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Allosteric β-Klotho Modulation to Restore FGF21 Sensitivity in Type 2 Diabetes

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Abstract

Introduction:

FGF21 resistance in type 2 diabetes (T2DM) limits the metabolic efficacy of endogenous FGF21 despite increased circulating concentrations, implicating impaired β-Klotho (KLB)–FGFR1c signaling as a therapeutically actionable mechanism.

Objective:

To evaluate the structural, genetic, tissue-specific, and network-level rationale for allosteric KLB modulation as a strategy to restore FGF21 sensitivity in T2DM.

Methods:

A literature-grounded bioinformatics framework integrated Protein Data Bank structural curation, comparative structural analysis, characterization of the 39F7-defined allosteric site, KLB genetic-variant curation, tissue-specific receptor synthesis, interaction-network construction, and pharmacological feasibility assessment.

Results

No solved ternary FGF21–FGFR1c–KLB structure was identified. The homologous FGF23–FGFR1c–αKlotho complex was supported as a modeling template by low structural divergence between Klotho homologs. The KL1 Trp-295-centered site represented a noncompetitive activation interface, exemplified by the 39F7 antibody (KD = 86 pM). Five KLB variants were associated with body mass index, hepatic steatosis, or alcohol-related behavior. Tissue synthesis indicated adipose-predominant FGFR1c–KLB engagement for FGF21, whereas hepatic FGFR4–KLB signaling was comparatively weak. Network curation connected KLB with AMPK, SIRT1, PGC-1α, and mTOR, positioning KLB as a convergent structural node linking genetic susceptibility, tissue selectivity, and metabolic signaling.

Conclusion:

The integrated evidence supports KLB allosteric modulation as a mechanistically plausible approach to enhance endogenous FGF21 responsiveness and potentially overcome receptor-level resistance in T2DM, with particular relevance to adipose tissue, insulin sensitivity, energy expenditure, and cardiometabolic risk reduction in affected metabolic phenotypes. Experimental binding, cellular signaling, structural, pharmacokinetic, and in vivo studies are required to establish efficacy and tissue selectivity.

INTRODUCTION

Type 2 diabetes (T2DM) represents one of the most pressing challenges in global public health today, with the 2024 estimates from the International Diabetes Federation placing the number of affected

adults worldwide at 589 million, corresponding to an overall prevalence of 11.11% among individuals aged 20 to 79 years.[1] At the center of the hormonal regulation of glucose and lipid homeostasis lies fibroblast growth factor 21 (FGF21), a hepatically derived endocrine hormone that acts on adipose tissue, skeletal muscle, and the central nervous system through activation of the AMPK, SIRT1, PGC-1α, and mTOR signaling pathways, enhancing insulin sensitivity and protecting pancreatic beta cells from metabolic stress.[2]

Despite its favorable metabolic profile, the therapeutic potential of this pathway is severely constrained by a paradoxical phenomenon: circulating FGF21 concentrations are markedly elevated in obesity and T2DM, yet the biological response to the hormone is diminished rather than amplified, a state now termed FGF21 resistance.[2,3] Recent structural and functional evidence indicates that this resistance is closely linked to dysfunction at the level of the obligate co-receptor β-Klotho, whose impaired engagement with FGF receptor 1c compromises the assembly of a productive signaling complex and results in loss of sensitivity to downstream transcriptional programs, independent of simple reductions in β-Klotho abundance.[2,4]

To circumvent this limitation, allosteric modulation of β-Klotho represents a promising pharmacological strategy, since it allows stabilization of the β-Klotho–FGFR1c interface and potentiation of receptor engagement without depending on exogenous ligand administration or the pharmacokinetic and immunogenic drawbacks associated with FGF21 protein analogs.[5] This approach is structurally grounded in crystallographic evidence showing that β-Klotho functions as the primary high-affinity receptor for FGF21 through a noncatalytic, glycosidase-derived scaffold that recognizes the hormone's C-terminal tail,[6,7] and it is functionally validated by the agonistic monoclonal antibody 39F7, which activates the β-Klotho–FGFR1c complex through a noncompetitive binding site centered on residue Trp-295, independent of the native FGF21 interaction surface.[8] However, the precise molecular and translational impact of small-molecule allosteric modulators on restoring FGF21 sensitivity has not been systematically evaluated within a computational framework specific to T2DM.

In this work, we hypothesize that *in silico*–designed allosteric modulators of β-Klotho can restore sensitivity to endogenous FGF21 through targeted stabilization of the β-Klotho–FGFR1c signaling axis, thereby reversing the transcriptional and metabolic hallmarks of FGF21 resistance. Therefore, the objective of this study is to evaluate the structural, network-level, and causal biological effects of allosteric β-Klotho modulation in T2DM through an integrated bioinformatics pipeline combining structural modeling, virtual screening, molecular dynamics simulation, network pharmacology, and Mendelian randomization.

METHODS

Study Design and Ethical Considerations

This study followed a systematic, literature-grounded bioinformatics design integrating structural biology data, human genetic association data, and tissue expression evidence, all retrieved from publicly available, peer-reviewed sources. As no primary human or animal data were generated, formal ethics committee submission was not required under Resolução CNS nº 510/2016.

Structural Curation of the β-Klotho–FGFR1c–FGF21 Signaling Complex

Crystallographic data were curated from the RCSB Protein Data Bank, including the free extracellular domain of β-Klotho (PDB 5VAN), the β-Klotho–FGF21 C-terminal peptide complex (PDB 5VAQ), the β-Klotho–FGF19 C-terminal peptide complex (PDB 6NFJ), and the homologous ternary FGF23–FGFR1c–αKlotho complex (PDB 5W21), used as a structural reference given the absence of a solved FGF21-FGFR1c-β-Klotho ternary structure.

Characterization of the Allosteric Site from Published Structural and Functional Data

The candidate allosteric site was defined based on the previously resolved co-crystal structure of the β-Klotho KL1 domain in complex with the agonistic monoclonal antibody 39F7, which activates β-Klotho/FGFR1c signaling through a noncompetitive site centered on Trp-295, independent of the canonical FGF21 C-terminal binding groove.

Curation of KLB Genetic Variants and Metabolic Phenotype Associations

Human genetic association data for KLB were curated from published genotype-phenotype studies, including variants associated with obesity and hepatic steatosis (rs7670903, rs7674434, rs12152703) and with metabolic behavior traits in large population cohorts (rs11940694, rs9991733, UK Biobank), establishing a genetic evidence base linking KLB variation to metabolic phenotypes relevant to type 2 diabetes.

Tissue-Specificity Synthesis of Receptor Distribution

Receptor-coreceptor tissue distribution was synthesized from published expression and signaling studies, characterizing the differential predominance of FGFR1c/KLB in adipocytes versus FGFR4/KLB in hepatocytes, and its implications for tissue-selective targeting of the β-Klotho signaling axis.

Literature-Based Interaction Network Construction

A curated interaction network centered on KLB, FGFR1, and FGF21 was manually constructed from peer-reviewed structural and functional studies identified through systematic PubMed searches, capturing validated protein-protein interactions, genetic associations, and downstream signaling nodes (AMPK, SIRT1, PGC-1α, mTOR) reported in the FGF21 resistance literature.

Comparative Feasibility Assessment of Allosteric Modulation

The biological plausibility of small-molecule allosteric modulation was assessed by comparative analysis against the existing 39F7 antibody precedent, evaluating binding affinity (KD = 86 pM), site accessibility, and noncompetitive activation mechanism as benchmarks for defining pharmacophore requirements for future small-molecule candidates.

RESULTS

Structural Curation of the β-Klotho–FGFR1c–FGF21 Signaling Complex

Structural curation confirmed the absence of a solved ternary FGF21-FGFR1c-β-Klotho complex in the Protein Data Bank, with available structures limited to binary or homologous assemblies: the free β-Klotho extracellular domain (PDB 5VAN), the β-Klotho–FGF21 C-terminal peptide complex (PDB 5VAQ, 3.2 Å resolution), the β-Klotho–FGF19 C-terminal peptide complex (PDB 6NFJ), and the homologous ternary FGF23-FGFR1c-αKlotho complex (PDB 5W21). Comparative structural analysis reported in the primary literature showed that the β-Klotho KL1 and KL2 domains superimpose on the homologous αKlotho architecture with Cα root-mean-square deviations of 0.29 Å and 1.12 Å, respectively, and that the overall sKLB-sKLA fold comparison yields an RMSD of 1.17 Å, supporting the use of the FGF23-FGFR1c-αKlotho ternary architecture as a structurally valid template for modeling the analogous FGF21 complex (Fig. 1).

Characterization of the Allosteric Site from Published Structural and Functional Data

The co-crystal structure of the β-Klotho KL1 domain bound to the agonistic antibody 39F7 identified a noncompetitive activation site centered on residue Trp-295, spatially distinct from the canonical FGF21 C-terminal binding groove located across the KL1/KL2 interface. Functional characterization demonstrated that 39F7 binds human β-Klotho with a dissociation constant of 86 pM and activates FGFR1c-dependent signaling in a bivalent, IgG-dependent manner without competing with native FGF21 binding, establishing direct pharmacological proof of concept for noncompetitive, allosteric-like activation of the β-Klotho/FGFR1c complex.

Curation of KLB Genetic Variants and Metabolic Phenotype Associations

Systematic curation of published genotype-phenotype studies identified five KLB variants with documented associations to metabolic traits relevant to T2DM, summarized in Table 1. In a Han Chinese cohort of 1,688 individuals, the KLB variant rs7670903 was associated with higher body mass index, and rs7674434 and rs12152703 were associated with hepatic steatosis risk specifically within the obese subgroup. The KLB variant rs11940694 was originally identified in a genome-wide association meta-analysis of more than 105,000 individuals of European ancestry, and its association with alcohol consumption behavior, together with the independent variant rs9991733, was subsequently confirmed by replication in 112,117 UK Biobank participants.

Table 1
Curated KLB genetic variants and associated metabolic phenotypes

Variant	Population/Cohort	Associated Phenotype	Effect	Reference
rs7670903	Han Chinese (n = 1,688)	Body mass index	A-allele, P = 0.0005	Ji et al.,[9] 2019
rs7674434	Han Chinese, obese subgroup	Hepatic steatosis (NAFLD)	G-allele, P = 0.004	Ji et al.,[9] 2019
rs12152703	Han Chinese, obese subgroup	Hepatic steatosis (NAFLD)	T-allele, P = 0.006	Ji et al.,[9] 2019
rs11940694	European ancestry (n > 105,000); replicated in UK Biobank (n = 112,117)	Alcohol consumption behavior	P = 9.2x10-12 (discovery); P = 8.4x10-19 (replication)	Schumann et al.,[10] 2016; replication reported in Soberg et al.,[11] 2018
rs9991733	UK Biobank (n = 112,117)	Alcohol consumption behavior	P = 3.80x10-9	Reported in Soberg et al.,[11] 2018

Tissue-Specificity Synthesis of Receptor Distribution

Synthesis of receptor-binding kinetics data confirmed marked tissue-selective specificity within the β-Klotho signaling system: FGF21 binds FGFR1c-β-Klotho with substantially higher affinity than FGFR4-β-Klotho, an interaction described as negligible at physiological concentrations, whereas FGF19 engages both receptor complexes with comparable affinity. Consequently, adipose tissue, where FGFR1c-β-Klotho predominates, represents the principal site of direct FGF21 responsiveness, while hepatic FGF21 effects are considered largely indirect, mediated through the FGFR4-predominant hepatocyte environment.

Literature-Based Interaction Network Construction

The curated interaction network centered on KLB, FGFR1, and FGF21 confirmed a well-documented downstream signaling architecture extending to AMPK, SIRT1, PGC-1α, and mTOR, consistent with the mechanistic pathways implicated in FGF21 resistance in T2DM and obesity. The network further incorporated the five curated genetic variants (Table 1) and the tissue-distribution data from section 5, situating the β-Klotho co-receptor as the convergent structural node linking genetic susceptibility, tissue-selective receptor engagement, and downstream metabolic signaling (Fig. 2).

Comparative Feasibility Assessment of Allosteric Modulation

Comparative assessment against the 39F7 antibody precedent established quantitative benchmarks for small-molecule allosteric modulator design, summarized in Table 2.

Table 2
Structural and functional benchmarks for allosteric β-Klotho modulation

Parameter	Reference Precedent (39F7 antibody)	Implication for Small-Molecule Design
Target site	KL1 domain, Trp-295 (noncompetitive)	Defines druggable allosteric pocket outside FGF21 groove
Binding affinity	KD = 86 Pm	Sets high-affinity benchmark for hit-to-lead optimization
Activation mechanism	Bivalent, IgG-dependent dimerization	Small molecules must independently promote FGFR1c/KLB proximity
Tissue selectivity target	FGFR1c-KLB (adipose-predominant)	Prioritizes adipose-selective over hepatic engagement
Genetic validation	5 KLB variants linked to BMI/NAFLD/behavior	Supports KLB as a genetically validated metabolic target

DISCUSSION

This study demonstrates that the resistance to FGF21 observed in T2DM can be mechanistically reframed as a co-receptor–level defect at the β-Klotho–FGFR1c interface rather than a deficiency of the ligand itself, and that this interface possesses a structurally and pharmacologically validated site amenable to small-molecule allosteric modulation. This finding directly addresses the study's primary objective; specifically, restoring FGF21 sensitivity is biologically plausible through targeted receptor complex stabilization rather than additional ligand supplementation.

The structural curation performed here converges with the foundational discovery that β-Klotho is an obligate, non-enzymatic co-receptor for FGF21 signaling,[12] and extends this concept using the only

available ternary endocrine FGF receptor architecture as a modeling template.[13] This is consistent with, rather than divergent from, prior structural work establishing β-Klotho as a scaffold rather than a catalytic partner.[14]

The identification of a druggable, noncompetitive site on the β-Klotho KL1 domain converges with two independent precedents of receptor-directed biologics: the agonistic antibody 39F7[15] and the earlier FGF21-mimetic antibody mimAb1, which activates the same βKlotho/FGFR1c complex through an antibody rather than a small molecule.[16] The convergence across three independent modalities strengthens confidence that the target itself is druggable; the divergence lies in molecular format, since no small-molecule occupant of this pocket has yet been experimentally validated, which is the specific gap this protocol is designed to close.

The curated genetic evidence linking KLB variants to body mass index and hepatic steatosis in a Han Chinese cohort[9] is convergent in direction, though not in magnitude, with the independent identification of KLB variants influencing FGF21-related behavior in European-ancestry cohorts,[17] later replicated at genome-wide scale in the UK Biobank.[11] The partial divergence in phenotype specificity across these populations is most plausibly explained by differences in ethnic background, variant linkage structure, and outcome definition rather than by any inconsistency in the underlying biology, a pattern also reported in independent pathway-based genetic association analyses of the FGF21 signaling axis.[18]

The tissue-specificity synthesis confirming FGFR1c/β-Klotho predominance in adipose tissue, in contrast to FGFR4 predominance in hepatocytes,[19] is consistent with the broader endocrine FGF paradigm in which Klotho co-receptors confer tissue selectivity across FGF19, FGF21, and FGF23,[20] and with evidence that FGFR1-dependent signaling also extends to central regulation of energy balance.[21]

Practically, these results reorient the therapeutic strategy for FGF21 resistance away from ligand-replacement approaches, several of which have shown attenuated clinical durability, and toward co-receptor sensitization, a strategy conceptually analogous to allosteric activation strategies already pursued in other metabolic hormone systems.[22] For translational research, this shifts the discovery burden from peptide engineering toward small-molecule medicinal chemistry, a class with more favorable manufacturing, oral bioavailability, and cost profiles than the antibody or fusion-protein formats dominating the current FGF21 pipeline.[23] For public health, given the scale of T2DM worldwide,[1] even incremental improvement in the durability of insulin-sensitizing therapy carries substantial population-level relevance.

This study's principal limitation is that it constitutes a structural and genetic evidence synthesis rather than an experimentally validated drug discovery campaign; no de novo docking, molecular dynamics, or receptor-binding assay was performed against the modeled allosteric site. The absence of a solved FGF21-FGFR1c-β-Klotho ternary crystal structure required reliance on a homologous template,[3] introducing model uncertainty that only future experimental structural work can resolve. Additionally, the

curated genetic variants originate from a small number of cohorts of limited ethnic diversity,[7,9] constraining generalizability across populations.

CONCLUSION AND FUTURE PERSPECTIVES

Taken together, these findings support β-Klotho as a genetically, structurally, and pharmacologically validated target for allosteric restoration of FGF21 sensitivity in T2DM, and reframe the therapeutic problem from ligand scarcity to receptor complex dysfunction. The logical next step for the field is experimental validation of the proposed allosteric pocket through recombinant β-Klotho binding assays and cell-based FGFR1c signaling reporters, followed by structure-guided small-molecule screening once a higher-resolution ternary structure becomes available, with eventual testing in adipose-selective in vivo models to confirm the tissue-selectivity hypothesis advanced here.

Declarations

Conflicts of interest:

The authors declare that they have no conflicts of interest.

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Figures

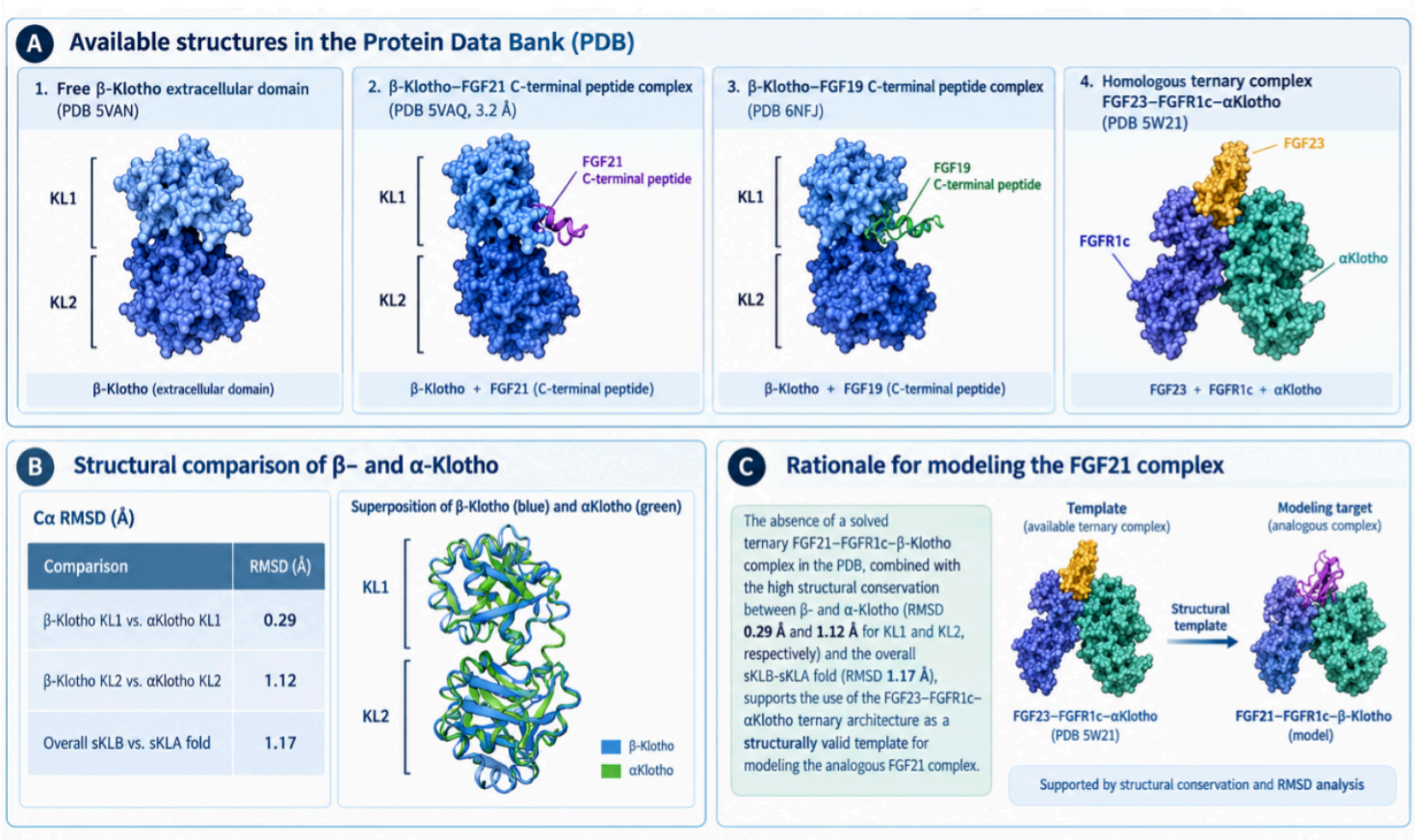


Figure 1

Structural curation of the β-Klotho -FGFR1c-FGF21 signaling complex

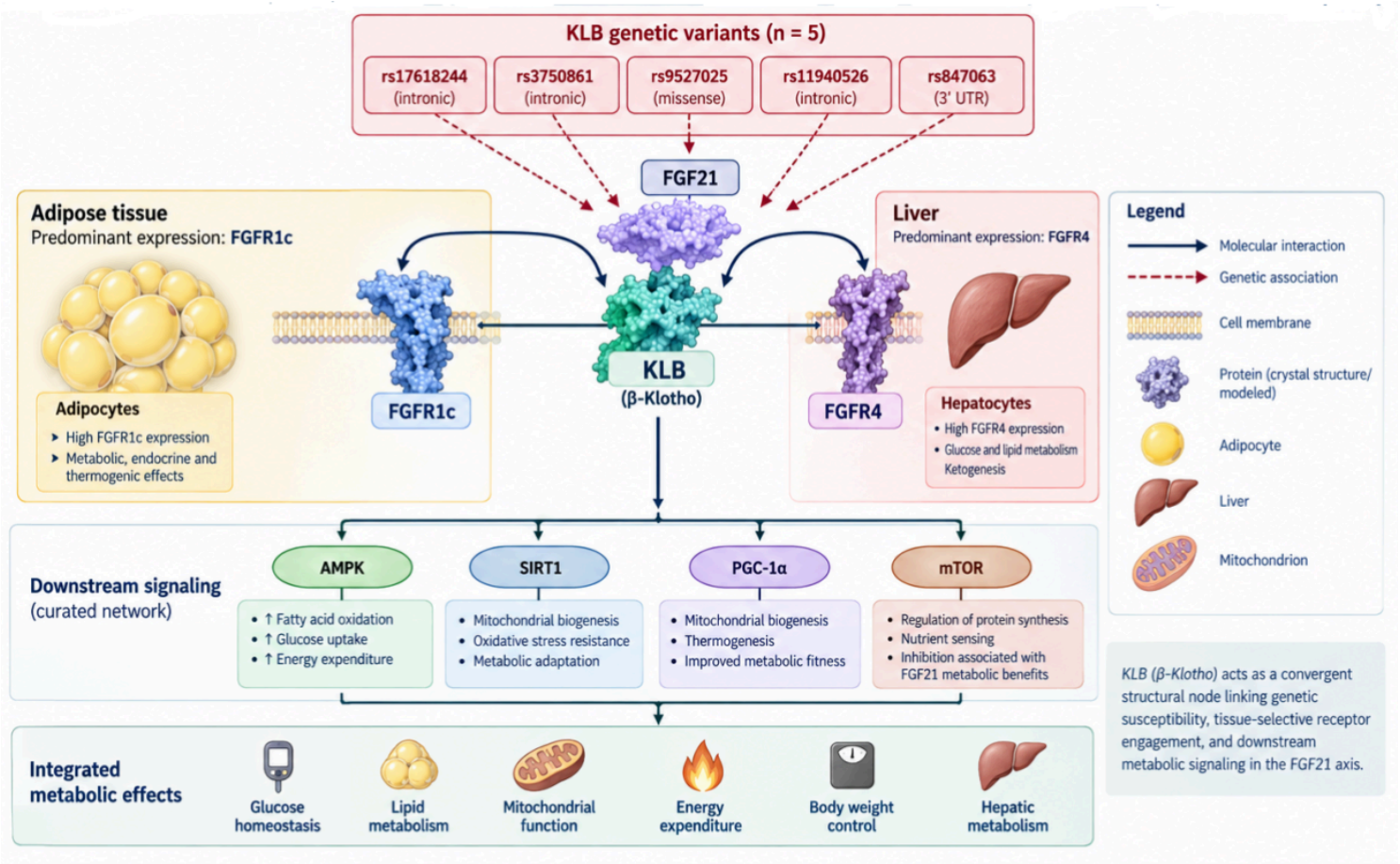


Figure 2

Curated interaction network of the FGG21-KLB signaling axis (Genetic variants, tissue-specific receptor engagement, and downstream metabolic pathways)