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Research Article

Exploring Missense Variants in the Human PNPLA3 Protein: An In Silico Analysis

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Abstract

Background: Missense variants in humans are genetic variations that lead to amino acid substitutions in protein-coding regions, which can modify protein structure, function, and phenotype. The Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene has received considerable attention because of its link to several metabolic disorders, such as non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD).

Methods: The PNPLA3 gene was extracted from the National Center for Biotechnology Information (NCBI) databases, and non-synonymous single-nucleotide polymorphisms (nsSNPs) were analyzed using computational software (SIFT, Polyphen-2, SNPs&GO, PhD-SNP, I-Mutant 3.0, MUpro, and Project Hope).

Results: A total of 108 nsSNPs were selected from the coding region for *Homo sapiens*. The SIFT server was used to distinguish between tolerant and intolerant nsSNPs. A total of 21 deleterious nsSNPs were identified, with a tolerance index ranging from 0.000 to 0.03. Polyphen-2 software predicted 19 damaging polymorphisms, with a score range of 0.970 to 1.000. Using Mupro and I-Mutant 3.0, 18 nsSNPs were identified as decreasing the stability of the mutated protein, while one nsSNP was found to increase the stability of the mutated protein. According to the SNPs&GO software, six nsSNPs were predicted to be disease-related, whereas the PhD-SNP software predicted 11 nsSNPs as disease-related. Six nsSNPs were selected for submission to the Project Hope software based on their prediction by SNPs&GO as the most damaging, with a score range of 0.998 to 1.000.

Conclusion: In this study, six deleterious mutations affecting the protein of the *PNPLA3* gene were detected, each with a high score, as indicated by a PSIC SD range of 0.998–1.000, implying pathological polymorphisms that alter the protein's structure, stability, and function. These mutations were regarded as significant nsSNPs for the PNPLA3 gene in relation to NAFLD. Computational tools have inherent limitations, including biases from training data and challenges in modeling complex biological systems, making experimental validation crucial for their implications and practical applications.

Keywords: missense variants, *PNPLA3* gene, in silico analysis, disease susceptibility, protein structure, functional impact

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1. Introduction

Non-synonymous single-nucleotide polymorphisms (nsSNPs), also known as missense variants, represent genetic variations that lead to amino acid substitutions within protein-coding regions of the genome [1, 2]. These variants have been implicated in various human diseases by altering protein structure, function, and interactions [3]. Located on chromosome 22q13.31, the *PNPLA3* gene is a member of the patatin-like phospholipase domain-containing protein family. It produces a protein involved in regulating lipid metabolism and storage [4, 5]. *PNPLA3* has emerged as a leading factor in the pathogenesis of metabolic disorders, particularly non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD), making it a prime target for genetic studies aimed at elucidating disease mechanisms and identifying therapeutic targets [6, 7]. The gene provides directions for producing the adiponutrin (ADPN) protein and calcium-independent phospholipase A2 epsilon acylglycerol. O-acyltransferase or calcium-independent phospholipase is an enzyme found in human fat cells (adipocytes) and liver cells (hepatocytes) [8, 9].

NAFLD is a buildup of excess fat in the liver that can cause liver damage, similar to the damage from alcohol abuse, but occurring in individuals who do not heavily consume alcohol. In some cases, NAFLD progresses to non-alcoholic steatohepatitis (NASH) and can result in permanent liver damage or cirrhosis [10].

This study aimed to perform an in silico analysis of nsSNPs in the human *PNPLA3* gene to elucidate their potential impact on protein structure, function, and disease susceptibility.

2. Methods

2.1. Data retrieval

Using the dbSNP, information regarding SNPs of the *PNPLA3* gene was collected. Various software, including Sorting Intolerant from Tolerant (SIFT), PolyPhen-2, I-Mutant 3.0, MUpro, SNPs&GO, PHD-SNPs, and Project Hope, were utilized to examine the impact of nsSNPs on the structure and function of the PNPLA3 protein (Figure **1**).

2.2. Protein function prediction

For studying the effect of mutations on the protein function, three software programs were used:

2.2.1. Sorting Intolerant from Tolerant (SIFT)

SIFT is a sequence homology-based tool that identifies intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect. SIFT is based on the premise that protein evolution is correlated with protein function. It involves a multistep procedure that searches for similar sequences, selects closely related sequences that may share similar functions with the query sequence, obtains the alignment of these selected sequences, and finally calculates normalized probabilities for all possible substitutions from the alignment [11]. The input nsSNPs' rs-IDs were submitted to the server for analysis; positions with normalized probabilities of <0.05 were predicted to be deleterious, while those ≥ 0.05 were expected to be tolerated [11].

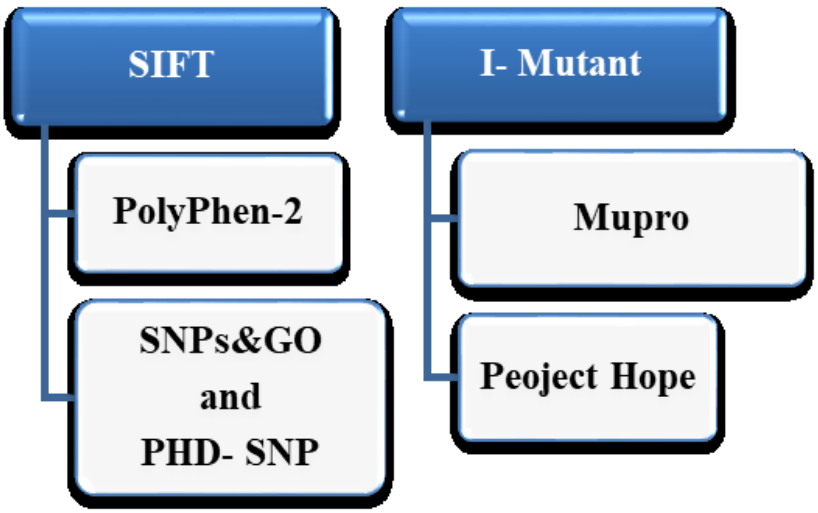

Figure 1: Software used in the study.

2.2.2. PolyPhen-2

Polymorphism Phenotyping v-2, or PolyPhen-2, predicts the potential impact of amino acid substitutions on the structure and function of human proteins using simple physical and evolutionary comparative considerations [12]. It estimates the position-specific independent count (PSIC) for each variant and then assesses the differences between them. The higher the PSIC, the greater the functional impact of the amino acid on protein function may be. Prediction outcomes are categorized as probably damaging, possibly damaging, or benign, based on scores ranging from 0 to 1.

2.2.3. SNPs&GO

Single-nucleotide polymorphism and gene ontology, or SNPs&GO, is an accurate method that, starting from a protein sequence, can predict whether a variation is disease-related or not by exploiting the corresponding protein functional annotation. SNPs&GO collects unique framework information derived from protein sequences and evolutionary information, as encoded in gene ontology terms, outperforming other available predictive methods [13]. The protein sequence is submitted in FASTA format obtained from UniproktB/ExPASY. After submitting the sequence, the mutations are entered in the XPASY format, where X and Y represent the wild-type and mutant residues, respectively. The result is indicated as neutral or disease. PHD-SNP results are included as part of the SNPs&GO output.

2.3. Protein stability prediction

To study the effect of mutations on protein stability, two software programs were used:

(i) I–Mutant 3.0: This software enables the automatic prediction of changes in protein stability resulting from single-site mutations, based solely on the protein sequence or the available protein structure, along with the Gibbs free energy change (DDG) reflecting either increased or decreased stability [14].

(ii) MUpro: This is a set of machine learning programs designed to predict the effect of a single-site amino acid mutation on protein stability. It is developed using two machine learning methods: Support vector machines (SVMs) and

neural networks [15]. The output indicates whether stability has increased or decreased.

2.4. Protein structure prediction

To study the effect of mutations on protein structure, the following software was used: Project Hope is an online web service that allows users to submit a sequence and a mutation. It collects structural information from various sources, including calculations of the 3D protein structure, sequence annotations in UniProt, and predictions from the Reprof software. Project Hope combines this information to analyze the impact of a specific mutation on protein structure. The software generates a report that includes text, figures, and animations [16].

3. Results

The *PNPLA3* gene contained a total of 110 SNPs, with 108 in the coding region and two in the non-coding region. According to SIFT, 21 nsSNPs were predicted to be damaging, while 87 were predicted as tolerated. Analysis using Polyphen-2 revealed that two nsSNPs were benign, four were possibly damaging, and 15 were probably damaging. Two additional software programs, SNPs&GO and PHD-SNPs, identified 6 and 11 nsSNPs with a disease effect, respectively (Table **1** and Figure **2**).

Overall, when using four different software programs (SIFT, Polyphen-2, SNPs&Go, and PHD-SNP) to study the functional and structural effects, a total of 19 SNPs were found to have a disease effect (Appendix **1**).

Regarding the effect on protein stability, 18 nsSNPs were predicted to reduce stability when using I-Mutant 3.0. In contrast, the MUpro software indicated that 18 nsSNPs diminish protein stability, while one nsSNP enhances it (Table **1**).

The structural impact of the SNPs on protein structure and function was examined using Project Hope. Six SNPs were assessed with this software (Appendix **2**). The software compares the wild and mutant residues, along with their size, charge, domain, and hydrophobicity values, for the six highest deleterious nsSNPs.

(i) rs370741805 (Gly 24 Try): The mutant residue leads to a G→W conversion at position 24. This mutant residue is larger than the wild-type residue, which could cause bumps. The wild-type residue is highly conserved. The torsion angles for this residue are unusual. Only glycine is flexible enough to make these torsion angles, and mutating it to a different residue will force the local backbone into an incorrect conformation, disrupting the local structure [16].

(ii) rs372035117 (Gly 112 Ser): The mutant residue leads to a G → S conversion at position 112. This mutant residue is larger than the wild-type residue and is situated on the protein's surface. Its mutation can disrupt interactions with other molecules or parts of the protein [16].

(iii) rs738409 (Cys 144 Ile): The mutant residue is larger than the wild-type residue, which may lead to bumps [16].

(iv) rs370175838 (Phe159 Arg): The mutant residue is larger than the wild-type residue, which may lead to the formation of bumps. There is a difference in charge between the wild-type and mutant amino acids. The mutation introduces a charge that could cause the repulsion of ligands or other residues with the same charge. The hydrophobicity of the wild-type and mutant residues varies. Hydrophobic interactions, whether in the core of the protein or on its surface, will be lost [16].

Table 1: Results from two different software programs.

Software	Result
SIFT	21 deleterious and 87 tolerated
Polyphen-2	15 probably damaging, 4 possibly damaging, and 2 benign
SNPs&GO	6 disease-related and 13 neutral
PHD-SNP	11 disease-related and 8 neutral
I-Mutant	18 decrease the protein stability and 1 increase the protein stability
MUpro	18 decrease the stability of the protein and 1 increase the protein stability
Project Hope	6 nsSNPs affect the protein function

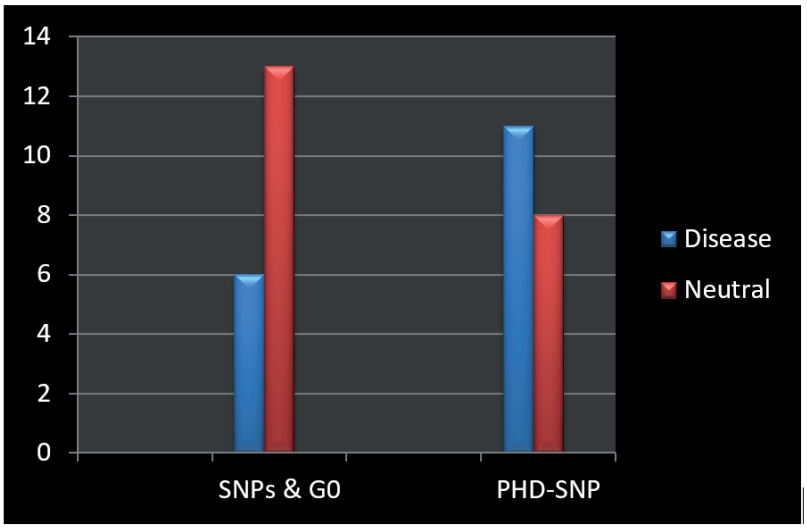

Figure 2: Results of SNPs & GO compared to PHD-SNP.

(v) rs369199264 (Pro 174 Ser): The mutant residue leads to P→S conversion at position 174 and is smaller than the wild-type residue, which may result in the loss of interactions. Hydrophobic interactions, whether in the core of the protein or on its surface, will be lost. Proline is known for its very rigid structure, sometimes forcing the backbone into a specific conformation. This mutation may convert a proline with such a function into another residue, thereby disturbing the local structure [16].

(vi) rs369199264 (Ser 170 Pro): The mutant residue is larger than the wild-type residue, which may lead to bumps. The hydrophobicity of the wild-type and mutant residues varies. The mutation introduces a more hydrophobic residue at this position. This can result in a loss of hydrogen bonds and/or disrupt proper folding [16].

4. Discussion

NAFLD is emerging as a common liver disease associated with obesity and insulin resistance, which can lead to type 2 diabetes and cardiovascular disease [17]. In this study, using six different software programs, six nsSNPs—namely rs370741805 (Gly 24 Try), rs372035117 (Gly 112 Ser), rs738409 (Cys 144 Ile), rs370175838 (Phe 159 Arg), rs369199264 (Pro 174 Ser), and rs369199264 (Ser 170 Pro)—were identified as being related to the disease. These results were not reported in ClinVar.

Studies by Luo *et al.* and Oh *et al.* reported that rs738409 is associated with lipid metabolism, which is the leading cause of NAFLD [18, 19]. Another study by Witzal *et al*. found that rs738409 causes lipolysis, leading to steatohepatitis [20]. The same mutation was identified by Färkkilä *et al*. as playing a role in primary sclerosing cholangitis (PSC), a chronic inflammatory disease characterized by bile duct damage, cholestasis, and biliary cirrhosis [21].

Gong *et al.* concluded that an rs2896019 was significantly associated with hepatocellular carcinoma (HCC) [22]. Further replication studies have demonstrated strong associations between *PNPLA3* and steatosis, fibrosis/cirrhosis, and HCC, particularly in the context of metabolic, alcoholic, and viral insults [23]. In 2008, genome-wide association studies (GWAS) performed in a population-based sample, where hepatic liver fat content was measured by magnetic resonance spectroscopy, indicated a strong association between a variant (rs738409 C > G, p.I148M) in the patatin-like phospholipase domain-containing 3 *(PNPLA3)* gene and NAFLD [6]. Additional genome-wide studies will be required to identify new variants associated with liver damage. The results of this study suggest that the deleterious and damaging variants of the PNPLA protein, along with obesity and alcohol intake, interact synergistically, leading to an increased risk of cirrhosis, HCC, and liver disease-related death. However, this research has its limitations, as its accuracy relies heavily on the quality and availability of biological and structural data. Computational tools often exhibit biases stemming from training data and struggle to model complex biological systems, resulting in inaccurate predictions. Additionally, the lack of high-resolution structural information for the full-length PNPLA3 protein and the impact of non-coding mutations must also be considered, alongside the critical need for experimental validation through in vitro and in vivo studies for accurate interpretation.

5. Conclusion

In conclusion, applying computational tools such as SIFT, PolyPhen-2, SNPs & GO, PhD-SNP, iMutant-3.0, MUpro, and Project Hope may offer an alternative approach for selecting target nsSNPs. It was found that the major mutations in the native protein of the *PNPLA3* gene were six nsSNPs that had high scores, with PSIC SD ranges (0.998–1.000), indicating pathological polymorphism changes in the amino acids. These mutations were predicted to alter the protein's structure, stability, and functions. The physicochemical properties affected by these nsSNPs are considered significant in causing NAFLD and can be used as diagnostic mutations.

Declarations

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Ethical Considerations

Not applicable.

Competing Interest

None.

Availability of Data and Material

Data are available within the submitted article.

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Abbreviations and Symbols

NAFLD: Non-alcoholic fatty liver disease

ALD: Alcoholic liver disease

NCBI: National Center for Biotechnology Information

nsSNPs: Non-synonymous single-nucleotide polymorphisms

ADPN: Adiponutrin

NASH: Non-alcoholic steatohepatitis

SIFT: Sorting Intolerant from Tolerant

PSIC: Position-specific independent count

SVMs: Support vector machines

PSC: Primary sclerosing cholangitis

HCC: Hepatocellular carcinoma

GWAS: Genome-wide association studies

Appendix 1

Table A.1: Results of SIFT and Polyphen analysis.

SNP	Amino acid change	Protein ID	SIFT score	SIFT prediction	Polyphen-2 prediction	Polyphen-2 score
rs370741805	G24W	ENSP00000216180	0	Deleterious	Probably damaging	1
rs141203357	G45V	ENSP00000216180	0.001	Deleterious	Probably damaging	1
rs201221697	G49R	ENSP00000216180	0.028	Deleterious	Possibly damaging	0.919
rs367826503	S74R	ENSP00000397987	0.024	Deleterious	Possibly damaging	0.746
rs367826503	S78I	ENSP00000216180	0.025	Deleterious	Probably damaging	0.994
rs200528261	R79W	ENSP00000216180	0.001	Deleterious	Probably damaging	1
rs200528261	R75K	ENSP00000397987	0.002	Deleterious	Benign	0.064
rs372035117	G112S	ENSP00000216180	0.014	Deleterious	Probably damaging	1
rs372035117	Q108G	ENSP00000397987	0.016	Deleterious	Possibly damaging	0.939
rs738409	I148M	ENSP00000216180	0.003	Deleterious	Probably damaging	0.994
rs738409	C144I	ENSP00000397987	0.003	Deleterious	Probably damaging	0.998
rs141106484	V162M	ENSP00000216180	0.018	Deleterious	Probably damaging	1
rs141106484	S158V	ENSP00000397987	0.021	Deleterious	Probably damaging	0.97
rs370175838	R163Q	ENSP00000216180	0.006	Deleterious	Probably damaging	0.97
rs370175838	F159R	ENSP00000397987	0.006	Deleterious	Probably damaging	1
rs369199264	P174S	ENSP00000216180	0	Deleterious	Probably damaging	1

Table A.1: Continued.

SNP	Amino acid change	Protein ID	SIFT score	SIFT prediction	Polyphen-2 prediction	Polyphen-2 score
rs369199264	S170P	ENSP00000397987	0	Deleterious	Probably damaging	1
rs369326583	D318H	ENSP00000216180	0.01	Deleterious	Probably damaging	0.565
rs369326583	D314E	ENSP00000397987	0.01	Deleterious	Benign	0.289
rs202021013	I316L	ENSP00000397987	0.027	Deleterious	Possibly damaging	0.807
rs202021013	L320F	ENSP00000216180	0.03	Deleterious	Probably damaging	0.976

Appendix 2

Table A.2: The effect of mutation on protein using the Project Hope prediction.

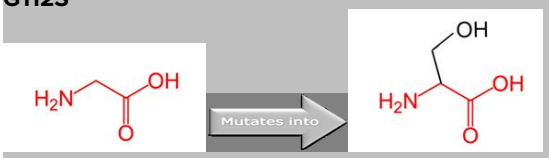
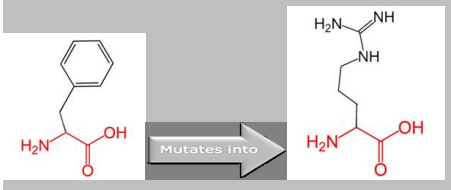
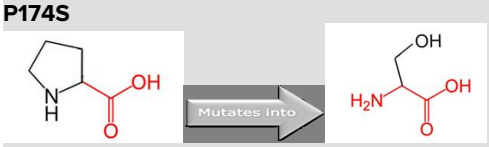
Rs	Wild and mutant variation
rs370741805	G24W 
rs372035117	G112S 
rs738409	C144I 
rs370175838	F159R 

Table A.2: Continued.

Rs	Wild and mutant variation
rs369199264	**P174S** 
rs369199264	**S170P** 

Appendix 3

Table A.3: Results of SNPs&GO and PhD-SNP.

Rs	Amino acid change	SNPs&GO prediction	RI	Probability	PhD-SNP prediction	RI	Probability
rs370741805	G24W	Disease	4	0.696	Disease	9	0.937
rs141203357	G45V	Neutral	1	0.454	Disease	6	0.8
rs201221697	G49R	Neutral	0	0.487	Disease	6	0.818
rs367826503	R74S	Neutral	4	0.281	Neutral	5	0.244
rs367826503	S78I	Neutral	4	0.321	Neutral	1	0.464
rs200528261	R79W	Neutral	1	0.475	Disease	1	0.545
rs200528261	R75K						
rs372035117	G112S	Disease	3	0.629	Disease	5	0.734
rs372035117	Q108G	Neutral	7	0.172	Neutral	6	0.183
rs738409	I148M	Neutral	4	0.305	Disease	2	0.623
rs738409	C144I	Disease	5	0.739	Disease	7	0.835
rs141106484	V162M	Neutral	7	0.15	Neutral	1	0.433
rs141106484	S158V	Neutral	1	0.449	Neutral	0	0.494
rs370175838	R163Q	Neutral	1	0.431	Disease	3	0.63
rs370175838	F159R	Disease	6	0.815	Disease	7	0.836
rs369199264	P174S	Disease	6	0.786	Disease	6	0.81
rs369199264	S170P	Disease	7	0.851	Disease	8	0.886

Table A.3: Continued.

Rs	Amino acid change	SNPs&GO prediction	RI	Probability	PhD-SNP prediction	RI	Probability
rs369326583	D318H	Neutral	8	0.121	Neutral	3	0.363
rs369326583	D314E						
rs202021013	I316L	Neutral	7	0.155	Neutral	3	0.336
rs202021013	L320F	Neutral	7	0.142	Neutral	6	0.193

Appendix 4

Table A.4: Results of Software, I -Mutant, and Mupro prediction.

Protein ID	Amino acid change	I -Mutant prediction	RI	DDG value (Kcal/mol)	Mupro prediction	DDG value	Confidence score
ENSP00000216180	G24W	Decrease	3	–0.19	Decrease	–0.042869786	–0.65390637
ENSP00000216180	G45V	Decrease	3	–0.26	Decrease	–0.08962408	0.065012029
ENSP00000216180	G49R	Decrease	3	–0.57	Decrease	–0.23534606	0.9047314
ENSP00000397987	R74S	Decrease	9	–1.46	Decrease	–0.49124976	–1
ENSP00000216180	S78I	Decrease	0	–0.12	Decrease	–0.27140407	0.51034774
ENSP00000216180	R79W	Decrease	6	–0.81	Decrease	–0.8556114	0.18118445
ENSP00000397987	R75K						
ENSP00000216180	G112S	Decrease	9	–1.40	Decrease	–0.18585132	–0.71701595
ENSP00000397987	Q108G	Decrease	7	–0.99	Decrease	–1.3655618	–0.68306945
ENSP00000216180	I148M	Decrease	9	–1.78	Decrease	–1.1283822	–0.53652383
ENSP00000397987	C144I	Decrease	4	–0.19	Increase	0.080044747	–0.32486298
ENSP00000216180	V162M	Decrease	9	–1.62	Decrease	–0.89227391	–0.025289674
ENSP00000397987	S158V	Increase	1	–0.09	Decrease	–0.34796299	0.84014597
ENSP00000216180	R163Q	Decrease	9	–1.45	Decrease	–1.3158497	–1
ENSP00000397987	F159R	Decrease	7	–1.31	Decrease	–1.8097122	–0.57676089
ENSP00000216180	P174S	Decrease	8	–1.66	Decrease	–1.2705549	–1
ENSP00000397987	S170P	Decrease	0	–0.58	Decrease	–0.76082466	0.078745668
ENSP00000216180	D318H	Decrease	2	–0.36	Decrease	–1.3487321	–0.50383528
ENSP00000397987	D314E						
ENSP00000397987	I316L	Decrease	7	–1.02	Decrease	–0.64031455	–0.64288551
ENSP00000216180	L320F	Decrease	6	–1.14	Decrease	–1.0150064	–1

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