Multimodal mapping of regional brain vulnerability to focal cortical dysplasia

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Abstract

Focal cortical dysplasia (FCD) type II is a highly epileptogenic developmental malformation and a common cause of surgically treated drug-resistant epilepsy. While clinical observations suggest frequent occurrence in the frontal lobe, mechanisms for such propensity remain unexplored. Here, we hypothesized that cortex-wide spatial associations of FCD distribution with cortical cytoarchitecture, gene expression and organizational axes may offer complementary insights into processes that predispose given cortical regions to harbor FCD.

We mapped the cortex-wide MRI distribution of FCDs in 337 patients collected from 13 sites worldwide. We then determined its associations with 1) cytoarchitectural features using histological atlases by Von Economo and Koskinas and BigBrain, 2) whole-brain gene expression and spatiotemporal dynamics from prenatal to adulthood stages using the Allen Human Brain Atlas and PsychENCODE BrainSpan and 3) macroscale developmental axes of cortical organization.

FCD lesions were preferentially located in the prefrontal and fronto-limbic cortices typified by low neuron density, large soma and thick gray matter. Transcriptomic associations with FCD distribution uncovered a prenatal component related to neuroglial proliferation and differentiation, likely accounting for the dysplastic makeup, and a postnatal component related to synaptogenesis and circuit organization, possibly contributing to circuit-level hyperexcitability. FCD distribution showed a strong association with the anterior region of the antero-posterior axis derived from heritability analysis of inter-regional structural covariance of cortical thickness, but not with structural and functional hierarchical axes. Reliability of all results was confirmed through resampling techniques.

Multimodal associations with cytoarchitecture, gene expression and axes of cortical organization indicates that prenatal neurogenesis and postnatal synaptogenesis may be key points of developmental vulnerability of the frontal lobe to FCD. Concordant with a causal role of atypical neuroglial proliferation and growth, our results indicate that FCD-vulnerable cortices display properties indicative of earlier termination of neurogenesis and initiation of cell growth. They also suggest a potential contribution of aberrant postnatal synaptogenesis and circuit development to FCD epileptogenicity.

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Introduction

Focal cortical dysplasia (FCD) type II is the most prevalent epileptogenic developmental brain malformation and a common cause of surgically amenable epilepsy.[1] This lesion is characterized

by cortical dyslamination, cytomegaly and cortical thickening,[2] likely due to atypical neuroglial proliferation, growth and migration.[3] At a molecular scale, studies in resected FCD tissue have established a causal role of somatic mutations in genes implicated in the mechanistic target of the rapamycin (mTOR) pathway[4-7]; mTOR hyperactivity disrupts neuronal migration and cortical lamination.[7] A recent multiomic study of somatic mutations in hemimegalencephaly and FCD also implicated genes related to calcium dynamics and synaptic function as potential contributors to epileptogenesis.[8]

Although FCD type II lesions may occur across the entire cortex, histopathological reports of surgically resected tissues in large cohorts[1, 9, 10] as well as a recent atlas of lesion location,[11] suggest a propensity for frontal lobe involvement. However, mechanisms underpinning this regional vulnerability remain unexplored. Notably, the developing cortex undergoes area-specific, genetically regulated neurogenesis, synaptogenesis and circuit development that give rise to variations in cytoarchitecture.[12] Given the strong genetic influence on regional cytoarchitecture,[13] it is conceivable that architectural features of the putative FCD-prone cortices may inform on the morphopathogenic characteristics of this malformation.[14] Likewise, given the substantial variability of gene expression profiles across the cortex,[15] their relation to FCD topology may provide insights into the molecular pathways contributing to the pathogenesis of this brain malformation. Furthermore, cortical organization is thought to be governed by graded macroscale axes, emerging from gene expression,[12, 16, 17] morphology and microstructure[18-21] as well as functional and structural connectivity.[22, 23] Specifically, the antero-posterior axis related to the prenatal timetable of neuroglial proliferation and growth,[24-26] results in a gradient of neuronal density, size and cortical thickness that persists throughout adulthood.[13, 20, 27] Another increasingly recognized axis marks the transition from sensory to transmodal association cortices.[17, 21, 23, 28, 29] Recapitulating classic accounts formulated in non-human primates,[30] this axis has been thought to mature during late prenatal and early postnatal stages[31] and reflect the hierarchical organization of neural function. In sum, cortex-wide spatial associations of FCD distribution with cortical cytoarchitecture, gene expression and organizational axes may offer complementary insights into the neurogenic processes that predispose given cortical regions to harbor this developmental malformation.[14, 29]

Whole-brain cross-modal associations are facilitated by the availability of human brain atlases based on histological features[32-34] and spatiotemporal gene expression profiles.[35, 36] The overall

purpose of this work was to investigate the intrinsic regional vulnerability of cortices harboring FCD. To this end, we mapped the cortex-wide lesional distribution of a multicentric dataset collected from epilepsy centers worldwide, determined cellular and genetic factors based on postmortem histology and transcriptomics, and examined the embedding of FCD lesions within the axes of neurogenic patterning and structure-function hierarchy. Specifically, after creating a topographic map of FCD type II lesions on MRI-derived cortical surface models, we cross-referenced it against histological taxonomies[32, 33] and a 3D high-resolution human brain histological model.[34] In parallel, we performed spatial correlation with whole-brain gene expression data from the Allen Human Brain Atlas[35] and examined spatiotemporal gene expression dynamics from prenatal to adulthood stages using the PsychENCODE BrainSpan, an independent development-targeted genetic dataset.[36, 37] Targeted gene enrichment analysis probed transcriptomic associations for previously known pathogenic FCD variants,[3, 38, 39] as well as non-FCD epilepsies[40] and other neurological disorders. Finally, we contextualized the FCD distribution within the antero-posterior axis previously associated with genetic cortical patterning and timetable of neurogenesis,[13, 24-26] contrasting these findings with hierarchical cortical axes derived from myelin-sensitive MRI[28] and resting-state MRI functional connectivity.[23]

Materials and methods

Study design and participants

We studied a consecutive retrospective cohort of 337 patients (153 females; mean±SD age = 22.2±12.7 years) with histologically verified FCD lesions collected from 13 tertiary epilepsy centers worldwide. All patients had been investigated for drug-resistant epilepsy with a standard presurgical workup including assessment of seizure history, routine MRI and video-EEG recordings. Histological examination of the surgical specimen[2] determined FCD type II as disrupted cortical lamination with dysmorphic neurons in isolation (IIA, n=134) or together with balloon cells (IIB, n=203). Site-specific demographics are summarized in **Table 1**. The Ethics Committees and Institutional Review Boards at all participating sites approved the study, and written informed consent was obtained from all patients.

MRI acquisition and processing

All patients had high-resolution 3D T1-weighted MRI (T1w) acquired as a part of the clinical presurgical investigation, consisting of images with isotropic 1x1x1 mm voxel resolution.[41] Data underwent intensity non-uniformity correction and normalization, and linear registration to the ICBM MNI152 symmetric template. To generate cortical surface models, we applied the Constrained Laplacian Anatomic Segmentation using Proximity algorithm, yielding GM-WM and GM-CSF surfaces with 41k surface points (or vertices) per hemisphere.[42] Surface-based registration, which aligns individual participants based on cortical folding, was performed to optimize vertex-wise anatomical correspondence across participants.[43]

Cortex-wide MRI mapping of FCD lesions

Two experts (AB, NB) independently segmented each FCD lesion on the 3D MRI registered onto the ICBM MNI152 template. The consensus labels (the union of the two segmentations; inter-rate Dice index: 0.94±0.13) was intersected with the cortical surfaces to generate surface-based FCD labels. To enhance regional sensitivity while retaining specificity, labels were minimally smoothed using a surface-based 4 mm full width at half maximum Gaussian kernel to maximize local specificity.[44] We then calculated for each vertex the FCD probability, defined as the percentage of patients whose lesion label coincided with that vertex. To assess *within-sample* reliability, we calculated bootstrap certainty at each vertex, defined by mean of lesion probability from the bootstrap subsamples divided by their standard deviation. Similarly, we assessed *cross-site* reliability as defined by the mean divided by the standard deviation from leave-one-site-out subsamples. We assessed the lobar distribution by counting the number of FCD lesions located within each lobe; to account for lobar size, we divided the lesion counts by the relative surface areas of each lobe, defined based on automated anatomical labelling parcellation atlas.[45]

Association analyses

Histological atlases

To assess associations of regional FCD probability with histological markers, we used the von Economo-Koskinas MRI atlas (http://dutchconnectomelab.nl) indexed with quantitative histological information (cell size, cell density and cortical thickness) of 43 cortical regions per hemisphere.[33] For independent validation, we leveraged the BigBrain atlas, a 3D reconstruction of a stained post-mortem human brain[34]; this histological data, mapped to intracortical surface models in standard space and to the Schaefer 400 parcellations,[46] were obtained from https://github.com/MICA-MNI/micaopen/tree/master/bigbrain.

Cortex-wide gene expression

To investigate the molecular properties of cortical vulnerability, we related the FCD distribution with the anatomically comprehensive gene expression data from Allen Human Brain Atlas (AHBA; six postmortem adult brains; 1 female; age = 42.5±13.4 years; https://human.brain-map.org),[35] which was mapped onto the 308 parcels of the Desikan-Killiany atlas (DKA).[47] The microarray data of these donors were acquired using ~500 samples per hemisphere, with each sample indexed with expression levels for ~60,000 genes from at least two probes. Following an established procedure,[48] the Maybrain package (https://github.com/rittman/maybrain) matched the closest AHBA sample in each donor to the centroids of 308 parcels of equal area (500 mm^2) averaged across donors. Notably, data were averaged across probes corresponding to the same gene, excluding those not matched to gene symbols in the AHBA data. To reduce inter-donor variability, expression data for each probe were normalized through z-transformations across the 308 DKA parcels within each donor. The final output was a matrix of z-scored expression values for each of 20,737 genes mapped onto the 308 DKA parcels.

Spatiotemporal gene expression

We determined how genes associated with the FCD distribution are spatially and temporally regulated throughout the pre- and postnatal development. To this purpose, we used

PsychENCODE BrainSpan (http://development.psychencode.org),[36] a dataset including tissue-level mRNA-sequencing of 607 samples across 16 anatomical brain regions of 41 postmortem human brains ranging from 8 postconceptional weeks to 40 postnatal years (18 females; postmortem interval = 12.9±10.4 hours; tissue pH = 6.5±0.3; RNA integrity number = 8.8±1). After bulk tissue mRNA-sequencing, this dataset has yielded expression levels for 60,154 genes. The final output consisted of a matrix of reads per kilobase million transcript expression level for each of 17,584 genes overlapping with the 20,737 genes from the AHBA atlas.

Developmental axes of cortical network organization

Gradient axes of cortical structural and functional network organization are shaped by gene expression and cytoarchitecture during the pre- and postnatal development. The antero-posterior axis relates to the prenatal timetable of neurogenesis and growth[24-26]; we derived this axis from a heritability analysis of structural covariance networks[13] mapped on the Schaefer 400 parcellations.[46] Structural and functional hierarchical axes are thought to mature during late prenatal and early postnatal circuit development[31]; we derived these axes from MRI-based covariance of microstructural profiles[28] and resting-state functional connectivity,[23] which we mapped to the Schaefer 400 parcellations using the BrainSpace toolbox (https://github.com/MICA-MNI/BrainSpace).[49] The FCD distribution and developmental axes were mapped to Schaefer 400 parcellations prior to correlation analysis to achieve anatomical correspondence between them.

Statistical analysis

Multivariate analysis

Cortex-wide linear models assessed associations of regional FCD probability with histological markers and neurodevelopmental axes. For the gene expression analysis, given the high dimensionality of AHBA data, we used partial least squares (PLS) regression, a multivariate linear model, to uncover weighted combinations of genes (or PLS components) that best explained the regional variance in FCD probability. The statistical significance of the variance explained by the

PLS components was tested based on 10,000 spin permutations of the FCD distribution, accounting for spatial autocorrelations.[50] The regional expression profile of each PLS component was defined as the average of the spatial expression profile of 20,757 genes, adjusted by their PLS weight; weight stability was estimated by dividing the PLS weight by the bootstrap SD.

Enrichment analysis

A web-based gene set analysis toolkit (https://webgestalt.org)[51] was utilized to uncover biological processes enriched in the list of genes whose bootstrap weights (absolute value) were ranked within the top 10 percentile of 20,757 genes. In other words, this analysis quantified the significance and enrichment ratio, namely the number of PLS-derived genes overlapping with each biological process divided by the number of genes expected to overlap by random permutations.

Spatiotemporal gene expression profiles

Using the PsychENCODE BrainSpan dataset, we calculated the spatiotemporal profile for each PLS component obtained in the gene expression analysis. This profile, defined as the regional average of each gene's expression level weighted by its bootstrap weight, was obtained across 16 cortical regions and timepoints based on major neurodevelopmental milestones derived from whole-brain transcriptomic signatures.[52] Student's t-tests compared the expression levels between time windows, and between different regions within time windows.

Specificity analysis

We assessed whether known genes of the pathways causing FCD via somatic mutations were enriched in the PLS components, including the PI3K-AKT-mTOR pathway,[5, 6, 38, 53, 54] PI3K-PTEN-AKT-TSC-RHEB pathway,[6, 53, 55-57] TSC1-TSC2 complex,[58-61] GATOR1 complex[6, 55, 57, 59, 62-65] and other reported variants (IRS1, RAB6B, ZNF337, RALA and HTR6).[61] These genes are listed in **Supplementary Table 1**. We also assessed associations with risk genes of focal epilepsy with hippocampal sclerosis, generalized epilepsy and all epilepsies as determined by a recent genome-wide association study,[40] neurodevelopmental conditions, namely autism[66] and bipolar

spectrum.[67] Finally, our specificity analysis included frontotemporal dementia[68] due to the preferential involvement of the frontal lobe.

For each PLS component, we quantified the enrichment ratio (defined as the difference between the mean bootstrap weight of the candidate genes and the mean bootstrap weight of the same number of randomly permuted genes), which was then divided by the standard deviation weight of the permutated genes. Significance was determined by percentile of the bootstrap weight of the candidate genes relative to the bootstrap weights of randomly selected genes from 10,000 permutations. Positive/negative ER of a given condition indicates that the risk genes are expressed to a higher/lower degree relative to the baseline expression level. In addition, the function of the risk genes needs to be considered when interpreting ER. For example, the FCD candidate genes are inhibitory regulators of mTOR pathway; thus, negative ER for these genes indicates activation of mTOR pathway.

Corrections for multiple comparisons

For all spatial correlation analyses, findings were corrected using spin permutation tests at $p_{spin}=0.05$.[50] Remaining results were corrected for multiple comparisons using false discovery rate (FDR) at 0.05.[69]

Data availability

The data supporting findings of this study are available from the corresponding author upon request. The datasets are not publicly available as they contain information that could compromise privacy of research participants.

Results

Cortex-wide MRI distribution of FCD

The vertex-wise MRI mapping of FCD lesions across the cortex (**Figure 1**) showed aggregation within the frontal lobe, particularly in prefrontal (dorsolateral, ventrolateral, dorsomedial and medial frontopolar; Brodmann areas 4, 9, 10, 44, 45, 46, 57) and cingulate (anterior-mid and pre-genual; Brodmann areas 24, 32, 33) cortices. The reliability of these areas was supported by higher within-sample and cross-site certainty as compared to the other regions. Lobar mapping also confirmed higher occurrence in the frontal lobe compared to other areas, even after normalizing for lobar surface area.

Association between FCD distribution and cytoarchitecture

With respect to the von Economo and Koskinas data (**Figure 2**), mapping 43 regions per hemisphere, we found a positive correlation between FCD distribution and cortical thickness (R=0.35, p_{spin}<0.05) and cell size (R=0.46, p_{spin}<0.05) and a negative correlation with cell density (R=-0.52, p_{spin}<0.001). We also found a negative correlation with cell density obtained from the BigBrain atlas (R=-0.34, p_{spin}<0.01). In other words, frontal lobe areas with the highest probability of lesions were those displaying lower neuronal density, larger neurons, and higher cortical thickness.

Transcriptomic associations and relation to spatiotemporal gene expression

Two PLS components explained 25% (PLS-1: p_{spin}<0.001) and 27% (PLS-2: p_{spin}=0.03) of the covariance between the FCD probability and AHBA gene expression (**Figure 3**). As shown by the gene enrichment analysis, PLS-1 reflected regulation at epigenetic, RNA and post-translational levels, as well as covalent chromatin modification and chromosome organization (FDR<0.05), both critical for mitotic cell division and differentiation. Conversely, PLS-2 was mainly

characterized by general synaptic organization and activity (*FDR*<0.05) and marginally by glutamate receptor signaling (*FDR*<0.1).

Evaluating the developmental spatiotemporal trajectories, the expression of genes associated with PLS-1 sharply increased from early to late fetal stages (*FDR*<0.05), plateaued during infancy and childhood and decreased thereafter (*FDR*<0.05). Conversely, the expression of genes associated with PLS-2 showed a monotonic increase from early fetal stage to adulthood (*FDR*<0.05). Expressions were more marked in the frontal lobe, with a fronto-occipital gradient for PLS-1 and a fronto-temporal gradient for PLS-2. We did not find differential associations between early and late onset lesional distribution and the PLS components.

Supplemental Table 1 lists the risk genes used for each condition. Specificity analysis revealed that PLS-1 and PLS-2 were enriched for the risk genes of all epilepsies (PLS-1: *FDR*=0.08; enrichment ratio, *ER*=-2.60; PLS-2: *FDR*<0.001, *ER*=-3.01), with PLS-1 additionally enriched for genes causing FCD via somatic mutations (p<0.05, *ER*=-1.99) and risk genes of generalized epilepsy (*FDR*=0.08, *ER*=-2.6). Neither PLS showed associations to genes for focal epilepsy with hippocampal sclerosis, frontotemporal dementia, bipolar or autism spectrum disorders; **Supplemental Table 2** provides uncorrected p values for the enrichment of the GWAS risk genes.

Relation to developmental axes of cortical organization (Figure 4)

The multisite-derived FCD distribution showed a strong positive association with the anterior region of the antero-posterior axis derived from heritability analysis of inter-regional structural covariance of cortical thickness (R=0.51, p_{spin}<0.001), but not with structural (R= 0.12, p=0.37) and functional (R=-0.07, p=0.92) hierarchical axes.

Discussion

We systematically investigated the cellular, genetic and organizational features of cortices harboring FCD. Mapping the cortex-wide MRI distribution of 337 histologically-verified lesions collected from 13 sites worldwide, we found a propensity for the frontal lobe. Associations with

histological markers derived from Von Economo and Koskinas and BigBrain atlases showed that in the healthy brain these areas display lower neuronal density, larger neurons and thicker cortices. Using whole-brain and spatiotemporal gene expression datasets, we identified two genetic factors related to FCD distribution: one defined by prenatal regulation of gene expression and chromosome organization and another related to postnatal synapse organization and activity driving neural circuits.[70] At macroscale, FCD distribution was associated with the antero-posterior organizational axis reflective of the timetable of neurogenesis. Concordant with a causal role of atypical neuroglial proliferation and growth, our results indicate that FCD-vulnerable cortices display cytoarchitectural, molecular and organizational properties indicative of earlier termination of neurogenesis and initiation of cell growth. Our findings also suggest a potential contribution of postnatal synaptogenesis and circuit development to FCD epileptogenicity.

While propensity for frontal lobe involvement is in keeping with previous observations,[1, 9-11] our multisite dataset refined this knowledge by demonstrating locoregional vulnerability of prefrontal and fronto-limbic cortices, the consistency of which was supported by high within-sample and cross-site reliability. Notably, normalizing for lobar surface did not modify results, attesting that such susceptibility is not merely due to the frontal lobe's larger size, but rather linked to intrinsic developmental, likely multifactorial vulnerability. With respect to cytoarchitectural markers, frontal cortices are typified by lower neuronal density, larger cell soma and thicker gray matter. Given that these are also key histopathological traits of FCD,[2, 71] the association we found may hint at potential pathophysiological developmental processes linked to intrinsic anatomical characteristics of the prefrontal and fronto-limbic cortices. In this context, the timetables of neurogenesis and synaptogenesis of the prefrontal cortices are distinct from other cortices,[72] as they undergo earlier initiation of proliferation, transition from symmetric (cloning) to asymmetric (differentiation) division, reduction of cell cycle rates and termination of neurogenesis, resulting in lower neuronal density. This is followed by early initiation of neuronal growth leading to larger soma and more complex dendritic arborization of frontal relative to occipital cortices.[24-26] Hence, although subtle somatic mutations can occur randomly throughout the developing cortex,[73] this tighter regulation of neurogenesis in the frontal cortex may explain its heightened susceptibility to harboring FCD. This longer period of cell growth sets the basis for the frontal neurons to undergo a longer period of synaptogenesis,[72, 74-76] resulting in the overproduction of synapses and a protracted period of pruning.[74, 75, 77, 78] Similarly, limbic cortices, marked by agranular or

dysgranular laminar patterns, develop earlier and undergo longer period of synaptic plasticity through adulthood relative to the isocortex.[79, 80] Fronto-limbic cortices have shown vulnerability for other developmental disorders, such as schizophrenia[81, 82] and autism,[83-86] while temporo-limbic cortices preferentially harbor neurodegenerative disorders, namely Alzheimer's and Parkinson's diseases.[87-90] Interestingly, tau pathology has been suggested to mediate premature neurodegeneration and cell injury in FCD,[91, 92] The frontal and limbic regions have been shown to become central hubs in the mature cortical network architecture, which also render themselves vulnerable to structural pathology in numerous lesional and degenerative conditions.[93, 94]

Contextualizing lesional distribution within axes of developmental cortical organization revealed that FCD preferentially occurs in the rostral portion of the anterior-posterior axis defined by genetically determined inter-regional synchrony of cortical development.[13, 95, 96] Given that this axis reflects the prenatal timetable of neurogenesis and cell growth, the rostral concentration of FCD supports the predisposing roles of aberrant neurogenesis and cell growth as contributors to the histopathological makeup of FCD. In contrast, FCD distribution was disassociated from the sensory-association axis established during late prenatal and postnatal neural circuit development,[31] a finding consistent with the prenatal occurrence of this malformation.[3] A potential genetic underpinning of FCD distribution was also suggested assessing associations to whole-brain gene expression. Indeed, transcriptomic associations based on data-driven PLS regression uncovered a component (PLS-1) reflecting regulation of gene expression at epigenetic, RNA and post-translational levels, as well as covalent chromatin modification and chromosome organization. Chromatin architecture is tightly coupled to mitotic cell cycle and fate. As such, its modification regulated by epigenetic, transcriptional and post-transcriptional mechanisms plays a key role in cell division[97] and differentiation.[98] Chromosome organization, which involves assembly, arrangement or disassembly of chromosomes, is the process that allows the parent cell to replicate its DNA such that each daughter cell receives a copy during mitosis.[99] Therefore, within the cortex, PLS-1 likely represents molecular mechanisms underpinning neuroglial proliferation and differentiation. On the other hand, PLS-2 was related to general synaptic organization and activity, circuit organization,[37] as well as glutamate receptor signaling. Evaluating the developmental spatiotemporal trajectories, PLS-1 expression sharply increased from the early fetal stage to late fetal stage, while PLS-2 expression showed steady increase from fetal stages to adulthood. The relevance of these PLS components was supported by the disease

specificity analysis. Indeed, while PLS-1 and -2 were both associated with risk genes for all epilepsies, PLS-1 was additionally associated with genes causing FCD via somatic mutations and risk genes of generalized seizures, Therefore, on one hand, it is conceivable that PLS-1 may indicate early cortical vulnerability to aberrant neurogenesis and cell growth, ultimately resulting in a dysplastic lesion. On the other hand, PLS-2 may account for the susceptibility to aberrant synaptogenesis and neurotransmitter systems that for hyperexcitable circuits during a latent period following the precipitating lesion,[100] thereby promoting epileptogenesis. Although synaptic and white matter maturation have been postulated to contribute to FCD occurrence,[101] the presented work is the first to provide evidence for the role of postnatal synaptogenesis and circuit development for FCD epileptogenesis.

Associations with cytoarchitecture, whole-brain and spatiotemporal gene expression, as well as macroscale organizational axes, collectively suggest a vulnerability continuum spanning from prenatal neurogenesis and cell growth to postnatal synaptogenesis. Although age at epilepsy onset has been postulated to account at least partly to variability in FCD histological features,[102] the link to molecular or cellular pathogenic processes remains still unclear. In our study, while we did not find differential associations between early and late disease onset lesional distribution with the PLS components, our findings clearly establish developmental underpinnings of FCD occurrence. To date, a plethora of molecular studies of resected FCD tissues have established a causal role of somatic variants that lead to hyperactivity of the mTOR pathway.[5, 38, 39, 57, 59, 61, 103-105] A recent large-scale multiomic study of somatic mutations suggested genes implicated in calcium dynamics and synaptic function as potential causes for epileptogenesis.[8] Nevertheless, given that the variant allelic frequency is typically below 5% in FCD, uncovering variants distinct from mTOR pathway may be difficult, even with a large sample of resected lesions,[59] Notably, the present study circumvents this logistical and statistical burdens by identifying the genetic fingerprints of the FCD-prone cortices based on noninvasive imaging and offers novel insights that may be difficult to obtain otherwise. It has been shown that somatic activating mutations in the mTOR pathway causes a continuum of malformations, spanning from hemimegaloencephaly to posterior quadrantic dysplasia. Although these malformations share some of the genetic determinants with FCD, the time of molecular insult, as well as additional genetic mutations, may lead to varying phenotypes, as suggested by the two-hit germline and somatic mechanisms in

hemimegaloencephaly.[57] As for the posterior quadrantic dysplasia, prolonged neurogenesis in the posterior isocortex involving higher number and rate of proliferation cycles translates to a greater amplification of abnormal founder cells lesion.[106] Subtle structural, possibly neurodevelopmental anomalies have been reported in generalized genetic epilepsy (GGE) and have been described as microdysgenesis in neuropathological studies [107, 108] that share histological similarity with FCD Type IA.[109] However, such reports have been sparse, as GGE patients generally do not undergo surgery. Furthermore, the replicability of identifying microdysgenesis in GGE has been limited, thereby not establishing it as a common feature of this condition.[110] In terms of genotype-phenotype associations, while the cellular mechanisms that drive the histopathological features of dysplasia are being elucidated,[7] those underlying circuit-level alterations that drive recurrent seizures in this condition remain elusive. Conceivably, mitigating the circuit-level alterations precipitated by FCD may reduce seizures.[100] Hence, future work should elucidate the molecular and cellular mechanisms of aberrant postnatal synaptogenesis that drive circuit hyperexcitability and identify novel therapeutic targets, possibly combined with mTOR inhibitors, for improved seizure control.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Cortex-wide FCD distribution. A. For each patient, the FCD lesion was manually segmented on MRI and mapped onto its cortical surface. **B.** Map of FCD distribution. **C.** Reliability analysis. Within-sample and cross-site robustness of regional FCD probability is high where the FCD probability is high. **D.** Lobar distribution. The spider plot of the FCD distribution across lobes demonstrates remarkable preference towards the frontal lobe, which holds after normalizing for the surface area of each lobe (dotted line).

Figure 2 Associations between FCD distribution and histological measures. Plots show correlations between FCD probability and cortical thickness, cell size, and cell density derived from the Von Economo-Koskinas atlas (**A**), as well as cell density (in arbitrary units, a.u.) indexed by optical density of silver-stained cells in the BigBrain atlas (**B**). In the scatterplots, x- and y-axes represent FCD probability (in %) and histological quantities, respectively; dots indicate 308 parcels of the Desikan-Killiany atlas. Color-coding is identical for brain maps and dots; p_{spin} indicates p value after adjusting for spatial autocorrelation.

Figure 3 Cortex-wide association between FCD topography and gene expression. A. Partial least squares (PLS) regression identified weighted combinations of genes, or PLS components, and their spatial expression profiles that best explained the regional variance in FCD distribution, or percent variance explained; p_{spin} indicates p value after adjusting for spatial autocorrelation). Inputs to PLS include the whole-brain gene expression data matrix (parcels by genes) and FCD distribution across parcels (in %). Outputs include gene weights (genes by components), gene spatial profiles (parcels by components) and percent variance explained by PLS components. **B.** Maps of gene expression. The color scale indicates the score for PLS-1 and 2, namely the weighted average expression level of 20,737. **C.** Gene enrichment analysis. Genes associated with PLS-1 were enriched for epigenetic, RNA and post-translational levels as well as covalent chromatin modification and chromosome organization; and PLS-2 for general synapse organization and activity. In the volcano plots, x-axis indicates $\log_2$ of enrichment ratio and y-axis indicates $-\log_{10}$

of FDR. Color codes indicate the number of genes related to the biological processes that overlap with the input list of top 10 percentile genes; upper/lower dotted lines indicate FDR=0.05/0.1. **D**. Developmental spatiotemporal trajectory. The expression of genes associated with PLS-1 sharply increased from early to late fetal stages, plateaued during infancy and childhood, and decreased thereafter. Conversely, PLS-2 showed monotonic increase from early fetal stage to adulthood. In both instances, expressions were more marked in the frontal lobe. Dots represent cortical samples at a given timepoint color-coded by lobes; dotted lines connecting dots correspond to the same region of interest. Thick colored lines connect the average of samples within each time window, thereby showing the overall trajectory. Asterisks indicate FDR<0.05. **E.** Specificity analysis. PLS-1 was significantly enriched for FCD pathogenic genes; the histogram shows bootstrap weights of 10,000 permutations; the dotted line indicates the bootstrap weight of the candidate genes. In relation to GWAS-risk genes, PLS-2 (blue) was enriched for genes associated with all epilepsies, while PLS-1 (red) was marginally enriched for those associated with all and generalized epilepsies. Top dotted line indicates FDR = 0.05; bottom dotted line indicates FDR = 0.1.

Figure 4 Relation to developmental axes of cortical organization. FCD distribution showed a strong association with the anterior region of the antero-posterior axis derived from heritability analysis of inter-regional structural covariance of cortical thickness (**A**), but not with structural (**B**) and functional (**C**) hierarchical axes. X and y- axes represent the FCD probability (in %) and the rank along the gradient axes, also represented as maps. The color scale represents the percentage of patients in whom the FCD is located at a given vertex.

Table 1 Site-specific demographics

	Sample size (n)	FCD IIA/IIB	Age (years)	Sex (female/male)	Age at onset (years)
All	337	134/203	22.2 ± 12.7	153/184	7.6 ± 6.7
S1	114	55/59	24.8 ± 10.5	56/58	9.1 ± 7.1
S2	8	3/5	10.5 ± 6.4	2/6	5.5 ± 4.2
S3	10	2/8	25.3 ± 14.2	5/5	7.2 ± 7.4
S4	43	6/37	24.3 ± 14.4	20/23	7.3 ± 7.6
S5	18	9/9	6.8 ± 5.6	8/10	5.6 ± 4.1
S6	22	13/9	17.4 ± 13.5	8/14	5.0 ± 4.8
S7	11	4/7	30.8 ± 14.0	7/4	4.1 ± 3.1
S8	14	3/11	29.1 ± 11.8	5/9	7.5 ± 5.6
S9	8	0/8	31.9 ± 15.3	3/5	8.9 ± 4.7
S10	14	7/7	25.3 ± 7.5	6/8	9.9 ± 5.6
S11	11	6/5	20.8 ± 6.8	7/4	6.8 ± 8.2
S12	42	17/25	17.0 ± 10.7	17/25	6.6 ± 5.8
S13	22	9/13	20.9 ± 15.5	9/13	7.1 ± 8.6

Data for age and age at onset indicate mean ± standard deviation.

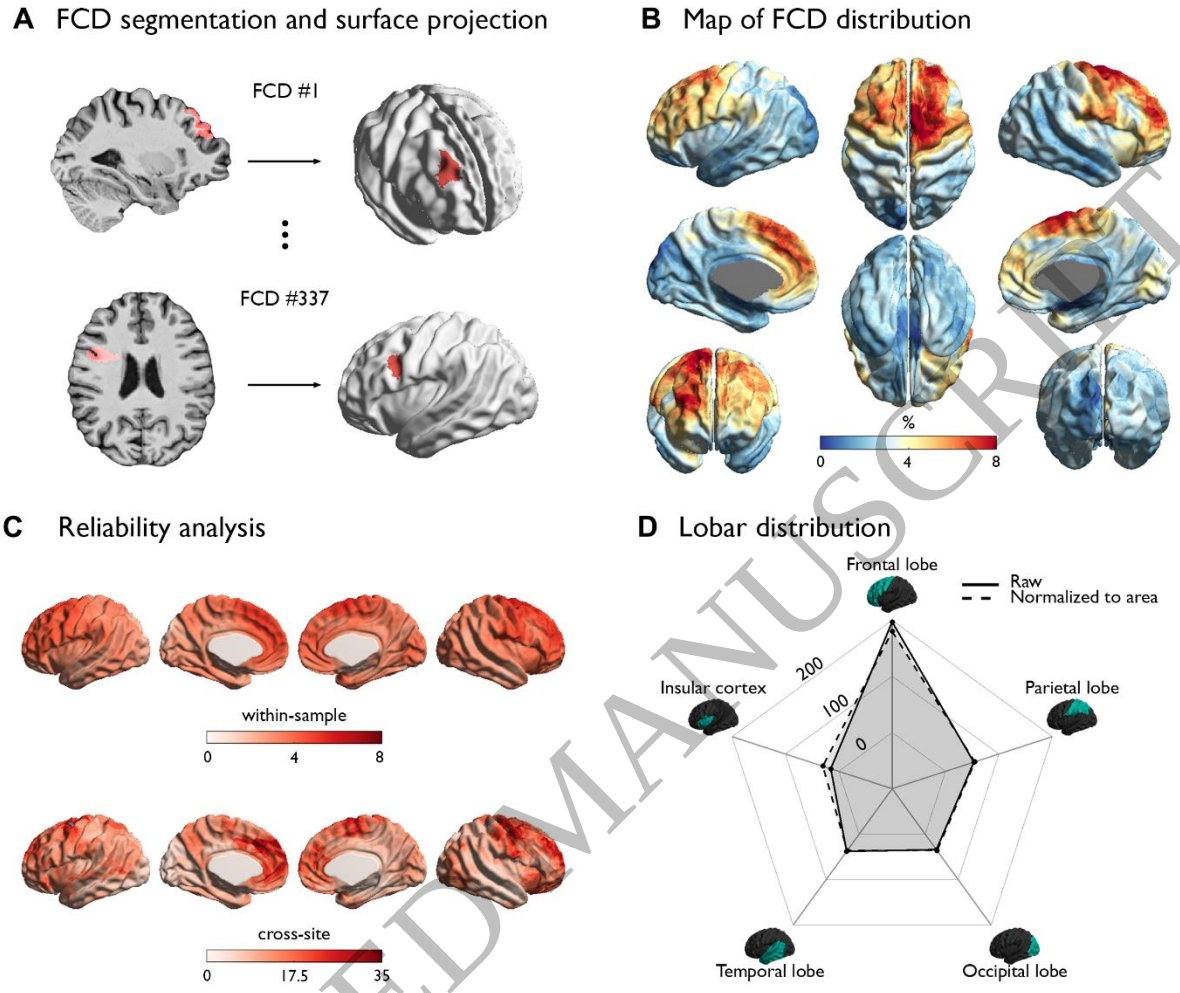

Figure 1
159x135 mm (x DPI)

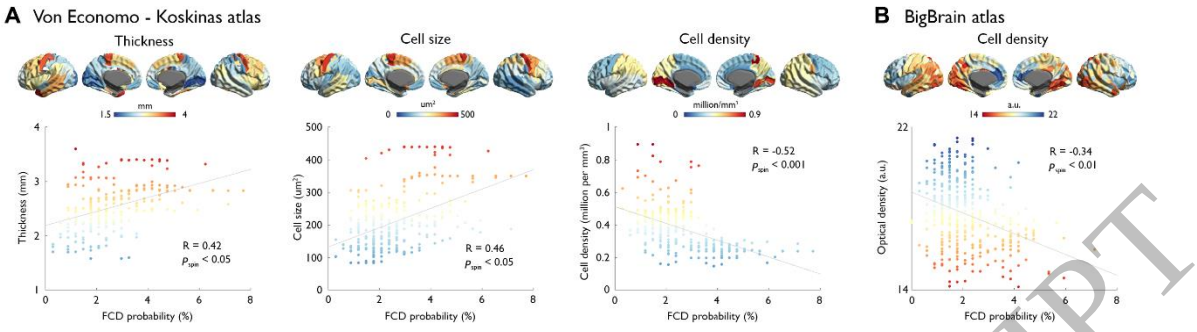

Figure 2
159x44 mm (x DPI)

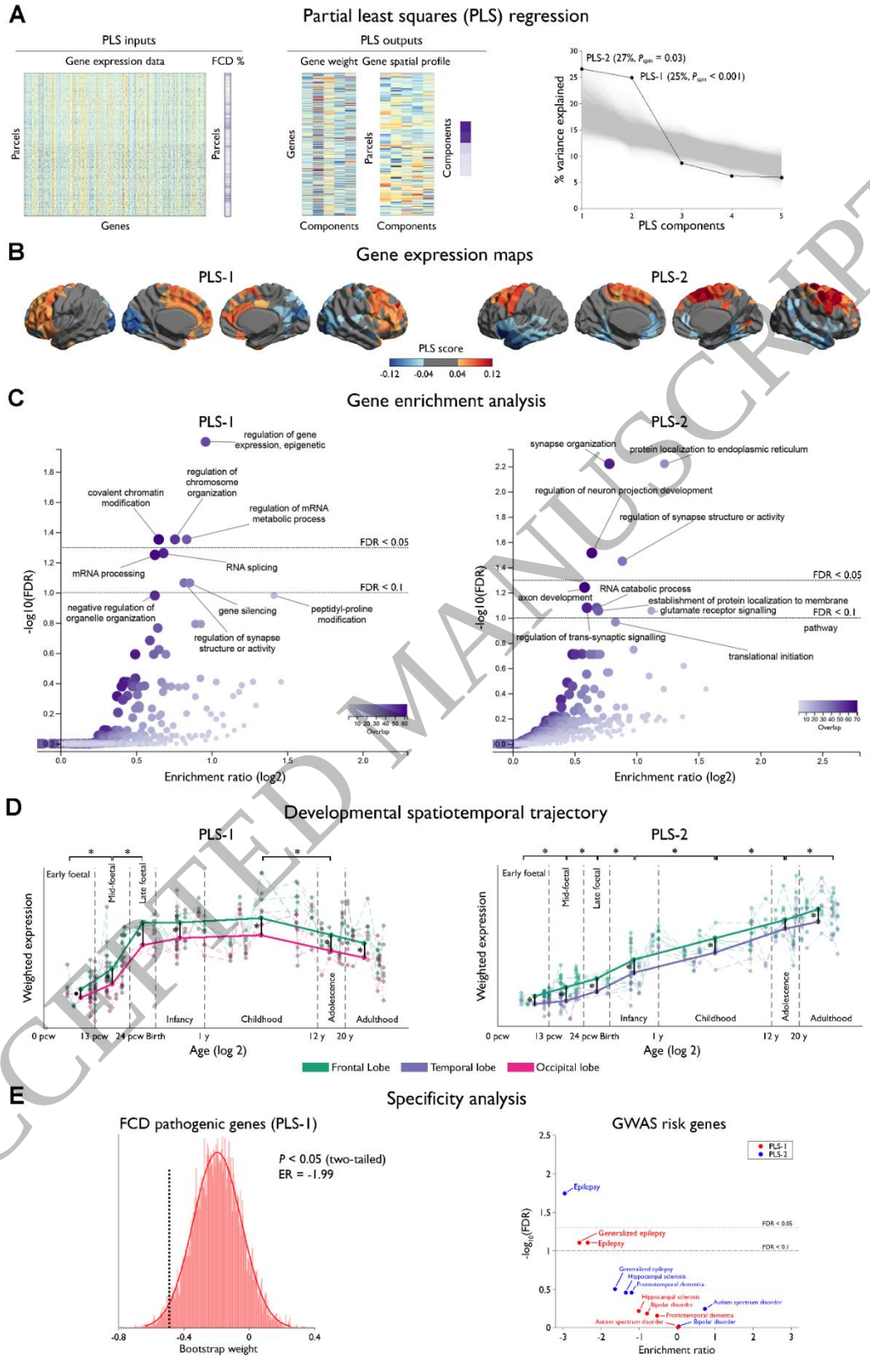

Figure 3
158x246 mm (x DPI)

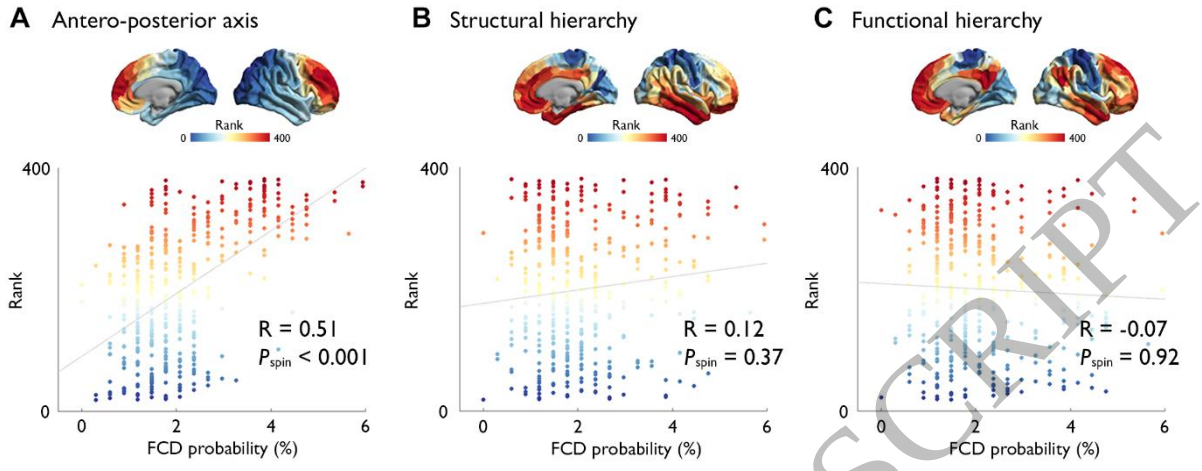

Figure 4
159x63 mm (x DPI)