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Unraveling the Pathogenicity of MAN2B1 Missense Single Nucleotide Polymorphisms: A Multi-Tool Computational Approach

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Abstract

Lysosomal alpha-mannosidosis is a rare genetic disorder caused by mutations in the MAN2B1 gene. This study performed a comprehensive *in silico* analysis of missense mutations within MAN2B1 to predict their pathogenicity and impact on protein function and stability. An initial screening of 114 missense variants located on chromosome 19 revealed that 34 were predicted to be deleterious by SIFT. To enhance prediction confidence, variants predicted as deleterious by SIFT were subjected to additional computational analysis using MutPred2, PHD-SNP, SNP&GO, and PROVEAN. This multi-tool approach identified five highly confident pathogenic missense variants: rs1054487 (T312N), rs387906261 (H72L), rs28934600 (H70L), rs199883559 (G212V), and rs374641984 (D196E). These five variants consistently received deleterious predictions across all five functional impact algorithms. Notably, four of these variants (rs1054487, rs28934600, rs374641984 and rs199883559) are currently not reported in the ClinVar database. These findings provide the first *in silico* evidence of their pathogenicity, suggesting their critical role in MAN2B1 dysfunction. Furthermore, protein stability predictions using MUpro and I-Mutant 2.0 indicated that rs1054487 and rs374641984 are likely to decrease protein stability, suggesting a potential mechanism of pathogenicity. While other strongly predicted deleterious variants showed mixed stability results, highlighting diverse pathogenic mechanisms, the consensus across multiple tools provides strong computational support for the likely disease-causing nature of these specific MAN2B1 missense mutations. These *in silico* findings serve as a crucial foundation for guiding future experimental validation studies.

Keywords: Alpha-mannosidosi, *in silico*, mutation, SIFT, variants.

Introduction

Lysosomal α-mannosidase, an enzyme encoded by the *MAN2B1* gene, plays a crucial role in the breakdown of N-linked glycans by hydrolyzing terminal non-reducing α-D-mannose residues within α-D-mannosides. This enzyme is a member of the α-mannosidase II family and is essential for the ordered degradation of these glycans (1). Deficiencies in this enzyme lead to alpha-mannosidosis, a rare autosomal recessive lysosomal storage disorder caused by mutations in the *MAN2B1* gene, located on chromosome 19 (19p13.13) (2). These mutations impede the degradation of glycoproteins and mannose recycling, resulting in a systemic accumulation of mannose-rich oligosaccharides (3).

Alpha-mannosidosis has a global presence, with an estimated prevalence of 1 in 500,000 live births, though its exact prevalence is not known (2) The disorder is typically catego-

rized into two forms based on severity: a severe form (Type 1) that often leads to early death due to infections and hepatomegaly, and a milder form (Type 2) characterized by hearing loss, intellectual disability, and a slower progression into adulthood (4). Diagnosis of alpha-mannosidosis is often suspected in patients presenting with a range of symptoms, including neurological anomalies, sensory defects, immune deficiency, skeletal abnormalities, and facial dysmorphism (5).Confirmatory diagnosis involves detecting low levels of acidic α-mannosidase enzyme activity in peripheral blood leukocytes or cultured skin fibroblasts; affected individuals typically exhibit only 5–15% of normal leukocyte activity. A molecular genetic test for pathogenic variants in *MAN2B1* is then performed to confirm the diagnosis (2).

Recent research on MAN2B1 mutations has predominantly focused on clinical characterization and the identification of novel variants through Whole Exome Sequencing (WES) in diverse populations. For instance, studies in Saudi Arabia and Pakistan identified pathogenic variants such as p.S802fs*129 and p.904Tyr>Ser associated with atypical phenotypes like glaucoma and complex neurological impairments (6-7). Similar clinical reports from Iraq and India have expanded the mutational spectrum, highlighting the presence of novel missense mutations like p.Pro277Leu (4-8). Furthermore, animal models have demonstrated that even single missense mutations, such as p.Asp104Gly, can lead to severe neurodegeneration and lysosomal dysfunction (9). However, these studies are reactive to existing clinical cases. There remains a critical lack of proactive, comprehensive *in silico* analyses that systematically screen the vast array of reported missense SNPs to prioritize those with high pathogenic potential.

This study utilizes a multi-tool *in silico* framework to prioritize MAN2B1 missense variants. By characterizing their functional and structural impacts, we aim to establish a high-confidence pathogenic landscape that facilitates clinical diagnosis and guides future experimental validation of lysosomal α-mannosidosis.

Materials and Methods

This study employed a comprehensive *in silico* bioinformatics pipeline to predict the pathogenicity of missense mutations in the human Mannosidase Alpha, Class 2B, Member 1 (*MAN2B1*) gene.

Variant data acquisition and initial screening of deleterious missense SNPS:

A total of 1,386 missense SNPs for the *MAN2B1* gene were initially retrieved from the NCBI dbSNP database. For each variant, details including SNP ID, chromosomal location, reference and alternative alleles, and the resulting amino acid change were collected retrieved from the single nucleotide polymorphism database (dbSNP) (http://www.ncbi. nlm.nih.gov/snp/) (10). These variants were subjected to functional screening using the Sorting Intolerant from Tolerated (SIFT) algorithm (https://sift.bii.a-star.edu.sg/). SIFT predicts how an amino acid substitution might affect protein function by analyzing sequence homology and the physical properties of amino acids (11). A SIFT score below 0.05 typically indicates a deleterious effect, while a score of 0.05 or higher suggests the change is tolerated (12) (Table 1). Out of the total pool, 114 variants were successfully analyzed, while the remaining variants were excluded as SIFT yielded a 'Not Found' result due to lack of sufficient evolutionary conservation data. Consequently, these 114 variants formed the final dataset for subsequent integrated *in silico* analysis.

Table 1: Bioinformatics tools and parameters used for MAN2B1 SNP analysis

Tool	Threshold (Cut-off)	Prediction category
SIFT	Score ≤0.05	Deleterious
MutPred2	Score>0.5	Moderate impact
	Score>0.75	Deleterious
PHD-SNP	Binary Classification	Disease/ Neutral
SNPs&GO	Score ≥0.5	Disease

PROVEAN	Score ≤-2.5	Deleteroius
I-MUTANT 2.0	DDG<0	Decrease in stability
	DDG>0	Increase in stability
MUpro	Score= 0	Decrease in stability
	Score >0	Increase in stability

Prediction of deleterious missense SNPS:

To enhance prediction confidence, variants predicted as deleterious by SIFT were subjected to additional computational analysis using MutPred2, PHD-SNP, SNP&GO, and PROVEAN.

MutPred2 (http://mutpred.mutdb.org/) utilizes a neural network to predict how amino acid changes might affect protein function and structure (13-14).

PHD-SNP (https://snps.biofold.org/phd-snp/phd-snp.html) employs support vector machines (SVMs) to classify mutations as either neutral or disease-causing, boasting an accuracy of 78% (15-16-17).

Single nucleotide polymorphism database and gene ontology (SNPs&GO) (https://snps.biofold.org/snps-and-go/snps-and-go.html) is a web server that predicts the disease relevance of mutations by combining sequence features, evolutionary data, and Gene Ontology (GO) annotations within an SVM model (18). The final prediction integrates results from Panther, PHD-SNP, and SNPs&GO. SNPs with scores ≥0.5 are categorized as disease-causing, while those <0.5 are considered neutral (19).

Protein variation effect analyzer (PROVEAN) (http://provean.jcvi.org/genome_submit_2.php?species=human) predicts the impact of single or multiple amino acid substitutions or indel mutations on protein function. It computes an alignment score by clustering BLAST hits with over 75% global sequence identity, using the top 30 clusters as the supporting sequence set. These scores are then averaged to obtain the final PROVEAN score (20). An amino acid substitution is considered 'Deleterious' if its PROVEAN score is less than or equal to -2.5, and 'Neutral' if it exceeds this threshold (21).

Prediction of protein stability

Two different computational tools were used to predict the effects of single amino acid substitution on the stability of the human α-mannosidase enzyme.

I-mutant 2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.htm) is an automated web server based on SVM, predicts changes in protein stability caused by single-point mutations. It quantifies the protein stability free energy change (DeltaDeltaG, or DDG) in kcal/mol. A negative DDG value indicates a decrease in stability, whereas a positive DDG value suggests an increase in stability (22-23).

MUpro (http://mupro.proteomics.ics.uci.edu/) employs both support vector machine (SVM) and neural network methods to predict protein stability changes resulting from single amino acid substitutions. Similar to I-Mutant, MUpro can predict stability changes using either protein sequence or structure. Protein sequence and substitutions were provided as inputs to the algorithm. A score of 0 indicates that the mutation reduces stability, while a score >0 suggests an increase in stability (24).

Results and Discussion

Analysis of missense mutations within the MAN2B1 gene identified a total of 117 variants. The analyzed Single Nucleotide Polymorphisms (SNPs) were located on chromosome 19 and within the Coding Sequence (CDS) region. Out of the 114 variants, a significant proportion, specifically 34 mutations, were predicted to be deleterious, indicating a high likelihood of adversely affecting protein function. These deleterious mutations were characterized by SIFT scores ranging from 0 to 0.047 (Table 2).

The remaining 80 variants were predicted to be tolerated, with SIFT scores ranging from 0.058 to 1, suggesting a lesser impact on protein function.

Table 2. Tolerated and deleterious missense SNPs predicted by SIFT

N	SNP ID	CHR	REF ALLELE	ALT ALLELE	AMINO ACID CHANGE	REGION	SIFT SCORE	SIFT PREDICTION
1	rs1054486	19	G	C	L278V	CDS	0.353	TOLERATED
2	rs1054487	19	G	T	T312N	CDS	0.001	DELETERIOUS
3	rs1133330	19	C	T	R337Q	CDS	0.227	TOLERATED
4	rs3745650	19	C	A	A250S	CDS	0.417	TOLERATED
5	rs11554970	19	C	T	R240Q	CDS	0.334	TOLERATED
6	rs34544747	19	C	A	A480S	CDS	0.621	TOLERATED
7	rs35836657	19	T	C	N412S	CDS	0.032	DELETERIOUS
8	rs59357922	19	G	C	Q581E	CDS	0.419	TOLERATED
9	rs61234887	19	C	T	G740R	CDS	0.004	DELETERIOUS
10	rs75029862	19	G	A	P668L	CDS	0.166	TOLERATED
11	rs80338680	19	G	A	R749W	CDS	0	DELETERIOUS
12	rs80338681	19	A	G	L808P	CDS	0.001	DELETERIOUS
13	rs112829030	19	C	T	Q385Q	CDS	0.568	TOLERATED
14	rs117843968	19	G	A	P248L	CDS	0.013	DELETERIOUS
15	rs121434333	19	G	C	P355R	CDS	0	DELETERIOUS
16	rs138349480	19	C	G	Q557H	CDS	0.1	TOLERATED
17	rs139041112	19	C	T	R949H	CDS	0.147	TOLERATED
18	rs139255957	19	G	T	T505K	CDS	0.55	TOLERATED
19	rs139281846	19	C	T	R140Q	CDS	0.082	TOLERATED
20	rs139290127	19	C	A	E203D	CDS	0.018	DELETERIOUS
21	rs139366493	19	C	T	E910K	CDS	0.705	TOLERATED
22	rs140281123	19	C	G	A835P	CDS	0.033	DELETERIOUS
23	rs140449678	19	C	G	Q698H	CDS	0	DELETERIOUS
24	rs140502524	19	G	A	T614M	CDS	0.1	TOLERATED
25	rs140843669	19	C	G	V770L	CDS	0.009	DELETERIOUS
26	rs141077530	19	G	A	L292L	CDS	1	TOLERATED
27	rs141212446	19	C	T	E753K	CDS	1	TOLERATED
28	rs141276889	19	G	A	P635S	CDS	0.482	TOLERATED
29	rs141391488	19	T	C	S801G	CDS	0.003	DELETERIOUS
30	rs141650075	19	C	A	V424L	CDS	0.818	TOLERATED
31	rs142210875	19	C	G	K726N	CDS	0.002	DELETERIOUS
32	rs142248782	19	G	A	R928C	CDS	0.15	TOLERATED
33	rs142702682	19	C	A	G800C	CDS	0.002	DELETERIOUS
34	rs142734279	19	T	G	K486N	CDS	0.254	TOLERATED
35	rs144119421	19	C	T	M172I	CDS	0.02	DELETERIOUS

36	rs144244650	19	C	G	G832R	CDS	0.002	DELETERIOUS
37	rs145062583	19	C	T	E607K	CDS	0.74	TOLERATED
38	rs145163643	19	G	A	P60L	CDS	0.08	TOLERATED
39	rs146725928	19	C	T	R637H	CDS	0.106	TOLERATED
40	rs148080695	19	T	C	N972D	CDS	1	TOLERATED
41	rs148134639	19	C	T	V720M	CDS	0.023	DELETERIOUS
42	rs148661421	19	C	G	A645P	CDS	0.012	DELETERIOUS
43	rs148724402	19	C	T	G96S	CDS	0.047	DELETERIOUS
44	rs150533763	19	G	A	R707W	CDS	0.011	DELETERIOUS
45	rs181476648	19	C	A	R598I	CDS	0.098	TOLERATED
46	rs185112259	19	G	A	R308C	CDS	0.001	DELETERIOUS
47	rs191035238	19	T	C	D653G	CDS	0.223	TOLERATED
48	rs199673719	19	C	G	A7P	CDS	0.32	TOLERATED
49	rs199690827	19	G	A	A300V	CDS	0.859	TOLERATED
50	rs199700264	19	G	A	R731C	CDS	0	DELETERIOUS
51	rs199967717	19	G	A	R731C	CDS	0.274	TOLERATED
52	rs200033151	19	G	T	H900N	CDS	0.393	TOLERATED
53	rs200164758	19	T	C	N152S	CDS	0.237	TOLERATED
54	rs200431869	19	A	G	L967P	CDS	0.004	DELETERIOUS
55	rs200704731	19	G	C	P69A	CDS	0.533	TOLERATED
56	rs201140883	19	C	T	V119M	CDS	0.001	DELETERIOUS
57	rs201318291	19	T	C	K726R	CDS	0.076	TOLERATED
58	rs201448121	19	G	A	R240W	CDS	0.001	DELETERIOUS
59	rs201600797	19	G	A	R780W	CDS	0.004	DELETERIOUS
60	rs202100368	19	G	A	R305W	CDS	0.007	DELETERIOUS
61	rs202174515	19	C	T	R997H	CDS	0.002	DELETERIOUS
62	rs202245970	19	C	T	R144H	CDS	0.138	TOLERATED
63	rs367852398	19	G	A	L810F	CDS	0.032	DELETERIOUS
64	rs368245289	19	G	A	P561L	CDS	0.723	TOLERATED
65	rs368899357	19	C	A	G450C	CDS	0	DELETERIOUS
66	rs368951229	19	G	T	L891M	CDS	0.032	DELETERIOUS
67	rs370036738	19	T	C	N436D	CDS	0.138	TOLERATED
68	rs370276057	19	G	A	R961C	CDS	0.177	TOLERATED
69	rs370382032	19	T	C	D616G	CDS	0.041	DELETERIOUS
70	rs370551216	19	C	T	R741H	CDS	0.453	TOLERATED
71	rs370760999	19	C	T	E401K	CDS	0.232	TOLERATED
72	rs371597285	19	C	T	A151T	CDS	0.133	TOLERATED
73	rs372011807	19	G	A	T784M	CDS	0.031	DELETERIOUS

74	rs372845403	19	G	C	P560R	CDS	0.573	TOLERATED
75	rs372860498	19	C	A	V75L	CDS	0.042	DELETERIOUS
76	rs373101689	19	T	C	S553G	CDS	0.521	TOLERATED
77	rs373174565	19	G	A	P986L	CDS	0.099	TOLERATED
78	rs373240866	19	C	T	R749Q	CDS	0	DELETERIOUS
79	rs373553609	19	C	A	G484V	CDS	0.192	TOLERATED
80	rs373636903	19	T	C	M316V	CDS	0.003	DELETERIOUS
81	rs374524357	19	A	G	F208F	CDS	1	TOLERATED
82	rs375598352	19	C	T	R780Q	CDS	0.098	TOLERATED
83	rs376110556	19	G	A	N691N	CDS	0.642	TOLERATED
84	rs376719984	19	G	A	S554L	CDS	0.252	TOLERATED
85	rs376856949	19	G	A	P981L	CDS	0.337	TOLERATED
86	rs377049583	19	C	T	R283H	CDS	0.33	TOLERATED
87	rs377104016	19	C	A	A571S	CDS	0.079	TOLERATED
88	rs377509266	19	C	T	G484S	CDS	0.363	TOLERATED
89	rs377567861	19	G	A	T948I	CDS	0.232	TOLERATED
90	rs377752596	19	G	C	Q919E	CDS	0.022	DELETERIOUS
91	rs387906261	19	T	A	H72L	CDS	0	DELETERIOUS
92	rs11554969	19	G	T	P872T	CDS	0.335	TOLERATED
93	rs28934600	19	T	A	H70L	CDS	0	DELETERIOUS
94	rs138817592	19	C	T	R731H	CDS	0.002	DELETERIOUS
95	rs139473562	19	G	T	H678N	CDS	0.457	TOLERATED
96	rs139596530	19	C	T	R394Q	CDS	0	DELETERIOUS
97	rs141965228	19	C	T	V715M	CDS	0.001	DELETERIOUS
98	rs142754988	19	C	G	L684F	CDS	0.058	TOLERATED
99	rs143829874	19	G	A	T723I	CDS	0.126	TOLERATED
100	rs145126470	19	G	T	A658D	CDS	0	DELETERIOUS
101	rs148926889	19	T	C	M327V	CDS	0.6	TOLERATED
102	rs151037468	19	C	T	V299M	CDS	0.089	TOLERATED
103	rs199588220	19	G	A	A558V	CDS	0.333	TOLERATED
104	rs199883559	19	C	A	G212V	CDS	0.002	DELETERIOUS
105	rs201109710	19	T	C	T254A	CDS	0.114	TOLERATED
106	rs267605291	19	G	A	L174F	CDS	0.333	TOLERATED
107	rs369174673	19	C	G	G821A	CDS	1	TOLERATED
108	rs371071601	19	A	G	S599P	CDS	0.372	TOLERATED
109	rs371812948	19	C	T	R707Q	CDS	0.453	TOLERATED
110	rs373767545	19	T	C	Q59R	CDS	0.155	TOLERATED
111	rs374417692	19	G	T	P933T	CDS	0.058	TOLERATED

112	rs374506711	19	C	T	G573D	CDS	0.001	DELETERIOUS
113	rs374641984	19	G	T	D196E	CDS	0	DELETERIOUS
114	rs375673126	19	C	G	K89N	CDS	0.71	TOLERATED

To further validate these findings and gain a more comprehensive understanding of their pathogenicity, variants predicted as deleterious by SIFT were subjected to additional computational analysis using MutPred2, PHD-SNP, SNP&GO, and PROVEAN (Table 3).

Table 3. Prediction of the pathogenicity of the missense SNPs using PANTHER, PolyPhen-2, and PHD-SNP servers

N	SNP ID	Mutpred2		PHD-SNP		SNP&GO		PROVEAN	
		Score	Prediction	Score	Prediction	Score	Prediction	Score	Prediction
1	rs1054487	0.791	deleterious	0	Disease	7	Disease	-2.56	Deleterious
2	rs35836657	-	-	4	Neutral	-	-	-2.09	NEUTRAL
3	rs61234887	0.607	moderate impact	3	Neutral	-	-	-5.55	Deleterious
4	rs80338680	0.803	deleterious	6	Disease	-	-	-7.38	Deleterious
5	rs80338681	0.886	deleterious	7	Disease	-	-	-6.18	Deleterious
6	rs117843968	0.468	neutral	5	Neutral	3	Neutral	-6.03	Deleterious
7	rs121434333	-	-	10	Disease	-	-	-8.46	Deleterious
8	rs139290127	0.59	moderate impact	1	Disease	5	Disease	-2.77	Deleterious
9	rs140281123	0.728	moderate impact	3	Disease	-	-	-2.01	NEUTRAL
10	rs140449678	-	-	5	Disease	-	-	-4.8	Deleterious
11	rs140843669	0.545	moderate impact	2	Disease	-	-	-2.03	NEUTRAL
12	rs141391488	0.612	moderate impact	0	Neutral	-	-	-3.23	Deleterious
13	rs142210875	0.671	moderate impact	6	Disease	-	-	-4.52	Deleterious
14	rs142702682	0.746	moderate impact	5	Disease	8	Disease	-8.76	Deleterious

15	rs144119421	0.688	moderate impact	4	Disease	4	Disease	-2.79	Deleterious
16	rs144244650	0.611	moderate impact	5	Neutral	-	-	-6.57	Deleterious
17	rs148134639	0.405	neutral	0	Disease	-	-	-2.25	NEUTRAL
18	rs148661421	0.723	moderate impact	7	Disease	-	-	-4.03	Deleterious
19	rs148724402	0.251	neutral	1	Disease	3	Disease	-4.84	Deleterious
20	rs150533763	0.556	moderate impact	2	Neutral	-	-	-5	Deleterious
21	rs185112259	0.703	moderate impact	8	Disease	9	Disease	-6.23	Deleterious
22	rs199700264	0.789	deleterious	9	Disease	-	-	-6.34	Deleterious
23	rs200431869	0.817	deleterious	7	Disease	-	-	-5.61	Deleterious
24	rs201140883	0.519	moderate impact	4	Disease	8	Disease	-2.92	Deleterious
25	rs201448121	0.688	moderate impact	6	Disease	4	Disease	-6.44	Deleterious
26	rs201600797	0.709	moderate impact	2	Disease	-	-	-6.78	Deleterious
27	rs202100368	0.41	neutral	5	Neutral	4	Disease	-3.78	Deleterious
28	rs202174515	0.472	neutral	9	Disease	-	-	-4.23	Deleterious
29	rs367852398	0.57	moderate impact	4	Neutral	-	-	-3.69	Deleterious
30	rs368899357	-	-	4	Disease	-	-	-	-
31	rs368951229	0.382	neutral	3	Neutral	-	-	-1.63	NEUTRAL
32	rs370382032	0.587	moderate impact	5	Disease	-	-	-4.8	Deleterious
33	rs372011807	0.234	neutral	1	Disease	-	-	-2	NEUTRAL
34	rs372860498	0.432	neutral	8	Disease	3	Disease	-2.59	Deleterious
35	rs373240866	0.675	moderate impact	0	Neutral	-	-	-3.69	Deleterious
36	rs373636903	0.675	moderate impact	2	Neutral	7	Disease	-3.93	Deleterious
37	rs377752596	0.678	moderate impact	8	Neutral	-	-	-1.82	NEUTRAL

38	rs387906261	0.865	deleterious	9	Dis-ease	9	Disease	-9.71	Deleterious
39	rs28934600	0.899	deleterious	9	Dis-ease	9	Disease	-9.77	Deleterious
40	rs138817592	0.735	moderate impact	8	Dis-ease	-	-	-4.07	Deleterious
41	rs139596530	-	-	9	Dis-ease	-	-	-3.76	Deleterious
42	rs141965228	0.631	moderate impact	3	Dis-ease	-	-	-2.85	Deleterious
43	rs145126470	0.873	deleterious	9	Dis-ease	-	-	-5.96	Deleterious
44	rs199883559	0.907	deleterious	8	Dis-ease	7	Disease	-8.31	Deleterious
45	rs374506711	0.752	moderate impact	8	Dis-ease	-	-	-6.93	Deleterious
46	rs374641984	0.837	deleterious	8	Dis-ease	8	Disease	-3.92	Deleterious

The convergent predictions from these multiple tools provided strong evidence for the deleterious nature of several missense variants. For instance, rs1054487 was consistently predicted as deleterious by MutPred2 (0.791), PHD-SNP (Disease), SNP&GO (Disease), and PROVEAN (-2.56, Deleterious), reinforcing its likely pathogenic effect. Similarly, rs80338680, rs80338681, rs199700264, rs200431869, rs387906261, rs28934600, rs145126470, rs199883559, and rs374641984 all showed strong deleterious predictions across a majority of the tools. Several other variants, while sometimes receiving a “moderate impact” or “neutral” prediction from one tool, demonstrated a clear trend towards deleteriousness when considering the consensus. For example, rs139290127 and rs142702682 received “moderate impact” from MutPred2 but were consistently predicted as “Disease” by PHD-SNP and SNP&GO, and “Deleterious” by PROVEAN. This highlights the importance of integrating results from various prediction algorithms.

Conversely, some variants, such as rs35836657 and rs368951229, showed mixed or predominantly neutral predictions across the tools, suggesting they might be tolerated despite an initial SIFT prediction. For example, rs35836657, initially predicted as deleterious by SIFT (0.032), showed a “Neutral” prediction from PHD-SNP and PROVEAN (-2.09, Neutral) and lacked data for MutPred2 and SNP&GO. Similarly, rs117843968 was predicted as neutral byMutPred2 and PHD-SNP, although PROVEAN indicated deleterious. It is important t note that for some SNPs (e.g., rs368899357, rs121434333, rs140449678, rs13959650), data were incomplete for certain prediction tools.

Specifically, five missense variants consistently received deleterious predictions across all five independent computational tools: SIFT, MutPred2, PHD-SNP, SNP&GO, and PROVEAN (Table 4). These highly confident pathogenic predictions include rs1054487 (T312N), rs387906261 (H72L), rs28934600 (H70L), rs199883559 (G212V), and rs374641984 (D196E).

These specific set of five variants represents the strongest candidates for further experimental validation as pathogenic mutations in the MAN2B1 gene.

Table 4. Common deleterious SNPs in MAN2B1 gene as predicted by *in silico* tools

N	SNP ID	SIFT	Mutpred2	PHD-SNP	SNP&GO	PROVEAN
1	rs1054487	DELETERIOUS	deleterious	Disease	Disease	Deleterious
2	rs387906261	DELETERIOUS	deleterious	Disease	Disease	Deleterious
3	rs28934600	DELETERIOUS	deleterious	Disease	Disease	Deleterious
4	rs199883559	DELETERIOUS	deleterious	Disease	Disease	Deleterious
5	rs374641984	DELETERIOUS	deleterious	Disease	Disease	Deleterious

To further investigate the potential impact of these five highly predicted pathogenic variants on protein stability, predictions were generated using MUpro (Neural Network, SVM, and combined) and I-Mutant 2.0 (Table 5). The results from these stability prediction tools provided additional insights into the structural consequences of these mutations.

For rs1054487 (T312N), all three MUpro methods (Neural Network: -0.91 confidence, SVM: -0.27 confidence, combined: -0.93 DDG) consistently predicted a decrease in protein stability. I-Mutant 2.0 also predicted a decrease in stability. This strong consensus suggests that the T312N mutation is highly likely to destabilize the MAN2B1 protein.

Similarly, rs387906261 (H72L) showed a predominant trend towards decreased stability. MUpro (Neural Network: -0.50 confidence, combined: -0.64 DDG) and I-Mutant 2.0 predicted a decrease, while MUpro (SVM) showed a slight increase (0.37 confidence). The overall consensus for H72L, driven by the majority of predictions, points towards reduced stability.

Conversely, for rs28934600 (H70L) and rs199883559 (G212V), the stability predictions were mixed or showed a tendency towards increased stability. rs28934600 (H70L) was predicted to increase stability by all three MUpro methods (Neural Network: 0.73 confidence, SVM: 0.63 confidence, combined: 0.09 DDG), although I-Mutant 2.0 predicted a decrease. For rs199883559 (G212V), MUpro methods indicated increased stability (Neural Network: 0.50 confidence, SVM: 0.22 confidence), while the combined MUpro DDG was -0.78 (decrease), and I-Mutant 2.0 also predicted an increase.

Finally, rs374641984 (D196E) consistently predicted a decrease in protein stability across all three MUpro methods (Neural Network: -0.95 confidence, SVM: -0.50 confidence, combined: -0.81 DDG). I-Mutant 2.0, however, predicted an increase in stability for this variant.

Table 5. The effect of missense SNPs on protein stability

N	SNP ID	I-Mutant 2.0		MUpro		MUpro (SVM)		MUpro (Neural Network)	
		Stability	DDG	Stability	DDG	Stability	Confidence score	Stability	Confidence score
1	rs1054487	DECREASE	1	DECREASE	-0.93	DECREASE	-0.27	DECREASE	-0.91
2	rs387906261	DECREASE	1	DECREASE	-0.64	INCREASE	0.37	DECREASE	-0.50

3	rs28934600	DE-CREASE	1	INCREASE	0.09	INCREASE	0.63	INCREASE	0.73
4	rs199883559	INCREASE	5	DECREASE	-0.78	INCREASE	0.22	INCREASE	0.50
5	rs374641984	INCREASE	5	DECREASE	-0.81	DECREASE	-0.50	DE-CREASE	-0.95

Discussion

The application of high-throughput in silico frameworks has recently gained traction in characterizing various lysosomal-related genes. For instance, previous report utilized a systematic computational approach to prioritize deleterious SNPs in the ARSA gene, which are critical for diagnosing Metachromatic Leukodystrophy, a classic lysosomal storage disorder (25). Beyond primary storage diseases, similar methodologies have been applied to other lysosomal enzymes like Cathepsin D (CTSD) to identify potential biomarkers for cancer progression (26).

While these studies demonstrate the robustness of multi-tool analyses for lysosomal proteins, the MAN2B1 gene has not yet been subjected to such a comprehensive, large-scale screening. This study addresses this significant gap by providing the first integrated pathogenicity and stability profile for the reported mutational spectrum of MAN2B1.

Statistical summary of the prediction concordance revealed that out of the 114 analyzed variants, 34 (29.8%) were initially identified as deleterious by SIFT. To enhance the confidence in identifying truly pathogenic variants, a multi-tool approach was adopted, integrating predictions from MutPred2, PHD-SNP, SNP&GO, and PROVEAN. A high degree of concordance was observed among the majority of the utilized *in silico* tools; however, a small subset of variants displayed conflicting predictions across different algorithms. This variation is primarily attributed to the diversity of the underlying computational architectures and the distinct biological parameters—such as evolutionary, biochemical, and structural data—prioritized by each server. To enhance the reliability of our findings, we implemented a rigorous Consensus-Based Selection strategy. Specifically, the variants that were consistently predicted as pathogenic by all the utilized tools were prioritized.

This strategy allowed for the identification of five critical missense variants (4.4% of the total; 14.7% of the SIFT-deleterious subset)– rs1054487 (T312N), rs387906261 (H72L), rs28934600 (H70L), rs199883559 (G212V), and rs374641984 (D196E). Such strong consensus across multiple prediction platforms suggests that these specific amino acid substitutions are not merely tolerated variations but are likely to significantly disrupt the normal function of the MAN2B1 protein, which is crucial for lysosomal α-mannosidase activity.

The biological significance of the five high-confidence variants (H70L, H72L, D196E, G212V, and T312N) is underscored by their strategic localization within the functional architecture of the MAN2B1 protein. According to the InterPro and Pfam database annotations (Accession: O00754) (https://www.ebi.ac.uk/interpro/protein/UniProt/O00754/entry/pfam/#table) (27-28), all five variants are situated within the Glycosyl hydrolases family 38 N-terminal domain (PF01074), which spans residues 64 to 381.

This specific domain represents the primary catalytic engine of the lysosomal alpha-mannosidase enzyme. The clustering of these mutations within the catalytic core provides a robust biological rationale for their predicted pathogenicity.

Interestingly, a search in the ClinVar da-

tabase revealed that rs28934600, rs1054487, rs374641984 and rs199883559 have not been previously reported or classified. By utilizing a multi-tool consensus approach, we have provided the first line of evidence for the pathogenicity of these variants in the MAN2B1 gene, which may assist in improving the genetic diagnosis of alpha-mannosidosis.

Further investigation into the impact of these five highly predicted pathogenic variants on protein stability, using MUpro and I-Mutant 2.0, offered a deeper mechanistic understanding. For rs1054487 (T312N) and rs374641984 (D196E), the majority of stability prediction tools indicated a significant decrease in protein stability. This suggests that the pathogenic mechanism for these two variants likely involves protein destabilization, potentially leading to misfolding, aggregation, or accelerated degradation, ultimately compromising the enzyme's function. Conversely, the stability predictions for rs28934600 (H70L) and rs199883559 (G212V) were mixed or even suggested an increase in stability by some tools. This intriguing observation suggests that while these mutations are strongly predicted to be pathogenic by functional impact tools, their mechanism of action may not primarily involve protein destabilization. Instead, they might exert their deleterious effects through other means, such as disrupting the active site geometry, altering substrate binding, interfering with protein-protein interactions, or affecting crucial catalytic residues. For rs387906261 (H72L), while some tools predicted a decrease in stability, there was a minor conflicting prediction, warranting careful consideration in the context of its robust functional impact prediction.

It is important to acknowledge the inherent limitations of *in silico* prediction methods. While these tools provide valuable insights and prioritize variants for further investigation, their predictions are computational and do not replace experimental validation. False positives and negatives can occur, and the complex interplay of protein structure, function, and cellular environment cannot be fully captured by algorithms alone. However, the use of multiple, complementary prediction tools, as demonstrated in this study, significantly increases the reliability of the predictions. The consistent deleterious predictions for a subset of *MAN2B1* missense variants across functional impact and, in some cases, stability prediction tools, provides a strong foundation for future experimental studies aimed at elucidating the precise molecular mechanisms underlying their pathogenicity and their role in lysosomal α-mannosidosis.

Conclusion

This *in silico* study provides a robust computational assessment of missense mutations within the *MAN2B1* gene, identifying several variants with a high likelihood of pathogenicity. Through the application of multiple, complementary prediction algorithms for both functional impact and protein stability, we have pinpointed five mutations (rs1054487, rs387906261, rs28934600, rs199883559, and rs374641984) that consistently predict a deleterious effect. Notably, this study highlights four pathogenic candidates (rs1054487, rs28934600, rs374641984 and rs199883559) that are currently not reported in the ClinVar database. By providing the first *in silico* characterization of these variants, our findings fill a critical gap in the genetic landscape of MAN2B1, offering a prioritized roadmap for future clinical diagnostics and functional validation. While *in silico* predictions offer invaluable guidance, the identified high-confidence pathogenic variants warrant urgent experimental validation to confirm their precise molecular mechanisms and clinical relevance.

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Authors' contributions

Concept and Design, D.J.; Data collection and analyzing, S.A., R.K., D.J.; Discussion, D.J.; Writing the manuscript and Supervision,

D.J. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

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