Volume Number 10 || Issue Number 2 || Year 2025 || Page 7 || * Corresponding Author: zafar@uswat.edu.pk||

ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746

WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY

Research Manuscript www.sciplatform.com Peer review

Molecular characterization of acromesomelic dysplasia type maroteaux: A homozygous nonsense mutation in NPR2

[a] Zafar Ali *, [a] Sami Ullah, [a] Najeebullah, [a] Fazal Akbar, [a] Itazaz Ul Haq, [a] Sania Fawad, [b] Shahid Mahmood Baig, [c] Niklas Dahl

[a] Centre for Biotechnology and Microbiology, University of Swat, Charbagh, Swat, Khyberpakhtunkhwa 19120,
[b] Faculty of Life Sciences, Health Services Academy, Park Road, Islamabad 44000, Pakistan,
[c] Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, BMC Box815, 751 08, Uppsala, Sweden

Contribution Material preparation, data collection and supervision were performed by **Z. Ali**, collection of samples and lab experiments were performed by **S. Ullah**, & **Najeebullah**, **F. Akbar** revise the manuscript. **Itazaz Ul Haq** & **S. Fawad** performed the computational simulation. **Z. Ali**, **S. Mahmood Baig** & **N. Dhal** supervised the overall study and validated the results.

ABSTRACT

Acromesomelic dysplasia, type Maroteaux (AMDM) is an autosomal recessive skeletal condition characterized by uneven growth plates, spines, and limbs. People with Acromesomelic dysplasia are often much shorter than average and have disproportionately short arms and legs. We investigated the cause of autosomal recessive skeletal disorder in a consanguineous Pakistani family comprises of four affected individuals across two generations. By utilizing whole exome sequencing, we identified a nonsense mutation in the exon 2 of *NPR2* gene (c.844 C>T, p.Q282X) as the possible cause of the disease. The nonsense *NPR2* gene mutation causes the premature termination of *NPR2* protein, resulting in a shortened protein, missing a significant structural component. Segregation of *NPR2* gene variant (c.844 C>T, p. Q282X) was confirmed by Sanger sequencing across the pedigree. Furthermore, bioinformatics analysis such as proteins secondary and 3D protein structures predictions were also carried out to assess their functional impact. The results of this study can help the clinicians in differential and better diagnosis, developing carrier screening and parental diagnosis tests for the family studied. It can also aid in our understanding of the pathophysiology of the disease caused by NPR2 mutations.

Keywords: Skeletal disorder, consanguineous family, whole exome sequencing, nonsense mutation, Sanger Sequencing

INTRODUCTION: Acromesomelic dysplasia, type Maroteaux (AMDM), is a rare genetic condition that effects bone growth. People with AMDM have disproportionately short limbs, hands and feet. Mutations in the natriuretic peptide receptor 2 gene (NPR2) cause the autosomal recessive inheritance pattern of the disease (Bartels *et al.*, 2004). A receptor is encoded by this gene for a protein called C-type natriuretic peptide (CNP). It is a hormone that regulates growth and development. Mutations in the *NPR2* gene disrupt CNP's binding to its receptor, causing growth and development deficiencies (Bartels *et al.*, 2004). Mutations in *NPR2* to be the cause of AMDM was first reported in a number of families in 2004 (Bartels *et al.*, 2004).

People affected with AMDM can be recognized during first year after their birth because they show a significant reduced skeletal growth in their spines and limbs, as well as irregular growth plates (Bartels *et al.*, 2004). The *NPR2* gene a is located on chromosome 9 between positions p13 and q12 (Kant *et al.*, 1998).The C-type natriuretic peptide (CNP) is bound by *NPR2*, which encodes the receptor NPR-B, which affects secondary messenger synthesis and action inside the cell, as well as cyclic GMP release and action (Bartels *et al.*, 2004; Schulz, 2005).

A receptor for C-type natriuretic peptides (CNPs) is located on the surface of cells and is encoded by the gene *NPR2*. It has 5 structural domains including CNP-binding domain, a transmembrane domain, a coil-coil domain, a tyrosine kinase domain and a guanylate cyclase domain (Olney *et al.*, 2006). It regulates vital cellular functions including differentiation and proliferation by converting extracellular signals into changes in cyclic guanosine monophosphate (cGMP) levels (Vasques *et al.*, 2017). Numerous investigations have demonstrated the importance of CNP-NPR2 signaling in endochondral ossification, and both humans and mouse develop dwarfism as a result of its deactivation.

OBJECTIVES: The current study describes the genetic investigation of a consanguineous Pakistani family. Whole exome sequencing (WES) followed by Sanger sequencing was used to identify a nonsense mutation in *NPR2* gene (c.844 C>T, p. Q282X) responsible for Acromesomelic dysplasia, type Maroteaux (AMDM).

MATERIALS AND METHODS: Ethical consideration and sample collection: The University of Swat's ethical committee authorized all of the experimental protocols and subject handling methods, in accordance with the Helsinki declaration. To protect their privacy, the guardians/elders of the affected provided a written consent form for their data and photographs to be used in the publication. A consanguineous family with several AMDM patients was identified from district Swat, Khyber Pakhtunkhwa (KPK) province, Pakistan. Blood samples of 5ml were collected from ten individuals, consist of four affected and 6 normal individuals. The blood samples were in stored in ethylenediaminetetraacetic acid (EDTA) containing tubes. Each affected individual's height and weight recorded. Photographs of affected individuals were taken and all the relevant clinical information were recorded.

Whole exome sequencing analysis: The WES was conducted on 50 ng of genomic DNA from a single family member who was affected. The Covaris tool was employed to shear the DNA. Additionally, the AB library builder system (Life technologies, Carlsbad, CA, USA) was used to construct fragment libraries from sheared DNA samples, and size chosen utilizing BluePeppin tool. Furthermore, the Ion AmpliSeq™ was used to enrich targets following manufacturer instructions. Exome capture was accomplished by incubating the DNA libraries with biotinylated RNA baits for 24 h before extracting them with streptavidin-coated magnetic beads. The Ion PI template OT2 200 kit chemistry and the Ion OneTouch 2 tool were used to amplify the captured DNA using emulsion PCR, after enrichment with Ion OneTouch ES, samples were placed onto an Ion PI chip and sequenced with the Ion PI sequencing 200 kit and Ion proton system. LifeScope™ (v 2.1) software was utilized to align reads to the human reference sequence (hg19 assembly) and detect variants SNP and indel data was maintained in an-house exome database, combined with variant annotation information collected from ANNOVAR. In addition, dbSNP135 custom R programs identified potentially hazardous mutations sheared by patients but were absent in the other 1000 exome in the in-house database.

Sanger sequencing and sequence alignment: To confirm the segregation of NPR2 gene variant across the pedigree, bidirectional sanger sequencing was performed on a genetic DNA analyzer (Applied Biosystems Big Dye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystem, Life Technologies, Carlsbad, CA, USA). Sanger sequencing results were analyzed using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). An amino acid sequence alignment was performed using *NPR2* protein sequences from nine different species downloaded from NCBI (National Centre for Biotechnology Information). ClustalW (Multiple Sequence Alignment - CLUSTALW) was used to perform multiple sequence alignments.

Secondary and tertiary structure analysis: The Robetta server (http://robetta.bakerlab.org) was used to generate 3D models of NPR2, using amino acid sequences obtained from NCBI (Haq *et al.*, 2025). In order to visualize 3D structure of wild-type and mutant *NPR2* proteins, UCSF Chimera server (Pettersen *et al.*, 2004) was used. PSIPRED predicted secondary structures. A Mutation in a protein can destabilize its structure, potentially leading to deformities in human body (Hassan *et al.*, 2017).

RESULTS AND DISCUSSION: Phenotype and clinical features: There were no complications reported during the pregnancy and all affected individuals were born after a normal delivery and full term pregnancy. The ages of affected individuals ranges from 4 to 33 years old at the time of investigation. The disease was congenital and symptoms of AMDM were present in the affected individual

after birth. In the affected members, the skin was redundant and the fingers were very short. There was a clear shortening of the distal nd middle portions of the legs. The bilateral metacarpals were small and stubby. Figure 1 summarizes the proband's clinical features, including height, age, head circumference, and arm and leg length measurements. AMDM is associated with these symptoms, as described previously (Mustafa *et al.*, 2020). AMDM patient did not exhibit any neurological impairment or persistent abnormality of any other organ system besides the skeleton (Mustafa *et al.*, 2020).

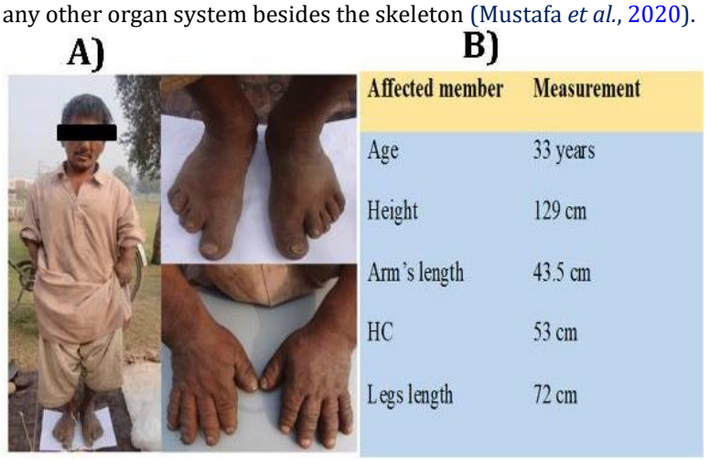

Figure 1: Phenotypic features (A) and measurements of an individual (B) (IV-4).

Whole exome sequence analysis result: We investigated the WES data for compound heterozygous and homozygous variants because the pedigree was autosomal recessive. Data analysis predicted a nonsense homozygous variant (c.844 C>T, p. Q282X) in exon 2 of *NPR2* gene (NM_003995) along with other 17 homozygous variants (figure 2).

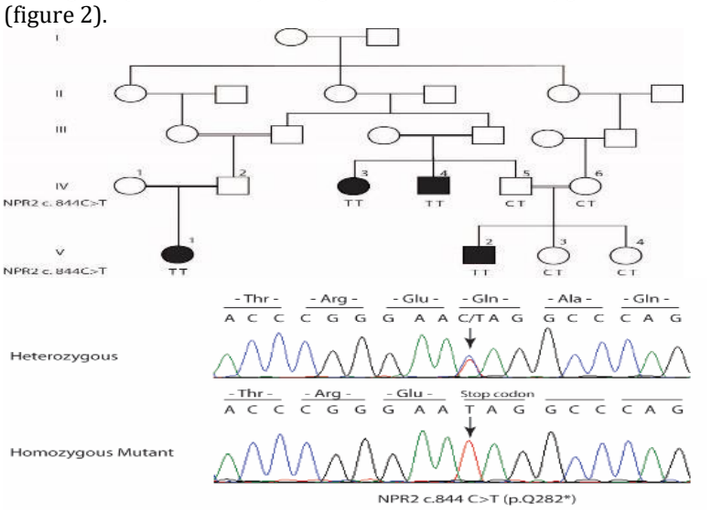

Figure 2: Pedigree shows clear segregation pattern of the Mutation after WES analysis and Chromatogram of genomic DNA presenting exon 2 of NPR2 gene obtained from healthy controls (top), heterozygous parents (middle), and homozygous affected individuals (bottom). A c.844C>T transition is indicated by an arrow.

The *NPR2* gene variant was considered to be the most likely candidate variant because of its previous association with AMDM. No other variant localized in any genes is previously associated with similar phenotype under investigation. Moreover, Mutation taster, an *in-silco* tool used to predict the pathogenicity of variants in the genome, predicted the *NPR2* gene variant (c.844 C>T, p. Q282X) to be pathogenic and a nonsense mediated decay of the protein. Furthermore, the variants were not found in the gnomAD database (https://gnomad.broadinstitute.org/), a database that consist of exome sequences and whole-genome sequences of 730,947 and 76,215 individuals respectively. In addition to these, bidirectional Sanger sequencing confirm that the nonsense NPR2 gene variant (c.844 C>T, p. Q282X) is segregated across the pedigree. All the affected individuals were homozygous mutant while all the phenotypically health individuals were heterozygous carries.

Multiple sequence alignment analysis: Clustal Omega program was used for alignment of sequences from multiple species to check whether the identified variant fall in conserved region or not. The alignment showed that glutamine (denoted by Q) and the amino acids surrounding it are highly conserved in that region among different species (**Error! Reference source not found.**), so any change/mutation in this region may have a significant impact on the structure of the protein which may leads to an abnormal human phenotype.

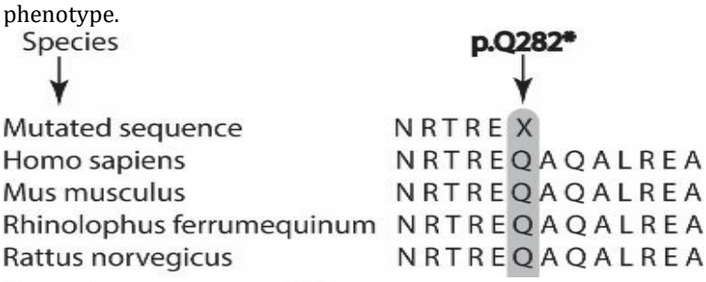

Figure 3: Alignment of NPR2 sequences from nine different organisms using clustalW reveals conservation of p.Q282X (shown in yellow) across species.

NPR2 protein structure analysis: The differences between wild and mutant type structures can be observed from secondary structure prediction. Wild type residues 16, 31, 38-40, 71-72, 27-30, 40-41, 102, 114, 128-129, 138-141, 171, 178, 179, 193-194 and 203 are predicted as coil structures, but mutant structures are not the same. Furthermore, residues 57-59, 124-126, 159, 210, and 280-282 which include our mutation showed helix structures in wild type that differed from those in mutants. Additionally, Residues 31, 71-72 and 203 were predicted as strand structures in mutant, which were expected to be coil structures as observed in wild type structure (**Error! Reference source not found.**).

Figure 4: Secondary structure prediction for wild-type and mutant proteins. PSIPRED 4.0 prediction of secondary structure of (A) wild-type and (B) mutant proteins.

Based on the 3D superimposed model results, when both proteins are intact, the predicted models align perfectly and match in terms of their structural components, which is not the case when mutant protein are used that is because mutant truncated protein lacks a significant structural component as compared to normal protein (**Error! Reference source not found.**) .

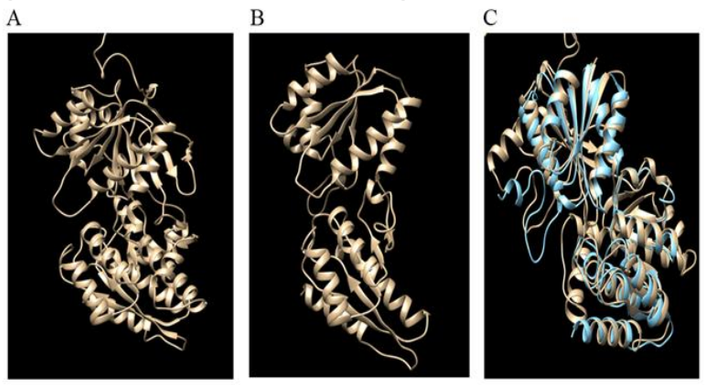

Figure 5: (A) Wild-type NPR2 protein predicted structure (B) Mutant NPR2 protein predicted structure (C) Superimposed structure of wild-type and mutant predicted models

The current study elucidated the genetic basis of a Pakistani family with several members who had short stature and bone abnormalities. AMDM in the family was diagnosed based on the

clinical characteristics and condition similar to what has been reported in other ethnic groups with the same disease (Olney *et al.*, 2006; Hachiya *et al.*, 2007). Using WES, we identified a nonsense mutation (c.844C>T, p. Q282X) in exon 2 of *NPR2* gene in the family segregating Acromesomelic dysplasia type Maroteaux. The substitution of C to A create a premature stop codon, so instead of coding another amino acid, the protein synthesis terminates prematurely. As a result, the protein is shorter than normal, containing only 282 amino acids. Based on secondary structure prediction, we can observe the differences between wild and mutant types. A coil structure is predicted for wild type residues 16, 31, 38-40, 71-72, 27-30, 40-41, 102, 114, 128-129, 138-141, 171, 178, 179, 193-194 and 203, which is not the case with mutant protein.

In addition, wild type and mutant helix structures differed between residues 57-59, 124-126, 159, 210, and 280-282. The mutant was predicted to have strand structures at residues 31, 71-72, and 203, which were expected to be coiled in the wild type protein structure. NPR-A, NPR-B, and NPR-C are the three types of natriuretic peptide receptors (NPRs). Among other functions, as well as regulating blood pressure and heart growth, these receptors regulate endochondral ossification (Kishimoto *et al.*, 2001; Tamura *et al.*, 2004). By activating cGMP-dependent protein kinase II and inhibiting MPK pathway, cyclic GMP (cGMP) is an intracellular secondary messenger produced by NRP receptors in the growth plate (Teixeira *et al.*, 2008; Schulz, 2005; Yasoda *et al.*, 2004; Chusho *et al.*, 2001). Based on a comprehensive examination of the literature, 71 pathogenic variations in NPR2 have been discovered to be linked with AMDM (Wu *et al.*, 2022). These changes were mostly found in the intracellular gunylyl cylase domain (28.2%) and extracellular CNP binding domain (47.9%). Different NPR2 variations are found in almost every family, indicating a significant degree of segregation within families. Finding the genotype-phenotype associations of discrete mutations in various NPR-B functional domains, or even within the same functional region, is difficult (Wu *et al.*, 2022).Furthermore, families with AMDM of various ethnicities, mutations in the *NPR2* gene have also previously been reported (Bartels *et al.*, 2004; Olney *et al.*, 2006; Hachiya *et al.*, 2007). Four Pakistani families were included in the DNA sequencing of 21 families affected with AMDM from different ethnicities, regions, and geographical locations (Bartels *et al.*, 2004). Apart from the nonsense mutations, eleven missense mutations, two splice-site mutations, and four frameshift mutations were discovered. It is common for AMDM patients to have shortened limbs with bowed forearms and a small stature. We noticed similar phenotypes in our family as well. In the functional assay, The activity of guanylyl cyclase was significantly reduced by missense mutations (Bartels *et al.*, 2004). Another study had reported a twenty-eight-year-old Japanese boy with notable dwarfism and found shortening in the intermediate and distal limb segment was enlisted. Both the patient and his parents were found to have homozygous and heterozygous variants of a missense mutation in NPR2 (L658F) in the intracellular kinase domain of NPR-B. The mutation resulted in typical CNP binding affinity but no detectable ligand-triggered cGMP synthesis. It was the first investigation to show that intact NPR-B KHD is required for skeletal growth (Hachiya *et al.*, 2007). Mustafa *et al.*, (2020) Seven heterozygous NPR2 missense or splice site variants were discovered in short stature patients from the United States. They discovered that NPR2 functional haploinsufficiency leads to the short stature. Few investigations on AMDM from Pakistan have previously been reported (Khan *et al.*, 2012). Six Pakistani families affected segregating AMDM were subjected to *NPR2* sequencing analysis. Along with abnormally small fingers with redundant skin, all patients presented with mesomelic shortening of the arms, metacarpals and phalanges. Intermediate and distal portions of the limbs showed significant shortening. These findings are in-line with the current study as similar symptoms were observed in our family as well. The researchers found a mutation affecting the splice donor site (c.2986 +2 T>G) in one of the families and a missense mutation in the other family (p.T907M).

CONCLUSION: In conclusion, we have discovered a nonsense mutation (c.C844T, p.Q282X) in exon 2 of the NPR2 gene in our family as the likely cause of the disease AMDM. This nonsense mutation create a premature stop codon causing nonsense mediated decoy of the protein coded by *NPR2* gene as predicted by Mutation taster (https://www.mutationtaster.org/). Similarly, nonsense mutation has been previously reported as well having adverse effect on the resultant phenotype.

FUNDING: This research was conducted without any funding.

CONFLICT OF INTEREST: All the authors declared no conflict of interest.

LIFE SCIENCE REPORTING: In current research article, no life science threat was reported.

ETHICAL RESPONSIBILITY: The authors declare that this study was conducted with the highest ethical standards, and all procedures were carried out in compliance with relevant laws and institutional guidelines. Written informed consent was obtained from all participants, and confidentiality was maintained throughout the study.

This is original research, and not submitted in whole or in parts to another journal for publication purpose.

INFORMED CONSENT: The author(s) have reviewed the entire manuscript and approved the final version before submission.

REFERENCES: Bartels, C. F., Bükülmez, H., Padayatti, P., Rhee, D. K., van Ravenswaaij-Arts, C., Pauli, R. M., Mundlos, S., Chitayat, D., Shih, L.-Y., & Al-Gazali, L. I. (2004). Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. The american journal of human genetics, 75(1), 27-34.

Buchan, D. W., & Jones, D. T. (2019). The PSIPRED protein analysis workbench: 20 years on. Nucleic acids research, 47(W1), W402-W407.

Chusho, H., Tamura, N., Ogawa, Y., Yasoda, A., Suda, M., Miyazawa, T., Nakamura, K., Nakao, K., Kurihara, T., & Komatsu, Y. (2001). Dwarfism and early death in mice lacking C-type natriuretic peptide. Proceedings of the national academy of sciences, 98(7), 4016-4021.

Hachiya, R., Ohashi, Y., Kamei, Y., Suganami, T., Mochizuki, H., Mitsui, N., Saitoh, M., Sakuragi, M., Nishimura, G., & Ohashi, H. (2007). Intact kinase homology domain of natriuretic peptide receptor-B is essential for skeletal development. The Journal of clinical endocrinology & metabolism, 92(10), 4009-4014.

Haq, I. U., Ullah, N., Rahiyab, M., Ali, S. S., Khan, I., Hussain, Z., & Iqbal, A. (2025). A computational immune-informatics approach to design multi-epitope vaccine against Guanarito virus targeting nucleoprotein and nucleo-capsid proteins. World Journal of Biology and Biotechnology, 10(1), 25-35.

Hassan, M., Abbas, Q., Raza, H., Moustafa, A. A., & Seo, S.-Y. (2017). Computational analysis of histidine mutations on the structural stability of human tyrosinases leading to albinism insurgence. Molecular bioSystems, *13*(8), 1534-1544.

Kant, S. G., Polinkovsky, A., Mundlos, S., Zabel, B., Thomeer, R. T., Zonderland, H. M., Shih, L.-y., van Haeringen, A., & Warman, M. L. (1998). Acromesomelic dysplasia Maroteaux type maps to human chromosome 9. The american journal of human genetics, 63(1), 155-162.

Khan, S., Ali, R. H., Abbasi, S., Nawaz, M., Muhammad, N., & Ahmad, W. (2012). Novel mutations in natriuretic peptide receptor-2 gene underlie acromesomelic dysplasia, type maroteaux. BMC medical genetics, 13, 1-8.

Kishimoto, I., Rossi, K., & Garbers, D. L. (2001). A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-A) inhibits cardiac ventricular myocyte hypertrophy. Proceedings of the national academy of sciences, 98(5), 2703-2706

Madeira, F., Madhusoodanan, N., Lee, J., Eusebi, A., Niewielska, A., Tivey, A. R. N., Lopez, R., & Butcher, S. (2024). The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. Nucleic acids research, 52(W1), W521-W525.

Mustafa, S., Akhtar, Z., Latif, M., Hassan, M., Faisal, M., & Iqbal, F. (2020). A novel nonsense mutation in NPR2 gene causing Acromesomelic dysplasia, type Maroteaux in a consanguineous family in Southern Punjab (Pakistan). Genes & genomics, 42, 847-854.

Najeebullah, Haq, I. U., Rahiyab, M., Ali, S. S., Khan, I., & Iqbal, A. (2025). Rational in-silico design of a multi-epitope vaccine against human Rhinovirus an immune simulation and molecular dynamics simulation approach. Vacunas, 500427.

Olney, R. C., Bükülmez, H. l., Bartels, C. F., Prickett, T. C., Espiner, E. A., Potter, L. R., & Warman, M. L. (2006). Heterozygous mutations in natriuretic peptide receptor-B (NPR2) are associated with short stature. The Journal of clinical endocrinology & metabolism, 91(4),

1229-1232.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera a visualization system for exploratory research and analysis. Journal of computational chemistry, 25(13), 1605-1612.

Schulz, S. (2005). C-type natriuretic peptide and guanylyl cyclase B receptor. Peptides, *26*(6), 1024-1034.

Suda, M., Ogawa, Y., Tanaka, K., Tamura, N., Yasoda, A., Takigawa, T., Uehira, M., Nishimoto, H., Itoh, H., Saito, Y., Shiota, K., & Nakao, K. (1998). Skeletal overgrowth in transgenic mice that overexpress brain natriuretic peptide. Proceding of natural academy of Sciences USA, 95(5), 2337-2342.

Tamura, N., Doolittle, L. K., Hammer, R. E., Shelton, J. M., Richardson, J. A., & Garbers, D. L. (2004). Critical roles of the guanylyl cyclase B receptor in endochondral ossification and development of female reproductive organs. Proceedings of the national academy of sciences, 101(49), 17300-17305.

Teixeira, C. C., Agoston, H., & Beier, F. (2008). Nitric oxide, C-type natriuretic peptide and cGMP as regulators of endochondral ossification. Developmental biology, 319(2), 171-178.

Vasques, G. A., Hisado-Oliva, A., Funari, M. F., Lerario, A. M., Quedas, E. P., Solberg, P., Heath, K. E., & Jorge, A. A. (2017). Long-term response to growth hormone therapy in a patient with short stature caused by a novel heterozygous mutation in NPR2. Journal of pediatric endocrinology and metabolism, *30*(1), 111-116.

Wu, J., Wang, M., Jiao, Z., Dou, B., Li, B., Zhang, J., Zhang, H., Sun, Y., Tu, X., Kong, X., & Bai, Y. (2022). Novel Loss-of-Function Mutations in NPR2 Cause Acromesomelic Dysplasia, Maroteaux Type. Frontiers in genetics.

Yasoda, A., Komatsu, Y., Chusho, H., Miyazawa, T., Ozasa, A., Miura, M., Kurihara, T., Rogi, T., Tanaka, S., & Suda, M. (2004). Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. Nature medicine, 10(1), 80-86.