

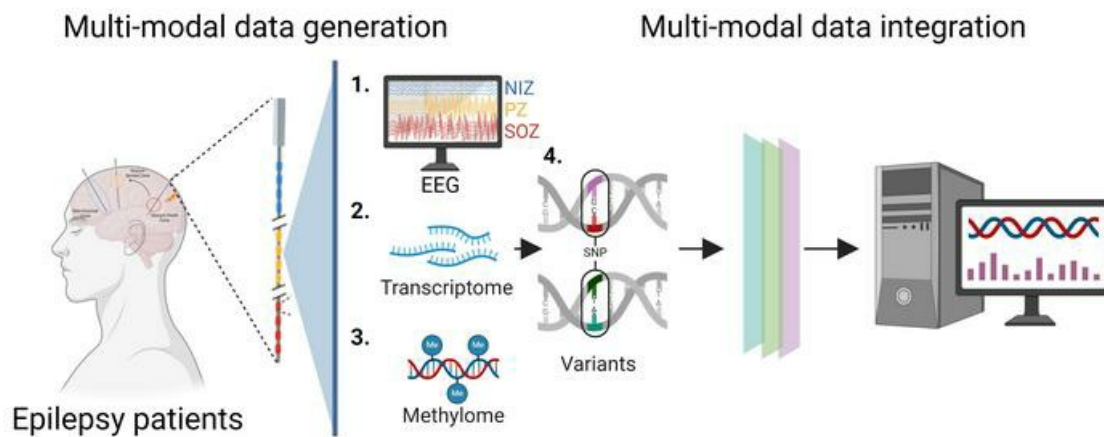
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High-Resolution Multimodal Profiling of Human Epileptic Brain Activity via Explanted Depth Electrodes

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Conflict of interest

PWW has been a paid speaker for Jazz Pharmaceuticals (epidiolex) and Angelini (ontozry).

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Abstract

The availability and integration of electrophysiological and molecular data from the living brain is critical to understand and diagnose complex human disease. Intracranial stereo electroencephalography (SEEG) electrodes used for identifying the seizure focus on epilepsy patients could enable the integration of such multimodal data. Here, we report MoPEDE (Multimodal Profiling of Epileptic Brain Activity via Explanted Depth Electrodes), a method that recovers extensive protein-coding transcripts, including cell-type markers, DNA methylation and short variant profiles from explanted SEEG electrodes matched with electrophysiological and radiological data allowing for high-resolution reconstructions of brain structure and function. We find gene expression gradients that correspond with the neurophysiology-assigned epileptogenicity index but also outlier molecular fingerprints in some electrodes, potentially indicating seizure generation or propagation zones not detected during electroclinical assessments. Additionally, we identify DNA methylation profiles indicative of transcriptionally permissive or restrictive chromatin states and SEEG-adherent differentially expressed and methylated genes not previously associated with epilepsy. Together, these findings validate that RNA profiles and genome-wide epigenetic data from explanted SEEG electrodes offer high-resolution surrogate molecular landscapes of brain activity. The MoPEDE approach has the potential to enhance diagnostic decisions and deepen our understanding of epileptogenic network processes in the human brain.

Introduction

An improved understanding of human brain function requires systems-level approaches that sample and integrate multimodal data spanning several orders of magnitude from single molecules, through individual cells to local networks and up to higher order organisation.(1,2) Combining RNA sequencing and genome architecture maps with *in vivo* recording technologies and brain imaging enables the production of high-resolution reconstructions of the structure and function of the mammalian brain in health and disease. Indeed, increasingly comprehensive taxonomies of the mammalian brain have been achieved by integrating sequencing approaches with other modalities. These studies have demonstrated neuronal phenotypes including morphology, location and electrophysiologic properties are strongly defined by cell type-specific transcriptional and epigenetic signatures for different neuron subtypes,(3-5) across different layers of the cortex,(3,4,6) and structures throughout the mouse brain including the hippocampus.(5-7) Transcription factors are a principle driver of cellular diversity, regional specialisation of function and neurotransmitter type which in turn establishes the electrophysiological properties of different cell types.(7) Equivalent mapping exercises for the human brain have emerged, with recent single-cell transcriptional and epigenetic studies revealing the distinct molecular programs that define neuronal and non-neuronal cell type and diversity, network and regional organisation, and complexity.(8-10)

Treatment-resistant epilepsies represent unique opportunities for access to the living human brain. *Ex vivo* electrophysiologic, pharmacologic and gene expression profiling of surgically-resected material has enabled important advances in brain function in health and disease.(11-13) The analysis from implanted electrodes, placed to guide surgical decisions has also enabled studies of human brain function(14), including elucidating network

behaviour during seizure onset,(15) and how epileptiform activity can interfere with memory.(16) Intracranial electroencephalography (EEG) recordings from stereotactically-implanted electrodes (known as stereoelectroencephalography or SEEG) are performed for a subset of patients with difficult-to-localize focal epilepsy to identify seizure-onset zones. The implantation of typically between 5 – 15 electrodes with multiple contact sites into deep brain structures yields necessary spatial and temporal mapping of hyperexcitable epileptogenic tissue, enabling localization of the seizure onset zone, the associated propagation zones and differentiation from the normal brain.(14,17) The neurophysiologic findings are then combined with imaging, computational tools and clinical information to guide surgical decisions, with operations performed upon explantation of SEEG electrodes.(17-19) Researchers recently reported that DNA present on explanted SEEG electrodes can be used to identify somatic mutations in pre-resection epilepsy patients.(20-22) Notably, a gradient of mosaic gene variation may be present in relation to recorded epileptiform activity.(21) Mapping epigenetic marks using such material may also have utility for subtyping malformations of cortical development such as focal cortical dysplasia (FCD).(23) Assembling comprehensive molecular architectures that comprise read-outs of gene activity, for example, seizure-regulated gene transcripts or inflammatory signals, in combination with recorded neurophysiology would offer powerful new insights into human brain function and causal mechanisms of epilepsy, and potentially support surgical decision-making. There are, however, several unknowns. What additional nucleic acids can be reliably obtained from explanted electrodes, in particular mRNA transcripts, and do these correspond to epigenetic marks that influence chromatin state? Do the signals retain information about cell types and implantation locations? Finally, epilepsies are heterogeneous in clinical presentation, semiology and underlying mechanism so can this

approach be applied across surgical candidates with different aetiologies and is it scalable from highly focal epilepsies through to those affecting larger structures and networks? Indeed, defining the extent of epilepsy with existing technology is one of the greatest challenges in epileptology and hence, novel methodological developments are urgently required.

Here we report a method called MoPEDE (Multimodal Profiling of Epileptic Brain Activity via Explanted Depth Electrodes) in which an extensive repertoire of protein-coding transcripts including immediate early genes and mediators of inflammation, DNA methylation and variant profiles can be recovered from explanted SEEG electrodes matched with recorded neurophysiological and radiological data. Our findings provide proof-of-concept that RNA profiles and genome-wide epigenetic surveillance can be obtained from explanted SEEG electrodes and these provide surrogate molecular landscapes of human brain activity at high resolution that may support diagnostic decisions as well as improve our understanding of the epileptogenic process in the live human brain.

Results

Multimodal profiling of SEEG electrode surfaces from epilepsy patients

The MoPEDE method utilizes intracranial SEEG electrodes to measure electrophysiological activity in various brain regions, including deeper structures such as the hippocampus, amygdala, and insula, providing a comprehensive sampling of these areas. Previous studies have demonstrated that these electrodes can be used to identify brain-specific somatic mutations in different epileptic brain regions.(20-22)

Here, we collected explanted SEEG electrodes from three patients with distinct epilepsy subtypes (Table 1 and see overview of workflow and analysis in Fig. 1). Based on EEG profiles, we stratified the electrodes into: 1) Seizure Onset Zone (SOZ), 2) Propagation Zone (PZ), and 3) Non-Involved Zone (NIZ). We then sectioned the electrodes from the SOZ, PZ, and NIZ for each patient and extracted total nucleic acid (RNA and genomic DNA (gDNA)) from the same sample. We then performed both transcriptome (RNA-seq) and epigenome (DNA methylation) profiling on these samples. Additionally, we identified short variants (SNPs and INDELS) using RNA-seq datasets (Fig. 1A).

We first analyzed the transcriptome data, focusing on the quality and quantity of material that could be recovered and on specific expression patterns (P) between the SOZ, PZ, and NIZ (i.e. up- or downregulated in SOZ/PZ vs NIZ comparisons) (Fig. 1B). Further, we then compared the detected transcripts and patterns to publicly available epilepsy data of transcriptome (bulk and single-cell/nucleus RNA-seq) and epilepsy-related genes.(24-27) Next, we conducted functional enrichment analysis to identify the biological processes (Fig. 1B). For DNA methylation analysis, we examined the same samples (SOZ, PZ, and NIZ) to identify differentially methylated regions (DMRs). We integrated the transcriptome data with

the methylome data to understand how DNA methylation profiles correlate with corresponding transcriptome profiles (Fig. 1C). Finally, we explored whether we could integrate our molecular data with the SEEG neurophysiology (visual analysis and epileptogenicity index score) (Fig. 1D).

Patient neurophysiology, neuroimaging, and neuropathological features

To assess the broad applicability of our method, we recruited three patients with epileptic foci of diverse etiology and extent: focal cortical dysplasia (FCD), non-lesional temporal lobe epilepsy (TLE), and Rasmussen's encephalitis (RE) (see Table 1). Each patient was discussed at an interdisciplinary patient management conference, where intracranial evaluation with SEEG was recommended exclusively based on clinical indication. All participants underwent robot-assisted SEEG monitoring. Depth electrodes with 12, 15 or 18 contacts were implanted based on a pre-operative hypothesis of the SOZ. By standard nomenclature, each depth is labelled with a letter, with electrode contacts numbered from mesial to lateral. The anatomic location of each electrode was confirmed by postoperative computed tomography (CT) co-registered with preoperative volumetric MRI (Figure 2). Continuous EEG recordings of seizures were conducted with concurrent video, and visual analysis classified cortical regions into the SOZ, PZ, or NIZ. For quantitative SEEG analysis, representative seizures with minimal artifacts occurring over 48 hours post-implantation were selected. The epileptogenicity index (EI) at each electrode pair was measured to assess changes in energy ratio and time delay from electrode contacts from seizure onset, estimating the epileptogenicity of cortical regions.(28,29).

Patient details and operational outcomes are in Table 1 (and see Methods). The first patient had medication-resistant focal epilepsy due to a right parietal FCD type IIA (Fig. 2A-C,

Supplementary Fig. S1A, Supplementary Table S1). During SEEG monitoring, thirty-three electroclinical seizures were recorded, with EEG onset from the lateral contacts of the depth electrodes at the parietal FCD (Table 1). Seizure propagation involved the supplementary motor area and temporo-occipital junction. We collected 18 electrode samples from NIZ, PZ and SOZ regions and then selected ~40% of electrodes covering the NIZ, PZ and SOZ regions for whole transcriptome and DNA methylome profiling (Fig. 2A).

The second patient had MRI-negative, medication-resistant left TLE (Fig. 2D-F, Table 1, Supplementary Fig. S1B-D, Supplementary Table S1). SEEG monitoring recorded six electroclinical seizures, with EEG onset from the anterior hippocampus and the temporal pole. Seizure propagation involved the middle temporal gyrus and inferior temporal gyrus. We collected 12 electrode samples from the NIZ, PZ, and SOZ regions. We then profiled approximately 75% of these electrodes, covering all three regions (NIZ, PZ, and SOZ), for whole transcriptome and DNA methylation (Fig. 2D).

The third patient was diagnosed with RE at age 15 years, treated medically (Fig. 2G-I, Supplementary Fig. S1E-G, Supplementary Table S1). Persistent medication-resistant epilepsy led to SEEG monitoring, during which 32 electroclinical seizures were recorded, primarily from the hippocampus and the gyrus rectus. Seizure propagation involved the cingulate and frontal operculum. We collected 20 electrode samples from the NIZ, PZ, and SOZ regions. We then profiled all three regions (NIZ, PZ, and SOZ), encompassing approximately 40% of the electrodes, for whole transcriptome and DNA methylation (Fig. 2G).

We first investigated whether the collected explanted SEEG contacts retained any intact cells. For this, electrodes were cut into small pieces and rinsed with PBS for collecting cells.

Trypan blue staining confirmed the presence of cells on these electrodes (Supplementary Fig. 2A). We then extracted total nucleic acids (both RNA and gDNA) from the eluate of rinsed electrode contacts and measured their quantity using a Nanodrop spectrophotometer. Remarkably, we obtained significant concentrations of nucleic acids from these electrodes, with amounts directly proportional to the number of metal contact points in the sample (Supplementary Fig. 2B). Next, we analyzed the quality of these nucleic acids using a high-sensitivity fragment analyzer, finding both gDNA and RNA in considerable concentrations (Supplementary Fig. 2C-E) (Supplementary Tables 2 and 3). We then divided these samples into equal portions and purified the gDNA and RNA separately and the libraries were prepared for sequencing (Supplementary Fig. 3F and G) (Supplementary Tables 4, 5 and 6). The purified RNA from most contacts exhibited acceptable RNA integrity (RIN) numbers (Supplementary Fig. 2G and 2A). These results indicate that SEEG electrodes used in the pre-surgical evaluation of epilepsy carry sufficient nucleic acid material from the implanted brain regions to enable the transcriptome and epigenome of living people with epilepsy.

Single-sourced multi-ome profiles of SEEG electrode contacts show high sequence coverage and mapping

To achieve comprehensive sequence coverage in the transcriptome of very low input RNA samples from the SEEG electrodes, we applied the Flash-seq method.⁽³⁰⁾ Originally developed for full-length single-cell RNA sequencing, we adapted Flash-seq for bulk RNA sequencing in our study.⁽³¹⁾ This allowed us to obtain full-length transcripts with high coverage from electrodes, performing both transcriptome and DNA methylome analysis (Fig 3A). Although some of the samples had low RIN numbers, it did not affect the coverage of varied range of transcripts, indicating sufficient RNA quality (Supplementary Fig. 3A, 3B, 4A

and 4B). Further, we observed a correlation between the electrode-derived transcriptome and the transcriptome of the subsequently removed tissue in which that electrode had previously been implanted (Supplementary Fig. 5A-D). This strongly indicates that explanted SEEG electrodes retain a representative transcriptional profile from the implantation site.

From the FCD case, we used seven different electrode samples, achieving an average of 100 million and 85 million reads per sample in DNA methylome and transcriptome sequencing, respectively. Of these, 91 million reads from the methylome and 76 million reads from the transcriptome were mapped to the human genome (Fig. 2D, 3B). From the TLE case, we used nine electrode samples, resulting in an average of 100 million and 106 million reads per sample in DNA methylome and transcriptome sequencing, respectively. Some 90 million methylome reads and 97 million transcriptome reads were mapped for the TLE case to the human genome (Fig. 3B). One of the RNA samples failed in QC and was excluded from sequencing (Fig. 2H, 3B). From the RE case, eight electrode samples were analysed, yielding an average of 117 million and 101 million reads per sample in DNA methylome and transcriptome sequencing, respectively. Of these, 105 million methylome reads and 97 million transcriptome reads were mapped to the human genome (Fig. 3B). One of the RNA samples failed in QC and was excluded from sequencing (Fig. 2L,3B).

We then performed genome-wide density analysis for mapped transcriptome reads across the transcriptional start (TSS) and end (TES) sites, covering a 3kb upstream and downstream flanking window (Fig. 3C left panel). Similarly, we analysed the density of mapped DNA methylation reads across the TSS and TES sites with a 3kb flanking window (Fig. 3C right panel). As expected, these density plots revealed a consistent negative correlation between the transcriptome and DNA methylome across all samples analysed

(Figs. 3C). Thus, it is possible to obtain high-coverage multiome profiles from SEEG electrode contacts from pre-surgical epilepsy patients.

Transcriptome analysis of SEEG electrodes reveals signatures of epileptic brain regions

To delineate transcriptional changes associated with epilepsy, we performed a differential expression analysis comparing SOZ, PZ and NIZ (Fig. 1B, Fig. 4A). To validate the transcriptome profile from SEEG electrodes, we analyzed previously published single nuclei RNA sequencing (snRNAseq) data,(26,27) from surgically obtained epilepsy samples and matched controls (Supplementary Fig. 6 A-C). We integrated epilepsy and healthy single nuclei profiles and annotated different clusters using markers for various excitatory and inhibitory neurons (Supplementary Figure 7A and B). Furthermore, we found our DEGs enriched in previous studies of TLE and RE bulk and scRNA-seq transcriptome including key epilepsy related genes (Supplementary Fig. 6D and E). (24-27)

For the FCD patient sample, only the PZ and NIZ samples were sequenced. We performed transcriptome correlations comparing PZ vs NIZ and found they were poorly correlated (Supplementary Fig. 7A). We identified 8 significantly upregulated and 36 downregulated genes in the PZ compared to the NIZ (Fig. 4A, Supplementary Fig. 7B and Supplementary Tables 7 & 8). GO enrichment analysis indicated that genes activated in PZ areas were involved in translational processes (Supplementary Figure 7C and Supplementary Table 9) whereas the downregulated genes were mostly involved in metabolic processes (Supplementary Figure 7D). We next compared the differentially expressed genes with known epilepsy-associated genes,(26) and found 2 genes in our list (Supplementary Fig. 7E and Supplementary Table 10). In contrast, some genes (*RPL4*, *MTPAP* and *SNX30*) that

are downregulated in the PZ also showed downregulation in epilepsy samples in the TLE vs healthy sc-RNA data set (27) (Supplementary Figure 7F).

For the TLE patient, we observed that the replicates from PZ and NIZ regions were anticorrelated, whereas a high correlation was observed within the replicates from the same regions (Fig. 4B). Next, we identified 174 significantly upregulated and 71 downregulated genes in the PZ compared to NIZ (Fig. 4A and 4C, and Supplementary Tables 11 & 12). The upregulated genes were enriched for processes related to translation, gene expression and peptide and macromolecule biosynthetic pathways (Supplementary Fig. 8A and Supplementary Table 13), while the downregulated genes were enriched for functions related to superoxide generation, cytokine production and various stress signalling pathways (Supplementary Figure 8B and Supplementary Table 14). Confirming these signatures held plausible biological relevance, we found a set of transcripts upregulated in PZ vs NIZ that were previously reported as enriched among upregulated genes in bulk and single nucleus RNAseq analyses of resected TLE tissue and contain known epilepsy-related genes (24-27) (Supplementary Fig. 6D).

Next, we compared the SOZ vs NIZ in TLE, and found 76 genes significantly upregulated and 67 genes downregulated, respectively (Fig. 4A, Supplementary Fig. 8D and Supplementary Tables 15 & 16). A correlation analysis for SOZ vs NIZ found they are highly correlated within the replicates from same regions (Supplementary Fig. 8C). We observed genes upregulated in the same comparison were enriched and upregulated in the previously published single nuclei profiles from the neocortex of TLE patients(27) (Supplementary Fig. 6D and 8E). Similarly, the upregulated genes were also found to be enriched in RNAseq data from NeuN-positive cells from the neocortex of TLE patients.(25) Further, upregulated genes were highly enriched for cellular responses to zinc, copper and cadmium ions

(Supplementary Fig. 8F and Supplementary Table 17). In contrast, the downregulated genes were enriched for immune response, cell junction disassembly and synapse pruning (Supplementary Fig. 8G and Supplementary Table 18).

We further compared the transcriptome of SOZ vs PZ for TLE, where 79 genes were significantly upregulated, and 81 genes were downregulated, respectively (Fig. 4A, 4K and Supplementary Tables 19 & 20). The upregulated genes were enriched for cellular responses to ions, dephosphorylation, cell fate commitment and stem cell differentiation (Supplementary Fig. 9A and Supplementary Table 21). In contrast, the downregulated genes were enriched for proteolysis, protein catabolic process and peptidase activity (Supplementary Fig. 9B and Supplementary Table 22). We also observed that epilepsy-associated genes *PDCC10*, *PHGDH*, *PSAT1* and *TBC1D24* showed higher expression levels in SOZ compared to PZ (Fig 4L-M).(26) Notably, two of them were upregulated and enriched in TLE versus healthy scRNA data (27) (Supplementary Fig. 6D and Figure 9M).

From the RE patient SEEG samples, we detected 207 significantly upregulated and 374 downregulated genes in the PZ compared to the NIZ (Fig. 4A, 4G and Supplementary Tables 23 & 24). When performing the correlation analysis, we found PZ vs NIZ replicates were less correlated (Fig. 4F). Epilepsy associated genes (*TPP1*, *BCKDK*, *ALG3* and *PACS1*) were upregulated and enriched in PZ and showed higher expression in the TLE as compared with the healthy control scRNA data (Fig. 4H and I, Supplementary Fig. 6D).(26,27) In addition, upregulated genes were enriched in TLE NEUN+, OLIG2+ and RE as compared to the healthy controls. (24,25) The upregulated genes were associated with processes such as nuclear export, amino acid transport, cell migration and positive regulation of gene expression (Supplementary Figure 10A and Supplementary Table 25), whereas the downregulated genes were enriched for translation, peptide and

macromolecule biosynthesis and gene expression (Supplementary Fig. 10B and Supplementary Table 26).

When we compared SOZ vs PZ in the RE patient we found that 2 and 6 genes were significantly up and downregulated, respectively (Fig. 4A, Supplementary Figure 10C-D and Supplementary Tables 27 & 28). The upregulated genes were associated with the negative regulation of the cell cycle, apoptotic process, regulation of necrotic cell death and cellular respiration (Supplementary Figure 9E and Supplementary Table 29), whereas the downregulated genes were associated with the positive regulation of amyloid-beta formation, catabolic process and ubiquitin-dependent proteolysis (Supplementary Fig. 9F and Supplementary Table 30).

In the SOZ compared to the NIZ, 5 genes were upregulated, and 8 genes were downregulated significantly (Fig. 4A and Supplementary Fig. 11D and Supplementary Tables 31 & 32) and they showed less correlation with each other (Supplementary Fig. 10C). Among the upregulated genes in the SOZ, the *QPCT* gene showed higher expression in epilepsy compared to healthy controls (Supplementary Fig. 10F).(27) GO enrichment analysis revealed that genes activated in the SOZ were involved in the negative regulation of cell cycle and cellular response to stress (Supplementary Fig. 10E and Supplementary Table 33). In contrast, downregulated genes were associated with amyloid beta response and cholesterol import (Supplementary Fig. 10G and Supplementary Table 34).

Overall, the SEEG transcriptional signatures from SOZ and PZ show a range of differential gene expression and shared as well as distinct biological processes for the three etiologies. Furthermore, our SEEG-recovered gene signatures highly correlated with tissue-based epilepsy bulk and scRNA-seq transcriptome data showing the potential of our approach in

capturing the molecular and cellular mechanisms underlying different epilepsy subtypes (24-27) (Supplementary Fig. 6D and E).

Integration of SEEG transcriptome and DNA methylome data

Aberrant DNA methylation has previously been reported in resected tissues from epilepsy patients(32-35). Here, we investigated DNA methylation maps derived from the explanted SEEG electrodes from the epilepsy patients. We began by comparing the differential DNA methylation regions (DMRs) between the SOZ, PZ and NIZ. For the FCD case, we observed an overall raised level of transcriptome and methylome in NIZ compared to PZ in the gene body and flanking regions and these were poorly correlated (Supplementary Fig. 11C, D and E). We found that 216 regions were hypermethylated and 1801 regions were hypomethylated between PZ and NIZ (Supplementary Fig. 11F). GO term analysis revealed that the hypomethylated regions were enriched for genes involved in morphogenesis and GTPase-mediated signal transduction (Supplementary Fig. 11G), whereas hypermethylated genes were enriched for cholesterol storage and nuclear membrane disassembly (Supplementary Fig. 11H). Notably, hypomethylated genes were enriched in epilepsy-related genes(26) (Supplementary Fig. 11B).

For the TLE case, we found an increase in methylation and transcriptome levels in NIZ compared to PZ and SOZ and they were less correlated with each other than was the PZ vs NIZ comparison (Fig. 5C, D, Supplementary Fig. 13A and B). DMR analysis of these regions showed 2649 and 2405 hyper- and hypomethylated regions, respectively (Fig 5E). We next investigated the link between gene expression and DNA methylation in PZ and NIZ regions in the TLE case by integrating single-source transcriptome and methylome data. This analysis revealed that methylation levels in PZ vs NIZ were negatively correlated with their transcriptome as expected (Fig. 5B and Supplementary Fig. 11A). In addition, hyper- and

hypo-methylated genes were enriched in genes related to epilepsy and those previously shown to have aberrant DNA methylation in drug-resistant TLE patients (Supplementary Fig. 11A and B). (26,36) Notably, transcriptome and methylome showed an inverse correlation across the gene body and flanking regions, with decreased DNA methylation levels accompanied by higher gene expression levels (Fig. 5C and D). Conversely, decreased transcription at the TSS and TES coincide with increased DNA methylation levels at these sites. The hypomethylated regions were enriched for GO terms including phosphorylation and negative regulation of DNA binding of transcription factors (Supplementary Figure 12B), whereas the hypermethylated regions were enriched for GTPase-mediated signal transduction, regulation of cell migration and phagocytosis (Supplementary Figure 12C). Using the example of epilepsy-associated gene *PTPRC*, we found a reduction in the DNA methylation (hypomethylation) level and an increase in transcription in the PZ compared to NIZ (Fig. 5F).

Comparison of the DNA methylome of SOZ and NIZ in the TLE case showed a poor correlation (Supplementary Fig. 12D). DMR analysis for these regions showed that 636 regions were hypermethylated and 475 regions were hypomethylated when comparing the SOZ to the NIZ area (Supplementary Fig. 12E). Notably, these hyper- and hypo-methylated genes have previously been found to be enriched in TLE patient brain tissue.(36,37) GO-term analysis of DMRs are provided in Supplementary Fig. 12F, G).

We found a positive correlation in DNA methylation levels among SOZ and PZ replicates (Supplementary Fig. 13C). DMR analysis for these regions showed that 608 regions were hypermethylated and 872 regions were hypomethylated when comparing the SOZ to the PZ region (Supplementary Fig. 13B). We observed that both hypermethylated and hypomethylated genes were significantly enriched in the data from the tissues of drug-

resistant TLE patients.(36,37) GTPase-mediated signal transduction, G-protein coupled receptor signalling and negative regulation of cell proliferation-related terms were enriched for the hypomethylated regions (Supplementary Fig. 13E). The hypermethylated regions were enriched for processes including phospholipid biosynthesis and CNS development (Supplementary Fig. 13F).

Tissue DNA methylation landscapes have recently been reported for RE(38). For the SEEG samples from the RE patient, we observed elevated transcriptome and DNA methylome levels in NIZ as compared to PZ and SOZ regions in gene body and flanking regions (Supplementary Fig. 14A-B). In addition, overall transcriptome and methylome levels were inversely proportional to each other in the gene body and flanking regions. Next, DNA methylation correlation analysis showed a high correlation among PZ and NIZ replicates (Supplementary Fig. 14C). DMR analysis for these regions showed that 31 regions were hypermethylated and 328 regions were hypomethylated when comparing the SOZ to the PZ region (Supplementary Fig. 14D). Further, we observed transcriptionally upregulated genes negatively correlating with their DNA methylation levels (hypo-methylated) in PZ as compared with NIZ (Supplementary Fig. 14E). GO-term analysis of hypomethylated regions was enriched for sulfate biosynthesis, mesenchymal cell migration and semaphorin-plexin signalling pathways (Supplementary Fig. 14F).

Combining neurophysiology with multiomic data from SEEG electrodes

Next, we explored the relationship between epileptogenicity index (EI) scores based on the recorded neurophysiologic signals and transcriptomic signatures. To accomplish this, we integrated the EI scores of the TLE and RE cases with transcriptome-derived signatures from NIZ, PZ, and SOZ and compared them with independent public epilepsy datasets.(27) This analysis revealed that SOZ transcriptome signatures with high EI scores displayed

elevated expression in epilepsy patient samples compared to healthy controls(27) (left panel in Fig. 6A and B). Similarly, transcriptional signatures linked with moderate EI scores in the PZ of the TLE and RE cases showed increased activation in epilepsy samples relative to healthy controls (left panel in Fig. 6A and B).

Last, we explored the activity of these transcriptional signatures in excitatory and inhibitory neurons from both epilepsy and control samples. Our findings indicated that gene signatures from TLE and RE were positively correlated with EI scores in both excitatory and inhibitory neurons of epilepsy patients compared to control counterparts (right panel Fig 6A-B). Overall, our analyses underscored that the expression profiles of gene signatures from SOZ and PZ of TLE, and RE patients closely mirrored those observed in existing epilepsy datasets. This shows the relevance of our approach to identifying epilepsy-related transcriptomic signatures from SEEG electrodes.

SEEG-derived transcriptome can predict distinct genomic variants

Last, we investigated whether genomic variants can be detected in our SEEG-derived RNA transcript sequences. We explored the presence of short variants (SNPs and Indels) in our transcriptome data using GATK(39) best practice workflows and annotated the high-quality variants through wANNOVAR (Supplementary Fig. 15A). We then mapped the chromosome-wide distribution of variants in RE where we compared the difference between NIZ and PZ (Supplementary Fig. 15B). Similarly, we performed a chromosome-wide distribution of variants in TLE where we compared the difference between NIZ, PZ and SOZ (Supplementary Fig. 15C).

Using the transcriptomic data from the TLE samples, we identified a total of 4396 genes harbouring the variants in NIZ and among these 2.8% were unique to the NIZ region. In the

SOZ region, we found a total of 4698 genes, among which were 9.5% specific to the SOZ region (Supplementary Fig. 16A and B). In the PZ region, we identified a total of 4226 genes and among these 1.6% were unique to the PZ. A total of 3588 genes (59.7%) were shared across NIZ, PZ and SOZ (Supplementary Fig. 16A and B). We then overlaid these variants with known epilepsy-associated genes (26) and this analysis revealed that 3.9%, 3.9% and 4.6% of genes are shared among NIZ, PZ and SOZ respectively (Supplementary Fig. 16A). Next, we performed GO-term analysis for the 3799 genes that were shared across the NIZ, PZ and SOZ. Interestingly, this analysis showed that these novel variants were enriched for RISC complex assembly, mRNA, pre-RNA processing and RNA secondary structure unwinding pathways (Supplementary Fig. 16C). NIZ-specific genes were enriched for negative regulation of inflammatory responses to antigens, negative regulation of DNA biosynthesis and regulation of intracellular signalling (Supplementary Figures 16D). For the PZ region, genes were enriched for the regulation of cellular localisation, apoptotic signalling pathways and protein deubiquitylation-related GO-terms (Supplementary Fig. 16E). The NIZ genes were enriched for intracellular pH elevation, nuclear pore organisation and assembly and glial cell differentiation GO-terms (Supplementary Fig. 16F). We then analysed the total number of genetic variants (INDELs and SNPs) present in SOZ, PZ and NIZ and found that SOZ harboured more genetic variants compared to PZ and NIZ (Supplementary Fig. 15D). We also overlapped genes harbouring the variants with known epilepsy associated genes and found that 3.6% of genes were commonly present in PZ and NIZ (Supplementary Fig. 17A). Similarly, in the RE brain, we found a total of 4095 genes harbouring the variants (517 unique) in the PZ area, and 4036 gene variants (458 unique) in the NIZ area among these variants 3578 are present in both NIZ and PZ (Supplementary Fig. 17B). We further annotated these genes using GO enrichment analysis (Supplementary Fig. 17C-E). In RE

samples, the shared genes were enriched for protein localisation to chromatin, cytoskeletal organisation, and ribonucleotides biosynthesis (Supplementary Fig. 17C), whereas the genes specific to the PZ regions were enriched for glycerophospholipid metabolism, lymphoid progenitor cell differentiation and axonal guidance-related processes (Supplementary Fig. 17D). Furthermore, for the NIZ-related genes, mRNA processing and phosphorylation-related GO-terms were enriched (Supplementary Fig. 17E). We then analysed the total number of genetic variants (Indels and SNPs) present in SOZ, PZ and NIZ and found the SOZ harboured more genetic variants compared to PZ and NIZ (Supplementary Fig. 15E). These observations suggest that SEEG electrode-derived transcripts can be used to successfully detect genomic variants and complement the matched data on gene expression, DNA methylation, electrophysiology and radiology from epilepsy patients.

Discussion

Unravelling the molecular mechanisms underlying the development and maintenance of the epileptic state presents several challenges due to its multifactorial nature and heterogeneity. Genetic complexity, including polygenic inheritance and gene-environment interactions, complicates the identification of causal factors.(40-43) The dynamic nature of epileptogenesis, the interplay between excitatory and inhibitory neurotransmission and the effects of recurring seizures on brain networks further obscure our understanding.(44-46) An incomplete understanding of seizure onset and network recruitment mechanisms and the lack of biomarkers for early diagnosis pose additional obstacles.(40,42,47,48) Addressing these challenges requires interdisciplinary collaboration, advanced experimental models and innovative technologies and data integration to decipher the intricate molecular landscape of epilepsy and develop targeted therapies and innovations in diagnosis.(49)

Treatment-resistant epilepsies offer a unique opportunity to access the living human brain via analysis of surgically-resected material. This has yielded significant advances in understanding brain function and mechanisms of epileptogenesis.(50-52) Intracranial EEG recordings from depth electrodes provides a further means of detailed spatial and temporal mapping of epileptogenic tissue, offering valuable insights into seizure onset and propagation. Recent studies have demonstrated the feasibility of extracting nucleic acids from depth electrodes, opening new avenues for molecular profiling of the human brain.(23,37,53,54) In this study, we demonstrate MoPEDE, a method to extract nucleic acids from explanted depth electrodes, enabling multimodal profiling of the transcriptome and epigenome in relation to the electrophysiological readings. Through meticulous experimental procedures, including electrode sectioning, nucleic acid extraction, and

comprehensive profiling, we demonstrate the feasibility of obtaining high-quality RNA and gDNA from SEEG electrodes, even from deep brain structures. The inclusion of FCD, non-lesional TLE, and RE demonstrates MoPEDE can in principle be applied across spatial scales ranging from highly localised (FCD) through to whole hemisphere (RE). We identified transcripts and DNA methylation profiles that correlate with recorded neurophysiological signals, highlighting the potential relevance of molecular profiling in understanding epilepsy aetiology. While the SEEG electrodes covered only selected regions of the brain, surgical outcomes are the gold standard for measuring successful identification and surgical removal of epileptogenic tissue. The excellent surgical outcome, along with accurate ictal recordings, support that the electrodes were in the epileptic foci (Table 1). Additionally, the identification of specific genes and pathways enriched in epileptic brain regions, validated through comparison with publicly available datasets and single-cell RNA sequencing data,(55,56) is an important validation of the findings. Additionally, our approach demonstrates the potential to derive information about clinical variants, such as SNPs and Indels, from a limited number of SEEG electrodes. This includes identifying both pathogenic and risk factor variants across various brain regions affected by epilepsy and their associated functions (22).

While the biological significance of the variation in numbers of differentially expressed genes in relation to epileptogenicity index and etiologies cannot be determined using the small number of cases in the present study, the trace nucleic acids on SEEG contacts may reflect processes that differ in the SOZ and PZ compared to NIZ. This included a number of genes associated with cell metabolism, processes increasingly recognised across the epilepsies. In both TLE and RE samples, we found differences in the SOZ compared to PZ that included transcripts regulating cell death. This could reflect the engagement of pathways that limit neurodegeneration in tissue actively generating seizures, processes

known to be evoked by repeated seizures(57). Analysis of SNPs and indels within the transcriptome data showed enrichment of many of the same pathways, as well as highlighting RNA processing and chromatin processes that are also implicated in the pathogenesis of epilepsy(58-61). The amount of these variants appears to scale with EI score implying potential diagnostic applications in distinguishing SOZ from PZ. Further, DNA methylation findings showed alignment with findings from tissue-based studies of FCD and TLE, in terms of numbers of hyper- and hypo-methylated genes(62,63) and biological processes influenced by this epigenetic mark. Correlations between SOZ and PZ suggest a proportion of epigenetic marks reflect the effects of seizures per se whereas others may distinguish seizure-triggering sites from recruited networks. Thus, the surface of explanted SEEG contacts appears to bear molecular traces reflective of cellular biology and pathology of the source tissue.

The source of the detected transcripts and epigenetic signals on the electrode surface is likely to be from the surrounding neurons, glia and perhaps vascular cells at that specific site or along the path of contact. Indeed, we detected transcripts representing multiple resident brain cell types in all three cases. Mechanical injury is the likely cause of the release of these nucleic acids due to insertion and/or withdrawal of the electrode, but controlled release is also possible. Indeed, both neurons and glia are capable of releasing packets of membrane-enclosed cellular material in the form of extracellular vesicles (64). While the extent and functional significance of such information-carrying paracrine signalling remains incompletely understood, experimentally evoked seizures have been reported to adjust the abundance and nucleic acid content of extracellular vesicles(65). The coherence between epigenetic and transcript signals suggests these materials had a similar cellular source, but it is possible that origin and release mechanisms differ between the two nucleic acid types.

The source of some nucleic acids may be from infiltrating immune cells, which deliver such material to resident brain cells, including neurons after seizures(66). While RE is most strongly associated with an infiltrating inflammatory cellular presence, both TLE,(67) and FCD(68), feature immune cell infiltration. Further studies may yield a more complete understanding of the basis of the detected signals and their relationship to pathophysiological communication from local and perhaps non-resident cellular sources.

There are a number of limitations to consider in the present study. Foremost, the small number of cases in our proof-of-concept study limits insights into disease mechanisms and etiological factors. Subsequent studies with larger patient numbers will be needed. Obtaining sufficient quantities of high-quality nucleic acids for NGS methods from a limited number of SEEG electrodes remains challenging. Indeed, we did not achieve uniform sample integrity with several samples failing to pass the QC in the NGS workflow. This may be addressed by further improvements in the workflow such as reducing the time to snap-freezing electrodes or proceeding to immediate extraction of nucleic acids. Additional or new extraction methods should may better ensure the preservation of sample integrity. Second, other information-containing material may also be recoverable that would complement MoPEDE. For example, histone modifications which play a major role in controlling gene expression and cellular identity(47,69).

Another caveat of the present study is that the implantation or explantation procedure may result in some gliosis as well as the carriage of nucleic acid material from one site to another, thereby obscuring the origin of the signal. A detailed evaluation of the extent of such material transfer and its impact on molecular information will be required in future studies. Also, our study also relied on comparisons to publicly available datasets which include data derived from postmortem samples. Future studies could explore matching explanted electrode

fingerprints to findings in matched post-resection tissue samples. Indeed, recent studies comparing the transcriptome profiles of fresh, frozen, and formalin-fixed, paraffin-embedded (FFPE) tissues have demonstrated that both frozen and FFPE tissues can be used to retrieve certain information on gene expression, provided proper quality control measures are in place (70,71)

It is important to note that SEEG has an inherent sampling bias with low spatial resolution, being able to cover only a limited area of the brain or epileptic network. Increasing numbers of depth electrodes must be balanced with limiting intra-operative and post-operative complications of multiple-depth electrode placement. Visual inspection of SEEG data is open to reader variability, and while still the clinical standard, has prompted the development of more automated techniques. EI scores, as used here, are more accurate with fast frequencies at seizure onset but are not as accurate with slower frequencies. For example, the temporal pole SOZ in patient B had a slow ictal onset frequency with a low EI score. EI does not uniformly correlate with visual inspection. For example, EI scores in the RE case were diffusely elevated, including in the NIZ, likely due to the expected diffuse epileptogenicity in the affected hemisphere.

In conclusion, we describe a multimodal methodology that has the potential to provide insights into disease mechanisms and prospects for improving the diagnosis and treatment of epilepsy. The ability to extract nucleic acids from depth electrodes provides a non-invasive means of molecular profiling, offering potential applications beyond epilepsy, such as studying other neurological disorders. Future research directions may involve exploring additional nucleic acids (e.g. long non-coding RNA, small RNAs), epi-transcriptome changes (RNA modifications) and epigenetic marks (histone marks) obtained from depth electrodes, as well as investigating the applicability of this approach across diverse epilepsy subtypes.

Methods

Sex as a biological variable

This study examined data from both male (N = 1) and female (N = 2) individuals. Due to the small number of subjects, the primary analyses in this study did not consider sex as a biological variable.

Neurophysiology data collection

All participants underwent robot-assisted implantation of intracranial depth electrodes and SEEG monitoring in the Epilepsy Monitoring Unit in Beaumont Hospital Dublin, Ireland. Depth electrodes (DIXI Medical, France) were implanted according to the pre-operative hypothesis of the SOZ. The anatomic location of each electrode was confirmed by postoperative CT co-registered with preoperative volumetric MRI (Figure 2). Continuous EEG was recorded at 1024Hz with concurrent video recording (Xitek EEG System, Natus Inc.). Two experienced clinical neurophysiologists/epileptologists performed visual inspection of seizures and classified each cortical region as part of the SOZ, PZ, or NIZ, based on summation of ictal EEG data. The SOZ is defined as the electrodes involved at the onset of the EEG seizure, and correlates with an epileptogenicity index (EI) >0.4 . The PZ is defined as the electrodes involved in early seizure propagation within 10 seconds, and correlates with an EI of 0.2-0.4. The NIZ is defined as electrodes not involved in the seizure and correlates with an EI <0.2 .

To measure EI, EEG was analysed using the AnyWave software (Marseille, France). (28,29) The EI measures the change in energy ratio and time delay from electrode contacts at seizure onset to estimate the epileptogenicity of a given cortical region. Representative seizures from each patient which occurred over 48 h from electrode implantation and with

minimal artefact were selected. Bipolar contacts in grey matter at the mesial or lateral point of each depth electrode represented each brain region under investigation. Contacts in white matter or outside of the brain were excluded.

The depth electrodes were explanted under general anaesthesia following standard clinical procedures. They were immediately placed in sterile, RNA, DNA and nuclease-free 15 ml tubes and frozen in dry ice for transport. The electrodes were then stored at -80°C until further processing.

Patient clinical characteristics

Table 1 summarizes information on the patients. Patient A is a 49-year-old male who underwent SEEG monitoring for medication-resistant focal epilepsy due to a right parietal focal cortical dysplasia (FCD). Thirty-three electroclinical seizures were recorded. The EEG onset for all seizures arose from the lateral contacts of the X electrode, at the parietal FCD. Seizure propagation involved the supplementary motor area, and temporo-occipital junction. The patient underwent resection of the FCD and achieved an Engel class 1A outcome, remaining seizure-free at one year. Histopathology confirmed a type IIa FCD.

Patient B is a 40-year-old female with MRI-negative, medication-resistant temporal lobe epilepsy who underwent SEEG monitoring. Six electroclinical seizures were recorded. EEG onset was from the anterior hippocampus, and the temporal pole. Seizure propagation involved the middle temporal gyrus and inferior temporal gyrus. The patient underwent a left temporal lobectomy with amygdalo-hippocampectomy, resulting in an Engel class 2A outcome at one year, initially seizure-free but experiencing rare seizures subsequently. Histopathology revealed Chaslin's subpial gliosis.

Patient C is a 24-year-old female with Rasmussen's encephalitis at the age of 15 years treated medically. Due to persistent medication resistant epilepsy, she underwent SEEG

monitoring. Thirty-two electroclinical seizures were recorded. Ictal onset of most seizures was from the hippocampus and the gyrus rectus. Seizure propagation involved the cingulate, and frontal operculum. The patient underwent right frontal and anterior temporal resection with an Engel class 2A outcome at one year, initially seizure-free with rare seizures subsequently. Initial brain biopsy at age 15 years showed chronic encephalitis with T-cell rich perivascular and parenchymal inflammation. Histopathology from the resection eight years later revealed severe hippocampal sclerosis and cortical gliosis, with no active inflammation. (see Table 1)

Total Nucleic acid extraction

For the total nucleic acid extraction, snap-frozen SEEG electrodes were cut into small pieces using sterile nuclease-free scissors into microfuge tubes and the total nucleic acids were extracted using PicoPure™ RNA Isolation Kit (Thermo KIT0204) according to the manufacturer's instructions with minor modifications. Briefly, 50 to 100 µl (up to the electrode pieces were completely immersed) extraction buffer was added to the cut electrodes followed by 30 minutes of incubation at 42°C. Meanwhile, the column was preconditioned with 250 µl of Conditioning Buffer. An equal amount (50 to 100 µl) of 70 % ethanol was added to the extracted samples and mixed, transferred to the preconditioned column centrifuge for 2 minutes at 100 x g, immediately followed by centrifugation at 16,000 x g for 30 seconds to remove flowthrough. Bound fractions were washed with wash buffer I without DNase to retain the genomic DNA in the same samples followed by wash buffer II and the samples were eluted with 11µl of elution buffer.

Samples were quantified in a nanodrop and the quality of the samples was analysed using an Agilent TapeStation system with high-sensitivity RNA tapes or in a fragment analyser. For

the separation of DNA and RNA, the eluted samples were divided into equal portions and one portion was used for RNA isolation and the other was used for DNA isolation.

RNA purification

For the RNA purification, samples were first treated with DNase I (Thermo EN0521) to remove the DNA and purified using GeneJET RNA Purification Kit (Thermo K0732) according to the manufacturer's instructions. Purified RNA samples were quantified using Qubit high-sensitivity RNA Quantification assay (Thermo Q32852). The quality of the RNA was analysed using Agilent TapeStation systems with high-sensitivity RNA tapes with RIN numbers.

Genomic DNA purification

For gDNA purification samples were added to nuclease-free water (Thermo AM9939) to the final volume of 100 μ l. Then 3 μ l of RNase A (Thermo R1253) was added to the samples and incubated for 20 minutes at 37°C. Further DNA was purified using Monarch® Genomic DNA Purification Kit (NEB T3010S) according to the manufacturer's instructions with minor modifications. 1 μ l of Proteinase K (Thermo E00491) was added to the samples and 100 μ l of lysis buffer was added and incubated for 5 min at 57°C followed by 400 μ l of binding buffer was added and loaded to the purification columns. Washed twice with wash buffer and eluted with 15 μ l of elution buffer. Purified DNA samples were quantified using Qubit high-sensitivity dsDNA Quantification assay (Thermo Q32854). The quality of the gDNA was analysed using an Agilent TapeStation system with high-sensitivity genomic DNA tapes.

Bulk FLASH-seq:

Following extraction, RNA samples were normalized to 1ng/uL and 1 μ L of RNA was input to prepare RNA libraries using a bulk input optimized FLASH-seq protocol. In brief, RNA was

converted to cDNA fragments using Maxima H Minus (#EP0753, Thermo Fisher Scientific) and amplified with KAPA HiFi HotStart (#KK2602, Roche). Note, double lysis mix volume used and 4uL RTPCR mix used to account for larger wells in 96 well plate. cDNA was then cleaned using a 0.8x ratio of homebrew SeraMag beads in 18% PEG (#GE24152105050250, CytiviaTm). cDNA concentrations and sizings were checked using Qubit (#Q33231, Thermo Fisher Scientific) and Agilent Bioanalyzer (#5067-4626 Agilent). cDNA were normalized to 200 pg/ μ L before tagmentation using 0.2 μ M of homemade Tn5 (EPFL). The reaction was halted with 0.2% SDS. Indexing PCR was performed to add Nextera index adapters (1 μ M final, Integrated DNA Technology) using KAPA HiFi reagents (#KK2102, Roche). Libraries were pooled in equal volumes and a final 0.8x cleanup was performed with homebrew SeraMag beads before measuring the sample concentration and sizing. The library pool was normalized and sequenced on Illumina NovaSeq SP flowcell (75-8-8-75) at approximately 25 million reads/sample. Basecalling and demultiplexing were performed with bcl2fastq (v2.20, Illumina Inc.).

Transcriptome quality check, mapping, quantification and variant analysis

Adapter sequences were removed from raw FASTQ files using Cutadapt (v4.9).(72) Subsequently, high-quality, adapter-trimmed reads were mapped against the human reference genome (GRCh38) using the STARlong utility within the STAR aligner.(73) Transcriptome assembly and quantification of gene expression was performed using StringTie2 at default parameters for each electrode from different brain regions in each case.(74) Then we performed differential gene expression for all regions and epilepsy brains using DESeq2.(75) We incorporated publicly available single-cell RNA-seq (scRNA-seq) data from epilepsy patients,(27) into our analysis. Quality checks, data reduction, and integration of healthy and epilepsy scRNA-seq data were performed using Seurat

(v4).(56,76) To identify variants using transcriptome data, we employed the GATK (v4.5.0.0) RNAseq short variant discovery (SNPs + Indels) pipeline.(56) The process began by marking duplicate reads with MarkDuplicates. Next, we used SplitNCigarReads to split reads with N in the CIGAR string into multiple supplementary alignments and hard clip mismatching overhangs. We then used AddOrReplaceReadGroups (Picard) to assign all reads in a file to a single new read group. Variants were called using HaplotypeCaller, followed by high-quality variant filtering with VariantFiltration, applying filters at QD < 2.0, FS > 60.0, MQ (number of reads supporting the variants) < 30.0, DP (reads coverage at variant site) < 20.0, and QUAL < 20.0.(55) Variants effect was determined using Ensembl Variant Effect Predictor (VEP) for high quality variants.(77) Further we intersect the variants present in all replicate for a region in epilepsy brains using BCFtools,(78) and annotated them using wANNOVAR.(79)

Methylation quality check, mapping, and differential Analysis

We performed quality checks using FastQC to assess the overall quality of the bisulfite-converted sequencing (BS-Seq) reads. Following quality control, adapter sequences were trimmed using TrimGalore. The human reference genome (GRCh38) was prepared using the bismark_genome_preparation utility from Bismark to account for bisulfite conversion. Subsequently, high-quality, adapter-trimmed reads were mapped to the prepared reference genome using Bismark (v0.22.3). Methylated cytosines were extracted from the mapped reads using MethylDackel, considering all three methylation contexts: CpG, CHG, and CHH (dpryan79/MethylDackel: A (mostly) universal methylation extractor for BS-seq experiments GitHub repository). Differentially methylated regions (DMRs) were identified using methylKit with a sliding window approach (window size = 200 bp, step size = 200 bp, minimum coverage = 10 bp, difference ≥ 5 & ≤ -5 and q value ≤ 0.05) using default parameters,

Methylated regions were further annotated using the bedtools intersect utility, to identify overlapping genes. Finally, we integrated methylation information at the gene level with their corresponding transcriptional expression data.

Visualization and statistical analysis

We used mapped BAM files to generate BigWig files for visualization of transcriptome and methylome density in gene bodies and flanking regions using deepTools,(80) various utilities available within SAMtools to prepare the file inputs for different analyses.(81) Statistical analyses and visualizations were performed using R packages (R Core Team, 2023). Finally, BioRender was used to create schematics and enhance the visual presentation of our figures.

Study approval

The present study was reviewed and approved by the Beaumont Hospital Medical Research Ethics Committee under study no. 20.58. All patients provided written informed consent.

Data availability

We have submitted raw fastq and count matrix for transcriptome and methylome data generated in this study to Gene Expression Omnibus (GEO) using accession numbers GSE268714 and GSE268715.

Author contributions

AD performed data analysis and wrote the manuscript. AM performed the experiments, and molecular analysis of samples and wrote the manuscript; AS and JL participated in sample procurement, neurophysiology and clinical data collection and analysis. KJS and DFO'B performed SEEG implantation procedures, and surgical resections and obtained

consent. PWW oversaw and interpreted the clinical, neurophysiological, radiological and pathological data collection and analysis, and edited the manuscript. RS and SP performed the FLASH-seq procedure on SEEG-derived RNA. VKT and DCH co-conceived the study, obtained funding, supervised the study, performed data interpretation, and co-edited the manuscript.

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Figure legends

Figure 1. Schematic framework for multimodal profiling of different epilepsy subtypes using SEEG electrodes. (A) Overview of multimodal data integration of single-source EEG, whole transcriptome, methylome and short variants profiles from electrodes collected from FCD, TLE, and RE brains. (B) We distinguished different sets of signatures based on differential gene expression and validated their patterns using multiple publicly available epilepsy data, followed by functional enrichment analysis. (C) Whole-methylome profiles were generated using the same samples and identified DMRs by investigating positive and negative correlations between the transcriptome and methylome data. (D) Snapshot of the integration of electrophysiology data with transcriptome signatures and a single-resolution map illustrating the correlation between transcriptome, methylome and variants levels for a known epilepsy risk-associated gene (*PACS1*) in both PZ and NIZ electrodes from an FCD brain.

Figure 2. Neurophysiology, neuroimaging, and neuropathological features of study participants. (A, E, I) Coronal, axial, and mesial sagittal volumetric MRI reconstruction showing the location of the implanted SEEG electrodes for patients A, B, and C. (B) Coronal T2-weighted MRI sequence of patient A. Arrow indicates the location of the FCD. (C) NeuN immunohistochemical staining for patient A demonstrated dysmorphic, haphazardly arranged and abnormally clustered cortical neurons consistent with FCD, ILAE Type IIa. Balloon cells were not identified. (D, H, L) Representative sample SEEG ictal recording with matched Epileptogenicity Index, epileptogenic network involvement based on visual inspection (VI) and presence or absence of contact points in our molecular analysis. Letters correspond to depth electrodes indicated in A, E, and I, bipolar recording. At ictal onset, there is fast repetitive spiking and paroxysmal fast activity. High pass filter 5Hz, low pass filter 80Hz, Notch 50Hz RNAseq: RNA sequencing; Met: Methylome sequencing; NIZ: Non-involved Zone, PZ: Propagation Zone, SOZ: Seizure Onset Zone, MTG: middle temporal gyrus, mHipp: mid-hippocampus, TOJ: temporo-occipital junction, pHipp: posterior hippocampus, aHipp: anterior hippocampus, SFG: superior frontal gyrus, MFG: middle frontal gyrus, IFG: inferior frontal gyrus, Orb: orbitofrontal, aCing: anterior cingulate, SMA: supplementary motor area, ITG: inferior temporal gyrus, aIns: anterior insula, MTP: mesial temporal pole, Op: opercular. (F) Coronal MRI FLAIR sequence of patient B demonstrating normal temporal lobe structures. (G) Haematoxylin and eosin staining for patient B showing Chaslin's subpial gliosis, indicated by arrow. (J) Coronal T2-weighted MRI sequence of patient C showing diffuse right hemisphere atrophy including the hippocampus, consistent with RE. (K) NeuN immunohistochemical staining for patient C showing severe hippocampal sclerosis with granular neuron depletion, dispersion and mossy fibre sprouting. No evidence of active encephalitis was present.

Figure 3. Single-source high throughput multi-omic profiling of EEG electrodes in epilepsy. (A) Selection of EEG electrodes: EEG electrodes were selected from SOZ, PZ and NIZ. The attached nucleic acid material was extracted from each electrode, and whole transcriptome and methylome data were generated. (B) Seven electrodes explanted from the brain of a patient with FCD were used. An average of 100 million and 85 million reads were sequenced for the methylome and transcriptome, respectively. Notably, 91 million and 74 million methylome and transcriptome reads, respectively, mapped to the human genome.

Similarly, nine electrodes explanted from the TLE patient were used. An average of 100 million and 95 million reads were sequenced for the methylome and transcriptome, respectively. Prominently, an average of 95 million and 87 million methylome and transcriptome reads, respectively, mapped to the human genome. Additionally, eight electrodes explanted from the RE patient were used. An average of 117 million and 101 million reads were sequenced for the methylome and transcriptome, respectively. Importantly, an average of 105 million and 92 million methylome and transcriptome reads, respectively, mapped to the human genome. (C) Genome wide transcriptome and methylome density: The transcriptome and methylome density of each electrode from all three patients were plotted across gene body and flanking regions (3kb). A consistent negative correlation between methylation and transcription was observed across all electrodes.

Figure 4. Distinct transcriptional signatures are activated in different epileptic brain regions. (A) Differentially expressed genes (DEGs; \log_2 fold change \pm 0.5 and P value \leq 0.05) identified in epileptic brains. (B) Plot showing the correlation between different replicates, and (C) the distribution of DEGs in PZ vs. NIZ brain regions for TLE patients. (D) Heatmap of upregulated genes enriched in epilepsy-related genes (P value \leq 0.05). (E) Dot plot showing upregulated genes enriched in upregulated (TLE vs. Control scRNA, P value \leq 0.05) interneurons and neurons in epilepsy patients compared to healthy controls and epilepsy-related genes (P value \leq 0.05). (F) Correlation between replicates and (G) volcano plot showing DEGs from the NIZ and PZ brain regions of RE patients. (H) Heatmap of upregulated genes enriched in epilepsy-related genes (P-value \leq 0.05). (I) Dot plot showing upregulated genes enriched in upregulated (TLE vs. Control scRNA, P-value \leq 0.05) genes of interneurons and neurons in epilepsy patients compared with healthy controls, as well as epilepsy-related genes (P-value \leq 0.05). (J) Plot showing the correlation between different replicates. (K) Distribution of DEGs in SOZ vs PZ brain regions for TLE patients. (L) Heatmap of upregulated genes enriched in epilepsy-related genes (P value \leq 0.05). (M) Dot plot showing upregulated genes enriched in TLE vs Control scRNA (P value \leq 0.05) of interneurons and neurons in epilepsy patients compared with healthy controls and epilepsy-related genes (P value \leq 0.05). Color code: Plasma: B-E/J-M, TLE PZ vs NIZ / TLE SOZ vs PZ; Viridis: F-I, RE PZ vs NIZ.

Figure 5. Insights into the epigenetic dysregulation in epilepsy. (A) Replicate wise correlation of methylation of CpG context from PZ and NIZ regions of TLE brain. (B and E) DMRs in PZ vs NIZ comparison with genes negatively correlated with their transcriptome and methylome level. (C and D) Transcriptome and methylome levels are elevated in NIZ electrodes compared to PZ electrodes in TLE brain. Notably, transcriptome and methylome show an antagonistic correlation across the gene body. Decreased methylome levels in these regions accompany increased transcriptome levels in the gene body and flanking regions. Conversely, decreased transcriptome levels at the TSS and TES coincide with increased methylome levels at these sites/regions. (E) A higher number of hypermethylated regions (2649) compared to hypomethylated regions (2405) were identified in PZ vs NIZ electrode comparisons. (F) Integration of transcriptome and methylome data revealed an inverse relationship between methylation and gene expression. A single-base resolution map was generated for *PTPRC* an epilepsy related gene, showing that depletion of

methylation levels increased transcriptome levels in PZ electrodes. Conversely, increased methylation levels resulted correlated with in decreased transcriptome levels in NIZ electrodes for *PTPRC*. In addition, we also observed *PTPRC* harboured short variants (SNPs/Indels) in PZ regions in TLE brain.

Figure 6. Transcriptional signatures associated with epileptogenicity index in epilepsy subtypes. (A and B) Relationship between the EI and transcriptional signatures in TLE and RE brains. This panel displays the relationship between the EI and transcriptional signatures across (set gene differentially expressed in the same direction in our and public data) different brain regions. We analyzed SOZ, PZ, and NIZ regions of TLE and RE. The results show that transcriptional signatures associated with a high epileptogenicity index (SOZ) in TLE and RE were also highly activated in epilepsy samples compared to healthy controls (data from an independent public dataset). Similarly, transcriptional signatures associated with a moderate epileptogenicity index (PZ) in non-lesional TLE and RE displayed increased activation in epilepsy compared to healthy controls (data from an independent public dataset). (A and B) The activity of transcriptional signatures in excitatory and inhibitory neurons present in epilepsy and healthy controls. The ridge plots show the activity of transcriptional signatures within excitatory and inhibitory neurons. We compared the scores of signature genes associated with a high epileptogenicity index in excitatory and inhibitory neurons from both epilepsy and healthy samples. The results revealed higher correlation between activity scores and epileptogenicity index for these signature genes in excitatory and inhibitory neurons of epilepsy samples compared to their counterparts in healthy controls.

Figure 1

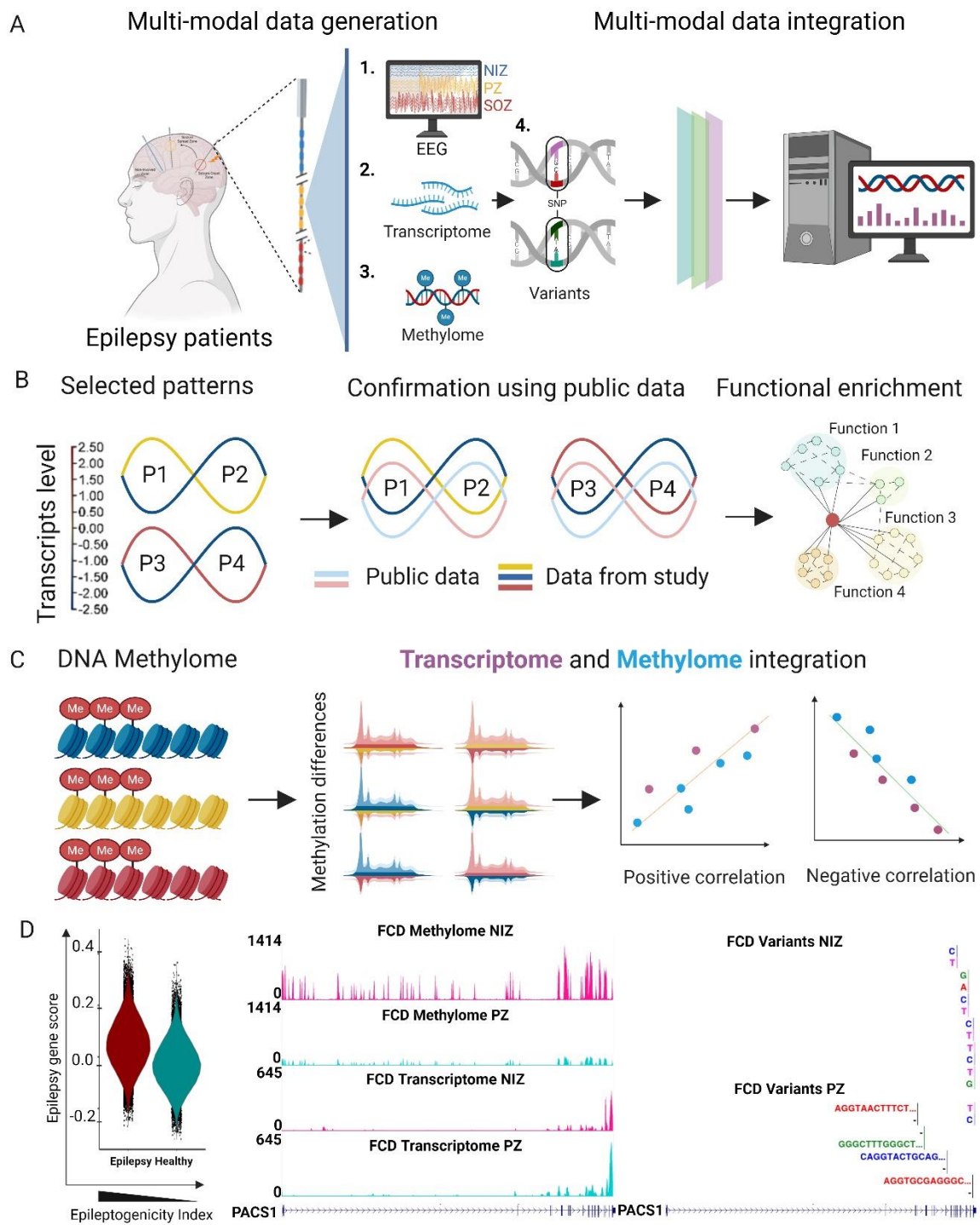


Figure 2

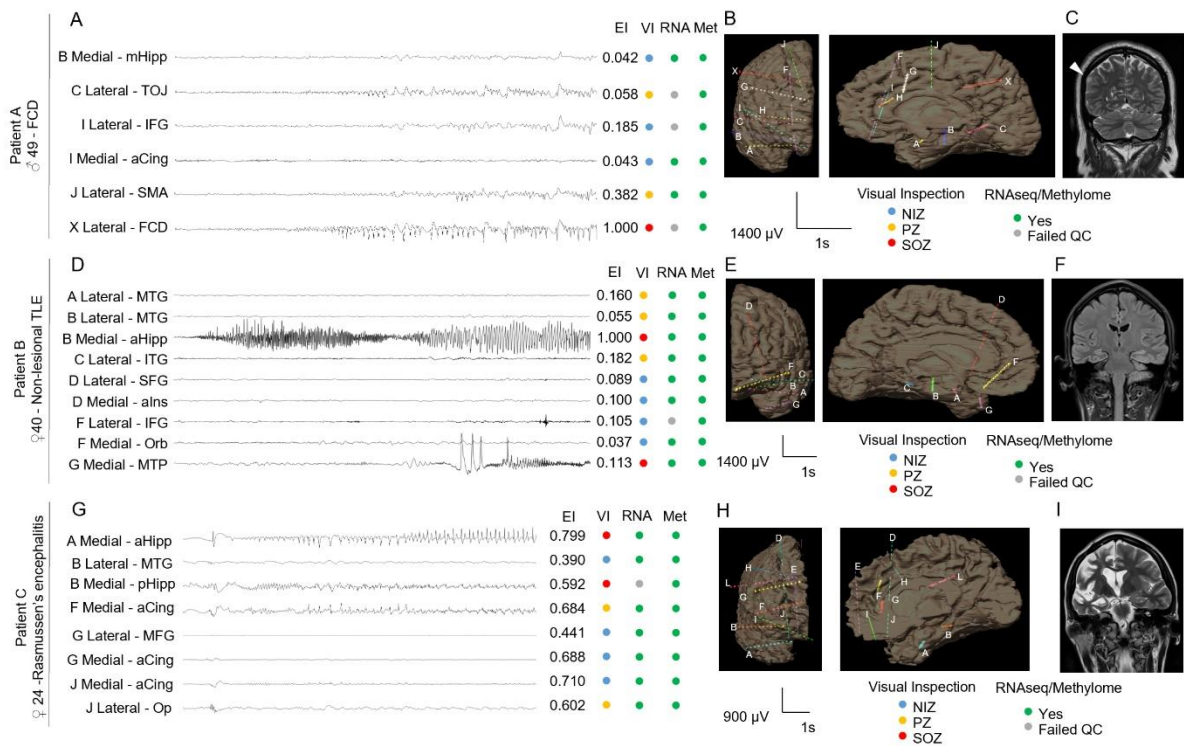


Figure 3

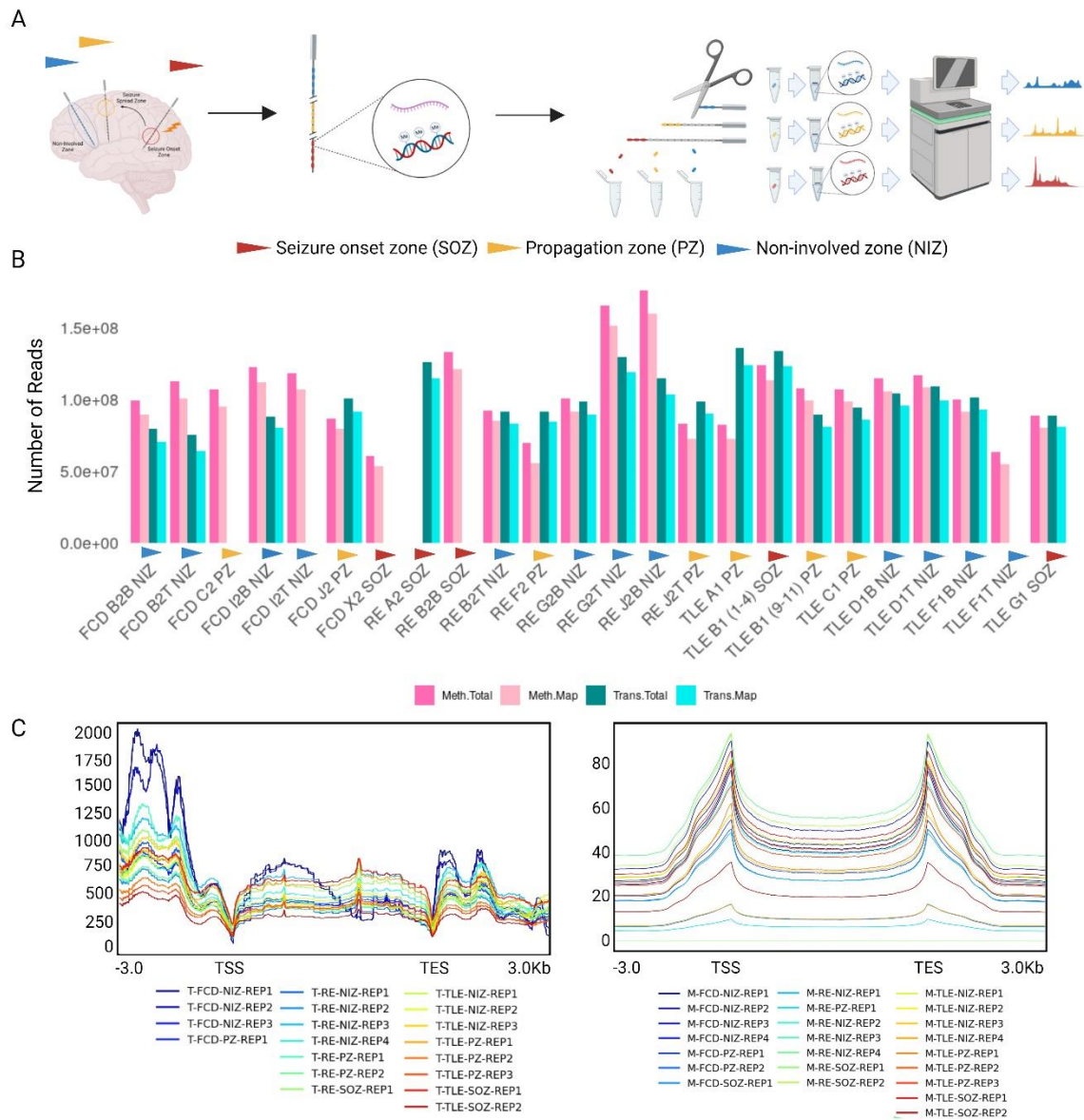


Figure 4

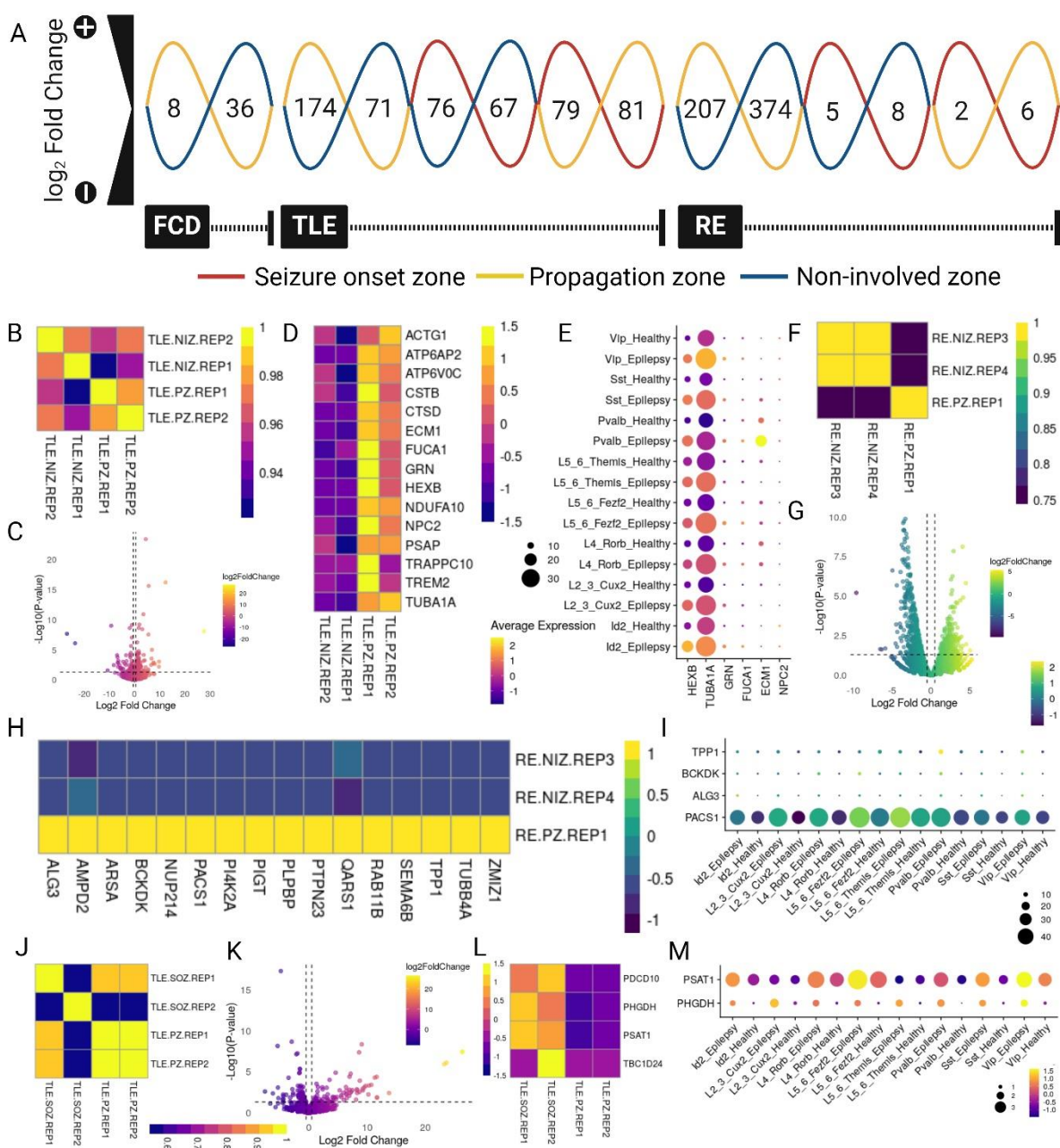


Figure 5

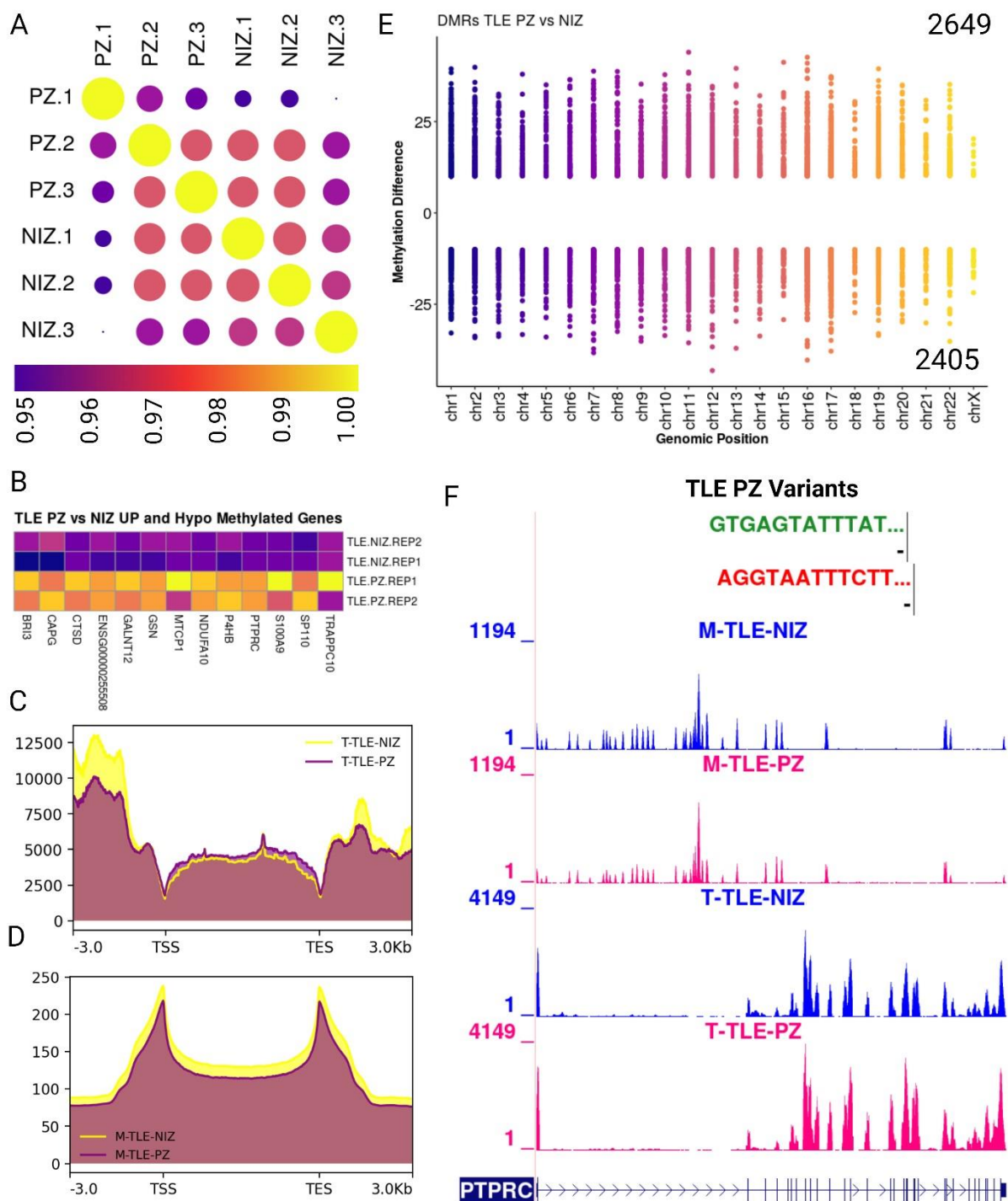


Figure 6

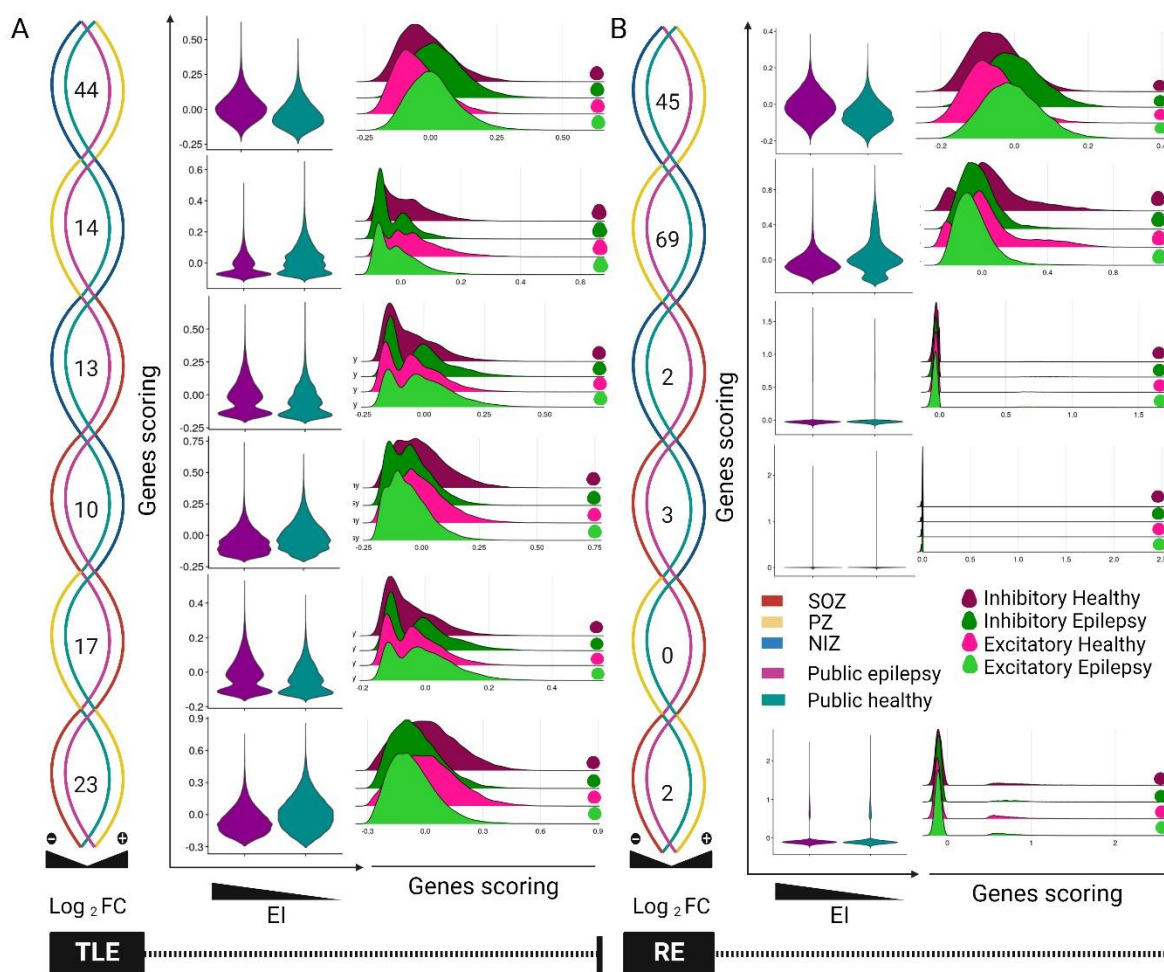


Table 1: Clinical characteristics of patients

	Patient A	Patient B	Patient C
Age (years)	49	40	24
Sex	M	F	F
Age at epilepsy onset (years)	15	32	15
Anti-seizure medications (prev. ASMs)	CAR, LEV, TOP (BRIV, GAB, LAC, LTG, PHEN, VAL, VIG)	CEN, ESL (BRIV, CLOB, CLON, LAC, LTG)	CLOB, LEV, PRE, ZON (CEN, CLON, ESL, LAC, LTG, PER, ZON, TOP)
Neuroimaging	FCD	Non-lesional	Diffuse right hemi-atrophy, gliosis
Duration of SEEG monitoring (days)	12	18	11
No. of depths	9	6	10
Surgery	Lesionectomy	Left ATL with AH	Right ATL with AH, inferior frontal resection with prefrontal disconnection
Histopathological diagnosis	FCD IIa	Chaslin's subpial gliosis	Hippocampal sclerosis, cortical gliosis, no inflammation
Surgical outcome, Engel Class	1A	2A	2A

FCD: focal cortical dysplasia, ATL: anterior temporal lobectomy, AH: amygdalo-hippocampectomy, CAR: carbamazepine, LEV: levetiracetam, TOP: topiramate, BRIV: brivaracetam, GAB: gabapentin, LAC: lacosamide, LTG: lamotrigine, PHEN: phenytoin, VAL: valproate, VIG: vigabatrin, CEN: cenobamate, ESL: eslicarbazepine, CLOB: clobazam, CLON, clonazepam, ZON: zonisamide, PRE: pregabalin, PER: perampanel.