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Gene expression analysis in untreated absence epilepsy demonstrates an inconsistent pattern

Markus von Deimling[a,*], Robert Häsler[b], Verena Steinbach[a], Paul-Martin Holterhus[c], Sarah von Spiczak[a,d], Ulrich Stephani[a,d], Ingo Helbig[a,e], Hiltrud Muhle[a]

[a] Department of Neuropediatrics, University Medical Center Schleswig-Holstein, Kiel University, Kiel, Germany
[b] Institute of Clinical Molecular Biology, Kiel University, Kiel, Schleswig-Holstein, Germany
[c] Department of Pediatrics, University Medical Center Schleswig-Holstein, Kiel University, Kiel, Germany
[d] Northern German Epilepsy Center for Children and Adolescents, Schwentinental-Raisdorf, Schleswig-Holstein, Germany
[e] Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

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ABSTRACT

Objective: Childhood and Juvenile Absence Epilepsy account for 30% of all genetic generalized epilepsies with a strong genetic contribution. At the current state the genetic background remains to be resolved. The aim of this study was to identify disease associated transcripts pinpointing potential underlying disease mechanisms in patients with CAE and JAE.
Methods: We performed gene expression analysis from peripheral blood mononuclear cells (PBMCs) in 30 patients with newly-diagnosed absence epilepsy prior to initiating treatment and 30 healthy age – and gender-matched pediatric controls. In a first group (group 1), 10 patients and controls we performed genome-wide transcriptome analysis using the Affymetrix HG U133 2.0+ microarray. 75 differentially expressed genes were followed up by qRT-PCR in two independent groups of 10 patients and controls (group 2 and 3). Furthermore, we analyzed 18 candidate genes by qRT-PCR in groups 2 and 3, which had previously been considered strong candidates for genetic epilepsies.
Results: Genome-wide gene expression analysis in group 1 revealed 601 differentially regulated genes. Independent validation of 75 group 1-derived genes by qRT-PCR in groups 2 and 3 confirmed candidate genes with a consistent, but non-significant pattern of up- or down-regulation across all groups (*ATP1B3*, *CAND1*, *PRPF6*, *TRIM8*). Previously known genes including *GABRA1*, *GABRB3*, *GABRG2*, and *RCN2* showed evidence for up- or down-regulation in individual experiments, but were not reliable across groups either.
Discussion: Gene expression analysis in absence epilepsy from PMBCs displayed a high degree of heterogeneity between different patient groups. Our study provides several potentially interesting candidate genes, while demonstrating the limits of using gene expression analysis from blood in the identification of novel pathogenic mechanisms. In particular, we found that gene expression levels vary in response to altered experimental conditions, representing a substantial challenge for the identification of disease-related gene expression signatures for neurological diseases from whole blood.

1. Introduction

Childhood Absence Epilepsy (CAE) and Juvenile Absence Epilepsies (JAE) are common epilepsy disorders in childhood and adolescence, formerly named Idiopathic Generalized Epilepsies (IGE). These conditions are now classified as Genetic Generalized Epilepsies (GGE) (Berg et al., 2010), emphasizing the presumed genetic etiology in these conditions. GGE account for about 30% of all epilepsies (Jallon and Loiseau, 2001) and within GGE absence epilepsies account for roughly 13% of all seizure disorders (Hauser et al., 1993). Twin studies have demonstrated a strong genetic com-

* Corresponding author at: Department of Neuropediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Building 9, Arnold-Heller-Str. 3, 24105 Kiel, Germany.
E-mail addresses: m.v.deimling@pedneuro.uni-kiel.de (M. von Deimling), r.haesler@mucosa.de (R. Häsler), vvolz@hotmail.de (V. Steinbach), holterhus@pediatrics.uni-kiel.de (P.-M. Holterhus), s.vonspiczak@pedneuro.uni-kiel.de (S. von Spiczak), stephani@pedneuro.uni-kiel.de (U. Stephani), helbigi@email.chop.edu (I. Helbig), muhle@pedneuro.uni-kiel.de (H. Muhle).

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ponent to GGE (Berkovic et al., 1998; Marini et al., 2004), but the genetic architecture of GGE largely remains to be resolved. Despite the identification of rare variants and single mutations of major effect, a heterogenic and multifactorial etiology of GGEs that follows a complex trait is frequently assumed, limiting the abilities to resolve GGEs genetically with currently available technologies (Berkovic et al., 2006; Helbig et al., 2008b; Kjeldsen et al., 2003).

Assessing the role of particular genes and pathways through the analysis of genome-wide gene expression is a commonly used technology in epilepsy and other neurologic conditions (Greiner et al., 2013; Helbig et al., 2008a; Hershey et al., 2008; Tang et al., 2004b). However, this technology is limited when assessing common neurological conditions in childhood. The accessibility of brain tissue in patients with absence epilepsy is limited and interindividual and interallelic gene expression may be highly variable (Cheung et al., 2003; Yan et al., 2002). However, previous studies showed that peripheral blood cells can be an adequate surrogate for brain tissue given the fact that some target genes are expressed in both brain and blood (Borovecki et al., 2005; Rollins et al., 2010; Sullivan et al., 2006; Tylee et al., 2013). Furthermore, gene expression studies have previously been performed in neurological disorders including absence epilepsies (Borovecki et al., 2005; Greiner et al., 2013; Helbig et al., 2008a; Hershey et al., 2012) and have suggested novel candidate genes. However, many antiepileptic medications including valproic acid can alter gene expression and may be potential confounding factors (Milutinovic et al., 2007; Tang et al., 2004a). So far, gene expression studies have not taken into account the dynamic course of the seizures in patients with absence epilepsy and not have examined gene expression profile in patients with active absence epilepsy prior to treatment.

In this present study, we perform gene expression analysis from whole blood in patients with untreated and newly diagnosed absence epilepsy compared to age and gender-matched controls. We follow up 75 candidate genes from an initial genome-wide gene expression screen in two independent groups. Hypothesizing that significant changes in gene expression would be detectable using different RNA extraction methods, we refrained from combining groups 2 and 3 and considered them as independent sample pools (see Section 2). Several genes showed a consistent pattern of up- or down-regulation across all groups and significant differential expression in two groups. Previously implicated candidate genes were confirmed to be differentially expressed in groups of 10 patients with absence epilepsy and age and gender-matched controls.

Taken together, our study emphasizes the inherent technological challenges in identifying candidate genes from blood-derived gene expression studies. Integrating data from previous studies, we outline a framework for the usefulness of gene expression studies in common neurodevelopmental disorders and suggest that in parallel to genetic association studies, reducing random noise through larger samples sizes will be the key to identifying reproducible gene expression signatures.

2. Methods

2.1. Study population

Our study population consisted of 30 pairs of unrelated patients with either CAE or JAE and controls matched for age and gender. Blood samples were obtained when patients were first diagnosed with absence epilepsy prior to anticonvulsive treatment with any antiepileptic medication. RNA was extracted from peripheral blood mononuclear cells (PBMC) using standard protocols. Each patient was assigned to a healthy, age- and gender-matched pediatric control participant. All patients were recruited at the Department of Neuropediatrics, University Medical Center Schleswig-Holstein, Kiel University in Kiel, Germany and participants and/or families gave full written informed consent. The study was approved by the local IRB of the Kiel University in Kiel, Germany.

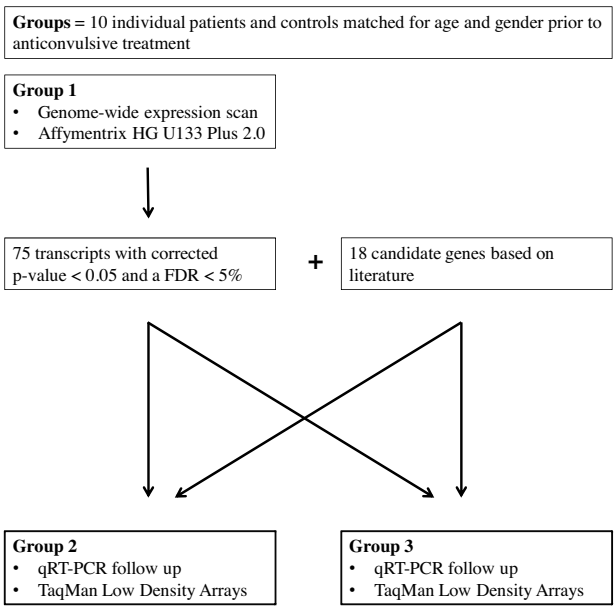

Fig. 1. Design of the overall study: The overall study was constructed as a three step approach. In a genome-wide transcriptome analysis hundreds of genes were identified to be differentially expressed with statistical significance. The first 75 transcripts after ranking all identified transcripts according to their *p*-value were followed up in two independent groups by qRT-PCR. In addition, 18 candidate genes that have previously been described as strong candidates for epilepsies were chosen for validation. Each group consisted of 10 patients with newly-diagnosed, untreated epilepsy and 10 age- and gender-matched controls.

2.2. Mircoarray hybridization (Group 1)

Total RNA was extracted using the RNeasy Mini Kit (Qiagen) and further processed for hybridization to an Affymentrix HG U133 Plus 2.0 (Fig. 1) following the manufacturer's guidelines as previously described (Häsler et al., 2012). Subsequent data normalization was performed using GCRMA (R, Bioconductor). Signals that were not present in at least 80% of all samples in at least one analytical group (patients or controls) were omitted from further analysis. Significant difference of transcript levels was determined by a Mann-Whitney *U* test with a Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995). Signed fold changes, which were based on ratios of medians were subjected to a Westfall and Young permutation using K = 5000 permutations (Westfall and Young, 1993) in order to generate false discovery rate (FDR). Transcripts with a corrected *p*-value < 0.05 and a FDR <5% were considered differentially expressed.

2.3. qRT-PCR (Groups 2 and 3)

Overall, 96 genes, including three housekeeping genes (*ACTB*, *B2 M* and *GAPDH*), were followed up by quantitative real time PCR (qRT-PCR) (Fig. 1, Supplementary Table 1 and Table 1) in groups 2 and 3 which were considered independently from each other due to different RNA extraction methods.

2.3.1. Hypothesis-based gene selection

Statistical work up of the genome-wide transcriptome analysis generated a list of all genes ranked by their *p*-value after correction for multiple testing. The first 75 genes showing dif-