

ARTICLE ID: BTS0326011

COULD RLIP76 BE THE KEY TO UNLOCKING NOVEL THERAPEUTIC AVENUES IN EPILEPSY?

Pratyush Porel | Khadga Raj Aran*

ISF College of Pharmacy, Moga-142001 Punjab, India.

Corresponding Author Email: bishalarann@gmail.com**Received:** 15 July 2025 | **Revised & Accepted:** 25 September 2025**Keywords:** RLIP76, Epilepsy, Oxidative stress, Neuroinflammation, Seizures

ABSTRACT

Epilepsy is a debilitating neurological disorder characterized by recurrent seizures that is associated with significant cognitive and physical impairments. Several important factors, including oxidative stress, metabolic imbalances, and genetic mutations, contribute to the etiopathology of epilepsy, and its gradual progression leads to neurodegeneration. The traditional antiepileptic drugs (AEDs) cannot cure drug resistance in epilepsy, which is a challenge in current research. Overcoming such difficulties creates a necessity to develop new therapeutic methods. Ral interacting protein 76 (RLIP76), an ATP-dependent transporter, is essential for cellular homeostasis through modulation of oxidative stress and promoting the removal of toxic metabolites like 4-hydroxynonrenal linked with neuronal injury. It controls proteins like c-Jun N-terminal kinases (JNK) and extracellular signal-regulated kinases (ERK), controlling cell survival, apoptosis, and drug resistance. It also regulates neuronal excitability and synaptic plasticity, crucial determinants of seizure susceptibility, along with its function in cell detoxification. Its multifaceted functions in neuron stability, protection against oxidative stress, and restraint of pathological excitability contribute to the therapeutic potential of RLIP76. Its modulatory activity may be beneficial to improve the efficacy of AEDs through increased availability of drugs and decreased multidrug resistance, providing promise to develop more novel and effective therapeutic approaches for the management of epilepsy, particularly drug-resistant epilepsy. This review emphasizes the organization, functions, and cellular processes of RLIP76, highlighting its role in epilepsy pathophysiology as a possible biomarker for epileptic conditions and its therapeutic potential in the development of targeted therapies, including small-molecule inhibitors and gene therapy, to improve its therapeutic outcomes.

ABBREVIATIONS

ABC: ATP-binding cassette transporter; LGI1/ADAM22: Leucine-rich: glioma inactivated 1/A disintegrin and metalloproteinase 22; AE: Acquired epilepsy; AED: Antiepileptic drugs; AP2: Adaptor protein complex 2; ARNO: ADP-ribosylation factor nucleotide binding site open; ATP: Adenosine triphosphate; BBB: Blood-brain barrier; CDE: Clathrin-dependent endocytosis; CDK1: Cyclin-dependent kinase-1; CNS: Central nervous system; CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; DNA: Deoxyribonucleic acid; EAAT1: Excitatory amino acid transporter 1.

INTRODUCTION

Epilepsy is a complex neurodegenerative disorder characterized by recurrent, unprovoked, and spontaneous cortical seizures along with sensory, motor, autonomic, and mental symptoms resulting in significant deterioration of life quality (Duncan *et al.*, 2024). Various mechanisms, including oxidative stress (OS), genetic mutations, metabolic dysfunction, and overactivation of neurons, produce recurrent episodes of seizures and facilitate neurodegeneration (Manole *et al.*, 2023). In the majority of cases, currently available antiepileptic drugs (AEDs) are unable to treat epilepsy effectively; therefore, healthcare requires more improved molecular insight and novel neuroprotective therapies to avoid neurodegeneration and disease progression (Rheims *et al.*, 2011). Ral interacting protein 76 (RLIP76), or RalA binding protein 1 (RALBP1), an ATP-dependent efflux transporter, eliminates toxic metabolites by removing 4-hydroxynonrenal (4-HNE) in addition to other electrophilic agents and regulating the balance. The protein affects both c-Jun N-terminal kinases (JNK) as well as extracellular signal-regulated kinases (ERK) stress pathways, allowing their activity in determining cellular survival rates while at the same time affecting drug resistance and apoptosis progression. RLIP76 is a multidrug resistance protein of 76 kDa, encoded by the RALBP1 gene present at chromosome 18, and acts as an indispensable mediator between OS and cellular homeostasis (Jullien-Flores *et al.*, 1995). As a stress-protective ATPase in the mercapturic acid pathway, it couples clathrin-dependent endocytosis (CDE) and glutathione-electrophile conjugate export (Singhal *et al.*, 2011). The transporter activity of RLIP76 enables critical

molecules to communicate, regulate plasticity, excitability, and amplify seizure susceptibility (Awasthi *et al.*, 2002). It is also critical in neuronal stability maintenance through regulation of seizure-induced OS damage, mitochondrial abnormalities, and minimizing excessive excitability, which is a feature of epilepsy (Awasthi *et al.*, 2021). Moreover, it is associated with multidrug resistance processes through xenobiotic transport activities. Thus, RLIP76 is an encouraging therapeutic target, and pharmacological modification of the protein has the potential to provide novel seizure treatment interventions with simultaneous alleviation of neurodegeneration severity. Modulation of RLIP76 would enhance AEDs serum bioavailability and efficacy by preventing efflux and ultimately improving response with pharmacotherapy among drug-resistant epilepsy patients (Awasthi *et al.*, 2007). Moreover, such therapies targeting RLIP76 have neuroprotective potential in maintaining synaptic protein levels and lowering seizure-induced neuronal loss. Targeting the interaction between other molecular pathways and RLIP76 in epilepsy can lead to new information and assist in planning new effective treatments, such as small molecule inhibitors, gene therapy, and targeted drug delivery systems (Kaneko *et al.*, 2008). This review aims to explore the structure, function, as well as cellular processes related to RLIP76 and highlight its significance in epilepsy. Additionally, it focuses on RLIP76 as a potential biomarker for epilepsies, emphasizing its expression levels and its connections with OS pathways in epileptic conditions. Furthermore, it also evaluates the clinical value of targeting RLIP76 for novel therapeutic approaches that, in turn, will create opportunities for innovative drugs to enhance epilepsy therapy.

RLIP76: structure and physiological functions

RLIP76 primarily consists of 655 residues, with two identifiable regions present in the center of the protein, i.e., RhoGAP and Ral binding domain (RBD), surrounded by N- and C-termini (Cantor *et al.*, 1995), as shown in **Figure 1**. The C-terminus has been projected to develop small coiled-coil between residues 393 and 446 that attaches firmly with the Ral, Repl1, and partially overlaps with BTB1 protein 1 (POB1), cyclin-dependent kinase 1 (CDK1), and heat shock factor 1 (HSF-1) proteins, which connect RLIP76 to several processes, such as clathrin-coated-pit-mediated receptor-ligand endocytosis of impulses through insulin and transforming growth factor-beta (TGF- β) receptors, mitosis mechanisms via mitotic spindle movement, CDK1 as well as neurotransmitter exocytosis across the exocyst complexities (Cornish *et al.*, 2021). The RhoGAP homologous domain, which contains residues 192-368, is found to enhance GTPase functioning of Rho family tiny GTPases Rac1 as well as Cdc42 *in vitro*, proteins that control cytoskeletal interactions, motility, cell cycle, and clathrin-dependent endocytosis (Rajasekar *et al.*, 2013). The RBD is located near the RhoGAP region, and it interacts with RalA and RalB. RLIP-RalA as well as RLIP-RalB form links that have been associated with essential functions, such as controlling mitochondrial fission during mitosis, localization of p27, clathrin-dependent endocytosis of receptors, cytoskeletal reconfiguration, and invadopodia formation (Cornish *et al.*, 2021; Neel *et al.*, 2012). RLIP has an N-terminal Eps 15 Homology (EH) domain that binds the adaptor protein complex 2 (AP2) clathrin adapter complex and with ADP-ribosylation factor nucleotide binding

site open (ARNO), enhancing endocytosis and cell migration functions, respectively. RBD binds to R-Ras with N $\rightarrow$ C orientation, whose interaction aids in the activation of ARNO. A region including both the AP2/ARNO interaction area as well as the R-Ras binding residues known as the ear domain binds to Epsin, a binding that is supposed to affect cell migration as well as clathrin-dependent endocytosis (CDE) inhibiting during mitosis (Coon *et al.*, 2010). RLIP has two ATP-binding regions present at N- and C-terminal domains, protein kinase C alpha (PKC α) phosphorylation sites, and vector peptides that share significant sequence similarity (Singhal *et al.*, 2006). In addition, using site-directed mutation research through substitution and elimination mutation analyses, it has been proven that RLIP76 possesses proteins connected to the membrane, which plays a crucial part in transport and membrane attachment alongside a cell surface domain. Mutating lysines K74 alongside K425 to methionines has been shown to inhibit RLIP's ATP hydrolysis efficacy while decreasing xenobiotic transport, along with the development of invadopodia declining within RLIP-forming cells via the K425M mutation in comparison to wild type (Neel *et al.*, 2012).

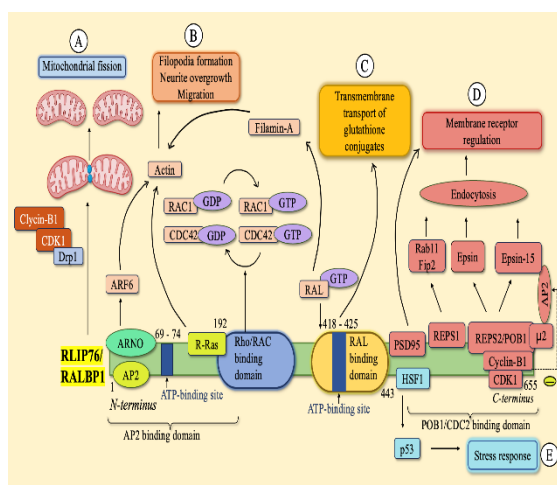


Fig (1): Molecular structure and functional interactions of RLIP76 in cellular processes.

RLIP76 in the central nervous system

RLIP76 is highly expressed in numerous human tissues, including the brain, specifically on luminal surfaces of endothelial cell membranes, with its expression in CNS tissues, such as cerebral cortex, hippocampus, cerebellum, and basal ganglia (Fan *et al.*, 2015). It was found to be largely distributed in the BBB tissues of resected epileptic foci. Research studies have established that the cellular expression of RLIP76 is in neurons, but also in other glial cells, such as microglial and astrocytic cells (Reddy *et al.*, 2023). The neuronal localization with glial cells implies neuroinflammation (Li *et al.*, 2022). It is specifically found at the cell membrane where it regulates various transport pathways dependent on ATP, and in intracellular organelles where it can interact with numerous signaling components such as Ral GTPases (Goldfinger and Lee 2013).

Epilepsy: Pathophysiology and molecular mechanisms

Epileptic seizures involve jerky or twitching movements throughout the body caused by unusual neuronal activity, which

harms the brain or other organs as well as produces emotional, behavioral, and neurological conditions in individuals. These may occur in several parts of the brain, and the degree of efficacy depends on the area and the different types of seizures, including focal as well as generalized seizures (Milligan 2021). Focal seizures are those that occur in a tiny, restricted area of the brain's cortex or a more extensive component of the cerebrum, and the key subgroups of focal seizures include awareness, diminished awareness, motor onset, non-motor onset, and localized to generalized tonic-clonic seizures (Leguia *et al.*, 2021). In contrast, generalized seizures involve both cerebral hemispheres of the brain. They were broadly categorized into two distinct groups: motor and non-motor. Motor convulsions are classified as tonic, clonic, tonic-clonic, myoclonic, myoclonic-tonic-clonic, atonic, and epileptic contractions. Non-motor convulsions consist of atypical, typical, and myoclonic, along with eyelid myoclonias (Raga *et al.*, 2021).

Epileptic seizures are exacerbated through several factors such as neurotransmitter imbalance, ion channel disruption, synaptic plasticity, inflammatory pathways, and cellular metabolism disruptions. This occurs due to primary imbalances along neuronal inhibition as well as excitation that generate additional abnormalities by inhibiting synapses and voltage-gated inhibitory conductance or enhancing synapses and voltage-gated excitatory conductance (Sumadewi *et al.*, 2023). The nervous system exhibits neurochemical activity altered from glutamate as well as gamma-aminobutyric acid (GABA) neurotransmitters alongside sodium, potassium, and calcium ion channels, altered ion concentrations, as well as imbalanced cell surface receptors and

inflammatory conditions because of OS (McKarns 2023). Current clinical studies show how epilepsy genes like synapsins, including stargazin and leucine-rich glioma inactivated 1/A disintegrin and metalloproteinase 22 (LGI1/ADAM22), control seizure development by managing excitatory/inhibitory ratios, and their absence results in an excitatory/inhibitory disproportion that leads to seizures. Alterations of genes of ion channels can be due to mutations, infections, and antibodies (Yamaguchi *et al.*, 2025). Moreover, epileptic seizures lead to improper synaptic plasticity along with hyperstability of neuronal connections. Synaptic plasticity refers to the capability of synapses to modify their degree of strength periodically, which might correspond to another cause of seizure production, which is also connected with inhibitory synapses (Liu *et al.*, 2024).

Inflammatory mediators, including interleukin-6 (IL-6) and macrophage inflammatory protein (MIP), are produced by astrocytes and microglia, resulting in hyperexcitability. Higher levels of IL-6 and TNF- α were identified along with enhanced synapse protein generation, neuronal inflammation, as well as neurogenesis in the adult hippocampus in mice lacking synapsin 2 throughout the epileptic phase (Bäckström *et al.*, 2023). Inflammation also results in BBB disruption that produces significant levels of immunoglobulin G (IgG), which can be observed in seizure-generating areas of the brain. Some targets in epileptic seizures involve P-glycoprotein (P-gp), mammalian target of rapamycin (mTOR), as well as mutations in voltage-gated ion channels such as ashyerpolarization-activated cyclic nucleotide-gated (HCN) channel, Na-K-2Cl cotransporter isoform 1 NKCC.,) and Kv7 channel (Sumadewi *et al.*, 2023).

RLIP76 in Epilepsy

RLIP76 contributes to seizure pathophysiology through modulation of OS, neuroinflammation, and epileptogenesis (Meda *et al.*, 2019). Excessive neuronal excitability and its activation in epilepsy lead to increased metabolic demand and production of ROS (Rho and Boison 2022). Under normal conditions, RLIP76 maintains redox homeostasis through the reduction of toxic lipid peroxidative breakdown products, including 4-hydroxy-2-nonenal (4-HNE) and MDA. The OS overload and inflammatory signals, together with loss of mutational changes of RLIP76, lead to a condition blocking the cellular exclusion of ROS, resulting in increased neuronal cell hyperexcitability and seizure susceptibility (Jaramillo *et al.*, 2014). RLIP76 regulates certain intracellular signaling processes, involving phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) as well as mitogen-activated protein kinase (MAPK) cascades, including their dysregulation that leads to mitochondrial dysfunction, glutamate excitotoxicity, and disruption of the BBB, all of which are known to worsen epilepsy (Bajalia *et al.*, 2022). Furthermore, RLIP76 has been associated with neuroinflammatory reactions, which increase gliosis and cytokine release, therefore promoting seizure formation. Hence, RLIP76 is a prospective target for new epilepsy treatments because of its complex function. Although suppression in some situations may alter abnormal cell signaling linked to the onset of seizures, improving RLIP76 activity may help reduce oxidative injury and neuroinflammation (Depondt and Shorvon 2006).

Role of RLIP76 in seizure-induced oxidative stress, mitochondrial dysfunction

RLIP76 is crucial in lowering seizure-induced oxidative injury along with mitochondrial dysfunction through its mechanisms for glutathione conjugate transport as well as stress response pathway modulation (Das *et al.*, 2011). Seizures cause increased neuronal excitability, which breaks down the metabolism of neuronal cells, resulting in OS, mitochondrial dysfunction, and neuronal damage (Aguiar *et al.*, 2012). The brain's natural antioxidant defense mechanism is unable to keep up with the overproduction of ROS and RNS caused by excessive neuronal excitability. The OS affects proteins, mitochondria, and DNA, triggering lipid peroxidation and further damaging neurons (Waldbaum and Patel 2010). ROS promotes oxidative degeneration of polyunsaturated fatty acids, resulting in the formation of 4-HNE, a genotoxic as well as proteotoxic peroxidation byproduct from omega-6 polyunsaturated fatty acids like arachidonic acid, linoleic acid (Zhong and Yin 2015). Both MRP1 and MRP2 detoxify the body by removing trace amounts of harmful chemicals, such as 4-HNE, when OS is low; however, in excessive OS, RLIP76 detoxifies toxic 4-HNE (Gasparovic *et al.*, 2017). RLIP transfers the end-product GS-HNE molecule out of the cell in an ATP-dependent way when 4-HNE is conjugated to GSH by glutathione S-transferase alpha 4 (GSTA4). However, dysregulated RLIP76 produces excessive ROS accumulation, which intensifies oxidative harm to neurons (Dratkiewicz *et al.*, 2021). This occurs due to gene mutations affecting ion channels such as potassium voltage-gated channel subfamily Q member 2 (KCNQ2), sodium channel,

voltage-gated, type I, alpha subunit (SCN1A), and synaptic proteins like gamma-aminobutyric acid receptor alpha subunit 1 (GABRA1), syntaxin binding protein 1 (STXBP1), as well as metabolic pathways, including mTOR signaling and mitochondrial function (Smolarz *et al.*, 2021). The dysfunction of voltage-gated sodium channels coded by SCN1A mutations results in hyperexcited inhibitory interneurons that allow less GABAergic inhibition to occur, thus leading to seizures (Ademuwagun *et al.*, 2021). Similarly, the damage to KCNQ2 genes interrupts potassium channel processes, leading to neuronal membrane failures that result in increased neuronal excitability (Steinlein 2012). Neurological cells affected by GABRA1 alterations become more likely to experience depolarization since their GABA receptor-mediated inhibitory signals decrease (Mele *et al.*, 2019). The dysfunction of synaptic vesicle fusion mechanisms caused by STXBP1 mutations creates a breakdown of neurotransmitter release and disrupts synapses from maintaining balance (Freibauer *et al.*, 2023). ROS amounts that exceed normal levels because of RLIP76 dysfunction trigger damage to the TSC1/TSC2 complex, which controls mTOR complex 1 (mTORC1) (Hranac 2013). The TSC1/TSC2 complex inhibits mTORC1 activity through Ras homolog enriched in brain (Rheb) inactivation, thereby preventing hyperactive protein synthesis and abnormal neuronal growth during normal conditions (Vasic *et al.*, 2021). However, OS resulting from RLIP76 dysregulation triggers oxidative alterations to deactivate TSC1/TSC2, which further activates mTORC1 through hyperactivation (Borović Šunjić *et al.*, 2024), as shown in **Figure 2**. Excessive activity of mTORC1 causes a combination

of increased protein production, autophagy impairment, and cell enlargement that leads directly to neural network disruption and seizure development. The dysfunctional mitochondrial dynamics that occur because of mTORC1 hyperactivation lead to more ROS production and worsen OS conditions (LaSarge and Danzer 2014). RLIP76 dysfunction induces OS that activates mTORC1, thus sustaining enhanced neuronal activity and generating epileptogenesis in the brain (Wiśniewska *et al.*, 2021). Synaptic plasticity changes due to mTORC1 activation, thereby altering essential synaptic protein levels (GABRA1, STXBP1), which results in inappropriate excitatory and inhibitory neurotransmission. The neuronal network becomes more prone to seizure tendencies because excitatory synapses increase while inhibitory pathways deteriorate (Poliquin and Kang 2022).

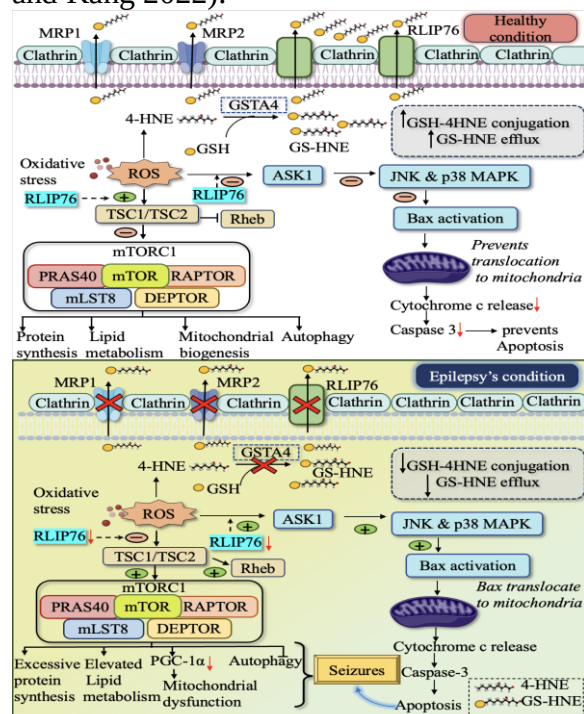


Fig (2): Impact of RLIP76 dysregulation on oxidative stress and mitochondrial dysfunction in epilepsy.

RLIP76 acts as a vital factor for mitochondrial functioning through diverse pathways by protecting mitochondrial integrity throughout seizures, which primarily generate ROS (Bachi *et al.*, 2013). RLIP76 is demonstrated to control mitochondrial membrane potentially via its interactions linked to apoptosis-associated proteins, including survival factor Bcl-2 and death-promoting protein Bax, triggering mitochondrial outer membrane permeabilization (MOMP) (Kim *et al.*, 2022). These interaction of RLIP76-regulated Bcl-2 and Bax proteins control and inhibits mitochondrial membrane depolarization along with cytochrome c release during OS-induced apoptosis (Gómez-Crisóstomo *et al.*, 2013). Moreover, RLIP76 also functions as a transporter to transport detoxification products bearing glutathione and other compounds across the mitochondrial membranes to maintain both redox balance and organelle internal stability (Franco *et al.*, 2007). Due to its ATP-dependent transporter activity, RLIP76 removes 4-HNE byproducts from lipid peroxidation, thereby preventing the progression of mitochondrial damage and neuronal deterioration (Poli *et al.*, 2008). The interaction between RLIP76 and JNK and p38 MAPK stress-responsive kinases allows control over mitochondrial OS responses along with apoptotic signaling processes. The degradation of upstream activators like apoptosis signal-regulating kinase 1 (ASK1), which initiates JNK and stimulation of p38, is facilitated by RLIP76 that prevents JNK, p38-induced apoptosis, and mitochondrial damage. Therefore, RLIP76 maintains healthy mitochondria by controlling excessive ROS production combined with its actions in controlling dynamic stability while enhancing detoxification processes, which preserves

neurons from loss following seizure activity (Fernandes *et al.*, 2010). However, during mitochondrial dysfunction and RLIP76 dysregulation, excessive ROS generation leads to severe degradation of lipids, proteins, and DNA structures, which results in abnormal epileptic development and neuronal death (Singhal *et al.*, 2022).

Role of RLIP76 in seizure-induced neuroinflammation and apoptosis

RLIP76 functions essentially in controlling OS processes along with cellular signaling and apoptosis, yet its imbalanced expression stimulates seizure-induced neuroinflammation and neuronal death (Li *et al.*, 2022). Excessive neuronal activity during seizures creates metabolic stress on neurons, which damages mitochondria while generating excessive ROS (Patel 2004). However, the dysregulation of RLIP76 in epilepsy patients causes increased ROS accumulation that results in severe oxidative harm to cell components and starts inflammatory signals and apoptotic processes (Niftullayev and Lamarche-Vane 2019). Nuclear factor-kappa B (NF- κ B) activation reaches higher levels among cells with RLIP76 dysfunction as a result of OS conditions, which activates I κ B kinase that further phosphorylates I κ B, resulting in its degradation. Thus, they regulate elevated production of pro-inflammatory cytokines TNF- α , interleukin-1 β (IL-1 β), and IL-6 (Sagai and Bocci 2011). The excessive levels of these cytokines intensify neuronal hyperexcitability because they alter the activity of ion channels, especially voltage-gated sodium (SCN1A) and potassium (KCNQ2), disturbing the balance between excitatory as well as inhibitory neurotransmission (Jaworski 2020). After NF- κ B activation occurs, it induces excessive production of inducible nitric

oxide synthase (iNOS), cyclooxygenase-2 (COX-2), enhancing the production of excessive prostaglandins and nitric oxide because of OS and neural damage (Sorokin 2016). Neuroinflammation becomes persistent because it activates microglia and astrocytes, releasing more ROS and inflammatory mediators, which establishes a recurrent cycle that allows neuroinflammation to continue and increase seizure frequency (Sanz and Garcia-Gimeno 2020). RLIP76 serves as a negative JNK regulator because it transfers JNK-phosphorylated substrates, which prevents apoptotic cascade activation (Wang *et al.*, 2013). The protective functioning of RLIP76 helps decrease neural cell deaths that develop from OS conditions (Mattson 2009). The activation of JNK begins when upstream kinases such as mitogen-activated protein kinase kinase 4 (MKK4), along with MKK7, respond to both OS and inflammatory signals. The activated JNK molecule transits to the nucleus before phosphorylating transcription factors like c-Jun, which then activate pro-apoptotic genes Bim and FasL (Uddin *et al.*, 2021), as shown in **Figure 3**. In addition, through JNK-mediated Bcl-2 family proteins, phosphorylation of Bim and Bax activity gets enhanced, which drives MOMP, eventually releasing cytochrome c and triggering caspase activation (Singh *et al.*, 2019). RLIP76 protects cells by removing phosphorylated JNK substrate proteins from the cytosol, which limits both JNK nuclear entry and its pro-apoptotic effects. Neuronal survival during seizures becomes protected because RLIP76 reduces both OS-induced JNK hyperactivation and damaging cellular effects on the neurons (Dalleau *et al.*, 2013).

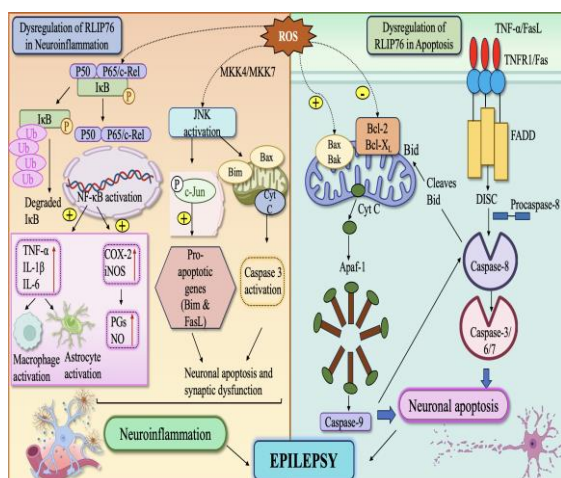


Fig (3): RLIP76 dysregulation in epilepsy: effects on neuroinflammation and apoptosis.

On the other hand, RLIP76 dysregulation results in seizure-induced neuronal apoptosis occurring through intrinsic (mitochondrial) and extrinsic (death receptor) mechanisms (Reddy *et al.*, 2023). Excessive accumulation of ROS triggers mitochondrial breakdown by activating death-promoting Bcl-2 proteins Bak and Bax, thereby antagonizing protective Bcl-2 elements Bcl-2 and Bcl-X_L simultaneously (Reed 2006). The alteration in mitochondrial membranes results in cytochrome c release into cytosol, which activates apoptotic protease activating factor-1 (Apaf-1) leading to production of apoptosome that activates caspase-9 from apoptosome, starts the caspase cascade that results in effector caspase activation, that is associated with vital protein cleavage and DNA fragmentation that finally causes apoptosis in neurons (Henshall *et al.*, 2001). Moreover, the ROS activation of poly (ADP-ribose) polymerase-1 (PARP-1) depletes cellular nicotinamide adenine dinucleotide (NAD⁺) while disrupting mitochondrial functioning and intensifying energy deficiencies in neurons, which subsequently increases their apoptotic

vulnerability (Khatri and Man 2013). The extrinsic apoptotic pathway becomes activated through the neuroinflammatory effects of seizures that result in increased numbers of membrane-bound TNF receptor-1 death receptors. Activation of caspase-8 begins after TNF-α or FasL ligand binds to receptors along with adapter proteins, including the Fas-associated death domain (FADD) (Henshall and Simon 2005). Stimulation of caspase-8 leads this protease to execute two different functions: it activates effector caspases directly, or it breaks down Bid (BH3-interacting domain death agonist), a BH3-only protein, linking exterior apoptotic pathologies with interior apoptotic pathways (Delgado 2014). Moreover, the elevated ROS production from RLIP76 simultaneously raises neuronal sensitivity to death receptor signals because it modifies key apoptotic proteins through oxidative alterations, which results in reduced resistance against programmatic cell death (Singhal *et al.*, 2011). The influence of RLIP76 on mTOR signaling represents an essential mechanism in seizure-induced neuronal apoptosis because this pathway functions as a major regulator for neuronal growth and survival and also plays a key role in epilepsy development (Zhang *et al.*, 2017). RLIP76 dysfunction during OS activates mTORC1 by oxidatively damaging the TSC1/TSC2 complex, which normally controls mTORC1 activity. This activation of the mTORC1 pathway results in increased protein synthesis while inhibiting autophagy, which degrades damaged components and prevents neuron apoptosis. Furthermore, the hyperactivated mTORC1 creates synaptic homeostasis disruptions through the regulation of essential synaptic proteins, such as GABRA1 and STXB1P1, thus producing abnormal excitatory and

inhibitory neurotransmission (Gourmaud *et al.*, 2022).

Role of RLIP76 in BBB dysfunction and drug resistance

In epilepsy, RLIP76 plays a crucial role in regulating both AED resistance and BBB integrity. Selective transport between the blood and brain is normally maintained by the blood-brain barrier (BBB), but epilepsy is characterized by its failure. Redox equilibrium is maintained by RLIP76, which increases endothelium export of ROS, lipid-peroxidation products such as 4 HNE and MDA, and GSH conjugated xenobiotics. Nevertheless, through RhoA/Rac1-mediated actin rearrangement, increased RLIP76 expression causes cytoskeletal and tight-junction protein (occludin, claudins) disruption, enhancing BBB permeability, neuroinflammation, and seizure susceptibility (Ganta *et al.*, 2010; Song *et al.*, 2020). Similar to an ABC transporter, RLIP76 effluxes AEDs from endothelial and neural cells via collaborating with P-gp and MRP proteins. Phenytoin, carbamazepine, and valproate are important AEDs whose intracellular concentrations are markedly decreased in vitro by RLIP76, which diminishes their therapeutic effectiveness. Interestingly, RLIP76 plays a dominating role in epileptic BBB endothelial cells, accounting for roughly 72% of phenytoin efflux, while P-gp contributes for 27%. This transport capacity is modulated by genetic variations in RLIP76, suggesting that AED resistance may be overcome by targeted RLIP76 inhibition (Soranzo *et al.*, 2005). Glutamate-conjugate efflux is also facilitated by RLIP76, which increases excitotoxicity and interferes with neuronal survival pathways. BBB disruption increases excitability and seizure thresholds by allowing serum proteins and peripheral inflammatory mediators to enter brain

tissue (Makrides *et al.*, 2017), diagrammatically explained in **Figure 4**.

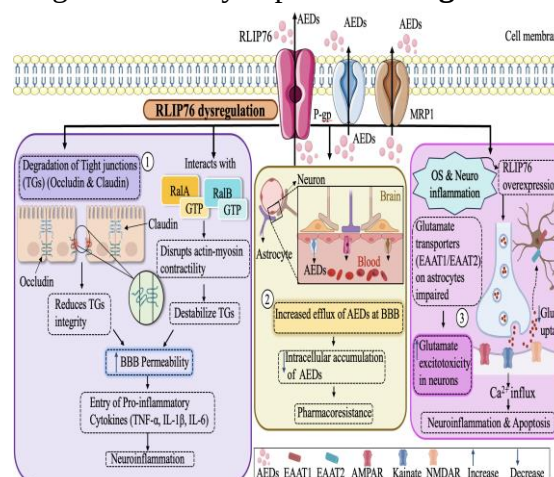


Fig 4: Role of RLIP76 in BBB dysfunction and drug resistance in epilepsy

RLIP76 as a potential biomarker for epilepsy

RLIP76 has been studied for its involvement in epilepsy, more notably drug resistance. High BBB endothelial cell expression of RLIP76 might be associated with lower AED bioavailability, inadequate seizure control, and increased doses or another drug (Ferraro *et al.*, 2006). Nevertheless, reduced RLIP76 expression may keep the AED in the brain to enhance seizure control. Hence, RLIP76 can act as a potential candidate for the prediction of AED response and individualization treatment selection (Kesavan *et al.*, 2011). The clinical relevance of RLIP76 as a biomarker consists of predicting response to AED, selection of drugs, and disease progression monitoring. Brain tissue or peripheral blood levels of RLIP76 can be quantified to evaluate response risk in typical AED treatment (Fricke-Galindo *et al.*, 2016). Clinicians must know that greater numbers of patients with higher RLIP76 expression are more susceptible to drug resistance and should initiate consideration of increased doses,

polytherapy, or other treatment modalities at the early stage of the disease, ideally before the treatment initiation (Alvarez *et al.*, 2007). The highly active patients would preferentially use AEDs less subject to the action of transporters such as levetiracetam and lacosamide, and if RLIP76 preferentially efflux drugs like phenytoin and carbamazepine (Panthan *et al.*, 2010). Biomarker-based selection of drugs would help control seizures and lower the number of inappropriate drug trials. Interpretation of serial measurement of RLIP76 expression may suggest how drug resistance changes over time and serve to monitor changes in drug resistance that may mandate treatment adjustments. Regular screening of RLIP76 levels in epileptic patients under AED treatment might be useful for optimal selection and dosage modification of AEDs. However, for life-threatening, severe, drug-resistant cases, epilepsy surgery (such as focal resection) may be considered. Measuring RLIP76 expression in resected brain tissue may be a valuable means to assess the post-surgical drug response to optimize postoperative AED doses (Cascino and Brinkmann 2021).

Therapeutic Implications of Targeting RLIP76

Researchers have focused on RLIP76 as an epilepsy drug target, especially for drug resistance mechanisms (Emich-Widera *et al.*, 2013). It acts as a transporter at the BBB, which moves out AEDs, including phenytoin and carbamazepine, from brain tissue, therefore promoting drug-resistant conditions and lowering intracellular concentrations. The collective evaluation of several studies that explore the function of RLIP76 in epilepsy, highlighting key findings and their associated limitations, is mentioned in **Table 1**. Experiments show that RLIP76 exists upon the luminal surface

of endothelial cells of the brain, particularly in regions that were removed from epileptic foci. Experimental assays confirmed that RLIP76 functions as an energy-dependent transport system for AEDs, and RLIP76-deficient mice responded more strongly to phenytoin treatment because of improper drug removal efficiency at the BBB (Singhal *et al.*, 2006). However, some recent research has produced findings that contradict RLIP76's involvement in epilepsy. Genetic studies have determined that RLIP76 genetic variations show no relationship to epilepsy vulnerability or AED medication effectiveness. The prediction models indicate that RLIP76 lacks both transmembrane behavior and ATPase activity, which are fundamental features of membrane-spanning transport proteins (Manguoglu *et al.*, 2011).

Further research must explore both drug resistance involvement and the therapeutic target potential of RLIP76. Despite these limitations, targeting RLIP76 is a promising avenue to enhance AED efficacy for resistant patients, who are typically non-responsive to conventional treatments (Kasperaviute and Sisodiya 2009). It may be feasible in promoting drug delivery across the BBB and elevating AED concentration in the CNS through RLIP76 activity (Edafiogho *et al.*, 2007). RLIP76 pharmacological inhibitors are promising as a treatment strategy to enhance treatment for epilepsy, especially in patients intolerant of standard AEDs. Small molecule RLIP76 inhibitors such as sunitinib, sorafenib, vincristine, doxorubicin, vinblastine, and colchicine offer an encouraging approach that enhances existing AEDs by inhibiting these AEDs from effluxing out of neuronal cells in various mechanisms (Singhal *et al.*, 2017). One of the methods is competitive inhibition, where inhibitors compete for

ARTICLE ID: BTS0326011

AED binding to RLIP76 sites and inhibit it from exporting these drugs out of the CNS. These inhibitors can be structured such that they can specifically inhibit the action of RLIP76 without affecting other essential transporters or cell functions (Kamel 2023). An increase in concentrations of AEDs in neuronal tissues can result in increased strength of their therapeutic effects. Allosteric inhibitors bind to sites located at the substrate binding sites of RLIP76 that cause conformational changes to reduce transport activity but will not compete with AEDs directly (Colabufo *et al.*, 2010). In addition, pharmacological agents downregulate leads to the degradation or internalization of RLIP76, resulting in a reduction of the cell surface and inhibiting its drug efflux functions (Singhal *et al.*, 2011).

Moreover, downregulation of RLIP76 in certain neuronal cells can be possible through using putatively revolutionary methods, such as RNA interference

together with clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology (Singhal *et al.*, 2021). They may increase the sensitivity of AED by reducing the capacity of the transporter for effluxing drugs from cells. Surgical removal of epileptic foci in non-responsive patients with elevated RLIP76 expression can be an option. Drug resistance patterns after surgery will assist in the analysis of RLIP76 expression in resected neuronal tissue. Synergistic interactions of combination therapy could be a target for therapeutic intervention for RLIP76. Conventional AEDs in combination with RLIP76 inhibitors could have synergistic action, particularly in the case of pharmacoresistance, since high levels of this transporter are observed. This strategy could be applicable for lower doses of AEDs without loss of efficacy (Boughrara and Chentouf 2022).

Table 1. Role of RLIP76 in Epilepsy

S.No	Type of study	Methods used	Major findings	References
1.	<i>In vivo</i> experimental study	Immunohistochemistry, Transport Assays, Knockout Mouse Model	RLIP76 is expressed in brain tissue, particularly on the luminal surface of endothelial cell membranes. Its expression is most prominent in BBB tissue from excised epileptic foci. It mediates the transport of AEDs like phenytoin and carbamazepine. RLIP76 knockout mice exhibited increased sensitivity to phenytoin, suggesting a role in drug resistance.	(Awasthi <i>et al.</i> , 2005)

ARTICLE ID: BTS0326011

2.	Genetic association study	Double-labeling fluorescent immunohistochemistry, population genetics, comparative protein structure modeling	Found no substantial evidence supporting RLIP76's role in drug resistance or epilepsy susceptibility. Immunoreactivity was cytoplasmic and did not localize with endothelial markers. No association was found between RLIP76 genetic variations and drug response phenotypes.	(Soranzo <i>et al.</i> , 2007)
3.	Experimental study	Immunohistochemistry, Western blot, pharmacokinetic assays	RLIP76 is the predominant transporter for phenytoin at the BBB, surpassing MDR1 (P-gp).	(Mancini <i>et al.</i> , 2005)
4.	Prospective cohort study	genotyping 23 common RLIP76 polymorphisms using PCR	There was also no effect in a subgroup of patients on carbamazepine, which is thought to be an RLIP76 substrate, although two polymorphisms were associated with time to first seizure.	(Leschziner <i>et al.</i> , 2007)
5.	Genetic Association Study	denaturing high-performance liquid chromatography (HPLC) and sequencing analysis techniques	RLIP76 gene variants were not associated with drug response in Turkish epilepsy patients.	(Manguoğlu <i>et al.</i> , 2011)
6.	<i>In vivo</i> experimental study	EEG, Immunohistochemistry, Western Blot,	CDDO-Me may ameliorate CA1 neuronal degeneration by accelerating RalBP1-mediated mitochondrial fission and 4-HNE efflux following SE suggesting that increased RalBP1 expression/activity may be one of the considerable targets to protect neurons from SE.	(Kim <i>et al.</i> , 2022)

7.	Observational cross-sectional pharmacogenetic study.	Genetic analysis	All studied alleles of the RALBP1 gene were present in the Mexican-Mestizo population. The allele and genotype frequencies of RALBP1 showed differences when compared to European, Asian, and African populations. Allele frequencies greater than 30% were observed in ten genes, including RALBP1.	(Fricke-Galindo <i>et al.</i> , 2016)
----	--	------------------	--	---------------------------------------

CONCLUSION AND FUTURE DIRECTIONS

This review concludes that due to its controls on neuronal functions and contributes to medication resistance, RLIP76 is important in epilepsy. Stress tolerance, clathrin-mediated endocytosis and exocytosis, neurotransmitter release, and redox balance are all processes that are necessary for preserving synaptic plasticity and neuronal health. These mechanisms frequently break down in epilepsy, increasing the risk of seizures. By facilitating glutathione conjugate transport, RLIP76 also lessens neuronal damage and oxidative stress. Research indicates that mice with RLIP76 deficiency are more prone to seizures, underscoring the potential for treatment. The CNS has high concentrations of RLIP76, particularly in neurons and at the blood-brain barrier. This makes it a potential biomarker for both acquired and hereditary causes of epilepsy. Targeting RLIP76 may enhance synaptic and cellular function, which could improve anti-epileptic drug (AED) responses, especially in drug-resistant instances. Its functions in drug transport, redox regulation, and cellular stress response imply a wider relevance in brain health,

notwithstanding the paucity of studies on RLIP76 in neurodegeneration. Its impact on synaptic transmission and brain excitability needs more research. Understanding the variety of AED response may be enhanced by looking into RLIP76 modulators and expression levels among patient groups. Combining RLIP76 with additional biomarkers could improve understanding of medication resistance and open the door to individualized care. In the end, RLIP76 targeting may make precision medicine approaches to epilepsy treatment possible.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Conflict of interest: No conflicts of interest to declare.

CRedit authorship contribution statement: **PP:** Writing – review & editing the manuscript. **KRA:** Conceptualization, review, editing, visualization.

Declaration of Competing Interest: The authors assert that they do not have any known financial interests or personal relationships that could be perceived as influencing the work reported in this paper.

Funding: There is no funding agency in this article.

Data availability: Not applicable.

Acknowledgements: Special thanks to Shri. Parveen Garg, Chairman, ISFCP, for providing this research platform.

REFERENCES

1. Ademuwagun IA, Rotimi SO, Syrbe S, Ajamma YU, Adebisi E. Voltage-gated sodium channel genes in epilepsy: mutations, functional studies, and treatment dimensions. *Frontiers in neurology*. 2021 Mar 24;12:600050. <https://doi.org/10.3389/fneur.2021.600050>
2. Aguiar CC, Almeida AB, Araújo PV, Abreu RN, Chaves EM, Vale OC, Macêdo DS, Woods DJ, Fonteles MM, Vasconcelos SM. Oxidative stress and epilepsy: literature review. *Oxidative medicine and cellular longevity*. 2012;2012(1):795259. <https://doi.org/10.1155/2012/795259>
3. Álvarez JC, Castro PJ, Fernández JM. Clinical implications of mechanisms of resistance to antiepileptic drugs. *The Neurologist*. 2007 Nov 1;13(6):S38-46. <https://doi.org/10.1097/NRL.0b013e31815bb403>
4. Awasthi S, Hindle A, Sawant NA, George M, Vijayan M, Kshirsagar S, Morton H, Bunquin LE, Palade PT, Lawrence JJ, Khan H. RALBP1 in oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Cells*. 2021 Nov 10;10(11):3113. <https://doi.org/10.3390/cells10113113>
5. Awasthi S, Sharma R, Yang Y, Singhal S, Pikula S, Bendorowicz-Pikula J, Singh S, Zimniak P, Awasthi Y. Transport functions and physiological significance of 76 kDa Ral-binding GTPase activating protein (RLIP76). *Acta Biochimica Polonica*. 2002;49(4):855-67. <https://doi.org/10.18388/abp.2002.3745>
6. Awasthi YC, Sharma R, Yadav S, Dwivedi S, Sharma A, Awasthi S. The non-ABC drug transporter RLIP76 (RALBP-1) plays a major role in the mechanisms of drug resistance. *Current drug metabolism*. 2007 May 1;8(4):315-23. <https://doi.org/10.2174/138920007780655414>
7. Bachi A, Dalle-Donne I, Scaloni A. Redox proteomics: chemical principles, methodological approaches and biological/biomedical promises. *Chemical reviews*. 2013 Jan 9;113(1):596-698. <https://doi.org/10.1021/cr300073p>
8. Bäckström F, Ahl M, Wickham J, Ekdahl CT. Reduced epilepsy development in synapsin 2 knockout mice with autistic behavior following early systemic treatment with interleukin-6 receptor antibody. *Epilepsy Research*. 2023 Mar 1;191:107114. <https://doi.org/10.1016/j.eplepsyres.2023.107114>
9. Bajalia EM, Azzouz FB, Chism DA, Giansiracusa DM, Wong CG, Plaskett KN, Bishayee A. Phytochemicals for the prevention and treatment of renal cell carcinoma: preclinical and clinical evidence and molecular

- mechanisms. *Cancers*. 2022 Jul 4;14(13):3278.
<https://doi.org/10.3390/cancers14133278>
10. Borović Šunjić S, Jaganjac M, Vlainić J, Halasz M, Žarković N. Lipid peroxidation-related redox signaling in osteosarcoma. *International journal of molecular sciences*. 2024 Apr 22;25(8):4559.
<https://doi.org/10.3390/ijms25084559>
 11. Boughrara W, Chentouf A. The ABCB1, ABCC2 and RALBP1 polymorphisms are associated with carbamazepine response in epileptic patient: a systematic review. *Acta Neurologica Belgica*. 2022 Aug;122(4):871-80.
<https://doi.org/10.1007/s13760-022-01920-5>
 12. Cantor SB, Urano T, Feig LA. Identification and characterization of Ral-binding protein 1, a potential downstream target of Ral GTPases. *Molecular and cellular biology*. 1995 Aug 1;15(8):4578-84.
<https://doi.org/10.1128/MCB.15.8.4578>
 13. Cárdenas-Rodríguez N, Carmona-Aparicio L, Pérez-Lozano DL, Ortega-Cuellar D, Gómez-Manzo S, Ignacio-Mejía I. Genetic variations associated with pharmacoresistant epilepsy. *Molecular medicine reports*. 2020 Apr;21(4):1685-701.
<https://doi.org/10.3892/mmr.2020.10999>
 14. Cascino GD, Brinkmann BH. Advances in the surgical management of epilepsy: drug-resistant focal epilepsy in the adult patient. *Neurologic clinics*. 2021 Feb 1;39(1):181-96.
<https://doi.org/10.1016/j.ncl.2020.09.010>
 15. Colabufo NA, Berardi F, Cantore M, Contino M, Inglese C, Niso M, Perrone R. Perspectives of P-glycoprotein modulating agents in oncology and neurodegenerative diseases: pharmaceutical, biological, and diagnostic potentials. *Journal of medicinal chemistry*. 2010 Mar 11;53(5):1883-97.
<https://doi.org/10.1021/jm900743c>
 16. Coon BG, Burgner J, Camonis JH, Aguilar RC. The epsin family of endocytic adaptors promotes fibrosarcoma migration and invasion. *Journal of biological chemistry*. 2010 Oct 22;285(43):33073-81.
<https://doi.org/10.1074/jbc.m110.124123>
 17. Cornish J, Owen D, Mott HR. RLIP76: A structural and functional triumvirate. *Cancers*. 2021 May 4;13(9):2206.
<https://doi.org/10.3390/cancers13092206>
 18. Dalleau S, Baradat M, Guéraud F, Huc L. Cell death and diseases related to oxidative stress: 4-hydroxynonenal (HNE) in the balance. *Cell Death & Differentiation*. 2013 Dec;20(12):1615-30.
<https://doi.org/10.1038/cdd.2013.138>
 19. Das A, Balan S, Banerjee M, Radhakrishnan K. Drug resistance in epilepsy and the ABCB1 gene: The clinical perspective. *Indian journal of human genetics*. 2011 May;17(Suppl

- 1):S12. <https://doi.org/10.4103/0971-6866.80353>
20. Delgado ME. *Determining the contributions of caspase-2 and caspase-8 to intracellular protease activities during apoptosis* (Doctoral dissertation, Royal College of Surgeons in Ireland). <https://doi.org/10.25419/rcsi.10809404.v1>
21. Depondt C, Shorvon SD. Genetic association studies in epilepsy pharmacogenomics: lessons learnt and potential applications. *Pharmacogenomics*. 2006 Jul 1;7(5):731-45. <https://doi.org/10.2217/14622416.7.5.731>
22. Dratkiewicz E, Simiczyjew A, Mazurkiewicz J, Ziętek M, Matkowski R, Nowak D. Hypoxia and extracellular acidification as drivers of melanoma progression and drug resistance. *Cells*. 2021 Apr 9;10(4):862. <https://doi.org/10.3390/cells10040862>
23. Duncan J, Sander J, Alim-Marvasti A, Balestrini S, Baxendale S, Bindman D, Chinthapalli K, Chowdhury F, Diehl B, Eriksson S, Foong J. *Epilepsy and Related Disorders*. *Neurology: A Queen Square Textbook*. 2024 May 13:247-318. <https://doi.org/10.1002/9781119715672.ch10>
24. Edafiogho IO, Kombian SB, Ananthalakshmi KV, Salama NN, Eddington ND, Wilson TL, Alexander MS, Jackson PL, Hanson CD, Scott KR. Enaminones: Exploring additional therapeutic activities. *Journal of pharmaceutical sciences*. 2007 Oct 1;96(10):2509-31. <https://doi.org/10.1002/jps.20967>
25. Emich-Widera E, Likus W, Kazek B, Niemiec P, Balcerzyk A, Sieroń AL, Żak I. CYP3A5* 3 and C3435T MDR1 polymorphisms in prognostication of drug-resistant epilepsy in children and adolescents. *BioMed research international*. 2013;2013(1):526837. <https://doi.org/10.1155/2013/526837>
26. Fan SY, Jiang JD, Qian J, Lu YC, Hu GH, Luo C, Hou WD, Wang Q. Overexpression of RLIP76 required for proliferation in meningioma is associated with recurrence. *PloS one*. 2015 May 20;10(5):e0125661. <https://doi.org/10.1371/journal.pone.0125661>
27. Fernandes C, Soni U, Patravale V. Nano-interventions for neurodegenerative disorders. *Pharmacological research*. 2010 Aug 1;62(2):166-78. <https://doi.org/10.1016/j.phrs.2010.02.004>
28. Ferraro TN, Dlugos DJ, Buono RJ. Challenges and opportunities in the application of pharmacogenetics to antiepileptic drug therapy. *Pharmacogenomics*. 2006 Jan 1;7(1):89-103. <https://doi.org/10.2217/14622416.7.1.89>
29. Franco R, Schoneveld OJ, Pappa A, Panayiotidis MI. The central role of glutathione in the pathophysiology of human diseases. *Archives of physiology and biochemistry*. 2007 Jan 1;113(4-5):234-58. <https://doi.org/10.1080/13813450701661198>

ARTICLE ID: BTS0326011

30. Freibauer A, Wohlleben M, Boelman C. STXBP1-Related Disorders: Clinical Presentation, Molecular Function, Treatment, and Future Directions. *Genes*. 2023 Dec 5;14(12):2179. <https://doi.org/10.3390/genes14122179>
31. Fricke-Galindo I, Ortega-Vázquez A, Monroy-Jaramillo N, Dorado P, Jung-Cook H, Peñas-Lledó E, LLerena A, López-López M. Allele and genotype frequencies of genes relevant to anti-epileptic drug therapy in Mexican-Mestizo healthy volunteers. *Pharmacogenomics*. 2016 Nov 1;17(17):1913-30. <https://doi.org/10.2217/pgs-2016-0078>
32. Fricke-Galindo I, Ortega-Vázquez A, Monroy-Jaramillo N, Dorado P, Jung-Cook H, Peñas-Lledó E, LLerena A, López-López M. Allele and genotype frequencies of genes relevant to anti-epileptic drug therapy in Mexican-Mestizo healthy volunteers. *Pharmacogenomics*. 2016 Nov 1;17(17):1913-30. <https://doi.org/10.2217/pgs-2016-0078>
33. Ganta S, Deshpande D, Korde A, Amiji M. A review of multifunctional nanoemulsion systems to overcome oral and CNS drug delivery barriers. *Molecular membrane biology*. 2010 Oct 1;27(7):260-73. <https://doi.org/10.3109/09687688.2010.497971>
34. Gasparovic AC, Milkovic L, Sunjic SB, Zarkovic N. Cancer growth regulation by 4-hydroxynonenal. *Free Radical Biology and Medicine*. 2017 Oct 1;111:226-34. <https://doi.org/10.1016/j.freeradbiomed.2017.01.030>
35. Goldfinger LE, Lee S. Emerging treatments in lung cancer—targeting the RLIP76 molecular transporter. *Lung Cancer: Targets and Therapy*. 2013 Nov 4:61-9. <https://doi.org/10.2147/LCTT.S53672>
36. Gómez-Crisóstomo NP, López-Marure R, Zapata E, Zazueta C, Martínez-Abundis E. Bax induces cytochrome c release by multiple mechanisms in mitochondria from MCF7 cells. *Journal of bioenergetics and biomembranes*. 2013 Oct;45:441-8. <https://doi.org/10.1007/s10863-013-9508-x>
37. Gourmaud S, Stewart DA, Irwin DJ, Roberts N, Barbour AJ, Eberwine G, O'Brien WT, Vassar R, Talos DM, Jensen FE. The role of mTORC1 activation in seizure-induced exacerbation of Alzheimer's disease. *Brain*. 2022 Jan 1;145(1):324-39. <https://doi.org/10.1093/brain/awab268>
38. Henshall DC, Bonislawski DP, Skradski SL, Araki T, Lan JQ, Schindler CK, Meller R, Simon RP. Formation of the Apaf-1/cytochrome c complex precedes activation of caspase-9 during seizure-induced neuronal death. *Cell Death & Differentiation*. 2001 Dec;8(12):1169-81. <https://doi.org/10.1038/sj.cdd.4400921>
39. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. *Journal of Cerebral Blood Flow & Metabolism*. 2005

ARTICLE ID: BTS0326011

- Dec;25(12):1557-72.
<https://doi.org/10.1038/sj.jcbfm.9600149>
40. Hranac M (2013). *Effects of Impaired Glucose Tolerance on Steady-State Expression Levels of Murine Cytohesin 2 and Cytohesin 3 mRNA in Liver* (Doctoral dissertation, University of Zurich).
https://doi.org/10.1007/164_2020_406
 41. Jaramillo NM, Galindo IF, Vázquez AO, Cook HJ, LLerena A, López ML. Pharmacogenetic potential biomarkers for carbamazepine adverse drug reactions and clinical response. *Drug metabolism and drug interactions*. 2014 Jun 1;29(2):67-79.
<https://doi.org/10.1515/dmdi-2013-0046>
 42. Jaworski T. Control of neuronal excitability by GSK-3beta: Epilepsy and beyond. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2020 Sep 1;1867(9):118745.
<https://doi.org/10.1016/j.bbamcr.2020.118745>
 43. Jullien-Flores V, Dorseuil O, Romero F, Letourneur F, Saragosti S, Berger R, Tavitian A, Gacon G, Camonis JH. Bridging Ral GTPase to Rho Pathways: RLIP76, A Ral effector with cdc42/RAC GTPase-activating protein activity(*). *Journal of Biological Chemistry*. 1995 Sep 22;270(38):22473-7.
<https://doi.org/10.1074/jbc.270.38.22473>
 44. Kamel FO. Drug-Resistant Epilepsy; An Overview on Management and Treatment. *International Journal of Pharmaceutical Research and Allied Sciences*. 2023;12(4-2023):76-90.
<https://doi.org/10.51847/d3wwhHbZsW>
 45. Kasperavičiūtė D, Sisodiya SM. Epilepsy pharmacogenetics. *Pharmacogenomics*. 2009 May 1;10(5):817-36.
<https://doi.org/10.2217/pgs.09.34>
 46. Kesavan R, Kukreti R, Adithan C. Genetic polymorphism of drug refractory epilepsy. *Indian Journal of Medical Research*. 2011 Sep 1;134(3):253-5.
<https://doi.org/10.1111/j.1528-1157.1999.tb05566.x>
 47. Khatri N, Man HY. Synaptic activity and bioenergy homeostasis: implications in brain trauma and neurodegenerative diseases. *Frontiers in neurology*. 2013 Dec 11;4:199.
<https://doi.org/10.3389/fneur.2013.00199>
 48. Kim JE, Lee DS, Kim TH, Kang TC. CDDO-Me Attenuates CA1 Neuronal Death by Facilitating RalBP1-Mediated Mitochondrial Fission and 4-HNE Efflux in the Rat Hippocampus Following Status Epilepticus. *Antioxidants*. 2022 May 18;11(5):985.
<https://doi.org/10.3390/antiox11050985>
 49. LaSarge CL, Danzer SC. Mechanisms regulating neuronal excitability and seizure development following mTOR pathway hyperactivation. *Frontiers in molecular neuroscience*. 2014 Mar 14;7:18.
<https://doi.org/10.3389/fnmol.2014.00018>
 50. Leguia MG, Andrzejak RG, Rummel C, Fan JM, Mirro EA,

ARTICLE ID: BTS0326011

- Tcheng TK, Rao VR, Baud MO. Seizure cycles in focal epilepsy. *JAMA neurology*. 2021 Apr 1;78(4):454-63.
<https://doi.org/10.1001/jamaneurol.2020.5370>
51. Leschziner GD, Jorgensen AL, Andrew T, Williamson PR, Marson AG, Coffey AJ, Middleditch C, Balding DJ, Rogers J, Bentley DR, Chadwick D. The association between polymorphisms in RLIP76 and drug response in epilepsy. *Pharmacogenomics*. 2007 Dec 1;8(12):1715-22.
<https://doi.org/10.2217/14622416.8.12.1715>
 52. Li Y, Zhao T, Li J, Xia M, Li Y, Wang X, Liu C, Zheng T, Chen R, Kan D, Xie Y. Oxidative stress and 4-hydroxy-2-nonenal (4-HNE): Implications in the pathogenesis and treatment of aging-related diseases. *Journal of immunology research*. 2022;2022(1):2233906.
<https://doi.org/10.1155/2022/2233906>
 53. Liu X, Yu Y, Wang Q. Dynamic epileptic seizure propagation based on multiscale synaptic plasticity. *Nonlinear Dynamics*. 2024 Nov 19:1-5.
<https://doi.org/10.1007/s11071-024-10590-2>
 54. Makrides V, Dolgodilina E, Virgintino D. Blood-Brain Barrier Transporters. *The Blood Brain Barrier and Inflammation*. 2017 Mar 29:103.
https://doi.org/10.1007/978-3-319-45514-3_6
 55. Manguoğlu E, Akdeniz S, Dündar NO, Duman ÖZ, Aktekin B, Haspolat ŞE, Bilge UĞ, Özel D, Lüleci G. RLIP76 gene variants are not associated with drug response in turkish epilepsy patients. *Balkan Journal of Medical Genetics: BJMG*. 2011 Jul 25;14(1):25.
<https://doi.org/10.2478/v10034-011-0014-3>
 56. Manole AM, Sirbu CA, Mititelu MR, Vasiliu O, Lorusso L, Sirbu OM, Ionita Radu F. State of the art and challenges in epilepsy—a narrative review. *Journal of Personalized Medicine*. 2023 Apr 1;13(4):623.
<https://doi.org/10.3390/jpm13040623>
 57. Mattson MP. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. *Experimental gerontology*. 2009 Oct 1;44(10):625-33.
<https://doi.org/10.1016/j.exger.2009.07.003>
 58. McKarns SC. A Review of Neuroreceptors for Clinical and Experimental Neuropharmacology in Central Nervous System Disorders. *Current Reviews in Clinical and Experimental Pharmacology Formerly Current Clinical Pharmacology*. 2023 Nov 1;18(3):192-241.
<https://doi.org/10.2174/2772432817666220301104118>
 59. Meda S, Singh S, Palade P, Tonk S, Awasthi S. Oxidative stress in intensive care unit patients: A review of glutathione linked metabolism and lipid peroxidation. *The Southwest Respiratory and Critical Care Chronicles*. 2019 Jan 18;7(27):7-35.

- <https://doi.org/10.12746/swrccc.v7i27.511>
60. Mele M, Costa RO, Duarte CB. Alterations in GABAA-receptor trafficking and synaptic dysfunction in brain disorders. *Frontiers in cellular neuroscience*. 2019 Mar 7;13:77. <https://doi.org/10.3389/fncel.2019.00077>
 61. Milligan TA. Epilepsy: a clinical overview. *The American Journal of Medicine*. 2021 Jul 1;134(7):840-7. <https://doi.org/10.1016/j.amjmed.2021.01.038>
 62. Neel NF, Rossman KL, Martin TD, Hayes TK, Yeh JJ, Der CJ. The RalB small GTPase mediates formation of invadopodia through a GTPase-activating protein-independent function of the RalBP1/RLIP76 effector. *Molecular and cellular biology*. 2012 Apr 1. <https://doi.org/10.1128/MCB.06291-11>
 63. Niftullayev S, Lamarche-Vane N. Regulators of Rho GTPases in the nervous system: molecular implication in axon guidance and neurological disorders. *International journal of molecular sciences*. 2019 Mar 25;20(6):1497. <https://doi.org/10.3390/ijms20061497>
 64. Patel M. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. *Free Radical Biology and Medicine*. 2004 Dec 15;37(12):1951-62. <https://doi.org/10.1016/j.freeradbiomed.2004.08.021>
 65. Pathan SA, Jain GK, Akhter S, Vohora D, Ahmad FJ, Khar RK. Insights into the novel three 'D's of epilepsy treatment: drugs, delivery systems and devices. *Drug discovery today*. 2010 Sep 1;15(17-18):717-32. <https://doi.org/10.1016/j.drudis.2010.06.014>
 66. Poli G, Schaur RJ, Siems WA, Leonarduzzi G. 4-Hydroxynonenal: A membrane lipid oxidation product of medicinal interest. *Medicinal research reviews*. 2008 Jul;28(4):569-631. <https://doi.org/10.1002/med.20117>
 67. Poliquin S, Kang JQ. Disruption of the ubiquitin-proteasome system and elevated endoplasmic reticulum stress in epilepsy. *Biomedicines*. 2022 Mar 11;10(3):647. <https://doi.org/10.3390/biomedicines10030647>
 68. Raga S, Rheims S, Specchio N, Wilmshurst JM. Electroclinical markers to differentiate between focal and generalized epilepsies. *Epileptic Disorders*. 2021(3):437-58. <https://doi.org/10.1684/epd.2021.1291>
 69. Rajasekar KV, Campbell LJ, Nietlispach D, Owen D, Mott HR. The structure of the RLIP76 RhoGAP-Ral binding domain dyad: fixed position of the domains leads to dual engagement of small G proteins at the membrane. *Structure*. 2013 Dec 3;21(12):2131-42. <https://doi.org/10.1016/j.str.2013.09.007>
 70. Reddy PH, Kshirsagar S, Bose C, Pradeepkiran JA, Hindle A, Singh SP, Reddy AP. Rlip overexpression reduces oxidative stress and mitochondrial dysfunction in Alzheimer's disease: Mechanistic insights. *Biochimica et Biophysica*

ARTICLE ID: BTS0326011

- Acta (BBA)-Molecular Basis of Disease. 2023 Oct 1;1869(7):166759. <https://doi.org/10.1016/j.bbadis.2023.166759>
71. Reddy PH, Kshirsagar S, Bose C, Pradeepkiran JA, Hindle A, Singh SP, Reddy AP, Baig J. Rlip reduction induces oxidative stress and mitochondrial dysfunction in mutant tau-expressed immortalized hippocampal neurons: Mechanistic insights. Cells. 2023 Jun 16;12(12):1646. <https://doi.org/10.3390/cells12121646>
 72. Reed JC. Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. Cell death & differentiation. 2006 Aug;13(8):1378-86. <https://doi.org/10.1038/sj.cdd.4401975>
 73. Rheims S, Perucca E, Cucherat M, Ryvlin P. Factors determining response to antiepileptic drugs in randomized controlled trials. A systematic review and meta-analysis. Epilepsia. 2011 Feb;52(2):219-33. <https://doi.org/10.1111/j.1528-1167.2010.02915.x>
 74. Rho JM, Boison D. The metabolic basis of epilepsy. Nature Reviews Neurology. 2022 Jun;18(6):333-47. <https://doi.org/10.1038/s41582-022-00651-8>
 75. Sagai M, Bocci V. Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress?. Medical gas research. 2011 Dec;1:1-8. <https://doi.org/10.1186/2045-9912-1-29>
 76. Sanz P, Garcia-Gimeno MA. Reactive glia inflammatory signaling pathways and epilepsy. International journal of molecular sciences. 2020 Jun 8;21(11):4096. <https://doi.org/10.3390/ijms21114096>
 77. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nature reviews Molecular cell biology. 2019 Mar;20(3):175-93. <https://doi.org/10.1038/s41580-018-0089-8>
 78. Singhal J, Chikara S, Horne D, Awasthi S, Salgia R, Singhal SS. Targeting RLIP with CRISPR/Cas9 controls tumor growth. Carcinogenesis. 2021 Jan 1;42(1):48-57. <https://doi.org/10.1093/carcin/bgaa048>
 79. Singhal J, Yadav S, Nagaprashantha LD, Vatsyayan R, Singhal SS, Awasthi S. Targeting p53-null neuroblastomas through RLIP76. Cancer prevention research. 2011 Jun 1;4(6):879-89. <https://doi.org/10.1158/1940-6207.CAPR-11-0025>
 80. Singhal SS, Figarola J, Singhal J, Reddy MA, Liu X, Berz D, Natarajan R, Awasthi S. RLIP76 protein knockdown attenuates obesity due to a high-fat diet. Journal of Biological Chemistry. 2013 Aug 9;288(32):23394-406. <https://doi.org/10.1074/jbc.m113.480194>
 81. Singhal SS, Garg R, Horne D, Singhal S, Awasthi S, Salgia R. RLIP: A necessary transporter

- protein for translating oxidative stress into pro-obesity and pro-carcinogenic signaling. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2022 Sep 1;1877(5):188803.
<https://doi.org/10.1016/j.bbcan.2022.188803>
82. Singhal SS, Nagaprashantha L, Singhal P, Singhal S, Singhal J, Awasthi S, Horne D. RLIP76 inhibition: A promising developmental therapy for neuroblastoma. *Pharmaceutical Research*. 2017 Aug;34:1673-82.
<https://doi.org/10.1007/s11095-017-2154-y>
 83. Singhal SS, Wickramarachchi D, Yadav S, Singhal J, Leake K, Vatsyayan R, Chaudhary P, Lelsani P, Suzuki S, Yang S, Awasthi YC. Glutathione-conjugate transport by RLIP76 is required for clathrin-dependent endocytosis and chemical carcinogenesis. *Molecular cancer therapeutics*. 2011 Jan 1;10(1):16-28.
<https://doi.org/10.1158/1535-7163.MCT-10-0699>
 84. Singhal SS, Yadav S, Singhal J, Awasthi YC, Awasthi S. Mitogenic and drug-resistance mediating effects of PKC α require RLIP76. *Biochemical and biophysical research communications*. 2006 Sep 22;348(2):722-7.
<https://doi.org/10.1016/j.bbrc.2006.07.118>
 85. Smolarz B, Makowska M, Romanowicz H. Pharmacogenetics of drug-resistant epilepsy (review of literature). *International journal of molecular sciences*. 2021 Oct 28;22(21):11696.
<https://doi.org/10.3390/ijms222111696>
 86. Soranzo N, Goldstein DB, Sisodiya SM. The role of common variation in drug transporter genes in refractory epilepsy. *Expert Opinion on Pharmacotherapy*. 2005 Jul 1;6(8):1305-12.
<https://doi.org/10.1517/14656566.6.8.1305>
 87. Sorokin A. Nitric oxide synthase and cyclooxygenase pathways: a complex interplay in cellular signaling. *Current Medicinal Chemistry*. 2016 Jul 1;23(24):2559-78.
<https://doi.org/10.2174/0929867323666160729105312>
 88. Steinlein OK. Ion channel mutations in neuronal diseases: a genetics perspective. *Chemical Reviews*. 2012 Dec 12;112(12):6334-52.
<https://doi.org/10.1021/cr300044d>
 89. Sumadewi KT, Harkitasari S, Tjandra DC. Biomolecular mechanisms of epileptic seizures and epilepsy: a review. *Acta Epileptologica*. 2023 Nov 15;5(1):28.
<https://doi.org/10.1186/s42494-023-00137-0>
 90. Uddin MB, Wang Z, Yang C. The m6A RNA methylation regulates oncogenic signaling pathways driving cell malignant transformation and carcinogenesis. *Molecular cancer*. 2021 Apr 4;20(1):61.
<https://doi.org/10.1186/s12943-021-01356-0>
 91. Vasic V, Jones MS, Haslinger D, Knaus LS, Schmeisser MJ, Novarino G, Chiocchetti AG.

- Translating the role of mTOR-and RAS-associated signalopathies in autism spectrum disorder: models, mechanisms and treatment. *Genes*. 2021 Oct 30;12(11):1746. <https://doi.org/10.3390/genes12111746>
92. Waldbaum S, Patel M. Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy?. *Journal of bioenergetics and biomembranes*. 2010 Dec;42:449-55. <https://doi.org/10.1007/s10863-010-9320-9>
 93. Wang Q, Wang JY, Zhang XP, Lv ZW, Fu D, Lu YC, Hu GH, Luo C, Chen JX. RLIP76 is overexpressed in human glioblastomas and is required for proliferation, tumorigenesis and suppression of apoptosis. *Carcinogenesis*. 2013 Apr 1;34(4):916-26. <https://doi.org/10.1093/carcin/bgs401>
 94. Wiśniewska W, Kopka M, Siemiątkowska K, Fudalej MM, Sobiborowicz A, Badowska-Kozakiewicz AM. The complexity of tumour angiogenesis based on recently described molecules. *Contemporary Oncology/Współczesna Onkologia*. 2021 Apr 15;25(1):33-44. <https://doi.org/10.5114/wo.2021.105075>
 95. Yamaguchi T, Okatsu K, Kubota M, Mitsumori A, Yamagata A, Fukata Y, Fukata M, Shibata M, Fukai S. Structural insights into heterohexameric assembly of epilepsy-related ligand–receptor complex LGI1–ADAM22. *bioRxiv*. 2025:2025-01.
 96. Zhang C, Cai Z, Liang Q, Wang Q, Lu Y, Hu L, Hu G. RLIP76 depletion enhances autophagic flux in U251 cells. *Cellular and molecular neurobiology*. 2017 Apr;37:555-62. <https://doi.org/10.1007/s10571-016-0410-z>
 97. Zhong H, Yin H. Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria. *Redox biology*. 2015 Apr 1;4:193-9. <https://doi.org/10.1016/j.redox.2014.12.011>