Network Science applied to Epilepsy

BioBytes Group

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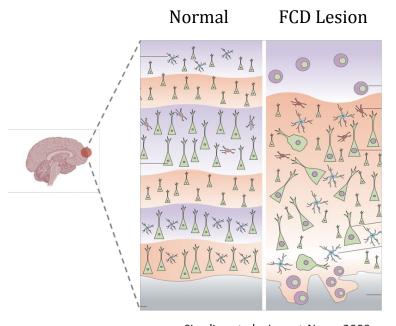
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Introduction

Epilepsy

○ Epilepsy is a disease caused by the **synchronous**, **excessive** or **abnormal** activity of neuronal cells in the brain.
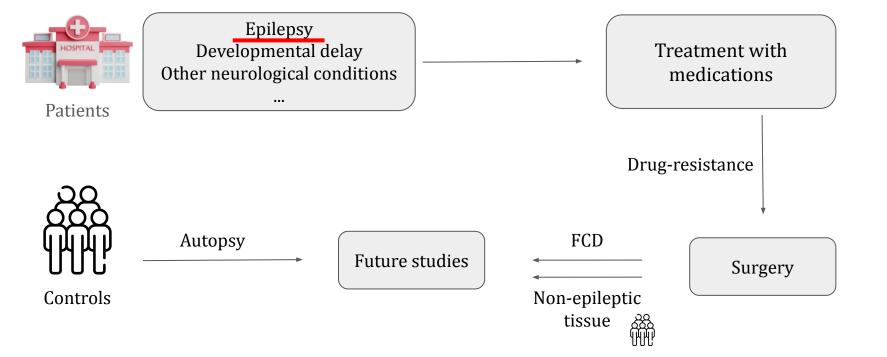
Focal Cortical Dysplasia

○ Pediatric epilepsy
○ Malformation of cortical development
○ Cortical and cellular abnormalities

Normal   FCD Lesion

Sisodiya et al., Lancet Neur., 2009

Samples

Patients

Epilepsy
Developmental delay
Other neurological conditions
...

Treatment with medications

Drug-resistance

Controls

Autopsy

Future studies

FCD
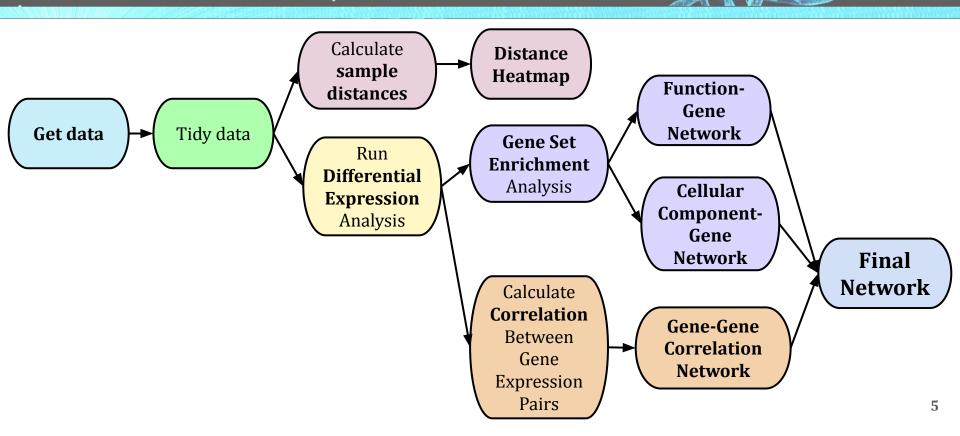
Non-epileptic tissue

Surgery

Proposal and Research Questions

Analyze the correlation between **genes** and **biological functions** in the analysis of differentially expressed genes in patients diagnosed with **epilepsy** caused by Focal Cortical Dysplasia type 2b (FCD 2b)

- What are the differentially expressed genes between **disease** and **control group**?
- Which **biological functions/cellular components** are most represented in the disease? ➜ **functional networks**
- How are these genes being regulated? ➜ **regulation networks**
- What are **the most important genes**, in the network, for FCD IIb, and how do they relate to the change in gene expression between the two groups?

Workflow

Get Data

GSE128300

- RNA-seq data
- Expression matrix
- n = 5 FCD IIb (collected during epilepsy surgery)
- n = 8 control (Autopsy + non-epileptic patients)
- Metadata obtained from the authors (.xlsx)
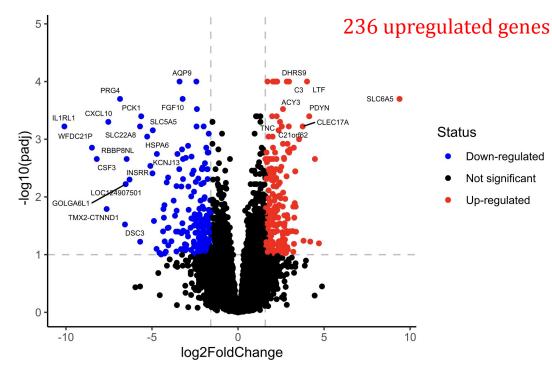

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128300
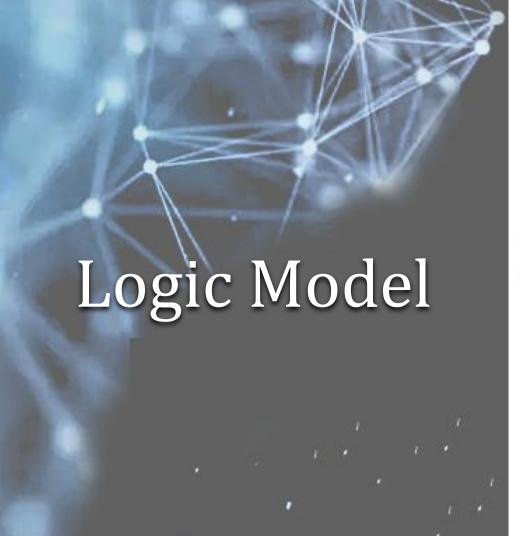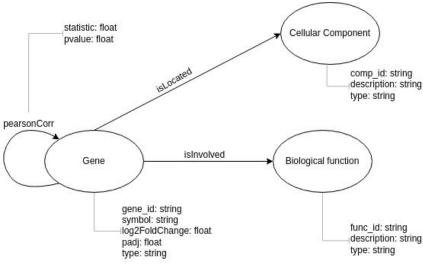
Differential Expression Analysis

Criteria:
- FC > 3
- padj < 0.1
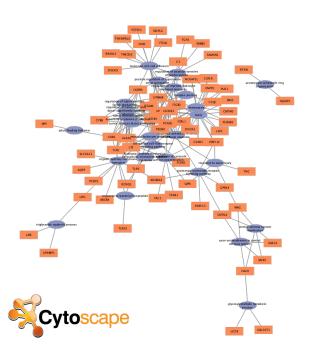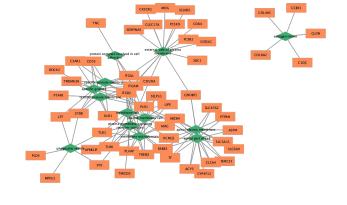
Logic Model

Gene Set Enrichment Analysis

ClusterProfiler
- EnrichGO
  - ont = "BP"
  - ont = "CC"

Function-Gene Network

Component-Gene Network
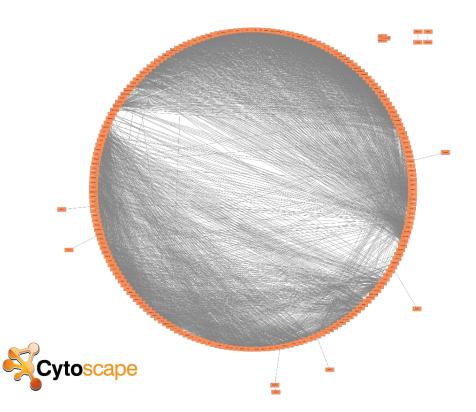
Correlation: Gene-Gene Network

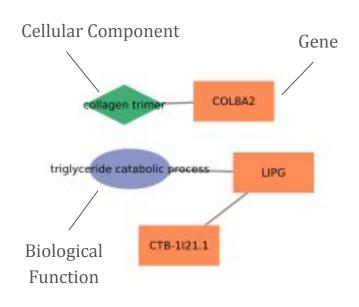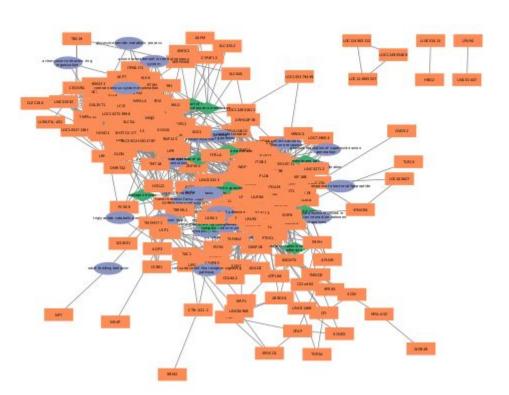Pearson Correlation
- P < 0.05
- statistic > 0.85

Final Network

Cellular Component

Gene

Biological Function

Statistical Analysis

Number of gene nodes	236
Number of function nodes	26
Number of cellular component nodes	13
Number of gene-gene edges	3650
Number of gene-function edges	210
Number of gene-cc edges	130

Number of nodes and edges

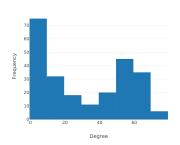|	
Av. deg. of genes	32.37
Av. deg. of functions	8.08
Av. deg. of cellular components	10.00
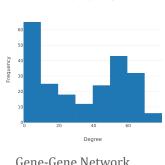
Average degree of genes, functions and cellular components

Statistical Analysis

Gene-Gene Network

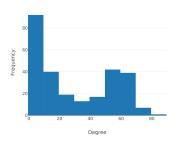Function-Gene Network
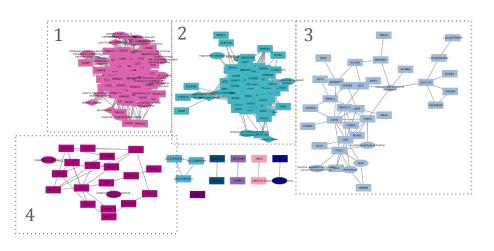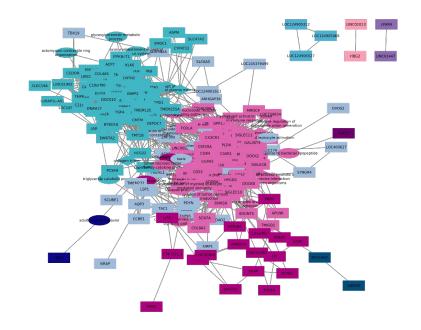
Component-Gene Network

Complete-Gene Network

13

Complete Network
12 communities

Communities Analysis: Leading EigenVector

Name	Degree	Comm
membrane microdomain	12	1
apical part of cell	15	2
external side of plasma membrane	15	4

Components with the highest degrees of each community
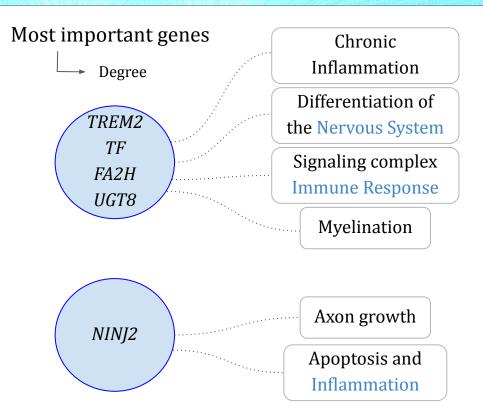
TREM2	82	1
TF	74	2
F2RL1	22	3
LTF	22	4

Genes with the highest degrees of each community

Name	Degree	Comm
leukocyte cell-cell adhesion	15	1
axon ensheathment in central nervous system	5	2
taxis	18	3
organic hydroxy compound transport	10	4

Functions with the highest degrees of each community

Most important elements

Most important genes

└──→ Degree

TREM2
TF
FA2H
UGT8

NINJ2

Chronic Inflammation

Differentiation of the Nervous System

Signaling complex Immune Response

Myelination

Axon growth

Apoptosis and Inflammation
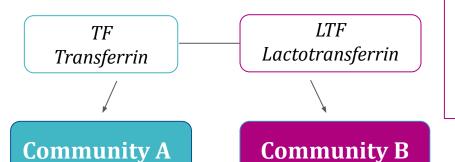
Most important functions	Degree
Chemotaxis	18
Leukocyte cell-cell adhesion	15
Myeloid leukocyte activation	14
Tumor necrosis factor production	12
Organic hydroxy compound transport	10

Most Important Component	Degree
External side of plasma membrane	15
Apical plasma membrane	15
Apical part of cell	15
Membrane raft	12
Membrane microdomain	12

Community Analysis

Transferrin family

- Iron capture for myelin sheath maintenance

TF Transferrin — *LTF Lactotransferrin*

- Anti-inflammatory activity
- Regulation of cellular growth and differentiation
- protection against reactive oxygen species

Community A

Community B

- Axon ensheathment in central nervous system
- Central nervous system myelination

- Response to axon injury
- Organic hydroxy compound transport

Fold-Change vs Degree
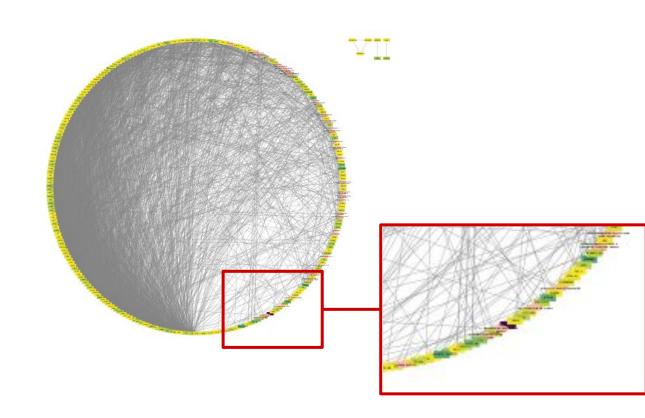
- Network important genes x Gene Expression

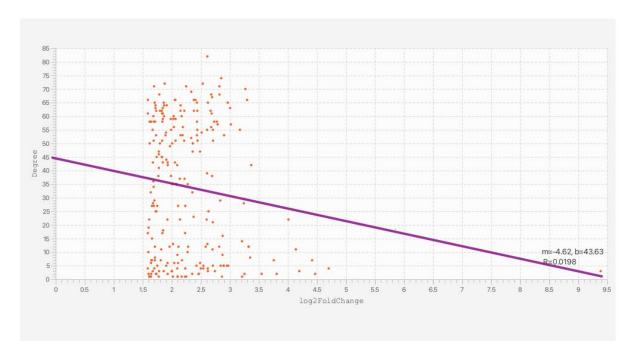| Highest degree	Highest Fold Change
TREM2	*SL6A5*
TF	*SERPINA5*
NINJ2	*LOC105379499*
FA2H	*LOC124901612*
UGT8	*ATP1B4*

Fold-Change vs Degree

- Network important genes x Gene Expression

Fold-Change vs Degree

- Network important genes x Gene Expression

Conclusions

Biological findings

- We found **236 up-regulated genes** (FC > 3; padj < 0.1), some of which have been **classically associated** with epilepsy/cortical malformations (e.g. CCBE1, ASPM) and some that had **not been reported** in the literature for the disease (e.g. NINJ2/LTF).
- Most of the biological functions overrepresented in the disease are linked to **immune system activation/inflammation**, which is in line with more recent findings in FCD.

Conclusions

Difficulties

- Networks assembling via Cytoscape (genes + functions + components)
  - It was necessary to create a new column to differentiate the type of node
- Robustness Analysis and Link Prediction with no clear biological effect
  - There was no biological interpretation for these kinds of analysis
- Community analysis using Cytoscape

Future Directions

- Experimental validation
- Exploratory study of disease-related genes not found in the literature
- Use of new technologies in this study

Thank You!
Questions?

FLORFINHA DA GRAFIDÃO

References

[1] - Patel P, Moshé SL. The evolution of the concepts of seizures and epilepsy: What's in a name? Epilepsia Open. 2020 Jan 10;5(1):22-35. doi: 10.1002/epi4.12375. PMID: 32140641; PMCID: PMC7049807.

[3] - Drugs.com: https://www.drugs.com/health-guide/seizure.html

[4] - Associations of B-Type Natriuretic Peptide and Its Coding Gene Promoter Methylation With Functional Outcome of Acute Ischemic Stroke: A Mediation Analysis - DOI:10.1161/JAHA.120.017499

[5] - DESeq2 (Bioconductor Software Package): https://bioconductor.org/packages/release/bioc/html/DESeq2.html

References

[6] - Gene Ontology: https://geneontology.org/

[7] - Cytoscape: https://cytoscape.org/

[8] - Transcriptomes distinguish human FCD subtypes:
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128300

[9] - Brain image theme: Getty Images (Yuichiro Chino)

[10] - DNA image theme: iStock (Shutter2U)