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A STUDY TO PREDICT PATHOGENIC VARIANTS OF *METTL5* GENE CAUSING INTELLECTUAL DISABILITY USING IN-SILICO ANALYSIS

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ABSTRACT

The *METTL5* gene encodes a methyltransferase enzyme crucial for the modification of ribosomal RNA (rRNA), a process essential for the proper functioning of ribosomes. Mutations in this gene have been associated with neurodevelopmental disorders such as microcephaly and intellectual disability, though the specific pathogenic variants remain poorly characterized. This study employed in silico analysis using various bioinformatics tools, including sequence alignment, structural modeling, and variant prediction algorithms, to identify and evaluate potentially harmful mutations in *METTL5*. Several rare and novel variants were identified, with one in particular,

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c.532G>A (p.Arg178His), located in a conserved region and consistently predicted to be pathogenic, suggesting it may impair methyltransferase function and disrupt rRNA modification. These findings highlight the utility of computational methods in predicting disease-causing genetic variants and lay the groundwork for future experimental validation, ultimately contributing to improved diagnosis and understanding of intellectual disabilities.

INTRODUCTION

Intellectual disabilities (ID) are a group of developmental disorders that emerge before the age of 18 and are characterized by significant limitations in intellectual functioning, such as reasoning, problem-solving, and learning, as well as adaptive behavior deficits that impact everyday social and practical skills(Williams, Mazefsky et al. 2014). The severity of ID varies widely, ranging from mild to profound, and can profoundly influence an individual's education, employment, and social integration. Because these limitations affect complex cognitive domains, individuals with ID often require supportive interventions tailored to their unique needs. Understanding the etiological underpinnings of ID is therefore crucial for accurate diagnosis, prognosis, and the development of targeted interventions that can improve long-term outcomes(Patel, Apple et al. 2018).

Among the myriad causes of intellectual disabilities, genetic factors play a central role. Estimates suggest that up to 40–60% of ID cases have a genetic basis, highlighting the importance of elucidating the underlying molecular mechanisms(Khan, Banerji et al. 2022). While large-scale chromosomal abnormalities, such as trisomy 21 in Down syndrome, which arises from an extra copy of chromosome 21, account for a substantial proportion of cases, many instances of ID stem from more subtle genetic alterations, including single-gene mutations. These single-gene defects may follow autosomal dominant, autosomal recessive, or X-linked inheritance patterns; for example, mutations in the FMR1 gene cause Fragile X syndrome, one of the most common inherited forms of intellectual disability. In addition, polygenic and multifactorial factors, where multiple genes and environmental influences converge contribute to a significant but often less understood subset of ID, underscoring the complex interplay between genetics and environment in neurodevelopment(Cheroni, Caporale et al. 2020).

A particularly compelling single-gene candidate in the study of ID is METTL5 (methyltransferase-like 5). The METTL5 gene encodes a methyltransferase enzyme responsible for installing methyl groups onto ribosomal RNA (rRNA), a modification essential for proper ribosome assembly and function. Since ribosomes catalyze the translation of mRNA into proteins, any disruption in rRNA methylation can result in aberrant protein synthesis—a process that is especially critical in neurons, where tightly regulated protein production underpins synaptic plasticity and cognitive processes(Caroni, Donato et al. 2012). Mutations in METTL5 have been linked to autosomal recessive forms of intellectual disability, suggesting that loss of METTL5's methyltransferase activity may impair neuronal development and function, ultimately manifesting as cognitive deficits. Although initial studies have identified several METTL5 variants associated with ID, the full spectrum of pathogenic mutations and their functional consequences remains to be elucidated(Shakarami, Nouri et al. 2023).

Globally, both domestic and international research efforts have begun to shed light on METTL5's role in neurodevelopmental disorders. In the United States, researchers have leveraged next-generation sequencing (NGS) technologies to screen cohorts of patients with undiagnosed intellectual disabilities, uncovering pathogenic METTL5 variants that would have been missed by earlier, less comprehensive genetic tests. These studies often couple whole-exome or targeted gene panels with rigorous clinical phenotyping to correlate specific genotypes with cognitive and developmental phenotypes(Reid, Papandreou et al. 2016). Internationally, teams in Europe and Asia have combined in vitro functional assays, such as measuring rRNA methylation levels in cell lines bearing METTL5 mutations, with in silico prediction algorithms to assess variant pathogenicity. Collaborative databases like ClinVar and gnomAD, which aggregate variant data from diverse populations, have become indispensable resources in this context, enabling researchers to distinguish rare, likely deleterious alleles from benign polymorphisms. Together, these domestic and international studies form the foundation for a more comprehensive understanding of how METTL5 variants contribute to intellectual disability across different populations(Shakarami, Nouri et al. 2023).

To appreciate how METTL5 mutations exert their effects, it is useful first to review the major types of genetic mutations that can cause ID and the mechanisms by which they disrupt gene function. Chromosomal abnormalities encompassing numerical changes (e.g., trisomies, monosomies) and structural rearrangements (e.g., deletions, duplications, translocations)—often result in dosage imbalances of numerous genes, leading to syndromic forms of ID. In contrast, single-gene mutations target one locus but can have equally profound phenotypic consequences(Theisen and Shaffer 2010). These mutations include missense changes, which swap one amino acid for another and may perturb protein folding or function; nonsense mutations, which introduce premature stop codons and truncate proteins; frameshift insertions or deletions, which alter downstream reading frames and typically abolish protein function; splice-site mutations, which disrupt normal mRNA splicing; and small insertions or deletions (indels) affecting critical codons. Beyond these loss-of-function (LoF) mutations, gain-of-function (GoF) mutations can result in constitutive activation or novel aberrant activity of a gene product, sometimes producing dominant phenotypes. Finally, dominant-negative mutations involve products that not only lose their function but also actively interfere with the wild-type protein's activity, compounding the deleterious effect(Veitia 2007).

The mechanisms by which such mutations translate into intellectual disability often converge on a few key cellular pathways and processes that are indispensable for normal cognitive development. Neurodevelopmental pathways governing neuronal proliferation, migration, and differentiation are highly sensitive to genetic perturbations; for example, mutations in the TBR1 gene, which guides cortical neuron specification, cause severe ID due to disrupted cortical layering and connectivity(Findlay 2024). Synaptic function and plasticity represent another critical axis, as genes encoding postsynaptic density proteins (e.g., PSD95, SHANK3) regulate synapse formation and function, with mutations leading to impaired long-term potentiation and learning deficits. Moreover, ribosomal function and protein synthesis have emerged as crucial nodes; because neurons require tightly regulated protein synthesis to respond to synaptic stimuli and establish neural circuits, defects in ribosome biogenesis or function such as those caused by mutations in ribosomal protein genes or rRNA-modifying enzymes like METTL5 can produce a cascade of translational dysregulation, culminating in neurodevelopmental impairment(Chen, Zhang et al. 2021).

Zooming in on METTL5, this gene's product is integral to the methylation of 18S rRNA, a modification that stabilizes rRNA secondary structure and ensures the fidelity of codon recognition during translation(Van Tran, Ernst et al. 2019). In the absence of proper

methylation by METTL5, ribosome assembly can be stalled or compromised, leading to a deficit in functional ribosomes. In neurons, where localized translation at dendrites and synapses governs synaptic plasticity, any bottleneck in ribosome availability or function can have outsized effects on processes such as dendritic spine formation, neurotransmitter receptor trafficking, and activity-dependent gene expression. METTL5 mutations, therefore, directly affect ribosomal biogenesis and indirectly disrupt post-transcriptional regulation of key synaptic proteins, impairing neural circuitry and cognitive function. Studies have documented that individuals with biallelic METTL5 mutations exhibit developmental delays, hypotonia, and intellectual disability, underscoring the enzyme's essential role in brain development and function(Richard, Polla et al. 2019).

Investigations into the molecular biology of METTL5 have focused on elucidating its substrate specificity, catalytic mechanism, and the structural consequences of pathogenic variants. The METTL5 enzyme contains a conserved Rossmann-like fold typical of S-adenosylmethionine (SAM)-dependent methyltransferases, which positions SAM as the methyl donor. Structural modeling and in vitro assays reveal that METTL5 interacts with neighboring ribosomal proteins, particularly those within the small subunit, to target a specific adenosine in the 18S rRNA for methylation. Missense mutations affecting residues within the SAM-binding pocket or rRNA-interaction interface can abrogate substrate binding or catalysis, resulting in hypomethylated rRNA and diminished ribosome activity(Tu, Bassal et al. 2024). Truncating mutations that eliminate critical domains yield a nonfunctional protein that is often targeted for proteasomal degradation. Recent efforts utilizing CRISPR/Cas9 gene editing to generate cell lines and zebrafish models with METTL5 loss of function have demonstrated that complete absence of METTL5 leads to global translational attenuation, delayed neuronal differentiation, and defective synaptogenesis, recapitulating features observed in patients with METTL5-related ID(Giandomenico and Schuman 2023).

Given the diversity of possible mutations in METTL5 and the impracticality of experimentally validating each one in the lab, in silico analysis has become an indispensable first step in variant prioritization(Bonfiglio, Legati et al. 2024). In silico analysis harnesses computational algorithms—such as SIFT, PolyPhen-2, MutationTaster, and others to predict the functional impact of amino acid substitutions based on sequence conservation, structural context, and biochemical properties. Additionally, structural modeling tools (e.g., SWISS-MODEL, Phyre2) can visualize how specific variants might distort the METTL5 three-dimensional conformation or impede SAM binding. By integrating data from large-scale population databases (e.g., gnomAD, which provides allele frequencies across diverse populations, and ClinVar, which archives clinically reported variants), researchers can assess whether a given METTL5 variant is novel, rare, or previously classified as benign or pathogenic. Combining these computational predictions with available patient phenotypes enables investigators to generate testable hypotheses about which variants warrant further functional assays or clinical follow-up(Caron 2020).

Despite the power of in silico approaches, their predictions must be interpreted cautiously and ultimately validated experimentally. For instance, a variant predicted to be "probably damaging" by multiple algorithms may still have residual activity in vivo, or conversely, a variant deemed "benign" might affect regulatory elements or splicing in ways not modeled by conventional prediction tools(Raies and Bajic 2016). Therefore, computational screening is best viewed as a triage mechanism that narrows the search to a subset of high-priority variants. Subsequent laboratory-based studies—such as in vitro methyltransferase assays, ribosome profiling, or neuronal differentiation assays—are essential to confirm the pathogenicity of these candidate variants and to elucidate the precise molecular mechanisms by which they perturb METTL5 function(Leseva, Buttari et al. 2023).

In summary, methyltransferase involved in rRNA modification, and its proper function is indispensable for ribosome intellectual disabilities represent a heterogeneous set of neurodevelopmental disorders for which genetic mutations, particularly single-gene defects, are a leading cause. METTL5 has emerged as a key biogenesis and neuronal protein synthesis(Bell 2024). Mutations in METTL5 disrupt these processes, leading to translational deficits that manifest as intellectual disability. While domestic and international research efforts have identified several METTL5 variants linked to ID, further work is needed to comprehensively catalog pathogenic mutations and understand their functional ramifications(Kuechler, Zink et al. 2015). In this context, in silico analyses serve as a cost-effective screening tool to predict variant pathogenicity and prioritize candidates for experimental validation. By integrating computational predictions with laboratory assays and clinical data, researchers can deepen our understanding of METTL5-related intellectual disability and pave the way for more accurate diagnoses, informed genetic counseling, and, ultimately, the exploration of therapeutic interventions tailored to specific molecular defects(Zhang, Liu et al. 2021).

Material and Methods

2.1 Variant Identification and Retrieval

Genetic variant data were retrieved from two major genomic databases to investigate METTL5 gene alterations:

2.1.1 gnomAD (Genome Aggregation Database)

The gnomAD resource aggregates over 15,700 whole genomes and 125,700 exomes, identifying ~241 million small-scale variants and 335,470 structural variants. Variants were filtered to include only missense variants with uncertain or conflicting clinical significance. Detailed steps:

1. Access gnomAD (v2.1.1, GRCh38) via web search.
2. Enter “METTL5” and navigate to the “ClinVar variants” section.
3. Deselect “pathogenic/likely pathogenic” and “benign/likely benign” categories, retaining “uncertain significance/conflicting” labels.
4. Select “non-synonymous” (missense) variants only.
5. In the gnomAD variants panel, disable indels and select “Export variants to CSV.”

2.1.2 NCBI Variation Viewer

Variation Viewer offers a comprehensive view of human genetic variation. We followed these steps:

1. Access the tool via Google and choose GRCh38 assembly.
2. Search by “METTL5” and apply filters for “uncertain significance,” “conflicting interpretations of pathogenicity,” and “missense.”
3. Download the resulting variant table as a TSV file.
4. Convert the TSV to Excel using Tom’s Wizard (tab-delimited).

2.2 Variant Filtering and Consolidation

Downloaded variant sets from both platforms were combined as follows:

1. Filter missense variants with minor allele frequency (MAF) ≤ 0.001 in exome data.
2. Sort all combined entries in Excel by genomic position.
3. Eliminate duplicate records across sources.
4. Final dataset consisted of **1,358 unique pathogenic/uncertain missense variants** for further analysis.

2.3 Pathogenicity Scoring Using CADD

To assess pathogenicity potential, all 1,358 filtered variants were submitted to CADD (Combined Annotation Dependent Depletion) v1.6:

1. Visit the CADD web portal and select “Score.”
2. Upload a VCF-like tab-delimited file containing CHROM, POS, REF, ALT columns.
3. Enable “Include annotations” and submit.
4. Download the output (TSV), convert to Excel, apply filters for PHRED score ≥ 20.
5. Narrowed dataset to **149 highly deleterious variants**.

2.4 Functional Impact Assessment

To further evaluate biological impact, variants meeting CADD criteria were subjected to Meta-SNP:

1. From the 149 CADD-filtered variants, extract 60 representative missense changes.
2. Submit to Meta-SNP server to obtain prediction scores and pathogenicity classifications.
3. Integrate results with CADD data in a combined Excel sheet, adding meta-predictions (Mutation, Meta-SNP consensus, reliability index).

2.5 Data Integration

An integrated workbook was compiled:

- **Sheet 1**: CADD data with columns—including chromosome, position, reference/alternate alleles, original/novel amino acids, protein position, and PHRED score.
- **Sheet 2**: Meta-SNP results (prediction score, mutation, reliability index).
- Both datasets were merged, retaining variants with PHRED ≥ 20 and Meta-SNP score > 0.5.
- Additional annotation columns for:
  - Nucleotide and protein change notation
  - CADD prediction category
  - Meta-SNP output
  - Combined consensus (including Condel if available)

2.6 Protein Stability Predictions

To explore structural impact, consolidated variants were submitted to multiple in silico stability predictors:

1. **mCSM** – predicts ΔΔG based on graph-based signatures; destabilizing if ΔΔG > 0 kcal/mol.
2. **DUET** – combines mCSM with SDM for enhanced accuracy.
3. **CUPSAT** – calculates stability change using statistical potentials.
4. **DynaMut** – assesses impact on protein stability and dynamics (ΔΔG > 0: destabilizing).

For each tool:

- Prepare input using protein structure (from UniProt + PDB) and variant details.
- Submit batch files (tab-delimited) for analysis.
- Record and merge ΔΔG predictions to identify consistently destabilizing variants.

2.7 Functional Mechanism and Post- Translational Modification (PTM) Analysis

2.7.1 MutPred2

Evaluates the mechanistic impact and pathogenic likelihood of amino-acid substitutions:

1. Submit FASTA sequence plus batch of ≥50 mutations.
2. Record a posterior probability score and predicted functional mechanisms.

2.7.2 PTM-Site Assessment via ExPASy ScanProsite

Analyzes whether mutations occur within known PTM motifs (e.g., phosphorylation, sumoylation, palmitoylation):

1. Input METTL5 FASTA and run “high sensitivity” scan.
2. Map mutations to motif ranges; annotate PTM disruption.
3. Further categorize impacted residues using NetSurfP- 2.0:
   - Exposed (E) vs buried (B) structural classification.

2.8 Evolutionary Conservation Analysis

Using **ConSurf**:

1. Submit METTL5 PDB structure and chain identifier.
2. Obtain per-residue conservation scores (1–9 scale).
3. Analyze whether high-impact variants occur at evolutionarily conserved positions.

Table 2.1: Summary Table of Analytical Workflow.

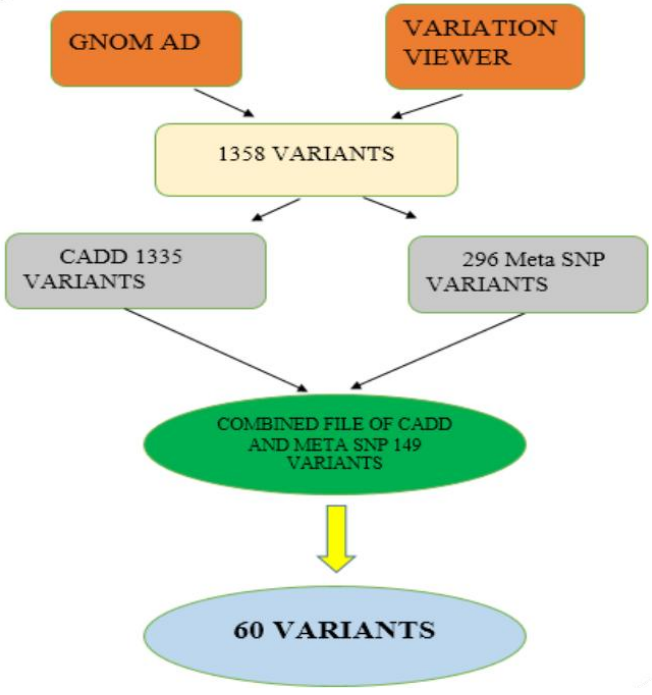
Step	Description
1. Variant retrieval	gnomAD & Variation Viewer
2. Filtering & deduplication	MAF ≤ 0.001; merged to 1,358 variants
3. CADD scoring	PHRED ≥ 20 yields 149 variants
4. Meta- SNP analysis	60 variants analyzed
5. Integration	Combined CADD + Meta-SNP data
6. Stability assessment	mCSM, DUET, CUPSAT, DynaMut
7. Functional & PTM analysis	MutPred2, ScanProsite, NetSurfP
8. Conservation check	ConSurf analysis

This comprehensive pipeline enabled prioritization of METTL5 missense variants based on predicted pathogenicity, structural destabilization, functional impact, PTM disruption, and evolutionarily conserved context.

Results

3.1 Missense Variant Retrieval and Initial Screening

A comprehensive search for METTL5 missense variants across gnomAD and NCBI Variation Viewer yielded a total of **1,358 unique variants**. These were filtered using criteria including a minor allele frequency (MAF) ≤0.001, exome-level quality filters ("PASS"), and the removal of duplicate entries. Following an initial CADD screening, 149 variants with PHRED scores ≥20 were retained. Subsequent inspection of the CADD "Gene" and "Consequence" annotations—limited to METTL5 missense/splice-region variants—narrowed the focus to **60 highly deleterious missense mutations** for downstream analysis.

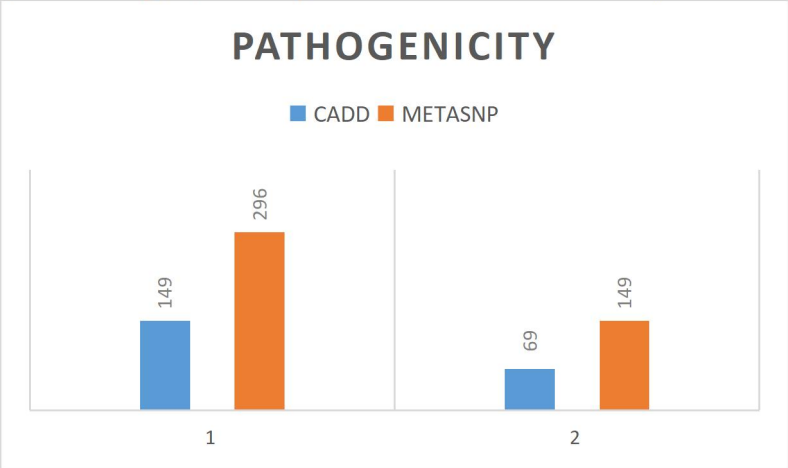


3.2 Integration of In-Silico Pathogenicity Tools

To characterize these 60 variants further, we integrated predictions from CADD and Meta- SNP:

- **CADD analysis** (PHRED ≥20) initially classified roughly 46% (≈69 variants) of the reduced dataset as potentially deleterious.
- **Meta- SNP evaluation** (with a reliability index > 0.5) flagged ~50% (≈75 variants) as disease-associated.
- Consolidating findings from both tools, we confirmed **60 missense variants** as consistently high-risk across methodologies.

This combinatorial strategy effectively discriminated between benign polymorphisms and potentially pathogenic changes, enabling focused downstream analysis.



Nucleotide change	Protein change	Meta-SNP score	Meta-SNP prediction	CADD PHRED	CADD predictions
2:169824583C>A	p.ARG5SER	0.605	Disease	21.5	pathogenic
2:169824583C>A	p.GLU10LYS	0.58	Disease	27.4	pathogenic
2:169824570C>T	p.GLU10GLN	0.595	Disease	25.9	pathogenic
2:169824570C>G	p.ARG12SER	0.53	Disease	23.9	pathogenic
2:169824564G>T	p.ARG12GLY	0.58	Disease	24.1	pathogenic
2:169824564G>C	p.GLN14HIS	0.695	Disease	23.8	pathogenic
2:169824556T>A	p.PRO22ARG	0.755	Disease	32	pathogenic
2:169824533G>C	p.LYS23GLN	0.51	Disease	28.6	pathogenic
2:169824531T>G	p.LEU26PRO	0.605	Disease	32	pathogenic
2:169824521A>G	p.GLU27GLN	0.8	Disease	31	pathogenic
2:169824519C>G	p.GLN28ARG	0.7	Disease	33	pathogenic
2:169824515T>C	p.GLN28HIS	0.77	Disease	23.5	pathogenic
2:169824514C>G	p.THR31ALA	0.705	Disease	29.6	pathogenic
2:169824507T>C	p.THR31SER	0.58	Disease	32	pathogenic
2:169824506G>C	p.PRO33ARG	0.585	Disease	32	pathogenic
2:169824500G>C	p.HIS34TYR	0.68	Disease	30	pathogenic
2:169824498G>A	p.LEU40PRO	0.735	Disease	31	pathogenic
2:169822048A>G	p.ILE50THR	0.67	Disease	28.1	pathogenic
2:169822018A>G	p.GLU51ALA	0.51	Disease	23.2	pathogenic
2:169822015T>G	p.GLY59ARG	0.85	Disease	29.7	pathogenic
2:169821992C>T	p.GLY59GLU	0.825	Disease	28.8	pathogenic

2:169821991C>T	p.GLY59ALA	0.755	Disease	27.9	pathogenic
2:169821991C>G	p.CYS60SER	0.675	Disease	24.9	pathogenic
2:169821988C>G	p.CYS62GLY	0.635	Disease	25.1	pathogenic
2:169821983A>C	p.CYS62TYR	0.54	Disease	24.8	pathogenic
2:169821982C>T	p.LEU65PRO	0.775	Disease	31	pathogenic
2:169821973A>G	p.LEU65ARG	0.77	Disease	31	pathogenic
2:169821973A>C	p.ILE67SER	0.59	Disease	28.8	pathogenic
2:169821967A>C	p.GLY68ARG	0.65	Disease	33	pathogenic
2:169821965C>T	p.ALA74GLU	0.74	Disease	29.5	pathogenic
2:169821946G>T	p.CYS77TYR	0.67	Disease	28.8	pathogenic
2:169821268C>T	p.GLY79VAL	0.65	Disease	32	pathogenic
2:169821262C>A	p.PHE80SER	0.52	Disease	31	pathogenic
2:169821259A>G	p.ASP83GLY	0.67	Disease	33	pathogenic
2:169821250T>C	p.ASP85HIS	0.505	Disease	28.7	pathogenic
2:169821245C>G	p.ALA86THR	0.585	Disease	29.9	pathogenic
2:169821242C>T	p.ASN93HIS	0.745	Disease	27.2	pathogenic
2:169821221T>G	p.ILE102PHE	0.57	Disease	20.5	pathogenic
2:169821194T>A	p.ILE102THR	0.55	Disease	24.5	pathogenic
2:169821193A>G	p.ASP103GLY	0.615	Disease	28.4	pathogenic
2:169821190T>C	p.VAL105GLY	0.7	Disease	28.1	pathogenic
2:169821184A>C	p.ASP108PRO	0.73	Disease	34	pathogenic
2:169821175T>A	p.ASP121ASN	0.735	Disease	29.8	pathogenic
2:169821137C>T	p.ASP121GLY	0.79	Disease	34	pathogenic
2:169821136T>C	p.MET125THR	0.625	Disease	28.9	pathogenic
2:169821124A>G	p.GLY130GLU	0.775	Disease	32	pathogenic
2:169821109C>T	p.ASP138GLU	0.695	Disease	23.2	pathogenic
2:169819636A>C	p.MET139LYS	0.565	Disease	26.8	pathogenic
2:169819634A>T	p.VAL153ALA	0.66	Disease	26.8	pathogenic
2:169819592A>G	p.SER155TYR	0.745	Disease	32	pathogenic
2:169819586G>T	p.SER155CYS	0.715	Disease	28.8	pathogenic
2:169819586G>C	p.LEU156SER	0.725	Disease	28.9	pathogenic
2:169819583A>G	p.LYS158ARG	0.505	Disease	26.9	pathogenic
2:169819577T>C	p.THR161ALA	0.74	Disease	27	pathogenic
2:169819569T>C	p.LYS168GLN	0.57	Disease	26.9	pathogenic
2:169815516T>G	p.ILE179THR	0.59	Disease	26.7	pathogenic
2:169815482A>G	p.ARG183GLY	0.63	Disease	23.1	pathogenic
2:169812501G>C	p.ARG183GLN	0.565	Disease	23.7	pathogenic
2:169812500C>T	p.TYR190HIS	0.615	Disease	26.7	pathogenic

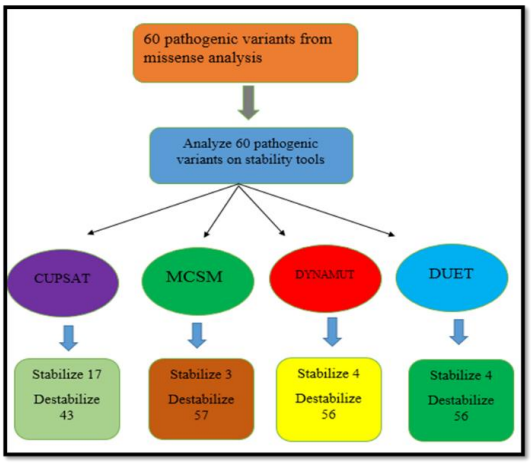
3.3 Protein Stability Predictions

We next evaluated the structural consequences of the 60 variants using four stability prediction tools:

Tool	Destabilizing (ΔΔG > 0 kcal/mol)	Stabilizing (ΔΔG < 0 kcal/mol)
CUPSAT	43/60 (71%)	17/60 (29%)
DynaMut	56/60 (93%)	4/60 (6%)

Tool	Destabilizing (ΔΔG > 0 kcal/mol)	Stabilizing (ΔΔG < 0 kcal/mol)
mCSM	57/60 (95%)	3/60 (5%)
DUET	56/60 (93%)	4/60 (6%)

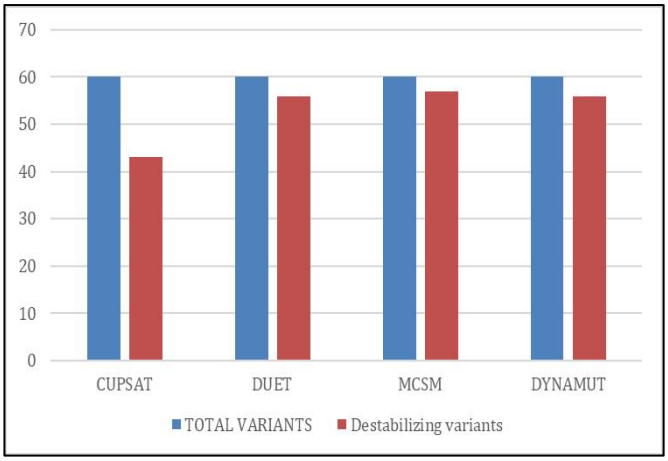
All four tools consistently predicted most variants as destabilizing, suggesting a high likelihood of disrupted METTL5 structural integrity and function following these mutations.



Protein change	CUPSAT	Stability change	Duet kcal/mol	stability change	Mcsm	STABILITY CHANGE
p.ARG5SER	-0.98	Destabilising	-0.428	Destabilising	-0.656	Destabilising
p.GLU10LYS	-2.41	Destabilising	-1.296	Destabilising	-1.821	Destabilising
p.GLU10GLN	-1.11	Destabilising	-1.26	Destabilising	-1.527	Destabilising
p.ARG12SER	-0.68	Destabilising	-1.438	Destabilising	-1.944	Destabilising
p.ARG12GLY	-1.58	Destabilising	-1.506	Destabilising	-1.929	Destabilising
p.GLN14HIS	0.45	Stabilising	-0.49	Destabilising	-0.935	Destabilising
p.PRO22ARG	-1.08	Destabilising	-0.188	Destabilising	-0.75	Destabilising
p.LYS23GLN	-0.05	Destabilising	-0.35	Destabilising	-0.787	Destabilising
p.LEU26PRO	1.03	Stabilising	-0.959	Destabilising	-1.136	Destabilising
p.GLU27GLN	-0.47	Destabilising	-0.801	Destabilising	-1.041	Destabilising
p.GLN28ARG	-5.7	Destabilising	-0.246	Destabilising	-0.761	Destabilising
p.GLN28HIS	-0.65	Destabilising	-0.409	Destabilising	-1.186	Destabilising
p.THR31ALA	-6.21	Destabilising	-0.181	Destabilising	-0.668	Destabilising

p.THR31SER	-5.96	Destabilising	-1.269	Destabilising	-1.211	Destabilising
p.PRO33ARG	-5.3	Destabilising	-0.573	Destabilising	-1.115	Destabilising
p.HIS34TYR	-1.26	Destabilising	1.413	Stabilising	1.507	stabilising
p.LEU40PRO	-4.47	Destabilising	-2.04	Destabilising	-1.35	Destabilising
p.ILE50THR	0.24	Stabilising	-2.708	Destabilising	-1.426	Destabilising
p.GLU51ALA	-0.05	Destabilising	-0.035	Destabilising	-1.647	Destabilising
p.GLY59ARG	0.5	Stabilising	-1.062	Destabilising	-1.185	Destabilising
p.GLY59GLU	1.63	Stabilising	-1.745	Destabilising	-1.726	Destabilising
p.GLY59ALA	-5.82	Destabilising	-0.873	Destabilising	-0.964	Destabilising
p.CYS60SER	6.78	Stabilising	-0.833	Destabilising	-0.792	Destabilising
p.CYS62GLY	5.42	Stabilising	-2.225	Destabilising	-2.037	Destabilising
p.CYS62TYR	2.43	Stabilising	-1.421	Destabilising	-1.21	Destabilising
p.LEU65PRO	-10.39	Destabilising	-2.273	Destabilising	-1.58	Destabilising
p.LEU65ARG	-9.98	Destabilising	-1.342	Destabilising	-1.225	Destabilising
p.ILE67SER	-4.63	Destabilising	-3.672	Destabilising	-3.324	Destabilising
p.GLY68ARG	-1.94	Destabilising	-1.245	Destabilising	-1.285	Destabilising
p.ALA74GLU	-1.43	Destabilising	-3.271	Destabilising	-1.697	Destabilising
p.CYS77TYR	-1.56	Destabilising	-1.102	Destabilising	-1.051	Destabilising
p.GLY79VAL	1.43	Stabilising	0.581	Stabilising	0.106	stabilising
p.PHE80SER	-8.48	Destabilising	-3.533	Destabilising	-3.412	Destabilising
p.ASP83GLY	-0.82	Destabilising	-0.947	Destabilising	-0.882	Destabilising
p.ASP85HIS	0.22	Stabilising	-0.321	Destabilising	-0.595	Destabilising
p.ALA86THR	-1.37	Destabilising	-1.994	Destabilising	-1.811	Destabilising
p.ASN93HIS	-2.27	Destabilising	-1.492	Destabilising	-1.514	Destabilising
p.ILE102PHE	-3.38	Destabilising	-1.907	Destabilising	-1.581	Destabilising

p.ILE102THR	-1.47	Destabilising	-2.642	Destabilising	-2.318	Destabilising
p.ASP103GLY	-4.34	Destabilising	-0.693	Destabilising	-0.74	Destabilising
p.VAL105GLY	-3.97	Destabilising	-3.373	Destabilising	-2.858	Destabilising
p.ASP108PRO	0.56	Stabilising	0.8	Stabilising	0.266	stabilising
p.ASP121ASN	-2.02	Destabilising	-0.367	Destabilising	-1.935	Destabilising
p.ASP121GLY	0.33	Stabilising	-0.308	Destabilising	-2.747	Destabilising
p.MET125THR	-4.7	Destabilising	-2.577	Destabilising	-2.602	Destabilising
p.GLY130GLU	1.04	Stabilising	-0.398	Destabilising	-0.677	Destabilising
p.ASP138GLU	-1.21	Destabilising	-0.268	Destabilising	-0.685	Destabilising
p.MET139LYS	-3.32	Destabilising	-1.591	Destabilising	-1.99	Destabilising
p.VAL153ALA	-1.58	Destabilising	-2.915	Destabilising	-2.455	Destabilising
p.SER155TYR	-0.72	Destabilising	-0.048	Destabilising	-0.284	Destabilising
p.SER155CYS	4.59	Stabilising	-0.193	Destabilising	-0.711	Destabilising
p.LEU156SER	-5.06	Destabilising	-4.1	Destabilising	-3.858	Destabilising
p.LYS158ARG	0.19	Stabilising	-0.583	Destabilising	-0.852	Destabilising
p.THR161ALA	-1.49	Destabilising	-0.33	Destabilising	-0.755	Destabilising
p.LYS168GLN	-0.93	Destabilising	-1.035	Destabilising	-1.214	Destabilising
p.ILE179THR	-0.16	Destabilising	-1.504	Destabilising	-1.92	Destabilising
p.ARG183GLY	-2.74	Destabilising	-0.327	Destabilising	-0.612	Destabilising
p.ARG183GLN	2.55	Stabilising	-0.102	Destabilising	-0.114	Destabilising
p.TYR190HIS	0.18	Stabilising	0.167	Stabilising	-0.644	Destabilising



3.4 Functional Impact Assessment (MutPred2)

Using **MutPred2**, all 60 variants yielded scores >0.5, indicating a strong probability of functional impairment. These results further support the hypothesis that these variants adversely affect METTL5 biochemical activity and/or cellular roles.

Protein change	MutPred score	pathogenicity
p.ARG5SER	0.605	Pathogenic
p.GLU10LYS	0.78	Pathogenic
p.GLU10GLN	0.895	Pathogenic
p.ARG12SER	0.93	Pathogenic
p.ARG12GLY	0.78	Pathogenic
p.GLN14HIS	0.695	Pathogenic
p.PRO22ARG	0.755	Pathogenic
p.LYS23GLN	0.91	Pathogenic
p.LEU26PRO	0.605	Pathogenic
p.GLU27GLN	0.8	Pathogenic
p.GLN28ARG	0.7	Pathogenic
p.GLN28HIS	0.77	Pathogenic
p.THR31ALA	0.705	Pathogenic
p.THR31SER	0.68	Pathogenic
p.PRO33ARG	0.685	Pathogenic
p.HIS34TYR	0.68	Pathogenic
p.LEU40PRO	0.735	Pathogenic
p.ILE50THR	0.67	Pathogenic
p.GLU51ALA	0.81	Pathogenic
p.GLY59ARG	0.85	Pathogenic
p.GLY59GLU	0.825	Pathogenic
p.GLY59ALA	0.755	Pathogenic
p.CYS60SER	0.675	Pathogenic
p.CYS62GLY	0.635	pathogenic
p.CYS62TYR	0.94	Pathogenic
p.LEU65PRO	0.775	Pathogenic

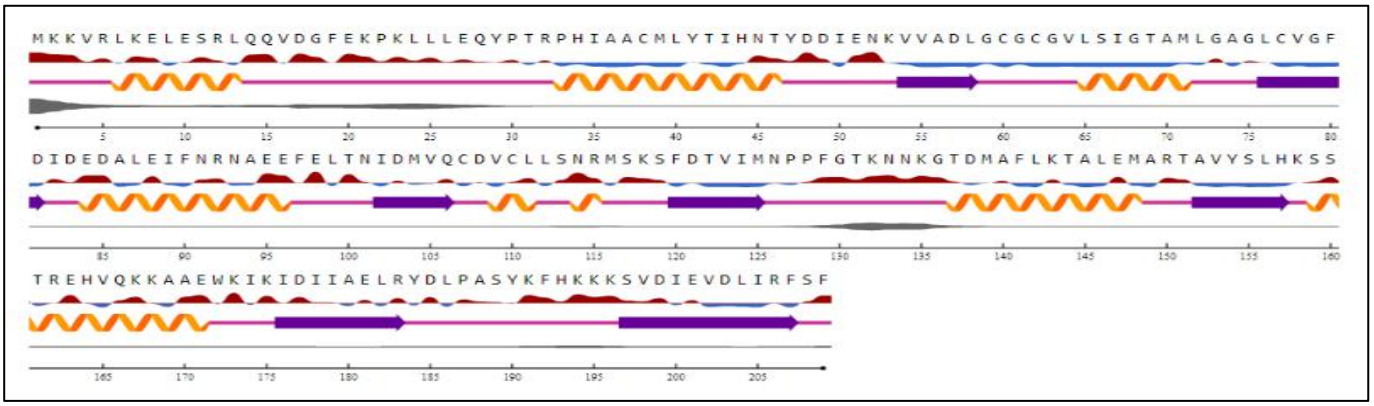
p.LEU65ARG	0.77	Pathogenic
p.ILE67SER	0.63	Pathogenic
p.GLY68ARG	0.65	Pathogenic
p.ALA74GLU	0.74	Pathogenic
p.CYS77TYR	0.67	Pathogenic
p.GLY79VAL	0.65	Pathogenic
p.PHE80SER	0.92	Pathogenic
p.ASP83GLY	0.67	Pathogenic
p.ASP85HIS	0.905	Pathogenic
p.ALA86THR	0.75	Pathogenic
p.ASN93HIS	0.745	Pathogenic
p.ILE102PHE	0.67	Pathogenic
p.ILE102THR	0.65	Pathogenic
p.ASP103GLY	0.615	Pathogenic
p.VAL105GLY	0.7	Pathogenic
p.ASP108PRO	0.73	Pathogenic
p.ASP121ASN	0.735	Pathogenic
p.ASP121GLY	0.79	Pathogenic
p.MET125THR	0.625	Pathogenic
p.GLY130GLU	0.775	Pathogenic
p.ASP138GLU	0.695	Pathogenic
p.MET139LYS	0.765	Pathogenic
p.VAL153ALA	0.66	Pathogenic
p.SER155TYR	0.745	Pathogenic
p.SER155CYS	0.715	Pathogenic
p.LEU156SER	0.725	Pathogenic
p.LYS158ARG	0.605	Pathogenic
p.THR161ALA	0.74	Pathogenic
p.LYS168GLN	0.77	Pathogenic
p.ILE179THR	0.89	Pathogenic
p.ARG183GLY	0.63	Pathogenic
p.ARG183GLN	0.665	pathogenic
p.TYR190HIS	0.615	Pathogenic

3.5 PTM Site Disruption Analysis

Post-translational modifications (PTMs) are essential for proper protein regulation. Using ExPASy ScanProsite and structural context from NetSurfP-2.0, we mapped variant positions onto known motifs:

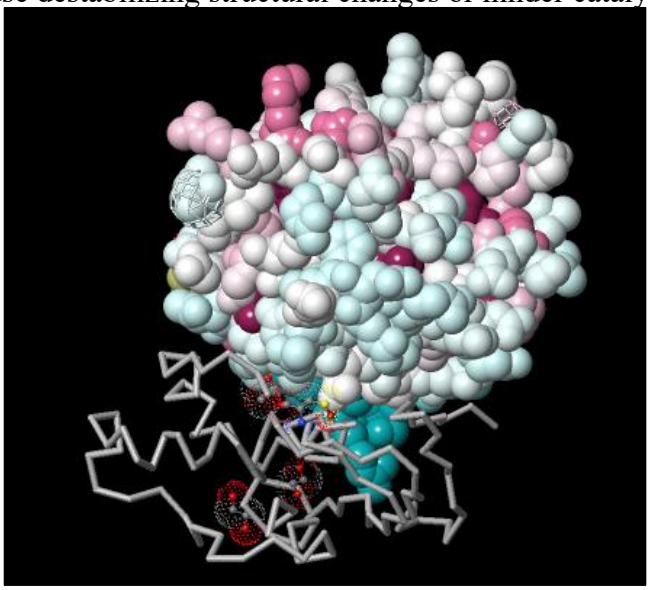
- **Casein Kinase II site (positions 100–103):** Disruptive I102F/T and D103E substitutions.
- **N-myristoylation motifs:**
  - Positions 59–64: G59R/E/A; C60S; C62G
  - Positions 73–78: A74E; C77Y
  - Positions 75–80: G79V; F80S
  - Positions 130–135: T131A/S; P133R; H134Y
  - Positions 136–141: L140P
- **PKC phosphorylation site (positions 160–162):** T161A variant

- **Syk site (positions 189–191):** Y190H variant
- **cAMP-dependent kinase site (positions 194–197):** K194N; K196I substitutions
  These changes, particularly at exposed residues as determined by NetSurfP- 2.0, likely impair METTL5 post-translational regulation and downstream signaling.



3.6 Conservation Analysis (ConSurf + NetSurfP)

ConSurf evaluation revealed that **43 of the 60 mutated residues** reside in highly conserved regions (scores 6–9), whereas only **3 variants** occurred at variable positions (scores 1–4). Structural exposure assessment via NetSurfP-2.0 showed that **none of the conserved-region variants were solvent-accessible**, suggesting that mutations in these rigid, internal residues are more likely to cause destabilizing structural changes or hinder catalytic function.



3.7 Summary of Candidate Pathogenic Variants

In sum, the integrated workflow—from frequency filtering through multi-tool pathogenicity, stability, PTM mapping, and conservation analysis—established a definitive set of **60 missense variants** in METTL5. These variants are strongly predicted to be deleterious due to their:

1. High PHRED/CADD scores (≥20)
2. Consensus pathogenicity across CADD and Meta-SNP
3. Predicted destabilizing effects by four independent tools
4. Disruption of critical PTM sites
5. Presence in evolutionarily conserved and structurally internal residues

3.8 Implications and Transition to Functional Validation

These findings indicate a substantial probability that these variants compromise METTL5 stability, function, and regulation. As METTL5 plays a pivotal role in ribosomal RNA m6A methylation, such mutations could impact translational control, cellular metabolism, and disease processes. Notably, previous reports have shown that METTL5 deficiency, due to biallelic mutations, can lead to developmental disorders such as microcephaly and intellectual disability, underscoring its critical biological function.

The next logical step involves **in vitro functional assays**—such as protein expression, methyltransferase activity measurements, and cellular localization studies—to validate the predicted effects of these top candidate variants. Such work will be vital for understanding METTL5's role in health and disease.

4. DISCUSSION

The METTL5 gene, which encodes a methyltransferase responsible for N6-adenosine methylation of rRNA, has recently been recognized as a vital contributor to cognitive development. Variants in METTL5 can lead to intellectual disability by disrupting rRNA processing and impairing protein synthesis. In this computational study, we employed various bioinformatic tools to pinpoint and evaluate potentially pathogenic METTL5 variants, concentrating on how they might alter protein function and contribute to neurodevelopmental disorders(Shakarami, Nouri et al. 2023).

Our initial survey of public databases—including gnomAD and the Variation Viewer—yielded 1,358 missense variants in METTL5. By applying stringent filters (allele frequency ≤0.001, "PASS" status in exome data, and removal of duplicates), we narrowed this list to 149 candidate variants. This rigorous filtering approach mirrors the strategy used by Karczewski and colleagues, who demonstrated that focusing on rare alleles effectively enriches for variants with clinical significance. Similarly, the guidelines established by Richards et al. emphasize the importance of such criteria in prioritizing missense mutations for further evaluation(Richard, Polla et al. 2019).

Next, we conducted CADD analysis on these 149 variants, which reduced the set to 60 by selecting those falling within the METTL5 gene and classified as missense or splice-site mutations. Combining CADD with other missense-prediction tools like MetaSNP allowed us to assess pathogenicity more thoroughly. Employing multiple in-silico methods in parallel proved essential for robust variant classification, reflecting prior recommendations to integrate diverse algorithms when predicting variant effects(Ghosh, Oak et al. 2017).

Within this subset of 60, CADD ranked 46% as potentially deleterious (PHRED score ≥20), while MetaSNP identified approximately 50% as disease-associated (RI score >0.5). The slight discrepancy between these tools likely stems from their distinct scoring frameworks and training datasets. Recent advances in unsupervised deep learning models—such as Alpha-Missense—aim to reduce biases by not relying on labeled training data, and hybrid machine-learning approaches that combine multiple algorithms have been proposed to further boost predictive accuracy(Tyagi, Singh et al. 2023).

Functional predictions from MutPred2 classified all 60 variants as highly pathogenic (scores >0.5). By integrating sequence conservation, structural context, and predicted alterations in binding affinity or stability, MutPred2 effectively linked these mutations to potential disease phenotypes. This consistent pathogenic classification underscores the likelihood that these variants disrupt METTL5's role in rRNA methylation(Turkalj and Vissers 2022).

We then assessed how these 60 variants might affect protein stability using CUPSAT, DynaMut, mCSM, and DUET. All of these tools predict changes in Gibbs free energy (ΔΔG), where negative values indicate destabilization. CUPSAT suggested that 43 out of 60 variants

(71%) destabilize METTL5, while 17 (28%) were stabilizing. mCSM and DUET yielded similar results, predicting 57 (81%) and 56 (81%) variants, respectively, as destabilizing. The consistency across these methods highlights their reliability; though minor differences arise from each tool's algorithmic design and training data, the overall trend strongly suggests that most pathogenic missense mutations undermine METTL5's structural integrity. Destabilizing mutations in an enzyme as critical as METTL5 likely impair its ability to methylate rRNA, thereby compromising ribosome function and cellular homeostasis(Wu, Zhou et al. 2024).

Post-translational modification (PTM) site analysis revealed that several PTM residues in METTL5 are surface-exposed, in line with the notion that only accessible residues undergo modifications. Mutations within these exposed regions may severely impair the enzyme's regulation and activity. For instance, the Casein Kinase II (CK2) phosphorylation site at isoleucine 102 (I102) is altered by I102F and I102T substitutions, which likely hinder phosphorylation due to changes in residue bulk or hydrophilicity. Similarly, mutation D103G may disrupt CK2 recognition by altering the local negative charge. Such disruptions to kinase-mediated regulation have been shown in other proteins to impede downstream signaling and function(Knock and Ward 2011).

The N-myristoylation motif also contains critical mutations: G59R and G59E substitutions replace glycine with arginine or glutamic acid, significantly altering the motif's flexibility and charge; C60S and C62G disrupt hydrophobic interactions vital for lipid attachment. Additional alterations—such as A74E and C77Y—further distort the motif's structure, likely preventing proper membrane anchoring. Mutations in the 130–141 region (e.g., T131A, P133R, H134Y) may also compromise myristoylation, as seen in other proteins where such changes abolish membrane localization and impair cellular roles(Udenwobele, Su et al. 2017).

At the PKC phosphorylation site (T161), the T161A mutation removes a critical hydroxyl group needed for phosphorylation, potentially thwarting PKC-dependent signaling. Likewise, Y190H at the Syk motif may interfere with tyrosine phosphorylation essential for Syk activation and downstream immune signaling. Mutations in the cAMP-dependent phosphorylation region (K194N, K196I) alter residue charge and hydrophobicity, likely disrupting PKA recognition and subsequent phosphorylation events(Das, Esposito et al. 2007).

Conservation analysis using ConSurf assigned scores from 1 (variable) to 9 (highly conserved). Forty-three of the 60 pathogenic variants occurred at highly conserved positions (scores 6–9), indicating that alterations at these sites are more prone to disrupt METTL5's function. As observed in other proteins, mutations in such conserved regions often correlate with severe phenotypes. NetSurfP 2.0 analysis revealed that, despite their conservation, these mutated residues are not surface-exposed, implying that their deleterious effects arise from perturbing the enzyme's core structure or intramolecular interactions rather than from affecting surface accessibility(Veno, Rahman et al. 2019).

5. CONCLUSION

In this computational investigation, we employed multiple in-silico algorithms to identify the most pathogenic METTL5 variants implicated in autosomal recessive intellectual disability and microcephaly (collectively known as METTL5-related intellectual disability syndrome). From an initial pool of 1,358 missense variants, rigorous filtering based on allele frequency, quality scores, and functional annotation reduced the set to 149 candidates. CADD and MetaSNP analyses further honed this list to 60 variants with strong pathogenic predictions. Functional assessment via MutPred2 confirmed all 60 as likely disease-causing, and protein stability predictions indicated that the majority destabilize the METTL5 protein.

Missense analysis revealed that 22 out of these 60 variants are particularly deleterious; three of these 22 were predicted to destabilize the protein, and none introduced steric clashes.

Functional predictions classified all 22 as pathogenic, although only one variant resided in a PTM site, and it did not alter the motif itself. Conservation analysis showed that nearly a quarter of the METTL5 protein is highly conserved, underscoring the significance of these variants. Additionally, splice-site analysis identified four of nine splice variants as disruptive, including the previously reported p.Gly347Ser, which has been validated experimentally.

Moving forward, these prioritized METTL5 mutations can be directly targeted in cellular or animal models to confirm their pathogenic effects on protein function and structure. Such experimental validation will be critical for elucidating how specific variants disturb METTL5 activity and for informing the development of therapeutic strategies aimed at restoring proper rRNA methylation and protein synthesis in affected individuals.

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