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SYSTEMS BIOLOGY AND NETWORK-BASED PATHWAY ANALYSIS OF LENNOX–GASTAUT SYNDROME USING FUNCTIONAL ENRICHMENT TOOLS

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Abstract:

Lennox–Gastaut syndrome is a serious developmental and epileptic encephalopathy marked by diverse seizure types, a characteristic slow spike-and-wave EEG pattern, and progressive cognitive impairment. LGS is associated with many genes and the functional relationship between these genes and biological pathway contribute to disease progression. This study utilizes a systems biology strategy to gain insight into the molecular processes involved in the disease. Gene disease association was collected from DisGeNET and analyzed using STRING and GeneMANIA to find functional interactions and network connections. Enrichment analysis was performed using Enrichr and mapped pathways through Reactome to understand the biological processes linked to the selected gene set. DisGeNET identified high-confidence genes like SCN1A, SCN2A, CACNA1A, GABRB3, DNM1, CHD2, POLG and CUX2 which have strong evidence linking them to seizure related neurodevelopmental disorders. STRING revealed a significantly enriched protein-protein interaction network (p < 1.0e−16) showing that there is meaningful biological connectivity. The enrichment and Reactome analyses consistently connected the genes to synaptic signaling, ion channel function and GABAergic neurotransmission pathways. The findings indicate that LGS results from overlapping disruptions in neuronal excitability and synaptic communication, highlighting the need for precise medicine strategies focused on these pathways.

Keywords: *Lennox- Gastaut Syndrome, DisGeNET, STRING, Epileptic Encephalopathy, Systems Biology.*

Introduction

Lennox- Gastaut syndrome (LGS) is one of the severe forms of developmental and epileptic encephalopathy identified as multiple seizure type, a characteristic EEG pattern and cognitive disability (1). As of 2022 definition, the EEG has been described as a slow spike and wave pattern of <3 Hz to <2.5 Hz (2). Considering epidemiology, the incidence rate is ~2.2- 6.5 per 100,000 while the prevalence rate is ~1.2- 6.5 per 100,000 people (3). Onset typically occurs in early childhood, with symptoms frequently extending into later stages of life. Developmental disability and cognitive decline may exist before onset of seizure and can continue to aggravate even after complete seizure control (4). Medical practitioners and caregivers noted that learning, verbal and non-verbal communication and developmental delay are severely affected in these cases (5). As the disease progresses, certain neurological observations like ataxia, movement impairment and behavioral disturbance can arise (6).

Resistant to drugs, poly therapy and adjusting treatment is common in patients prescribed with two or more anti-seizure medications (7). Here, rather than focusing on complete control over seizures, the main objective of the treatment is to minimize the frequency of seizures and thereby increase the quality of life (8). New treatments include Epidyolex which contains cannabidiol and is used as adjunct therapy to treat seizures related to LGS (9). In a study by (10) , dexamethasone was found efficient against refractory epileptic encephalopathy characterized as continuous spike and wave patterns during sleep. Other medications for LGS including valproic acid are frequently used off label (11). Usually the 'start low, go slow' approach is chosen to reduce the risk of side effects and improve patient adherence (12).

Genetic studies over the past years have changed the perception of LGS from a symptomatic syndrome to a heterogeneous condition with multiple genetic contributions. According to (13) , ion channel coding genes have strongly linked to many epilepsy subtypes like epileptic encephalopathy, generalized and focal epilepsy whereas other discovered genes are specific to different epilepsy and showcase their genetic basis for different types of epilepsy. Particularly genes like CACNA1A, CHD2, IQSEC2 and SCN10A shows association with LGS and DNAJC5, FRRS1L, SHANK3, SYN1, SYN2 genes are related to epilepsy (14).

The absence of pathway level insight limits the precision medicine approaches and continues the trial and error treatment strategies. At a research level, it slows the identification of disease modifying mechanisms. Though the multi-model modelling approaches are developing at a faster pace, their clinical and research oriented applications are still at an elementary stage (15).

Recent advances in bioinformatics and systems biology offer a way forward. In silico approaches allow for the integration of genetic data with gene ontology, pathway enrichment analysis, and protein - protein interaction networks enabling researchers to move from single gene interpretations toward a broader view of disease biology.

Materials and Methods

Retrieval of gene disease association data using DisGeNET

Gene disease association data for Lennox–Gastaut syndrome (LGS) was retrieved from DisGeNET database in December 2025. The database curates data from expert sources like UniProt, CTD and PsyGeNET (16). This database includes information on various human diseases along with traits and phenotypes that appear as part of disease or effect of drug (17). No manual filtering based on DisGeNET score was applied; all available gene associations were included. The highest gene–disease association score observed in the dataset was 0.75.

STRING analysis

The selected genes were then analysed using STRING which has a collection of physical and functional protein-protein interactions (18). The organism selected was *Homo sapiens*. Default parameters were used for obtaining the network. Observed interaction scores reached up to 0.75, indicating high-confidence associations among several proteins.

Prediction of gene interaction and association using GeneMANIA

GeneMANIA was used to explore their genetic association and predict additional genes linked through co - expression and shared pathways. GeneMANIA identifies genes that are strongly connected in the network (19).

Functional enrichment analysis using Enrichr

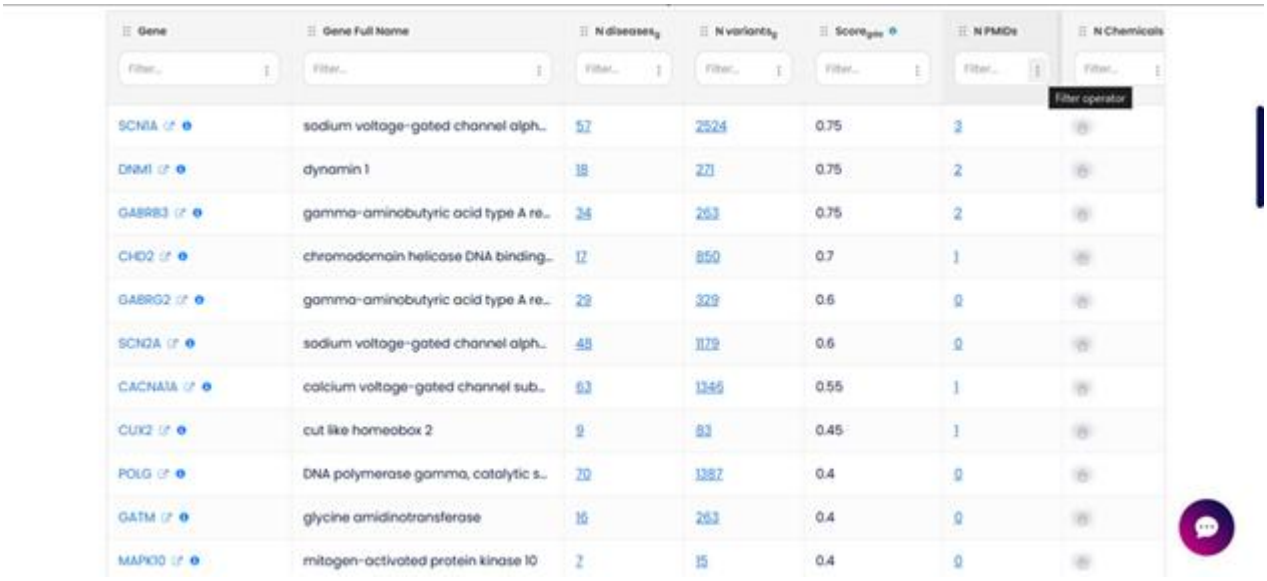
The curated gene set was subjected to enrichment analysis using Enrichr in order to identify the biological processes and cellular pathways. Enrichment was performed for multiple pathways and a transcription database under default parameters. No p-value cutoff was applied and the result was thoroughly visualized using Clustergram.

Reactome pathway analysis

Reactome pathway analysis was performed to map the genes to disease-related pathways. Analysis was conducted using *Homo sapiens* as a reference organism. Result interpretation of the molecular mechanisms contributing to LGS was done without manual adjustments.

Results

DisGeNET



Gene	Gene Full Name	N diseases	N variants	Score	N PMIDs	N Chemicals
SCN1A	sodium voltage-gated channel alph...	57	2524	0.75	3	
DNM1	dynamin 1	18	271	0.75	2	
GABRB3	gamma-aminobutyric acid type A re...	34	263	0.75	2	
CHD2	chromodomain helicase DNA binding...	17	850	0.7	1	
GABRG2	gamma-aminobutyric acid type A re...	29	329	0.6	0	
SCN2A	sodium voltage-gated channel alph...	48	1179	0.6	0	
CACNA1A	calcium voltage-gated channel sub...	63	1346	0.55	1	
CUX2	cut like homeobox 2	9	83	0.45	1	
POLG	DNA polymerase gamma, catalytic s...	70	1387	0.4	0	
GATM	glycine amidinotransferase	16	263	0.4	0	
MAPK10	mitogen-activated protein kinase 10	7	15	0.4	0	

Figure 1: DisGeNET derived gene disease association results showing high confidence LGS genes

The standardized MeSH term was used to search genes associated with LGS on DisGeNET and a structured and curated evidence for genes associated with Lennox Gastaut syndrome (LGS). This provided strongly connected genes on the basis of their gene-disease association score. Highest score obtained was 0.75 which is a part of default database output and not applied cutoff score. The genes obtained at highest confidence of 0.75 were SCN1A, GABRB3 and DNM1. This shows their strong connection to Seizures, Sodium/ Calcium Channelopathies and Neurodevelopmental Disorders. Other than these CHD2 (850 variants), POLG (1387 variants), CUX2 (83

variants), SCN2A (1179 variants) and CACNA1A (1346 variants) were seen, indicating mutation in these genes can lead to epilepsy like disorders. Genes that are related to sodium/calcium ion channel activity shows that altered neuronal excitability is a key mechanism in LGS. Few genes were involved in GABAergic inhibitory neurotransmission that indicate improper inhibitory signaling that leads to neural firing during seizures. Overall, the integration of MeSH vocabulary to search relevant disease and data collected through evidence-based gene associations supports the finding of multiple genetic basis of LGS.

STRING

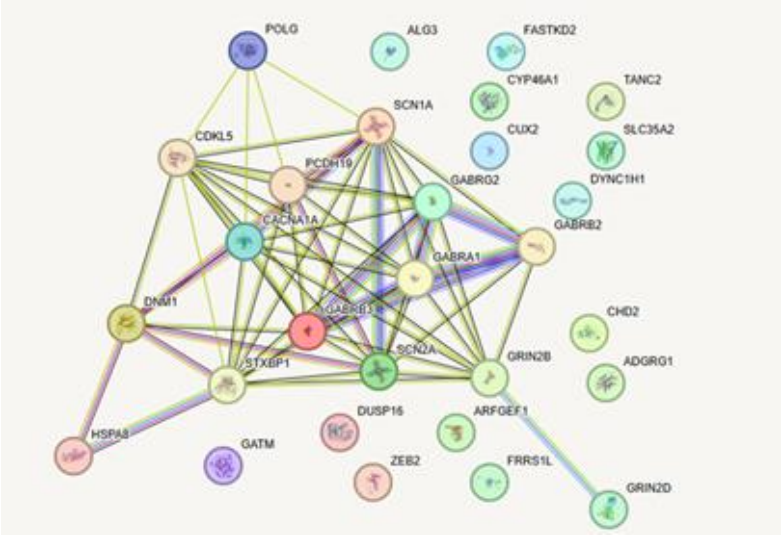


Figure 2: PPI network demonstrating significant functional connectivity among LGS genes

The STRING protein-protein interaction (PPI) analysis revealed a network among genes associated with Lennox Gastaut syndrome indicating a strong functional relationship at the protein level. The network represented 29 nodes and 61 edges which is significantly higher than the expected number of interactions (5 edges) and shows significant PPI enrichment PPI enrichment p-value < 1.0×10^{-16}. This suggests that the identified proteins do not interact randomly but are biologically connected. Central proteins such as SCN1A, SCN2A, CACNA1A, GABRB3, GABRA1, and GABRG2 showed considerable interactions which focus on their key role in regulating excitability, neurotransmission and synaptic transmission.

Table 1: Gene ontology enrichment terms identified by STRING PPI network in LGS.

Theme (Biological process)	**GO terms and pathways**
Synaptic organization and synaptic transmission	Inhibitory synapse assembly, chemical synaptic transmission, regulation of post synapse organization.
GABAergic neurotransmission	Synaptic transmission, GABA signaling pathway
Excitatory neurotransmission	Excitatory postsynaptic potential, positive regulation of excitatory postsynaptic potential
Ion transport and membrane potential regulation	Regulation of membrane potential, Ion transmembrane transport
Nervous system development	Cell to cell signaling, nervous system process

GeneMANIA

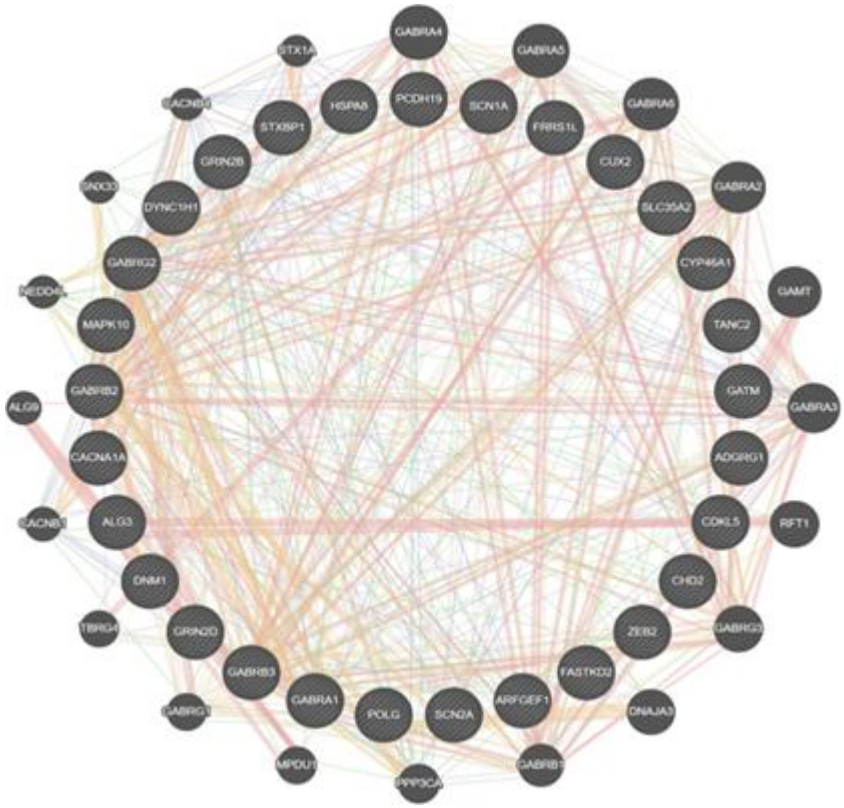


Figure 3: Functional gene network revealing co-expression, pathway sharing and predicted interaction in LGS.

GeneMANIA network analysis supports the strong functional relation between genes affecting Lennox Gastaut syndrome. The network shows high linkage between the genes involved in neurotransmission and ion channel activity. Genes such as GABRA1, GABRB3, GABRG2, SCN1A, SCN2A, CACNA1A and CDKL5 show extensive links indicating their central role in inhibitory neurotransmission and neural signaling. Importantly the consistent network structure seen across the orientations strengthens the reliability of these interactions. Overall, the GeneMANIA results complement the DisGeNET and STRING analyses by demonstrating that LGS-associated genes form a highly connected functional network rather than acting individually.

Enrichr

Across the different pathway and transcription databases used (Reactome Pathway 2024, KEGG 2021, ChEA 2022, and WikiPathways 2024 Human respectively), a common pattern is seen. Most of the genes are repeatedly linked to brain signaling, synapse activity and seizure-related pathways. Many of them, especially the GABA receptor genes (GABRA1, GABRB2, GABRB3, GABRG2) appear together in pathways related to neural signaling which is important for controlling brain activity. The results also show links to ion channel activity and synaptic transmission involving genes such as SCN1A, SCN2A, CACNA1A and STXBP1. These genes help regulate how signals pass between nerve cells. Some pathways are associated with known neurological and developmental disorders, supporting the clinical features seen in LGS patients. Overall, the Enrichr results suggest that the studied

genes mainly affect neuronal communication explaining their role in the development of Lennox Gastaut syndrome.

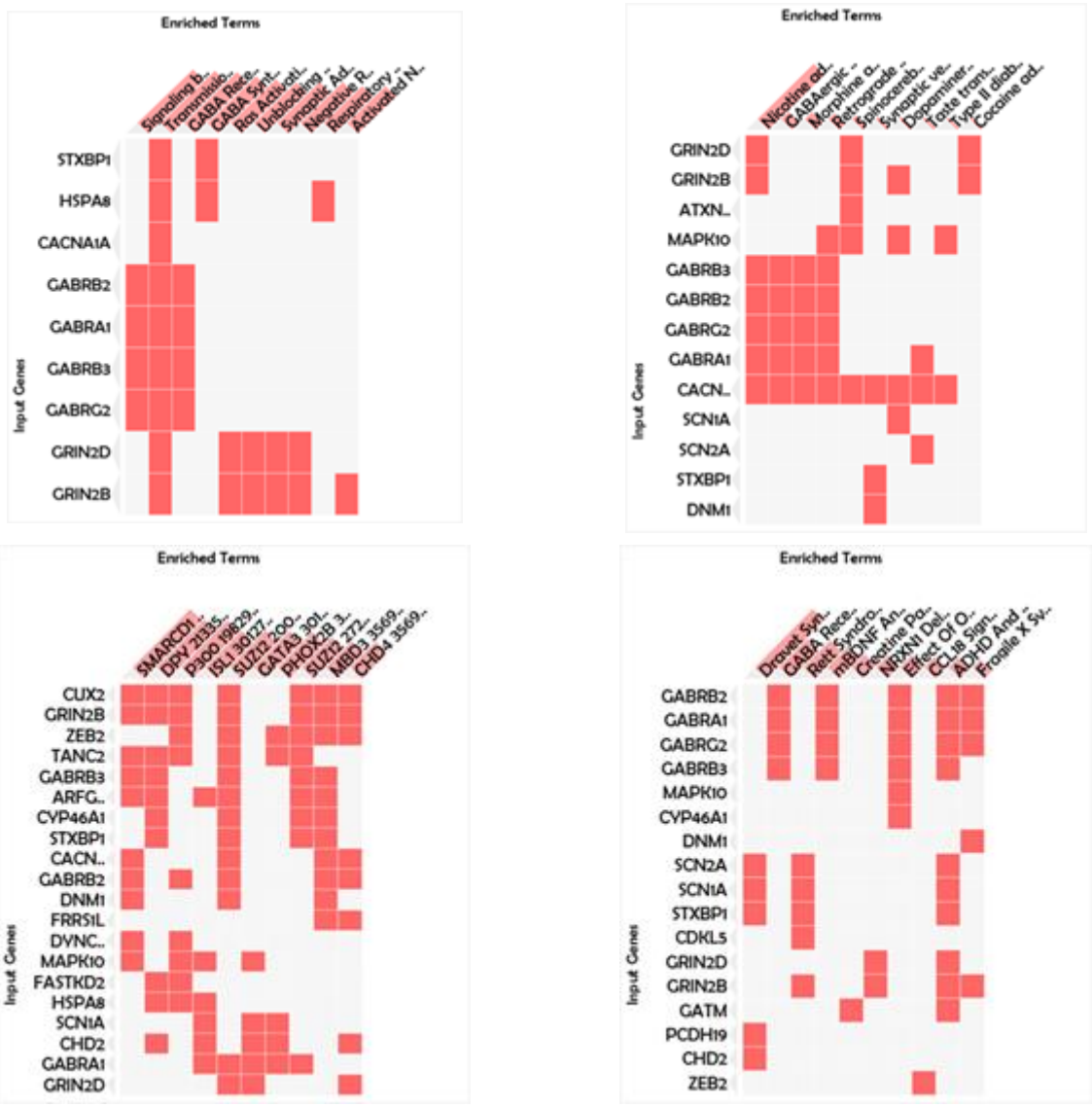


Figure 4: Enrichr output showed overrepresented biological processes and signaling pathway linked to LGS

Table 2: Hub-genes identified by multi database Enrichment Analysis

Gene	Reactome Pathway2024	KEGG 2021	ChEA 2022	WikiPathways 2024 Human	Count
STXBP1	✓	✓	✓	✓	4
GABRB2	✓	✓	✓	✓	4
GABRG2	✓	✓	☐	✓	3
SCN1A	☐	✓	✓	✓	3
DNM1	☐	✓	✓	✓	3

Reactome

Figure 5: Reactome pathway mapping of LGS genes emphasizing synaptic transmission, neuronal signaling and neurotransmitter regulation

The Reactome pathway analysis helped to understand biological activities that are most affected by the genes. From the results, most of the highlighted pathways are related to brain cell communication. Several pathways involve the release of chemical signals between neurons and how these signals are received at synapses. This is important because proper communication between nerve cells is essential for normal brain function. The results also point toward pathways involved in signal transmission and regulation of nerve activity, which means these genes help control the signalling in neurons. When this balance is disturbed, it can lead to excessive brain activity, which is a key feature of epilepsy and Lennox Gastaut syndrome.

Overall, the Reactome analysis suggests that the genes studied are mainly involved in brain signaling, synaptic communication and regulation of neuronal activity. This supports the idea that control signals play a major role in the development of LGS.

Discussion

Lennox–Gastaut syndrome (LGS) is widely recognized as a complex developmental and epileptic encephalopathy, where seizures and neurodevelopmental impairment arise from disrupted brain network function rather than a single abnormality. The bioinformatics findings support this model by showing linked genes and pathways involved in inhibitory neurotransmission, ion channel activity and synaptic signaling.

At the gene level, DisGeNET identified key LGS-associated genes such as SCN1A, SCN2A, CACNA1A, GABRB3, DNM1, CHD2, POLG and CUX2. A clear pattern is visible in this that several genes are directly involved in neuronal excitability and synaptic communication. Sodium channel genes such as SCN1A and SCN2A are central to action potential generation, while calcium channel genes such as CACNA1A influence neurotransmitter release patterns. Other than this KCNQ2, KCNT1, IQSEC2, Glutamate Receptor Dysfunction including NMDA and AMPA are commonly associated with LGS [20]. The convergence of sodium channel, calcium channel, and GABA receptor genes suggests that LGS may represent a disorder of excitation–inhibition imbalance at the network level.

A convincing explanation is that different genetic variants may still converge on the same interacting synaptic and ion-channel network, producing a similar clinical syndrome. This idea aligns with the precision-therapeutics perspective in LGS, where the goal is to move from a symptom based definition toward a pathway based understanding that explains shared clinical outcomes across different genetic backgrounds.

GeneMANIA also highlighted extensive links among GABRA1, GABRB3, GABRG2, SCN1A, SCN2A, CACNA1A, and CDKL5 reinforcing the centrality of inhibitory neurotransmission and neural signaling in LGS. The repeated appearance of GABA receptor genes across multiple tools is particularly meaningful, because impaired GABAergic signaling is strongly associated with seizure susceptibility and network instability. Clinically, this connects well with modern management strategies in LGS like corpus callosotomy, dietary therapy, VNS and DBS [21].

Enrichment results from Enrichr supported the same biological direction across Reactome, KEGG, WikiPathways, and transcription factor databases. Reactome results further emphasized synapse-level mechanisms, with multiple pathways linked to neurotransmitter release, synaptic reception, and neuron to neuron communication. Finally, while analysis mainly highlights molecular signaling pathways, it also supports the broader framework of treatment. CBD is approved by the European Medicines Agency (EMA) as adjunctive therapy combined with CLB for LGS in patients of more than 2 years of age [22].

This study has certain limitations. The analysis relies on publicly available curated databases, which may introduce bias toward well-studied genes while underrepresenting novel or rare contributors. Protein–protein interaction networks represent predicted and literature-supported associations rather than experimentally validated interactions specific to LGS. Additionally, the study does not incorporate transcriptomic or proteomic expression data from LGS patients, which could provide context-specific validation. Future studies integrating multi-omics datasets and experimental validation would strengthen pathway-level insights.

Conclusion

This study shows that Lennox- Gastaut syndrome is best seen as a genetically diverse disorder that revolves around a shared functional network involving synaptic transmission, ion channel regulation, and weakened inhibitory signaling. They form a tightly connected molecular network enriched in seizure related neurobiological pathways. The consistent grouping of sodium channel genes (SCN1A, SCN2A), calcium channel genes (CACNA1A), and GABA receptor subunits strongly indicates that disrupted neuronal excitability and diminished inhibitory control are key mechanisms behind LGS. These results help clarify why LGS causes severe, treatment-resistant epilepsy and lasting neurodevelopmental problems.

References

1. Samanta, D. (2025). Sleep in Lennox–Gastaut syndrome: A scoping review. *Children, 12*(12), 1676. https://doi.org/10.3390/children12121676
2. Nelson, J. A., & Knupp, K. G. (2023). Lennox-Gastaut syndrome: Current treatments, novel therapeutics, and future directions. *Neurotherapeutics, 20*(5), 1255–1262. https://doi.org/10.1007/s13311-023-01397-x
3. Andrews, J. S., Shah, D., Nacson, A., Symonds, T., Hughes, S., Asgharnejad, M., Benitez, A., & Sams, L. (2025). Development and refinement of the Clinical Global Impression of Improvement for non-seizure symptoms measure in Dravet syndrome and Lennox-Gastaut syndrome. *Journal of Patient-Reported Outcomes, 9*(1), 24. https://doi.org/10.1186/s41687-024-00829-2

4. Specchio, N., & Curatolo, P. (2021). Developmental and epileptic encephalopathies: What we do and do not know. *Brain, 144*(1), 32–43. https://doi.org/10.1093/brain/awaa371
5. Shah, D., Villanueva, V., Dlugos, D., Benitez, A., Taylor, Y., Olsen, S., Connolly, H., Lai, S., & Andrews, J. S. (2025). Nonseizure symptoms and broader seizure impacts in patients with Dravet syndrome and Lennox–Gastaut syndrome in clinical practice settings: Results from a multinational survey. *Epilepsia Open, 10*(6), 1783–1797. https://doi.org/10.1002/epi4.70118
6. Vetri, L., Calì, F., Saccone, S., Vinci, M., Chiavetta, N. V., Carotenuto, M., Roccella, M., Costanza, C., & Elia, M. (2024). Whole exome sequencing as a first-line molecular genetic test in developmental and epileptic encephalopathies. *International Journal of Molecular Sciences, 25*(2), 1146. https://doi.org/10.3390/ijms25021146
7. Cross, J. H., Benítez, A., Roth, J., Andrews, J. S., Shah, D., Butcher, E., Jones, A., & Sullivan, J. (2024). A comprehensive systematic literature review of the burden of illness of Lennox–Gastaut syndrome on patients, caregivers, and society. *Epilepsia, 65*(5), 1224–1239. https://doi.org/10.1111/epi.17932
8. Riva, A., D'Onofrio, G., Amadori, E., Arzimanoglou, A., Auvin, S., Bagnasco, I., Barabino, P., Biagioli, V., Brambilla, I., Cangemi, G., Coppola, A., De Lillo, A., Di Bonaventura, C., Di Gennaro, G., Ferlazzo, E., Gil-Nagel, A., Gobbi, G., Lattanzi, S., Kluger, G., … Striano, P. (2025). Lennox–Gastaut syndrome unveiled: Advancing diagnosis, therapies, and advocacy—Insights from the Genoa international workshop. *Epilepsia*, epi18696. https://doi.org/10.1111/epi.18696
9. Goudarzi, Z., Lotfi, F., Shahtaheri, R. S., Moradi, N., Taghizadeh, M., & Keshavarz, K. (2025). Value of cannabidiol as adjunctive treatment for Lennox Gastaut syndrome: Cost-effectiveness and budget impact analysis. *BMC Medicine, 23*(1), 135. https://doi.org/10.1186/s12916-025-03972-9
10. Li, J., Gao, Y., Cao, J., Cai, F., & Zhai, X. (2023). Efficacy analysis of oral dexamethasone in the treatment of infantile spasms and infantile spasms related Lennox–Gastaut syndrome. *BMC Pediatrics, 23*(1), 255. https://doi.org/10.1186/s12887-023-04062-6
11. Warren, A. E. L., Patel, A. D., Cross, J. H., Clarke, D. F., Dalic, L. J., Grinspan, Z. M., Conecker, G., & Knowles, J. K. (2025). Mobilizing a new era in Lennox-Gastaut syndrome treatment and prevention. *Epilepsy Currents, 25*(3), 236–243. https://doi.org/10.1177/15357597251321926
12. Seiden, L. G., & Connor, G. S. (2022). The importance of drug titration in the management of patients with epilepsy. *Epilepsy & Behavior, 128*, 108517. https://doi.org/10.1016/j.yebeh.2021.108517
13. Epi25 Collaborative, Chen, S., Abou-Khalil, B. W., Afawi, Z., Ali, Q. Z., Amadori, E., Anderson, A., Anderson, J., Andrade, D. M., Annesi, G., Arslan, M., Auce, P., Bahlo, M., Baker, M. D., Balagura, G., Balestrini, S., Banks, E., Barba, C., Barboza, K., … Berkovic, S. F. (2024). Exome sequencing of 20,979 individuals with epilepsy reveals shared and distinct ultra-rare genetic risk across disorder subtypes. *Nature Neuroscience, 27*(10), 1864–1879. https://doi.org/10.1038/s41593-024-01747-8
14. Yang, J. O., Choi, M. H., Yoon, J. Y., Lee, J. J., Nam, S. O., Jun, S. Y., Kwon, H. H., Yun, S., Jeon, S. J., Byeon, I., Halder, D., Kong, J., Lee, B., Lee, J., Kang, J. W., & Kim, N. S. (2021). Characteristics of genetic variations associated with Lennox-Gastaut syndrome in Korean families. *Frontiers in Genetics, 11*, 590924. https://doi.org/10.3389/fgene.2020.590924

15. Zeibich, R., Kwan, P., O'Brien, T. J., Perucca, P., Ge, Z., & Anderson, A. (2023). Applications for deep learning in epilepsy genetic research. *International Journal of Molecular Sciences, 24*(19), 14645. https://doi.org/10.3390/ijms241914645
16. Marchesin, S., & Silvello, G. (2022). TBGA: A large-scale gene-disease association dataset for biomedical relation extraction. *BMC Bioinformatics, 23*(1), 111. https://doi.org/10.1186/s12859-022-04646-6
17. Piñero, J., Saüch, J., Sanz, F., & Furlong, L. I. (2021). The DisGeNET Cytoscape app: Exploring and visualizing disease genomics data. *Computational and Structural Biotechnology Journal, 19*, 2960–2967. https://doi.org/10.1016/j.csbj.2021.05.015
18. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research, 51*(D1). https://doi.org/10.1093/nar/gkac1000
19. Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G. D., & Morris, Q. (2018). GeneMANIA update 2018. *Nucleic Acids Research, 46*(W1), W60–W64. https://doi.org/10.1093/nar/gky311
20. Samanta, D. (2025). Precision therapeutics in Lennox–Gastaut syndrome: Targeting molecular pathophysiology in a developmental and epileptic encephalopathy. *Children, 12*(4), 481. https://doi.org/10.3390/children12040481
21. Knowles, J. K., Warren, A. E. L., Mohamed, I. S., Stafstrom, C. E., Koh, H. Y., Samanta, D., Shellhaas, R. A., Gupta, G., Dixon-Salazar, T., Tran, L., Bhatia, S., McCabe, J. M., Patel, A. D., & Grinspan, Z. M. (2024). Clinical trials for Lennox–Gastaut syndrome: Challenges and priorities. *Annals of Clinical and Translational Neurology, 11*(11), 2818–2835. https://doi.org/10.1002/acn3.52211
22. Auvin, S., Arzimanoglou, A., Falip, M., Striano, P., & Cross, J. H. (2025). Refining management strategies for Lennox–Gastaut syndrome: Updated algorithms and practical approaches. *Epilepsia Open, 10*(1), 85–106. https://doi.org/10.1002/epi4.13075