Leukocyte Zinc Finger Protein Expression Changes With Baseline Seizure Frequency In Patients With Intractable Temporal Lobe Epilepsy

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Introduction

Baseline seizure frequency (BSF) is a clinical measure of epileptogenicity.\(^1\) High seizure frequency has even been correlated with worse clinical outcomes following surgery.\(^2\) Low seizure frequency has also been correlated with better post-operative seizure control.\(^3\)

Epilepsy is an inflammatory disease with systemic effects, including modulation of the immune system.\(^4\) Effects on the immune system may be measured through dysregulation of certain genes and pathways inside of immune cells.\(^5-7\)

The pattern of gene expression within expression immune cells is representative of epilepsy severity.\(^1\) Therefore, genetic expression within immune cells may offer a window into the dominant mechanisms associated with an individual’s seizure pathology.

Zinc finger genes encode for an enormous superfamily of proteins involved in many different cellular processes.\(^8,9\) Zinc finger proteins can recognize and bind specific nucleotide sequences making them particularly important for altering the expression of other genes.\(^10\)
Methods

6 patients with intractable TLE were chosen from a larger cohort of 16 because they had the highest and lowest seizure frequencies. The patients were separated into a High Seizure Frequency (HSF) group and a Low Seizure Frequency (LSF) group.

Peripheral blood samples were taken immediately before epilepsy surgery and RNA was extracted from the leukocytes.

Gene expression counts were normalized and genes showing differential expression between groups were identified.

qRT-PCR analysis was performed on the differentially expressed genes to eliminate any false positives.

Pathway analyses were performed using the Reactome and Ingenuity Pathway Analysis databases.

A separate pathway analysis was performed with only the significant zinc finger genes using the Reactome Database.
Results – Demographics and BSF

Due to the small sample sizes, normality of the demographic variables could be assumed. A Mann-Whitney U test was used to compare demographic variables between the HSF and LSF groups.

There was a significant difference between BSF in the HSF and LSF groups ($p = 0.0463$).

There was not significant difference in age ($p = 0.2752$) or duration of epilepsy ($p = 0.5127$) between the HSF and LSF groups.

A Chi-Squared analysis showed gender had no influence on BSF ($p = 0.2733$).
Results – Significantly Dysregulated Genes

Three zinc finger genes showed significantly more expression in the HSF group vs the LSF group.

- **ZNF43** – Encodes for a zinc finger protein that is likely involved regulating expression of other genes through nucleic acid binding.
- **ZNF75A** – Encodes for a zinc finger protein that is likely involved in regulating the expression of other genes through nucleic acid binding.
- **ZNF91** – Encodes for a zinc finger protein that represses the transcription of the FCGR2B gene by binding to its promotor.\(^\text{11}\)

The FCGR2B gene codes for an inhibitory receptor found on immune cells. FCGR2B was not significantly dysregulated between groups but the FCGR2A and other related genes were.

### Zinc Finger Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>HGNC Description</th>
<th>Gene Biotype</th>
<th>P-Value</th>
<th>FDR</th>
<th>Fold Change</th>
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### Genes Related to FCGR2B

<table>
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<tr>
<th>Gene</th>
<th>HGNC Description</th>
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<th>P-Value</th>
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Analyses utilizing the Reactome and IPA databases with all the significantly dysregulated genes implicated pathways affected by the genetic dysregulation. The relative direction of activation/deactivation between groups could be determined in some cases.

The significant zinc finger genes were not involved in any pathways identified by the pathway analyses.

- A separate Reactome pathway analysis of only the zinc finger genes showed that ZNF43 and ZNF75A share 3 common pathways: genetic transcription pathway, RNA polymerase II transcription pathway, and gene expression pathway.

Pathways involving Fc Gamma Receptor phagocytosis and signaling were found in both pathway analyses.

- **IPA:** *Fc Gamma Receptor-mediated phagocytosis in macrophages and monocytes* pathway showed less activation in the HSF group. This pathway involves FCGR2A, FCGR3A, and FCGR3B.
- **Reactome:** *FCGR dependent phagocytosis* and *FCGR activation* pathways both showed negative Enrichment Scores, indicating less activation in the HSF group.
ZNF43 and ZNF75A shared 3 common pathways in the Reactome database. It is unclear what downstream changes occur due to the increased expression of these genes.

ZNF91 has been found to repress transcription of the inhibitory receptor FCGR2B. Both FCGR2A and FCGR2B bind the Fc portion of IgG. When activated, FCGR2A initiates a stimulatory response in immune cells while FCGR2B initiates an inhibitory response.

- Decreased activation of the FCGR-related pathways is likely a product of the observed genetic dysregulation.
- It is likely that ZNF91 is causing some degree of transcriptional repression of FCGR2B in the HSF group. However, this repression did not significantly reduce FCGR2B expression.
- Repressing transcription of FCGR2B may help maintain the balance between stimulatory and inhibitory signals within immune cells. Here, it seems the balance was tipped toward inhibition in the HSF group as the FCGR2A (stimulatory) receptor expression was reduced while the FCGR2B (inhibitory) receptor expression was not.
- These results could indicate a degree of leukocyte suppression in the HSF group that is not present in the LSF group.

Discussion
BSF is a valid measure of epileptogenicity and correlates with clinical outcomes of various epilepsy treatments. Differences in BSF also correlates with changes in genetic expression, not only in the brain, but in peripheral leukocytes. These changes can be measured and used as a widow into and individual’s epilepsy pathology.

Zinc finger genes code for a wide variety of proteins, most of which function as transcription factors that regulate the expression of other genes. Changes in the expression of zinc finger genes can be used to find links between seemingly unrelated pathways and to explain the differential expression of other functional genes.

More research to determine the cellular functions of the zinc finger genes discussed here may lead to greater insights about the complex relationship between epilepsy and the immune system.
References


