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Molecular Heterogeneity of WNT and mTOR Signaling Pathways in Mild Malformation of Cortical Development with Oligodendroglial Hyperplasia and Epilepsy (MOGHE)

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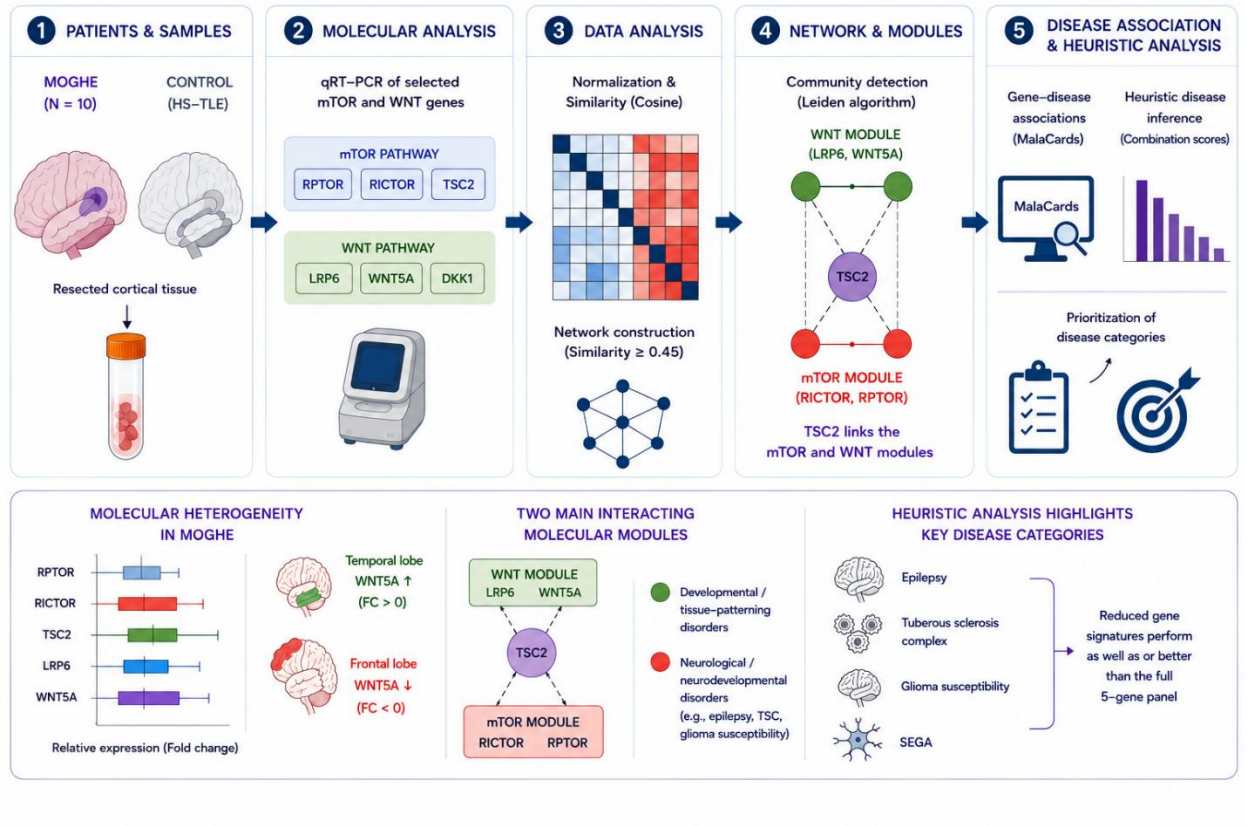
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Graphical Abstract. Molecular and network-based analysis of MOGHE cortical tissue revealed a heterogeneous transcriptional profile involving genes from both the mTOR and WNT signaling pathways. Integration of gene expression data with disease-association and network analyses identified two biologically distinct but interconnected molecular modules linked by TSC2, suggesting functional crosstalk between mTOR and WNT signaling. These findings support the concept that MOGHE is not a conventional mTORopathy, but rather a complex developmental disorder characterized by interacting molecular programs potentially shaped by somatic mosaicism within the epileptogenic cortex.

Abstract

Mild Malformation of Cortical Development with Oligodendroglial Hyperplasia and Epilepsy (MOGHE) is a recently recognized developmental lesion associated with drug-resistant epilepsy. Although its clinical and neuropathological features are increasingly understood, the molecular mechanisms underlying MOGHE remain largely unknown. Given the roles of mTOR and WNT signaling in cortical development and epileptogenesis, we investigated the expression of selected genes from these pathways in surgically resected MOGHE tissue and explored their biological associations through computational network analysis.

Cortical samples from 12 patients with histopathologically confirmed MOGHE were analyzed by quantitative real-time PCR. Gene expression levels were compared with control cortical tissue from patients with temporal lobe epilepsy associated with hippocampal sclerosis. Bioinformatic analyses included MalaCards-derived gene–disease associations, molecular network modeling, community detection, and heuristic disease-prioritization approaches.

Gene expression analysis revealed marked molecular heterogeneity among MOGHE samples. Despite substantial interindividual variability, several genes showed a consistent reduced expression, including RICTOR, RPTOR, TSC2, LRP6, and WNT5A. Stratification by cortical location identified region-specific expression patterns, particularly for WNT5A, which was upregulated in right temporal lesions and markedly downregulated in frontal lesions. Network analyses identified two major functional modules: one centered on RICTOR, RPTOR, and TSC2, associated with epilepsy and neurodevelopmental disorders, and another centered on LRP6 and WNT5A. TSC2 occupied a central position connecting both modules, suggesting biological interaction between mTOR and WNT signaling. These findings indicate that MOGHE is a molecularly heterogeneous developmental disorder involving coordinated alterations in mTOR and WNT pathways, providing new insights into its pathogenesis and potential therapeutic targets.

Keywords: MOGHE; epilepsy surgery; cortical malformations; mTOR signaling; WNT signaling; epileptogenesis.

Introduction

Malformations of cortical development (MCDs) comprise a spectrum of neurodevelopmental abnormalities associated with disturbances in neuronal proliferation, migration, and cortical organization, representing one of the major structural causes of drug-resistant epilepsy in children and young adults (Schurr et al. 2017; Liu et al. 2023) .

Mild Malformation of Cortical Development with Oligodendroglial Hyperplasia and Epilepsy (MOGHE) has recently been recognized as a distinct neuropathological entity characterized by subtle cortical abnormalities associated with oligodendroglial hyperplasia and high epileptogenic potential (Verentzioti et al. 2019). It was first delineated in 2017, when review of surgical specimens previously regarded as histopathologically non-lesional revealed a recurrent pattern of increased oligodendroglial density that was proposed as a discrete clinic-pathological entity, and its status was consolidated when the 2022 ILAE consensus update of the focal cortical dysplasia classification formally incorporated MOGHE within a multilayered, integrated genotype–phenotype diagnostic scheme (Najm et al. 2022).

MOGHE is characterized by a diffuse or focal increase in oligodendrocyte density within the subcortical white matter and/or the cortico-subcortical junction, frequently occurring in the absence of marked cortical dyslamination or dysmorphic neurons typically observed in classical focal cortical dysplasia (Schurr et al. 2017; Najm et al. 2022). Clinically, patients present with early-onset focal seizures that are often refractory to antiseizure medications, resulting in significant impairment of neurodevelopment and quality of life (Verentzioti et al. 2019; Bonduelle et al. 2021). The lesion shows a predilection for the frontal lobe, and its MRI signature is age-dependent, evolving from laminar T2/FLAIR hyperintensity at the cortico-medullary junction in young children (subtype I) toward reduced cortico-subcortical differentiation in older patients (subtype II) (Hartlieb et al. 2019), which contributes to frequent under-recognition on presurgical imaging.

Despite increasing recognition of MOGHE, its underlying pathophysiological mechanisms remain poorly understood. Recent studies suggest that, beyond a static structural abnormality, MOGHE may represent a dynamic disorder of the cortical microenvironment involving abnormal interactions among neurons, oligodendrocytes, and local inflammatory components (Crino 2015; Schurr et al. 2017). A decisive advance came with the discovery that brain-restricted somatic (mosaic) pathogenic variants in SLC35A2 are present in roughly 45% of cases and now serve

as a molecular marker for the entity (Bonduelle et al. 2021). SLC35A2 is an X-linked gene encoding the UDP-galactose transporter; loss of function impairs Golgi galactosylation and yields truncated glycans, linking MOGHE to a defect in protein and lipid glycosylation (Kang et al. 2022).

In parallel, cellular signaling pathways that are essential for central nervous system development and homeostasis, particularly the mammalian target of rapamycin (mTOR) and WNT/β-catenin pathways, have been implicated in several cortical malformations associated with drug-resistant epilepsy (Crino 2011; Galvão et al. 2025; Shakerzadeh et al. 2025). Alterations in these pathways have been linked not only to neuronal dysfunction but also to glial proliferation, aberrant myelination, and persistent inflammatory responses, all of which may be relevant to the pathobiology of MOGHE. Importantly, this glycosylation-centered signature distinguishes MOGHE from the mTOR-driven type II focal cortical dysplasia (Najm et al. 2022); nonetheless, roughly half of histologically confirmed cases carry no identifiable SLC35A2 variant, and the downstream engagement of canonical developmental cascades such as mTOR and WNT in MOGHE has not been systematically examined, representing a substantive gap given the disease's defining oligodendroglial expansion and white-matter involvement.

Considering the emerging role of mTOR and WNT signaling pathways in cortical development, glial proliferation, and neuroinflammatory processes, dysregulation of these pathways represents a plausible molecular mechanism underlying MOGHE and therefore constitutes the primary focus of the present investigation.

Accordingly, the aim of this study was to characterize the clinical, radiological, and molecular features of a cohort of patients with histopathologically confirmed MOGHE who underwent epilepsy surgery, with particular emphasis on the analysis of cellular signaling pathways and inflammatory processes potentially involved in the epileptogenic mechanisms associated with this entity.

Methods

Patients

This study included 12 patients with drug-resistant epilepsy who were treated at the Epilepsy Surgery Program of Hospital São Lucas, Pontifical Catholic University of Rio Grande do Sul (PUCRS), and had a diagnosis of cortical malformation with suspected MOGHE. The control group consisted of patients with temporal lobe epilepsy associated with hippocampal sclerosis. All patients underwent brain magnetic resonance imaging (MRI) and prolonged video-electroencephalographic monitoring as part of the presurgical evaluation. MRI examinations were performed using a 3.0-Tesla GE Signa HDx scanner (General Electric, Milwaukee, WI, USA), including at least one volumetric FLAIR sequence with isotropic 1 mm^3 resolution and a 512 × 512 acquisition matrix, as well as a non-volumetric T2-weighted sequence with 5-mm slice thickness and a 512 × 512 matrix. All imaging studies were retrospectively reviewed by an experienced neuroradiologist.

Human Ethics and Consent to Participate

The study was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (CAAE: 19776619.9.0000.5336; approval number 3.577.035). All participants or their legal guardians provided written informed consent prior to inclusion. All procedures were conducted in accordance with current ethical guidelines and the principles outlined in the Declaration of Helsinki and Brazilian National Health Council Resolution No. 466/12. For participants younger than 18 years of age, consent was obtained from parents or legal guardians.

Tissue Collection

All patients underwent a Patient Management Conference (PMC) at PAESP to determine the best approach for epilepsy surgery. In the control group, the decision to perform anterior temporal lobectomy rather than selective amygdalohippocampectomy was made exclusively by the clinical team and was independent of the research protocol. All participants or their legal representatives authorized the use of biological samples for research purposes. No additional cortical tissue was removed for the study. Following surgical resection, histopathological examination was performed to confirm the diagnosis of MOGHE in the study group and hippocampal sclerosis in the control group according to current neuropathological criteria.

Molecular Analysis

Selected cortical tissue samples were mechanically homogenized, and total RNA was extracted using the SV Total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer's instructions. Extracted RNA was subsequently reverse transcribed into complementary DNA (cDNA) using a Thermo Fisher Scientific reverse transcription kit following the manufacturer's protocol. cDNA concentration and quality were assessed using NanoDrop spectrophotometry to ensure standardized input concentrations for quantitative real-time polymerase chain reaction (qRT-PCR) analyses. qRT-PCR assays were performed using the SYBR Select Master Mix (Applied Biosystems) according to the manufacturer's instructions. Each reaction was conducted in duplicate using an initial input of 40 ng cDNA. Real-time PCR amplification was performed on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, Massachusetts, USA). Primers for the genes of mTOR pathway (AKT1, AKT2, AKT3, RPTOR, RICTOR, MLST8, TSC1, and TSC2 and WNT/β-catenin pathway (GSK3B, DKK1, LRP6, and WNT5A) were designed using the NCBI Primer-BLAST platform. Gene expression assays were performed in 96-well plates, with NSE and ACTB used as endogenous reference genes for normalization.

Bioinformatics Analysis

All data processing and analytical procedures were developed using the Python programming language. Data manipulation, organization, and integration were performed using the Pandas library, while numerical and matrix-based operations were conducted using NumPy. Association scores were standardized using Z-score transformation implemented through the StandardScaler class from the Scikit-learn library. Gene similarity was subsequently calculated using cosine similarity metrics. Molecular network modeling was performed using the igraph package, allowing genes to be represented as nodes and similarity measures as weighted edges. Functional community detection and module identification were carried out using the Leiden algorithm implemented in the Leidenalg package. Exploratory visualizations, including bubble plots and network representations, were generated using Plotly Express, enabling graphical exploration of gene–disease associations and molecular network architecture.

Disease and Gene Selection

An exploratory analysis was performed using the MalaCards database (Rappaport et al. 2013) to investigate associations between previously selected genes and neurological diseases. For each of the 12 analyzed genes, the internal semantic descriptor "Neurological Disease" was combined with the Boolean operator "AND" to retrieve disease associations specifically related to neurological conditions. Associated diseases and their respective global MalaCards scores were extracted. Association prioritization was based on the global score provided by the platform, which was used as a measure of gene–disease relevance. Genes presenting higher scores were considered more strongly associated with the neurobiological context under investigation. Selected genes were subsequently used for exploratory connectivity analyses and molecular network visualization.

Molecular Network Construction

After defining the gene pool, a molecular network was constructed based on the similarity of the disease-gene association profiles. Initially, a gene vs. disease matrix was generated, where each row represented a gene and each column a neurological disease identified in MalaCards. The matrix values corresponded to the global association scores provided by the platform. To reduce scaling effects between different diseases, the matrix values were standardized using Z-score transformation. Subsequently, the similarity between all gene pairs was calculated using the cosine similarity metric: $S_i m_{ij} = cos\theta = \frac{(A \cdot B)}{(\|A\|\|B\|)}$, where A and B represent the disease-gene association vectors of the genes analyzed.

Bubble Plot Construction

Duplicate gene–disease associations were consolidated while retaining the highest observed score for each pair. Bubble size was defined according to the MalaCards global score following nonlinear normalization to enhance visual discrimination between low- and high-relevance associations.

Probabilistic Disease Inference

To develop an exploratory disease inference model, the data underwent cleaning and standardization steps,

including normalization of gene and disease nomenclature, removal of incomplete records, and consolidation of duplicate associations, maintaining the highest observed score for each gene-disease pair. For each disease present in the database, a composite score was calculated considering three complementary components: Gene Coverage $C = \frac{Number\ of\ shared\ genes}{Total\ number\ of\ disease\ associated\ genes}$, intensity of association (Intensity) $I = \Sigma(scoregene - disease)$, Genetic specificity $S = \ln(1 + N)^{-1}$, where N represents the number of diseases associated with the gene. Genes associated with a large number of diseases received less weight, while more specific genes contributed proportionally more to the final score.

Composite Score

The three components were integrated through a weighted linear combination $FinalScored = \alpha Cd + \beta Id + \gamma Sd$. Weights were empirically defined to balance the contributions of gene overlap, association strength, and biological specificity.

Conversion to Relative Probabilities

Final scores were converted into relative probabilities using the Softmax function $P_i = \frac{\exp(FinalScore_i)}{\sum_{j=1}^{N} \exp(FinalScore_j)}$. This procedure normalized the results into a probabilistic distribution in which all probabilities summed to one, enabling direct comparison among candidate diseases. Diseases were subsequently ranked according to their estimated probabilities, generating a hierarchy of biological hypotheses compatible with the analyzed gene signature. Final ranking was performed in descending order of probability values, such that diseases with higher estimated probabilities received greater interpretative priority.

Accordingly, the final classification simultaneously incorporated observed gene overlap, association strength reported by MalaCards, and the biological specificity of the genes involved.

Statistical Analysis

Data was organized and analyzed using appropriate statistical software. Distribution normality was assessed using standard normality tests. Comparisons between groups were performed using parametric or non-parametric statistical tests as appropriate. Statistical significance was defined as a two-sided p-value < 0.05.

Results

Radiological and Clinical Profile

Radiological classification of MOGHE subtypes was performed according to the criteria previously described by Hartlieb et al. (2019). Lesional patterns could be defined in six patients. Among these, five were classified as MOGHE subtype II, characterized by cortico-subcortical blurring, predominantly involving the frontal lobe in four cases and the temporal lobe in one case. The temporal lesion displayed imaging features closely resembling focal cortical dysplasia. Only one patient exhibited a subcortical T2/FLAIR hyperintense lesion within the parietal lobe, consistent with MOGHE subtype I. Among the remaining six patients, no focal lesion was identified in three cases, while preoperative imaging studies were unavailable for retrospective review in the other two patients (Supplementary Table 1).

Molecular Analysis

Gene expression analysis revealed marked molecular heterogeneity across cortical samples obtained from patients with MOGHE. Despite substantial interindividual variability, several genes demonstrated a relatively consistent pattern of reduced expression compared with the control group. Among the most notable findings were the mTOR pathway-related genes RPTOR (mean fold change: −1.297 ± 1.089), RICTOR (−1.640 ± 1.008), and TSC2 (−1.407 ± 0.431), as well as the WNT signaling genes LRP6 (−0.709 ± 1.510) and WNT5A (−0.805 ± 2.713). Overall, these findings suggest coordinated dysregulation of both mTOR- and WNT-related signaling pathways in MOGHE cortical tissue, although the magnitude and direction of expression changes varied considerably among individual

patients.

Regional Gene Expression Patterns

Stratification according to cortical location revealed distinct regional expression profiles. The most prominent finding involved WNT5A expression, which was increased in samples obtained from the right temporal lobe (fold change: 1.919 ± 0.907), whereas frontal lobe samples exhibited marked downregulation (fold change: −2.942 ± 0.387). Similar regional trends were observed for RPTOR and RICTOR, particularly when comparing patients who underwent right temporal versus frontal cortical resections. Although exploratory in nature, these observations suggest that different cortical regions affected by MOGHE may harbor distinct molecular signatures.

Stratification according to disease duration revealed comparatively more homogeneous expression patterns only for RICTOR and DKK1. In patients with disease duration ranging from 9 to 22 years, RICTOR (1.810 ± 0.939) and DKK1 (−1.162 ± 0.673) displayed relatively stable expression profiles, suggesting that alterations involving these genes may remain preserved throughout the clinical course of epilepsy (Figure 1).

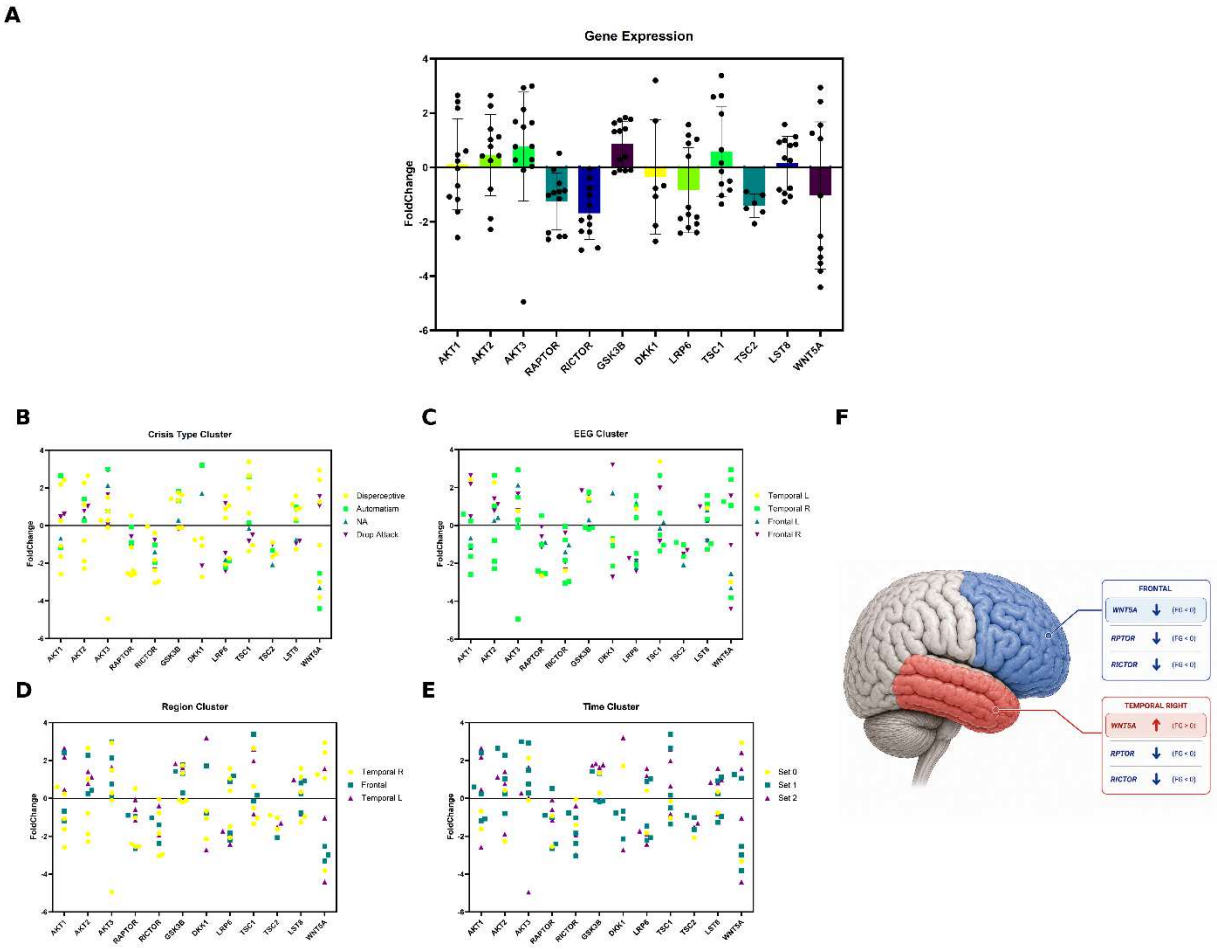


Figure 1. Relative gene expression analysis. (A) Gene expression profiling across all MOGHE samples revealed substantial heterogeneity among cortical specimens, highlighting differential fold-change patterns in RPTOR, RICTOR, TSC2, LRP6, and WNT5A. Stratified analysis according to seizure type (B) did not demonstrate significant differences in gene expression levels. Analyses according to cortical region based on EEG findings (C) and resection

area (D) showed increased WNT5A expression in right temporal lobe samples and reduced expression in frontal lobe specimens, whereas RPTOR and RICTOR predominantly exhibited negative fold-change values across both anatomical regions. Stratification according to disease duration (E) revealed relatively homogeneous gene expression patterns among samples.

Gene–Disease Association Networks and Heuristic Score Analysis

Integrated analysis of gene–disease associations, molecular interaction networks, and heuristic disease scores revealed that the differentially expressed genes identified in MOGHE were organized into at least two distinct functional modules. The first module, composed of RICTOR, RPTOR, and TSC2, showed predominant associations with neurological and neurodevelopmental disorders, including epilepsy, tuberous sclerosis complex, glioma susceptibility, and subependymal giant cell astrocytoma. In contrast, the second module centered on LRP6 and WNT5A was primarily associated with diseases related to tissue development, skeletal abnormalities, and retinal disorders.

Analysis of the 26 possible gene combinations demonstrated that reduced gene signatures frequently performed similarly to, or better than, the complete five-gene signature. The LRP6–WNT5A combination yielded the highest overall Combination Score (0.227), followed closely by the RICTOR–RPTOR–TSC2 signature (0.225), whereas the complete five-gene signature produced an intermediate score (0.206). Gene signatures dominated by WNT-related genes preferentially identified diseases associated with developmental processes, whereas mTOR-centered signatures enriched for conditions such as tuberous sclerosis complex, glioma susceptibility, and subependymal giant cell astrocytoma. Notably, TSC2 occupied an intermediate position within the molecular network, linking the mTOR- and WNT-centered modules. This pattern is consistent with the established role of the TSC1/TSC2 complex as a key regulator of mTOR signaling and with previous evidence supporting functional crosstalk between the mTOR and WNT/β-catenin pathways. Importantly, the spontaneous convergence of the algorithm toward epilepsy-related disorders, despite the absence of explicit epilepsy weighting during model construction, provides independent biological support for the relevance of the identified gene set. Collectively, these findings support the existence of two major molecular modules corresponding to the mTOR and WNT signaling axes. The interaction between these modules may contribute to the neurodevelopmental abnormalities, oligodendroglial proliferation, and epileptogenic mechanisms observed in MOGHE (Figure 2).

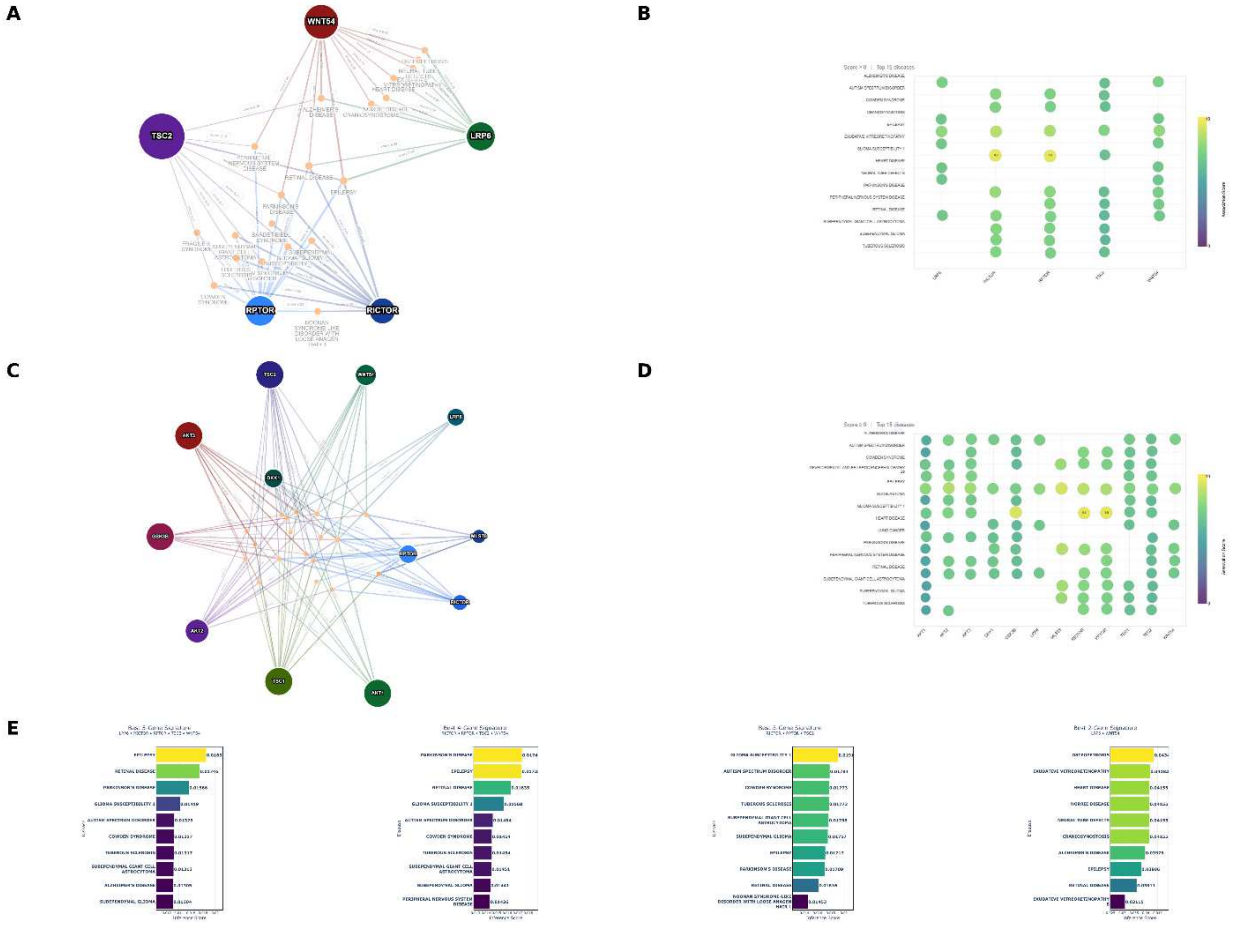


Figure 2. Integrated multi-scale analysis of gene–disease associations and inferred disease signatures. (A) Interactive gene–disease network depicting the relationships between the selected genes and their associated diseases. Gene nodes are represented by large colored circles, whereas disease nodes are shown as smaller orange circles. Edge color corresponds to the originating gene, and edge thickness is proportional to the association score. The network highlights the highly interconnected structure of the molecular landscape and reveals diseases simultaneously linked to multiple genes. (B) Bubble plot summarizing the distribution of gene–disease associations across the top-ranked diseases. Bubble size and color intensity represent the association score, allowing visualization of disease convergence and the relative contribution of individual genes to each phenotype. (C) Network representation restricted to the final optimized molecular signature composed of LRP6, RICTOR, RPTOR, TSC2, and WNT54, emphasizing the subset of diseases retained after combinatorial optimization and illustrating the preservation of highly connected and biologically coherent disease clusters. (D) Bubble plot corresponding to the optimized signature, showing that a reduced set of genes preserves a broad disease coverage while concentrating associations within neurological, developmental, retinal, and proliferative disorders. (E) Inference scores of the best-performing signatures after extensive exploration composed with five, four, three, and two genes' combinations.

Discussion

The present study investigated the expression of genes related to the mTOR and WNT signaling pathways in cortical tissue resected from patients with neuropathologically confirmed MOGHE. The principal finding was the identification of a markedly heterogeneous molecular profile characterized by substantial variability among individuals and across different cortical regions. These observations suggest that MOGHE represents a biologically complex entity whose molecular diversity may reflect both the mosaic nature of the disease and the spatial distribution of pathological cellular alterations within the epileptogenic cortex.

Radiological characterization revealed a predominance of MOGHE subtype II among evaluable patients, with five of six classified cases exhibiting cortico-subcortical blurring predominantly involving the frontal lobe. This finding is consistent with the original description by Schurr et al. (2017), who identified MOGHE as a clinicopathological entity frequently associated with frontal lobe epilepsy. The identification of only a single subtype I lesion characterized by T2/FLAIR subcortical hyperintensity further supports the hypothesis that subtype II may represent either a more advanced stage or a biologically more aggressive form of tissue disorganization. The subtle cortico-subcortical blurring observed in subtype II likely reflects deeper architectural disturbances associated with somatic mosaicism and may contribute to the high rate of drug resistance observed in affected patients. Because our cohort was predominantly subtype II and assessed cross-sectionally, we cannot distinguish whether subtype II represents a more advanced, more aggressive lesion or simply an older-age presentation; the interpretation of subtype II as a more aggressive or chronic stage therefore remains a hypothesis to be tested longitudinally rather than a conclusion supported by the present data.

Molecular analysis demonstrated substantial heterogeneity in gene expression profiles across cortical samples, a finding that is highly consistent with the current understanding of MOGHE as a disorder driven by brain-restricted somatic mosaicism. Recent studies employing deep sequencing approaches have demonstrated that pathogenic variants, particularly in SLC35A2, may exhibit variant allele frequencies ranging from 1.4% to 22.9% within resected brain tissue (Bonduelle et al. 2021; Kang et al. 2022). Under this framework, variability in gene expression should not be interpreted solely because of limited sample size but rather as a biological hallmark of the disease itself. Distinct proportions of mutant cells across different cortical regions may generate localized molecular microenvironments, thereby contributing to the heterogeneous transcriptional signatures observed in our cohort.

Although several malformations of cortical development are commonly regarded as “mTORopathies” resulting from hyperactivation of the mTOR pathway (Pang et al. 2008; Crino 2015), the expression profile observed in the present study suggests a more complex biological scenario. The reduced expression of TSC2, RICTOR, and RPTOR is not readily compatible with a simplistic model of global mTOR pathway activation. Instead, these findings raise the possibility that MOGHE may involve alterations in the balance between different mTOR complexes rather than uniform pathway hyperactivation.

Particularly intriguing was the reduction of RICTOR expression. As a core component of the mTORC2 complex, RICTOR plays a critical role in cytoskeletal organization, neuronal migration, and cellular polarity. While previous studies have largely focused on excessive mTOR signaling as a central pathogenic mechanism in cortical malformations (Pang et al. 2008; Crino 2011; Bonduelle et al. 2021), our findings raise the alternative possibility that impaired mTORC2 stability or signaling may contribute to the migrational abnormalities that characterize MOGHE. Although the present data do not allow direct functional assessment of mTOR activity, they support the hypothesis that dysregulation of the equilibrium between mTORC1 and mTORC2 may represent an underrecognized component of MOGHE pathophysiology.

Beyond the mTOR pathway, alterations involving WNT signaling genes were also identified, particularly LRP6, DKK1, and WNT5A. The WNT/β-catenin pathway plays a central role during cortical development by regulating neural progenitor proliferation, neuronal migration, differentiation, and glial maturation (Shakerzadeh et al. 2025). Consequently, disturbances affecting this signaling network may have profound consequences for cortical architecture and connectivity.

One of the most striking observations emerged from the regional stratification analysis. WNT5A expression differed substantially according to anatomical location, showing increased expression in right temporal lesions and marked downregulation in frontal lesions. This finding suggests that molecular heterogeneity in MOGHE extends beyond interindividual variability and may also reflect region-specific biological programs. Such regional differences could contribute to the variability in radiological appearance, clinical presentation, and epileptogenic behavior reported across MOGHE cohorts.

Importantly, gene expression analyses performed in bulk tissue likely capture global tissue states resulting from the combined contribution of multiple cellular populations. In contrast, recent single-cell and single-nucleus studies have demonstrated that molecular abnormalities in MOGHE may be concentrated within specific cellular subpopulations (Galvão et al. 2025). Therefore, the apparent discrepancies between bulk transcriptomic findings and cellular-resolution studies may not necessarily represent conflicting observations but rather complementary perspectives on the same biological process. The regional differences observed for WNT5A expression further suggest that molecular signatures may evolve according to the local burden and distribution of somatic mutations.

Recent molecular characterization studies have demonstrated abnormal protein expression patterns and loss of white matter-associated proteins in MOGHE lesions, directly linking molecular dysregulation to the cortico-subcortical blurring that defines the disease histologically (Cecchini et al. 2025). In this context, the heterogeneous alterations observed in both mTOR and WNT signaling pathways support the concept that MOGHE should not be

viewed as a static malformation but rather as a dynamic developmental disorder characterized by regionally variable molecular programs.

A particularly relevant finding of the present study emerged from the integrated network and heuristic analyses. Rather than forming a single homogeneous disease-associated cluster, the differentially expressed genes organized into two distinct but interconnected molecular modules. The first module, composed of RICTOR, RPTOR, and TSC2, showed predominant associations with neurological and neurodevelopmental disorders, including epilepsy, tuberous sclerosis complex, glioma susceptibility, and subependymal giant cell astrocytoma. In contrast, the second module, centered on LRP6 and WNT5A, was preferentially associated with developmental and tissue-patterning disorders.

A central question raised by these findings is how alterations in mTOR and WNT signaling relate to the glycosylation defect that defines the genetically characterized subset of MOGHE. SLC35A2 encodes the UDP-galactose transporter required for Golgi galactosylation, and its loss yields truncated glycans on membrane and secreted proteins (Bonduelle et al. 2021; Kang et al. 2022). Several WNT-pathway components are themselves glycoproteins whose function depends on glycosylation: N-glycosylation of the co-receptor LRP6, for instance, controls its trafficking to the plasma membrane and the strength of downstream Wnt/β-catenin signaling (Xu et al. 2023). It is therefore plausible that a galactosylation defect converges on WNT — and on receptor-dependent growth-factor/mTOR — signaling by impairing the maturation and surface presentation of pathway receptors, positioning the transcriptional changes we observed as potential downstream consequences of, or compensatory responses to, the primary glycosylation lesion. This hypothesis is directly testable by combining SLC35A2 genotyping with glyco-proteomic and pathway-activity assays in the same tissue.

This segregation suggests that distinct subsets of genes may capture complementary dimensions of MOGHE biology. While the mTOR-centered module appears more closely related to epileptogenic and neurodevelopmental processes, the WNT-centered module may reflect broader mechanisms involved in tissue organization, cellular differentiation, and developmental patterning. The intermediate position occupied by TSC2 within the molecular network further reinforces this interpretation, as this gene emerged as a potential bridge between both functional modules. Such a configuration is biologically plausible given the established regulatory role of the TSC1/TSC2 complex over mTOR activity and the growing evidence supporting crosstalk between mTOR and WNT/β-catenin signaling pathways.

Another noteworthy observation was the performance of reduced gene signatures. In several analyses, smaller signatures achieved equal or superior predictive performance compared with the complete five-gene model. This finding suggests that combining genes from biologically distinct modules may increase overall biological diversity while simultaneously reducing the specificity of disease-associated signatures. The superior performance of focused signatures such as LRP6–WNT5A and RICTOR–RPTOR–TSC2 supports the notion that biologically coherent molecular modules may provide more informative representations of disease-associated processes than larger composite signatures (Guo et al. 2015).

Finally, the recurrent identification of epilepsy-related disorders among the highest-ranked disease associations provides independent biological validation of the analytical strategy employed. Importantly, epilepsy was not explicitly weighted during model construction, yet the algorithm consistently converged toward disorders sharing epileptogenic mechanisms. This spontaneous convergence strengthens the biological relevance of the identified gene network and supports the interpretation that the observed molecular alterations are not random transcriptional events but rather reflect disease-relevant biological processes.

Several limitations should be acknowledged. The relatively small sample size limits statistical power and precludes definitive conclusions regarding genotype–phenotype correlations or regional molecular signatures. In addition, bulk tissue analysis does not permit identification of cell type-specific transcriptional alterations. SLC35A2 (and other somatic variants) genotyping was not performed, so expression could not be related to mutational burden. Nevertheless, given the rarity of MOGHE and the limited availability of surgically resected tissue, the present study provides an exploratory molecular characterization that generates biologically plausible hypotheses for future investigation.

In conclusion, our findings demonstrate that MOGHE exhibits a heterogeneous molecular profile involving genes associated with both mTOR and WNT signaling pathways. The observed variability across individuals and cortical regions suggests that biological heterogeneity is a central feature of the disease, likely reflecting the spatial distribution of somatic mosaicism within the epileptogenic cortex. Furthermore, network-based analyses support the existence of two major interacting molecular modules corresponding to the mTOR and WNT axes. Future studies employing spatial transcriptomics, single-nucleus RNA sequencing, and proteomic approaches will be necessary to clarify the cellular mechanisms underlying epileptogenesis and cortical dysregulation in MOGHE.

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Statements & Declarations

Author contributions

Conceptualization: VK and DRM; Methodology: VK, BL, PFPL, SRO, WAM, EP, RP, TTRP, and DBP; Formal analysis: VK, BL, PFPL, and DBP; Investigation: TMF, VK, AP, and TTRP; Writing – original draft preparation: VK, AP, DBP; Writing-review and editing: TMF, EP, DRM, and JCC; All authors read and approved the final manuscript.

Competing interests

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Ethics and Consent to Participate

The study was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (CAAE: 19776619.9.0000.5336; approval number 3.577.035). All participants or their legal guardians provided written informed consent prior to inclusion. All procedures were conducted in accordance with current ethical guidelines and the principles outlined in the Declaration of Helsinki and Brazilian National Health Council Resolution No. 466/12. For participants younger than 18 years of age, consent was obtained from parents or legal guardians.

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