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Original Article

PREDICTION OF FUNCTIONAL, STRUCTURAL AND STABILITY CHANGES IN *PMM2* GENE ASSOCIATED WITH NEPHROTIC SYNDROME USING COMPUTATIONAL ANALYSIS

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ABSTRACT

Objective: Nephrotic syndrome defines as a disorder with a group of symptoms like proteinuria, hypoalbuminemia, hyperlipidemia, and edema. *PMM2* encodes phosphomannosemutase protein enzyme involved in the synthesis of N-glycan.

Methods: Different Insilico analysis tools: SIFT, PolyPhen, PROVEAN, SNPandGO, MetaSNP, PhDSNP, MutPred, I-Mutant, STRUM, PROCHECK-Ramachandran, COACH and ConSurf, were used to check the effect of nsSNP on protein structure and function.

Results: The genetic polymorphism in the *PMM2* gene was retrieved from NCBI ClinVar and UniProtKB. Total 20 SNPs were predicted most significant and responsible for disease-causing and decrease protein stability.

Conclusion: This study helps to discover disease-causing deleterious SNPs with different computational tools and gives information about potent SNPs.

Keywords: nsSNP, *PMM2*, Nephrotic syndrome, Insilico analysis

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INTRODUCTION

Nephrotic syndrome (NS) defines as a heterozygous group of disorders with clinical features like proteinuria, hypoalbuminemia, hyperlipidemia, and edema [1, 2]. The prevalence of nephrotic syndrome ranges from 12-16 per 100,001, and the annual impact in children ranges from 2-7 per 100,000 in India [3]. Based on steroid therapy, nephrotic syndrome is classified as steroid-sensitive nephrotic syndrome (SSNS) and steroid resistance nephrotic syndrome (SRNS) [4]. Aebi and his colleague found that Phosphomannosemutase (*PMM2)* is a causative gene responsible for steroid-resistant nephrotic syndrome [5]. Phosphomannosemutase is a homodimeric protein enzyme, which synthesizes a critical component for N-glycan. It is located on chromosome number 16 and carried out N or C glycosylation of protein [6]. This metabolic enzyme is activated by glucose 1-6 bisphosphate and is involved in the isomerization of mannose 6 phosphate into mannose 1 phosphate in the cytosol of the podocyte cell. The end product of the isomerization process is GDP-mannose and required for the biogenesis of N-glycans. Protein glycosylation is required for cell attachment to the glomerular basement membrane and podocyte morphology [7]. Several animal studies reveal the importance of glycosylation in nephron maturation. Membrane channels present in podocyte is excessively made up of n-linked glycosylated protein. It is also reported that these channels perpetuate the tubular structure of the nephron and affect the filtration process of the kidney [8]. Single nucleotide polymorphism (SNPs) is a DNA sequencing variation at a definite location in the genome [9]. It is an interchange of a single DNA building block and happens once in every 1,000 nucleotides in the genome. There are around 4 to 5 million SNPs in a person's genome [10]. There were approximately 500,000 SNPs that fall in the coding region of the human genome. Recent studies show that about 50% of genetic mutation is due to nsSNP involved in various genetic diseases [11, 12]. There were>9 million SNPs reported in the public database [13]. To screen out these SNPs experimentally is time-consuming, costly, and very tedious. The computational analysis narrows down these SNPs and can be used as an alternative method. The Insilico analysis technique draws up the most affecting and significant nsSNP from the large dataset, and it is authentic and cost-effective [14].

MATERIALS AND METHODS

Data mining

The genetic polymorphism in the *PMM2* gene was retrieved from NCBI ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and UniProtKB (<https://www.uniprot.org/>). Swissport database was used for retrieving the protein sequence database of this gene.

Functional analysis of prediction tools

SIFT (https://sift.bii.a-star.edu.sg/www/)

Sorting Intolerant from Tolerant (SIFT) software is used to predict amino acid substitution on protein function [15]. It signifies a score ranging from 0.0 to 1.0 for evaluating change in an amino acid deleterious or tolerated. It is utilizing the rsID of SNPs as input queries from NCBI and SwissProt databases.

PolyPhen (genetics.bwh.harvard.edu/pph2/)

Polymorphism and phenotype (PolyPhen) work in a physical and comparative way to study the impact of amino acid substitution in the coding part of the gene. The predicted result was obtained in probability score classifying changes as 'probably damaging,' 'possibly damaging,' and 'benign' [16].

PROVEAN (provean.jcvi.org/index.php)

Protein Variation Effect Analyzer (PROVEAN) is a sequence homology-based tool to estimate the nsSNP variation effect on protein function. These SNP variations are predicted according to the cutoff score of-2.5. If the cutoff score is<-2.5, it is "deleterious," and the cutoff score is>-2.5, it is "neutral" [17].

SNPandGo (https://snps.biofold.org/snps-and-go/snpsandgo.html)

SNP and Go is a tool used for the prediction of disease-related mutation. It is required the FASTA sequence of the protein as input

and gives the result as a disease (RI>5) or neutral (RI<5) based on RI (reliability index) [18].

Meta SNP (snps.biofold.org/meta.snp)

Meta SNP is used to predict a single nucleotide variation in protein sequence, and these prediction results are from different tools like PANTHER, PhD-SNP, SIFT, and SNAP [19]. The output value>0.5 is considered as a disease, and<0.5 is neutral.

PhD SNP (https://snps.biofold.org/phd-snp/phd-snp.html)

Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP) is a tool used to predict the effect of nsSNP on protein function. It interprets results like disease or neutral [20].

Mutpred (http://mutpred.mutdb.org/)

Mutpred requires a FASTA file and amino acid variants as input. It gives the result in the form of the molecular mechanism and the probability of change in the protein structure and function [21].

Analysis of protein stability

I-Mutant (folding.biofold.org/i-mutant/I mutant2.0.html/)

I-Mutant is supporting vector-based software which predicts the effect of a single nucleotide change in protein stability. It is determining an increase or decrease in protein stability [22]. It results in free energy change (ddG) and the sign of prediction value as positive or negative.

STRUM (https://zhanglab.ccmb.med.umich.edu/STRUM/)

Structure-based prediction of protein stability change upon single-point mutation (STRUM) is a tool that predicts change instability of a single nucleotide change. It gives the ddG value on change in a single nucleotide [23].

Protein modelling

I-Tasser (https://zhanglab.ccmb.med.umich.edu/I-TASSER/)

Iterative Threading Assembly Refinement (I-TASSER) is software used for protein 3D modeling from the FASTA sequence of the protein. It is generating five different models of the protein having different c-score. Based on the c-score, the appropriate model is selected. C-score is a confidence score in the range of-5 to 2. Higher the c-score, which means a high confidence level and vice-versa [24].

Discovery studio (http://accelrys.com/products/collaborative-science/biovia-discovery-studio)

Discovery Studio is software used for protein modeling and its targets. It is used for generating the 3D structure of the model from the protein PDB file [25].

Structure validation

PROCHECK-Ramachandranplot (https://servicesn.mbi.ucla.edu/PROCHECK/)

Based on the c-score of the model, the PDB structure was selected. This model was verified by the Ramachandran plot using the PROCHECK server. A two-dimensional plot is used to distribute amino acids in the different conformation of the ψ and φ angles [14].

Identification of ligand binding site

COACH (https://zhanglab.ccmb.med.umich.edu/COACH/)

The ligand-binding site of *PMM2* protein was estimated by using the COACH server. It is a free software used for the prediction of the ligand-binding site of *PMM2* protein. COACH is a meta-server-based software and works on different methods like S-SITE and TM-SITE. It requires a PDB file of the protein and generated a 3D protein structure [26].

Phylogenic analysis

ConSurf (ConSurf.tau.ac.il/)

For the determination of evolutionarily conserved regions within *PMM2* protein, the ConSurf web-server was used. After submitting the FASTA sequence in the ConSurf, position-specific conservation scores were calculated using an empirical Bayesian algorithm. These conservation scores having well-defined scales of nine grades, i.e., 1-9. A score near 9 represent more conserved, and near one, it means variable.

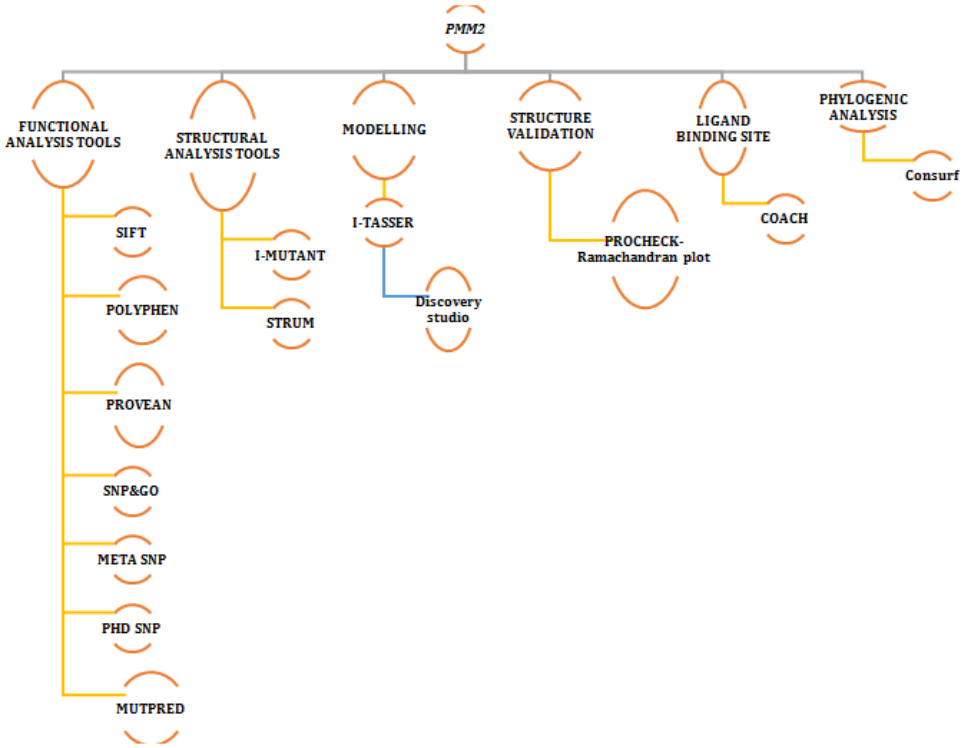


Fig. 1: Diagrammatic representation of computational tools used for *Insilico* analysis of the *PMM2* gene

RESULTS

SNPs for the *PMM2* gene were retrieved from uniport and ClinVar. In ClinVar, there were 356 SNPs in which 15 frameshift variants, 91 missense variants, 12 nonsense variants, 16 splice region variants, and 80 in the UTR region. We have selected only missense variants for this study.

Functional analysis tools

In this study total of seven functional analysis tools (fig. 1) were used. These tools included: SIFT, PolyPhen, PROVEAN, SNPandGO, MetaSNP, PhDSNP, and MutPred. According to SIFT results, a total of 53 variants were identified as affect protein function, and others were tolerated. This prediction was based on the SIFT prediction score. All 53 variants were further analyzed by PolyPhen, PROVEAN, SNP and GO, METASNP, PhDSNP, and MutPred tools. PolyPhen predicted variants as probably damaging, possibly damaging and benign. Out of 53 variants, 43 variants were predicted as probably damaging, 17 were possibly damaging, and 16 were benign. PROVEAN predicts SNPs either deleterious or neutral. Out of 53 variants, 52 were predicted as deleterious by SNP and GO and 49 by MetaSNP. PhDSNP is a very accurate SVM-based prediction method. It revealed only 34 SNPs were diseased ones, and the rest 22 SNPs were neutral. MutPred further validated these 34 variants. Mutpred predicts the association between deleterious variation and disease condition. These SNPs were predicted as loss of strand, altered stability, an altered transmembrane protein, and loss of Acetylation. Mutpred predicts a deleterious effect of these variants, given in the table (table 1).

Table 1: nsSNP predicted to be functionally significant in *PMM2* protein using functional analysis tools

rsID	Amino acid change	Sift	Polyphan	Provean	Snpand GO	Metasnp	PhD SNP	Mutpred
rs758340382	R21W	APF	PD	Deleterious	D	D	D	Loss of Acetylation
rs398123312	L32R	APF	PD	Deleterious	D	D	D	Loss of Acetylation; Altered Stability
rs755402538	G42R	APF	PD	Deleterious	D	D	D	Altered DNA binding
rs104894534	V44A	APF	PD	Deleterious	D	D	D	Altered Transmembrane protein; Altered Stability
rs770458492	L104V	APF	PD	Deleterious	D	D	D	-
rs387906824	Y106K	APF	PD	Deleterious	D	D	D	Gain of Acetylation; Altered Transmembrane protein
rs80338700	P113L	APF	PD	Deleterious	D	D	D	Altered Transmembrane protein
rs104894530	G117R	APF	PD	Deleterious	D	D	D	Loss of Strand; Altered Transmembrane protein
rs1057517110	F119L	APF	PD	Deleterious	D	D	D	Loss of Strand
rs368582085	I120T	APF	PD	Deleterious	D	D	D	Loss of Strand; Altered Stability
rs190521996	F157S	APF	PD	Deleterious	D	D	D	Loss of Acetylation; Altered Stability
rs941830625	G175R	APF	PD	Deleterious	D	D	D	Altered Metal-binding; Gain of ADP-ribosylation
rs780581250	F183S	APF	PD	Deleterious	D	D	D	Altered Transmembrane protein; Altered Stability; Loss of Proteolytic cleavage
rs80338704	D188G	APF	PD	Deleterious	D	D	D	Gain of Acetylation; Altered DNA binding; Altered Transmembrane protein
rs532870929	F207S	APF	PD	Deleterious	D	D	D	Altered Stability; Loss of Acetylation; Loss of Allosteric site
rs398123309	G208A	APF	PD	Deleterious	D	D	D	Loss of Allosteric site; Altered Transmembrane protein
rs78290141	N216S	APF	PD	Deleterious	D	D	D	Altered Metal-binding; Altered Transmembrane protein
rs752614554	D217E	APF	PD	Deleterious	D	D	D	Altered Metal-binding; Altered Transmembrane protein
rs80338706	T226S	APF	PD	Deleterious	D	D	D	-
rs558826439	G228R	APF	PD	Deleterious	D	D	D	Altered Metal binding
rs80338708	V231M	APF	PD	Deleterious	D	D	D	Altered Ordered interface; Loss of Relative solvent accessibility
rs80338708	T237R	APF	PD	Deleterious	D	D	D	Loss of Relative solvent accessibility; Altered Metal binding

APF: Affect protein function, PD: Probably damaging, D: Disease

Table 2: Stability prediction analysis of *PMM2* protein by I-Mutant and STRUM

rsID	Amino acid change	I-mutant		strum
		Stability	Free energy change	ddG
rs758340382	R21W	Decreases	-0.21	-0.42
rs398123312	L32R	Decreases	-1.05	-4.58
rs755402538	G42R	Decreases	-1.81	-1.92
rs104894534	V44A	Decreases	-2.27	-1.82
rs770458492	L104V	Decreases	-0.58	-0.38
rs387906824	Y106K	Decreases	-0.57	-1.23
rs80338700	P113L	Decreases	-1.02	-0.2
rs104894530	G117R	Decreases	-1.83	-1.23
rs1057517110	F119L	Decreases	-2.34	-0.99
rs368582085	I120T	Decreases	-1.26	-1.71
rs190521996	F157S	Decreases	-2.24	-2.42
rs941830625	G175R	Decreases	-2.36	-0.39
rs780581250	F183S	Decreases	-2.99	-2.31
rs80338704	D188G	Decreases	-2.07	-0.53
rs532870929	F207S	Decreases	-3.74	-2.25
rs398123309	G208A	Decreases	-2.18	-1.23
rs78290141	N216S	Decreases	-0.74	-0.9
rs80338706	T226S	Decreases	-0.23	-1.0
rs558826439	G228R	Decreases	-1.39	-0.4
rs80338708	V231M	Decreases	-2.26	-1.51

Analysis of protein stability

I-Mutant and STRUM were used to predict the variant's stability. I-Mutant indicates RI and free energy change value for nsSNP. STRUM is also giving the ddG (free energy change) value for a single nucleotide change. Out of 22 SNPs, 20 SNPs show decreased protein stability for both I-Mutant and STRUM. The result of I-Mutant and STRUM is shown in the table (table 2).

Protein modeling

The 3D structure of the protein was derived from I-Tasser. The 3D structure of *PMM2* protein was generated by submitting the FASTA sequence of protein after changing its amino acid to its altered amino acid. I-Tasser generates a 3D model of the protein and based on the c-score, the most stable structure was selected.

Structure validation

Ramachandran plot (fig. 2.) is used to validate the structural stability of the protein. Out of all the amino acids, 186 amino acids (85.7%) were found to be in the favored region, 28 (12.9%) amino acids were in the additional allowed region, 3(1.4%) were in generously allowed regions, and 0% were in the disallowed region. From the Ramachandran plot, the *PMM2* protein structure can be considered appropriate.

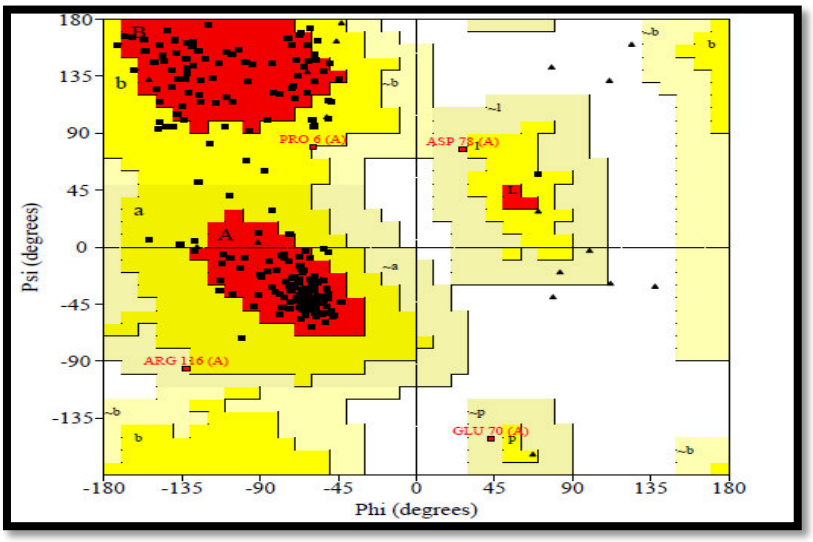


Fig. 2: Ramachandran plot of modeled *PMM2* protein

Identification of ligand-binding site

The most stable structure of the protein is used to determine the ligand-binding site of the enzyme. Those SNPs present in the Ligand binding site of *PMM2* protein were Arg21, Gly175, Asn216, Thr226, and Gly228. The Asn216 and Arg21 were involved in the binding of Mannose 6 phosphate and Mannose 1 phosphate, which are the substrate of protein glycosylation. These five binding sites are shown in Fig.3. by using pymol. Table 3 shows the results of the COACH server.

Phylogenic analysis

ConSurf

Conserved and variable regions of *PMM2* protein were predicted by the ConSurf server (table 3). The R21W, G175R and N216S have a conservation score of 9 and T226S and G228R have 8. All the amino acid residues fall in conserved regions, showing more possibilities to alter the protein structure [21]. The 3D structure and ligand-binding sites present in different conserved regions of *PMM2* protein mentioned in fig. 4.

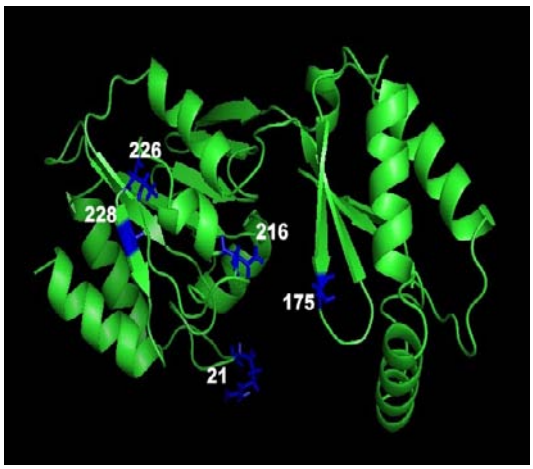
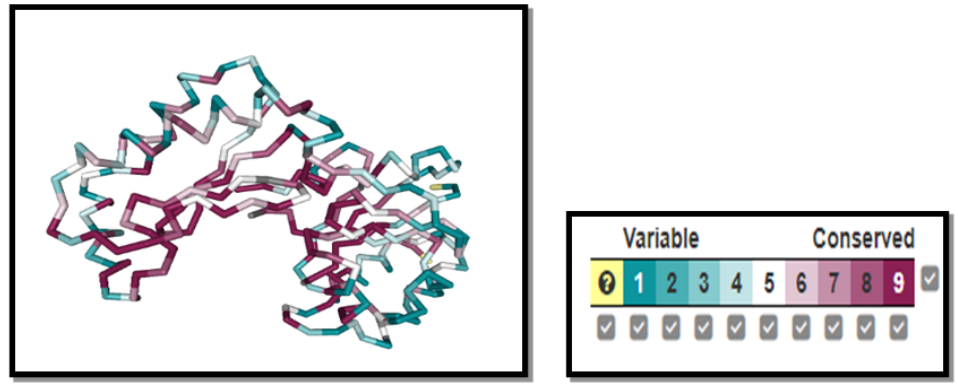


Fig. 3: Ligand binding sites within *PMM2* protein

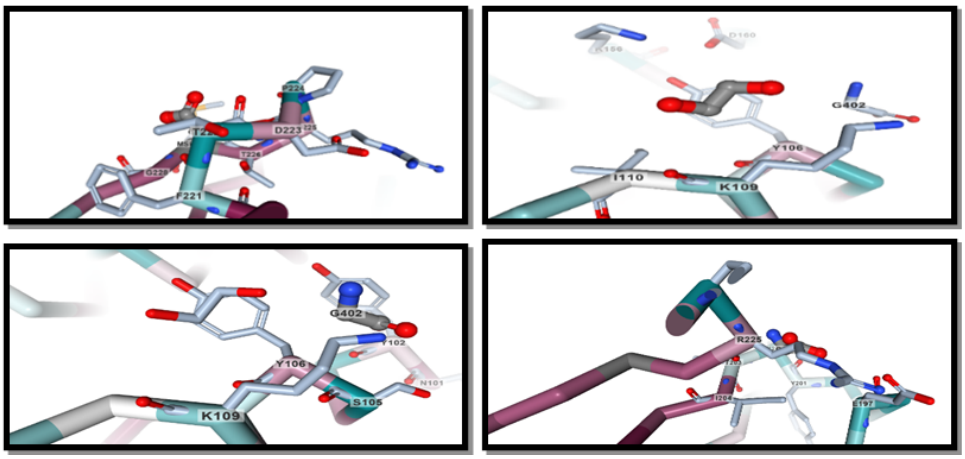
Table 3: Prediction of ligand binding sites and phylogenetic conservation within *PMM2* protein

rsID	Amino acid change	Ligand name (coach)	ConSurf conservation score
rs758340382	R21W	M1P	9/conserved
rs941830625	G175R	M6P	9/conserved
rs78290141	N216S	PO4, M6P, MG	9/conserved
rs80338706	T226S	GLY, MG	8/conserved
rs558826439	G228R	GLY, MG	8/conserved

M1P: mannose 1 phosphate, M6P: mannose 1 phosphate, PO4: phosphate, MG: magnesium, GLY: glycine



(a)



(b)

Fig. 4: (a) Conserved region in *PMM2* protein (b) Ligand binding sites in different conserved regions of *PMM2* gene

DISCUSSION

PMM2 is a metabolic protein and is mainly involved in the glycosylation of protein. Protein glycosylation requires for normal structure and function of protein which are involved in kidney morphogenesis. Glycoproteins are also required in cell adhesion in the basement membrane and cell migration [27]. *In vitro* study reveals that glycosylation also requires for functional growth of nephron. SNPs are genetic variations involved in various genetic diseases. Single nucleotide polymorphisms are linked to phenotypic and genotypic traits of individuals. Monogenic and inherited diseases are also correlated with single nucleotide change [28]. *PMM2* gene variants have also been linked to other diseases like a congenital disorder of glycosylation [29]. Not all nsSNP have a deleterious effect on protein function, so different bioinformatic tools were used to identify the effect of these SNPs. These tools are SIFT, PolyPhen, PROVEAN, SNPandGO, MetaSNP, PhDSNP, MutPred, and I-Mutant. After functional analysis, a total of 22 variants were found to be deleterious (table 1) in human *PMM2* protein. Despite p. Thr237Arg and p. Asp217Glu variants, all other SNPs have decreased the stability of the protein. Arg21, Gly175, Asn216, Thr226, and Gly228 were involved in the enzyme's ligand-binding site, and they are conserved and have a structural and functional effect on *PMM2* protein by the ConSurf server (table 3). Single nucleotide change in the ligand-binding site may affect interaction and interfere in the normal function of the enzyme and affect its stability. Several genetic studies reveal the role of the *PMM2* gene in a disease condition. Sarah and his colleagues found two mutations (p. Arg141His and p. Val231Met) in patients having a congenital disorder of glycosylation-Ia [30]. Two distinct variants (p. Arg141His and p. Arg215Leu) of the *PMM2* gene were reported in the Argentinean population [31]. Different *PMM2* variants were enlisted according to the Insilico analytical study of the congenital disorder of glycosylation, i.e., p. Asp65Tyr, p. Ile132Asn, p. Ile132Thr, and p. Phe183Ser [32]. Casado and his team discovered two mutations in two different patients with a congenital glycosylation disorder; they were p. Cys241Ser, p. Arg123Gln and p. Gly722Cys, p. Phe157Ser, respectively [29]. Several variants were also characterized in a proteolytic expression system. They were affecting protein stability (p. Arg123Gln and p. Arg141His). These two variants were disrupting dimer interface (p. Pro113Leu and p. Thr118Ser) and few others involved in misfolding changes (p. Leu32Arg, p. Val44Ala, p. Asp65Tyr, p. Phe157Ser, p. Pro184Thr, p. Phe207Ser, p. Thr237Met, and p. Cys241Ser) [33]. Patricia and his colleague found nine different mutations in the *PMM2* gene. Out of nine, six mutations (p. Val44Ala, p. Asp65Tyr, p. Arg162Trp, p. Thr237Met, p. Phe207Ser, and p. Cys241Ser) retained some residual activity of the protein. Two of them (p. Arg123Gln, and p. Arg141His) affected protein folding and catalytic property of *PMM2* protein. Mutation position p. Pro113Leu is associated with the dimerization of *PMM2* protein [34]. A study on hyperinsulinemic hypoglycemia and congenital polycystic kidney disease revealed promoter region mutation (p. Gly167Thr) on the *PMM2* gene [35]. This study helps to understand the effect of nsSNP on *PMM2* protein and suggesting that computer-based analysis help to select SNPs responsible for altering protein and affecting protein phenotype. This Insilico analysis is helpful for further laboratory experiments, i.e., these variants are further validated by lab-based experiments.

CONCLUSION

PMM2 protein is mainly involved in the synthesis of membrane channels of the nephron. In this study, nsSNP of the *PMM2* gene was determined by various bioinformatics tools. Total twenty significant SNPs were predicted as disease-causing. Among the most significant SNPs, Arg21, Gly175, Asn216, Thr226, and Gly228 were associated

with the protein's ligand-binding site. These SNPs can affect protein and are considered vital components that are causing diseases related to *PMM2* malfunction and help in drug discovery. This variation has been utilized for diagnosis as well as a therapeutic target for genetic diseases. Bioinformatic outcomes may be helpful for further lab-based experiments to study the effect of polymorphism on protein function.

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ABBREVIATION

Phosphomannosemutase (*PMM2*), Steroid resistance nephrotic syndrome (SRNS), Steroid sensitive nephrotic syndrome (SSNS), Single nucleotide polymorphism (SNPs), Nonsynonymous SNP (nsSNP)

AUTHORS CONTRIBUTIONS

Conceptualization: Kinnari N. Mistry, Sishir Gang, Dharamshibhai N. Rank, Chaitanya G. Joshi

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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