Large-scale expression study of human mesial temporal lobe epilepsy: evidence for dysregulation of the neurotransmission and complement systems in the entorhinal cortex

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Human mesial temporal lobe epilepsies (MTLE) are the most frequent form of partial epilepsies and display frequent pharmacoresistance. The molecular alterations underlying human MTLE remain poorly understood. A two-step transcriptional analysis consisting in cDNA microarray experiments followed by quantitative RT–PCR validations was performed. Because the entorhinal cortex (EC) plays an important role in the pathophysiology of the MTLE and usually discloses no detectable or little cell loss, resected EC and each corresponding lateral temporal neocortex (LTC) of MTLE patients were used as the source of disease-associated and control RNAs, respectively. Six genes encoding (i) a serotonin receptor (HTR2A) and a neuropeptide Y receptor type 1 (NPY1R), (ii) a protein (FHL2) associating with the KCNE1 (minK) potassium channel subunit and with presenilin-2 and (iii) three immune system-related proteins (C3, HLA-DR-γ and CD99), were found consistently downregulated or upregulated in the EC of MTLE patients as compared with non-epileptic autopsy controls. Quantitative western blot analyses confirmed decreased expression of NPY1R in all eight MTLE patients tested. Immunohistochemistry experiments revealed the existence of a perivascular infiltration of C3 positive leucocytes and/or detected membrane attack complexes on a subset of neurons, within the EC of nine out of eleven MTLE patients. To summarize, a large-scale microarray expression study on the EC of MTLE patients led to the identification of six candidate genes for human MTLE pathophysiology. Altered expression of NPY1R and C3 was also demonstrated at the protein level. Overall, our data indicate that local dysregulation of the neurotransmission and complement systems in the EC is a frequent event in human MTLE.

Keywords: epilepsy; entorhinal cortex; microarray; NPY1R; complement

Abbreviations: EC = entorhinal cortex; LTC = lateral temporal neocortex; MAC = membrane attack complex; MTLE = mesial temporal lobe epilepsies; NPY1R = neuropeptide Y receptor type 1


Introduction

The epilepsies represent one of the most common neurological disorders. It is usual to distinguish between generalized and partial epilepsies (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Mesial temporal lobe epilepsies (MTLE) are the most frequent form of partial epilepsies and display frequent...
resistance to anti-epileptic drugs (Lösch, 2002), thus representing a major medical and scientific issue as well as a major health care problem.

Generally, epilepsy may develop as a consequence of a brain-damaging insult, such as head trauma, stroke, brain infection, or febrile seizures (Hauser, 1997). For example, there is high frequency of often complex febrile seizures in the history of patients with MTLE (Baulac et al., 2004; Cendes, 2004). The epilepsies, MTLE particularly, typically proceed through three distinct phases (Mathern et al., 1996; Pitkanen and Sutula, 2002): (i) initial insult, (ii) latency period (epileptogenesis) and (iii) recurrent spontaneous seizures (epilepsy). Epileptogenic insults can cause a number of neurobiological changes underlying the remodelling of neuronal networks. These include the loss of selective neuronal populations, gliosis, neurogenesis, axonal and/or dendritic plasticity and modification of the extracellular matrix (Bazan and Serou, 1999; Clark and Wilson, 1999; Coulter and De Lorenzo, 1999; Ben-Ari, 2001). It is thus very likely that a large number of genes contribute to these different phenomena. Consistent with this idea, it has been suggested that up to 1000 genes could influence seizure susceptibility (Frankel, 1999). While an increasing number of genes encoding neurotransmitter- or voltage-gated ion channel subunits have proved to be mutated in various idiopathic human epilepsies (Roll and Szepetowski, 2002; de Guzman et al., 2004). In particular, the entorhinal cortex (EC), which is situated at a key-point linking neocortical and hippocampal areas, provides the main excitatory input to the hippocampus and is strongly involved in normal brain function as well as in pathological mechanisms. A hyperexcitable EC might deliver synchronous excitatory signals to the hippocampus and spontaneous seizures have indeed been recorded in the EC of patients and animal models (Jones et al., 1992; Heinemann et al., 1993; Barbosie and Avoli, 1997). Neuropathological and neuroradiological data have indicated alteration of EC in some patients with MTLE (Bernasconi et al., 1999, 2000; Yilmazer-Hanke et al., 2000; Jutila et al., 2001). Moreover, surgical resection of anterior temporal lobe structures and including the EC is an effective treatment in MTLE (Siegel et al., 1990). Altogether, these arguments strongly suggest that genes displaying altered expression in the EC of MTLE patients might be associated with the pathogenesis and evolution of MTLE.

In the present study, we have performed a two-step transcriptional analysis of human MTLE in a series of resected EC taken from patients with typical MTLE. Genes encoding two neurotransmitter receptors [the serotonin receptor HTR2A and the neuropeptide Y receptor type 1 (NPY1R)], a potassium channel-associating protein (FHL2) and three proteins of the immune system (C3, CD74 and CD99), were found consistently and significantly dysregulated at the transcriptional level. Two out of the six, C3 and NPY1R, were studied by immunohistochemistry and immunoblotting experiments, respectively. Decreased expression of NPY1R was confirmed at the protein level. Consistent with the participation of immune mechanisms, stig mata of complement activation in the EC (perivascular infiltration of C3 positive leukocytes and membrane attack complex (MAC) deposition on neurons) were found in most MTLE patients.

Material and methods

Patient and autopsy brain samples

MTLE samples

Brain samples were obtained from MTLE subjects who underwent surgical treatment consisting in a standard anterior temporal lobectomy at the Neurosurgical Department of AP-HM, La Timone Hospital, Marseille. The study was reviewed and approved by the local ethical committee (CCPRPB No. 03-309) and written informed consent from each patient was obtained prior to any sample recovery. All patients had a comprehensive presurgical evaluation in the Epilepsy Unit, including detailed history and neurological examination, neuropsychological testing, routine MRI study, surface EEG and long-term video-EEG monitoring. In three patients, a stereoelectroencephalography (SEEG, depth electrodes) recording of seizures was also performed. All patients had converging data toward the diagnosis of typical mesial temporal lobe epilepsy (Table 1).
<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender/Age</th>
<th>Medical history</th>
<th>Drugs</th>
<th>Delay S-FPS (y)</th>
<th>Aura</th>
<th>Ictal semiology (initial part)</th>
<th>MRI</th>
<th>Depth recordings (SEEG)</th>
<th>Type of epilepsy</th>
<th>Post-surgical results</th>
<th>Engel class (follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1</td>
<td>F/32</td>
<td>Simple FS (13 months)</td>
<td>LTG OXC</td>
<td>17</td>
<td>Déjà vu-dejà vécu</td>
<td>Bimanual automatisms LOC OAA</td>
<td>H-T2 and atrophy of RH</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (3 y.)</td>
<td></td>
</tr>
<tr>
<td>P 2</td>
<td>F/59</td>
<td>Meningitis (1 y.)</td>
<td>CBZ LTG</td>
<td>45</td>
<td>–</td>
<td>Bimanual automatisms LOC OAA rubefaction, mydriasis</td>
<td>H-T2 and atrophy of RH</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (2.5 y.)</td>
<td></td>
</tr>
<tr>
<td>P 3</td>
<td>M/42</td>
<td>Complex FS (3 y, following anti-smallpox vaccine)</td>
<td>CBZ VPA</td>
<td>35</td>
<td>–</td>
<td>GA LOC OAA Vocalization</td>
<td>H-T2 and atrophy of LH</td>
<td>No</td>
<td>L-MTLE</td>
<td>IA (3 y.)</td>
<td></td>
</tr>
<tr>
<td>P 4</td>
<td>F/42</td>
<td>Simple FS (4 y, following otitis)</td>
<td>TPM LVT</td>
<td>12</td>
<td>ES</td>
<td>Ictal dystonia GA LOC OAA</td>
<td>H-T2 and atrophy of LH</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (2.5 y.)</td>
<td></td>
</tr>
<tr>
<td>P 5</td>
<td>F/53</td>
<td>Complex FS (encephalitis, 16 months, followed by transient right hemiparesis)</td>
<td>CBZ CLZ</td>
<td>44</td>
<td>Anxiety ES</td>
<td>Chewing ictal dystonia (right superior limb)</td>
<td>H-T2 and atrophy of LH</td>
<td>Yes</td>
<td>L-MTLE</td>
<td>IB (3 y.)</td>
<td></td>
</tr>
<tr>
<td>P 6</td>
<td>F/45</td>
<td>Simple FS (9 months)</td>
<td>CBZ TPM</td>
<td>41</td>
<td>Anxiety ES</td>
<td>LOC GA</td>
<td>H-T2 and atrophy of RH</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (4 y.)</td>
<td></td>
</tr>
<tr>
<td>P 7</td>
<td>M/39</td>
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<td>CBZ</td>
<td>37</td>
<td></td>
<td></td>
<td>H-T2 and atrophy of LH</td>
<td>No</td>
<td>L-MTLE</td>
<td>IA (2 y.)</td>
<td></td>
</tr>
<tr>
<td>P 8</td>
<td>M/40</td>
<td>None</td>
<td>LVT</td>
<td>19</td>
<td>Anxiety Déjà vu’</td>
<td>LOC OAA</td>
<td>Slight atrophy LH</td>
<td>Yes</td>
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<td>IA (3 y.)</td>
<td></td>
</tr>
<tr>
<td>P 9</td>
<td>M/45</td>
<td>Simple FS</td>
<td>LVT LTG CBZ</td>
<td>16</td>
<td>–</td>
<td>Staring, LOC GA</td>
<td>Left head rotation, LOC automatisms</td>
<td>H-T2 and atrophy of RH</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (2 y.)</td>
</tr>
<tr>
<td>P 10</td>
<td>H/35</td>
<td>None</td>
<td>CBZ LVT TPM</td>
<td>32</td>
<td>ES acid taste Anxiety Déjà vu</td>
<td>LOC OAA</td>
<td>H-T2 and atrophy of LH</td>
<td>Yes</td>
<td>L-MTLE</td>
<td>IB (2 y.)</td>
<td></td>
</tr>
<tr>
<td>P 11</td>
<td>M/37</td>
<td>None</td>
<td>CBZ TPM CLZ</td>
<td>6</td>
<td>ES oppression Déjà vu</td>
<td>LOC OAA</td>
<td>Staring, LOC automatisms</td>
<td>Slight atrophy RH</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (2 y.)</td>
</tr>
<tr>
<td>P 12</td>
<td>M/43</td>
<td>IC</td>
<td>LVT CBZ TPM</td>
<td>38</td>
<td>ES oppression</td>
<td></td>
<td>Normal</td>
<td>No</td>
<td>L-MTLE</td>
<td>IA (1.5 y.)</td>
<td></td>
</tr>
<tr>
<td>P 13</td>
<td>M/31</td>
<td>Simple FS (9 months)</td>
<td>CBZ LVT</td>
<td>16</td>
<td>ES oppression</td>
<td>Staring, ± LOC OAA</td>
<td>H-T2 and atrophy of LH</td>
<td>No</td>
<td>L-MTLE</td>
<td>IA (1.5 y.)</td>
<td></td>
</tr>
<tr>
<td>P 14</td>
<td>F/24</td>
<td>IC</td>
<td>LTG PHT CLZ</td>
<td>21</td>
<td>Bizarre sensation</td>
<td></td>
<td>Staring, GA</td>
<td>Right amygdala hypersignal (T2)</td>
<td>No</td>
<td>R-MTLE</td>
<td>IA (0.7 y.)</td>
</tr>
<tr>
<td>P 15</td>
<td>M/43</td>
<td>IC</td>
<td>VPA OXC</td>
<td>42</td>
<td></td>
<td></td>
<td>Right hand dystonia LOC</td>
<td>H-T2 and atrophy of LH</td>
<td>No</td>
<td>L-MTLE</td>
<td>IA (2 y.)</td>
</tr>
</tbody>
</table>

Abbreviations: FS: febrile seizures; IC: infantile convulsions (undetermined whether febrile or not); R(right)/L(left)-MTLE: mesial temporal lobe epilepsy; LH: left hippocampus; RH: right hippocampus; GA: gestural automatisms; H-T2: hypersignal T2; Delay S-FPS: delay between surgery and first partial seizure; ES: epigastric sensation; LOC: loss of contact; OAA: oro-alimentary automatisms; CBZ: carbamazepine; TPM: topiramate; LVT: levetiracetam; VPA: valproate; OXC: oxcarbazepine; PHT: phenytoin; CLZ: clonazepam; LTG: lamotrigine. y.: year(s). MRI features consisting of hypersignal T2 and/or atrophy of the hippocampus are suggestive of hippocampal sclerosis. The patients’ post-surgical outcomes were determined using Engel’s outcome scores, as previously described (Engel et al., 1993).
For each patient, two types of brain samples were used for analysis: (i) the EC as part of the ictal onset network (epileptogenic zone, EZ) (Bartolomei et al., 2004) and (ii) the part of the lateral temporal neocortex (LTC) that is removed for surgical purposes and corresponds to the non-epileptogenic tissue. This latter does not belong to the ictal onset network and was used as an internal control (non-epileptogenic zone; NEZ). Using an internal control ensured that the epileptogenic (EZ) and non-epileptogenic (NEZ) samples of a given patient had been subjected to the same environment and drugs throughout their life. Tissue samples were recovered immediately after surgery, frozen with liquid nitrogen vapours and stored at −80°C at Marseille Biological Resource Centre (BRC) until use.

Autopsy samples
Entire brains were removed at autopsies from one male and three females who were 51, 53, 55 and 70 years old, had no history of brain-related disease and suffered sudden deaths without associated brain damage. Autopsies were all performed ≤10 h after death. From these brains, the regions homologous to those removed in the MTLE patients, i.e. the LTC and the EC, were dissected, frozen in liquid nitrogen and stored at −80°C at the Netherlands Brain Bank (NBB). Comparing those two areas in the autopsy controls ensured that any significant discrepancy found between the EC and the LTC of the patients, is not due to an inherent (physiological) difference in gene expression between the two brain territories. Working with biopsy samples and autopsy samples is in good agreement with the necessary items to match between samples (Babbe et al., 2000). The autopsies performed by the NBB are rapid autopsies with short post-mortem delay and the quality of the samples is constantly kept by using the pH of the brain samples as parameter (Ravid et al., 1992; Kingsbury et al., 1995).

Isolation of RNA
Each sample was carefully checked at the histological level (12 μm section perpendicular to cortex surface, Microm cryostat, Nissl-staining). The grey matter was microdissected and separated from white matter at −21°C under nuclease free conditions, providing 50–400 mg of wet weight per sample. The volume density of neuronal and non-neuronal cells was then estimated by stereological point-counting and did not reveal any statistically significant difference between each microdissected grey matter of the EC and LTC of a given patient or autopsy control (data not shown).

Total RNAs were isolated from the grey matter samples using the TRIZOL reagent according to the manufacturer’s instructions (Gibco BRL Life Technologies, USA) and purified with the ‘Qiagen RNasy™ kit (Qiagen, UK) following the RNA cleanup protocol. All RNAs displayed a 260/280 OD ratio >1.9. The absence of degradation and of DNA contamination was demonstrated by RNA microelectrophoresis on a 2100 Bioanalyser apparatus (Agilent Technologies, UK) and using the RNA 6000 Nano labchip technology kit. Protein extracts were obtained from the same samples according to the manufacturer’s instructions (Gibco BRL).

cDNA microarray analysis
Microarray experiments were performed using the MICROMAX™ Microarrays Human cDNAI according to the manufacturer’s instructions (Perkin-Elmer Life Sciences, USA) and following the Microarray Gene Expression Data (MGED) Society guidelines (http://www.mged.org/). The microarray included 2400 human cDNAs spotted in duplicate (4800 spots) onto glass slides. Human cDNAs had been taken from the AlphaGene database without any prior selection (Supplementary Material online). The microarrays also included 28 internal control spots corresponding to various Arabidopsis thaliana genes as well as eight negative control (empty) spots.

For each patient 1 μg of total RNA from each area (EC and LTC) was used for competitive hybridization. Briefly, first-strand cDNA synthesis and labelling were done by using either biotin (EC) or fluorescein (LTC) labelled nucleotides. An equal amount of total Arabidopsis thaliana RNA was added to each human RNA sample prior to the cDNA synthesis and served as an internal control of cDNA synthesis, hybridization and data analysis all along the microarray experiment. Both types of labelled cDNAs were mixed and simultaneously hybridized at 65°C for 14 h to a MICROMAX™ human cDNA glass slide. Fluorescein and biotin labelled cDNAs were then sequentially detected with a series of conjugate reporter molecules according to the tyramide signal amplification (TSA) process.

Microarrays were scanned using a 4000 XL confocal GeneArray laser scanner (Hewlett Packard) and the resulting images were analysed with the Genepix Pro 4.0™ software (Axon Instruments, USA). The intensity of each spot, quantified after background correction (see below), reflected the level of expression of each gene. Spots of poor quality were flagged and removed from further analysis. Raw data generated with the GenePix Pro 4.0™ software, i.e. median intensities and background of each spot in each fluorescence, were exported to the GeneSpring 5.0™ software and then normalized using the default settings for the parameters (Supplementary material online), according to the manufacturer’s recommendations (Silicon Genetics, USA). Normalized log transformed data were then further processed through a two-step analysis. First, the differential expression cut-off was calculated in order to confirm that recommended by the manufacturer (PerkinElmer). This was done by hybridizing probe mixtures generated from two aliquots of the same RNA source to the microarray. The cutoff was then statistically calculated using a Z-score as described (Quackenbush, 2002). Increases or decreases in mRNA levels were thus considered significant for a given spot at the 95% confidence level (|Z| > 1.96) when at least a 2.56-fold change was obtained. In addition, the level of statistical significance was drastically increased as only the genes with their two corresponding spots displaying significant differential expression were selected for further analysis (Table 2). The issue of low intensity signals (Tran et al., 2002) was also addressed in order to define two subgroups of genes on the basis of their expression, or absence of expression, in a given sample. Using the model proposed by Durbin and Rocke (2003), background levels were estimated based on fluorescent signals produced by the empty spots (negative controls). Each gene could thus be classified on a binary basis as ‘expressed’ or ‘non-expressed’ for each fluorescence signal. ‘Non-expressed’ genes correspond to spots with signal intensities statistically indistinguishable from the background. Spot intensities of ‘expressed’ genes are above the statistical threshold, i.e. 2 SD from the estimated background. Genes were then classified qualitatively (Table 2) as downregulated (↓) or upregulated (↑) when they were defined as ‘non-expressed’ in at least one out of the two areas (EC or LTC). The three layers variation (Churchill, 2002) were also tested in order to avoid some of the problems encountered with relative measurements of gene expression in two-colour competitive hybridizations. For this purpose, the spots corresponding to plant cDNAs were checked in order to ensure that the corresponding signal ratios never exhibited any differential expression as defined above.

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### Table 2 Microarray identification of 16 dysregulated genes in the entorhinal cortex (EC) of five MTLE patients (P1–P5)

<table>
<thead>
<tr>
<th>Gene name and symbol</th>
<th>GenBank</th>
<th>Locus</th>
<th>Normalized ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P1</td>
</tr>
<tr>
<td><strong>Upregulated genes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-1-antichymotrypsin (AACT, SERPINA3)</td>
<td>NM_001085</td>
<td>14q32</td>
<td>↑ 32.6</td>
</tr>
<tr>
<td>Aquaporin 1 (AQP1)</td>
<td>NM_198098</td>
<td>7p14</td>
<td>1048.4</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>215.7</td>
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<td></td>
<td></td>
<td></td>
<td>183.30</td>
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<td></td>
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<td>293.69</td>
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<tr>
<td>Complement component 3 (C3)</td>
<td>NM_000064</td>
<td>19p13</td>
<td>3.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.24</td>
</tr>
<tr>
<td>Tenascin C (TNC)</td>
<td>NM_002160</td>
<td>9q33</td>
<td>6.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.95</td>
</tr>
<tr>
<td>CD74 antigen (CD74)</td>
<td>NM_004107</td>
<td>19q13</td>
<td>3.47</td>
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<td></td>
<td></td>
<td>8.58</td>
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<td></td>
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<td>9.73</td>
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<td>Myelin basic protein (MBP)</td>
<td>NM_002385</td>
<td>18q23</td>
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<td></td>
<td></td>
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<tr>
<td>LPS-induced TNF-alpha factor (LUTAF)</td>
<td>NM_004862</td>
<td>16p13</td>
<td>20.39</td>
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<td></td>
<td>21.13</td>
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<tr>
<td><strong>Downregulated genes</strong></td>
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<tr>
<td>Four-and-a-half LIM domains 2 (FHL2)</td>
<td>NM_001450</td>
<td>2q12</td>
<td>4.65</td>
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<td></td>
<td></td>
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<td>4.26</td>
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<td></td>
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<td></td>
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<td>Carbonic anhydrase IV (CA4)</td>
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<td>Glutamate receptor, ionotropic, NMDA, subunit 2A (GRIN2A)</td>
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<td>Neuropeptide Y receptor Y1 (NPY1R)</td>
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<td>Rad51 homologue C (RAD51C)</td>
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<td>5-Hydroxytryptamine receptor 2A (HTR2A)</td>
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<td>7.09</td>
</tr>
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</table>

Genes were considered as differentially expressed if they displayed significant difference in the same direction (upregulated or downregulated) in the EC (epileptogenic zone) versus the lateral temporal neocortex (non-epileptogenic zone) in at least four out of the five MTLE patients (P < 4 × 10⁻⁴ for each series of a given spot). All fold changes that are significant (cut-off: 2.56) for a given spot are in boldface.

For each gene, two spots were analysed; the first line corresponds to the first spot and the second line to the second spot. For each patient, differential expression of a given gene was validated only when both the two corresponding spots displayed significant changes in the same direction. Whenever needed, qualitative classification (see Material and methods) was used and is indicated as follows: (↑): upregulated; (↓): downregulated; (ND): not determined. LPS: lipopolysaccharide; TNF: tumour necrosis factor; NMDA: N-methyl-D-aspartate.

### Real-time quantitative RT–PCR

Aliquots of total RNAs were extracted from the patients’ samples and from the autopsy samples after separation of the white and grey matters, as described above. One microgram of total RNA was reverse-transcribed using random hexamers and the Superscript® II RNase H1 reverse transcriptase (Invitrogen, UK), according to the manufacturer’s instructions. PCR primers (Table 3) were designed for all the 16 genes selected on the basis of the cDNA microarray data (Table 2) as well as for the two control genes (GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MRPL-28: mitochondrial ribosomal protein L28), using the ABI Primer Express software (Applied Biosystems). GAPDH and MRPL-28 were used because they represent classical controls for quantitative RT–PCR experiments and because their expression levels as assessed in our microarray experiments did not vary significantly in any of the five MTLE patients. Quantitative PCR was carried out using SYBR Green PCR Master Mix Plus (Eurogentec, Belgium) in the ABI Prism 7000 sequence detection system (Applied Biosystems). All primer pairs were optimized to ensure the specific amplification of the PCR product and the absence of any primer dimer. Quantitative PCR standard curves were set up for all. Real-time PCR data were analysed using the ABI Prism® 7000 sequence detection system software v1.0. Values of fold changes in the EC sample versus the LTC sample represent averages from triplicate measurements, using the...
2−ΔΔCT method. Relative quantification was performed using GAPDH as reference gene; the other control gene MRPL-28 did not show any significant variation, as expected (data not shown). Data were analysed by Mann–Whitney test to statistically significant differences between patients and autopsy samples (P-value < 0.05).

**SDS–PAGE and western blotting**

Forty micrograms of proteins were separated by 10% SDS–PAGE and then transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel) for western blot analysis using a Bio-Rad apparatus (Bio-Rad, USA). Non-specific epitopes were blocked with 5% casein solution/Tris-buffered saline (TBS) (10 mM Tris and 150 mM NaCl, pH 8.0) and the membranes were incubated for 1 h 30 min at room temperature with each of the following primary antibodies: rabbit anti-NPY1R antibodies (NPY1R12-A, Alpha Diagnostic International, USA; dilution 1:1000) and mouse anti-GAPDH as control antibodies (MAB374, Chemicon, USA; dilution 1:1000) and mouse anti-GAPDH as control antibodies (MAB374, Chemicon, USA; dilution 1:1000) and mouse anti-GAPDH as control antibodies (MAB374, Chemicon, USA; dilution 1:1000) and mouse anti-GAPDH as control antibodies (MAB374, Chemicon, USA; dilution 1:1000). Membranes were then washed three times with TBS and incubated for 1 h 30 min with either anti-rabbit HRP-conjugated secondary antibody (Amersham, USA; 1:5000) or anti-mouse HRP-conjugated secondary antibody (Jackson Immunoresearch, USA; 1:5000) followed by chemiluminescence ECL detection (Amersham, USA) and exposure to autoradiography film (Kodak, France). Images were collected with a scanner (Agfa, Snapscan) and optical density of gel bands analysed using NIH Image software and statistically significant differences between patients and autopsy samples were estimated with the Mann–Whitney test (P-value < 0.01).

**Immunohistochemistry**

Immunohistochemistry experiments were performed on serial sections of human adult brain samples. Briefly, sections of 12 µm were made from frozen brain samples using a cryostat (Microm, France). Sections were dried for at least 30 min, fixed in 4% paraformaldehyde for 20 min and then washed for 10 min in phosphate-buffered saline (PBS), dehydrated in graded ethanol and then frozen at −80°C at least overnight and no more than 2 weeks. For each experiment, slides were left 1 h at room temperature before incubation in PBS–glycine 1% for 15 min, then in permeabilizing and blocking buffer [0.3% Triton X-100/normal goat serum (NGS) 10%/TBS] for 10 min. Incubation with the primary antibody was next performed overnight at 4°C. The following antibodies were used as primary antibodies diluted in blocking buffer: Rabbit polyclonal anti-C3 (1:500; Thomas et al., 2000), mouse monoclonal anti-CD45 (1:50, M0701; Dako, USA), mouse monoclonal anti-MAP2 (1:100, M4403; Sigma), rabbit polyclonal anti-human C5b-9 (final concentration 10 µg/ml; 204903, Calbiochem, USA). Immunofluorescent studies were performed using fluorescently labelled antibodies. Briefly, CD45 and MAP2 were detected using Alexa 488-anti-mouse antibodies (1:400; Molecular Probes). C3 and C5b-9 were detected using F(ab’2) donkey anti-rabbit biotinylated antibodies (1:200; Jackson Immunoresearch, Laboratories, USA) and then with streptavidin–Texas Red™ (Molecular Probes, USA; 1 h, final concentration 5 µg/ml). For those individuals with enough amount of brain tissue (patients P3, P10–P12, P14, P15; autopsy individuals A3, A4), immunoenzymatic staining was also performed as follows: after three washes, sections were incubated at room temperature for 1 h in biotinylated F(ab’2) donkey anti-rabbit IgG or anti-mouse IgG (1/200; Jackson Immunoresearch Laboratories) diluted in blocking buffer. After three additional washes, sections were then incubated 1 h at room temperature with alkaline phosphatase (AP)-linked streptavidin followed by Vector Red AP substrate (Vector Laboratories, USA) incubation. All sections were counterstained with DAPI (25 ng/ml) and then mounted with Vectashield® (Vector Laboratories). Fluorescent and Nomarski DIC (differential interference contrast) images were captured with a CoolSnap camera (Princeton, USA) connected to a microscope (Leica Microsystems, Germany). In each experiment, blocking buffer was used for washes and non-immune IgG (from rabbit or mouse, obtained from Jackson Laboratories) were used as negative controls (Figs 3–6; Supplementary Figures 1–5 online).

**Results**

**Gene expression profile**

As a first step to identify genes that may contribute to the pathophysiology of human MTLE, gene expression profiles of EC (EZ) from MTLE patients were compared within each
individual with the expression profiles of samples from the corresponding LTC (NEZ) taken from the same patients and necessarily removed during the surgery. Several studies have pointed to the very limited number of genes that show consistent differential expression between two given brain territories (Sandberg et al., 2000; de Chaldee et al., 2003) and the transcriptome of the human cerebral cortex differs more between individuals than among regions within an individual (Khaitovich et al., 2004). Such an internal comparison ensured that both the EZ and the NEZ were submitted to similar environmental and pharmaceutical stimuli throughout their life.

A first series of five MTLE patients was selected (Table 1). Genes were considered as differentially expressed if they displayed significant difference (as defined in the Material and methods section) in the same direction (upregulated or downregulated) between the EC and the LTC in at least four out of the five patients ($P < 4.10^{-6}$ for each series of a given spot). Two genes (FHL2 and AQP1) were represented twice on the microarray (2 x 2 spots) and similar results were obtained for each corresponding duplicate. cDNA microarray profiling thus provided an initial list of 16 differentially expressed genes in the entorhinal cortices of human MTLE (Table 2).

Confirmation of microarray data by quantitative RT–PCR

In order to confirm the data obtained by microarray analyses, we used a second and independent method of RNA quantification. An independent set of RNAs extracted from the same samples that had been used for the microarray experiments, was analysed by quantitative RT–PCR experiments with specific primers (Table 3). The expression profiles of all 16 selected genes from the initial list (Table 2) could thus be compared between the EC and the LTC of the five MTLE patients. Overall, microarray data were confirmed by quantitative RT–PCR for 9 of the 16 genes. In contrast, microarray and RT–PCR data (not shown) were not consistent for the remaining seven genes (AQP1, CA4, CCKBR, FCGR, GRIN2A, MBP, RAD51C) and these were thus excluded from further analyses. Because the differences in the expression profiles between the EC and the LTC could reflect inherent differences between the two brain territories that were compared, rather than reflect pathological processes, quantitative RT–PCR was also used to compare the expression profiles of the nine selected genes between the EC and the LTC of four non-epileptic autopsy brains. Three genes (AACT, LITAF, TNC) displayed expression profiles in the autopsy samples similar to those obtained in MTLE samples (data not shown); this may reflect physiological differences between the EC and the LTC. Finally, quantitative RT–PCR validated six genes displaying no significant difference between the EC and LTC of non-epileptic control brains and significant upregulation or downregulation ($P < 0.05$) in the EC of MTLE patients (Fig. 1).

The three validated and upregulated genes all encoded proteins of the immune system: MHC2 encodes a cell surface molecule (CD99 antigen) involved in T-cell adhesion processes (Gelin et al., 1989); HLADG encodes the HLA-DR gamma invariant chain (CD74) participating in antigen presentation (Topilski et al., 2002); C3 encodes the human complement component C3 (Fong et al., 1990). Among the three validated and downregulated genes, one (FHL2) encodes a protein associating with the KCNE1 potassium channel subunit (Krishnamurthy et al., 2004) and with the Alzheimer’s disease protein presenilin-2 (Tanahashi and Tabira, 2000), while the two others encode neurotransmitter receptors: HTR2A encodes the serotonin receptor type 2 (Sparkes et al., 1991) and NPY1R the neuropeptide receptor type 1 (Herzog et al., 1993).

NPY1R protein levels are decreased in human MTLE

Because of the limited amount of human tissue available, not all six genes could be analysed at the protein level. One of our observations by microarray and quantitative PCR analyses was a consistent change in the transcriptional expression of NPY1R. Neuropeptide Y (NPY) is well known as an endogenous modulator of synaptic transmission and of epileptic activity in experimental animals and in humans (Baraban, 2004). We thus decided to further analyse NPY1R and performed semi-quantitative western blot analysis to ascertain that the changes in NPY1R mRNA levels were also reflected at the protein level. Eight MTLE patients (four from the first series and four additional; Table 1) were analysed together with four autopsy controls. Western blot analysis (Fig. 2A) revealed that the NPY1R antibody reacted with a band migrating at approximately 44 kDa while the GAPDH (control) antibody revealed a band at 36 kDa, as expected. The two bands were scanned for statistical analysis of which results are plotted in Fig. 2B. No significant difference was observed in NPY1R levels between the EC and the LTC of all four autopsy samples (A1–A4). In contrast, significant decreases ($P < 0.01$) in NPY1R levels, ranging from 2.4- to 28.9-fold were found in all eight EC from MTLE patients, as compared with their respective LTC (Fig. 2). These data demonstrated decreased expression of NPY1R in the EC of MTLE patients.

Perivascular infiltration of C3 positive cells and neuronal MAC detection in human MTLE

Complement factor C3 is a major component of the immune complement system. Complement activation can be due to diverse mechanisms and has been associated with several diseases of the central nervous system (van Beek et al., 2003). As C3 was upregulated in the EC of the MTLE patients that had been tested at the transcriptional level, we performed immunohistochemistry experiments to detect the C3 fragments using anti-C3c antibodies. These react with native C3, C3b,
iC3b and C3c (Thomas et al., 2000; MF, unpublished data). Samples corresponding to the EC and LTC of 11 MTLE patients (two from the first series and nine additional; see Table 1) were analysed and the data compared with those obtained with the corresponding autopsy areas (EC and LTC) taken from four non-epileptic control individuals. In seven MTLE patients, C3 positive cells were detected in the EC as perivascular infiltrates, while no such pattern was ever detected in the corresponding LTC of the same patients (Figs 3 and 4; Table 4). Infiltrating cells were not detected in the EC and LTC of the four autopsy samples (Figs 3 and 4; Table 4). Anti-CD45 antibody revealed that the infiltrating cells corresponded to leucocytes (Figs 3 and 4). Apart from the perivascular areas in the EC of the seven patients, no microgliosis was observed in either tissue sample (≤2 CD45 positive cells/750 μm² microscopy field). This confirmed the data obtained by stereological quantitative analysis (see Material and methods). The detection of native C3 in leucocytes was reminiscent of complement activation associated with inflammatory processes. To further indicate complement activation, we used antibodies specific to the C5b-C6-C7-C8-C9 (C5b-9) MAC (Xiong et al., 2003). No MAC was detected either in the LTC of 11 MTLE patients or in all four control EC (Figs 5 and 6; Table 4). In contrast,
MAC deposits were detected on microtubule-associated protein 2 (MAP2) positive neuronal cell bodies in the corresponding EC of 7 out of the 11 patients (Figs 5 and 6; Table 4), among which five also had detectable C3 positive infiltrates. These data were also consistent with the C3 enzymatic staining of a subset of neuronal cells (Supplementary Figure 5 online) in the EC of three (P12, P14, P15) out of the six patients (P3, P10–P12, P14, P15) that could be retested by immunoenzymatic reaction (see Material and methods), a feature that had escaped detection by immunofluorescence and could be related to higher signal intensities obtained by immunoenzymatic detection. Moreover, immunofluorescence data on MAC deposition were also independently confirmed by immunoenzymatic staining experiments in the six patients (P3, P11, P14, P15: presence of MAC deposits; P10, P12: absence of MAC deposits) that were retested (Table 4). Overall, evidence for complement system activation was detected in nine EC of the 11 MTLE patients that were tested (Table 4).

**Discussion**

We have used an expression microarray approach to identify differentially expressed genes in surgical specimens removed from MTLE patients. Such samples provide a nearly unique opportunity to study human non-tumoral, non-autopsy tissue of the central nervous system. Only few studies describing the use of microarrays in experiments on human or animal models of MTLE have been published so far (Lukasiuk and Pitkanen, 2004). Most, if not all studies, focused on the hippocampus. While the hippocampus clearly plays an important role in the pathogenesis of MTLE, sclerosis occurs frequently and may prevent the identification of more subtle defects at the molecular level. Parahippocampal structures and the EC particularly, undoubtedly play an important role in the pathophysiology of human MTLE as well (Cohen et al., 2002; Bartolomei et al., 2004, de Guzman et al., 2004) but display less dramatic histological changes (Dawodu and Thorn, 2005). As a first step, 2400 human genes were analysed in the EC of MTLE patients; this represents about one-tenth of the entire human genome content. Because we identified six genes consistently dysregulated in human MTLE and because no biased selection of the genes spotted onto the microarray had been made, the theoretical number of genes consistently dysregulated in the EC of human MTLE should be around 60, on the basis of the criteria and methodology used in the present study. Association studies also suggested the participation of numerous genes in MTLE (Baulac et al., 2004). It is noteworthy that additional genes playing an important role in the pathophysiology of MTLE might remain undetectable, either because important pathogenic mechanisms may rely on slight transcriptional disturbances, or because altered expression may occur in only a minority of the cells that compose the EZ.

Gene expression profiling may serve as an important tool to identify candidate genes in multifactorial diseases. The identification of six genes displaying altered expression thus provide novel insights into the potential molecular mechanisms that are associated with human MTLE as well as help identify new candidate genes for MTLE. Moreover, expression data on two genes, *NPY1R* and C3, were confirmed by quantitative Western blot and by immunohistochemistry, respectively. This further argued in favour of the reliability and accuracy of the transcriptome approach that was undertaken here. An open issue obviously relies on the distinction between the

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**Fig. 2** Semi-quantitative western blot analysis of *NPY1R*. (A) 40 μg of proteins from the entorhinal cortex (EC) and lateral temporal neocortex (LTC) of eight MTLE patients (P1–P3 and P5–P9) and four autopsy controls (A1–A4) were separated by 10% SDS-PAGE. As expected, a single band of 44 or 36 kDa was visualized with the *NPY1R* and GAPDH (control) antibodies, respectively. (B) Semi-logarithmic representation of relative NPY1R amounts in the EC of MTLE patients. Fold changes in values in the EC versus the LTC were represented as bar graphs. In each sample, GAPDH was used as control for experimental variations. Quantification of band intensities revealed significant decrease in NPY1R levels in the EC of the patients compared with the EC of the autopsy controls (P-value < 0.01).
genes predisposing to the disease and those whose altered expression is a downstream consequence of the disease. Although we cannot exclude that the dysregulated expression of a subset of genes in patients with MTLE might be a consequence of epilepsy rather than being constitutive, secondary events are also highly suspected to participate in the pathophysiological evolution of human MTLE.

**HTR2A** encodes the serotonin receptor type 2A. Serotonin is one of the neurotransmitters influencing the cortical and subcortical excitatory/inhibitory balance and participates in many physiological and pathological processes of the brain, including the epilepsies. HTR2A itself acts as viral receptor for human polyomavirus JCV that causes progressive multifocal leucoencephalopathy in immunodeficient patients (Elphick et al., 2004). The HTR2A gene has also been implicated in schizophrenia, obsessive-compulsive disorder, affective disorder, alcohol dependence and anorexia nervosa (see the OMIM database at NCBI: http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=182135). In contrast with other receptor subtypes such as HTR1A, which displays decreased availability in patients with MTLE (Merlet et al., 2004), the possible role of HTR2A in epilepsy had been hardly considered so far.

**Fig. 3** Perivascular infiltration of C3 positive leucocytes in the entorhinal cortex (EC) of patient P3. Immunofluorescence staining. Sections of lateral temporal cortex (LTC) and of EC of patient P3 and autopsy control A4. (A–D) Detection of complement C3 using anti-C3c antibodies and Texas red-coupled secondary antibodies. (E–H) Detection of CD45 antigen using anti-CD45 antibodies and fluorescein isothiocyanate-coupled secondary antibodies. (I–L) Merge pictures of red and green signals. (M–P) DAPI staining. In each picture, the top right insert is a 2-fold enlargement of the boxed area. Bar: 100 μm. The EC of patient P3 contains C3 positive, CD45 positive cells (B, F, J). No C3 signal was detected in either the LTC of patient P3 (A, I) or the EC (D, L) and LTC (C, K) of autopsy control A4. Lipofuscin pigments (see arrowheads) gave green and red autofluorescent background signals (see also Fig. 5). In each experiment, non-immune rabbit (Q, S) or mouse (R, T) IgG were used as negative controls on the corresponding DAPI-stained (U–X) serial sections (see also Supplementary Figure 1 online). Lipofuscin pigments only are detected in the negative controls (Q–T).
In addition to the serotonergic system, alterations in the expression of NPY and its receptors have also received increased interest in the recent years (Baraban, 2004). Decreased expression of NPY1R was demonstrated here at both the transcriptional and protein levels. Decreased NPY1R expression would trigger neuronal hyperexcitability because the overall action of NPY is to inhibit seizure activity (Baraban, 2004). As a matter of fact, NPY actually inhibits in vitro epileptiform activity in the EC of mice (Woldbye et al., 2002). Our data on the EC of MTLE patients are also consistent with previous findings showing that Y1R binding sites are significantly reduced in epileptic patients with hippocampal sclerosis (Furtinger et al., 2001) and in epileptic rats (Kofler et al., 1997). In the amygdala rat model, the
Table 4 Summary of immunohistochemistry experiments

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<td>C3+ perivascular leucocytes</td>
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<td>C5b-9 deposits on neurons</td>
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The presence (+) or absence (−) of perivascular infiltration of C3 positive (C3+) leucocytes and of deposition of membrane attack complexes (C5b-9) on cortical neurons, respectively, was assessed by fluorescent immunodetection in the entorhinal cortex (EC) and lateral temporal neocortex (LTC) of eleven MTLE patients (P) and four non-epileptic, autopsy controls (A). The data were confirmed independently in the six patients (P3, P10–P12, P14 and P15) and two autopsy controls (A3 and A4) that could be retested by immunoenzymatic reaction.

Fig. 5 Deposition of membrane attack complexes (MAC) on cortical neurons in the entorhinal cortex (EC) of patient P11. Immunofluorescence staining. Sections of lateral temporal cortex (LTC) and of EC of patient P11 and autopsy control A3. (A–D) Detection of MAC with anti-C5b-9 antibodies and Texas red-coupled secondary antibodies. (E–H) Detection of neurons using anti-MAP2 (microtubule-associated protein 2) antibodies and Alexa488-coupled secondary antibodies. (I–L) Merge pictures of red and green signals. (M–P) DAPI staining. Bar: 65 μm. The EC of patient P11 contains C5b-9 positive neurons (B, F, J; see arrows). No C5b-9 signal was detected in either the LTC of patient P11 (A, I) or the EC (D, L) and LTC (C, K) of autopsy control A3. Lipofuscin pigments (see arrowheads) gave green and red autofluorescent background signals that appeared as yellow dots in merged pictures and specifically appeared as light purple dots in DAPI pictures. In each experiment, non-immune rabbit (Q, S) or mouse (R, T) IgG were used as negative controls on the corresponding DAPI-stained (U–X) serial sections (see also Supplementary Figure 3 online). Lipofuscin pigments only are detected in the negative controls (Q–T).
process of kindling has been associated with downregulation of NPY1R (Husum et al., 2004), although no difference in kindling development has been observed in NPY1R knock-out mice (Benmaamar et al., 2003). Cell-specific loss of NPY-containing interneurons in the hippocampus of patients with mesial temporal lobe sclerosis has also been shown (de Lanerolle et al., 1989). The same process could occur here; although recent (Dawodu and Thorn, 2005) as well as the present histological data (see Material and methods) clearly confirmed the absence of major neuronal loss in the EC of MTLE patients, a specific and subtle loss of NPY1R and/or HTR2A-expressing neurons cannot be excluded. Serotonin and NPY are central modulators of seizure activity and HTR2A and/or NPY1R may be important targets for anti-epileptic drugs. For instance, levetiracetam increases hippocampal NPY1R levels (Husum et al., 2004) and valproic acid leads to enhanced activity of HTR2A (Sullivan et al., 2004). Pharmacoresistance depends at least partly on active drug efflux transporters of the ATP-binding cassette gene family (Lösch and Potschka, 2002; Siddiqui et al., 2003) and is likely to be a multifactorial process. Decreased levels of HTR2A or NPY1R targets within the EZ as demonstrated in this study, would obviously lead to decreased activity of anti-epileptic drugs and hence would also participate in the pharmacoresistance that is classically associated with MTLE.

FHL2 encodes a protein with double zinc finger domains (LIM domains). How the downregulation of FHL2 might be associated with human MTLE remains an open question. On the one hand, the FHL2 protein associates with the KCNE1 (minK) potassium channel (Krishnamurthy et al., 2004) in

Fig. 6 Deposition of membrane attack complexes (MAC) on cortical neurons in the entorhinal cortex (EC) of patient P14. Immunoenzymatic staining (alkaline phosphatase, Nomarski DIC). Serial sections of lateral temporal cortex (LTC) and of EC of patient P14 and autopsy control A3. (A-D) Detection of MAC with anti-C5b-9 antibodies. (E-H) DAPI staining. (I-L) Detection of neurons using anti-MAP2 (microtubule-associated protein 2) antibodies. (M-P) DAPI staining. The EC of patient P14 contains C5b-9 positive neurons (B, J). No C5b-9 signal was detected in either the LTC of patient P14 (A) or the LTC (C) and EC (D) of autopsy control A3. In each experiment, non-immune rabbit (Q, S) or mouse (R, T) IgG were used as negative controls on the corresponding DAPI-stained (U-X) serial sections (see also Supplementary Figure 4 online). Bar: 65 μm.
cardiac tissue and appears to participate in the generation of cardiac IsK currents (Kupershmidt et al., 2002). The alteration of inward currents may lead to absence epilepsy associated with cardiac dysrhythmia (Ludwig et al., 2003) and KCNE1 itself interacts with the KCNQ2/KCNQ3 tetrameric potassium channel (Yang et al., 1998) that underlies benign neonatal familial convulsions (Gutierrez-Dellicado and Serratosa, 2004). FHL2 may thus be involved in epileptic seizures by interfering with potassium currents in the brain. Indeed, acquired potassium channelopathy has been demonstrated in experimental temporal lobe epilepsy (Bernard et al., 2004). On the other hand, FHL2 associates with the androgen receptor (Muller et al., 2000) and androgens interact with neuronal functioning including seizure susceptibility (Reddy, 2004). FHL2 also associates with the insulin-like growth factor-binding protein 5 (IGFBP5) and with the Alzheimer’s disease protein presenilin-2 (PS2). Both IGFBP5 (Zhong et al., 2002) and PS2 (Wolozin et al., 1998) that underlies benign ne-onatal familial convulsions (Gutierrez-Dellicado and Serratosa, 2004). On the other hand, FHL2 associates with the androgen receptor (Muller et al., 2000) and androgens interact with neuronal functioning including seizure susceptibility (Reddy, 2004). FHL2 also associates with the insulin-like growth factor-binding protein 5 (IGFBP5) and with the Alzheimer’s disease protein presenilin-2 (PS2). Both IGFBP5 (Zhong et al., 2002) and PS2 (Wolozin et al., 1998) have proapoptotic effects and a link between apoptotic processes and the epilepsies is well recognized (Ben-Ari, 2001). It was also recently shown that FHL2 is capable of inhibiting FOXO1-induced apoptosis (Yang et al., 2005).

CD99 is a T-cell surface antigen and its activation also leads to apoptosis (Petersen et al., 2001). Alternatively, abnormalities of T-cell mediated immunity have been described in epileptic patients (Eeg-Olofsson et al., 1985). CD74 encodes the invariant gamma chain (HLA-DR gamma) of class II antigens of the major histocompatibility complex (MHC). The role of HLA class I and II antigens has long been suspected in human epilepsies including MTLE and in febrile seizures and association studies have yielded contradictory results with increased or decreased frequency of antigens or haplotypes (Eeg-Olofsson, 2003). Overall, CD99 and CD74 upregulations both confirm the existence of local alteration of the immune system in MTLE (McNamara, 1999; Eeg-Olofsson, 2002).

The complement system attacks diseased and dysplastic cells—and normally spares normal cells. When C3 convertase is activated, C3a, C3b and then the terminal C5b–9 (MAC) complex are produced. Components of the complement system may participate in the genetic susceptibility to various neurodegenerative pathologies, as recently shown for age-related macular degeneration (Klein et al., 2005) and complement activation also occurs in several pathogenic conditions of the brain (van Beek et al., 2003). In particular, experimental evidences for a role of the complement system in epileptic processes have been reported (Whitney and McNamara, 2000; Xiong et al., 2003). The complex process of neuroinflammation involves various components of the immune system and of the complement cascade particularly. Our data demonstrate the presence of perivascular infiltrates composed of C3-producing leucocytes as well as the presence of MAC deposits on neurons, in the EC of most human MTLE tested. Two MTLE patients (P12, P13; Table 4) displayed C3 immunoreactive leucocytes but no detectable neuronal MAC deposits. Complement activation may not always proceed further than C3, as shown for instance in Alzheimer’s disease (Veerhuis et al., 1995; Eikelenboom and Veerhuis, 1996). Conversely, two patients (P7, P9; Table 4) had neuronal MAC immunoreactivity but no detectable C3 positive leucocytes. This may be due to inaccessibility of the epitope to the antibody, to a more pronounced degradation of C3 (C3d fragment, not detected by our anti-C3c antibody) in these samples, or to the presence of C3 in a section not selected for immunohistochemistry experiments. Such artefacts may also apply to the above situation (patients P12, P13) and have indeed already been reported in complement studies on the epilepsies (Xiong et al., 2003). Similarly, immunoenzymatic staining experiments revealed C3 positive neurons in three (P12, P14, P15) of the six patients (P3, P10–12, P14, P15) that were retested. Again, the lack of C3 neuronal detection in patients P3 and P11 could be due to the same causes as described above. Patient P10 never exhibited any C3 or MAC immunoreactivity, whether fluorescent or enzymatic. Based on the analysis of human epileptic hippocampi, the role of inflammatory processes in neuronal excitability has already been suggested in MTLE (Crespel et al., 2002). The observations presented here suggest that inflammatory reactions are also present in the EC of MTLE patients.

Although little is known about the possible role of inflammation in epilepsy, it may be speculated that activation of the innate immune system and the associated inflammatory reactions in brain mediate some of the molecular and structural changes associated with seizure activity. Perivascular abnormalities may also reflect and/or participate in the alteration of the blood/brain barrier involved in pharmacoresistance (Abbott et al., 2002). A participation of NPY1R in the development of neurogenic inflammation has even been suggested (Naveilhan et al., 2001). Generally, the possible role of the immune system in epilepsy has long been debated (McNamara, 1999; Aarli, 2000; Levine; 2002; Eeg-Olofsson, 2003; Billiau et al., 2005). Numerous and sometimes contradictory reports point to the existence of various immunological alterations in epileptic patients (Billiau et al., 2005). Although the immune mechanisms involved in the pathogenesis of epilepsies remain largely unknown, their possible role is also clinically supported by the effectiveness of immunomodulatory treatment such as steroids and intravenous immunoglobulins in some particularly severe epilepsies (Villani and Avanzini, 2002; Verhelst et al., 2005). Our data may provide a molecular basis for such therapeutic strategies in human MTLE.

To summarize, we have used a large-scale expression study on the EC of MTLE patients to identify six candidate genes for human MTLE pathophysiology. Two of the genes encode neurotransmitter receptor subtypes. Our data also indicate that local dysregulation of the immune and complement systems in the EC is a frequent event in human MTLE.

Supplementary material
Supplementary data are available at Brain Online.
Acknowledgements

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