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In Silico Structural Analysis and Molecular Docking of Human *NPR2* Gene Causing Acromesomelic Dysplasia, Type Maroteaux

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ABSTRACT

Acromesomelic dysplasia maroteaux type is a severe skeletal disorder that is caused due to loss of function mutations in the *NPR2* gene which leads to the dysfunction of the Natriuretic Peptide Receptor (*NPR2*). Skeletal growth in AMDM patients falls off sharply after birth causing abnormal growth plate and short misshapen bones in the extremities and carrier parents of AMDM children are shorter than average. This protein comprises four domains: The extracellular ligand-binding domain, carboxyl-terminal guanylyl cyclase catalytic domain, intracellular protein kinase homology domain, and single membrane-spanning region. The missence mutation occur in chromosome 9p13.q12 with accession ID: P20594, result amino acid changes at 907 of Threonine into Methionine. The aims of this study were to find out the underlined missense mutation p.T907M in Pakistani family effect with acromesomelic dysplasia, type maroteaux. In this study a missense mutation determines by in Pakistani family which cause acromesomelic dysplasia, type maroteaux. Whole exome sequencing identified by the missense mutation was carried out by the causative gene. Bioinformatics tools were used for confirmation of pathogenicity of the identified mutations. The novel missense mutations, c. 2720C>T; p.T907M in *NPR2* gene were identified in Pakistani family, respectively. Whole exome sequencing confirmed co-segregation of the mutations with disease phenotypes in Pakistani family. *In Silico* analysis of the sequence variants confirmed their pathogenicity. The report of *NPR2* gene mutations in Pakistani family, which support the frequent involvement of *NPR2* sequence variants in acromesomelic dysplasia, type maroteaux.

Keywords: Acromesomelic dysplasia; Type maroteaux; Missense variants; *NPR2*; Structural analysis; Molecular docking

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INTRODUCTION

Acromesomelic dysplasia moroteaux type is a severe skeletal disorder that is caused due to loss of function mutations in the *NPR2* gene which leads to the dysfunction of the Natriuretic Peptide Receptor (*NPR2*) [1]. The idiopathic short stature occurs due to heterozygous mutations in *NPR2* gene [2]. Natriuretic Peptide Receptor B (*NPR2*) is encoded by the *NPR2* gene. This protein comprises four domains: The extracellular ligand-binding domain, carboxyl-terminal guanylyl cyclase catalytic domain, intracellular protein kinase homology domain, and single membrane-spanning region [3]. It acts as a receptor for C-type Natriuretic Peptide (CNP). In order to activate the *NPR2* gene, it forms a homodimer that starts producing cytoplasmic cyclic GMP from GTP upon binding of their extracellular ligand, called CNP [4]. Such binding enhances the guanylyl cyclase activity of the Natriuretic Peptide (*NPR2*) Receptor which subsequently, boosted the cGMP synthesis which further stimulates typeII cGMP-dependent protein kinase. This led to the formation of CNP/NPR2 cascade which induces cartilage homeostasis, differentiation and proliferation of osteoclasts and osteoblasts by regulating endochondral ossification. To determine the impact of mutations on protein structure and their subsequent relationship with disorders development, it is pertinent to carry out *in silico* modeling of the structural and functional analysis of protein. In this regard, bioinformatics tools help us to understand the structural and functional analysis of novel mutations and subsequently, their impact on changes in ligand binding interaction, structural changes and show the relationship between phenotypes and mutations. The *NPR2* gene causes acromesomelic dysplasia, type maroteaux. A sequence variant (c.2720 C>T; p.T907M) in the *NPR2* cause acromesomelic dysplasia, type maroteaux located on the chromosome 9p13.q12. Acromesomelic dysplasia, type Maroteaux, which is autosomal recessive, may be impacted by this protein's ability to function. The NPR2 gene contains 4248 bp which encode 1047 residues amino acid. The *NPR2* gene is expressed in a variety of tissues and cell types, including the uterus, brain, heart, and blood vessels. The *NPR2* gene sequence variant (c.2720 C>T; p.T907M) to analyze it computationally by different bioinformatics tools [6-10].

MATERIALS AND METHODS

Pathogenicity of Sequence Variant

To study the sequence variant which cause phenotypically cromesomelic dysplasia, type maroteaux. A syndrome in human being the sequence variant was packed from the previous research study of the *NPR2* gene. The target variant of *NPR2* gene protein FASTA sequence was retrieved from the uniprot database with accession ID: P20594. The variation table for the *NPR2* gene in the ensemble genome browser has confirmation of the mutation. For more studying, the impact of sequence variant which cause protein structure and function to confirm the target variant through various online tools. MAPP, Predict SNP, Panther, SIFT, SNAP2, SNP and GO, Polyphen-2, I-Mutant to find the structural and functional consequence of variant (c.2720C>T lead in to point mutation p.Thr907 Met).

Phylogenetic Analysis

To determine the greatest similarity and confirm the related gene family in other species, a phylogenetic evolutionary tree was constructed using the *NPR2* gene and 14 other closed related species found by the NCBI-BLAST tool. In order to construct the phylogenetic relationship between protein sequences, the MEGA X programme used neighbour-joining algorithms, verifying the statistical significance of the tree.

Structural Analysis and Validation

The complete 3D structure of *NPR2* gene is not present in the online tools PDB (protein data bank). The *NPR2* gene retrieved the FASTA sequence from uniprot database. The multi protein template structure was predicted through online web server Alpha Fold and Swiss model. The CASP 10 method, a verified refinement technique. Ramachandran plot and ERRAT were generated with procheck to verify the phi and psi angles in order to validate the 3D model of the *NPR2* gene. For more studying the, validated structure was refined through RAMPAGE and galaxy refine tools.

Mutagenesis of *NPR2* and Structure Comparison with Wild Type *NPR2*

The 3-dimensional structure of the mutant protein from the wild type protein through chimera. The mutant structure was retrieved from the wild type protein. In addition to having a graphical user interface, the Chimera interface also accepts user commands that are script-based and executes them. To demonstrate several rotamers in chimera during mutagenesis. The mutation percentage valve for each rotamer illustrates how likely each rotamer's chances. The possible clashes in the structure was then cleared and remove. Through chimera, the protein structures of the wild-type and mutant type were compared.

Energy Minimization and Impact of Deleterious

To study the variant analysis, that will examine the mutation alteration lying the novel structure through Hope server. Hope server was used to draw residue to residue comparison. The 3-dimensional structure energy minimization was carried out through SWISSPDB. The *NPR2* mutagenesis compared with wild type.

Molecular Docking

The *NPR2* gene was docked by various computational methods for further investigation. ChemBl, Pymol, autodock vina, HPEPDOCK and Biovia discovery studio. To study how two or more molecular structure are fit together. It is technique how a protein interacts with small molecules (LIGAND).

RESULTS

Pathogenicity of Sequence Variant

The *NPR2* gene sequence variant were retrieved from ensemble genome browser and uniprot databases. The *NPR2* gene identified a missense mutation (c.2720 C>T; p.Thr907Met) located on the chromosome 9p13-q12. In the result threonine are converted in to methionine after the mutation. For more studying various online tools for the structural and functional annotation of sequence variant. MAPP is affected the protein sequence with deleterious score as 86% accuracy that cause the disease. Predict SNP affect the disease with a score as 87% accuracy. Panther show probably damaging with a score as 0.85. SIFT is affect the protein function with a score as 0.00 mutation can the disease SIFT. SNP and GO affect the structure with score as =52 and show 75% accuracy. Polyphen2 indicate the probably damaging with a score as 1.000. PROVEAN was deleterious that affect the protein structure with a score as -5.744. I-mutant affects the protein structure stability (**Table1**).

Table 1: Effect of sequence p.T907M on protein function by different tools with mutational score.

Mutation	MAPP	*SNAP2*	Provean	Panther	SNP and GO	PredictSNP
p.T907M	Deleterious	Effect	Deleterious	Possibly damaging	Disease effect	Deleterious
Mutation score	87% accuracy	52 accuracy 75%	-5.744	0.85	80% accuracy	87% accuracy

Secondary Structure Prediction and Mutagenesis

The secondary structure prediction was retrieved from PDBsum tool. There are 7 sheets, 11 beta hairpin, 32 strands, 12 beta bulges, 38 helices, 32 Helix-Helix interactions, 76 beta turn, 13 gamma turn and 2 disulphide. To enhance the secondary prediction, compare the solvent accessibility of the wild-type and mutant-type through RaptorX tools. The results indicate that the *NPR2* gene, which causes acromesomelic dysplasia, type maroteaux, can decrease and increase solvent accessibility.

Phylogenetic Analysis

The 14 species sequence has been blasted with the *NPR2* gene, to confirm a relationship among the species and to show the distance among all these species with expected threshold value of against the NCBI domain database. The conserved domain programme was carried out using several sequence alignment approaches; 14 species resembled for *NPR2* gene were classified in to two clusters on the basis of closely relationship. Based on BLAST results, the phylogenetic tree was also constructed to confirm the conservation of *NPR2* with these 14 different species belonging to the *NPR2* gene family as highly conserved (**Figure 1**).

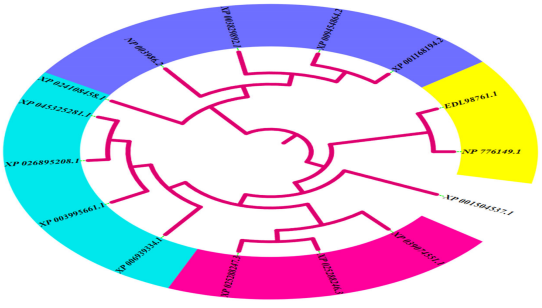

Figure 1: Phylogenetic tree analyses of 15 different protein sequences with different species using the Neighbor-Joining method by MEGA X with the branch length.

Structural Analysis and Validation

The *NPR2* gene protein sequence was retrieved from uniprot database. The 3-dimensional structure was obtained from online web based tool AlphaFold and Swiss model. The validation of 3-dimensional structure using various methods used to examine the characteristics of proteins for structure validation. ERRAT and Ramachandran plot were used for structure validation. Ramachandran plot show that 91.7% of residues are most favored region and 7.6% residues in additionally allowed region and no residues fall in the outlier region. The quality factor score according to ERRAT is 97.1%.To further enhance the reliability, RAMPAGE and Galaxy Refine tools were used for validation the 3D-structure (**Figures 2A-C**).

Mutagenesis of *NPR2* and Structure Comparison with Wild type *NPR2*

The mutant structure of *NPR2* aligned with the wild type of *NPR2*. Both the structures demonstrate how the residues differ from one another. Structure of mutant and wild type of the gene were minimized before comparison. The difference between the side chains of the residues that threonine is conserved in to methionine was shown by structural comparison (**Figures 2D,E**). The comparison of residues reveals that the mutant type residue is larger than the wild type.

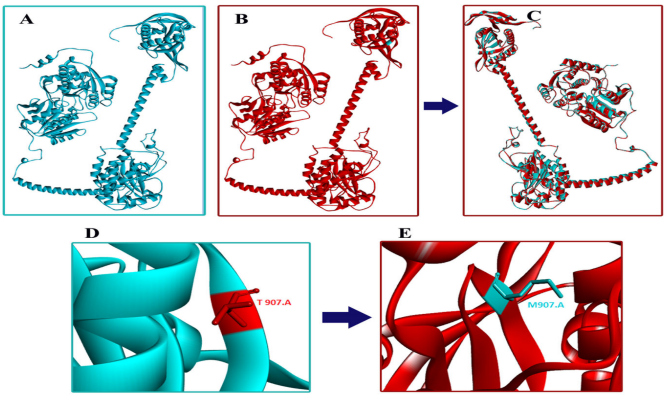

Figure 2: Structure analysis and modeled of normal and mutant *NPR2* protein. (A) Structure of wild type *NPR2* protein is highlighted in cyan colour. (B) Structure of mutant type *NPR2* protein is highlighted in red colour. (C) Superimposition of wild (cyan) and mutant (red). (D) Wild type p.T907 residue is shown in red colour. (E) Mutant type p. T907M residue is shown in cyan colour.

Energy Minimization and Impact of Deleterious

The *NPR2* gene variant analysis check through the online server hope server, demonstrated that the mutation occurs in the single amino acid at the position of T907M. The mutation region is larger than the region of the wild type. The backbone of each amino acid is red and change the mutation backbone is black. These mutations have a disease severity annotation. The wild and mutant type energy minimization demonstrates that protein is unstable reveal a huge difference between wild and mutant type (**Table 2**).

Table 2: Total energy of wild type and mutant type structure after energy minimization.

Amino acid variant	Total energy after minimization kj/mol	Electrostatics constraint
Wild type	-48919.2	-32688.9
Mutant type	-48904.1	-32674.8

Molecular Docking

The 3D model structure of *NPR2* predicted and characterized by online computational tools. Ramachandran plot and ERRAT show that approximately 90-97% residues area in allowed region. Analyses of molecular docking chimera were used to create the mutant type structure. For molecular docking analysis ligand molecules are retrieved from ChEMBL database (CHEMBL387059). By using molecular docking analysis, the interaction of the normal and mutant types of the *NPR2* gene was determined. The interaction between (CHEMBL387059) with normal (p.T907) is Leu71,Glu72, Ala74, Cys75, Glu77, Thr175 (**Figures 3A,B**), while in case of mutant (p.907M) is Leu71, Glu72, Ala74, Cys75, Glu77, Thy38, Thr175 (**Figures 3C,D**).

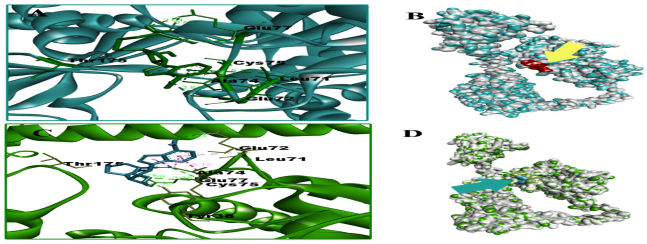

Figure 3: Visualization of the best docked *NPR2* gene complex normal and mutant. (A) Cartoon view of the normal *NPR2* compound with H-bond interaction. (B) Surface view is shown in atomic representation with normal (C) Cartoon view of the mutant NPR2 compound with H-bond interaction. (D) Surface view is shown in atomic representation with mutant.

The complex compound between the normal and mutant to show change in residues and change some distance between surfaces around ligand (**Figure 4**). Furthermore to check the confirmation of binding interaction through Pymol and hpepdock for ligand preparation.

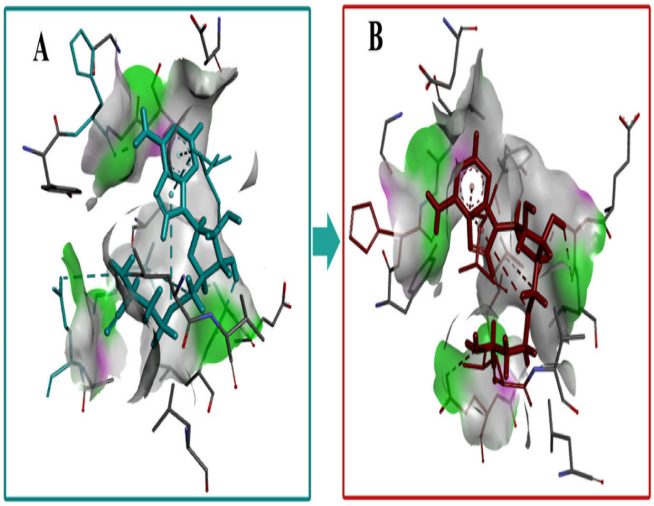

Figure 4: Molecular interaction surface around ligand *NPR2* protein. (A) Normal *NPR2* with surface around ligand (B) Mutant *NPR2* with surface around ligand.

DISCUSSION

Acromesomelic dysplasia moroteaux type is a severe skeletal disorder that is caused due to loss of function mutations in the *NPR2* gene which leads to the dysfunction of the natriuretic peptide receptor (*NPR2*). Skeletal growth in AMDM patients falls off sharply after birth causing abnormal growth plate and short misshapen bones in the extremities and spine [11]. Carrier parents of AMDM children are shorter than average. This protein comprises four domains: The extracellular ligand-binding domain, carboxyl-terminal guanylyl cyclase catalytic domain, intracellular protein kinase homology domain, and single membrane-spanning region. The *NPR2* gene encode 1047 amino acid contain 22exons. Sequence analysis of the gene revealed (c.2720C>T; p.Thr907Met) effect the acromesomelic dysplasia, type maroteaux. The *NPR2* gene's sequence variation in humans has been linked to acromesomelic dysplasia of the type Maroteaux [12-14]. In addition, the sequencing of the available proteome information now allows for a better understanding of the relationship between phenotype and genotype. This phenotype-genotype correlation studies has now been made easy by the bioinformatics. To determine the pathogenicity of the amino acid alteration, the sequence variant is assessed using a variety of bioinformatics tools. MAPP, PredictSNP, Panther, SIFT, SNAP2, SNP and GO, Polyphen-2, I-Mutant, detected the sequence variant disease causing. The mutation identified to be harmful using through different tools in variant table. The study of the relationship between phenotype and genotype and the impact of single nucleotide stability [15,16]. The analysis of human genetic variation of *NPR2* gene in combination with computational method to predict the possible functional impact *NPR2* gene sequence variant and their effect on functional characteristics of protein. The stability of native protein is tested by homology modeling both structure of the *NPR2* gene variant to compare the wild type and mutant types. For the *NPR2* variation, the RMSD valve, TM score, and total energy in kcal/mol of the normal and mutant 3D models are compared. The decrease in protein stability was shown to be caused by a single amino acid substitution [17,18]. The variant analysis and the total energy of protein show that protein is unstable and huge difference between the wild type and mutant type of protein. A significant conformational alteration in the active binding site of the mutant *NPR2* was discovered by molecular docking analysis, which affected the interaction with the compound [19,20].

CONCLUSION

In conclusion we have reported that *in silico* sequence variant analysis of acromesomelic dysplasia, type maroteauxis autosomal recessive as a protein folding diseases perform through computational analysis. The sequence variant of *NPR2* (c.2720C>T lead in to point mutation p.Thr907Met) is predicted to be disease causing by MAPP, PredictSNP, Panther, SIFT, *SNAP2*, SNP and GO, Polyphen-2, I-Mutant predicted that there is decrease in the stability of protein structure. The variation analysis and energy calculation showed that the total energy of protein differs by kj/mol. The mutant protein's structure analysis using the Chimera tool reveals that the threonine ring is at the centre of the strand and that the substituted methionine is hanging outside. In docking, the active binding site of mutant *NPR2* is change the stability of the protein because of change some distance surface around ligand. According to the findings, the sequence variant (p.Thr907Met) is altering the structural confirmation and decreasing the stability of the protein, which results in acromesomelic dysplasia of type Maroteaux.

AUTHOR CONTRIBUTIONS

SI, OH, AA, RT: Conceptualization, methodology, software, data curation, writing-original draft preparation. RT, SI, AA, S.A.U, N.G: Helped in write-up and editing, validation, methodology, visualization, validation. Y.A: Help in reading manuscript.

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Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Not applicable.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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