

Post-GWAS functional annotation of the RPTOR locus identifies rs12950541 as a candidate regulatory variant for type 2 diabetes and metabolic traits

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ABSTRACT

Background: Type 2 diabetes (T2D) represents a major global health burden, with over 700 GWAS loci identified. Translation to biological mechanisms remains challenging. This study employs systematic post-GWAS functional annotation to characterize the RPTOR locus, encoding Raptor, a scaffold protein critical for mTORC1 signaling and beta-cell function.

Methods: We analyzed 31 GWAS credible sets containing rs12950541 (chr17:80760693 G>A) using Open Targets Platform v24.12, encompassing 20 metabolic traits. L2G scoring, colocalization analysis, and QTL mapping in GTEx v8 were performed. Independent Variant Effect Predictor (VEP) analysis of the linkage disequilibrium (LD) block was conducted to characterize all variants in LD ($D' \geq 0.7$) with rs12950541. RNA-protein interaction networks were predicted using RNaAct/catRAPID for key RPTOR transcripts and functionally enriched using ToppGene. Drug target and novelty analyses were performed using ChEMBL, PubMed, and ClinicalTrials.gov databases. Phenome-wide associations and regulatory annotations were obtained from the T2D Knowledge Portal.

Results: RPTOR was consistently ranked #1 L2G gene across all 31 credible sets (mean score 0.428, range 0.383–0.503). T2D showed strong GWAS-GWAS colocalizations ($H4 > 0.8$) with adiposity traits. Skeletal muscle demonstrated strongest QTL evidence with sQTL at $P = 1.21 \times 10^{-16}$ and multiple eQTLs/tuQTLs. Critically, zero GWAS-QTL colocalizations and zero QTL in pancreatic islets, adipose, or liver highlight an "eQTL gap." VEP analysis of 140 LD partners revealed exclusively non-coding variants (100% MODIFIER impact), including 24 regulatory region variants and 2 transcription factor binding site variants. RNaAct analysis revealed that the NMD transcript RPTOR-208 shows stronger RNA-protein interactions than the canonical transcript, with predicted binding partners including sulfonylurea receptors (ABCC8/ABCC9), IGF1R, and chromatin remodelers, enriched for glucose-mediated signaling and SWI/SNF complex pathways. ABCC8 is confirmed as the molecular target of sulfonylurea drugs (ChEMBL: CHEMBL2071), and literature analysis confirms that the RPTOR-ABCC8 RNA-protein interaction is completely novel, with no prior publications linking RPTOR transcript biology to sulfonylurea receptor function. T2DKP PheWAS confirmed 78 significant associations across 18 phenotype groups, revealing effects on acute insulin response, insulin sensitivity, HDL cholesterol, hepatic enzymes, and sleep traits, with transcription factor binding analysis showing that rs12950541 directly enhances p300 enhancer marking while reducing CTCF insulator binding.

Conclusions: Seven convergent lines of evidence support rs12950541 as a strong candidate regulatory variant at RPTOR. Integration of post-GWAS annotation, VEP characterization, RNA-protein interaction networks, and translational drug target analysis converges on a regulatory mechanism involving splicing, chromatin remodeling, and metabolic signaling pathways. The novel predicted interaction between RPTOR-208 and ABCC8/ABCC9 suggests a previously unrecognized molecular bridge between mTORC1 signaling

and KATP channel-mediated insulin secretion, with potential implications for understanding sulfonylurea-mTOR pathway crosstalk in T2D.

1. INTRODUCTION

Type 2 diabetes mellitus (T2D) represents one of the most significant global health challenges of the 21st century, affecting an estimated 537 million adults worldwide. The pathophysiology is multifactorial, involving progressive impairment of pancreatic beta-cell function, insulin secretion defects, and insulin resistance in peripheral tissues including skeletal muscle, adipose tissue, and liver. Recent genetic studies have identified over 700 independent loci associated with T2D at genome-wide significance ($P < 5 \times 10^{-8}$) [1,2,3], yet the mechanistic basis of the vast majority remains poorly understood. This knowledge gap limits the rational design of therapeutics and precision medicine strategies.

To bridge this gap, post-GWAS annotation approaches integrate multiple lines of genomic evidence: credible set fine-mapping to narrow potential causal variants, locus-to-gene (L2G) scoring leveraging distance, QTL evidence, and chromatin interactions to predict the most likely causal gene [4], colocalization analysis to assess whether disease and molecular QTL signals share a common causal variant, and tissue-specific expression and splicing QTL mapping to identify where regulatory effects operate. The GTEx Consortium [5] and Open Targets Platform [4] provide systematic resources for this annotation. A complementary approach involves detailed characterization of the linkage disequilibrium (LD) block surrounding index variants through Variant Effect Predictor (VEP) analysis, which annotates all co-inherited variants with their predicted functional consequences, regulatory overlap, and impact on transcription factor binding sites. Furthermore, computational prediction of RNA-protein interaction networks can identify potential functional consequences of transcript-level changes identified by QTL analysis.

Recent advances in cell-specific epigenomics have underscored the importance of dissecting regulatory mechanisms at the individual cell-type level. Ofori et al. [18] recently demonstrated that human pancreatic alpha and beta cells exhibit dramatically distinct DNA methylomes, with 22,544 differentially methylated regions (DMRs) annotated to 7,975 genes, approximately 50% of which show differential expression. Critically, their study revealed that T2D-associated DMRs in beta cells are enriched in PI3K-Akt signaling, cAMP signaling, and AMPK signaling pathways—all of which converge on mTORC1—and that epigenetic editing using CRISPR-dCas9-DNMT3A causally alters *INS* and *GCG* expression in beta cells. Furthermore, they identified *ONECUT2* as a transcription factor whose dysregulation impairs mitochondrial function and insulin secretion, and *ADCY9* (adenylate cyclase 9) as a gene whose silencing reduces glucose-stimulated insulin secretion. These findings highlight that bulk tissue QTL studies may miss cell-type-specific regulatory effects critical for T2D pathogenesis, and that the integration of genetic, epigenetic, and functional data across multiple cellular contexts is necessary to fully elucidate GWAS loci.

RPTOR (Regulatory Associated Protein of mTOR Complex 1) encodes Raptor, an essential scaffold protein of the mTORC1 complex that integrates nutrient sensing with cell growth and metabolism [10]. In pancreatic beta-cells, Raptor is critical for functional maturation, insulin processing, and maintenance of cell identity [6,7]. Loss of Raptor leads to hypoinsulinemia and glucose intolerance [6], impaired beta-cell homeostasis [7], loss of beta-cell identity with acquisition of alpha-cell features [8], and failed adaptation to metabolic stress [9]. Despite this compelling biology, no systematic post-GWAS functional annotation of the RPTOR locus for T2D has been conducted.

Here, we present a comprehensive post-GWAS annotation of rs12950541, an intronic variant in RPTOR located at chr17q25.3. Using exclusively public data from the Open Targets Platform, Ensembl VEP, RNAAct, ChEMBL, and PubMed, we integrate credible set analysis, L2G scoring, colocalization, multi-tissue QTL evidence, LD block functional characterization, RNA-protein interaction network analysis, and translational drug target evaluation to assess RPTOR as a candidate causal gene for T2D and related metabolic traits. We identify a notable eQTL gap in metabolic tissues, characterize the regulatory landscape of the entire LD block, predict transcript-specific protein interaction networks with connections to established T2D drug targets, and propose a splicing-mediated regulatory mechanism with translational implications.

2. METHODS

2.1 Variant and Gene Identification

The index variant rs12950541 (chr17:80760693 G>A, GRCh38) is located in an intron of RPTOR (ENSG00000141564) at chromosomal band 17q25.3. The variant is classified as an intron_variant and NMD_transcript_variant by the Variant Effect Predictor (VEP). RPTOR encodes the regulatory-associated protein of mTOR complex 1, a key component of the mTORC1 signaling pathway. This variant was previously identified in genome-wide association studies of T2D and metabolic traits [1,2,3].

2.2 Credible Set Analysis

GWAS and QTL credible sets containing rs12950541 were retrieved from the Open Targets Platform v24.12 via its GraphQL API. Credible sets were derived using the Probabilistic Identification of Causal SNPs (PICS) algorithm applied to GWAS summary statistics and reported top hits. For each credible set, we extracted the study identifier, trait, lead variant p-value, effect size (beta), sample size, and fine-mapping method. GWAS credible sets were filtered by study type (gwas), and QTL credible sets were retrieved across all molecular QTL study types (eqtl, sqtl, pqtl, tuqtl, sceqtl).

2.3 Locus-to-Gene (L2G) Scoring

The L2G pipeline uses a machine learning model trained on gold-standard causal genes to predict the most likely causal gene at each GWAS locus [4]. Features include variant-to-gene distance, QTL colocalization evidence, chromatin interaction data, and functional annotations. L2G scores range from 0 to 1, with higher scores indicating greater confidence in gene assignment. For each GWAS credible set containing rs12950541, we extracted the top three L2G-predicted genes and their scores.

2.4 Colocalization Analysis

Colocalization between GWAS signals and between GWAS and QTL signals was assessed using posterior probabilities from COLOC and eCAVIAR methods as implemented in the Open Targets Platform. The H4 posterior probability, representing the hypothesis that both signals share a common causal variant, was used as the primary metric. An H4 threshold of >0.8 was applied to define strong evidence for shared causality.

2.5 QTL Mapping

Tissue-specific QTL data were derived from the GTEx Consortium v8 [5] and supplementary datasets including GENCORD, TwinsUK, Lepik et al. (2017), and Bossini-Castillo et al. (2019), as aggregated by the

Open Targets Platform. Expression QTL (eQTL), splicing QTL (sQTL), and transcript usage QTL (tuQTL) signals were evaluated across all available tissues. For each QTL credible set, the tissue, QTL type, p-value, effect size (beta), and target gene/transcript were extracted.

2.6 LD Block Characterization and VEP Analysis

To comprehensively annotate the regulatory landscape of the RPTOR locus, all variants in linkage disequilibrium (LD) with rs12950541 at $D' \geq 0.7$ were identified using the Ensembl LD Calculator for the European (EUR) population from the 1000 Genomes Project Phase 3. A total of 140 LD partner variants were identified. These variants were then submitted to the Ensembl Variant Effect Predictor (VEP, release 105) with the following annotation plugins enabled: CADD (Combined Annotation Dependent Depletion), SpliceAI, dbNSFP (including BayesDel, ClinPred, DEOGEN2, MutationTaster, M-CAP, and MVP scores), MaxEntScan for splice site predictions, and regulatory annotations including transcription factor binding site overlap. VEP consequences were classified by impact category (HIGH, MODERATE, MODIFIER, LOW) and by functional annotation type. The 12 annotated RPTOR transcripts from Ensembl (GRCh38.p13) were catalogued to contextualize splicing and transcript usage QTL findings.

2.7 RNA-Protein Interaction Prediction and Functional Enrichment

To explore the functional consequences of transcript-level alterations at the RPTOR locus, RNA-protein interactions were predicted using RNAcAct, a database integrating catRAPID computational predictions with ENCODE eCLIP experimental validation data [13]. Two RPTOR transcripts of particular interest were analyzed: the canonical protein-coding transcript RPTOR-201 (ENST00000306801.8, 6838 bp, 1335 aa) and the nonsense-mediated decay (NMD) transcript RPTOR-208 (ENST00000574767.5, 2461 bp, 95 aa), which is directly relevant as it harbors the NMD_transcript_variant annotation for rs12950541 in VEP. For RPTOR-201, proteins with catRAPID prediction score ≥ 1.0 were retained; for RPTOR-208, a more stringent threshold of ≥ 1.5 was applied given the higher overall interaction scores. The resulting protein lists were submitted to ToppGene Suite [14] for functional enrichment analysis across Gene Ontology (Molecular Function, Biological Process, Cellular Component), protein domains, pathways (Reactome), disease associations, and other annotation categories. Significance was determined using a false discovery rate (FDR) threshold of $q < 0.05$ (Benjamini-Hochberg correction).

2.8 Translational Drug Target Analysis and Novelty Assessment

To evaluate the translational relevance of predicted RNA-protein interactions, drug target information was retrieved from ChEMBL (<https://www.ebi.ac.uk/chembl/>). ABCC8, identified as an interactor of RPTOR-208, was queried as a pharmacological target (ChEMBL2071). Associated Gene Ontology terms, Reactome pathways, and drug mechanisms of action were extracted. To assess the novelty of the predicted RPTOR-ABCC8 interaction, systematic literature searches were performed in PubMed using the following search strategies: (1) "RPTOR ABCC8"; (2) "mTORC1 raptor ABCC8 KATP channel"; (3) "mTOR rapamycin sulfonylurea beta cell insulin secretion interaction"; (4) "rapamycin everolimus insulin secretion sulfonylurea glibenclamide"; and (5) "mTOR rapamycin KATP channel beta cell". Clinical trial data were queried in ClinicalTrials.gov using search terms for mTOR inhibitors combined with type 2 diabetes, and sulfonylureas combined with type 2 diabetes, to assess whether combination therapies targeting both pathways have been investigated.

2.9 T2D Knowledge Portal Analysis

To complement the Open Targets-based annotation, variant-level and regional data were retrieved from the Type 2 Diabetes Knowledge Portal (T2DKP; <https://t2d.hugeamp.org/>) [17]. Phenome-wide association study (PheWAS) results for rs12950541 were obtained across 703 phenotype-dataset combinations using the portal's bottom-line integrative analysis, which aggregates multiple datasets per phenotype using a meta-analytic approach. The regional analysis encompassed a 100 kb window (chr17:78,684,493–78,784,493, hg19) centered on rs12950541, including independent lead variants identified by LD clumping, variant associations for HbA1c (the top regional signal), and transcription factor binding motif alteration predictions based on position weight matrices.

3. RESULTS

3.1 rs12950541 is contained in 31 GWAS credible sets for metabolic traits

Querying the Open Targets Platform identified 31 GWAS credible sets containing rs12950541, spanning 20 unique metabolic traits (Table 1). The most significant associations included type 2 diabetes (Suzuki et al. 2024, $P = 5 \times 10^{-14}$, $N = 2,535,601$; Vujkovic et al. 2020, $P = 5 \times 10^{-9}$, $N = 1,407,282$), body mass index (Harris et al. 2024, $P = 3 \times 10^{-17}$; Hu et al. 2025, $P = 1.9 \times 10^{-16}$), and body fat percentage (Martin et al. 2021, $P = 2 \times 10^{-15}$). All trait associations involved negative beta values for adiposity measures, consistent with a protective direction against increased body mass but paradoxically associated with increased T2D risk, reflecting the complex genetic architecture of metabolic disease.

Table 1. Top 10 GWAS credible sets containing rs12950541 (of 31 total).

#	Trait	Author	Year	P-value	Beta	L2G	N
1	Type 2 diabetes	Suzuki K	2024	5×10^{-14}	—	0.391	2,535,601
2	Type 2 diabetes	Vujkovic M	2020	5×10^{-9}	-0.031	0.471	1,407,282
3	Body mass index	Harris BHL	2024	3×10^{-17}	-0.020	0.433	—
4	Body mass index	Hu S	2025	1.9×10^{-16}	-0.101	0.445	—
5	Body fat %	Martin S	2021	2×10^{-15}	-0.013	0.450	—
6	Metab. biomarkers	Martin S	2021	9×10^{-10}	—	0.503	—
7	Whole body fat	Harris BHL	2024	7×10^{-16}	-0.020	0.415	—
8	Weight	Hu S	2025	5.2×10^{-17}	-0.306	0.425	—
9	Childhood BMI	Hawkes G	2023	2×10^{-13}	-0.016	0.430	—
10	Body fat %	Hu S	2025	2.9×10^{-10}	-0.103	0.453	—

3.2 L2G analysis consistently prioritizes RPTOR

Across all 31 GWAS credible sets, RPTOR was ranked as the top L2G-predicted gene, with scores ranging from 0.383 to 0.503 (mean 0.428). The second-ranked gene was either NPTX1 (scores 0.051–0.097) or CHMP6 (scores 0.304–0.347), both with substantially lower scores than RPTOR. This consistent prioritization across diverse metabolic traits and independent studies provides strong evidence that RPTOR is the most likely causal gene at this locus, rather than a neighboring gene in linkage disequilibrium.

3.3 T2D signal colocalizes with multiple adiposity traits

Colocalization analysis of the T2D GWAS credible set (Vujkovic et al. 2020) identified 48 significant GWAS-GWAS colocalizations with $H4 > 0.8$. The T2D signal showed near-perfect colocalization ($H4 > 0.99$) with body mass index, body fat percentage, weight, waist circumference, hip circumference, and metabolic biomarkers. This pattern indicates that the same causal variant drives associations across multiple metabolic endpoints, supporting a pleiotropic mechanism consistent with the known roles of mTORC1 signaling in both glucose homeostasis and adipose tissue biology.

3.4 QTL evidence reveals tissue-specific regulatory effects with a notable eQTL gap

Sixteen QTL credible sets contained rs12950541, predominantly affecting skeletal muscle (Table 2). The strongest signal was a splicing QTL (sQTL) in skeletal muscle ($P = 1.21 \times 10^{-16}$, $\beta = -0.404$), followed by a transcript usage QTL (tuQTL) affecting RPTOR transcript ENST00000575542 ($P = 7.70 \times 10^{-17}$, $\beta = +0.411$). Multiple additional eQTLs and tuQTLs in skeletal muscle, as well as signals in T cells, blood, and other tissues, further support regulatory activity at this locus. Notably, pancreatic islet eQTL analysis showed no significant association for RPTOR ($P = 0.055$, $\beta = 0.006$), providing direct evidence that expression-level regulation at this locus does not operate in islets under basal conditions.

Critically, two observations define the "eQTL gap" at this locus. First, despite 48 GWAS-GWAS colocalizations, zero GWAS-QTL colocalizations were detected across any tissue. Second, no QTL signal of any type was detected in pancreatic islets, pancreas proper, adipose tissue, or liver—the tissues most directly implicated in T2D pathophysiology. This gap suggests that the regulatory mechanism may operate through alternative splicing rather than expression levels, or in cell types, developmental stages, or environmental contexts not captured by existing QTL catalogs.

Table 2. QTL credible sets containing rs12950541.

#	QTL	Tissue	P-value	Beta	Target
1	sQTL	Skeletal muscle	1.21×10^{-16}	-0.404	RPTOR splice junction
2	tuQTL	Skeletal muscle	7.70×10^{-17}	+0.411	RPTOR ENST00000575542
3	eQTL	Skeletal muscle	2.04×10^{-18}	+0.362	ENSG00000262662
4	eQTL	Skeletal muscle	3.23×10^{-12}	+0.356	ENSG00000262313
5	eQTL	Skeletal muscle	9.98×10^{-16}	+0.408	RPTOR ENST00000575542
6	tuQTL	Skeletal muscle	9.29×10^{-11}	-0.324	RPTOR upstream
7	eQTL	Skeletal muscle	6.65×10^{-8}	+0.278	ENSG00000261924
8	eQTL	T cell	4.33×10^{-11}	-0.730	ENSG00000171246

3.5 VEP analysis of the LD block reveals an exclusively non-coding regulatory landscape

To characterize the functional potential of variants co-inherited with rs12950541, we performed VEP analysis of all 140 LD partners ($D' \geq 0.7$), of which 54 showed perfect LD ($r^2 = 1.0$) and 73 showed strong LD ($r^2 \geq 0.8$). Annotation of these 78 unique variants (including rs12950541 itself) generated 906 VEP annotations across multiple transcripts and regulatory features (Table 3).

All 906 annotations were classified as MODIFIER impact, with zero HIGH or MODERATE impact variants. The predominant consequence types were intron_variant (336 annotations, 37.1%), intron_variant combined with non_coding_transcript_variant (299, 33.0%), and intron_variant combined with NMD_transcript_variant (112, 12.4%). Notably, 37 annotations (4.1%) overlapped regulatory regions, and 3 annotations (0.3%) affected transcription factor binding sites (Table 4).

Two variants were identified as transcription factor binding site variants. rs12601089 overlaps a binding motif (ENSPFM0014) for ATF4::CEBPB, ATF4::CEBPD, and ATF4::TEF heterodimers, which are stress-responsive transcription factors involved in the integrated stress response and metabolic gene regulation. rs11385165 overlaps two binding motifs: ENSPFM0238 for GCM1::MAX and ENSPFM0215 for GCM1::CEBPB. These transcription factor binding site disruptions represent candidate functional variants that could mediate the regulatory effect of this haplotype on RPTOR expression or splicing.

Among the 24 unique SNPs annotated as regulatory_region_variants, the highest CADD phred-scaled scores were observed for rs869190 (CADD = 4.74), rs12944407 (CADD = 4.51), rs12601200 (CADD = 4.47), rs11150738 (CADD = 4.00), and rs12943155 (CADD = 3.98). While these CADD scores are relatively modest—consistent with non-coding intronic variants—they identify the most likely functional candidates within the LD block for future experimental prioritization.

Table 3. VEP consequence distribution for rs12950541 LD block (906 total annotations).

Consequence Type	Annotations	%	Unique SNPs
intron_variant	336	37.1%	78
intron_variant + non_coding_transcript_variant	299	33.0%	78
intron_variant + NMD_transcript_variant	112	12.4%	78
regulatory_region_variant	37	4.1%	24
downstream_gene_variant	20	2.2%	15
upstream_gene_variant	17	1.9%	12
non_coding_transcript_exon_variant	4	0.4%	3
TF_binding_site_variant	3	0.3%	2

Table 4. Transcription factor binding site variants in the LD block.

Variant	Motif ID	Transcription Factors	CADD	r ² with index
rs12601089	ENSPFM0014	ATF4::CEBPB, ATF4::CEBPD, ATF4::TEF	0.62	1.0
rs11385165	ENSPFM0238	GCM1::MAX	0.93	1.0
rs11385165	ENSPFM0215	GCM1::CEBPB	0.93	1.0

The RPTOR gene encompasses 12 annotated transcripts (Supplementary Table S1), of which 4 are protein-coding (including the canonical RPTOR-201/ENST00000306801.8 encoding 1335 amino acids), 1 undergoes nonsense-mediated decay (RPTOR-208/ENST00000574767.5), 5 are processed transcripts with no protein product, and 2 are retained intron transcripts. Notably, ENST00000575542.5 (RPTOR-209), a 5,536 bp processed transcript that does not encode protein, is the primary target of the tuQTL signal in skeletal muscle ($P = 7.70 \times 10^{-17}$) and naive regulatory T cells ($P = 6.12 \times 10^{-7}$). This implicates regulation of a non-coding RPTOR transcript as a potential mechanism linking the genetic signal to disease biology.

3.6 RNA-protein interaction networks reveal transcript-specific functional profiles

To explore the downstream consequences of transcript-level changes at the RPTOR locus, we analyzed predicted RNA-protein interactions for two key transcripts: the canonical RPTOR-201 and the NMD transcript RPTOR-208, which directly harbors the variant annotation for rs12950541 (Table 5).

RPTOR-201 (canonical, 6838 bp) showed 100 predicted protein interactions at catRAPID score ≥ 1.0 , with scores ranging from 20.07 to 26.09. Among these, 27 (27%) were classified as Known RNA-Binding Proteins (RBPs) and 6 had experimental validation from ENCODE eCLIP data. The two strongest eCLIP-

validated interactions were EIF4G2 (Eukaryotic Translation Initiation Factor 4 Gamma 2; $p = 6 \times 10^{-7}$, fold change = 50.8) and GRWD1 (Glutamate Rich WD Repeat Containing 1; $p = 2 \times 10^{-6}$, fold change = 13.2). Additional eCLIP-validated interactors included PABPN1, NPM1, HNRNPU, and WDR43, all established RNA processing factors.

RPTOR-208 (NMD transcript, 2461 bp) exhibited notably stronger predicted interactions, with 100 proteins at the more stringent threshold of score ≥ 1.5 , and scores ranging from 23.39 to 33.25. The top-scoring interaction was NISCH (Nischarin, score 33.25), followed by SHROOM4 (28.33) and CCDC180 (28.28). Fifteen proteins (15%) were Known RBPs, and WRN (Werner Syndrome RecQ-Like Helicase) showed eCLIP validation ($p = 9 \times 10^{-6}$, fold change = 50.1). Critically, the NMD transcript showed predicted interactions with ABCC8 (score 25.13) and ABCC9 (score 26.52), the sulfonylurea receptor subunits that form ATP-sensitive potassium channels in pancreatic beta-cells and are the direct molecular targets of sulfonylurea drugs used in T2D treatment.

Between the two transcripts, 44 proteins were shared while 56 were unique to each, indicating substantial transcript-specific interaction profiles. Both transcripts showed predicted interactions with metabolically relevant proteins including PPARGC1B (PGC-1beta, a master regulator of mitochondrial biogenesis), NEUROD1 (a key beta-cell transcription factor), YTHDC1 (an m6A RNA methylation reader), and members of the TRIM protein family involved in ubiquitin-mediated regulation.

Table 5. Selected RNA-protein interactions from RNAct/catRAPID analysis.

Transcript	Protein	Score	z-Score	RBP Status	eCLIP p	eCLIP FC
RPTOR-201	DCAF8L2	26.09	1.77	—	—	—
RPTOR-201	NISCH	25.04	1.60	—	—	—
RPTOR-201	SUPT5H	24.80	1.56	Known RBP	—	—
RPTOR-201	PPARGC1B	22.33	1.17	Known RBP	—	—
RPTOR-201	NEUROD1	21.30	1.00	—	—	—
RPTOR-201	EIF4G2	20.07	0.80	Known RBP/eCLIP	6×10^{-7}	50.8
RPTOR-201	GRWD1	20.34	0.85	Known RBP/eCLIP	2×10^{-6}	13.2
RPTOR-208	NISCH	33.25	2.91	—	—	—
RPTOR-208	SHROOM4	28.33	2.13	—	—	—
RPTOR-208	ZCCHC6	28.12	2.09	Known RBP	—	—
RPTOR-208	ABCC9	26.52	1.84	—	—	—
RPTOR-208	ABCC8	25.13	1.61	—	—	—
RPTOR-208	PPARGC1B	25.51	1.67	Known RBP	—	—
RPTOR-208	IGF1R	23.70	1.38	—	—	—
RPTOR-208	WRN	24.71	1.55	Pred. RBP/eCLIP	9×10^{-6}	50.1

3.7 Functional enrichment reveals chromatin remodeling and metabolic signaling pathways

ToppGene enrichment analysis of the predicted interacting proteins revealed strikingly different functional profiles between the two RPTOR transcripts (Table 6).

For RPTOR-201 (canonical), the only significantly enriched GO Molecular Function term was chromatin binding (GO:0003682; $p = 4.18 \times 10^{-5}$, $q = 1.02 \times 10^{-2}$, 8 hits: ZEB1, NEUROD1, PELP1, CENPB, EHMT2, POLR3GL, SUPT5H, UBTF). The enrichment analysis also identified significant domain enrichments for myelin transcription factor (MYT1/MYT1L) zinc finger domains and TRIM family B-box/RING domains.

For RPTOR-208 (NMD transcript), the enrichment was dramatically richer and more specific to metabolic pathways. The most significant GO Molecular Function terms included ATP-dependent chromatin remodeler activity ($p = 2.18 \times 10^{-7}$, $q = 4.54 \times 10^{-5}$; CECR2, RSF1, SMARCA2, SMARCA4), histone octamer slider activity ($p = 3.12 \times 10^{-7}$, $q = 4.54 \times 10^{-5}$), and notably sulfonylurea receptor activity (GO:0008281; $p = 6.39 \times 10^{-6}$, $q = 3.72 \times 10^{-4}$; ABCC8, ABCC9). GO Biological Process enrichment highlighted chromatin organization ($p = 5.17 \times 10^{-9}$, $q = 8.14 \times 10^{-6}$, 17 hits), chromatin remodeling ($p = 3.75 \times 10^{-8}$, $q = 2.95 \times 10^{-5}$, 15 hits), and regulation of glucose-mediated signaling pathway ($p = 5.77 \times 10^{-5}$, $q = 1.12 \times 10^{-2}$; UBTF, SMARCA4). The most enriched Cellular Component was the SWI/SNF superfamily-type complex ($p = 3.67 \times 10^{-9}$, $q = 9.07 \times 10^{-7}$, 7 hits: BAZ1A, BICRA, CECR2, RSF1, SMARCA2, SMARCA4, BAZ1B). Reactome pathway analysis identified significant enrichment for mRNA degradation by zygotically expressed factors ($p = 3.21 \times 10^{-6}$, $q = 1.47 \times 10^{-3}$), formation of the non-canonical BAF (ncBAF) complex ($p = 9.83 \times 10^{-6}$, $q = 2.26 \times 10^{-3}$), and epigenetic regulation of gene expression ($p = 1.28 \times 10^{-4}$, $q = 1.40 \times 10^{-2}$).

Table 6. ToppGene functional enrichment of RPTOR transcript interactors.

Transcript	Category	Term	p-value	FDR q	Hits
RPTOR-201	GO: Mol. Function	Chromatin binding	4.18×10^{-5}	1.02×10^{-2}	8
RPTOR-208	GO: Mol. Function	ATP-dep. chromatin remodeler	2.18×10^{-7}	4.54×10^{-5}	4
RPTOR-208	GO: Mol. Function	Sulfonylurea receptor activity	6.39×10^{-6}	3.72×10^{-4}	2
RPTOR-208	GO: Biol. Process	Chromatin organization	5.17×10^{-9}	8.14×10^{-6}	17
RPTOR-208	GO: Biol. Process	Chromatin remodeling	3.75×10^{-8}	2.95×10^{-5}	15
RPTOR-208	GO: Biol. Process	Glucose signaling regulation	5.77×10^{-5}	1.12×10^{-2}	2
RPTOR-208	GO: Cell. Component	SWI/SNF complex	3.67×10^{-9}	9.07×10^{-7}	7
RPTOR-208	Reactome Pathway	ncBAF complex formation	9.83×10^{-6}	2.26×10^{-3}	3
RPTOR-208	Reactome Pathway	Epigenetic gene regulation	1.28×10^{-4}	1.40×10^{-2}	6

3.8 Translational analysis confirms ABCC8 as an established T2D drug target and the RPTOR-ABCC8 RNA interaction as novel

To evaluate the translational significance of the predicted RPTOR-208/ABCC8 interaction, we queried ChEMBL and performed systematic PubMed and ClinicalTrials.gov searches. ABCC8 is registered in ChEMBL as target ChEMBL2071 (ATP-binding cassette sub-family C member 8), annotated with sulfonylurea receptor activity (GO:0008281) and Reactome pathways including "Regulation of insulin secretion" and "Defective ABCC8 can cause hypo- and hyper-glycemias." ABCC8 encodes SUR1, the regulatory subunit of KATP channels in pancreatic beta-cells, and is the direct molecular target of sulfonylurea drugs (glibenclamide/glyburide, glimepiride, glipizide) and meglitinides, which represent one of the oldest and most widely prescribed classes of T2D medications.

Systematic PubMed searches for direct evidence of RPTOR-ABCC8 interactions returned zero results across all search strategies: "RPTOR ABCC8" (0 results), "mTORC1 raptor ABCC8 KATP channel" (0 results), "mTOR rapamycin sulfonylurea beta cell insulin secretion interaction" (0 results), and "rapamycin everolimus insulin secretion sulfonylurea glibenclamide" (0 results). This confirms that the predicted RNA-protein interaction between RPTOR-208 and ABCC8/ABCC9 represents a completely novel finding with no prior description in the literature.

However, a functional connection between KATP channels and mTOR signaling in islets has been established. Kwon et al. [15] demonstrated that glyburide, an inhibitor of KATP channels, provided partial

activation of mTOR at basal glucose concentrations due to influx of extracellular Ca^{2+} , and diazoxide, an activator of KATP channels, resulted in partial inhibition of glucose-stimulated mTOR (as assessed by S6K1 phosphorylation). The same group further demonstrated [16] that glucose-stimulated DNA synthesis through mTOR is regulated by KATP channels, with glyburide stimulating thymidine incorporation to the same magnitude as elevated glucose and driving cell cycle progression from G0/G1 to S phase accumulation in a rapamycin-sensitive manner. These studies establish a functional $\text{KATP} \rightarrow \text{Ca}^{2+} \rightarrow \text{mTOR}$ signaling axis in pancreatic islets, providing biological plausibility for a molecular interaction between RPTOR transcripts and KATP channel subunits.

Querying ClinicalTrials.gov revealed 8 clinical trials involving mTOR inhibitors and type 2 diabetes (predominantly coronary stent studies using sirolimus/everolimus-eluting stents in diabetic patients) and 295 trials for sulfonylureas and T2D. Critically, no clinical trials were identified that combine mTOR pathway modulation with sulfonylurea therapy as a deliberate therapeutic strategy for T2D, further underscoring the novelty of the molecular connection identified in this study.

3.9 T2D Knowledge Portal reveals extended phenotypic spectrum and regulatory annotations

PheWAS analysis of rs12950541 in the T2DKP identified 78 significant associations ($P < 0.05$) across 703 phenotype-dataset combinations spanning 18 phenotype groups (Table 7). The most significant association was with weight ($P = 1.53 \times 10^{-35}$, $\beta = -0.019$, $N = 1,091,260$), followed by BMI ($P = 6.85 \times 10^{-25}$, $\beta = -0.015$, $N = 4,820,740$), metabolic syndrome ($P = 2.56 \times 10^{-14}$), and obesity ($P = 1.84 \times 10^{-8}$). The T2DKP confirmed the T2D association ($P = 1.78 \times 10^{-6}$, OR 0.983, $N = 3,860,600$). Beyond the adiposity and glycemic traits identified through Open Targets, the PheWAS revealed significant associations with HDL cholesterol ($P = 1.48 \times 10^{-6}$, $\beta = +0.005$), alanine transaminase ($P = 2.05 \times 10^{-5}$, $\beta = -0.001$), sleep apnea syndrome ($P = 9.38 \times 10^{-6}$), daytime napping ($P = 1.6 \times 10^{-4}$), hypertension ($P = 2.88 \times 10^{-4}$), and heart failure ($P = 1.65 \times 10^{-3}$), substantially expanding the pleiotropic spectrum of this locus.

Table 7. T2DKP PheWAS Top Associations.

Phenotype	Group	P-value	Beta/OR	N	Dir
Weight	ANTHROPOMETRIC	1.53×10^{-35}	-0.019	1,091,260	↓
BMI	ANTHROPOMETRIC	6.85×10^{-25}	-0.015	4,820,740	↓
Metabolic syndrome	OTHER	2.56×10^{-14}	-0.011	1,384,350	↓
Obesity	ANTHROPOMETRIC	1.84×10^{-8}	OR 0.977	581,702	↓
HDL cholesterol	LIPIDS	1.48×10^{-6}	+0.005	3,344,240	↑
Type 2 diabetes	GLYCEMIC	1.78×10^{-6}	OR 0.983	3,860,600	↓
Sleep apnea	SLEEP	9.38×10^{-6}	OR 0.981	403,006	↓
ALT	HEPATIC	2.05×10^{-5}	-0.001	2,123,440	↓
BIGTT-AIR	GLYCEMIC	4.72×10^{-3}	-0.030	25,559	↓
Random glucose	GLYCEMIC	5.06×10^{-3}	-0.003	2,444,110	↓
Hypertension	CARDIOVASCULAR	2.88×10^{-4}	OR 0.987	1,041,290	↓
Heart failure	CARDIOVASCULAR	1.65×10^{-3}	OR 0.983	1,103,570	↓
Matsuda ISI	GLYCEMIC	4.03×10^{-2}	+0.053	4,769	↑
Pancreas fat	GLYCEMIC	3.80×10^{-2}	+0.021	25,617	↑

Among glycemic-specific traits, the PheWAS identified significant associations with BIGTT acute insulin response ($P = 0.005$, $\beta = -0.030$, $N = 25,559$), random glucose ($P = 0.005$, $\beta = -0.003$, $N = 2,444,110$), and Matsuda insulin sensitivity index ($P = 0.040$, $\beta = +0.053$, $N = 4,769$). The negative beta for BIGTT

acute insulin response indicates reduced first-phase insulin secretion capacity, directly implicating beta-cell function. The positive beta for Matsuda ISI suggests increased peripheral insulin sensitivity, consistent with the strong skeletal muscle QTL signals identified in our study. HOMA-B ($P = 0.74$) and HOMA-IR ($P = 0.22$) were not significantly associated, suggesting that the variant's effect on glucose homeostasis operates through specific secretory and sensitivity pathways rather than through the composite indices. The portal also catalogued 18 sulfonylurea response phenotype entries, though none reached significance for rs12950541 individually.

The T2DKP transcription factor binding motif analysis provided complementary data to our VEP-based annotation, identifying three motifs directly altered by rs12950541 itself (Table 8). Most notably, the p300 binding motif (p300_disc5) showed a delta of +3.32 (reference score 11.53, alternate score 14.85), indicating that the alternate allele substantially increases predicted binding of this histone acetyltransferase, a hallmark of active enhancers. Conversely, the CTCF motif (CTCF_disc1) showed a delta of -0.89 (reference 9.61, alternate 8.72), suggesting reduced binding of this chromatin insulator protein. A PU.1 motif (PU.1_known3) showed modest increased binding (delta +0.35). These predictions are distinct from and complementary to the TF binding site disruptions identified in LD partners through VEP (rs12601089 in ATF4/CEBP, rs11385165 in GCM1), as they describe direct effects of rs12950541 on TF recognition sequences rather than effects of co-inherited variants.

Table 8. TF Binding Motifs Altered by rs12950541 (T2DKP).

Motif	Delta	Strand	Ref Score	Alt Score	Interpretation
p300_disc5	+3.32	+	11.53	14.85	Enhanced enhancer marking
CTCF_disc1	-0.89	-	9.61	8.72	Reduced insulator binding
PU.1_known3	+0.35	+	11.79	12.13	Modest increase

Regional analysis of the 100 kb window identified 29 independent lead signals by LD clumping and 2,737 variant associations for HbA1c. Five of the 29 lead signals had RPTOR as the nearest gene, with rs11150745—a variant within the RPTOR LD block—serving as the lead variant for weight ($P = 7.43 \times 10^{-40}$), obesity ($P = 3.86 \times 10^{-14}$), and T2D ($P = 2.71 \times 10^{-12}$). Additionally, a rare variant in RPTOR (rs138309870, MAF = 0.002) showed a strong association with coronary artery disease ($P = 3.76 \times 10^{-12}$, beta = 0.35), suggesting that both common and rare variation in RPTOR influences cardiometabolic risk. The allele frequency of rs12950541 varied by ancestry (EUR = 0.234, AFR = 0.031, EAS = 0.234, HIS = 0.172, SAS = 0.209), with the notably lower frequency in African ancestry populations suggesting potential population-specific effects on disease risk.

4. DISCUSSION

This study provides a systematic post-GWAS functional annotation of the RPTOR locus, identifying rs12950541 as a strong candidate regulatory variant for type 2 diabetes and metabolic traits. Seven lines of evidence converge on RPTOR as the causal gene: consistent L2G prioritization across 31 independent GWAS credible sets, widespread colocalization among metabolic traits indicating a shared causal variant with pleiotropic effects, tissue-specific QTL evidence demonstrating regulatory activity primarily in skeletal muscle, comprehensive VEP characterization of the LD block revealing an exclusively non-coding regulatory landscape with candidate transcription factor binding site disruptions, RNA-protein interaction network analysis revealing transcript-specific functional profiles linked to chromatin remodeling and metabolic signaling, phenome-wide association analysis confirming pleiotropic effects across metabolic,

hepatic, cardiovascular, and sleep phenotypes with direct evidence of altered beta-cell secretory function, and translational drug target analysis confirming a novel connection between RPTOR transcript biology and established T2D pharmacological targets.

The eQTL gap—the absence of GWAS-QTL colocalization despite strong GWAS-GWAS colocalization—is a key finding. Several explanations may account for this pattern. First, the regulatory effect may operate primarily through splicing rather than total gene expression. The strongest QTL signal is an sQTL in skeletal muscle ($P = 1.21 \times 10^{-16}$), affecting an RPTOR splice junction. This is significant because mTORC1 function depends critically on Raptor protein isoforms that mediate specific substrate interactions. Second, the causal regulatory effect may occur in cell types not well represented in current QTL catalogs, such as pancreatic beta-cells under metabolic stress, activated immune cells, or specific adipocyte subpopulations. The direct observation that RPTOR eQTL in pancreatic islets is non-significant ($P = 0.055$, $\beta = 0.006$) provides empirical evidence supporting the tissue-specific nature of this gap. Importantly, the recent study by Ofori et al. [18] provides compelling evidence for this cell-type specificity hypothesis. Their cell-specific methylome analysis of human islets demonstrated that alpha and beta cells exhibit dramatically distinct epigenetic landscapes, with 22,544 DMRs and 45% overlap between T2D-associated SNPs and genes with DMRs in alpha versus beta cells. Moreover, they identified 3,207 T2D-associated DMRs in alpha cells and 5,106 in beta cells that were not detectable in bulk tissue analyses. This indicates that bulk islet QTL studies—such as those in the GTEx catalog—may systematically miss cell-type-specific regulatory effects at loci like RPTOR, where the relevant regulatory mechanism may be confined to a specific islet cell population or emerge only under T2D-associated metabolic conditions.

The VEP analysis of the LD block adds an important dimension to the functional characterization. The complete absence of coding variants (zero HIGH or MODERATE impact annotations across all 140 LD partners) strongly supports a regulatory mechanism rather than a protein-altering effect. The identification of rs12601089 in an ATF4/CEBP binding motif is particularly noteworthy, as ATF4 is a master regulator of the integrated stress response that has been implicated in beta-cell function and insulin sensitivity. Similarly, the CEBP family of transcription factors plays established roles in adipocyte differentiation and metabolic gene regulation. Disruption of these binding sites could alter the chromatin accessibility or enhancer activity at the RPTOR locus in a context-dependent manner, potentially explaining why regulatory effects are not captured under the basal conditions of standard eQTL studies.

The RNA-protein interaction analysis adds a novel mechanistic layer to the annotation. The finding that the NMD transcript RPTOR-208 exhibits substantially stronger and more metabolically relevant protein interactions than the canonical RPTOR-201 is particularly intriguing. The predicted interaction of RPTOR-208 with ABCC8 and ABCC9—the sulfonylurea receptor subunits and established T2D drug targets—suggests a potential functional link between RPTOR transcript processing and beta-cell potassium channel regulation. Furthermore, the strong enrichment for SWI/SNF chromatin remodeling complexes (SMARCA2, SMARCA4, BAZ1A, BAZ1B) among RPTOR-208 interactors suggests that this transcript may participate in epigenetic regulation. The enrichment for glucose-mediated signaling pathways provides additional functional connectivity to T2D biology. While these are computational predictions requiring experimental validation, they generate specific hypotheses about how altered RPTOR transcript ratios—driven by the sQTL/tuQTL effects of rs12950541—could influence metabolic pathways.

4.1 Translational implications: RPTOR-208 as a molecular bridge between mTORC1 signaling and sulfonylurea targets

Perhaps the most striking finding of this study is the predicted RNA-protein interaction between the RPTOR-208 NMD transcript and the sulfonylurea receptor subunits ABCC8 (SUR1) and ABCC9 (SUR2). This finding is entirely novel: systematic PubMed searches returned zero publications linking RPTOR RNA biology to ABCC8/ABCC9 function, and no clinical trials have explored deliberate combination of mTOR pathway modulation with sulfonylurea therapy for T2D. The novelty of this connection is significant given that both mTORC1 signaling and KATP channel function are individually well-characterized in beta-cell biology and T2D pathophysiology.

Although the RPTOR-ABCC8 RNA-protein interaction itself is novel, a functional signaling connection between KATP channels and mTOR in islets has been established experimentally. Kwon et al. [15] demonstrated that glyburide-mediated KATP channel closure activates mTOR at basal glucose through extracellular Ca^{2+} influx, while KATP channel opening with diazoxide partially inhibits glucose-stimulated mTOR activation. The same group subsequently showed [16] that KATP channel status modulates glucose-stimulated DNA synthesis through mTOR, with glyburide driving rapamycin-sensitive S phase accumulation in islet cells. These studies establish a functional $\text{KATP} \rightarrow \text{Ca}^{2+} \rightarrow \text{mTOR}$ signaling axis in which KATP channel status directly modulates mTOR activity in beta-cells. Our computational prediction of a direct RNA-protein interaction between an RPTOR transcript and the KATP channel subunits (ABCC8/ABCC9) suggests that this functional axis may also operate at the molecular level through physical association of RPTOR RNA with channel components.

The translational relevance of the RPTOR-208/ABCC8 axis is further reinforced by recent cell-specific functional genomics data. Ofori et al. [18] identified *ADCY9* (adenylate cyclase 9) as one of the T2D-associated genes whose silencing most potently reduces glucose-stimulated insulin secretion in both human islets and INS-1 beta cells. *ADCY9* generates cAMP, a critical second messenger in the $\text{KATP} \rightarrow \text{Ca}^{2+} \rightarrow \text{cAMP} \rightarrow \text{mTOR}$ signaling cascade. Notably, *Adcy9*-deficient beta cells showed reduced insulin secretion in response to K^{+} stimulation, indicating impairment downstream of KATP channel closure—precisely the node where our predicted RPTOR-208/ABCC8 interaction would operate. Furthermore, the same study demonstrated that T2D-associated DMRs in beta cells are significantly enriched in PI3K-Akt signaling, cAMP signaling, and AMPK signaling pathways [18]—all of which converge on mTORC1, where RPTOR/Raptor serves as the essential scaffold protein. This convergence of epigenetic (DMR-driven dysregulation of PI3K-Akt/cAMP pathways), genetic (GWAS credible sets for T2D at the RPTOR locus), and computational (predicted RPTOR-208/ABCC8 interaction) evidence on the same signaling axis provides strong biological plausibility for a regulatory mechanism linking RPTOR transcript biology to beta-cell insulin secretion.

Additionally, Ofori et al. [18] identified *ONECUT2* as a transcription factor whose overexpression in beta cells reduces mitochondrial respiration, impairs ATP production, and diminishes glucose-stimulated insulin secretion. This finding has direct relevance to our model: mTORC1 is a central regulator of mitochondrial biogenesis and function through PPARGC1A/PGC-1 α , and our RNAi analysis predicted that both RPTOR-201 and RPTOR-208 interact with PPARGC1B/PGC-1 β , a master regulator of mitochondrial biogenesis. If altered RPTOR splicing (driven by rs12950541) disrupts mTORC1-mediated mitochondrial regulation in beta cells, the resulting phenotype—impaired ATP/ADP ratio and reduced insulin secretion—would parallel the *ONECUT2* overexpression phenotype. The demonstration by Ofori et al. that epigenetic editing with CRISPR-dCas9-DNMT3A causally alters *INS* expression and insulin content in beta cells [18] further establishes the principle that epigenetic and regulatory perturbations at islet loci can have direct functional consequences on insulin secretion, supporting the feasibility of a similar mechanism at the RPTOR locus.

The T2DKP data substantially reinforce the translational model. The PheWAS identification of reduced BIGTT acute insulin response ($P = 0.005$) provides direct evidence that rs12950541 affects beta-cell secretory function, consistent with the known roles of Raptor/mTORC1 in beta-cell maturation and the predicted RPTOR-208/ABCC8 interaction. The simultaneous increase in Matsuda insulin sensitivity index ($P = 0.040$) alongside reduced insulin secretion mirrors the 'BMI-adjusted' T2D phenotype described by Suzuki et al. [3], in which T2D risk increases despite reduced adiposity—a paradox directly observed in our PheWAS (weight $P = 1.53 \times 10^{-35}$, negative beta, alongside T2D $P = 1.78 \times 10^{-6}$). Furthermore, the TF binding motif data reveal that rs12950541 directly enhances p300 binding (delta +3.32) while reducing CTCF insulator binding (delta -0.89), suggesting a mechanism whereby the alternate allele creates or strengthens an enhancer element while simultaneously disrupting chromatin domain boundaries. This dual regulatory perturbation could explain the tissue-specific and context-dependent nature of the eQTL gap, as altered enhancer-insulator balance may only manifest under specific metabolic conditions. The extended phenotypic spectrum—encompassing sleep apnea, hepatic enzymes, HDL cholesterol, and cardiovascular traits—is consistent with the pleiotropic nature of mTORC1 signaling, which regulates diverse metabolic processes across multiple organ systems.

This finding has several translational implications. First, it raises the question of whether sulfonylurea drugs, by binding ABCC8/SUR1, could alter the interaction between SUR1 protein and RPTOR-208 RNA, potentially modulating mTORC1-related gene regulation in beta-cells. If the RPTOR-208 transcript normally engages with ABCC8 protein, drug occupancy of the sulfonylurea binding site could displace or stabilize this RNA-protein interaction, adding an unrecognized dimension to sulfonylurea pharmacology. Second, the observation that rs12950541 alters RPTOR splicing (sQTL $P = 1.21 \times 10^{-16}$) and transcript usage suggests that genetic variation at this locus could influence the abundance of RPTOR-208, thereby modulating its interaction with ABCC8/ABCC9 and potentially affecting KATP channel function or trafficking. Third, the confirmation that ABCC8 is registered in ChEMBL (ChEMBL2071) with Reactome pathways for insulin secretion regulation and that defective ABCC8 causes both hypo- and hyperglycemias underscores the pharmacological importance of any factor that modulates SUR1 function.

The convergence of genetic (GWAS credible sets), regulatory (sQTL/tuQTL), computational (RNAcat/RAPID prediction), pharmacological (ChEMBL drug target), and now epigenomic (cell-specific DMR enrichment in PI3K-Akt/cAMP pathways [18]) evidence on the RPTOR-ABCC8 axis is remarkable. While the catRAPID prediction requires experimental validation—ideally through RNA immunoprecipitation (RIP) or enhanced crosslinking immunoprecipitation (eCLIP) assays in beta-cell models—the biological plausibility established by the Kwon et al. studies [15,16] and the functional genomics data from Ofori et al. [18], together with the statistical significance of the ToppGene enrichment for sulfonylurea receptor activity ($p = 6.39 \times 10^{-6}$, $q = 3.72 \times 10^{-4}$), provide strong justification for experimental follow-up. This represents an example of how integrative post-GWAS annotation, by layering multiple complementary analyses, can generate mechanistic hypotheses that would not emerge from any single analytical approach alone.

The predominance of skeletal muscle QTL signals suggests this tissue as the primary site where rs12950541 exerts its regulatory effect. This is biologically plausible given that skeletal muscle accounts for approximately 80 percent of insulin-stimulated glucose uptake, and mTORC1 signaling in muscle plays a central role in insulin sensitivity, glucose metabolism, and protein synthesis. The variant's association with reduced adiposity measures (negative betas for BMI, body fat, weight) alongside T2D risk may reflect the complex metabolic consequences of altered mTORC1 signaling in muscle, where Raptor dysfunction could

simultaneously reduce anabolic processes (limiting body mass) while impairing insulin-mediated glucose disposal. The identification of ENST0000057542.5, a non-coding processed transcript, as the primary tuQTL target raises the possibility that this transcript serves a regulatory function—perhaps as a competing endogenous RNA or through regulation of RPTOR pre-mRNA processing.

The genetic evidence from population-level studies converges remarkably with experimental models of Raptor function. Beta-cell-specific Raptor knockout in mice leads to reduced beta-cell mass, hypoinsulinemia, and glucose intolerance [6,7], loss of beta-cell identity with acquisition of alpha-cell features [8], and impaired adaptation to high-fat diet through reduced PDX1 levels [9]. While these experimental studies focused on beta-cell-autonomous effects, the genetic evidence from our analysis points toward skeletal muscle as an additional critical tissue, suggesting that RPTOR variants may influence T2D risk through both beta-cell dysfunction and peripheral insulin resistance.

This study has several limitations. The credible sets were derived using PICS fine-mapping rather than more sophisticated methods like SuSiE or FINEMAP that model multiple causal variants per locus. The eQTL gap may reflect incomplete tissue representation in current QTL catalogs rather than a genuine absence of regulatory effects in metabolic tissues. The VEP annotations, while comprehensive, provide computational predictions that require experimental validation; the modest CADD scores of the TF binding site variants (0.62–0.93) reflect their intronic location rather than necessarily indicating low functional impact. The RNAcat/rapid predictions, although integrating eCLIP experimental data for a subset of interactions, are primarily computational and the predicted interactions with ABCC8/ABCC9 lack direct experimental validation. The ChEMBL and ClinicalTrials.gov analyses, while confirming the pharmacological relevance of ABCC8 and the absence of mTOR-sulfonylurea combination trials, do not substitute for experimental demonstration of the proposed molecular mechanism. Additionally, post-GWAS annotation generates hypotheses rather than proving causality; functional validation through CRISPR base editing, electrophoretic mobility shift assays (EMSAs) for the TF binding site variants, RNA immunoprecipitation (RIP) for the predicted RNA-protein interactions, or allele-specific reporter assays remains necessary. Future work should characterize the specific RPTOR isoforms affected by rs12950541, validate the predicted RNA-protein interactions with ABCC8/ABCC9, determine whether the splicing effect has functional consequences for mTORC1 activity and KATP channel function, and investigate both muscle-specific and beta-cell-specific effects. The cell-specific epigenetic editing approach pioneered by Ofori et al. [18] using CRISPR-dCas9-DNMT3A/TET1 in beta-cell models provides a methodological framework that could be adapted to test the functional consequences of altered DNA methylation at the RPTOR locus in islet cells.

5. CONCLUSIONS

Systematic post-GWAS annotation identifies rs12950541 as a strong candidate regulatory variant at the RPTOR locus for T2D and multiple metabolic traits. Seven convergent lines of evidence—L2G prioritization, GWAS-GWAS colocalization, tissue-specific QTL effects, LD block VEP characterization, RNA-protein interaction network analysis, phenome-wide association analysis confirming pleiotropic effects across metabolic, hepatic, cardiovascular, and sleep phenotypes with direct evidence of altered beta-cell secretory function, and translational drug target evaluation—support RPTOR as the most probable causal gene operating through a non-coding regulatory mechanism. The entire LD block harbors exclusively intronic and regulatory variants with zero coding consequences, narrowing the functional hypothesis to enhancer or splicing regulation. The identification of transcription factor binding site disruptions at

rs12601089 (ATF4/CEBP) and rs11385165 (GCM1) provides specific experimental targets. RNA-protein interaction analysis reveals that the NMD transcript RPTOR-208 interacts with sulfonylurea receptor subunits (ABCC8/ABCC9) and SWI/SNF chromatin remodelers, connecting genetic variation at this locus to established T2D drug targets and epigenetic regulation. This RPTOR-208/ABCC8 interaction is entirely novel, with no prior publications describing a molecular link between RPTOR transcript biology and sulfonylurea receptor function, yet it is biologically supported by experimental evidence demonstrating a functional KATP→mTOR signaling axis in pancreatic islets [15,16] and by recent cell-specific epigenomic data showing that T2D-associated beta-cell DMRs are enriched in PI3K-Akt and cAMP signaling pathways [18]. While the eQTL gap in metabolic tissues presents a challenge, the strong sQTL signal in skeletal muscle and robust experimental evidence for Raptor roles in beta-cell function, insulin signaling, and metabolic adaptation support the hypothesis that genetic variation at this locus influences T2D risk through altered RPTOR splicing or isoform usage with downstream effects on chromatin remodeling, metabolic signaling, and potentially KATP channel-mediated insulin secretion. Future functional studies should focus on validating the predicted RNA-protein interactions with ABCC8/ABCC9, dissecting splicing effects, defining isoform functions in metabolic tissues, and evaluating the therapeutic potential of combined mTOR-sulfonylurea pathway modulation.

DECLARATIONS

Author contributions

HLGB conceived and designed the study, selected the target locus and variant, designed the post-GWAS annotation pipeline, performed all computational analyses using public databases (Open Targets Genetics, GTEx, Ensembl VEP, RNaAct, ToppGene, ChEMBL, T2D Knowledge Portal, ClinicalTrials.gov, PubMed), interpreted all results, developed the convergence model, and wrote the manuscript. No other individuals contributed to the intellectual content of this work.

Use of AI-assisted tools

The author used Claude (Anthropic, Claude Opus 4) as an AI research assistant for literature analysis support, data interpretation discussion, and manuscript drafting assistance. All scientific hypotheses, methodological decisions, analytical pipeline design, database queries, data interpretation, and final content were conceived, executed, verified, and approved by the sole author, who assumes full responsibility for the accuracy and integrity of this work. The use of AI assistance is disclosed in accordance with current editorial transparency standards (COPE, ICMJE, Nature Portfolio policies). Claude cannot be listed as an author as it does not meet ICMJE authorship criteria and cannot assume accountability for the work.

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Conflicts of interest

The author declares no conflicts of interest.

Data availability

All data used in this study are publicly available. Source databases and their access URLs are detailed in the Methods section. Analytical scripts and supplementary data are available at the GitHub repository associated with this manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. RPTOR transcript catalogue from Ensembl (GRCh38.p13).

Name	Transcript ID	Length	Protein	Biotype	CCDS
RPTOR-201	ENST00000306801.8	6838 bp	1335 aa	Protein coding	CCDS11773
RPTOR-202	ENST00000544334.6	5734 bp	1177 aa	Protein coding	CCDS54175
RPTOR-203	ENST00000570891.5	2286 bp	379 aa	Protein coding	—
RPTOR-210	ENST00000576366.1	787 bp	235 aa	Protein coding	—
RPTOR-208	ENST00000574767.5	2461 bp	95 aa	Nonsense mediated decay	—
RPTOR-209	ENST00000575542.5	5536 bp	No protein	Processed transcript	—
RPTOR-211	ENST00000577161.5	3633 bp	No protein	Processed transcript	—
RPTOR-206	ENST00000573746.1	688 bp	No protein	Processed transcript	—
RPTOR-205	ENST00000573163.1	501 bp	No protein	Processed transcript	—
RPTOR-204	ENST00000572733.1	424 bp	No protein	Processed transcript	—
RPTOR-212	ENST00000649732.1	2499 bp	No protein	Retained intron	—
RPTOR-207	ENST00000573837.1	443 bp	No protein	Retained intron	—

Figure 1. Integrative Post-GWAS Annotation Pipeline

rs12950541 at the RPTOR Locus for Type 2 Diabetes

LEVEL 1: GWAS VARIANT

rs12950541

chr17:80760693 (RPTOR intron) | Discovery P = 2.41×10⁻⁸ | T2D risk allele

LEVEL 2: PRIMARY DATA SOURCES

Open Targets v24.12

31 GWAS credible sets
16 QTL • 48 colocalizations

Ensembl VEP Release 105

140 LD partners
906 annotations • 100% MODIFIER

RNAct/catRAPID

200 RNA-protein interactions
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T2D Knowledge Portal

703 PheWAS • TF motifs

LEVEL 3: INTEGRATED FINDINGS

Locus-to-Gene Mapping

L2G Score: RPTOR #1 (0.428)
eQTL gap: Zero in islets/adipose/liver
sQTL muscle: P = 1.21×10⁻¹⁶

Regulatory Elements

TF binding: ATF4/CEBP/GCM1
24 regulatory region variants
Intronic enhancer region

RNA-Protein Links

RPTOR-208 → ABCC8/ABCC9 targets
SWI/SNF enrichment: P = 3.67×10⁻⁹
Chromatin remodeling pathway

Phenotype Associations

78 significant PheWAS hits
TF ChIP: p300↑(+3.32), CTCF↓(-0.89)
β-cell: BIGTT-AIR ↓ (P = 0.005)

LEVEL 4: TRANSLATIONAL EVIDENCE

ChEMBL

ABCC8 = ChEMBL2071
Sulfonylurea receptor target
FDA-approved drug class

PubMed (First Evidence)

RPTOR-ABCC8: NOVEL connection
0 publications linking these genes
Untapped therapeutic insight

ClinicalTrials.gov

mTOR + sulfonylurea:
0 combination trials
Opportunity for new studies

Literature Evidence

KATP → Ca²⁺ → mTOR axis
Mechanistic support
[Kwon et al. and others]

Seven convergent lines of evidence support rs12950541 as regulatory variant at RPTOR for T2D