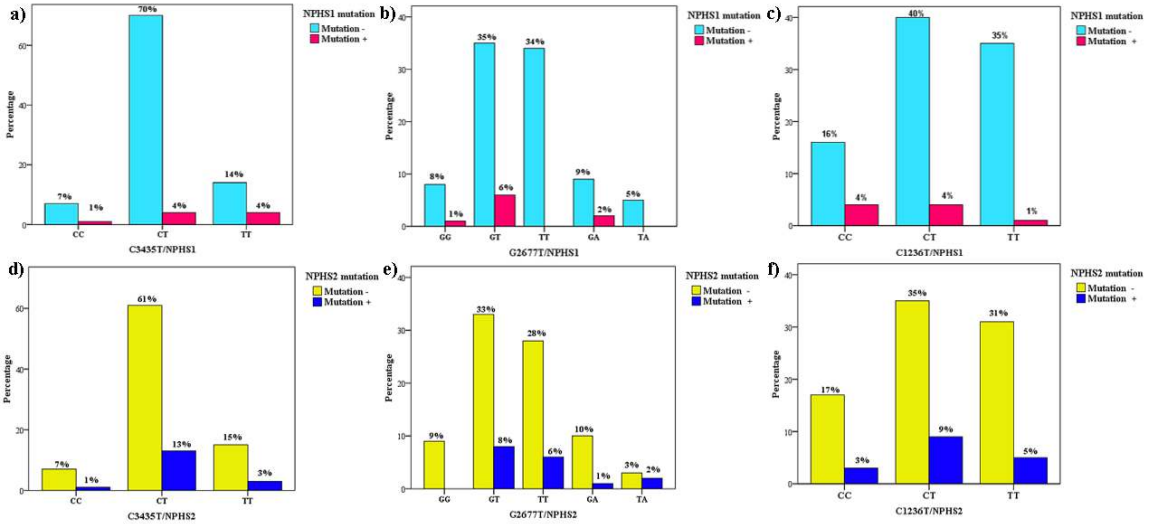

Figure 1: Comparative analysis of *MDR1* polymorphisms with (a-c) *NPHS1* mutation and (d-f) with *NPHS2* mutation.

Gene-Gene interactions and functional profiling of *NPHS1, NPHS2* and *MDR1* genes:

The BioGRID dataset revealed several interactions, physical and/or genetic, for *NPHS1, NPHS2* and *MDR1 (ABCB1)* genes. In case of *NPHS1*, the analysis revealed 22 physical interactions and 7 genetic interactions with several genes, including *KIRREL* (kin of IRRE like) and *NPHS2*. The analysis also revealed that *NPHS1* had both physical and genetic interactions with *NPHS2* exhibiting the property of phenotypic enhancement, suggesting that a mutation in *NPHS1* would result in the enhancement of any phenotype associated with mutation of *NPHS2*. On the contrary, *NPHS1* was found to have a phenotypic suppression property with *CDC42*. The list of genes with physical and genetic interactions is given in the Table 1. In addition to the gene-gene interactions, the dataset also provided information on the key functions of *NPHS1* in terms of biological process, molecular function and cellular component. In case of *NPHS2,* the analysis revealed 9 physical interactions with genes such as *CD2AP* (CD2-associated protein), *NPHS1* and *SH3KBP1* (SH3-domain kinase binding protein-1). The analysis did not reveal any genetic interaction between *NPHS2* and another gene. In case of *MDR1 (ABCB1)*, the analysis revealed 24 physical interactions with several genes. No genetic interaction was observed with *MDR1* gene. The functions of *NPHS1, NPHS2* and *MDR1* (*ABCB1*) genes in terms of biological process, molecular function and cellular component are shown in Table 2.GeneMania database revealed similar interactions between the 3 genes (*NPHS1, NPHS2* and *MDR1*) and various important functions related to nephrotic syndrome. Table 3 shows the list of functions for the *NPHS1:NPHS2:MDR1* gene interaction. In addition to the gene functions, interactions between *NPHS1, NPHS2* and *MDR1* genes and with

other related target genes were assessed. The network analysis reveals that *NPHS1* and *NPHS2* had strong interaction and *ABCB1* had strong interaction with *NPHS2* .The gene-gene network is illustrated in the Figure 2. *NPHS1* and *NPHS2* are found to play an important role in glomerular visceral cell differentiation, glomerular epithelial cell differentiation, glomerular epithelial development, renal filtration cell differentiation ,cell-cell junction ,epithelial cell differentiation are found to be involved in kidney development. Whereas *ABCB1* gene was found to function mainly in cell-cell junction in Nephrotic syndrome (NS).

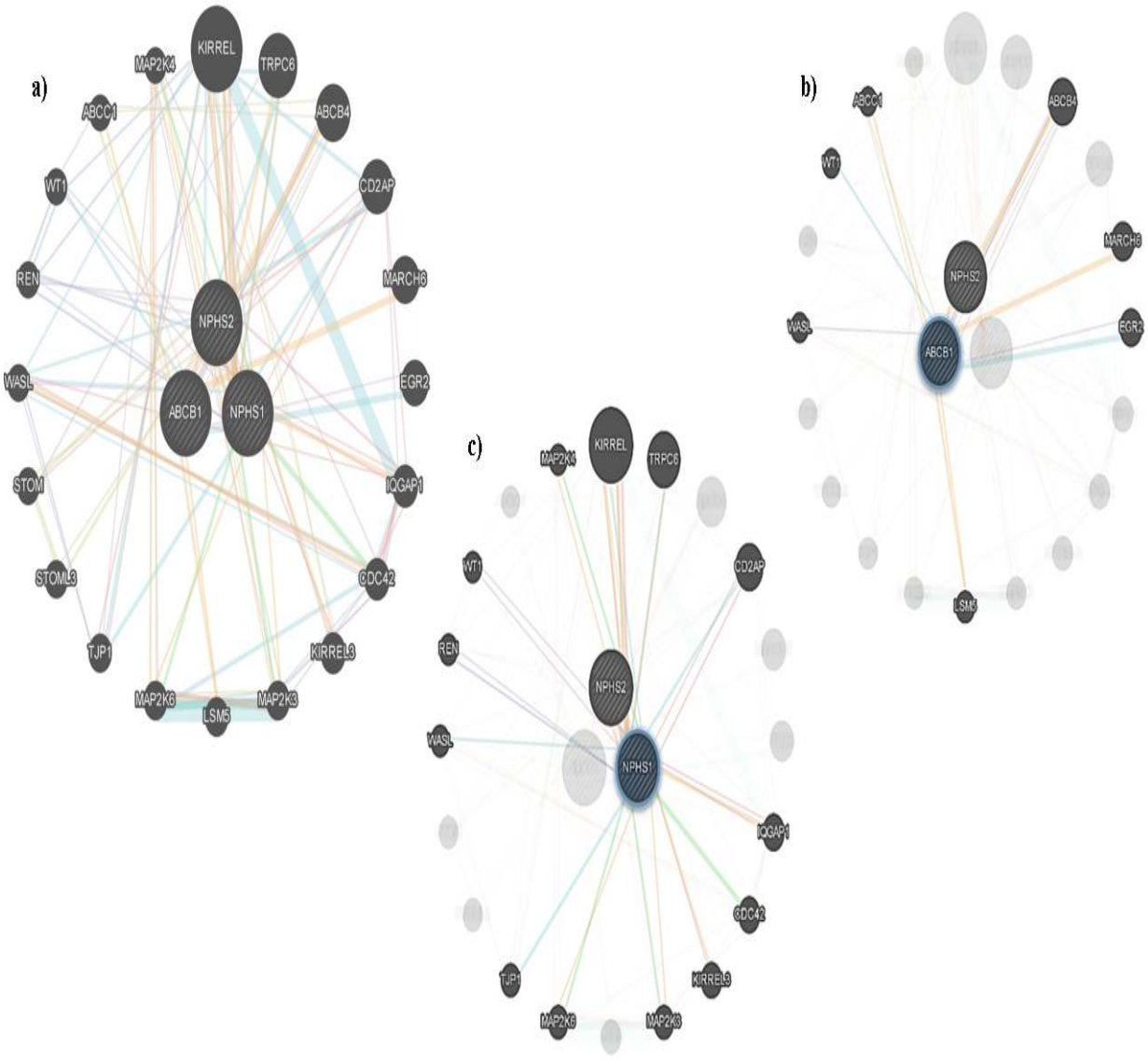

Figure 2: Genetics interactions between *NPHS1:NPHS2:MDR1(ABCB1*) network obtained from GeneMania database.

Table 1: List of physical and genetic interactions of *NPHS1* gene analysed using BioGRID database

Query Gene	Physical interaction	Genetic interaction
NPHS1	*ALB*	*CDC4*
	CANX	*MAP2K3*
	CD2AP	*MAP2K4*
	FYN	*MAP2K6*
	IQGAP1	*NPHS2*
	KIRREL	*RAC1*
	NPHS2	*RHOA*
	SH3KBP1	
	YES1	

Table 2: Functionality assessment of *NPHS1, NPHS2*and*MDR1*genes analysed using BioGRID database

Biological Process	Molecular Function	Cellular Component
NPHS1		
Excretion	Myosin binding	Extracellular vesicular exosome
Glomerular basement membrane development	Protein binding	Integral component of plasma membrane
Glomerular visceral epithelial cell development		Plasma membrane
		Slit diaphragm
NPHS2		
Actin cytoskeleton reorganization	Protein binding	Cell-cell junction
Metanephric glomerular visceral epithelial cell development		Extracellular vesicular exosome
		Intrinsic component of the cytoplasmic side of the plasma membrane
		Membrane raft
		Plasma membrane
		Protein complex
		Slit diaphragm
MDR1		
G2/M transition of mitotic cell	ATPase activity, coupled to transmembrane movement of substances	Cell surface
Drug transmembrane transport	Protein binding	Extracellular vesicular exosome
Response to drug	Transporter activity	Integral component of membrane
Small molecule metabolic process		Membrane
Stem cell proliferation		Plasma membrane
Transmembrane transport		

Table 3: Functionality assessment of the interactions betweenNPHS1:NPHS2:MDR1 using GeneMania database

Function	FDR	Genes in network	Genes in genome
glomerular visceral epithelial cell differentiation	5.02772E-06	4	12
glomerular epithelial cell differentiation	5.02772E-06	4	13
glomerular epithelium development	5.02772E-06	4	13
renal filtration cell differentiation	5.02772E-06	4	12
cell-cell junction	5.88679E-06	7	189
epithelial cell differentiation involved in kidney development	0.000109966	4	29
cell differentiation involved in kidney development	0.000161888	4	33
glomerulus development	0.000251913	4	39
kidney epithelium development	0.000251913	4	39
kidney development	0.000422691	5	117
renal system development	0.000534586	5	125
nephron development	0.000593384	4	52
urogenital system development	0.000593384	5	132
epithelial cell development	0.000882277	4	59
toll-like receptor 10 signaling pathway	0.068023777	3	65
toll-like receptor 5 signaling pathway	0.068023777	3	65
cellular response to gonadotropin stimulus	0.068023777	2	10
toll-like receptor TLR1:TLR2 signaling pathway	0.071624385	3	71
toll-like receptor TLR6:TLR2 signaling pathway	0.071624385	3	71
metanephric epithelium development	0.071624385	2	11
response to gonadotropin	0.071624385	2	11
metanephric glomerulus development	0.074000729	2	12
toll-like receptor 9 signaling pathway	0.074000729	3	74
TRIF-dependent toll-like receptor signaling pathway	0.076648631	3	76
toll-like receptor 2 signaling pathway	0.076648631	3	77
MyD88-independent toll-like receptor signaling pathway	0.079529609	3	79
MyD88-dependent toll-like receptor signaling pathway	0.079529609	3	80
toll-like receptor 3 signaling pathway	0.082615595	3	83
MAP kinase kinase activity	0.082615595	2	14

DISCUSSION

Genetic interactions have been studied years in model organisms as a means of identifying there functional relationships among genes or their corresponding gene products and also, with the nature of these relationships depending on the types of interactions.[10] Sometimes mutations in two genes produce a phenotype that is surprising in light of each mutation's individual effects. This phenomenon, which defines genetic interaction, can reveal functional relationships between genes and pathways. Gene interaction between multiple genes has an impact on the expression of an organism's phenotype. The genetic interplay between podocyte genes and their influence on the clinical phenotype of NS has been previously studied as for instance, both nephrin and podocin may interact directly or indirectly and are important for maintenance of the glomerular capillary permeability barrier.[11-14] To the best of our knowledge, there has been no report so far on interactions between podocyte genes (*NPHS1, NPHS2*) in combination with the genes involved in regulating fluid circulation (*ACE*) and drug traffic (*MDR*1) at the target cell. Hence, the study has extended the analysis on the interactions of podocyte encoding genes in combination with *MDR1* SNPs, towards steroid responsiveness in NS patients.

The findings of the present study suggests that this SNP both independently and in combination with mutations in the *NPHS1/NPHS2* genes is a potential genetic marker to detect drug resistance and responsiveness to steroids. If our hypothesis is correct, then one can expect a mutation in the candidate genes (*NPHS1, NPHS2*) in all the SRNS patients. However, only 27% of the patients showed mutation despite the entire candidate gens (29 exons in *NPHS1* and 8 exons in *NPHS2*) were sequenced. Of which, 17% are novel and has not been reported in any population. It supports the notion that the type and frequency of mutation may depends upon the life-style and/or ethnic variation; in addition to those candidate genes other genes such as *PLCE1, CD2AP, LAMB2, TRPC6* and many other genes might play an equally important role in regulating the renal physiology and dysregulation could results in NS. 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 perceptive 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.

CONCLUSION

The present study has investigated the interactions between *NPHS1* and *NPHS2* mutations in combination with 3 SNPs in *MDR1* gene. In addition, the bioinformatics analysis of the 3 genes revealed genetic interactions with other genes such as *CD2AP, KIRREL* and *TRPC6* which play critical roles in glomerular development and slit

diaphragm. The findings of the study revealed that determining these genetic interactions will help in reducing the risk of resistance to steroid responsiveness in children in NS.

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