INTRODUCTION

Nephrotic syndrome (NS) defines as a hereditary group of disorders with clinical features like proteinuria, hypoalbuminemia, hyperlipidemia, and edema. PMM2 encodes phosphomannomutase protein enzyme involved in the synthesis of N-glycan.

METHODS

Different Insilico analysis tools: SIFT, PolyPhen, PROVEAN, SNPandGO, MetaSNP, 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 determine 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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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.ccb.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-Ramachandran plot** (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.ccb.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].

**Phylogenetic 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](image-url)
RESULTS

SNPs for the PMM2 gene were retrieved from uniport and ClinVar. In ClinVar, there were 356 SNPs in which 15 frameshift variants, 9 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, SNP and GO, 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 disease 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 (1).

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

| rsID          | Amino acid change | Sift | PolyPhen | Provean | Snupand GO | MetaSNP | PhD SNP | MutPred   |
|---------------|-------------------|------|----------|---------|------------|---------|--------|-----------|
| rs75340382    | R21W              | PD   | Deleterious | D       | D          | D       | D      | Loss of Acetylation |
| rs398123312   | L32R              | APF  | Deleterious | D       | D          | D       | D      | Loss of Acetylation; Altered Stability |
| rs755402538   | G42R              | APF  | Deleterious | D       | D          | D       | D      | Altered DNA binding |
| rs104894534   | V44A              | APF  | Deleterious | D       | D          | D       | D      | Altered Transmembrane protein; Altered Stability |
| rs750498949   | L104W             | APF  | Deleterious | D       | D          | D       | D      | - |
| rs387906824   | Y106K             | APF  | Deleterious | D       | D          | D       | D      | Gain of Acetylation; Altered Transmembrane protein |
| rs80338700    | P113L             | APF  | Deleterious | D       | D          | D       | D      | Altered Transmembrane protein |
| rs104894530   | G117R             | APF  | Deleterious | D       | D          | D       | D      | Loss of Strand; Altered Transmembrane protein |
| rs1057517110  | F119L             | APF  | Deleterious | D       | D          | D       | D      | Loss of Strand |
| rs3658582085  | I120T             | APF  | Deleterious | D       | D          | D       | D      | Loss of Strand; Altered Stability |
| rs190521996   | F157S             | APF  | Deleterious | D       | D          | D       | D      | Altered Metal-binding; Gain of ADP-ribosylation |
| rs941830625   | G175R             | APF  | Deleterious | D       | D          | D       | D      | Altered Metal-binding; Altered Transmembrane protein; Altered Stability; Loss of Proteolytic cleavage |
| rs780581250   | F183S             | APF  | Deleterious | D       | D          | D       | D      | Altered Transmembrane protein; Altered Stability; Loss of Allosteric site |
| rs80338704    | D188G             | APF  | Deleterious | D       | D          | D       | D      | Gain of Acetylation; Altered DNA binding; Altered Transmembrane protein |
| rs532870929   | F207S             | APF  | Deleterious | D       | D          | D       | D      | Altered Stability; Loss of Acetylation; Loss of Allosteric site |
| rs398123309   | G208A             | APF  | Deleterious | D       | D          | D       | D      | Loss of Alloster; Altered Transmembrane protein |
| rs782901411   | N216S             | APF  | Deleterious | D       | D          | D       | D      | Altered Metal-binding; Altered Transmembrane protein |
| rs752614554   | D217E             | APF  | Deleterious | D       | D          | D       | D      | Altered Metal-binding; Altered Transmembrane protein |
| rs80338706    | T226S             | APF  | Deleterious | D       | D          | D       | D      | - |
| rs558862439   | G228R             | APF  | Deleterious | D       | D          | D       | D      | Altered Metal binding |
| rs80338708    | V231M             | APF  | Deleterious | D       | D          | D       | D      | Altered Ordered interface; Loss of Relative solvent accessibility |
| rs80338708    | T237R             | APF  | Deleterious | D       | 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 analysis | STRUM   |
|---------------|-------------------|-------------------|---------|
| rs75340382    | R21W              | Decreases         | -0.21   |
| rs398123312   | L32R              | Decreases         | -1.05   |
| rs755402538   | G42R              | Decreases         | -1.81   |
| rs104894534   | V44A              | Decreases         | -2.27   |
| rs750498949   | L104W             | Decreases         | -0.58   |
| rs387906824   | Y106K             | Decreases         | -0.57   |
| rs80338700    | P113L             | Decreases         | -1.02   |
| rs104894530   | G117R             | Decreases         | -1.83   |
| rs1057517110  | F119L             | Decreases         | -2.34   |
| rs3658582085  | I120T             | Decreases         | -1.26   |
| rs190521996   | F157S             | Decreases         | -2.24   |
| rs941830625   | G175R             | Decreases         | -2.36   |
| rs780581250   | F183S             | Decreases         | -2.99   |
| rs80338704    | D188G             | Decreases         | -2.07   |
| rs532870929   | F207S             | Decreases         | -3.74   |
| rs398123309   | G208A             | Decreases         | -2.18   |
| rs782901411   | N216S             | Decreases         | -0.74   |
| rs80338706    | T226S             | Decreases         | -0.23   |
| rs558862439   | G228R             | Decreases         | -1.39   |
| rs80338708    | V231M             | Decreases         | -2.26   |

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

Phylogenetic 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.

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

Fig. 3: Ligand binding sites within PMM2 protein

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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, PhD SNP, 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, 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.
Leu52Arg, p. Val44Ala, p. Asp65Tyr, p. Phe157Ser, p. Pro194Thr, 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

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

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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