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Cortical gene expression correlates of temporal lobe epileptogenicity

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ABSTRACT

Introduction: Despite being one of the most common neurological diseases, it is unknown whether there may be a genetic basis to temporal lobe epilepsy (TLE). Whole genome analyses were performed to test the hypothesis that temporal cortical gene expression differs between TLE patients with high vs. low baseline seizure frequency.
Methods: Baseline seizure frequency was used as a clinical measure of epileptogenicity. Twenty-four patients in high or low seizure frequency groups (median seizures/month) underwent anterior temporal lobectomy with amygdalohippocampectomy for intractable TLE. RNA was isolated from the lateral temporal cortex and submitted for expression analysis. Genes significantly associated with baseline seizure frequency on likelihood ratio test were identified based on >0.90 area under the ROC curve, P value of <0.05.
Results: Expression levels of forty genes were significantly associated with baseline seizure frequency. Of the seven most significant, four have been linked to other neurologic diseases. Expression levels associated with high seizure frequency included low expression of Homeobox A10, Forkhead box A2, Lymphoblastic leukemia derived sequence 1, HGF activator, Kelch repeat and BTB (POZ) domain containing 11, Thanatos-associated protein domain containing 8 and Heparin sulfate (glucosamine) 3-O-sulfotransferase 3A1.
Conclusions: This study describes novel associations between forty known genes and a clinical marker of epileptogenicity, baseline seizure frequency. Four of the seven discussed have been previously related to other neurologic diseases. Future investigation of these genes could establish new biomarkers for predicting epileptogenicity, and could have significant implications for diagnosis and management of temporal lobe epilepsy, as well as epilepsy pathogenesis.

1. Introduction

Epilepsy affects 2–4 million people in the United States. The disorder affects 1 in 50 children and 1 in 100 adults, making it one of the most common neurologic disorders [1,2]. Partial epilepsies such as temporal lobe epilepsy (TLE) predominate in adults, most commonly in the mesial form, with ictal onset occurring in the hippocampus, amygdala, and parahippocampal cortex [1,3,4].

Anti-epileptic drugs (AEDs) are the mainstay of epilepsy therapy. Patients whose seizures are resistant to two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) are referred to as having "intractable epilepsy" and become candidates for other treatments [5]. Amygdalohippocampectomy, with or without anterior temporal lobectomy (ATL), is the most commonly employed operation for those with TLE who fail to achieve good control of their seizures on two first-line AEDs [2,6,7]. Selective amygdalohippocampectomy has the benefit of preserving lateral cortical brain structures and results in positive outcomes for seizure freedom despite a possible small increased risk of recurrent seizures compared to ATL [7]. Seizure freedom can be achieved in

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approximately 65% of intractable TLE patients with temporal lobectomy with amygdalohippocampectomy (ATL/AH) [8]. Patients with intractable temporal lobe epilepsy who have undergone ATL/AH make up the sample group for this study.

To-date there is limited data regarding the molecular mechanisms underlying temporal lobe epilepsy or temporal lobe epileptogenesis, except for those cases in which there is an apparent lesion – such as in the cases of head trauma, strokes or neoplasms [1,9–11]. Long-term temporal cortical cerebral blood flow (CBF) recording has shown that a direct linear relationship exists between epileptic temporal cortical CBF and seizure interval (frequency^{-1}), a clinical measure of epileptogenicity [12]. Based on this finding that temporal lobe epileptogenicity is correlated with lateral temporal cortical physiology, the current study was designed to investigate whether lateral temporal cortical gene expression contributes to the pathophysiology of temporal lobe epileptogenicity as expressed by seizure frequency. The purpose of this study is to compare the gene expression profiles of temporal lobe brain tissue obtained from patients who have undergone ATL/AH with patient baseline seizure frequency. Our possession of human epileptic brain tissue presented a critical opportunity to evaluate molecular profiles associated with practical clinical markers of epileptogenicity. Knowing which patients may be genetically more likely to have increased epileptogenicity will help inform treatment choices.

2. Methods

This study was performed in accordance with protocol and research consents approved by the University of Arizona Institutional Review Board. Brain tissue samples were obtained from subjects who underwent ATL/AH at University Medical Center (Tucson, AZ) based on previously described clinical selection criteria and neurosurgical operative technique for unilateral intractable temporal lobe epilepsy [8]. The extent of cortical resection was 4.5 cm in length from anterior to posterior in the temporal (middle) fossa on the left side and at least 5.0 cm in length from anterior to posterior in the temporal (middle) fossa on the right side. All tissue samples were stored in RNAlater RNA stabilization Solution (Qiagen, Valencia, CA) at −80 °C until RNA extraction was performed. RNA was extracted using the RNeasy lipid tissue mini kit (Qiagen, Valencia, CA) following manufacturer's instructions. RNA was then stored at −80 °C until RNA analysis was performed.

The isolated total RNA samples were used to produce labeled targets, hybridized to Affymetrix GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA), and read using the Agilent/Affymetrix 2500A (Affymetrix, Santa Clara, CA) scanner according to manufacturer's protocols.

2.1. Statistical analysis

Descriptive statistics were conducted using R v. 3.2.2 [13]. Comparisons were made using Fisher's exact test for all demographics except age. Student *t*-test was used to analyze the significance of age among the groups. The level of significance was established at $P < 0.05$. Comparisons were also made of individual medication use across the two seizure frequency groups.

Analysis of gene expression data was performed utilizing the bioinformatics and statistical tools provided by the R programming and BioConductor projects (www.r-project.org and www.bioconductor.org.) The analysis included assessment of data quality, positive and negative controls on the Affymetrix ST1.0 microarrays, probe annotation, and analysis of pathways and biological function (gene ontologies). Whole genome data were analyzed for predictive value for baseline seizure frequency preceding ATL/AH by logistic regression using the likelihood ratio test. Baseline seizure frequency was a useful parameter because it is a clinical measure obtainable through patient history. Median baseline seizure frequency (four seizures per month) was used as the cut-off between high and low seizure frequency groups, with four or more seizures per month defining the high seizure frequency group and less than four defining the low seizure frequency group. Logistic regression criteria for significance were defined by a high quality regression model with an area under the receiver operator curve (AUC) > 0.9 and *P* values ≤ 0.05 for the slope coefficient of the logistic regression gene models. A 'leave one out' cross validation (LOOCV) was performed for these probes. For each gene, individual samples were removed in turn and a logistic regression model was built. The model was then used to score the removed observation for validation. Conditional density plots of each gene were produced, which indicate the predictive value of increasing expression cutoff values for either epilepsy group.

3. Results

3.1. Subject characteristics

Twenty-five patients underwent ATL/AH for medically intractable temporal lobe seizures. All patients had lateral temporal cortex removed during routine course of the surgery and this brain tissue was preserved for genetic analysis. One of these patients was excluded from the analysis. The excluded patient's array (array 10) stood out noticeably with regards to three separate array quality control measures. Array 10 had universally the lowest correlation with the other arrays (correlation coefficients of 0.91-0.95). All other arrays correlated much more with each other (coefficients of 0.97–1.0). Additionally, a noticeable difference existed in both control and expression value density for array 10 compared to the others. Based on this, the decision was made to remove array 10 from the analysis. The remaining 24 subjects were divided into two groups, baseline seizure frequency of 4 seizures per month or more (N = 16) or fewer than 4 seizures per month (N = 8). Table 1 shows comparison of the demographic data for all studied patients in the two seizure frequency groups. Comparison was also made of pre-operative medication use across the two groups, as described in Table 2.

3.2. Array quality control

RNA A260/A280 was 1.9–2.1 and yield was 3.7–30.2 μg from 100 mg of brain tissue. The density plot does not show any outlying arrays. Positive and Negative control probes are well spread between the two groups and show good separation in values. No array showed significant degradation. Overall, this represents consistently high quality data. Positive and negative controls were left in the data to be analyzed to assess further the role of gene probes in distinguishing the two groups.

3.3. Predictive genes

Logistic regression analysis identified 40 genes that met the criteria for having significantly different expression levels between high seizure frequency and low seizure frequency groups (AUC > 0.9 & $P < 0.05$, Table 3).

High seizure frequency was associated with significant relative down-regulation of most genes according to the analysis. Of the forty genes in Table 3, only five were associated with high seizure frequency at increased expression levels. The top seven genes in Table 3 represent those that were most significant in our analysis. These make up the body of our discussion. Conditional