Abstract

Nephrotic syndrome (NS) is one of the commonly diagnose kidney disease in children and its progressive forms can lead to end stage renal disease (ESRD). In this study we deliberate the potential role of two important podocyte genes (i.e. TRPC6 and NPHS2) involved in childhood nephrotic syndrome (NS). Both the genes seem to be allied with the slit pore, where it is perhaps concerned in slit diaphragm signaling. Single nucleotide polymorphisms (SNPs) in targeted gene are known to be associated in children with NS. It was observed that the SNPs in TRPC6 and NPHS2 gene may critically altered the functions of transient receptor potential cation channel-6 (TRPC6) and podocin protein. We performed in silico analysis and next generation sequencing (NGS) analysis to characterize the targeted gene polymorphisms associated with NS

SNP data for in silico analysis was retrieved from dbSNP-NCBI and further used to investigate damaging effect using SIFT, PolyPhen, SNP&Go, PROVEAN, and PANTHER. The comparative analysis predicted 5 SNPs, p.A404V and p.N157T (TRPC6 gene) and p.P20L, p.G92C and p.D160G (NPHS2 gene) showing damaging effect (score of 0.096-1.00). SNPs that may possibly undergo post-translation modifications were also identified in TRPC6 and podocin protein. It was found that polymorphism p.N157T can lead to alteration in Glycation sites of TRPC6 protein whereas polymorphisms p.A404V and p.G92C were present at ligand binding sites of podocin protein. Additionally, the energy minimization values and docking score of mutant and native molecule confirmed the pathogenic effect of mutant molecule in compare to wild type, where the p.P20L showed more prominent effect in regulating protein structural stability.

Sequencing analysis predictions using GATK identified 70 different SNPs in the intronic, missense, synonymous 3’ UTR, 5’UTR and splice regions of targeted genes (50 in TRPC6 and 20 in NPHS2). 55.71% of these identified SNPs were reported for the first time in the present study, which were further accepted by ClinVar-NCBI. The remaining 44.28 % of these SNPs were already reported in different groups over the worldwide. Fold expression analysis at transcript level of TRPC6 and podocin protein using RT-qPCR shows up-regulation of TRPC6 and down-regulation of podocin protein in cases compared to controls. Thus, our results signifies the fact that SNPs of targeted genes might affect the protein fold expression levels and adds up the development of NS in Indian children.