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Analysis of the GNB1 L95P Mutation and its Functional Impact on the Gβγ Complex Associated with GNB1 Encephalopathy

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Anna-Sophie Grasl BSc

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Abstract

GNB1 encephalopathy is an extremely rare genetic disorder caused by pathogenic variants in the GNB1 gene. This gene encodes the $G\beta_1$ subunit of heterotrimeric G proteins, which play a central role in intracellular signal transduction cascades.

Disruptions in these signaling pathways can lead to a broad spectrum of neurodevelopmental abnormalities with considerable variability in clinical manifestations among affected individuals.

This study specifically focuses on the pathogenic L95P variant. Molecular docking experiments were performed targeting the $G\beta_1\gamma_2$ complex to identify potential pharmacological modulators. A library of FDA-approved compounds was screened using the GOLD software, and the top-ranked candidates were subsequently analyzed and visualized in PyMOL to investigate their predicted binding modes and key interactions within the $G\beta_1\gamma_2$ complex.

This in silico screening represents an initial step toward identifying promising molecules for subsequent molecular dynamics (MD) simulations. The docking analysis revealed several compounds exhibiting favorable predicted binding affinities and stable interaction networks within functionally relevant regions of the $G\beta_1\gamma_2$ interface. These findings provide a preliminary framework for identifying molecules that may compensate for or restore signaling disturbances caused by the L95P mutation.

Further MD simulations and pathway-oriented analyses will be required to validate these results and to assess their therapeutic potential.

Zusammenfassung

GNB1-Enzephalopathie ist eine äußerst seltene genetische Erkrankung, die durch pathogene Varianten im GNB1-Gen verursacht wird.

Dieses Gen kodiert die $G\beta_1$-Untereinheit heterotrimerer G-Proteine, welche eine zentrale Rolle in intrazellulären Signaltransduktionskaskaden einnehmen. Störungen in diesen Signalwegen können zu einem breiten Spektrum neuroentwicklungsbedingter Auffälligkeiten führen, wobei sich die klinischen Manifestationen zwischen betroffenen Individuen erheblich unterscheiden. Diese Arbeit konzentriert sich spezifisch auf die pathogene Variante L95P.

Im Rahmen dieser Arbeit wurden molekulare Docking-Experimente durchgeführt, die auf den $G\beta_1\gamma_2$-Komplex abzielen, um potenzielle pharmakologische Modulatoren zu identifizieren.

Eine Bibliothek FDA-zugelassener Verbindungen wurde mithilfe der GOLD-Software gescreent und die höchstbewerteten Kandidaten wurden anschließend in PyMOL analysiert und visualisiert, um ihre Bindungsmodi sowie zentrale Interaktionen innerhalb des $G\beta_1\gamma_2$-Komplexes zu untersuchen.

Dieses in silico-Screening stellte einen ersten Schritt dar, um vielversprechende Moleküle für nachfolgende Molekulardynamik-(MD)-Simulationen zu identifizieren.

Die Docking-Analyse zeigte mehrere Verbindungen mit günstigen Bindungsaffinitäten und stabilen Interaktionsnetzwerken in funktionell relevanten Bereichen der $G\beta_1\gamma_2$-Schnittstelle. Diese Ergebnisse bilden einen vorläufigen Ansatz zur Identifizierung von Molekülen, die möglicherweise die durch die L95P - Mutation verursachten Signalstörungen kompensieren oder wiederherstellen könnten.

Weitere MD-Simulationen sowie signalwegorientierte Analysen werden erforderlich sein, um diese Befunde zu validieren und ihr therapeutisches Potenzial zu bewerten.

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List of abbrevations

A_1	Adenosine A1 Receptor
BBB	Blood-Brain Barrier
CCDC	Cambridge Crystallographic Data Centre
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
DA	Dopamine
FDA	Food and Drug Administration
GA	Genetic Algorithm
GABA	Gamma-Aminobutyric Acid
$GABA_B$	$GABA_B$ Receptor
Gα	G Protein Alpha Subunit
Gβ	G Protein Beta Subunit
Gβγ	G Protein Beta-Gamma Dimer
Gγ	G Protein Gamma Subunit
GDD	Global Developmental Delay
GDP	Guanosine Diphosphate
GEFs	Guanine-Nucleotide Exchange Factors
GIRK	G Protein-Coupled Inwardly Rectifying Potassium Channel
GNB1	Guanine Nucleotide-Binding Protein β1
GNB1-E	GNB1 Encephalopathy
GPCR	G Protein-Coupled Receptor
GRKs	G Protein-Coupled Receptor Kinases
GTP	Guanosine Triphosphate
$5\text{-}HT_{1A}$	5-Hydroxytryptamine (Serotonin) Receptor 1A
ID	Intellectual Disability
Kir	Inwardly Rectifying Potassium Channel
M2	Muscarinic Acetylcholine Receptor 2
MD	Molecular Dynamics
NDDs	Neurodevelopmental Disorders
PDB	Protein Data Bank
PLIP	Protein-Ligand Interaction Profiler
PLCβ	Phospholipase Cβ
PLP	Piecewise Linear Potential
WT	Wild-Type

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1. Introduction

Neurodevelopmental disorders (NDDs) are a genetically and clinically heterogeneous group of diseases often characterized by global developmental delay (GDD), intellectual disability (ID), seizures and motor dysfunction. With the rapid progress in genomic sequencing technologies, de novo mutations in genes involved in neuronal signaling have been increasingly recognized as key contributors to these conditions.

Among these, mutations in genes encoding guanine nucleotide-binding proteins (G proteins), such as GNB1, have been associated with severe forms of neurodevelopmental impairment (1).

The GNB1 gene encodes the G protein subunit β_1 ($G\beta_1$), which forms, together with the Gα and Gγ subunits, the heterotrimeric G protein complex that mediates signal transduction from G protein-coupled receptors (GPCRs) to various intracellular effectors. Mutations in GNB1 can disrupt this signaling cascade and lead to GNB1 encephalopathy, a rare but severe neurodevelopmental disorder characterized by hypotonia, movement abnormalities and seizures. One such mutation, L95P, affects a highly conserved residue within the β-propeller domain of $G\beta_1$ that is critical for maintaining the structural integrity of the Gβγ complex and for its interaction with effector proteins. However, the precise functional consequences of this mutation and its potential as a therapeutic target remain largely unknown (2).

In this study, molecular docking analyses were performed to assess whether FDA-approved drugs can interact with the Gβγ complex containing the GNB1 L95P mutation. This in silico approach serves as a first screening step to identify compounds capable of binding to the mutant protein and with suitable blood-brain barrier permeability. Such findings could provide an initial foundation for the repurposing of existing drugs in the future treatment of GNB1 encephalopathy.

1.1 G protein-coupled receptors

G protein-coupled receptors (GPCRs) represent the largest and most diverse receptor superfamily in the human genome, comprising more than 800 distinct genes that encode receptors for neurotransmitters, peptides, hormones, sensory stimuli, metabolic signals and chemokines (3,4).

As seven-transmembrane (7TM) receptors, GPCRs share a conserved topological framework consisting of seven α-helical membrane-spanning segments connected by extracellular and intracellular loops, an extracellular N-terminus and an intracellular C-terminus (5).

Despite their structural conservation, GPCRs exhibit extraordinary functional diversity, enabling them to regulate nearly every physiological system, including neuronal signaling, cardiovascular function, development, immune processes and endocrine regulation (6).

1.1.1 Activation mechanism

Ligand binding to the GPCR extracellular domain induces a series of conformational rearrangements in transmembrane helices (particularly in helices III, V, VI and VII) which collectively reshape the intracellular cavity and allow coupling to heterotrimeric G proteins (7). Crystal and cryo-EM structures reveal that GPCRs do not behave merely as binary switches but instead sample dynamic ensembles of active and inactive conformations (8).

1.1.2 GPCR signaling diversity and classification

GPCRs are traditionally classified into five families - rhodopsin-like (class A), secretin-like (class B1), adhesion (class B_2), glutamate (class C) and frizzled/taste2 - based on sequence homology and functional properties (9). Class A GPCRs form the largest group and include most neurotransmitter receptors, such as dopamineric, serotonineric, adrenergic and muscarinic receptors (3). Importantly, many Class A GPCRs couple to Gαi/o proteins and signal via the release of Gβγ dimers, including the $G\beta_1\gamma_2$ complex, which plays a central role in neuronal ion channel regulation (10).

1.1.3 Heterotrimeric G proteins

Heterotrimeric G proteins are central components of signal transduction pathways that couple extracellular stimuli detected by G protein-coupled receptors (GPCRs) to intracellular effector systems. These proteins act as molecular switches that translate receptor activation into precisely regulated biochemical and electrophysiological responses. Due to their ubiquitous

expression and involvement in numerous signaling pathways, heterotrimeric G proteins play a critical role in neuronal development, synaptic transmission and homeostatic regulation of cellular excitability (7,11).

Heterotrimeric G proteins are composed of three distinct subunits: Gα, Gβ and Gγ. In the inactive state, these subunits form a tightly associated heterotrimer (Gαβγ), as shown in Figure 1. This complex is anchored to the inner leaflet of the plasma membrane (7).

The membrane association is mediated primarily by lipid modifications: Gα subunits are myristoylated or palmitoylated, while Gγ subunits are prenylated at their C-termini (11).

In humans, heterotrimeric G proteins display substantial molecular diversity. At least 16 genes encode Gα subunits, which can be grouped into four major families (Gαi/o, Gαs, Gαq/11 and Gα12/13). Each family activates distinct downstream signaling pathways (12). In contrast, there are five known Gβ isoforms ($G\beta_1$-$G\beta_5$) and twelve Gγ isoforms, allowing for a wide range of possible Gβγ dimer combinations (13).

These combinations yield a large array of functionally distinct G protein dimers and trimers, with biochemical properties shaped by subunit pairing, expression levels and post-translational modifications (11,12).

Among the five Gβ isoforms, $G\beta_1$ is the most ubiquitously expressed and forms highly stable complexes with multiple Gγ isoforms, particularly $G\gamma_2$ (14,15). This makes $G\beta_1\gamma_2$ the predominant Gβγ dimer in the brain and a critical component in neuronal signaling pathways relevant for GNB1-associated disease (15–17).

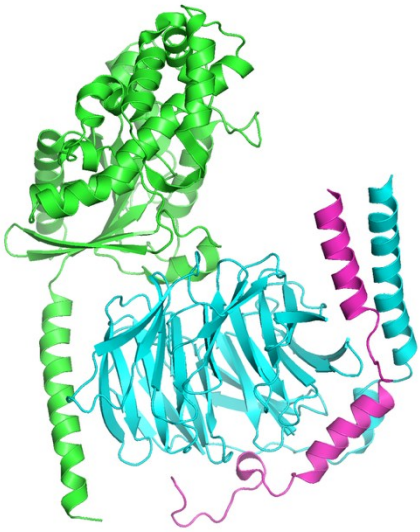

Figure 1: $G\alpha_1\beta_1\gamma_2$ complex

Shown in PyMOL, PDB ID: 1GP2, green: $G\alpha_1$, turquoise: $G\beta_1$, pink: $G\gamma_2$

The Gα subunit consists of a Ras-like GTPase domain and an α-helical domain that together form a nucleotide-binding pocket (7). In the inactive state, Gα binds guanosine diphosphate (GDP) and remains associated with the Gβγ dimer. Upon GPCR activation, the receptor acts as a guanine nucleotide exchange factor (GEF), promoting the release of GDP and binding of guanosine triphosphate (GTP) to Gα. GTP binding induces conformational changes in switch regions of Gα, leading to dissociation from the Gβγ dimer. The activated Gα-GTP complex can then interact with downstream effectors such as adenylyl cyclases, phospholipase Cβ (PLCβ) and Rho guanine nucleotide exchange factors, depending on the Gα subtype (12).
A schematic representation of this signaling mechanism is shown in Figure 2 of Rutz et al. (2024) (18).
Signal termination occurs through intrinsic GTP hydrolysis by Gα, a process that can be accelerated by regulators of G protein signaling (RGS proteins) (19).

Unlike Gα subunits, Gβ and Gγ function as an obligate dimer. Gβ subunits adopt a highly conserved WD-repeat β-propeller structure, composed of seven WD40 motifs that form a rigid scaffold for protein-protein interactions (20). Gγ subunits are smaller and largely α-helical, stabilizing the Gβ fold and anchoring the dimer to cellular membranes via prenylation (13).
The Gβγ dimer is not merely a structural support for Gα but acts as an active signaling unit. Upon heterotrimer dissociation, the dimer can directly regulate a wide range of downstream effectors including ion channels, kinases and enzymes (14).
Importantly the structural integrity of the Gβ subunit is essential for proper dimer formation, membrane targeting and effector binding, making it particularly sensitive to destabilizing mutations such as those observed in GNB1-E (16).

1.1.4 G protein activation cycle

In the resting state, GDP-bound Gα associates with Gβγ to form an inactive heterotrimer that can interact with GPCRs. Ligand binding to a GPCR induces conformational changes that promote nucleotide exchange on Gα (21). Following GTP binding, the heterotrimer dissociates into Gα-GTP and free Gβγ, both of which propagate signaling independently (7).
A representation of this activation cycle is shown in Figure 2 of Waelbroeck et al.(2012) (22).
Activated Gα and Gβγ regulate distinct but often complementary signaling pathways.
While Gα subunits classically control second-messenger production, Gβγ dimers modulate ion channel activity, kinase recruitment and receptor desensitization (9).

Termination of signaling requires GTP hydrolysis by Gα, reassociation of the heterotrimer and receptor desensitization mechanisms involving GPCR kinases (GRKs) and arrestins (23).

1.1.5 Gβγ-mediated effector regulation

One of the most extensively studied roles of Gβγ dimers is the regulation of ion channels. The dimer directly activates G protein-gated inwardly rectifying potassium (GIRK) channels by binding to their cytoplasmic domains, leading to membrane hyperpolarization and reduced neuronal excitability (24). Additionally, Gβγ inhibits voltage-gated calcium channels, thereby decreasing neurotransmitter release at presynaptic terminals (9).
Beyond ion channels, the dimer regulates key signaling enzymes, including PLCβ and phosphoinositide 3-kinase γ (PI3Kγ), influencing calcium signaling, cytoskeletal dynamics and cell survival pathways (15). It also recruits GRKs to activated GPCRs, facilitating receptor phosphorylation and desensitization (23). These diverse functions underscore the central role of Gβγ in maintaining signaling fidelity and preventing cellular overstimulation.

Especially in the nervous system, heterotrimeric G proteins are indispensable for neuromodulation. GPCRs coupled to G proteins mediate the actions of neurotransmitters such as dopamine, serotonin, GABA, acetylcholine and neuropeptides (12). Through coordinated regulation of ion channels and intracellular signaling cascades, G proteins shape neuronal excitability, synaptic plasticity, and network synchronization. Disruption of this signaling has profound neurological consequences. Mutations affecting Gα, Gβ or Gγ subunits have been linked to epilepsy, movement disorders, intellectual disability and neuropsychiatric diseases (16,17).

1.1.6 Relevance to GNB1-associated disorders

The functional dependence of Gα subunits and GPCRs on intact Gβγ dimers explains why mutations in GNB1 result in widespread signaling deficits. Destabilization of the $G\beta_1$ WD-repeat structure impairs dimer formation, reduces effector regulation and alters receptor coupling efficiency (16).
Consequently, GNB1-associated encephalopathy represents a disorder of global GPCR signal integration rather than a defect in a single signaling pathway (16).

The molecular biology of heterotrimeric G proteins is therefore essential for interpreting the pathogenic mechanisms of GNB1 mutations and for developing rational therapeutic strategies aimed at restoring signaling balance in affected neurons (13,16).

1.1.7 GPCR signaling pathways

GPCR-dependent signaling represents one of the most versatile and evolutionarily conserved mechanisms for transmitting extracellular cues into intracellular responses. Upon agonist binding, GPCRs undergo conformational rearrangements that allow them to function as guanine-nucleotide exchange factors (GEFs) for heterotrimeric G proteins, thereby initiating a broad spectrum of downstream signaling branches (3–5).

Because $G\beta_1\gamma_2$ is one of the most abundant Gβγ dimers in the mammalian brain, alterations of its structure or effector interactions, as observed in GNB1-associated encephalopathy, produce severe disturbances in neuronal physiology (9,15,16).

1.1.8 Gβγ activation of GIRK channels

G protein-gated inwardly rectifying potassium (GIRK) channels (Kir3) constitute one of the most well-characterized direct effectors of Gβγ dimers. Structural and electrophysiological studies demonstrated that Gβγ directly binds to cytoplasmic domains of GIRK channels, stabilizing their open configuration and promoting K^+ efflux (24,25).

This induces neuronal hyperpolarization, thereby, decreasing action potential firing, dampening synaptic excitation and shaping network inhibition downstream of receptors such as $GABA_B$, M_2, $5\text{-}HT_{1A}$ and A_1 receptors (24).

The $G\beta_1\gamma_2$ dimer shows particularly high potency in activating GIRK channels, due to the structural compatibility between the $G\beta_1$ β-propeller surface and GIRK's cytosolic binding pockets (9,25).

1.1.9 $G\beta_1\gamma_2$-GIRK binding

GIRK activation relies on precise structural features in both the Gβ subunit and the Gγ-dependent membrane anchoring mechanism. The seven-bladed WD-repeat β-propeller of $G\beta_1$ provides a curved interaction surface that interfaces with basic and hydrophobic residues of GIRK N-terminal and C-terminal cytoplasmic domains (9,26).

Disruption of the β-propeller-through mutations, such as GNB1 Leu95Pro, reduces the stability of the Gβγ dimer, weakens GIRK engagement, and can lead to reduced channel opening probability (15,16).

Additionally, $G\gamma_2$ contributes to GIRK activation by targeting the dimer to the plasma membrane via C-terminal prenylation, creating optimal spatial positioning for channel interactions (9). If $G\beta_1$ cannot correctly associate with $G\gamma_2$, the dimer is inefficiently targeted to the plasma membrane, leading to impaired GIRK channel activation (17).

Because GIRK channels participate in inhibitory signaling across key neurotransmitter systems, including GABAergic, dopaminergic, serotonergic and cholinergic pathways, impaired $G\beta_1\gamma_2$ - GIRK coupling has widespread consequences (24,25).
Deficient activation of GIRK channels is expected to produce increased neuronal firing, susceptibility to hyperexcitability and seizures, altered neuromodulatory tone and dysregulated network oscillations. These pathophysiological features correspond to the neurological manifestations observed in GNB1 encephalopathy, particularly early-onset epilepsy and neurodevelopmental delay (16).

1.2 GNB1 encephalopathy

Because $G\beta_1$ plays a central role in GPCR-mediated pathways, through the formation of $G\beta_1\gamma_2$ dimers, mutations disrupt multiple levels of neuronal signaling, leading to broad dysfunction across neural networks. Pathogenic variants in GNB1 (the gene encoding the $G\beta_1$ subunit) have emerged as a cause of a severe neurodevelopmental disorder characterized by global developmental delay, intellectual disability, hypotonia, epileptic seizures, movement abnormalities and behavioral disturbances (16).

1.2.1 Mechanistic consequences of GNB1 mutations

Variants in GNB1 are predominantly de novo heterozygous missense mutations (16). The majority cluster within conserved regions of the WD-repeat β-propeller, which is essential for proper folding and interaction with Gγ subunits as described above.
Mechanistic consequences include impaired folding of the β-propeller structure which is stabilized by hydrophobic interactions and inter-blade hydrogen bonds. Mutations that disrupt these cores impair folding efficiency, resulting in unstable or misassembled protein (9,26).
Furthermore several patient-derived variants, including L95P, K78R and E241K, cause impaired binding to Gγ, leading to reduced Gβγ dimerization and incorrect cellular localization (16).
If the $G\beta_1\gamma_2$ dimer is reduced or dysfunctional, the heterotrimeric G protein cannot efficiently bind GPCRs or undergo nucleotide exchange (7,8).

Certain GNB1 variants reduce Gβγ-mediated GIRK activation, suggesting weakened effector interaction which leads to diminished inhibitory signaling and increased excitability (16,24).

Unlike monogenic channelopathies, where defects affect a single ion channel, GNB1 mutations disrupt a central signaling node, creating dysfunction across many pathways simultaneously. Affected pathways include GABAergic signaling, dopamine (D2) receptor signaling, serotonergic pathways, mAChR (M_2/M_4) signaling and adenosine A_1 receptor pathways.
Consequently, because $G\beta_1\gamma_2$ participates in all of these systems, single-point mutations can produce network-wide dysregulation (16).

1.2.2 Genotype-phenotype correlations and mutational hotspots

Clinical studies reveal that mutations within the WD-repeat blades 1-3, including codons 75-100, are associated with the most severe phenotypes, such as early-onset epileptic encephalopathy and profound hypotonia (16). Variants within these blades interfere with early assembly and folding, leading to near-complete loss of function.
Mutations in external loops, by contrast, often yield partial loss-of-function phenotypes, presenting as moderate developmental delay, treatable epilepsy, improved motor function and fewer behavioral abnormalities (1).

1.2.3 Structural vulnerability of the Gβ$_1$-propeller domain

The $G\beta_1$ protein folds into a seven-bladed β-propeller structure composed of WD40 repeats, each forming one blade of the propeller (9). These repetitive motifs facilitate extensive intramolecular hydrogen bonding and packing interactions, producing a rigid scaffold that stabilizes the Gβγ dimer and provides the binding surface for Gα subunits (9,13). Mutations within the WD40 repeats particularly those affecting hydrophobic core residues such as L95P can destabilize the propeller domain (27).
As shown in Figure 2 the L95P variant, located at a hydrophobic core position within blade 2 of the β-propeller, is predicted to severely destabilize the fold, representing a key example of a high-impact pathogenic mutation (1).

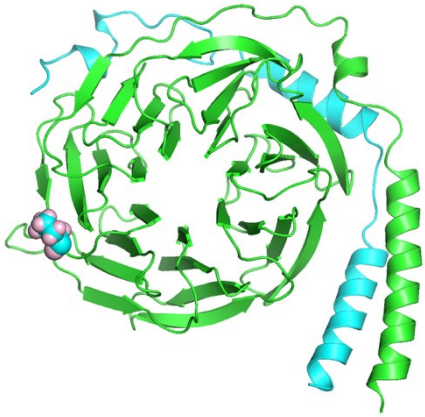

Figure 2: $G\beta_1\gamma_2$ subunit

Shown in PyMOL with the L95P mutation (on the left, displayed in spheres)

1.2.4 Clinical manifestation and prognosis

Clinical manifestations of GNB1-related neurodevelopmental disorder are broad but follow common recurring patterns. The majority of affected individuals present with global developmental delay, intellectual disability, early-onset hypotonia and variable motor impairment (16). Epilepsy is highly prevalent, with seizure types ranging from infantile spasms to generalized tonic-clonic seizures and focal epileptic events (16,28).

The long-term prognosis of individuals with GNB1-related encephalopathy is generally poor, reflecting the essential and universal role of $G\beta_1$ in neuronal GPCR signaling. Most affected individuals exhibit lifelong neurodevelopmental impairment. Although clinical severity varies among specific variants, pathogenic missense variants located within structurally constrained WD40 repeats, such as L95P, tend to result in a more severe phenotype, including profound developmental delay, persistent hypotonia, and refractory epilepsy (16). Although some individuals achieve partial developmental progress, such as basic communication or assisted walking, most remain significantly impaired and seizure burden correlates with poorer outcomes (28). Longitudinal data remain limited due to the rarity of the disorder (29).

1.3 L95P variant

The L95P variant in GNB1, shown in Figure 3, is among the most structurally deleterious mutations identified to date. It disrupts a highly conserved hydrophobic residue within WD40 blade 2 of $G\beta_1$, leading to profound impairment of protein folding, Gβγ dimerization and effector signaling. Due to its destabilizing effect on the β-propeller architecture, L95P is

classified as a severe loss-of-function variant with predicted widespread impairment across multiple GPCR pathways (16,30,31).

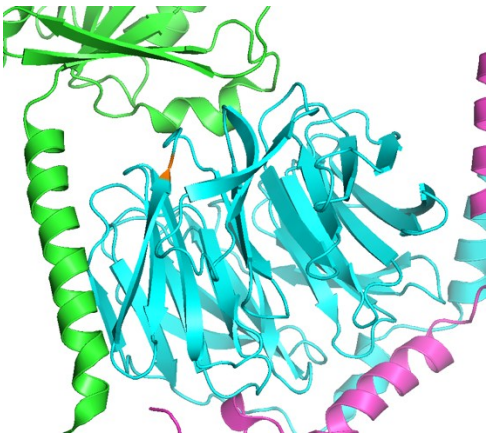

Figure 3: Residue L95 within $G\alpha_1\beta_1\gamma_2$ complex

shown in PyMOL, L95: colored in orange, $G\alpha_1$ in green, $G\beta_1$ in turquoise and $G\gamma_2$ in pink, PDB ID: 1GP2

Leucine 95 (L95) is located in the second WD40 repeat of $G\beta_1$, forming part of the hydrophobic core that stabilizes the internal β-strands of blade 2 (9). WD40 repeats create a radial arrangement of antiparallel β -sheets that depend on tightly packed hydrophobic resides for structural rigidity (32). L95 is evolutionarily conserved across vertebrates and invertebrates, indicating strong selective pressure to maintain its physicochemical properties (28).

Residues at this position are shielded from solvent exposure and contribute to inter-blade packing interactions, which are essential to maintain the geometry of the seven-bladed propeller. Even subtle alterations in side-chain properties can result in partial unfolding or misalignment of adjacent blades (9).

1.3.1 Biochemical consequences of the L95P substitution

The substitution of leucine (a flexible, hydrophobic aliphatic residue) with proline introduces a rigid pyrrolidine ring that imposes structural constraints on the local backbone (33). Proline disrupts β-strand formation due to its inability to donate backbone amide hydrogen bonds, a structural requirement for the stability of the β-sheet architecture characteristic of WD40 repeats (32).

The missense mutation L95P has recently been studied in detail for its functional consequences, providing new mechanistic insight into GNB1 encephalopathy.

A recent study used the heterologous expression system in Xenopus laevis oocytes to compare wild-type (WT) Gβ, and Gβ-L95P mutant, co-expressed with $G\gamma_2$ and neuronal inwardly-

rectifying potassium channels (GIRK2 or GIRK1/2), to test both membrane expression and channel activation. The data revealed a significant reduction in Gβ-L95P surface expression compared to WT. These findings indicate that the L95P mutation causes a dual defect. First a reduced protein expression/stability or trafficking and second the loss of functional coupling between Gβγ and effector channels, even when expression is normalized (2).

The loss of expression combined with impaired function of Gβ-L95P provides a plausible cellular mechanism for the neurological and epileptic phenotype observed in patients carrying this variant. Given the role of GIRK channels in regulating neuronal excitability, impaired GIRK activation could alter inhibitory control and contribute to hyperexcitability or network dysregulation. Previous functional studies of other GNB1 disease-associated variants (e.g. K78R, I80N and 180T) demonstrated variant-specific effects on GIRK regulation, including gain-of-function for some mutations and loss-of-function for others, depending on the mutation and channel subtype (34).

By adding L95P to the spectrum, the emerging picture underscores a wide mechanistic heterogeneity among GNB1 mutations, including variants that may disrupt protein folding/stability rather than directly impair binding interfaces.

This in turn increases the complexity of genotype-phenotype correlations in GNB1-E and suggests that rescuing impaired function may require different strategies depending on the underlying defect (e.g. promoting proper folding/stability, enhancing membrane trafficking, or modulating downstream effectors) (2,34).

While compelling evidence for the functional deficit induced by L95P exists, several caveats apply. The experiments were conducted in a heterologous Xenopus system rather than human neurons, only GIRK channels were tested as effectors, leaving open whether other Gβγ-dependent signaling pathways (e.g. other ion channels, GPCR effectors) are similarly affected. In-cell validation, ideally in patient-derived cells, remains to be done (2).

1.3.2 Treatment strategies

Hemati et al. reported in their study, refining the phenotype associated with GNB1 mutations, clinical and molecular data from 18 newly identified patients and reviewed previously published cases (28). Notably, the recurrent L95P missense mutation was identified among affected individuals and was associated with a severe neurodevelopmental phenotype, including early-onset seizures and profound developmental impairment. Current treatment strategies for patients carrying the L95P mutation, as for other GNB1 variants, are limited to

symptomatic management. Antiepileptic drugs are frequently prescribed (e.g. valproate, levetiracetam), yet seizure control is often incomplete and variable. Likewise, treatment of movement disorders and behavioral symptoms remains empirical and is not tailored to the underlying molecular defect (28). Current clinical treatments such as antiseizure medications only target downstream electrophysiological consequences but do not modify the molecular defect itself (16).

Hemati et al. explicitly emphasized the lack of targeted therapeutic options for GNB1-related disorders and highlighted the urgent need for novel approaches addressing the pathogenic mechanisms at the protein level (28).

Emerging therapeutic strategies for monogenic neurodevelopmental disorders include gene replacement therapy, antisense oligonucleotides and genome editing technologies.

While these approaches are promising, they currently face substantial challenges related to delivery, safety and long-term efficacy in the brain (3).

While current therapeutic approaches remain largely symptomatic, recent advances in structural biology and computational chemistry provide new opportunities to explore potential strategies for molecular stabilization or functional modulation of disease-associated proteins. In particular, structure-based in silico docking has emerged as a powerful tool to evaluate interactions between small molecules and protein targets, allowing the identification of compounds that may bind to destabilized regions, alter protein conformation, or modulate protein-protein interactions (35). In contrast drug repurposing strategies based on FDA-approved compounds offer a more immediately translatable alternative, particularly for rare disorders with limited patient populations.

Given the central role of Gβ in neuronal GPCR signaling, small molecules capable of binding to or stabilizing structurally compromised $G\beta_1$ variants, such as L95P, may exert indirect modulatory or compensatory effects on disrupted signaling pathways. Although such compounds are unlikely to fully restore native protein function, even partial stabilization or alteration of interaction dynamics could have functional relevance. In this context, structure-based in silico screening of FDA-approved, blood-brain barrier permeable compounds represents a rational exploratory approach to identify candidate molecules for further experimental evaluation (36).

The present study employs computational docking of FDA-approved drugs onto a structural model of the $G\beta_1$ protein harboring the L95P mutation. This strategy aims to assess whether

existing pharmacological compounds can bind to the mutant protein with favorable predict affinities and defined interaction modes. By focusing on FDA-approved molecules, this approach leverages compounds with established safety profiles, thereby increasing the translational relevance of any identified interactions (36).

1.4 Molecular docking - principle and theory

Molecular docking is a computational technique widely used in structure-based drug design to predict the preferred orientation, binding mode and affinity of a small molecule (ligand) when interacting with a macromolecular target, typically a protein or nucleic acid. The fundamental goal of docking is to estimate the most energetically favorable ligand-receptor complex based on molecular recognition principles such as shape complementarity, electrostatic interactions, hydrogen bonding and hydrophobic effects (35,37).

Docking algorithms generally consist of two main components: a search algorithm and a scoring function. The search algorithm explores the conformational and positional space of the ligand within the binding site, accounting for ligand flexibility. The scoring function evaluates each generated pose by approximating the binding free energy and ranking poses accordingly (38).

1.4.1 GOLD docking program

The program used in this study was GOLD. GOLD (Genetic Optimization for Ligand Docking) is a well-established molecular docking program that employs a genetic algorithm (GA) to efficiently explore ligand conformational space within a defined binding site. The GA mimics evolutionary processes such as mutation, crossover and selection to optimize ligand binding poses. This approach allows extensive sampling of ligand flexibility, including rotatable bonds and ring conformations, while the protein is typically treated as rigid or semi-flexible (39).

GOLD supports multiple scoring functions, including GoldScore, ChemScore, ASP and PLP, each emphasizing different interaction components such as hydrogen bonding, van der Waals' forces, metal coordination and ligand strain energy (39,40).

The availability of multiple scoring functions enables consensus scoring approaches, which can improve docking reliability and reduce false-positive predictions. Additionally, GOLD allows user-defined constraints, such as hydrogen bond or distance constraints, to incorporate experimental knowledge into the docking process (41).

1.4.1.1 Scoring functions and binding evaluation

Scoring functions in docking aim to approximate the binding free energy of a ligand-receptor complex. However, due to simplifications in force fields and solvation models, docking scores are best interpreted in a relative rather than absolute manner (38).

Consequently, docking results are often used for pose prediction, virtual screening and qualitative ranking of ligands rather than precise affinity estimation. Post-docking analysis typically involves visual inspection of binding modes, evaluation of key intermolecular interactions and comparison with known reference ligands or experimental structures (37).

1.4.2 PyMOL

PyMOL is a widely used molecular visualization system designed for the analysis and graphical representation of three-dimensional biomolecular structures. In docking workflows, PyMOL is commonly employed to visualize docked ligand poses generated by programs such as GOLD, allowing detailed inspection of ligand-receptor interactions, hydrogen bonds, steric clashes, and binding site geometry. PyMOL supports high-quality rendering, scripting and measurement tools, making it suitable for the preparation of publication-quality figures and structural analysis. While PyMOL does not perform docking calculations itself, it plays a crucial role in interpreting and communicating docking results in structure-based studies (42).

1.4.3 PLIP

Protein-ligand interactions were analyzed using the Protein-Ligand Interaction Profiler (PLIP), an automated web-based tool developed at TU Dresden for the systematic identification of non-covalent interactions in protein-ligand complexes (43).

The following sections present the workflow of molecular docking studies and later the outcomes of this docking analysis.

2. Materials and Methods

2.1 GOLD setup

In this study, molecular docking studies were performed using GOLD, version Hermes 2025.1.0 (Cambridge Crystallographic Data Centre, CCDC), which employs a GA to explore ligand conformational space and optimize ligand-protein interactions (39). Later on, Structural visualization and interaction analysis were conducted using PyMOL, version 3.1.6.1. (42).
Molecular docking was performed using the GOLD docking wizard (CCDC).

The workflow of docking studies consisted of following steps:
First, the protein structure carrying the L95P mutation was loaded into the wizard tool using the ‚load protein' option. Hydrogen atoms were then added to the protein structure.
The binding site was defined using the ‚define binding site' option by specifying a list of residues rather than a geometric radius. This approach allowed for a precise and biologically relevant definition of the binding site, focusing on residues known or predicted to be involved in ligand interactions. It ensured that docking was restricted to the relevant interaction region and reduced the likelihood of non-specific or spurious binding poses. For this purpose, a predefined residue list file (residuelisttopgbeta.txt.) was imported to accurately define the binding region. The binding site was further refined by selecting key amino acid residues.
The selected residues included Tyrosine59 (Tyr59), Alanine231 (Ala231), Threonine274 (Thr274), Methionine 188 (Met188) and Serine 316 (Ser316). These residues are located on the top surface of the Gβ WD-repeat β-propeller in proximity to the L95P mutation and are predicted to contribute to ligand recognition and interaction.

For the docking setup, the default configuration template (chemscore_kinase) was retained. Ligand selection was performed by loading the compound library ‚FDA.sdf'.
Docking was initially carried out using one GA run per ligand.
As the fitness function, the ChemPLP-scoring function was selected and kept unchanged.
The GA search efficiency was initially set to fast (least accurate) to reduce computational time during the first screening. Subsequently, ligands predicted to be blood-brain barrier permeable and showing the highest docking scores were re-docked using the slow (more accurate) GA search option.

Docking calculations were executed and the results were saved. After completion, docking poses were inspected using the ‚view solutions' option and ligand ranking was evaluated based on the ChemPLP fitness score.

2.2 Selection of the scoring function and GA settings

The ChemPLP scoring function was selected for molecular docking due to its proven reliability in predicting ligand binding poses and its strong performance in virtual screening applications (39,44). ChemPLP is a piecewise linear potential (PLP) specifically optimized for pose discrimination and ranking, incorporating terms that describe hydrogen bonding, steric complementarity and lipophilic interactions. Previous benchmarking studies have demonstrated that ChemPLP performs favorably compared to other scoring functions implemented in GOLD, particularly for identifying biologically relevant binding conformations across chemically diverse ligand sets (44).

For conformational sampling, the GA implemented in GOLD was employed (45). During the initial screening of the FDA-approved drug library, the fast (least accurate) GA search setting was used to minimize computational cost while enabling efficient high-throughput docking. This approach is commonly applied in early virtual screening stages to rapidly identify promising candidates (45). Subsequently, ligands exhibiting the highest scores and predicted blood-brain barrier permeability were re-docked using the slow (more accurate) GA search setting, which increases sampling depth and optimization rigor. This step improves confidence in the predicted binding poses of prioritized compounds by enhancing convergence and reducing stochastic variability (45).

Overall, this two-step docking strategy represents a balanced compromise between computational efficiency and predictive accuracy, ensuring broad ligand coverage during initial screening while providing refined and reliable docking results for selected top-ranking compounds.

2.3 Protein structure preparation and docking

The structural model of the human $G\beta_1\gamma_2$ subunit was based on the crystal structure of the G protein heterotrimer αβγ (PDB ID: 1GP2) (46).

A representative molecular dynamics (MD) snapshot, provided as a PDB structure, containing the L95P point mutation (L95P.pdb), provided by the supervising research group, was used as the receptor model for all docking calculations.

It was also used to define the receptor conformation and docking region. The receptor was treated as rigid during docking, while full ligand flexibility was allowed.

2.4 Ligand selection

An initial set of FDA-approved compounds was docked against the Gβγ-L95P model. To ensure neurological relevance, only compounds predicted to cross the blood-brain barrier were selected for further analysis. BBB permeability was assessed based on annotations from DrugBank and SwissADME (47,48).

Compounds without BBB penetration were excluded from detailed evaluation, as central nervous system (CNS) exposure is essential for therapeutic relevance in GNB1-related encephalopathy.

2.5 Refined docking of high-scoring compounds

As described above, following the initial docking screen, compounds achieving a PLP-Fitness score ≥ 60 and predicted to cross the BBB were selected for refined docking analysis. This threshold was chosen to prioritize ligands with comparatively favorable predicted binding while maintaining a manageable number of candidates for detailed evaluation.

Selected compounds were subsequently re-docked using 100 independent GA runs per ligand, generating up to 100 distinct binding poses for each compound. Increasing the number of GA runs enhanced conformational sampling and allowed for improved assessment of pose reproducibility and interaction stability across multiple docking solutions (37,40).

2.6 Docking parameters

For all docking calculations, default GOLD parameters were applied unless otherwise stated.

As shown in Table 1 , ligands were treated as fully flexible, while the receptor structure was kept rigid throughout the docking procedure.

Table 1: GOLD docking parameters

Parameter	**Value**
Software	GOLD 2025.1.0
Scoring function	ChemPLP
Initial GA runs	1 per ligand
Refined GA runs	100 per ligand

Protein flexibility	rigid
Ligand flexibility	flexible
Binding site	list of atoms or residues

2.7 Pose analysis and visualization

Docking poses were ranked based on PLP-FitnessScore. The highest-scoring pose from the cluster was selected for each compound. Ligand-protein interactions were visualized and analyzed in PyMOL, focusing on hydrogen bonding, hydrophobic interactions and aromatic stacking.

2.8 PLIP protein-ligand interaction analysis

For this purpose, the protein-ligand complex corresponding to the top-ranked docking pose was selected. The docking pose with the highest PLPFitness-score was exported from the docking software as a PDB file, containing both the Gβγ L95P mutant structure and the bound ligand.

The PDB file was uploaded to the PLIP webserver, where the complex was analyzed using default geometric criteria. PLIP automatically detects and classifies non-covalent protein-ligand interactions, including hydrogen bonds, hydrophobic contacts, π-stacking interactions, cation-interactions, salt bridges and halogen bonds, based on predefined distance and angle thresholds (43).

As output, PLIP generated a detailed interaction report, including an interaction table summarizing the identified contacts and their geometric parameters, as well as PSE files. The resulting PLIP-generated files were subsequently loaded into PyMOL for visualization and figure preparation. PyMOL was used to display the ligand binding pose, interacting residues, and interaction networks in a consistent graphical representation across all analyzed compounds. These visualizations served as the basis for qualitative interpretation of binding modes and interaction patterns but were not used for quantitative comparison of binding affinities (42).

In the subsequent analysis, only drug molecules exhibiting the highest docking scores and predicted ability to cross the blood-brain barrier were selected for further evaluation. These selected compounds are presented in the following sections of this work, where their binding

modes within the mutated Gβ subunit are analyzed in detail. Visualization and structural interpretation of the docked complexes were performed using PyMOL, enabling detailed inspection of ligand orientation, binding pocket occupancy and key intermolecular interactions (42).

3. Results

Among the screened FDA-approved compounds, six BBB-permeable molecules achieved PLPFitness-scores ≥ 60 and were selected for detailed analysis.

3.1 Scores from docking studies

Table 2: BBB-crossing compounds with PLP scores ≥ 60

ZINC ID	Compund name	BBB permeability	PLPFitness-score
ZINC000001530579	Carvedilol	yes	66.1229
ZINC000034608502	Umeclidinium	yes	63.1477
ZINC000019632668	Doxapram	yes	62.3986
ZINC000072267023	Eliglustat	yes	61.9824
ZINC000001996117	Darifenacin	yes	61.2001
ZINC000016159083	Pimavanserin	yes	61.1596

ZINC identifiers were obtained from the ZINC database (49). Blood-brain barrier permeability was annotated based on DrugBank and SwissADME (47,48). Docking scores were calculated in this study using GOLD and represent predicted relative binding affinities rather than experimental binding energies.

3.2 Docking of BBB-permeable compounds to $G\beta_1$ L95P

To identify pharmacologically relevant interactions with the GB1 L95P variant, molecular docking was performed using a library of FDA-approved compounds. Following initial screening, compounds predicted to cross the blood-brain barrier (BBB) were prioritized for further analysis, as central nervous system exposure is required for potential relevance in GNB1-related encephalopathy.

Using a two-step docking strategy, all ligands were initially docked with a single genetic algorithm (GA) run. Compounds achieving PLPFitness-scores ≥ 60 were subsequently subjected to refined docking using 100 GA runs per ligand to improve sampling and pose reproducibility. Based on this approach, six BBB-permeable compounds fulfilled the selection criteria and were retained for detailed evaluation shown in Table 2.

Among these compounds, Carvedilol achieved the highest PLP score (66.12), followed by umeclidinium (63.15) and doxapram (62.40). Eliglustat, darifenacin and pimavanserin also displayed PLP scores slightly above the applied threshold.

For all selected compounds, the refined docking runs produced reproducible binding poses located on surface-exposed regions of the $G\beta_1$ protein in proximity to the L95P mutation site. The predicted binding modes were primarily stabilized by hydrophobic interactions and in some cases, additional hydrogen bonds to neighboring residues within the WD-repeat domain. No deeply buried binding pockets were observed, consistent with the known role of $G\beta_1$ as a protein-protein interaction scaffold rather than a classical ligand-binding protein (27).

Overall, the docking results indicate that a small subset of BBB-permeable, FDA-approved compounds can bind to the $G\beta_1\gamma_2$ L95P structure with moderate predicted affinity. These findings provide a basis for further structural analysis and experimental validation but do not imply direct therapeutic efficacy.

3.2.1 Carvedilol

Carvedilol is a synthetic antihypertensive drug belonging to the carbazole class (50,51). It is a racemic mixture of two enantiomers with distinct pharmacological properties. The S(-)-enantiomer exhibits non-selective β_1/β_2-drenergic and α_1-adrenergic receptor antagonism, whereas the R(+)-enantiomer selectively blocks α_1-adrenergic receptors. Carvedilol acts primarily as a non-cardioselective β-blocker with additional α_1-adrenergic blocking activity, resulting in reduced heart rate, decreased myocardial contractility and peripheral vasodilation without reflex tachycardia (52).

Clinically, carvedilol is used in the treatment of hypertension, heart failure, left ventricular dysfunction following myocardial infarction and angina pectoris (50,52). Its dual α- and β-adrenergic antagonism provides therapeutic advantages in cardiovascular disease management. Carvedilol was approved by the FDA in 1995 and is generally well tolerated, although rare cases of clinically apparent liver injury have been reported (52,53).

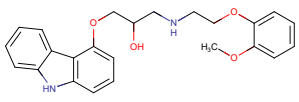

Figure 4: 2D-structure of carvedilol (51)

Carvedilol: structural visualization of the best-scoring binding pose

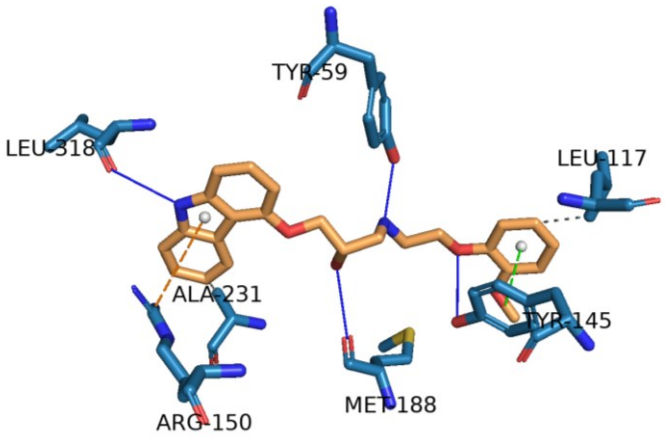

Figure 5: Molecular docking pose of carvedilol within the L95P mutant of the Gβ protein

Unless stated otherwise, ligand and interaction representations are consistent across all docking figures.

In all 3D structural visualizations the ligand is shown in orange, while interacting protein residues are displayed in blue. Hydrogen bonds are shown as solid blue lines, while hydrophobic interactions and aromatic interactions are indicated by colored dashed lines (π-stacking dashed green line and π-cation interaction orange dashed line).

Visualization of the docking pose revealed a network of stabilizing non-covalent interactions between carvedilol and the L95P mutant binding site, including hydrogen bonds (blue lines), hydrophobic interactions (dashed grey line) and aromatic stacking interactions with surrounding residues (Figure 5).

The visualization highlights key residues involved in ligand stabilization, including TYR59, TYR145, ARG150, MET188, ALA231, LEU318 and LEU117, illustrating the interaction network.

The docking results indicate that carvedilol occupies a well-defined binding pocket and adopts a stable orientation within the protein. Visualization of the top-ranked docking pose reveals that ligand stabilization is mediated by a combination of hydrophobic contacts and polar interactions. In particular, hydrogen bonds (blue lines) and π-stacking (green lines) interactions with aromatic residues contribute to the anchoring of the ligand, while hydrophobic interactions support its positioning within the binding cavity (37).

3.2.2 Umeclidinium

Umeclidinium is a long-acting muscarinic acetylcholine receptor antagonist with high affinity for M1-M5 subtypes, particularly M3, mediating bronchodilation by competitively inhibiting acetylcholine at airway smooth muscle receptors. Its pharmacological action is antagonistic at

muscarinic GPCRs, with slow reversible binding that supports once-daily dosing in chronic obstructive pulmonary disease (COPD). Common adverse effects include anticholinergic effects such as dry mouth, constipation and headache. Systemic side effects are minimized due to low systemic exposure following inhalation (54,55).

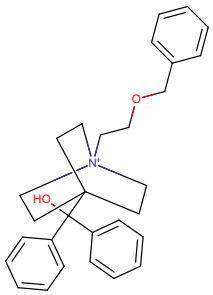

Figure 6: 2D-structure of umeclidinium (54)

Umeclidinium: structural visualization of the best-scoring binding pose

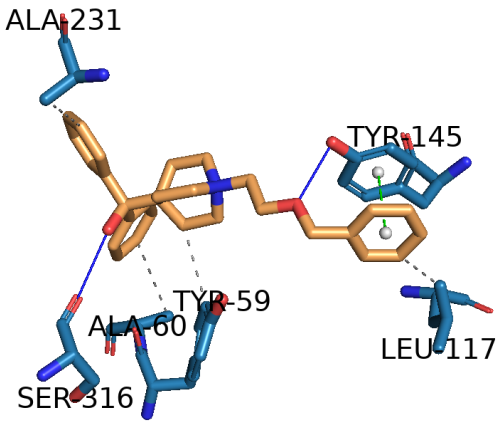

Figure 7: Molecular docking pose of umeclidinium within the L95P mutant of the Gβ protein

The docking pose shows that umeclidinium occupies a defined binding pocket within the protein and adopts a stable orientation. It reveals that ligand stabilization is mediated by a network of non-covalent interactions involving both polar and hydrophobic residues within the binding site.

Umeclidinium forms hydrogen bonds with polar residues, including TYR145 and SER316. In addition, hydrophobic interactions with surrounding residues such as TYR59, ALA60, LEU117, and ALA231 support ligand positioning within the cavity. Aromatic interactions, including π-stacking with TYR145, further stabilize the ligand orientation.

Overall umeclidinium is accommodated within the L95P mutant binding site of the Gβ protein.

3.2.3 Doxapram

Doxapram is a central and peripheral respiratory stimulant that enhances ventilatory drive primarily via inhibition of background potassium channels (KCNK3, KCNK9) in carotid body chemoreceptors, thereby increasing respiratory rate and tidal volume. This action is not mediated via classical GPCRs but rather through ion channel modulation.

Adverse events can include hypertension, tremor, tachycardia, anxiety and convulsions at high doses. Its clinical use is in acute respiratory depression and not in chronic neurologic disease (56,57).

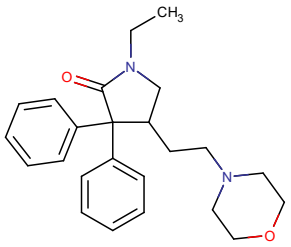

Figure 8: 2D-Structure of doxapram (57)

Doxapram: structural visualization of the best-scoring binding pose

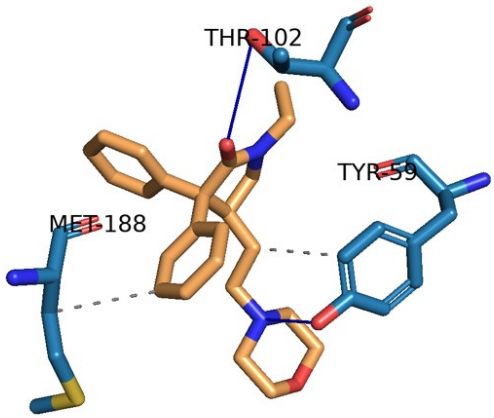

Figure 9: Molecular docking pose of doxapram within the L95P mutant of the Gβ protein

The best-scoring binding pose of doxapram shows that it occupies the defined region of the binding pocket and adopts a stable orientation within the protein. Ligand stabilization is achieved through a combination of polar and hydrophobic interactions with surrounding residues in the binding site.

Protein-ligand interaction analysis reveals that doxapram forms hydrogen bonds with

TYR59 and THR102, contributing to ligand anchoring within the binding pocket. In addition, hydrophobic contacts with residues TYR59 and MET188, support the positioning of the ligand within the cavity. Overall, the observed interaction network suggests that doxapram can be accommodated within the L95P mutant binding site of the Gβ protein.

3.2.4 Eliglustat

Eliglustat is a glucosylceramide synthase inhibitor used as substrate-reduction therapy for type 1 Gaucher disease. Its mechanism involves inhibition of a lipid-metabolizing enzyme. Eliglustat significantly reduces glycosphingolipid synthesis and accumulation, ameliorating systemic features of Gaucher disease. Common side effects include gastrointestinal symptoms and fatigue. Cardiac conduction changes have been theorized at supratherapeutic levels but are not observed with normal dosing (58–60).

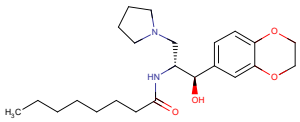

Figure 10: 2D-structure of eliglustat (58)

Eliglustat: structural visualization of the best-scoring binding pose

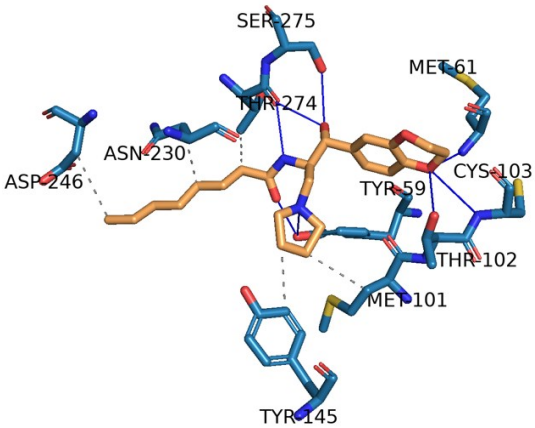

Figure 11: Molecular docking pose of eliglustat within the L95P mutant of the Gβ protein

Eliglustat occupies an extended binding pocket and adopts a stable orientation within the protein. Structural visualization reveals that ligand stabilization is mediated by a dense

network of non-covalent interactions involving both polar and hydrophobic residues distributed along the binding cavity.

Protein-ligand interaction analysis shows that the ligand forms multiple hydrogen bonds with key residues, including TYR59, MET61, THR102, CYS103, THR274 and SER275, contributing significantly to ligand anchoring and orientation within the binding site. In addition, hydrophobic interactions with residues such as MET101, TYR145, ASN230, ASP246 and THR274 further support ligand stabilization.

Collectively, the observed interaction pattern suggests that Eliglustat is well accommodated within the L95P mutant binding site of the Gβ protein.

3.2.5 Darifenacin

Darifenacin is a selective muscarinic M3 receptor antagonist indicated for overactive bladder. It functions through competitive antagonism at the M3 GPCR, reducing involuntary bladder contractions. Its antimuscarinic profile is similar to other M3 antagonists, with side effects such as dry mouth, constipation and blurred vision secondary to cholinergic blockade (61).

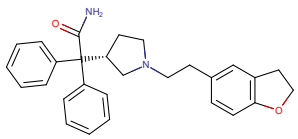

Figure 12: 2D-structure of darifenacin (61)

Darifenacin: structural visualization of the best-scoring binding pose

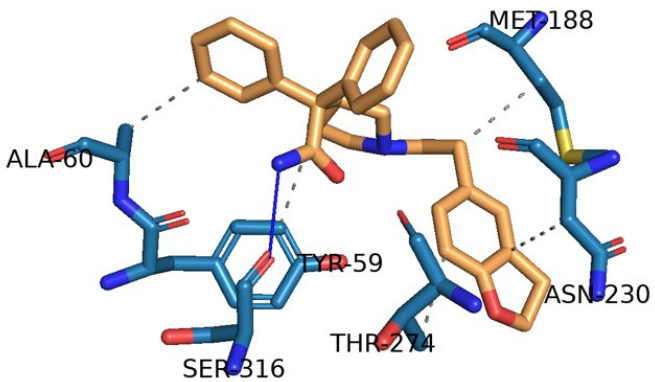

Figure 13: Molecular docking pose of darifenacin within the L95P mutant of the Gβ protein

Darifenacin occupies a well-defined binding pocket and adopts a stable orientation within the protein. Structural visualization shows that ligand stabilization is mediated by a combination of hydrophobic contacts and polar interactions as well.

Protein-ligand interaction analysis reveals that darifenacin forms a hydrogen bond with SER316. Hydrophobic interactions with surrounding residues, including TYR59, ALA60, MET188, ASN230 and THR274, support ligand positioning and stabilization.

Taken together, Darifenacin can be accommodated within the L95P mutant binding site of the Gβ protein.

3.2.6 Pimavanserin

Pimavanserin is an atypical antipsychotic that functions as a selective inverse agonist/antagonist at serotonin 5-HT_{2A} receptors (and to a lesser extent 5-HT_{2C}), without significant D2-receptor antagonism. Common adverse effects include somnolence, headache and QT-interval prolongation risk (62,63).

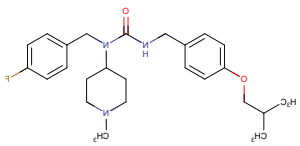

Figure 14: 2D-structure of pimavanserin (62)

Pimavanserin: structural visualization of the best-scoring binding pose

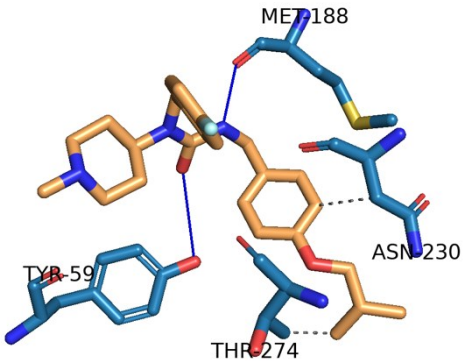

Figure 15: Molecular docking pose of pimavanserin within the L95P mutant of the Gβ protein

The ligand pimavanserin occupies a defined region of the binding pocket and adopts a stable orientation within the protein. Structural visualization shows that ligand stabilization is also mediated by a combination of hydrogen bonding and hydrophobic interactions.

The compound forms hydrogen bonds with TYR59 and MET188. In addition, hydrophobic interactions with ASN230 and THR274 support ligand positioning within the binding cavity.

Therefore, pimavanserin can be accommodated within the L95P mutant binding site of the Gβ protein.

3.3 Binding mode analysis

All BBB-permeable compounds were predicted to bind within surface grooves adjacent to the L95P mutation site. Binding poses were predominantly stabilized by hydrophobic interactions with WD-repeat residues and occasional hydrogen bonding to polar side chains. None of the compounds occupied a deeply buried pocket, consistent with the protein-protein interaction nature of $G\beta_1$ surfaces (9).

Carvedilol exhibited the highest score among BBB-permeable ligands and showed a stable binding orientation near the mutated WD-repeat blade, suggesting potential for local structural stabilization.

Other compounds, including umeclidinium, doxapram, eliglustat, darifenacin and pimavanserin, displayed weaker but reproducible binding poses, indicating possible allosteric interactions rather than direct functional rescue.

Table 3: Pharmacological overview of best-scoring BBB-permeable FDA-approved drugs (47,48)

Compound	**BBB-permeable**	**Indication**	**Effects**	**Special side effects**
Carvedilol	yes	heart failure, high blood pressure, reducing risk and severity of heart attacks in risk-patients	is a non-selective beta-adrenergic antagonist	dizziness, headaches, heart failure, low blood pressure, weakness
Umeclidinium	yes	maintenance treatment for symptoms of COPD	long-acting muscarinic antagonist	nasopharyngitis, upper respiratory tract infection
Doxapram	yes	temporary measure in hospitalized patients with acute respiratory insufficiency superimposed on COPD	short acting respiratory stimulant mediated through the peripheral carotid chemoreceptors	tremors, increased restlessness, seizures
Eliglustat	yes	long-term treatment of type 1 Gaucher disease	glucosylceramide synthase inhibitor	dyspepsia, fatigue, headache, nausea
Darifenacin	yes	overactive bladder with symptoms of urge urinary incontinence, urgency and frequency	M3-muscarinic receptor blocker	dry mouth, obstipation
Pimavanserin	yes	treatment of hallucinations and delusions caused by Parkinson's disease	mechanism of action is unknown	peripheral edema and confusion, prolongs the QT-interval

4. Discussion

Although this study represents an initial docking-based screening without comparison to the wild-type (WT) protein, the identified binding poses and interaction profiles support the suitability of all six FDA-approved ligands as a candidate for further computational and experimental investigation targeting Gβ protein modulation.

The results are limited to predicted binding poses and interaction patterns and require further validation by molecular dynamics simulations and experimental assays.

The present study employed a structure-based molecular docking approach to explore whether FDA-approved, BBB-permeable compounds can bind to the $G\beta_1\gamma_2$ dimer carrying the pathogenic L95P mutation. Given the absence of disease-modifying therapies for GNB1-related encephalopathy, this exploratory strategy aimed to assess whether pharmacological repurposing could represent a potential avenue for future therapeutic development.

4.1 Interpretation of docking scores

The docking results revealed that a limited subset of BBB-permeable compounds achieved PLPFitness-scores ≥ 60, indicating moderate predicted binding affinity to the $G\beta_1$ L95P structure. Among these, carvedilol displayed the highest docking score and a reproducible binding pose in proximity to the L95P mutation site. Other compounds, including eliglustat, pimavanserin and darifenacin, exhibited slightly lower but consistent scores and binding orientations.

It is important to emphasize that the observed PLP scores fall within a moderate range, which is expected given the nature of the $G\beta_1$ protein. $G\beta_1$ does not possess a classical ligand-binding pocket but instead presents relatively flat, solvent-exposed surfaces optimized for protein-protein interactions (9). Consequently, small molecules are unlikely to bind with high affinity comparable to enzyme active sites or GPCR orthosteric pockets. The docking results therefore suggest potential allosteric or stabilizing interactions, rather than strong binding.

Compared to emerging approaches such as gene replacement or CRISPR-based correction, small-molecule docking represents a lower-risk and more immediately translatable strategy, particularly when focusing on FDA-approved compounds (64).

Docking studies such as this provide valuable initial insights and help prioritize compounds for experimental testing.

4.2 Limitations of structure-based docking

While molecular docking provides valuable insights into potential ligand-protein interactions, several limitations must be considered when interpreting the present results. First, docking simulations rely on static protein structures and do not account for protein dynamics, conformational flexibility or induced-fit effects that may occur upon ligand binding (35). This limitation is particularly relevant for WD-repeat proteins such as $G\beta_1$, which exhibit dynamic surface interactions during protein-protein complex formation.

Second, the ChemPLP scoring function used in this study provides relative ranking of ligand poses rather than quantitative binding affinities. Consequently, docking scores cannot be directly translated into experimental dissociation constants or therapeutic efficacy (65). Third, the receptor was treated as rigid during docking, which may underestimate potential binding modes requiring local structural rearrangements, especially near the destabilized L95P region (35).

Additionally, predicted blood-brain barrier permeability does not guarantee sufficient brain exposure, target engagement or pharmacodynamic effect in vivo (66). Consequently, the docking results should be regarded as hypothesis-generating, rather than as evidence of therapeutic efficacy. Experimental validation using biochemical binding assays, cellular models or electrophysiological readouts is required (67,68).

4.3 Neurological relevance of identified compounds

GNB1 encephalopathy is a primary neurodevelopmental disorder characterized by severe intellectual disability, epilepsy, hypotonia and movement disorders, reflecting widespread dysfunction of GPCR-dependent neuronal signaling pathways. Consequently, any candidate compound considered for repurposing must be evaluated primarily through a neurological safety lens. Many of the compounds identified in the present screening were originally developed for non-neurological indications, including cardiovascular, pulmonary, metabolic or infectious diseases (as shown in Table 3). Nevertheless, several possess known central nervous system (CNS) penetration or documented neurological effects, which is a prerequisite for targeting neuronal $G\beta_1$ function but simultaneously raises safety concerns.

Importantly, none of the top-ranked compounds occupied deeply buried binding pockets on $G\beta_1$, which is consistent with the predominantly protein-protein interaction-mediated surface topology of Gβ subunits (9,13). This observation suggests that any potential therapeutic effect

would likely arise from subtle modulation of protein interfaces or conformational stabilization rather than classical high-affinity active-site inhibition.

Many of the identified compounds have documented neurological side effects, including dizziness, headache, sedation, tremor, dyskinesia or seizures. Such adverse effects are particularly relevant in individuals with GNB1 encephalopathy, who often already suffer from epilepsy, abnormal movement patterns and impaired autonomic regulation. Drugs that modulate cholinergic, adrenergic or dopaminergic signaling may exacerbate these symports due to downstream interactions with GPCR pathways that are already compromised in this disorder (16,29).

Systemic side effects also warrant careful consideration. Cardiovascular instability, respiratory suppression, gastrointestinal dysmotility and metabolic disturbances have all been reported for several repurposable agents identified in the screen. Given the high prevalence of feeding difficulties, respiratory vulnerability and autonomic dysfunction in GNB1 encephalopathy, such adverse effects could significantly outweigh any hypothetical benefit (16).

From a clinical perspective, the use of these compounds in GNB1-E would currently be experimental and off-label. While drug repurposing has proven successful in other conformational or genetic disorders, such as transthyretin amyloidosis or cystic fibrosis, these successes were supported by extensive in vitro, in vivo and clinical validation prior to long-term administration (69,70).

Long-term use is particularly problematic in pediatric neurodevelopmental disorders. Chronic exposure during critical periods of brain development may lead to unpredictable effects on synaptogenesis, neuronal excitability and cognitive outcomes. Moreover, many FDA-approved drugs lack long-term safety data in children with severe neurological impairment. Therefore, prolonged administration of such compounds cannot be recommended without rigorous preclinical validation, including functional assays, neuronal models and toxicity studies (71,72).

The findings of this study should be interpreted as a starting point rather than a therapeutic recommendation. Structure-based docking provides valuable insight into potential molecular interactions but does not account for cellular context, protein expression levels, compensatory signaling or developmental effects. Future work should focus on validating candidate

compounds in cellular systems expressing mutant GNB1, followed by functional readouts of GPCR signaling, GIRK channel activity and neuronal excitability.

In the longer term, advances in structure-guided drug design and precision medicine may enable the development of safer, variant-specific modulators of $G\beta_1$ function. Until such strategies are realized, any pharmacological intervention in GNB1 encephalopathy must remain cautious, individualized and firmly grounded in symptomatic management.

5. Conclusion

In summary, this study identifies several BBB-permeable, FDA-approved compounds capable of binding to the $G\beta_1$-L95P structure with moderate predicted affinity, supporting the applicability of this approach for the identification of potential Gβ modulators.

While none of the compounds are expected to directly correct the underlying molecular defect, their binding profiles suggest possible stabilizing or allosteric effects that warrant further investigation.

Future studies should focus on experimental validation using biochemical binding assays, cellular models of GNB1 deficiency and electrophysiological readouts of GPCR signaling. Ultimately, integrating structure-based drug discovery with genetic and functional approaches may enable the development of targeted therapies for GNB1-related encephalopathy.

6. References

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