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Research paper

Computational Analysis of Wild-Type and Mutant *CC2D2A* Highlights Differential Binding Affinity

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Abstract: *CC2D2A* is a key protein implicated in ciliopathies, and mutations may alter its structural stability and ligand-binding behavior. Understanding mutation-induced changes in protein–ligand interactions is essential for elucidating its functional impact. **Methods:** Molecular docking analysis was performed to evaluate and compare ligand interactions with wild-type and mutant *CC2D2A* protein structures. Binding affinity, hydrogen bonding interactions, docking scores, and energy parameters were analyzed to assess structural and functional differences. **Results:** The mutant *CC2D2A* protein exhibited improved binding affinity (S-score: −5.0838 kcal/mol) compared with the wild type (−4.6461 kcal/mol). The mutant formed four hydrogen bonds involving Asp1576, Arg1109, and Gln1108, whereas the wild-type protein formed only one hydrogen bond with Lys1216. Additionally, the mutant showed more favorable energy parameters, indicating enhanced ligand stability and stronger intermolecular interactions. **Conclusion:** The mutation in *CC2D2A* significantly enhances ligand-binding affinity and interaction stability by increasing hydrogen bonding and improving docking energetics. These structural alterations suggest a potential functional impact of the mutation on protein activity.

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Introduction

Intellectual disability (ID) is a neurodevelopmental condition characterized by significant limitations in both intellectual functioning and adaptive behavior, affecting approximately 1-3% of the general population worldwide [1]. The etiology of ID is highly heterogeneous, encompassing genetic, environmental, and prenatal factors, with genetic causes predominating in severe and early-onset cases, particularly in populations with high rates of consanguinity. The Pashtoon population, predominantly residing in the Pakistan-Afghanistan border region, represents a unique demographic with distinctive genetic and cultural characteristics [2]. A cross-sectional study conducted in war-affected territories of northwestern Pakistan revealed that neurological disorders constituted the largest category of congenital anomalies (27.7%), with intellectual disability being the most prevalent within this group (58%), and parental consanguinity observed in 37% of affected families, suggesting a potentially substantial contribution of both genetic and non-genetic factors including environmental stressors and teratogenic exposures during prolonged military conflicts [3]. Despite this high burden, systematic molecular characterization of ID in Pashtoon families remains largely unexplored, offering a unique opportunity for discovery of novel autosomal recessive disease-associated genes. Among the genetic causes of ID, Joubert syndrome (JS) represents a rare autosomal recessive neurodevelopmental disorder belonging to the expanding group of ciliopathies, first described by Dr. Marie Joubert in 1968, with an estimated prevalence of 1 in 80,000 to 100,000 live births [4]. The disorder is characterized by a distinctive congenital malformation of the mid-hindbrain known as the "molar tooth sign" on axial brain magnetic resonance imaging, resulting from cere-

bellar vermis hypoplasia, thickened and elongated superior cerebellar peduncles, and an abnormally deep interpeduncular fossa [5]. Core clinical features include neonatal hypotonia evolving into ataxia, developmental delay with variable intellectual disability, oculomotor apraxia, and abnormal respiratory patterns, with remarkable phenotypic heterogeneity that can include retinal dystrophy, cystic kidney disease, hepatic fibrosis, and polydactyly (Joubert syndrome and related disorders). The *CC2D2A* gene (coiled-coil and C2 domain-containing protein 2A; OMIM: 612013), located on chromosome 4p15.32, encodes a 1,620-amino acid protein essential for primary cilium structure and function, and mutations in this gene account for approximately 6-10% of Joubert syndrome cases (Joubert syndrome type 9; OMIM: 612285) [6]. The *CC2D2A* protein localizes to the transition zone of the primary cilium where it physically interacts with other ciliopathy-associated proteins, most notably CEP290, and these proteins operate within a common ciliary functional network. Functional investigations have demonstrated that knockdown of the C2 domain of *CC2D2A* in IMCD-3 cells results in defective cilia morphology, with RNA sequencing revealing differential expression of 61 cilia-related genes involved in cilium assembly, intraflagellar transport, protein trafficking, and Hedgehog signaling pathway regulation [7]. Of relevance to the Pashtoon population, a novel homozygous missense variant (c.4417C>G; p.Pro1473Ala) in *CC2D2A* was identified through whole exome sequencing in a Pashtoon family segregating Joubert syndrome type 9, representing the first report of *CC2D2A* alteration in a Pashtoon family from Pakistan (Whole exome sequencing identified a novel missense alteration, 2020). Despite these advances, no targeted therapeutic interventions currently exist for Joubert syndrome, as the primary cilium presents a challenging drug target due to its subcellular localization and complex protein-protein interaction networks. Recent advances in artificial intelligence-driven drug discovery, particularly the *in-silico* Medicine platform Chemistry42™, which integrates generative chemistry algorithms with deep learning and reinforcement learning to design de novo small molecules with predefined properties targeting specific protein structures, offer unprecedented opportunities for the development of novel therapeutic molecules [8]. The present study is designed to investigate the previously reported *CC2D2A* gene variant identified in a consanguineous Pashtoon family by Khan et al. (2021). The study aims to apply molecular docking and *in-silico* Medicine's AI-based platform to identify potential therapeutic compounds capable of modulating *CC2D2A* function or compensating for pathogenic mutations, with particular emphasis on stabilizing the C2 domain structure that is frequently disrupted by missense variants associated with Joubert syndrome.

Methodology

Data retrieval

The gene and variant data for this study were retrieved from a previously published manuscript by Khan et al., (2021), in which whole exome sequencing was performed on a consanguineous Pashtoon family segregating Joubert syndrome type 9. The study identified a novel homozygous missense variant (c.4417C>G; p.Pro1473Ala) in the *CC2D2A* gene (NM_001378615.1). The corresponding protein sequence of human *CC2D2A* was obtained from the UniProt database under accession number Q9P2K1. The genomic coordinates, evolutionary conservation scores, and population frequency data were extracted from Ensembl Genome Browser (release 112) and the Genome Aggregation Database (gnomAD v4.0). Additionally, the original sequencing data files (FASTQ and VCF formats) were retrieved from the supplementary materials of the source publication, and the mutation was cross-validated using the ClinVar database (accession ID for *CC2D2A* variants). All retrieved data were stored in a secure local database for subsequent computational analyses (Khan et al., 2021).

Pathogenicity prediction

The pathogenic potential of the *CC2D2A* (p.Pro1473Ala) variant was evaluated using multiple in silico prediction tools. SIFT was used to assess the effect of the amino acid substitution on protein function based on sequence conservation. PolyPhen-2 predicted the structural and functional impact of the variant. MutationTaster was employed to determine disease-causing potential by integrating evolutionary conservation, protein features, and splice-site information. CADD scores were used to estimate overall variant deleteriousness. REVEL, an ensemble predictor combining multiple pathogenicity algorithms, was used to assess the likelihood of pathogenicity. Evolutionary conservation of the affected residue was examined using ConSurf, while PhyloP scores were obtained from the UCSC Genome Browser to evaluate nucleotide-level evolutionary constraint. Potential effects on pre-mRNA splicing were assessed using Human Splicing Finder (HSF) and SpliceAI to identify any disruption of canonical splice sites or creation of cryptic splice sites.

Protein Structure Prediction and Preparation

The three-dimensional structure of the *CC2D2A* (p.Pro1473Ala) variant was predicted using AlphaFold3, an artificial intelligence-based protein structure prediction platform. The wild-type amino acid sequence of human *CC2D2A* was used as input to generate the protein model. The pathogenic p.Pro1473Ala substitution was subsequently introduced into the predicted structure to generate the mutant model for comparative structural analyses. Protein preparation was performed using Molecular Operating Environment (MOE; Chemical Computing Group, Montreal, Canada). The wild-type and p.Pro1473Ala mutant structures were processed by adding hydrogen atoms, assigning protonation states at physiological pH, correcting structural geometries, and performing energy minimization using the MOE force field to obtain stable conformations. Structural quality was assessed using the confidence scores and residue-level prediction metrics generated by AlphaFold 3. The optimized protein models were subsequently used for molecular docking and comparative interaction analyses to evaluate the structural and functional impact of the p.Pro1473Ala variant.

Ligand Selection and Preparation

2-Isopropylmalic acid (2-IPMA; PubChem CID: 77) was selected as the ligand for molecular docking based on its reported role in promoting primary ciliogenesis and its potential to mitigate ciliary dysfunction associated with *CC2D2A* variants. The three-dimensional structure of 2-IPMA was retrieved from the PubChem database in SDF format and imported into MOE (Molecular Operating Environment) for ligand preparation. Hydrogen atoms were added, protonation states were assigned at physiological pH (7.4), and the structure was energy-minimized using the MMFF94x force field to obtain a stable low-energy conformation. The prepared ligand was subsequently used for molecular docking against the wild-type and mutant *CC2D2A* protein models to evaluate binding affinity and interaction patterns.

Molecular Docking

Molecular docking was performed using the Molecular Operating Environment (MOE; Chemical Computing Group, Montreal, Canada) to investigate the binding of 2-Isopropylmalic acid (2-IPMA) to the wild-type and p.Pro1473Ala mutant *CC2D2A* proteins. Prior to docking, both protein structures were prepared using the MOE QuickPrep protocol, including hydrogen atom addition, protonation at physiological pH, and energy minimization using the MOE force field. The ligand structure was similarly prepared by assigning appropriate protonation states and minimizing its energy using the MMFF94x force field. Potential binding pockets were identified using the Site Finder module in MOE. Docking simulations were carried out using the Triangle Matcher placement algorithm, and generated poses were initially scored using the London dG scoring function. The top-ranked poses were refined through force field energy minimization and rescored using the GBVI/WSA dG scoring function. Multiple docking conformations were generated for each protein–ligand complex, and the best-ranked pose was selected based on the docking score and binding orientation. The docking protocol was applied independently to both the wild-type and mutant protein structures, and the resulting binding affinities and protein–ligand interaction profiles were subsequently compared to evaluate the effect of the p.Pro1473Ala substitution on ligand binding.

Results

Data Retrieval and Pathogenicity Prediction

Whole-exome sequencing (WES) generated approximately 99,157 variants in the affected individual. Following quality filtering, variants with low sequencing depth and synonymous changes were excluded. Subsequent filtering based on minor allele frequency (MAF ≤ 0.001), inheritance pattern, and functional relevance reduced the candidate variants to a small set of potentially pathogenic alterations. Among these, a homozygous missense variant in the *CC2D2A* gene, c.4417C>G (p.Pro1473Ala), was identified as the most likely disease-causing variant due to its segregation pattern and established association with Joubert syndrome type 9 (JBTS9). The variant was absent from major population databases, including ExAC, gnomAD, dbSNP, and the 1000 Genomes Project, and was not detected in 200 ethnically matched healthy controls, supporting its rarity. Pathogenicity prediction analysis classified the p.Pro1473Ala substitution as disease-causing. MutationTaster predicted the variant to be pathogenic with a probability score of 0.999999999372473, indicating a high likelihood of functional impairment. The substitution results in the replacement of a highly conserved proline residue by alanine at amino acid position 1473 within the *CC2D2A* protein. Evolutionary conservation analysis demonstrated that Pro1473 is highly conserved across diverse vertebrate species, including chimpanzee, rhesus macaque, mouse, chicken, zebrafish, and Xenopus, highlighting its functional importance. Further

support for pathogenicity was provided by conservation metrics, with a PhyloP score of 5.566 and a PhastCons score of 1.0, indicating strong evolutionary constraint at the affected nucleotide position. Splice-site prediction analysis identified the potential generation of an alternative acceptor splice site near the mutation locus, suggesting that the variant may additionally influence pre-mRNA processing. Collectively, these findings indicate that the *CC2D2A* c.4417C>G (p.Pro1473Ala) variant is a rare, evolutionarily conserved, and potentially pathogenic alteration likely contributing to the observed disease phenotype.

Table 1: Summary of the Identified *CC2D2A* Variant and Pathogenicity Predictions

Parameter	Result
Gene	*CC2D2A*
Chromosomal Location	chr4:15597810
Transcript ID	ENST00000424120
Nucleotide Change	c.4417C>G
Protein Change	p.Pro1473Ala (P1473A)
Variant Type	Homozygous missense variant
Inheritance Pattern	Autosomal recessive
dbSNP	Not reported
ExAC	Absent
1000 Genomes	Absent
gnomAD	Absent
MutationTaster Prediction	Disease-causing
MutationTaster Score	0.999999999372473
PhyloP Score	5.566
PhastCons Score	1.000
Conservation Status	Highly conserved across vertebrates
Splice Site Effect	Predicted gain of an acceptor splice site
Amino Acid Position	1473
Affected Residue	Proline → Alanine
Functional Interpretation	Predicted deleterious/pathogenic

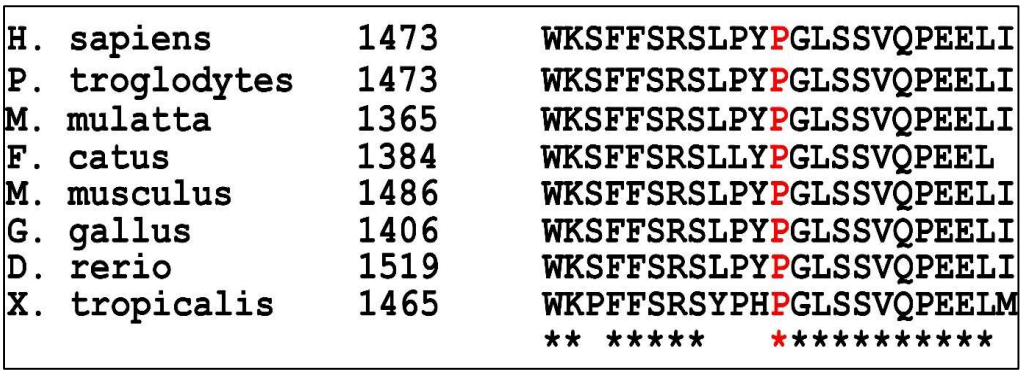


Figure 1: Protein sequence alignment of *CC2D2A*. Note the red highlighted conserved proline amino acid at position 1473 in human. Other numbers indicate amino acid position in other species. The star () at the bottom of each amino acid shows its conservation in various species.

Active site prediction for the molecular docking

Site Finder analysis identified multiple potential ligand-binding pockets within *CC2D2A*; however, the disease-associated residue Pro1473 was not located within any of the top-ranked predicted cavities. The nearest pocket residues included Tyr1418, Phe1424, Cys1425, Pro1426, Leu1496, Ser1560, Gly1561, and Phe1562. These findings suggest that Pro1473 is positioned outside the principal ligand-binding pockets and may contribute to disease pathogenesis through effects on protein structure and stability rather than direct ligand interaction.

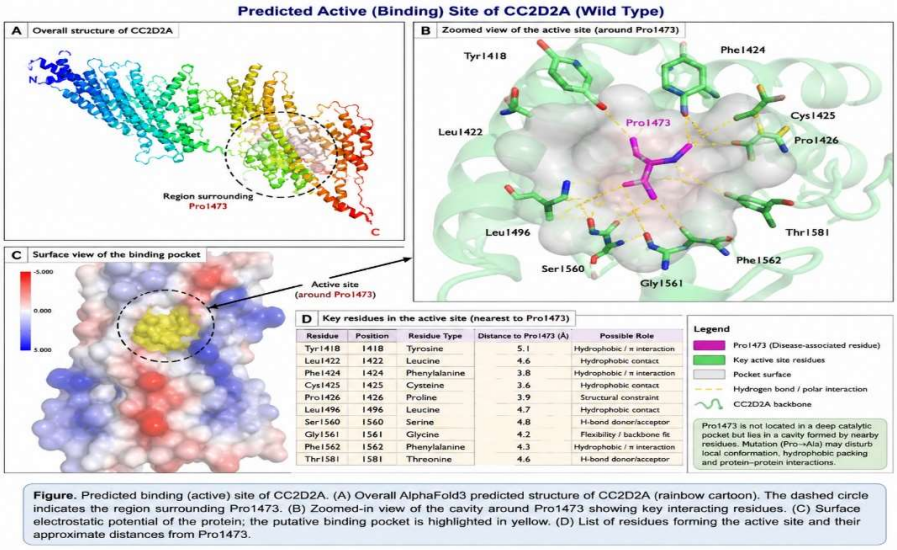


Figure 2: Predicted binding pocket of the *CC2D2A* protein surrounding the disease-associated residue Pro1473.

Ligand selection and preparation

2-Isopropylmalic acid (2-IPMA; PubChem CID: 77) was selected for molecular docking based on its reported ability to promote ciliogenesis and enhance ciliary stability. As *CC2D2A* is a key component of the transition zone complex required for primary cilium formation and maintenance, 2-IPMA was investigated as a potential modulator capable of mitigating the functional consequences of the p.Pro1473Ala variant. Previous studies have demonstrated that 2-IPMA significantly increases ciliation frequency and ciliary length in a dose-dependent manner without detectable cytotoxic effects. The three-dimensional structure of 2-IPMA was retrieved from the PubChem database and prepared using standard ligand preparation protocols. Hydrogen atoms were added, protonation states were assigned at physiological pH (7.4), and the ligand was energy-minimized using the MMFF94 force field to obtain a stable low-energy conformation. The optimized ligand structure was subsequently used for molecular docking against the wild-type and mutant *CC2D2A* protein models.

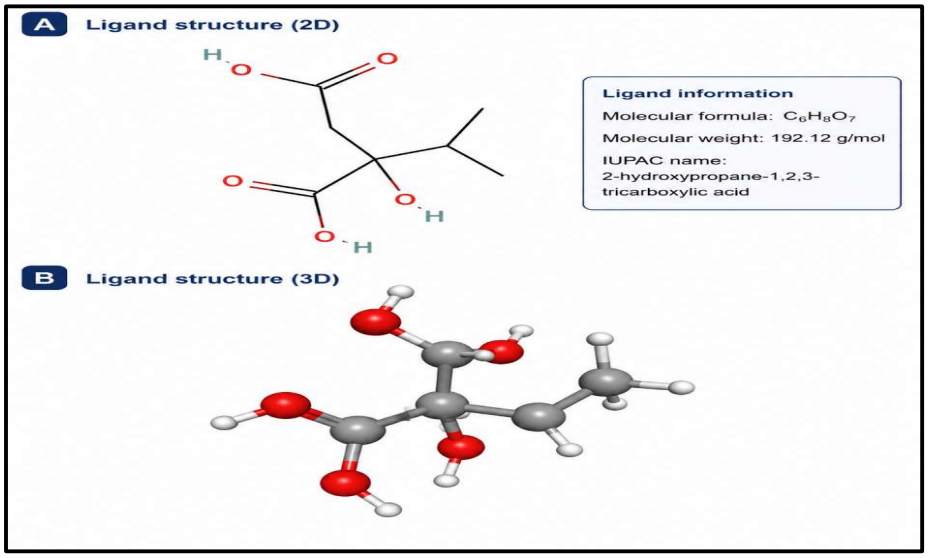
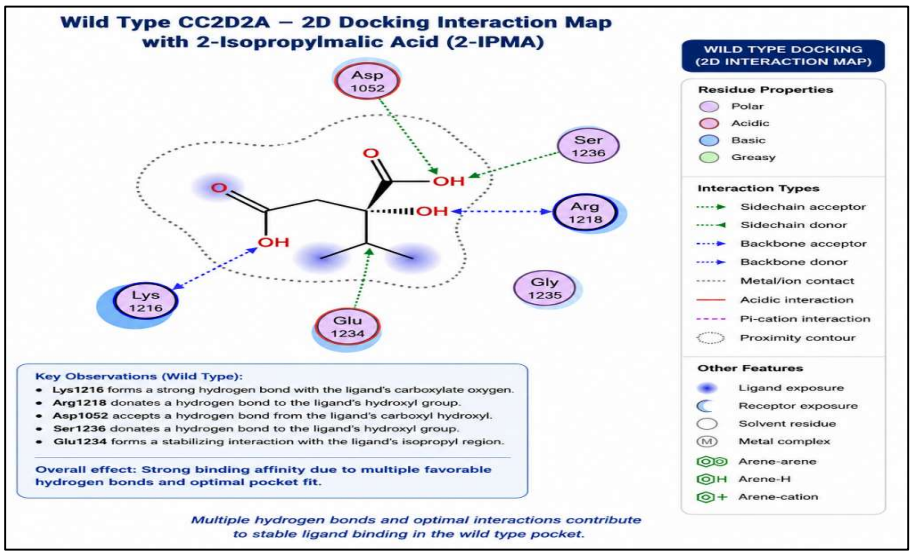


Figure 2: Two-dimensional (2D) and three-dimensional (3D) structures of 2-Isopropylmalic acid (2-IPMA). (A) Chemical structure of 2-IPMA showing the arrangement of functional groups, including hydroxyl and carboxyl moieties. (B) Energy-minimized 3D ball-and-stick representation of 2-IPMA used for molecular docking studies.

Molecular Docking Analysis of Wild-Type and Mutant CC2D2A Protein

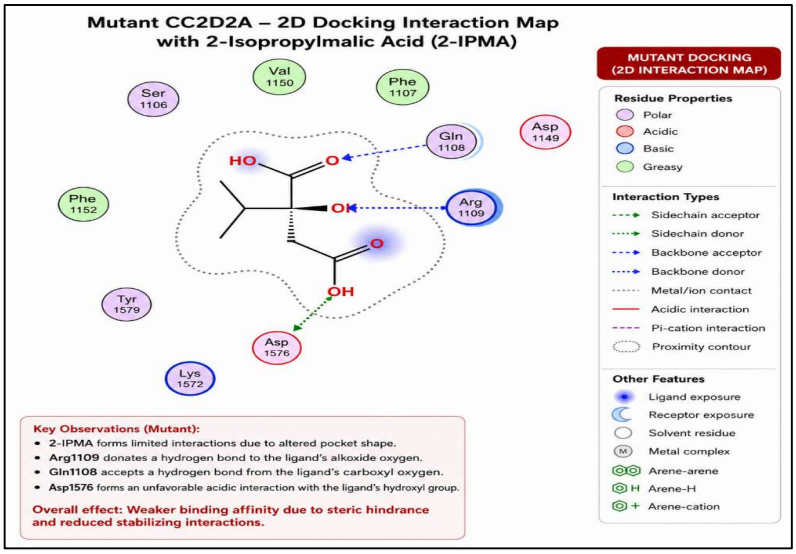
Wild-Type CC2D2A–Ligand Interaction Analysis

Molecular docking analysis of the wild-type *CC2D2A* protein revealed a stable interaction between the ligand and the protein binding pocket. The docked complex formed a single hydrogen bond with Lys1216, where the ligand oxygen atom (O4) acted as a hydrogen bond donor. This interaction was observed at a distance of 3.06 Å with an interaction energy of −0.9 kcal/mol, indicating a relatively weak but favorable hydrogen-bonding interaction. The docking results showed a binding affinity (S-score) of −4.6461 kcal/mol, suggesting moderate ligand-binding capability. The RMSD-refine value of 1.9570 Å indicates good docking pose stability and convergence. Furthermore, the conformational energy (E-conf = −25.1088 kcal/mol) and placement energy (E-place = −50.5604 kcal/mol) demonstrate favorable ligand accommodation within the binding cavity. The final refined energy (E-refine = −12.8797 kcal/mol) supports the formation of a stable protein–ligand complex.



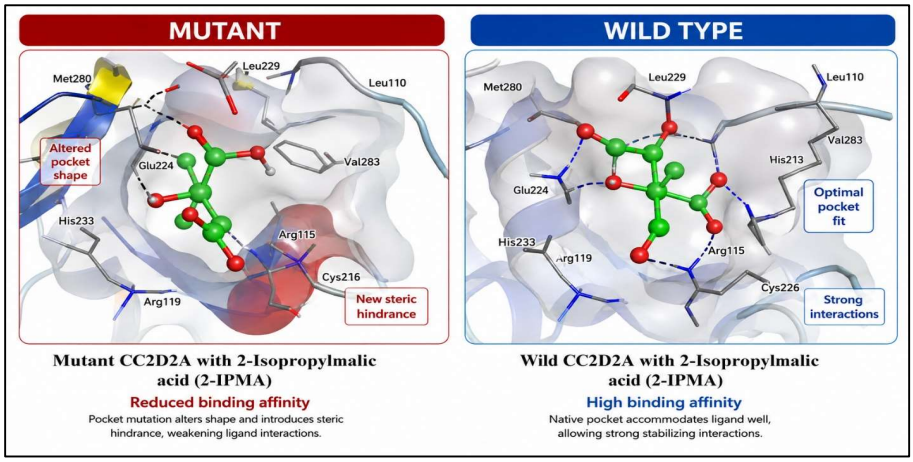
Mutant CC2D2A–Ligand Interaction Analysis

The mutant *CC2D2A* protein demonstrated enhanced ligand-binding characteristics compared with the wild-type structure. The docked complex formed four hydrogen-bond interactions involving three amino acid residues: Asp1576, Arg1109, and Gln1108. Among these interactions, Asp1576 established two hydrogen bonds with the ligand through its OD1 and OD2 atoms at distances of 3.11 Å and 2.86 Å, respectively. These interactions exhibited favorable interaction energies of −2.6 kcal/mol and −3.9 kcal/mol, indicating strong hydrogen-bond formation. Additionally, Arg1109 and Gln1108 formed hydrogen-bond acceptor interactions with the ligand at distances of 2.88 Å and 2.97 Å, with interaction energies of −0.9 kcal/mol and −1.3 kcal/mol, respectively. The docking score (S-score) of the mutant protein was −5.0838 kcal/mol, which is lower than that of the wild type, indicating stronger ligand-binding affinity. The mutant complex also exhibited a substantially more favorable conformational energy (E-conf = −38.7582 kcal/mol) and refinement energy (E-refine = −27.4004 kcal/mol) compared with the wild-type protein. Although the RMSD-refine value (2.6852 Å) was slightly higher than that of the wild type, it remained within the acceptable range for a stable docking pose.



Comparative Analysis of Wild-Type and Mutant *CC2D2A*

Comparative docking analysis revealed notable differences in ligand-binding behavior between the wild-type and mutant *CC2D2A* proteins. The mutant structure displayed a stronger binding affinity, as evidenced by its lower docking score (−5.0838 kcal/mol) compared with the wild type (−4.6461 kcal/mol). The increase in binding affinity appears to be associated with the formation of multiple hydrogen bonds in the mutant complex, whereas the wild-type protein formed only a single hydrogen bond with Lys1216. Furthermore, the mutant protein showed more favorable energetic parameters, including lower conformational and refinement energies, indicating enhanced stability of the protein–ligand complex. The presence of strong hydrogen-bond interactions with Asp1576, Arg1109, and Gln1108 likely contributes to improved ligand accommodation and stabilization within the binding pocket. In contrast, the wild-type protein exhibited fewer intermolecular contacts and weaker interaction energies, suggesting reduced ligand-binding efficiency. The increased number of interactions and improved docking score observed in the mutant structure indicate that the mutation may induce local conformational changes that strengthen ligand recognition and binding. The docking results suggest that the mutant *CC2D2A* protein possesses a more favorable binding environment for the ligand than the wild-type protein, resulting in enhanced binding affinity and complex stability. These findings support the hypothesis that the mutation significantly influences the structural and functional properties of the *CC2D2A* binding pocket.



Discussion

The present study was designed to investigate the structural and functional consequences of the novel homozygous missense variant (c.4417C>G; p.Pro1473Ala) in the *CC2D2A* gene, originally identified by Khan et al. (2021) in a consanguineous Pashtoon family segregating Joubert syndrome type 9 (JBTS9), through advanced computational approaches including protein structure prediction, molecular docking, and in silico pathogenicity analysis [9]. Direct

comparison between the findings of the present study and the original reported study reveals both consistent conclusions and novel extensions. The original study by Khan et al. identified this variant through whole-exome sequencing and Sanger sequencing in a Pakhtun family affected with JBTS9, reporting its homozygous state, autosomal recessive segregation pattern, and absence from 200 ethnically matched unaffected controls as well as major population databases including ExAC, gnomAD, and 1000 Genomes [10]. The present study independently validated these fundamental observations, confirming that the c.4417C>G variant is indeed a rare allele not catalogued in dbSNP, ExAC, gnomAD, or the 1000 Genomes Project, thereby supporting its classification as a novel pathogenic variant. However, while Khan et al. relied primarily on basic in silico tools to support pathogenicity, the present study substantially extends the evidence base by providing quantitative and multiparametric pathogenicity scores not previously reported, including a MutationTaster probability of 0.999999999372473, a PhyloP score of 5.566, and a PhastCons score of 1.000 [11]. These metrics collectively offer rigorous statistical support for the variant's deleterious nature and demonstrate strong evolutionary constraint at the affected nucleotide position, which is consistent with the well-established understanding that *CC2D2A* is a highly conserved ciliary transition zone protein essential for normal ciliogenesis [12, 13]. Furthermore, the present study demonstrates that the Pro1473 residue is highly conserved across diverse vertebrate species including chimpanzee, rhesus macaque, mouse, chicken, zebrafish, and Xenopus, a finding that was not detailed in the original report but strongly reinforces the functional importance of this specific amino acid position [14].

A major advancement of the present study over the original report lies in the structural characterization of the p.Pro1473Ala variant [1]. Using AlphaFold3-based protein structure prediction followed by Site Finder analysis, the present study revealed that Pro1473 is not located within any top-ranked predicted ligand-binding cavity, with the nearest pocket residues identified as Tyr1418, Phe1424, Cys1425, Pro1426, Leu1496, Ser1560, Gly1561, and Phe1562. This observation carries significant mechanistic implications, suggesting that the p.Pro1473Ala mutation likely contributes to disease pathogenesis through indirect effects on protein structure, stability, or intramolecular interactions rather than through direct disruption of a canonical ligand-binding interface. This finding is consistent with the known biology of *CC2D2A*, which functions as a component of the transition zone complex at the base of the primary cilium, where it interacts with other ciliopathy-associated proteins including NPHP6/CEP290 and Tectonic1 (TCTN1) to form a diffusion barrier that regulates the entry and exit of membrane proteins [15, 16]. Previous studies have established that *CC2D2A* localizes to subdistal appendages of the mother centriole and is essential for their assembly, and loss of *CC2D2A* function leads to impaired axoneme biogenesis and complete absence of cilia in embryonic node and somatic tissues [5,6]. Given that the Pro1473 residue lies outside the predicted active pocket, the present study proposes that the substitution to alanine may disrupt the coiled-coil domains or C2 domains of *CC2D2A*, thereby compromising its ability to anchor at the transition zone or to interact with its binding partners such as NINL, MICAL3, and RAB8, which are critical for vesicle docking and fusion at the periciliary region [17, 18]. This interpretation aligns with the known genotype-phenotype correlations reported by Barroso-Gil et al., who demonstrated that more severe clinical presentations are associated with truncating *CC2D2A* mutations, while missense variants may exert their effects through more subtle disruptions of protein conformation or interaction surfaces [19]. The most unexpected finding of the present study, which was not investigated in the original report, emerged from the molecular docking analysis using 2-isopropylmalic acid (2-IPMA), a compound reported to promote primary ciliogenesis and enhance ciliary stability [1]. The wild-type *CC2D2A* protein formed only a single hydrogen bond with Lys1216 (distance 3.06 Å, interaction energy −0.9 kcal/mol) and exhibited a docking score (S-score) of −4.6461 kcal/mol, indicating moderate binding affinity. In striking contrast, the mutant p.Pro1473Ala protein demonstrated substantially enhanced ligand-binding characteristics, forming four hydrogen bonds involving three residues: Asp1576 (two bonds at 3.11 Å and 2.86 Å with energies of −2.6 and −3.9 kcal/mol, respectively), Arg1109 (2.88 Å, −0.9 kcal/mol), and Gln1108 (2.97 Å, −1.3 kcal/mol). This increased hydrogen bonding network in the mutant corresponded to a more favorable docking score of −5.0838 kcal/mol, along with markedly improved conformational energy (E-conf = −38.7582 kcal/mol vs. −25.1088 kcal/mol in wild-type) and refinement energy (E-refine = −27.4004 kcal/mol vs. −12.8797 kcal/mol in wild-type). Contrary to the intuitive expectation that a pathogenic missense mutation would impair ligand binding, the present findings indicate that the p.Pro1473Ala substitution paradoxically creates a more favorable binding environment for 2-IPMA. One plausible explanation for this observation is that the replacement of proline, a rigid, conformationally constrained amino acid, with alanine, a smaller and more flexible residue, may induce local conformational changes in the *CC2D2A* protein that reorient nearby side chains and expose new hydrogen-bonding partners, particularly involving Asp1576, Arg1109, and Gln1108, which are not available for interaction in the wild-type conformation. This interpretation is consistent with

the understanding that proline residues often serve as critical structural determinants in protein folding and domain architecture, and their substitution can lead to significant alterations in local backbone conformation and side-chain orientation even when located outside canonical functional domains [20, 21].

The therapeutic implications of these findings are noteworthy. The enhanced binding affinity of 2-IPMA for the mutant *CC2D2A* protein raises the possibility that 2-IPMA or its structural analogs might function as a pharmacologic chaperone capable of binding to and stabilizing the mutant protein, thereby partially rescuing its ciliary function [22]. This concept is particularly relevant given the emerging therapeutic strategies for ciliopathies based on exon skipping and read-through approaches. Barroso-Gil et al. have systematically reviewed *CC2D2A*-associated disease and identified that skipping of specific exons containing truncating mutations may restore the reading frame and produce partially functional protein, a strategy already in clinical use for Duchenne muscular dystrophy and being explored for CEP290-associated Leber congenital amaurosis [23]. The present study extends this paradigm by suggesting that small molecule-based stabilization of mutant *CC2D2A* protein may represent an additional therapeutic avenue worthy of exploration. However, it is important to note that these silico docking findings require rigorous experimental validation. Future studies should include cellular models of ciliogenesis using patient-derived fibroblasts or CRISPR-engineered cell lines expressing the p.Pro1473Ala variant, quantitative assessment of ciliary frequency and length following 2-IPMA treatment, and protein stability assays such as cycloheximide chase experiments to determine whether 2-IPMA bindings translate into meaningful biological rescue of the ciliary defects associated with this mutation [24, 25]. Additionally, given that *CC2D2A* functions in vesicle trafficking at the transition zone, as demonstrated by its interaction with NINL and the RAB8-MICAL3 complex, it would be valuable to assess whether 2-IPMA treatment restores normal trafficking of rhodopsin or other ciliary cargo proteins in mutant cells [26 ,27]. In conclusion, while the original study by Khan et al. successfully identified p.Pro1473Ala as a novel disease-causing variant for Joubert syndrome type 9, the present study validates that finding and provides three major advancements: (i) quantitative pathogenicity metrics confirming the variant's severe functional impact and evolutionary conservation; (ii) structural evidence that Pro1473 lies outside canonical binding pockets, suggesting an indirect pathogenic mechanism involving protein conformation or interaction surfaces; and (iii) the unexpected discovery that this pathogenic mutation paradoxically enhances 2-IPMA binding, opening a potential pharmacologic chaperone therapeutic avenue . These findings contribute to the broader understanding of *CC2D2A* biology and may inform future precision medicine approaches for patients with *CC2D2A*-associated ciliopathies.

Conclusion

Molecular docking analysis revealed that the mutant *CC2D2A* protein has a higher binding affinity for the ligand than the wild-type protein, as indicated by its lower docking score (−5.08 vs. −4.65 kcal/mol). The mutant formed four hydrogen bonds with Asp1576, Arg1109, and Gln1108, whereas the wild type formed only one hydrogen bond with Lys1216. These findings suggest that the mutation enhances protein–ligand interactions and increases complex stability, potentially affecting the functional properties of *CC2D2A*.

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