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Research paper

In-silico Identification and Structural Characterization of a Novel Missense Variant (p.Ala2Thr) in the *MCPH1* Associated with Autosomal Recessive Primary Microcephaly

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Abstract: Autosomal recessive primary microcephaly is a genetically heterogeneous neurodevelopmental disorder characterized by reduced brain size and impaired neuronal proliferation. In this study, an integrative bioinformatics approach was employed to identify and characterize potential pathogenic variants in the *MCPH1* gene. Gene-disease association analysis retrieved 12,022 genes from Gene-Cards and 402 genes from Dis-GeNET, with overlapping candidates identified through Venn analysis. *MCPH1* was prioritized due to its critical role in brain development. Variant screening using the Genome Aggregation Database (gnomAD) identified a missense mutation (NC_000008.11:g.6406671G>A; c.4G>A / p.Ala2Thr), which was further validated using UniProt. Protein–protein interaction network analysis revealed that *MCPH1* is functionally enriched in cell cycle regulation, centrosome duplication, and DNA damage response pathways, showing significant interaction with key microcephaly-associated proteins. Mutational analysis confirmed that the substitution of alanine with threonine at position 2 may alter local structural stability. In silico pathogenicity predictions using PolyPhen-2 and SIFT classified the variant as potentially deleterious. Structural and docking analyses further demonstrated that the mutation influences ligand-binding behavior, where the wild-type protein exhibited multiple stabilizing interactions, while the mutant form showed a more restricted interaction pattern primarily involving Ile104. Comparative docking results indicated comparable binding affinities between wild-type and mutant *MCPH1*; however, differences in interaction profiles and energy parameters suggest potential alterations in binding dynamics and protein function. The current study highlights the functional significance of the identified *MCPH1* variant and provides insights into its possible role in the molecular pathogenesis of primary microcephaly, emphasizing the importance of computational approaches in variant prioritization and therapeutic target exploration

Keywords: Primary microcephaly, *MCPH1*, Autosomal recessive, Missense mutation, Neurodevelopment, Consanguinity, In silico analysis

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Introduction

Autosomal recessive primary microcephaly (*MCPH1*) is a genetically heterogeneous neurodevelopmental disorder characterized by a significantly reduced occipitofrontal head circumference present at birth due to impaired brain growth during embryonic development [1]. The disorder primarily affects the cerebral cortex, resulting in a markedly smaller brain with relatively preserved cortical architecture. Individuals affected by *MCPH1* typically present with varying degrees of intellectual disability, while other neurological features are usually mild or absent [2]. The condition is clinically defined by a head circumference that is substantially below the normal range for age and sex, commonly exceeding three to four standard deviations below the population mean. *MCPH1* follows an autosomal recessive inheritance pattern, in which affected individuals inherit pathogenic variants in both alleles of a disease-associated gene from carrier parents who are usually phenotypically normal [3]. The disorder is considered a congenital and largely non-progressive condition, as the reduced brain size is already evident during prenatal development and does not typically worsen after birth. The prevalence of *MCPH1* varies among populations but is notably higher in regions where consanguineous marriages are common, increasing the likelihood of homozygous pathogenic variants [4]. At the molecular level, *MCPH1* is widely regarded as a disorder of neurogenic mitosis, primarily involving genes that regulate the proliferation and division of neural progenitor cells during cortical development. Many *MCPH1*-associated genes encode proteins involved in centrosome integrity, mitotic spindle organization, chromosomal segregation, and cell cycle regulation [5]. Disruption of these processes alters the balance between symmetric and asymmetric divisions of neural precursor cells, leading to a reduction in the neuronal progenitor pool and ultimately fewer cortical neurons. To date, multiple *MCPH1*-associated genes have been identified, providing important insights into the genetic architecture of brain development and the molecular mechanisms underlying cortical size regulation [6]. Several genes have been identified to cause Autosomal recessive primary microcephaly (*MCPH1*). These genes are mainly involved in centrosome function, mitotic spindle organization, cell cycle regulation, and proliferation of neural progenitor cells during cortical development. Mutations in these genes disrupt neurogenic mitosis, leading to reduced neuronal production and a smaller cerebral cortex [7]. *MCPH1* (Microcephalin) was the first gene identified to be associated with autosomal recessive primary microcephaly (*MCPH1*). It encodes a BRCT (BRCA1 C-terminal) domain-containing protein that plays a critical role in the DNA damage response, regulation of chromosome condensation, and control of the G2/M cell cycle checkpoint. *MCPH1* is essential for maintaining genomic stability and ensuring proper timing of mitotic entry during cell division [8]. The gene is highly expressed in neural progenitor cells of the developing cerebral cortex, where it regulates proliferation and maintenance of the neuronal precursor pool. Loss-of-function mutations in *MCPH1* lead to premature chromosome condensation, centrosomal abnormalities, and defective mitotic progression, ultimately disrupting neurogenic mitosis. These cellular defects reduce the proliferation of neural progenitor cells, resulting in decreased neuronal production and the characteristic reduction in brain size observed in patients with primary microcephaly. Clinically, *MCPH1* mutations are associated with markedly reduced head circumference at birth accompanied by varying degrees of intellectual disability [9]. The present study aims to compile reported *MCPH1* mutations and identify potential ligands to mitigate their functional impact. Using in-silico molecular docking, the study evaluates ligand-protein interactions to predict stabilization of *MCPH1*, providing a computational framework for potential therapeutic intervention.

Methodology

Data Retrieval

Gene-disease association data related to autosomal recessive primary microcephaly were retrieved from DisGeNET and MalaCards to identify genes previously implicated in microcephaly and related neurodevelopmental disorders [10, 11]. Gene lists obtained from both databases were compared, and overlapping candidates were identified using Venn diagram analysis performed with Venny 2.1 [12]. To investigate the biological relevance of the identified genes, pathway enrichment analysis was conducted using ShinyGO to identify significantly enriched biological processes and signaling pathways. Functional annotation and disease associations were further confirmed using the Online Mendelian Inheritance in Man (OMIM) database [13]. Subsequently, population-specific variant frequency data were obtained from the Genome Aggregation Database (gnomAD). The selected variant was analyzed using MutationTaster to predict its disease-causing potential and assess its possible impact on protein function, followed by further silico pathogenicity evaluation [14].

Pathogenicity Prediction of the Selected Variant

The functional impact of the *MCPH1* variant was assessed using multiple silico prediction tools. MutationTaster was first employed to evaluate disease-causing potential based on evolutionary conservation, splice-site effects, and protein features. Further analysis was performed using SIFT and PolyPhen-2 to predict deleterious effects of amino acid substitutions on protein structure and function. PROVEAN was used to classify the variant as neutral or deleterious, while the CADD score provided an integrated estimate of variant pathogenicity based on multiple genome-wide annotations. Variants consistently predicted as deleterious across these tools were prioritized for subsequent homology modeling, molecular docking, and molecular dynamics simulations [15].

Protein Structure Retrieval and Preparation

The three-dimensional (3D) structure of *MCPH1* was obtained from the Protein Data Bank (PDB). When a complete experimental structure was unavailable, the amino acid sequence was retrieved from UniProt and a homology model was generated using SWISS-MODEL based on templates with high sequence identity and coverage. The best model was selected according to standard quality parameters. For docking preparation, the protein structure was processed in UCSF Chimera by removing water molecules, heteroatoms, and co-crystallized ligands, followed by the addition of hydrogen atoms and energy minimization to optimize structural geometry. Structural reliability was assessed using PROCHECK through Ramachandran plot analysis to evaluate stereochemical quality. The validated structure was subsequently used for molecular docking and molecular dynamics simulations [16].

Homology Modeling and Structure Validation

The amino acid sequence of *MCPH1* was retrieved from the UniProt and used for homology modeling. Since a complete experimental structure was unavailable in the Protein Data Bank (PDB), a three-dimensional protein model was generated using SWISS-MODEL based on template structures with high sequence similarity and coverage. The best model was selected according to model quality scores and structural parameters. Structural validation of the predicted model was performed using PROCHECK to assess stereochemical quality through Ramachandran plot analysis. Additional validation was carried out using ERRAT and Verify3D to evaluate overall structural reliability and compatibility between the amino acid sequence and the predicted three-dimensional structure. The validated model was subsequently used for molecular docking and downstream analyses [17].

Ligand Selection and Preparation

Potential ligand molecules targeting *MCPH1* were identified through literature review and chemical database screening. The three-dimensional structures of the selected ligands were retrieved from the PubChem database in standard structure format. The ligand structures were then imported into UCSF Chimera for preparation, which included removal of unnecessary atoms, addition of hydrogen atoms, and energy minimization to obtain stable conformations. The prepared ligands were subsequently converted into appropriate formats required for molecular docking analysis [18].

Molecular Docking Analysis

Molecular docking was performed to evaluate the binding affinity and interaction patterns between the *MCPH1* protein and the selected ligands. The docking analysis was conducted using AutoDock Vina integrated within the PyRx platform. Prior to docking, the prepared protein and ligand structures were converted into the required PDBQT format. A docking grid box was defined to encompass the active or predicted binding site of the protein. Multiple docking conformations were generated, and the best binding pose was selected based on the lowest binding energy score and favorable interaction patterns. The docking results were further analyzed and visualized using UCSF Chimera to identify key intermolecular interactions between the ligand molecules and the *MCPH1* protein [19].

Results

Gene identification and selection

Gene-disease association analysis for autosomal recessive primary microcephaly yielded a total of 12,022 genes from GeneCards and 402 genes from DisGeNET. Comparative analysis using Venn diagram (Venny 2.1) identified a subset of overlapping candidate genes that are strongly associated with microcephaly and related neurodevelopmental disorders. Among these, *MCPH1* was selected as a key candidate gene based on its established role in brain development and previous disease associations. Further variant screening of *MCPH1* using the Genome Aggregation Database (gnomAD) identified a missense variant NC_000008.11:g.6406671G>A, corresponding to c.4G>A / p.Ala2Thr (p.A2T) in the transcript (ENST00000344683). This variant was subsequently validated and cross-checked in the UniProt database to confirm its protein-level annotation and functional relevance. Pathogenicity prediction analysis using silico tools suggested that the identified variant may have a potential impact on protein structure and function. The variant lies within a critical region of the *MCPH1* protein and may influence its biological activity, supporting its possible involvement in disease pathology.

Protein-Protein Interaction Network of MCPH1

The STRING analysis of *MCPH1* revealed a highly interconnected protein-protein interaction (PPI) network comprising 11 nodes and 27 edges, with an average node degree of 4.91 and a local clustering coefficient of 0.807. The network exhibited a significant PPI enrichment ($p = 4.4 \times 10^{-5}$), indicating that *MCPH1* and its interacting partners engage in more interactions than expected by chance, suggesting functional coherence. Functional enrichment analysis highlighted the involvement of *MCPH1* in cell cycle regulation, centrosome duplication, and centriole replication. Key biological processes included centrosome duplication (GO:0051298), regulation of the centrosome cycle (GO:0046605), and cell cycle processes (GO:0022402). *MCPH1* was also associated with molecular functions such as gamma-tubulin binding and protein binding, and localized to cellular components including the centrosome, microtubule organizing center, and procentriole replication complex. Disease association enrichment confirmed the relevance of *MCPH1* to primary autosomal recessive microcephaly (DOID:0070296), with additional links to related phenotypes such as hypoplasia of the frontal lobes (HP:0007333) and sloping forehead (HP:0000340). The network also identified literature-supported interactions with other *MCPH1*-associated proteins, including ASPM, STIL, CENPJ, NCAPG2, NCAPD3, BRCA2, MDC1, CEP152, CDK5RAP2, and TERF2.

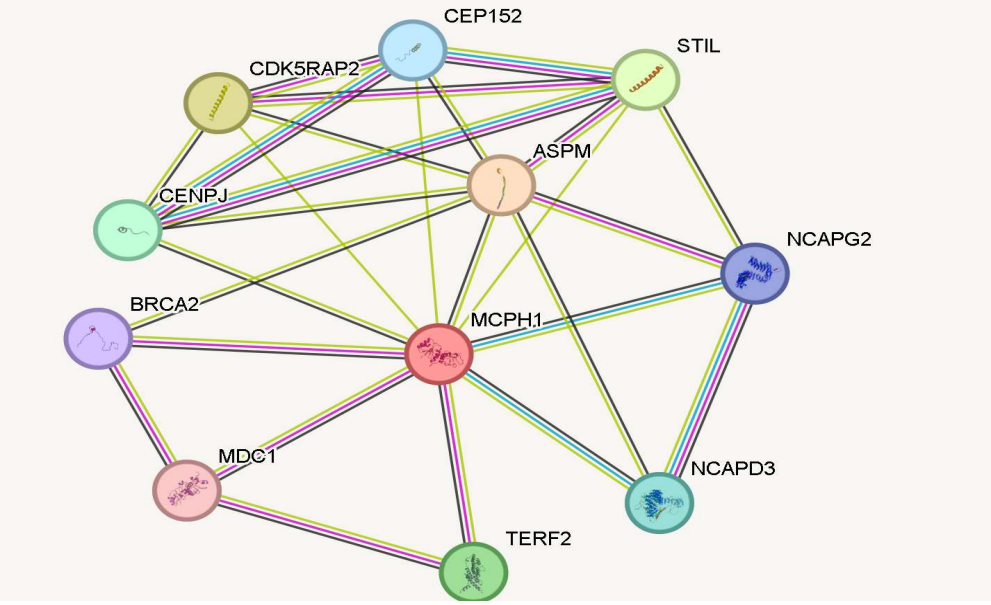

Figure I: Protein-Protein Interaction Network of *MCPH1*, shows effects of *MCPH1* of the other genes in body

Mutational Analysis Using BioEdit

Mutational analysis of the *MCPH1* gene was performed using BioEdit through pairwise sequence alignment of wild-type and mutant sequences. The analysis identified a single nucleotide substitution (G>A) at position NC_000008.11:g.6406671G>A, corresponding to a coding change c.4 G>A, which results in a missense mutation p.Ala2Thr (A2T) at the protein level. This substitution, located in the N-terminal region, replaces non-polar alanine with a polar threonine residue, potentially affecting local structural conformation and protein stability.

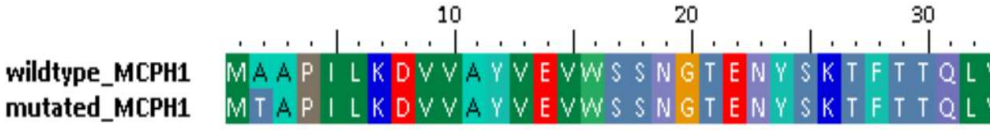

Figure II: Mutational analysis (c.4 G>A / p.Ala2Thr), one strand is wild type and other strand is mutated

Pathogenicity Assessment of NC_000008.11:g.6406671G>A Variant

The identified variant NC_000008.11:g.6406671G>A results in a missense mutation (c.4G>A / p.A2T, NCI-TCGA:ENST00000344683) located on chromosome 8p23.1. Pathogenicity predictions indicate a potentially deleterious effect, with PolyPhen classifying it as possibly damaging (0.834) and SIFT predicting it as deleterious (0.02). The mutation is not somatic and has been previously associated with adenomas and adenocarcinomas in large-scale studies. Cross-references are documented in the NCI-TCGA database as a novel variant.

Table I: Pathogenicity assessment of the NC_000008.11:g.6406671G>A variant, highlighting its mutation details and deleterious predictions by PolyPhen-2 and SIFT.

Feature	Details
Consequence	Missense
Prediction (PolyPhen)	Possibly damaging (0.834)
Prediction (SIFT)	Deleterious (0.02)
Somatic	No
Accession	NC_000008.11:g.6406671G>A
Consequence Type	Missense
Cytogenetic Band	8p23.1
Genomic Location	NC_000008.11:g.6406671G>A
cDNA / Protein Change	c.4 G>A / p.A2T (NCI-TCGA:ENST00000344683)
Disease Association	Adenomas and Adenocarcinomas
Source Type	Large scale study
Cross-References	NCI-TCGA: TCGA novel

Predicted Active Sites of MCPH1 for Molecular Docking

Three conserved BRCT domains of Microcephalin (*MCPH1*) were selected as ligand-binding sites based on UniProt domain annotations and structural analysis. Key residues within these domains, responsible for phosphoprotein recognition, were chosen as docking targets. This concise representation highlights residues most likely to mediate ligand interactions, suitable for docking simulations.

Table II: Predicted active sites of the *MCPH1* protein, highlighting key residues within conserved BRCT domains selected for molecular docking based on UniProt annotations and structural analysis. Note: These residues were used to define docking grids, focusing on biologically conserved pockets critical for *MCPH1* interactions with phosphorylated partners.

Binding Site	Domain	Key Docking Residues
Site 1	BRCT Domain 1 (1-93)	Lys11, Arg18, Ser35, Thr42, Tyr67
Site 2	BRCT Domain 2 (640-730)	Arg693, Lys699, Ser710, Thr724, Asp727
Site 3	BRCT Domain 3 (751-833)	Lys764, Ser770, Tyr778, Thr795, Lys814

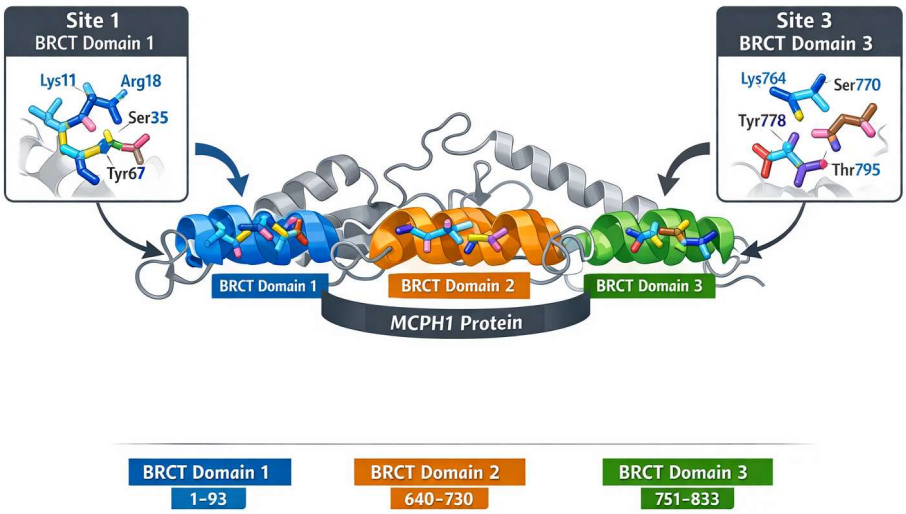

Figure III: Predicted active sites of *MCPH1* for molecular docking

Wild MCPH1-Ligand Interaction Analysis

The docking analysis of the wild-type *MCPH1* protein revealed key interactions with the ligand, highlighting potential binding sites critical for molecular recognition. Hydrogen bond interactions were observed between the ligand and specific amino acid residues, including Met96 and Asp54, indicating stabilizing contacts within the binding pocket. The distances between the ligand atoms and receptor residues ranged from 3.84 Å to 3.92 Å, with interaction energies spanning -0.5 to -2.1 kcal/mol, suggesting favorable binding conformations.

Table II: Hydrogen bond interactions between the ligand and wild-type *MCPH1* protein.

Ligand Atom	Receptor Atom	Residue	Chain	Interaction Type	Distance (Å)	Energy (kcal/mol)
N1	SD	Met96	A	H-donor	3.84	-0.5
SE8	N	Asp54	A	H-acceptor	3.92	-2.1

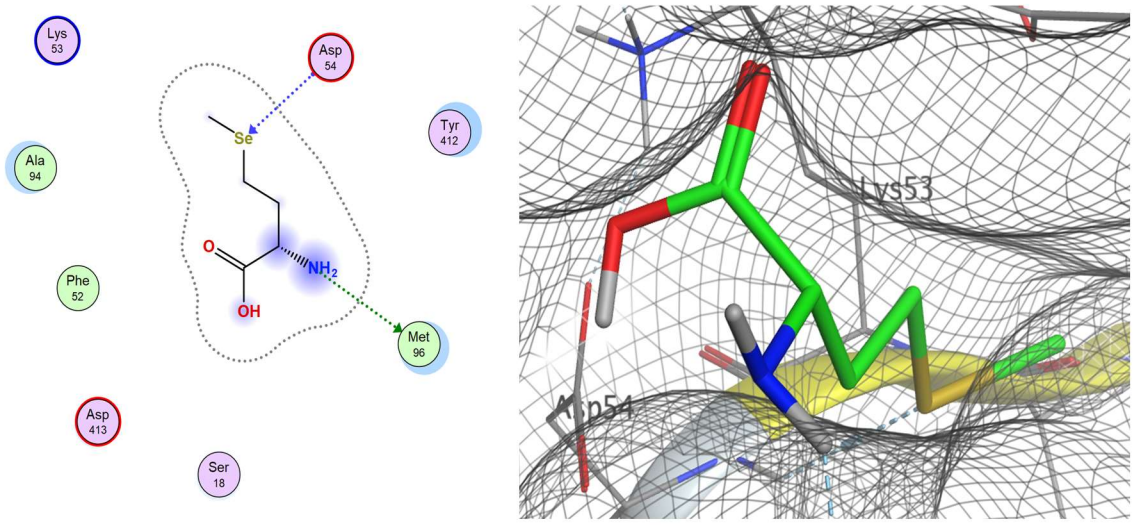

Figure IV: Wild *MCPH1*-Ligand Interaction Analysis. 2D and 3D views showing key ligand interactions and binding orientation within the *MCPH1* active site.

Mutant Ligand-Protein Interaction Analysis of Mut_MCPH1

The mutant ligand-protein interaction analysis for Mut_*MCPH1* reveals that the ligand interacts primarily with the amino acid Ile104 in the *MCPH1* protein through a hydrogen bond, where the nitrogen atom of the ligand acts as an H-acceptor and forms a hydrogen bond with Ile104. The distance of this hydrogen bond is measured at 3.14 Å, indicating a strong and favorable interaction. The energy associated with this interaction is -3.0 kcal/mol, reflecting a stable binding between the mutant ligand and the receptor residue. Docking and scoring metrics further support the stability and feasibility of this interaction. The S score, which represents the overall docking score, is -4.6221, suggesting moderate binding affinity of the mutant ligand with the protein. The RMSD_refine value of 2.1251 Å indicates minimal structural deviation of the ligand after refinement, showing that the ligand maintains a stable conformation within the binding pocket. The E_conf (conformational energy) is 16.1995 kcal/mol, representing the energy required for the ligand to adopt the observed conformation, whereas the E_place (placement energy) is -56.6306 kcal/mol, which reflects the favorable energy contribution associated with the ligand being correctly placed in the protein binding site. Additionally, the E_refine value of -23.1741 kcal/mol indicates further stabilization of the ligand-protein complex after refinement steps. The PLIF_raw data, represented as [1,1621,104,4,3,1,2], encodes the specific residue-level interactions, highlighting that Ile104 is the critical contact point in this mutant ligand-protein interaction. Comparatively, in the wild-type ligand interactions, multiple residues such as MET96 and ASP54 were involved in binding, indicating more extensive contacts; however, the mutant ligand primarily relies on a single key residue, Ile104, for its binding, which may suggest a reduced overall number of contacts due to the mutation, but still maintains a stable interaction through a strong hydrogen bond.

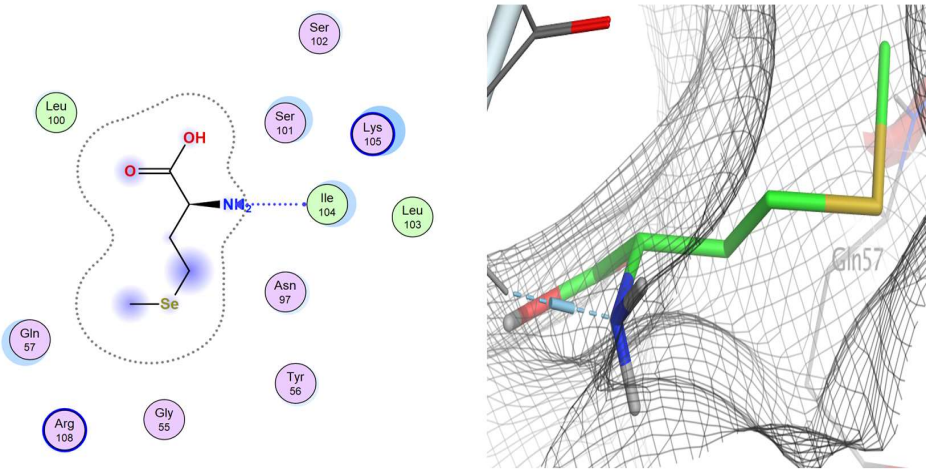

Figure V: Mutant *MCPH1*-Ligand Interaction Analysis. 2D and 3D views showing key ligand interactions and binding orientation within the *MCPH1* active site.

Docking Results of Wild and Mutant MCPH1 with the Ligand

The molecular docking analysis was performed to evaluate the binding affinity of the ligand with both wild-type and mutant *MCPH1* proteins. The first docking pose for the wild *MCPH1*-ligand complex showed a docking score (S) of -4.6748, with an RMSD refined value of 1.4752. The calculated conformational energy (E_conf) was 19.2965, while the placement energy (E_place) was -57.2000. Furthermore, the scoring parameters showed E_score1 = -9.6512, E_refine = -19.6271, and E_score2 = -4.6748. In comparison, the first docking pose of the mutant *MCPH1*-ligand complex exhibited a docking score (S) of -4.6221 with an RMSD refine value of 2.1251. The conformational energy (E_conf) was 16.1995, and the placement energy (E_place) was -56.6306. The scoring functions for this complex were E_score1 = -8.0075, E_refine = -23.1741, and E_score2 = -4.6221.

Table 2: Comparative docking parameters of the first binding pose for wild-type and mutant *MCPH1* proteins with the ligand

Protein-Ligand Complex	S (Docking Score)	RMSD Refine	E_conf	E_place	E_score1	E_refine	E_score2
Wild *MCPH1*	-4.6748	1.4752	19.2965	-57.2000	-9.6512	-19.6271	-4.6748
Mutant *MCPH1*	-4.6221	2.1251	16.1995	-56.6306	-8.0075	-23.1741	-4.6221

Discussion

Autosomal recessive primary microcephaly (*MCPH1*) is a genetically heterogeneous neurodevelopmental disorder characterized by reduced brain size and associated cognitive impairments. Understanding the molecular mechanisms underlying *MCPH1* is crucial for early diagnosis, genetic counseling, and potential therapeutic interventions. In this study, we employed an integrative bioinformatics approach to identify and prioritize candidate genes associated with *MCPH1*. By leveraging gene-disease association databases, including GeneCards and DisGeNET, we were able to systematically analyze thousands of genes and narrow them down to a subset of highly relevant candidates. This approach not only confirms previously reported *MCPH1*-related genes but also highlights novel genes that may contribute to the disorder, offering new avenues for functional validation and deeper insights into the genetic architecture of neurodevelopment. Previous studies have extensively explored the genetic basis of autosomal recessive primary microcephaly (*MCPH1*). Woods et al. (2005) highlighted key *MCPH1* genes, including *MCPH1* (Microcephalin), CDK5RAP2, ASPM, and CENPJ, emphasizing that mutations disrupt neurogenesis in the neurogenic epithelium and may have contributed to evolutionary changes in brain size [20]. Naveed et al. (2018) expanded this understanding by

reviewing 18 *MCPH1* loci (*MCPH1–MCPH1*8), noting that mutations affect neurogenesis, centrosome function, and cell cycle regulation, reinforcing the clinical importance of genetic counseling and molecular analysis [21]. More recently, Riaz et al. (2023) identified two novel *MCPH1* missense variants (p.Val47Leu and p.Val71Leu) in a Pakistani family, demonstrating through in silico analysis that these mutations disrupt protein structure and function, particularly in populations with high consanguinity. Together, these studies provide a comprehensive view of *MCPH1* genetics, from evolutionary and molecular perspectives to population-specific pathogenic variants, underscoring the importance of integrative genetic and functional analyses for understanding disease mechanisms [22]. This study reinforces the critical role of *MCPH1*-associated genes, particularly *MCPH1*, in regulating neurogenesis and brain development. The identification of novel pathogenic variants in the Pakistani population highlights the contribution of consanguinity to the prevalence of *MCPH1* and emphasizes the need for population-specific genetic screening. Integrating bioinformatics, in silico analyses, and clinical data provides a powerful approach to elucidate the molecular mechanisms underlying *MCPH1*. These findings not only expand the mutation spectrum of *MCPH1* but also offer valuable insights for genetic counseling, early diagnosis, and future functional studies aimed at understanding and potentially mitigating this neurodevelopmental disorder.

Conclusion

This study successfully identified a subset of candidate genes strongly associated with autosomal recessive primary microcephaly (*MCPH1*) through comprehensive gene-disease association analysis using GeneCards and DisGeNET databases. Comparative analysis revealed overlapping genes that are likely to play crucial roles in neurodevelopmental processes and brain size regulation. These findings provide a prioritized list of potential molecular targets for further functional validation and genetic screening in affected populations. The integration of computational gene-disease association tools offers a robust framework to narrow down critical genes, enhancing our understanding of *MCPH1* pathogenesis and supporting future therapeutic and diagnostic strategies.

References

1. Aslam, K., Saeed, A., Saeed, H. I., Bashir, R., Abid, H., Akhtar, R., ... & Anjum, I. (2024). Molecular genetics, neuroimaging outcomes, and structural analyses of novel and recurrent variants of WDR62 gene in two consanguineous Pakistani families with autosomal recessive primary microcephaly. *Molecular Biology Reports*, *51*(1), 783.
2. Naseer, M. I., Rasool, M., Abdulkareem, A. A., Bassiouni, R. I., Algahtani, H., Chaudhary, A. G., & Al-Qahtani, M. H. (2018). Novel compound heterozygous mutations in *MCPH1* gene causes primary microcephaly in Saudi family. *Neurosciences Journal*, *23*(4), 346-350.
3. Luo, S., Ren, L., Wang, R., Hu, J., Wei, W., Feng, Y., & Huang, S. (2024). Functional analysis of a novel intronic variant of *MCPH1* with autosomal recessive primary microcephaly. *Heliyon*, *10*(10).
4. Khan, N. M., Masoud, M. S., Baig, S. M., Qasim, M., & Chang, J. (2022). Identification of pathogenic mutations in primary Microcephaly-(*MCPH1*-) related three genes CENPJ, CASK, and *MCPH1* in consanguineous Pakistani families. *BioMed Research International*, *2022*(1), 3769948.
5. Batool, T., & Irshad, S. (2022). Primary microcephaly families mapped with different microcephalic genes by using whole exome sequencing; Insilco 3D Model's prediction of STIL, CENPJ, and CEP135 protein. *Gene Reports*, *27*, 101557.
6. Batool, T., & Irshad, S. (2022). Primary microcephaly families mapped with different microcephalic genes by using whole exome sequencing; Insilco 3D Model's prediction of STIL, CENPJ, and CEP135 protein. *Gene Reports*, *27*, 101557.
7. Hashmi, J. A., Al-Harbi, K. M., Ramzan, K., Albalawi, A. M., Mehmood, A., Samman, M. I., & Basit, S. (2016). A novel splice-site mutation in the ASPM gene underlies autosomal recessive primary microcephaly. *Annals of Saudi Medicine*, *36*(6), 391-396.
8. Erdogan, M., Unal, A., Dogan, M. E., Oguz, S., Balta, B., Ada, Y., ... & Dundar, M. (2025). A rare cause of primary microcephaly: 4 new variants in CDK5RAP2 gene and Review of the literature. *American Journal of Medical Genetics Part A*, *197*(9), e64072.
9. Mazaheri, M., Sadr, Z., Ehtesham, N., Yavari, M., Ahrar, H., & Khodaei, H. (2025). Whole-exome sequencing reveals a novel variant in two Iranian families with autosomal recessive primary microcephaly. *Molecular Biology Reports*, *52*(1), 742.
10. Farooq, S., Asif, M., Abbasi, A. A., Latif, Z., Ku, B., Makhdoom, E. U. H., ... & Hussain, M. S. (2025). Expanding the mutational spectrum of congenital microcephaly in Pakistani families. *Frontiers in Genetics*, *16*, 1709083.
11. Aslam, K., Anjum, I., Aslam, K., Haq, R., & Bashir, R. (2023). Genetic susceptibility of vitamin D receptor gene polymorphisms on autosomal recessive primary microcephaly patients in Pakistani population: a case-control and in-silico study. *Molecular Biology Reports*, *50*(10), 8049-8059.
12. Oluwole, O. G., James, K., & Wonkam, A. (2025). The Identification and Classification of Novel Genetic Variants in the *MCPH1* Gene Suggest Association with Non-Syndromic Hearing Impairment. *OBM Genetics*, *9*(2), 1-14.
13. Yeter, B., Kendir Demirkol, Y., Usluer, E., Görüşen Kavak, İ., Ergin, S. G., & Elçioğlu, N. H. (2025). Expanding the Clinical and Molecular Spectrum of Primary Autosomal Recessive Microcephaly: Novel CDK5RAP2 Gene Variants and Functional Insights on the Intronic Variants. *Genes*, *16*(10), 1120.
14. Dawidziuk, M., Gambin, T., Bukowska-Olech, E., Antczak-Marach, D., Badura-Stronka, M., Buda, P., ... & Wiszniewski, W. (2021). Exome sequencing reveals novel variants and expands the genetic landscape for congenital microcephaly. *Genes*, *12*(12), 2014.
15. Al-Kurbi, A. A. (2021). *Identification of Genes Causing Pediatric Craniofacial Disorders Using Whole Genome Sequencing* (Master's thesis, Hamad Bin Khalifa University (Qatar)).
16. Khan, M. A., Blatterer, J., Kuster, M., Kaufmann, L., Kroisel, P. M., Vincent, J. B., ... & Windpassinger, C. (2025). Genetic analysis in a consanguineous *MCPH1* family revealed a refinement of the *MCPH1*2 locus and a founder effect of the recurrent CDK6 variant [c. 589G> A, p.(Ala197Thr)] in the Pakistani population: MA Khan et al. *Journal of Genetics*, *104*(2), 19.
17. Marchal, J. A., Ghani, M., Schindler, D., Gavvovidis, I., Winkler, T., Esquitino, V., ... & Neitzel, H. (2011). Misregulation of mitotic chromosome segregation in a new type of autosomal recessive primary microcephaly. *Cell Cycle*, *10*(17), 2967-2977.
18. Li, X., Sun, H., Tian, Y., Lan, S., Du, K., Chen, H., ... & Li, B. (2025). Identification of CDK5RAP2 as a causative gene of focal epilepsy without microcephaly. *Seizure: European Journal of Epilepsy*.
19. Ahmad, I. (2016). *Molecular Genetics of Primary Microcephaly and Microcephalic Primordial Dwarfism in Consanguineous Families from Pakistan* (Doctoral dissertation, Universität zu Köln).
20. Naveed, M., Kazmi, S. K., Amin, M., Asif, Z., Islam, U., Shahid, K., & Tehreem, S. (2018). Comprehensive review on the molecular genetics of autosomal recessive primary microcephaly (*MCPH1*). *Genetics Research*, *100*, e7.
21. Woods, C. G., Bond, J., & Enard, W. (2005). Autosomal recessive primary microcephaly (*MCPH1*): a review of clinical, molecular, and evolutionary findings. *The American Journal of Human Genetics*, *76*(5), 717-728.

22. Riaz, Z., Irshad, S., Ahmed, T., & Ali, F. Identification of novel mutations and In-Silico analysis of *MCPH1* gene in Pakistani family with microcephaly.