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Gene expression variance in hippocampal tissue of temporal lobe epilepsy patients corresponds to differential memory performance

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ABSTRACT

Temporal lobe epilepsy (TLE) is a severe brain disorder affecting particularly young adults. TLE is frequently associated with memory deterioration and neuronal damage of the hippocampal formation. It thereby reveals striking parallels to neurodegenerative disorders including Alzheimer's disease (AD). TLE patients differ with respect to their cognitive performance, but currently little is known about relevant molecular–genetic factors. Here, we correlated differential memory performance of pharmacoresistant TLE patients undergoing neurosurgery for seizure control with in-vitro findings of their hippocampal tissues. We analyzed mRNA transcripts and subsequently promoter variants specifically altered in brain tissue of individuals with 'very severe' memory impairment. TLE patients (n = 79) were stratified according to preoperative memory impairment using an established four-tiered grading system ranging from 'average' to 'very severely'. Multimodal cluster analyses revealed molecules specifically associated with synaptic function and abundantly expressed in TLE patients with very impaired memory performance. In a subsequent promoter analysis, we found the single nucleotide polymorphism rs744373 C-allele to be associated with high mRNA levels of bridging integrator 1 (BIN1)/Amphiphysin 2, i.e. a major component of the endocytotic machinery and located in a crucial genetic AD risk locus. Using in vitro luciferase transfection assays, we found that BIN1 promoter activation is genotype dependent and strongly increased by reduced binding of the transcriptional repressor TGIF. Our data indicate that poor memory performance in patients with TLE strongly corresponds to distinctly altered neuronal transcript signatures, which – as demonstrated for BIN1 – can correlate with a particular allelic promoter variant. Our data suggest aberrant transcriptional signaling to significantly impact synaptic dynamics in TLE resulting in impaired memory performance and may serve as basis for future therapy development of this severe comorbidity.

1. Introduction

Memory disruption is a major feature in TLE patients that has been extensively studied during the last decades. Multiple factors were found to influence memory performance such as the type of epilepsy, effects of drug treatment, seizure onset, comorbidity conditions and alterations of the hippocampal structures i.e. gliosis and cell loss (Bell et al., 2011). However, the relationship between memory impairment and genetic variability in TLE patients has largely been unexplored. In patients with pharmacoresistant temporal lobe epilepsy, the APOE ε4 allele affects memory performance, both verbal and nonverbal, in interaction with longstanding seizures (Busch et al., 2007). Genome-wide association studies (GWAS) confirmed the ε4 allele of the apolipoprotein E (APOE) gene as the main genetic risk factor for late-onset AD (LOAD). APOE ε4 carriers show enhanced AD pathology, accelerated age-dependent cognitive decline and worse memory performance than non-carriers (Raber et al., 2004). APOE ε4 was, however, not found to be associated with early onset TLE (Blümcke et al., 1997). GWA-studies recently suggested new susceptibility loci associated with LOAD including gene loci of phosphatidylinositol binding clathrin assembly protein (PICALM), Clusterin (CLU) or Amphiphysin 2 (BIN1) (Hollingworth et al., 2011; Harold et al., 2009). With regard to the shared cognitive and structural alterations in AD and TLE, such risk loci may potentially impact the clinical outcome of TLE patients as well. In TLE and AD, neurodegeneration that leads to synaptic failure is a central condition. Thus, it may not be surprising that severe memory deficits characterize both diseases, which in TLE patients manifest

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depending on the lateralization of the epileptogenic focus: The language-dominant left hemisphere is particularly sensitive to verbal memory impairment whereas the non-dominant right hemisphere is more susceptible to compromised figural memory. Altered neuronal cell densities as molecular correlates of memory disturbance were found in hippocampal specimens of TLE patients but only in the left hippocampus for verbal memory parameters (Witt et al., 2014).

Here, we used our unique access to human hippocampal tissue from pharmacoresistant TLE patients with distinct memory performance in order to characterize potentially altered gene expression signatures with this comorbidity. We therefore started with fresh frozen hippocampal tissue gained after epilepsy surgery for seizure control to conduct genome wide expression array analysis. We used a four-tiered classification system of memory performance including patients with very severe (group 1), severe (group 2), borderline (group 3) and average (group 4) memory performance. Our data show similar alterations of transcript patterns in left and right hippocampi specifically associated with neuronal structures in individuals with very severe memory performance. Subsequent promoter analysis revealed that SNPs are differentially distributed according to memory performance with striking influence on respective mRNA levels as for BIN1, a key component of the endocytotic machinery. These data indicate that distinct single nucleotide polymorphisms (SNPs) located in promoter regions contribute to a significant extent to an individual's cognitive performance in human TLE.

2. Materials and methods

2.1. Surgical specimens and memory assessment

Hippocampal biopsy specimens of 136 Caucasian patients with chronic mesial TLE who underwent surgical treatment in the Epilepsy Surgery Program at the University of Bonn Medical Center were included in the study. Seizure onset in the mesial temporal lobe was confirmed by presurgical invasive and non-invasive procedures in every case (Kral et al., 2002).

Informed consent for use of their tissue was obtained from every patient and all procedures were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the University of Bonn Medical Center. Comprehensive clinical characteristics of patients were available for our analyses as described in detail previously (Grimminger et al., 2013; Pernhorst et al., 2011).

All patients underwent a comprehensive neuropsychological examination of verbal and nonverbal declarative memory before undergoing epilepsy surgery assessed by the 'Verbaler Lern- und Merkfähigkeitstest' ('verbal learning and memory test'; VLMT) (Helmstaedter et al., 2001) and a revised version of the 'Diagnosticum für Cerebralschädigung' ('diagnosticum for cerebral damage'; DCS-R) (Helmstaedter et al., 1991) as previously described (Witt et al., 2014). The VLMT consists of five learning trials with immediate free recall of 15 words followed by free recall after an interval period and a final free recall and recognition trial after a delay of 30 min. Figural memory is assessed by the DCS-R, which includes five consecutive trials comprising learning and reconstruction of nine geometric figures. After a 30-minute delay a recognition trial follows. The overall performance of accurately remembered items of all trials was taken into account. Memory parameters were standardized and age corrected to a co-normalization sample of healthy individuals. According to the test results, patients were classified into the following groups according to the relative impairment: very severely (1), severely (2), borderline (3) and average (4). Classification into three groups for joint verbal and figural memory performance was based on the same scoring systems but combining the results for both types of memory as follows: 'Group a' includes all patients with very severely impaired memory performance in one type or severely impaired memory performance in both types of memory. In 'group b', all patients with marginal or marginal and severely impaired memory performance are included. 'Group c' contains patients with overall average or average and marginal memory performance. Based on this scheme, all patients could be further stratified according to the following memory performance impairment classes: very severely (a), borderline (b) and average (c). Tissue samples were neuropathologically analyzed by an experienced neuropathologist (AJB) following international standards and only hippocampi with representation of the individual subfields were included (Blümcke et al., 2007). The biological specimens for the present study included an equivalent number of left ($n = 42$) and right ($n = 37$) hippocampal tissues for respective analyses on verbal and nonverbal memory.

2.2. Real-time quantitative (q)RT-PCR

Sections up to 20 μm were used for mRNA isolation from frozen human hippocampal tissue samples and total mRNA was extracted from the hippocampus of wild type adult mouse and NG108-15 neuroblastoma cells using the Dynabeads mRNA Direct Micro Kit (Dynal, Oslo, Norway) according to the manufacturer's protocol. For real-time quantitative (q)RT-PCR confirmation experiments we prepared complementary cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA) by reverse transcription following the manufacturer's protocol.

Transcript quantification of *BIN1*, *TGIF*, *β-actin* and *synaptophysin* by real-time (q)RT-PCR was performed using TaqMan Gene Expression Assays (*synaptophysin*: Hs00300531_m1; *BIN1*: Hs01120886_m1; *TGIF*: Hs00231483_m1, Mm01227699_m1; *β-actin*: Mm00607939_s1; Life Technologies, Carlsbad, Ca, USA). Due to high BIN1 expression levels in neurons and previous transcript analyses confirming reliable quantification results in epileptic brain tissue, we used the neuronally expressed reference gene *synaptophysin* for (q)RT-PCR validation experiments. *Synaptophysin* was amplified from all human samples and *β-actin* from mouse samples and NG108-15 neuroblastoma cells for normalization of the analyzed mRNAs as described before (Wierschke et al., 2010; Becker et al., 2008; Pernhorst et al., 2011; Chen et al., 2001). The ABI Prism 9700HT sequence detection system (Life Technologies) and the $\Delta\Delta C_t$ quantification method were applied.

2.3. mRNA microarray analysis

Total RNA for gene expression microarray analyses was isolated from 20 μm human hippocampal tissue sections using the AllPrep DNA/RNA Mini Kit (Qiagen, Hilden) according to the manufacturer's protocol. To synthesize cDNA and in vitro transcription to biotin-labeled cRNA, Ambion Illumina TotalPrep-96 RNA Amplification Kit (Life Technologies Corporation, Carlsbad, CA, USA) was used following the manufacturer's instructions. For mRNA expression analysis, a total amount of 750 ng cRNA was then hybridized to Illumina HumanHT12v4 Expression BeadChips using the Illumina Direct Hybridization Assay Kit (Illumina, San Diego, CA) according to the manufacturer's protocol. Overnight Incubation was carried out to hybridize the cRNA to beads with respective complementary oligonucleotides. For detection of differential expression signals, Cy3–Streptavidin was added to the hybridized samples. The Illumina BeadArray Reader was applied to scan the fluorescent signals and obtained gene expression data were normalized using Illumina's GenomeStudio Gene Expression Module by means of quantile normalization with background subtraction. Visualization and clustering of gene expression data were conducted using the corresponding software package. Using the software R (version 2.15.2) subsequent data analyses, i.e. correlations of genotype and expression BeadChip data, were performed.

2.4. DNA microarray analysis

For our DNA microarray analysis, we used human hippocampal biopsy tissue from patients with hippocampal sclerosis (HS) pattern