

## MRS shows syndrome differentiated metabolite changes in human-generalized epilepsies

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Received 15 April 2003; revised 13 August 2003; accepted 14 August 2003

**Objective:** While it is generally accepted that the thalamo-cortical loop is abnormal in idiopathic generalized epilepsy (IGE), it is uncertain whether this loop is similarly affected among different IGE syndromes. We recently demonstrated reduced frontal lobe levels of *N*-acetyl aspartate (NAA) in patients with juvenile myoclonic epilepsy (JME). The present follow-up study investigates if similar or other types of changes exist in subjects with pure primarily generalized tonic clonic epilepsy (GTCS). **Method:** Twenty patients with GTCS, 26 patients with JME, and 10 matched healthy controls were investigated with quantitative single voxel MR spectroscopy (MRS) measurements of NAA, choline (Cho), creatine (Cr), and myo-inositol (mI) at 1.5 T scanner. The voxels were placed over the right cerebellum, right thalamus, prefrontal, occipital cortex, and over a spherical phantom above the subject's head. **Results:** Patients with JME had reduced frontal lobe NAA (mmol/l) in relation to controls ( $9.8 \pm 1.1$  vs.  $10.8 \pm 0.7$ ,  $P = 0.01$ ), as well as GTCS patients ( $9.8 \pm 1.1$  vs.  $10.6 \pm 0.7$ ,  $P = 0.007$ ), whose values were normal. Patients with GTCS, on the other hand, showed significantly lower thalamic NAA than controls ( $9.7 \pm 1.0$  vs.  $10.8 \pm 0.9$ ,  $P = 0.002$ ), and both groups of patients had reduced thalamic Cho, and mI; [Cho:  $2.0 \pm 0.4$  (control) vs.  $1.61 \pm 0.3$  (JME)  $P = 0.001$ , and vs.  $1.57 \pm 0.3$  (GTCS)  $P = 0.0005$ ; mI:  $4.8 \pm 1.5$ , (control) vs.  $3.3 \pm 1.4$  (JME)  $P = 0.003$ , and vs.  $3.2 \pm 1.5$  (GTCS),  $P = 0.002$ ]. No other regional changes were observed. **Conclusion:** The present MRS data emphasize the involvement of thalamus in IGE. They also show partly differentiated alterations within the thalamo-cortical loop in JME vs. GTCS. The various clinical expressions of IGE may, thus, be associated with more localized neuroanatomical substrates than generally believed.

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**Keywords:** Primarily generalized epilepsy; Juvenile myoclonic epilepsy; Generalized tonic clonic epilepsy; MR spectroscopy; Thalamo-cortical circuits

### Introduction

Idiopathic generalized epilepsy (IGE) is a common and complex condition with a predominantly genetic etiology and variable phenotypes (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Clinically, IGE can be divided into different syndromes. Juvenile myoclonic epilepsy (JME), juvenile absence epilepsy (JAE), and epilepsy with generalized tonic clonic seizures (GTCS) are three common syndromes of IGE of adolescent onset included in the classification of epilepsy syndromes of the International League Against Epilepsy (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). These syndromes differ in their predominant seizure types, but share some important clinical features, such as infrequent occurrence of generalized tonic and clonic seizures, absence of pathology on routine neuroradiology (CT scan and MRI), and a relatively early age of seizure onset (Engel, 1989; Gastaut, 1970; Janz, 1997). Furthermore, one IGE syndrome may evolve out of another IGE syndrome, and even families with a single gene defect can manifest more than one of these syndromes (Andermann and Berkovic, 2001), thus giving rise to questions about phenotypic overlap and purity, and the corresponding pathophysiological mechanisms and neuroanatomical substrates.

Although electrophysiological data infer thalamo-cortical dysfunction as the major mechanism of IGE (Gloor, 1978), it is generally believed that this dysfunction is not associated with specific morphological or chemical changes in thalamus or cortex, and that IGE is not associated with tissue pathology (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). This concept is, however, contradicted by an increasing number of studies: neuropathological data show migrational disturbances in IGE (Meencke and Janz, 1984; Meencke and Veith, 1992), cerebral and cerebellar distortions have been reported when comparing patient and control MRIs with computerized brain atlas (Savic et al., 1998), and widespread structural changes have been revealed with tissue segmentation programs (Woermann et al., 1998). Furthermore, it has been reported that patients with JME have elevated binding to benzodiazepine receptors (Koepp et al., 1997), and increased gray matter fraction in the inferior–medial frontal lobe (Woermann et al., 1999). We recently offered additional arguments for a specific

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Table 1

(A) Patient data, JME

Patient	Age (years)	Duration (years)	EEG	Seizure type	Present seizure type and frequency <sup>a</sup>	Medication	Last seizure	No of GM seizures in lifetime	Thalamus NAA (mM)	Frontal lobe NAA (nM)
JME 1	26	13	Bil spike-wave activity	Myoclonies, gm	Myoclonus (2–3 per mo), gm (5 per yr)	Valproate	Myoclonus, 3 day	65	10.4	10.0
JME 2	27	12	Bil spike-wave activity	Myoclonies, gm	Myoclonus (3 per mo), gm (1 per yr)	Valproate	Myoclonus, 3 wk	12	9.0	10.2
JME 3	40	27	Bil spike-wave activity	Myoclonies, gm	No seizures for 8 yr	Valproate	Gm, 8 yr	21	10.3	10.1
JME 4	35	24	Bil spike-wave activity	Myoclonies, gm	No seizures for 6 yr	Valproate, Lamotrigin	Gm, 5 yr	10	10.7	8.6
JME 5	34	21	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per mo), Gm (0.2 per yr)	Valproate	Myoclonus, 1 wk	21	9.8	8.1
JME 6	29	17	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per mo)	Valproate	Myoclonus, 1 mo	1	10.6	9.2
JME 7	31	19	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per wk), sporadic absences, Gm 0.2 per yr	Valproate	Myoclonus, 1 mo	5	10.9	9.4
JME 8	32	19	Polyspike wave activity	Myoclonies, gm	Myoclonus (1 per mo), Gm (1 per yr)	Carbamaz-epine	Myoclonus, 1 wk	19	9.6	9.2
JME 9	37	22	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per mo), Gm (1 per yr)	Valproate	Myoclonus, 1 mo	22	9.8	9.6
JME 10	27	16	Bil polyspike-wave activity	Myoclonies, gm	Myoclonus (1 per yr)	No medication	Myoclonus, 1 yr	6	9.4	8.6
JME 11	42	29	Bil spike-wave activity	Myoclonies, gm	Myoclonus (2 per mo), Gm (0.5 per yr),	Valproate	Myoclonus, 1 mo	12	10.2	11.4
JME 12	26	13	Bil spike-wave activity	Myoclonies, gm	No seizures for 6 yr	Valproate	Gm, 6 yr	10	10.8	11.3
JME 13	35	17	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per mo)	Clonazepam	Myoclonus, 2 mo	5	11.0	9.8
JME 14	28	14	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per wk), Gm (6 per yr)	Valproate	Myoclonus, 1 wk	65	8.3	8.8
JME 15	25	18	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per mo)	Valproate	Myoclonus, 1 mo	10	<sup>b</sup>	8.5
JME 16	29	13	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per wk)	Valproate	Myoclonus, 1wk	7	10.8	10.6
JME 17	20	7	Bil spike-wave activity	Myoclonies, gm	Gm (1 per yr)	Valproate	Gm, 1 yr	7	10.6	9.2
JME 18	33	27	Bil spike-wave activity	Myoclonies, gm, sporadic absences	Myoclonus (1 per mo), Absence 2 yr ago	Valproate	Myoclonus, 1 wk	10	10.2	9.0
JME 19	43	31	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per wk), Gm (1 per yr)	Valproate	Myoclonus, 1 day	10	<sup>b</sup>	9.3
JME 20	34	17	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per mo), Gm (2 per yr)	Valproate	Myoclonus, 1 mo	25	10.6	10.0
JME 21	21	5	Bil polyspike-wave activity	Myoclonies, gm, sporadic absences	Myoclonus (2 per wk), Gm (1 per yr)	Valproate, Lamotrigin	Myoclonus, 1 day	5	9.5	10.9

JME 22	18	5	Bil spike-wave activity	Myoclonies, gm	Myoclonus (1 per wk), Gm (1 per yr)	Valproate	Myoclonus, 1 wk	4	11.0	12.3
JME 23	46	32	Bil polyspike-wave activity	Myoclonies, gm	Myoclonus 8 mo ago, Gm (1 per yr)	Valproate	Gm and myoclonus, 8 mo	30	7.2	10.6
JME 24	21	5	Bil polyspike-wave activity	Myoclonies, gm	Myoclonus (1 per wk), Gm (0.5 per yr)	Valproate	Myoclonus, 1 wk	3	12.0	9.1
JME 25	19	6	Bil spike-wave activity	Myoclonies, gm	Myoclonus (2 per mo), Gm (0.2 per yr)	Valproate	Myoclonus, 1 day	2	11.3	10.6

## (B) Patient data, GTCS

Patient	Age (years)	Duration (years)	EEG	Seizure type	Present seizure type	Medication	Last seizure	No of GM seizures in lifetime	Thalamus NAA (mM)	Frontal lobe NAA (mM)
GTCS 1	45	30	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2 yr	23	8.3	10.1
GTCS 2	35	9	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2 yr	3	10.3	10.6
GTCS 3	34	24	Bil spike-wave activity	Gm	No seizures for 11 yr	Valproate	Gm, 11 yr	15	9.9	12.2
GTCS 4	51	37	Bil spike-wave activity	Gm	No seizures for 6 yr	Phenytoin	Gm, 6 yr	55	10.3	<sup>b</sup>
GTCS 5	33	19	Bil spike-wave activity	Gm	Gm (0.3 per yr)	No medication	Gm, 3 yr	8	10.0	10.7
GTCS 6	35	16	Bil spike-wave activity	Gm, sporadic absences	Gm (0.3 per yr) Absence (1 yr)	Valproate	Absence, 3 mo	5	8.9	10.3
GTCS 7	19	2	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2 yr	3	10.5	<sup>b</sup>
GTCS 8	21	7	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 1 mo	80	10.0	9.4
GTCS 9	38	25	Bil spike-wave activity	Gm	Gm 3 mo ago (first in 7 yr)	Carbamazepine	Gm, 3 mo	3	10.2	11.6
GTCS 10	50	32	Sharp wave and bil spike-wave activity	Gm	Gm 3 yr ago	Valproate	Gm, 3 yr	20	9.0	11.1
GTCS 11	24	12	Bil spike activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2yr	25	10.0	10.8
GTCS 12	32	15	Bil spike-wave activity	Gm	Gm (5 per yr)	Carbamazepine	Gm, 1 mo	75	9.2	11.0
GTCS 13	32	20	Bil spike-wave activity	Gm	No seizures for 8 yr	Valproate	Gm, 8 yr	8	10.7	11.1
GTCS 14	31	7	Bil sharp wave activity	Gm	Gm (1 per yr)	Valproate	Gm, 1 yr	7	8.1	9.7
GTCS 15	35	12	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Carbamazepine	Gm, 1 yr	6	7.6	10.0
GTCS 16	27	10	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2 yr	3	10.3	11.4
GTCS 17	24	12	Normal	Gm	Gm (0.5 per yr)	Valproate	Gm, 2yr	15	10.0	10.8
GTCS 18	27	16	Bil spike-wave activity	Gm	Gm (0.2 per yr)	Valproate	Gm, 5 yr	10	9.5	10.8
GTCS 18	26	11	Bil spike activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2 yr	5	10.7	10.2
GTCS 19	51	39	Bil spike-wave activity	Gm	Gm (0.5 per yr)	Valproate	Gm, 2 yr	20	9.2	10.4
GTCS 20	29	14	Bil spike-wave activity	Gm	Gm (1 per yr)	Valproate	Gm, 0.5 yr	4	11.9	9.9

gm: generalized tonic clonic seizures, yr: year, mo: month, wk: week.

<sup>a</sup> Frequency of seizures (no/year) averaged over the last five years; nothing is indicated if the particular seizure type did not occur for the last 5 years.

<sup>b</sup> Excluded due to poor spectral quality.

frontal lobe affection in JME by demonstrating reduced concentrations of *N*-acetyl aspartate (NAA) with MR spectroscopy (MRS) (Savic et al., 2000).

While offering initial evidence for neurochemical and morphological substrate/s in IGE, these reports provide no information about the possible IGE syndrome-related changes. Yet, such information is of particular importance, as it may offer clues for better treatment strategies. The present study was therefore undertaken to specifically investigate whether possible syndrome-associated metabolite changes can be detected by quantitative MRS, which in our earlier study was capable of detecting frontal lobe reductions in *N*-acetyl aspartate (NAA) in JME. Regional concentrations of NAA, cholines (Cho), creatine (Cr), and myo-inositol (mI) were, therefore, specifically compared between patients with GTCS and JME. These forms of IGE are clinically well defined, comparable with respect to anticonvulsive therapy, and frequently encountered in adult patients in whom MRS studies are easier to motivate from an ethical perspective.

The following issues were particularly addressed:

- Does the preliminary observation of low frontal lobe NAA in JME stand also when extending the number of patients?
- Is frontal lobe NAA also reduced in patients with GTCS, or do these patients have abnormalities in other regions along the cerebello-thalamo-cortical loop?

## Materials and methods

### Patients

Forty-six long-term consecutive patients from the epilepsy clinic at Huddinge University Hospital participated. All of them were controlled at this hospital from the year of their seizure onset, and were well known to the responsible neurologist (I.S.). Only patients whose seizure phenomenology was reliably assessed with the aid of close relatives were included in the study. The seizure reports were thus deemed to be reliably correct. All the patients were also investigated with high resolution MRI according to the epilepsy protocol. All had normal findings.

The major clinical data are presented in Table 1. The diagnosis of the respective syndrome was based on seizure history, seizure semiology as described by relatives or recorded during hospitalization, and results from scalp EEG recordings. Twenty-five patients were diagnosed with JME. They had late childhood-or-teenage onset of awakening myoclonic jerks, most often in the upper, but sometimes also in the lower extremities. All had a history of myoclonies as well as GTCS. Four of them also experienced rare absences. None of these patients had a progressive condition. Twenty other patients had primarily generalized tonic clonic seizures only (one experienced occasional absences), and no experience of myoclonic seizures. These patients were, as recently suggested (Andermann and Berkovic, 2001; Reutens and Berkovic, 1995; Unterberger et al., 2001), regarded as a separate entity. They were classified as the group with ‘other idiopathic generalized epilepsies’ (Commission on Classification and Terminology of the International League Against Epilepsy, 1989), and will here be denoted as patients with GTCS, as opposed to those with JME. The majority of patients with GTCS reported seizures at random, although some experienced them most frequently on awakening.

One JME patient was diagnosed with diabetes, all the other patients were healthy apart from having seizures. None had a history of status epilepticus, drug intoxication, or drug-related encephalopathy, and the computed tomography and routine magnetic resonance of the brain was normal in all the subjects.

The control group consisted of 10 unmedicated, right-handed, healthy volunteers ( $28 \pm 7$  years, six females) without family history of neuropsychiatric disorders.

The study was approved by the local human subject protection committee, and informed written consent was obtained from each subject.

### MRI protocol and choice of volumes of interest (VOIs)

MR examinations were carried out on a 1.5-T clinical MR system (Signa Advantage, General Electric Medical Systems, Milwaukee, WI) using the standard quadrature birdcage head coil. To minimize variation in positioning of the head, the subjects were positioned by the same investigator using the orbito-meatal line as landmark. Incidence of possible seizures during the scan was evaluated from patient reports and by continuous supervision by an assistant. All the scans were interictal.

The MRI protocol consisted of axial T2-weighted Fast Spin Echo (FSE) images (effective TE = 56 ms, TR = 2500 ms, FOV = 24 cm, 23 slices of 3-mm thickness), and axial T1 weighted three-dimensional spoiled GRASS images (SPGR, TE = 7 ms, TR = 23 ms, FOV = 24 cm, flip angle 50°, 124–156 slice partitions of 1-mm thickness, 2 NEX). In three cases, the latter sequence was not carried out due to poor patient compliance. The volumes of interest (VOIs) were adjusted to individual anatomy on FSE images covering the right thalamus, right cerebellum, prefrontal cortex, and occipital cortex (Fig. 1) as described previously (Savic et al., 2000). Only one side was analyzed to reduce the scanning time.

### MRS measurement and data analysis

For single volume proton MRS, a stimulated echo sequence (STEAM) was used with a short echo time (TE = 30 ms) and a long repetition time (TR = 6 s) to reduce relaxation weighting. Care was taken to correct for individual experimental variation. The 64 acquisitions were individually phase corrected before averaging to reduce motion effects (Helms and Piringer, 2001). The signal was corrected for signal variations due to coil load and heterogeneity of the radio-frequency field (Helms, 2000). The prefrontal and occipital VOIs were corrected for the fractional CSF volume using the difference in T2 of the localized water signal for segmentation (Ernst et al., 1993; Helms, 2000). The spectra were analyzed by fitting a linear combination of model spectra and a smooth baseline using commercial software (“LCModel”, S.W. Provencher Inc., Oakville, ON, Canada) (Provencher, 1993) which fits a linear combination of model spectra and a smooth baseline. Concentrations in absolute units of mmol/l (mM) were estimated by calibration on a solution of known concentration (Helms, 1999). Data acquisition and evaluation was kept consistent with a previous study (Savic et al., 2000).

Since motion was found to affect particularly the pre-frontal regions, data from the controls and the JME patients who were included in our previous study (Savic et al., 2000) were system-

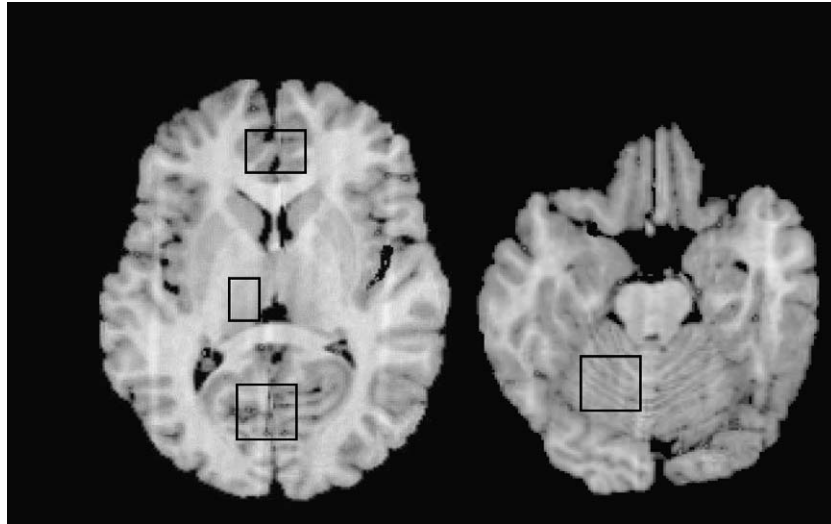


Fig. 1. Template with volumes of interest presented on a standard MR brain.

atically re-evaluated to correct for physiologic and subject motion (Helms and Piringer, 2001). Spectra showing abnormal baseline due to major artifact resonances (“nuisance peaks”) or unrecoverable excessive line width (altogether six spectra) were omitted from the statistical analysis because this may affect the results of the LCModel analysis in an uncontrollable manner.

Experimental parameters were checked for systematic differences to assess experimental confounds of the quantitative results (Table 2). The ‘MR water content’ of tissue was determined from the relaxation measurement performed for tissue or CSF segmentation (Ernst et al., 1993). The spectral quality is reflected by the line width (FWHM in parts-per-million of the proton frequency of 63.8 MHz) and the signal-to-noise ratio (SNR) provided by the LCModel program. SNR depends on the tissue volume ( $V_{\text{tissue}}$ ) estimated by correcting the size of the VOI by its individual percentage of CSF.

#### Neuropsychological tests

For a preliminary survey of possible cognitive and particularly frontal lobe dysfunctions (Devinsky et al., 1997; Reitan, 1986; Swartz et al., 1996), a minor battery of neuropsychological tests was employed. It consisted of Trailmaking test, part A and B (TMT-A and TMT-B), digit span, and word fluency tests (Devinsky et al., 1997; Reitan, 1986; Swartz et al., 1996). The neuropsychological tests were carried out by an investigator who was blinded to the clinical diagnosis.

#### Statistics

Regional differences in absolute concentrations of NAA, Cho, Cr, and mI, respectively, were tested among the three groups of subjects in four separate one-way ANOVAs using Fisher’s post hoc

Table 2  
Various experimental parameters in patients and controls

	MR water content (%)	FWHM (ppm)	Signal-to-noise ratio	VOI size (cm <sup>3</sup> )	V tissue (cm <sup>3</sup> )	CSF (%)
<i>Healthy controls</i>						
Frontal cortex	74.0 ± 2.0	5.7 ± 1.2	7.3 ± 1.6	8.7 ± 1.2	8.6 ± 2.3	6.6 ± 1.8
Cerebellum	69.0 ± 3.7	5.3 ± 0.5	6.7 ± 1.1	8.2 ± 0.8	8.0 ± 0.6	1.4 ± 0.4
Thalamus	67.5 ± 3.2	5.0 ± 1.0	3.8 ± 0.9	3.6 ± 1.0	3.5 ± 0.7	2.1 ± 1.0
Occipital cortex	68.0 ± 2.3	4.3 ± 0.4	10.2 ± 1.5	8.1 ± 1.0	7.2 ± 0.4	8.4 ± 1.7
<i>Patients with JME</i>						
Frontal cortex	68.3 ± 3.0	5.9 ± 1.2	7.3 ± 2.3	9.6 ± 2.3	8.9 ± 2.1	7.5 ± 2.4
Cerebellum	66.0 ± 2.4	5.8 ± 0.8	6.7 ± 1.0	8.0 ± 1.0	7.8 ± 1.0	2.5 ± 2.2
Thalamus	64.4 ± 2.2	6.0 ± 1.0**	4.5 ± 1.7	4.3 ± 1.7	4.3 ± 1.7	1.5 ± 0.9
Occipital cortex	63.4 ± 2.6	4.6 ± 0.4	9.4 ± 2.1	8.0 ± 1.2	7.4 ± 1.2	8.0 ± 2.3
<i>Patients with GTCS</i>						
Frontal cortex	69.0 ± 3.4	6.9 ± 2.1	6.9 ± 1.8	9.8 ± 2.2	9.0 ± 2.0	7.8 ± 2.8
Cerebellum	68.7 ± 3.6	5.9 ± 0.9	5.7 ± 1.3	7.7 ± 1.1	7.7 ± 1.1	1.6 ± 1.3
Thalamus	65.5 ± 2.7	6.0 ± 0.8**	4.0 ± 0.9	4.4 ± 1.0	4.4 ± 1.0	1.5 ± 0.5
Occipital cortex	64.6 ± 3.1	4.8 ± 0.6	8.4 ± 2.4	7.8 ± 1.8	7.1 ± 1.6	8.2 ± 2.5

Patients had significantly larger thalamus FWHM than controls ( $P = 0.002$  for GTCS and  $P = 0.003$  for JME).

This increase was not accompanied by a shortening of water T2 (ctrl: 74.2 ± 3.6 ms; JME: 72.5 ± 2.3 ms; GTCS: 72.2 ± 2.7 ms).

Hence, changes in line width are likely due to slightly larger VOIs in patients.

\*\*  $P < 0.01$ .

analysis. Given that concentrations of measured metabolites vary among different regions (Helms, 1999; Savic et al., 2000), only values from the same region (VOI) were compared between patients and controls in the post hoc analysis. Bonferroni correction was employed to account for the four separate ANOVAs (one per each metabolite). The significance values after correction was  $P < 0.013$ . Regional group differences in the content of tissue water (TWC), spectral line width (FWHM), and signal-to-noise ratio were tested accordingly ( $P < 0.010$  after Bonferroni correction for the five tests, see also Table 2).

Patients were defined to have abnormal regional concentrations of a given metabolite if the respective value was outside the 95% confidence interval of corresponding normal values ( $P < 0.05$ ). Differences between the two groups of patients with respect to age, age at seizure onset, duration of seizure history, the total number of generalized tonic clonic seizures during lifetime as well as the frequency of GTCS were tested with MANOVA ( $P < 0.05$ ).

The concentrations of metabolites showing abnormal values were related to the total number of GTCS seizures over lifetime, as well as the frequency of GTCS and myoclonic seizures, using Pearson's correlation coefficient ( $r$ ) ( $P < 0.05$ ).

## Results

### Metabolic concentrations

A synopsis of metabolite concentrations (mean and standard deviation, SD) in different groups and regions is given in Table 3.

JME patients showed significantly lower concentrations of frontal lobe NAA than controls ( $9.8 \pm 1.1$  vs.  $10.8 \pm 0.7$ ,  $P = 0.010$ ), as well as patients with GTCS ( $10.6 \pm 0.7$ ,  $p = 0.007$ ) (Fig. 2A). In contrast, the frontal lobe values in patients with GTCS were not different from normal (Table 3). To test whether the

Table 3  
Measured metabolites in patients and controls

	NAA (mM)	Cho (mM)	Cr (mM)	MI (mM)
<i>Healthy controls</i>				
Frontal cortex	$10.8 \pm 0.7$	$1.6 \pm 0.3$	$6.9 \pm 0.6$	$5.7 \pm 1.0$
Cerebellum	$9.7 \pm 0.8$	$2.0 \pm 0.4$	$8.2 \pm 0.7$	$5.3 \pm 1.6$
Thalamus	$10.8 \pm 0.9$	$2.0 \pm 0.4$	$6.1 \pm 1.1$	$4.8 \pm 1.5$
Occipital cortex	$11.3 \pm 0.7$	$0.9 \pm 0.2$	$6.5 \pm 0.6$	$4.6 \pm 1.0$
<i>Patients with JME</i>				
Frontal cortex	$9.8 \pm 1.1^*$	$1.6 \pm 0.2$	$6.7 \pm 0.9$	$4.3 \pm 1.2$
Cerebellum	$9.9 \pm 0.8$	$2.1 \pm 0.5$	$8.5 \pm 1.1$	$4.9 \pm 1.5$
Thalamus	$10.2 \pm 1.0$	$1.61 \pm 0.3^*$	$6.4 \pm 0.7$	$3.3 \pm 1.4^*$
Occipital cortex	$11.0 \pm 0.7$	$0.9 \pm 0.1$	$6.7 \pm 0.7$	$4.3 \pm 0.8$
<i>Patients with GTCS</i>				
Frontal cortex	$10.6 \pm 0.7$	$1.8 \pm 0.3$	$7.0 \pm 0.9$	$5.0 \pm 1.4$
Cerebellum	$10.1 \pm 0.6$	$2.1 \pm 0.5$	$8.7 \pm 0.8$	$5.8 \pm 2.0$
Thalamus	$9.7 \pm 1.0^*$	$1.57 \pm 0.3^{**}$	$5.9 \pm 0.6$	$3.2 \pm 1.5^*$
Occipital cortex	$11.1 \pm 1.4$	$1.0 \pm 0.1$	$6.9 \pm 0.7$	$4.0 \pm 0.7$

The significance level with Bonferroni correction was  $P < 0.01$ .

\* $P < 0.01$  indicates comparisons of the respective group of patients in relation to controls.

\*\* $P < 0.001$ .

distribution of subjects with low concentrations of frontal lobe NAA differed between the JME and the GTCS group, a post hoc analysis with chi-square statistics and Fisher's exact test was applied ( $P < 0.05$ ). The two types of IGE differed significantly also with respect to the number of subjects with low frontal lobe NAA (12 of 25 in the JME group, and 1 of 18 in the GTCS group,  $P < 0.001$ ) (Table 1). Notably, in the GTCS patient with low frontal lobe NAA, this value was at the limit for normal.

Patients with GTCS, on the other hand, had significantly reduced concentration of NAA in thalamus compared with controls ( $9.7 \pm 1.0$  vs.  $10.8 \pm 0.9$ ,  $P = 0.002$ ) (Fig. 2B). The corresponding value in patients with JME was also lower ( $10.2 \pm 1.0$ ), but the difference did not reach the level of significance ( $P = 0.09$ ). Both patient groups had reduced thalamic concentrations in Cho and mI (Table 3). They did not differ with respect to thalamic metabolites, including the NAA (Table 3). The cerebellum occipital VOIs showed normal values, independently of the specific metabolite.

### Clinical parameters

None of the patients showed morphological abnormalities on the routine MRI examination.

Patients with JME and GTCS did not differ with respect to age at examination ( $30.6 \pm 7.7$  vs.  $33.8 \pm 9.4$  years,  $P = 0.22$ ), duration of epilepsy history ( $17.2 \pm 8.2$  vs.  $17.6 \pm 10.2$  years,  $P = 0.78$ ), or the total number of generalized tonic clonic seizures over lifetime ( $16 \pm 17$  vs.  $19 \pm 23$  seizures). The two groups were also matched for antiepileptic medication. As expected, the age at onset of epilepsy (defined as the age at the first seizure (absence, myoclonic or GTCS)), was, however, lower in JME patients ( $13.6 \pm 3.0$  vs.  $17.6 \pm 6.1$  years,  $P = 0.006$ ).

Neither the frequency of myoclonic nor the frequency of generalized tonic clonic seizures or their number over lifetime was correlated with frontal lobe NAA in JME patients. Furthermore, JME patients with low frontal lobe NAA did not differ from those with normal NAA with respect to age at seizure onset, duration of epilepsy, frequency, recency (time between the last GTCS and the MRS scan), or the number of generalized tonic clonic seizures over lifetime.

JME patients with low NAA showed, however, a poorer performance in TMT-A and TMT-B tests than JME patients with normal NAA, patients with GTCS, and the controls (Table 4). No difference in neuropsychological tests was found among the three latter groups, and no group differences were observed in the digit span or word fluency tests (Table 4).

The thalamic concentration of NAA, Cho, and mI was not correlated with the frequency of GTCS or the number of lifetime GTCS. On the other hand, when classifying patients into a group with high (>10) vs. low number of lifetime GTCS ( $\leq 10$ ), a significantly lower thalamic NAA was found in patients with many seizures ( $9.5 \pm 0.7$  vs.  $10.2 \pm 1.4$ ,  $P = 0.04$ ). The cut off level was arbitrarily chosen, but based on clinical experience that >10 GTC seizures per year is regarded as a rather high frequency. No similar difference was detected in Cho and mI. No difference was observed in thalamus metabolites when arbitrarily classifying patients in a group with long (>5 years) vs. short ( $\leq 5$ ) duration of seizures.

### Experimental parameters

The water content of tissues in the respective VOI did not differ among any of the three groups. Neither did the percentage of CSF,

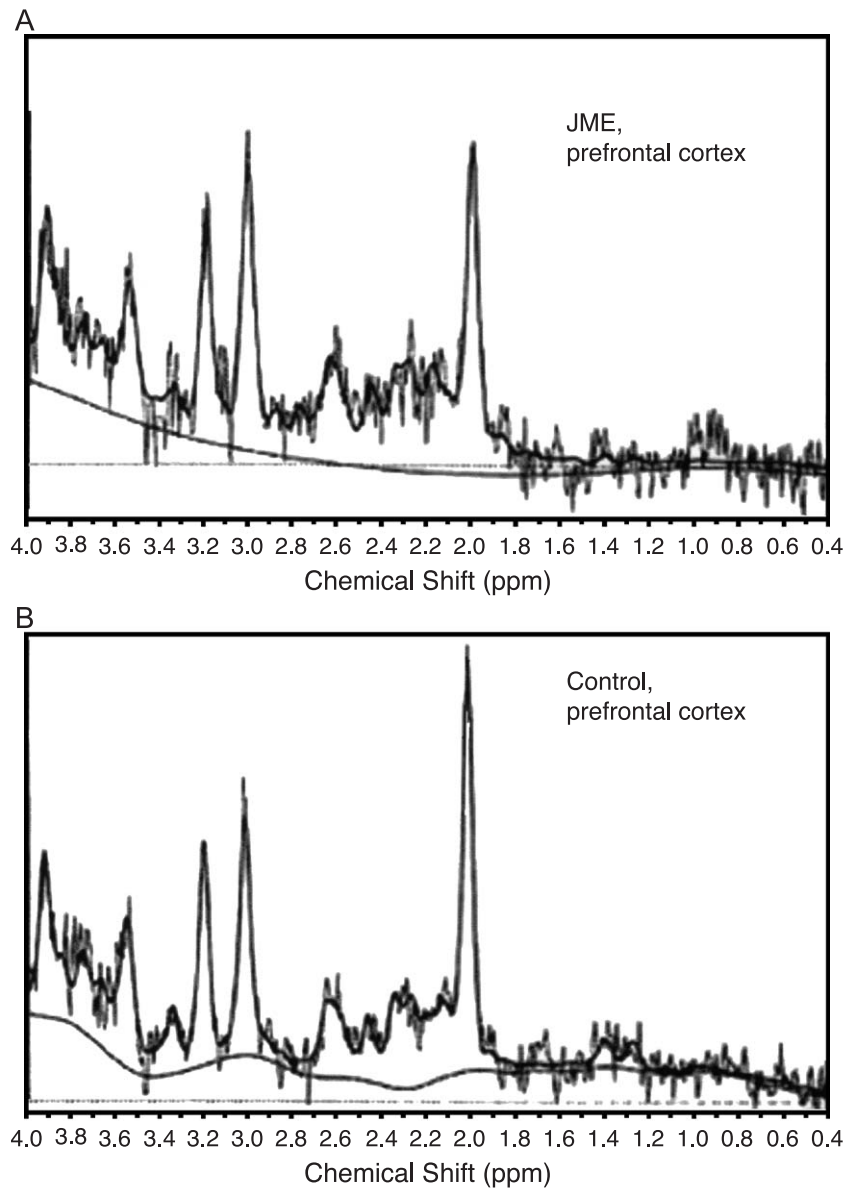


Fig. 2. Example of individual spectra. The noise is indicated. NAA at 2.0 ppm, Cr at 3.0 ppm, Cho at 3.2 ppm, and mI at 3.5 ppm. (A) Prefrontal spectrum in a patient with JME. Prefrontal spectrum in a control subject. (B) Thalamus spectrum in a patient with GTCS. Thalamus spectrum in a control subject. Thalamus spectrum from a patient with JME.

the tissue volume, nor the signal-to-noise ratio (Table 2). The line width of thalamus spectra was larger in both patient groups than age-matched controls (Table 2). However, when repeating group

comparisons using line width as covariate, the thalamic NAA reductions in patients with GTCS ( $P = 0.01$ ), as well as the Cho and mI reductions in the both groups of patients, remained

Table 4  
Results from neuropsychological tests

Patient group	TMT-A (s)	TMT-B (s)	Digit span forward (no)	Digit span backward (no)	Word fluency (no)
JME patients with reduced frontal lobe NAA ( $n = 12$ )	43 ± 13 <sup>a</sup>	85 ± 28 <sup>b</sup>	6.4 ± 1.0	4.7 ± 1.5	33 ± 9
JME patients with normal frontal lobe NAA ( $n = 14$ )	23 ± 6	60 ± 9	6.6 ± 0.5	5.3 ± 1.0	40 ± 14
Patients with GTCS ( $n = 20$ )	27 ± 8	59 ± 13	6.7 ± 0.5	5.0 ± 0.9	43 ± 13
Controls ( $n = 10$ )	28 ± 8	66 ± 6	7.0 ± 1.0	5.0 ± 1.0	46 ± 20

<sup>a</sup> TMT-A: JME patients with reduced NAA showed poorer performance than JME patients with normal NAA ( $P < 0.0001$ ), patients with GTCS ( $P < 0.0001$ ), and controls ( $p = 0.0009$ ).

<sup>b</sup> TMT-B: JME patients with reduced NAA showed poorer performance than JME patients with normal NAA ( $p = 0.002$ ), patients with GTCS ( $p = 0.0004$ ), and controls ( $p = 0.01$ ).

significant ( $P < 0.01$  for both metabolites and patient groups). This correction implied that the observed reductions in metabolites were not due to differences in line width.

## Discussion

The main observation in the present study was the significant difference between patients with JME and patients with GTCS with respect to the frontal lobe concentrations of NAA. Whereas the NAA was normal in patients with GTCS, it was significantly reduced in subjects with JME. These data suggest that the two forms of IGE have, at least to a certain extent, different pathophysiologies.

The second finding was the reduced thalamic concentration of mI and Cho in JME as well as GTCS, and the additional reduction in thalamic NAA in GTCS. Thus, the two groups of patients had abnormal thalamic concentrations as a common feature, but were dissimilar in their frontal lobe values. With respect to epilepsy, they only differed in the age at seizure onset and the seizure type.

### Methodological aspects

The applied method (single-volume MR spectroscopy using stimulated echoes at short TE and long TR and LCMoDeL evaluation) has been applied in numerous studies, yielding reproducible results, which were in accordance with *in vitro* data (Michaelis et al., 1993; Tedeschi et al., 1995). The data were corrected for subject movement (Helms and Piringer, 2001), which is particularly important for the prefrontal cortex. When comparing with the previously obtained data without scan-wise phase correction (Savic et al., 2000), considerable differences were seen in some subjects (see JME 1 in Table 1). They were probably due to subject compliance during the acquisition. Both water content in cortical grey matter and concentrations of measured metabolites correspond well to previously published data (Ernst et al., 1993; Michaelis et al., 1993; Pouwels and Frahm, 1998; Savic et al., 2000; Tedeschi et al., 1995), including the thalamus. Choice of the VOIs involved a compromise between defining the anatomical structures and sufficient signal-to-noise ratio. The issue of “partial volume effects” (McLean et al., 2000; Pouwels and Frahm, 1998) of different tissues, like grey and white matter (Fig. 1), was not specifically assessed. Metabolic differences between grey and white matter have been found for tCr, mI, and to a minor degree for tNAA by correlating voxel-based segmentation with MRS imaging (Pouwels and Frahm, 1998). While the influence of systematic partial volume changes cannot be ruled out conclusively, the present reduction of tNAA in the prefrontal cortex is too large to be explained by an increased partial volume of white matter. Furthermore, considering that the frontal lobe grey matter fraction of patients with JME is reported to be significantly increased, it seems highly unlikely that frontal lobe reductions in our JME patients were due to elevated WM fraction on the VOIs. Moreover, the VOIs were positioned using identical approach in all three populations. A partial inclusion of putatively unaffected regions, like those around the lateral thalamic border (globus pallidus and the internal capsule), could, thus, only have diminished but not enhanced the existing differences.

Only patients with a careful and complete documentation of seizure phenomenology and frequency, and with continuous con-

trol at the Huddinge University Hospital’s Epilepsy Clinic were included in the present study. Nevertheless, GTCS can sometimes be difficult to differentiate from epilepsy with secondary generalized tonic and clonic seizures, which may be a potential methodological bias. Several circumstances make it, however, highly improbable that the selected patients here had a secondarily generalized epilepsy. First of all, the age of seizure onset was in the teen ages or earlier in the majority of patients. Secondly, almost all had bilateral synchronous spike and wave activity. Finally, all had a normal MRI. Therefore, the classification of patient groups (JME vs. GTCS) is regarded as a reliable basis for the discussion about possible general differences in measured metabolites among the respective syndromes.

### Frontal lobe alterations in patients with JME

Our initial observation of reduced frontal lobe NAA in JME (Savic et al., 2000) was confirmed when investigating a larger cohort of patients. Furthermore, in the present study, we improved the reliability of the detected low NAA by carefully employing motion correction, which rendered the results more robust with respect to individual compliance. The generated data are in accordance with reports of increased prefrontal grey matter fraction (Woermann et al., 1999) and reduced prefrontal glucose metabolism (Swartz et al., 1996), as well as with the observation of prefrontal electromagnetic current sources to the epileptogenic activity in JME (Santiago-Rodriguez et al., 2002).

Frontal lobe reductions were not found in GTCS, and the two groups differed significantly with respect to both the mean NAA values and the number of affected patients. Certain overlaps can, however, not be excluded.

The reduced NAA concentration could simply reflect neuronal loss. This seems, however, less probable when considering that neither increase in tissue water content nor decrease in other metabolites was found. Alternatively, it could reflect neuronal dysfunction associated with an impaired NAA-precursor pool, a specific mitochondrial dysfunction, or a neuronal lesion leading to release of *N*-acetyl-L-aspartate aminohydrolase resulting in a degradation of NAA.

The 12 JME patients who had reduced frontal lobe NAA also showed poor performance on trailmaking tests, of which at least part B more specifically measures frontal lobe function (Reitan, 1986). This was not found in JME patients with normal frontal lobe NAA (Table 4). The observed bimodal distribution in JME is in accordance with the reports of Devinsky et al. (1997) and Woermann et al. (1999), who found abnormalities in frontal lobe tests and the frontal lobe grey matter fraction only in a portion of investigated JME patients.

Thus, JME may be a nonuniform condition. This is of note when taking into account that a candidate gene for JME has been reported both on chromosome 6 and 15 (Delgado-Escueta et al., 1999), and that a JME gene on chromosome 6 was recently found to encode a brain specific lysosomal membrane protein (Suzuki et al., 2002).

### The thalamic reductions in JME and GTCS

Our knowledge about the pathophysiology of IGE is mainly based on electrophysiological experiments, the results of which have led to centrencephalic and corticoreticular theories (Gloor et

al., 1982). The former attributes IGE to pathological changes in the thalamic nuclei, whereas the latter postulates that ascending impulses from the thalamus impinge upon a diffusely hyperexcitable cortex. Thus, in both theories, a neuroanatomical substrate is hypothesized (thalamus, cerebral cortex), but no such substrate has hitherto been shown. In this context, the observed thalamic reductions deserve special attention, especially as they were found in patients with both JME and GTCS.

The common feature for both groups was the occurrence of generalized tonic clonic seizures (absences were experienced only by four patients in the JME and one in the GTCS group). It is, therefore, of interest that the total number of these seizures was related to the thalamic concentration of NAA (even when each group was evaluated separately). This relationship further emphasizes the association between GTCS and thalamus—Although it does not explain whether the observed changes reflect a pathology which makes thalamus generate GTCS as indicated by the early ictal discharges in centromedian nucleus during IGE (Velasco et al., 1989), or if they simply reflect a GTCS-associated necrosis in thalamic relay neurons (Nevander et al., 1985).

The observed reduction in ml and Cho is congruent with these alternatives. The choline signal is generally believed to reflect membrane synthesis and degradation, and ml is an organic osmolyte mainly present in glia. While NAA is predominantly located in neurons, Cr (i.e., the sum of creatine and phosphocreatine) is ubiquitous for several cell types due to its role in energy metabolism. The combined reduction in NAA, Cho, and ml has been reported only in a few diseases—In glutamine intoxication, hepatic encephalopathy (Ross et al., 1994), and dysplastic cerebellar gangliocytoma (Klisch et al., 2001). In our patients, this reduction could be an effect of cell loss (although less probable as the tissue water concentration was normal), excitotoxicity (Nevander et al., 1985), or both. One possibility is that dystopic thalamus neurons of patients with IGE (Meencke and Janz, 1984) have a greater vulnerability to seizures, leading to a more rapid age-dependent decrease in neuronal density.

The present data do not allow further speculations about the cause–effect issue. Nevertheless, they show the reliability of the MRS method to detect thalamic changes in human epilepsy. Follow-up MRS experiments comparing patients with primary and secondary generalized tonic and clonic seizures are, therefore, under development and will, hopefully, further elucidate whether the thalamic changes are primary or secondary to the generalized seizures.

## Conclusions

The present results further substantiate the view that thalamo-cortical circuits represent the seizure-associated network in several subtypes of IGE. They suggest existence of both common and syndrome related anatomical substrates. The thalamic affection, repetitively shown in absence epilepsy (Bartenstein et al., 1993) and now also in JME and GTCS, could, thus, be more widespread among IGE. In contrast, the frontal lobe dysfunction seems to be primarily associated with JME. This reasoning is in line with data from genetic mapping of IGE, suggesting that different clinical IGE syndromes may depend on specific modifying genes along with shared IGE genotype (Durner et al., 2001). It is also congruent with reports about several different candidate genes in JME, potentially leading to different clinical expressions (Delgado-

Escueta et al., 1999). The bimodal distribution of frontal lobe NAA in JME along with unimodal distribution of seizure frequency suggests that JME can have different forms, despite similar seizure phenomenology. These forms may have different genetics and pathophysiology, perhaps underlying the clinically observed differences in prognosis, personality characteristics (Janz and Christian, 1957), and frontal lobe dysfunctions (Reitan, 1986). Combined imaging and neuropsychological and genetic studies of human IGE are therefore highly encouraged.

## Acknowledgments

This study was supported by the Swedish Medical Research Council (grant 98-14X-12599-01A), Karolinska Institute, the Captain Ericssons, the Åke Wiberg, and the Magnus Bergvall's foundation. We would like to acknowledge Dr. Dan Greitz for his professional evaluation of anatomical MR images, and Dr. Simon Kyaga for collection of some neuropsychological data and editorial help.

## References

- Andermann, F., Berkovic, S.F., 2001. Idiopathic generalized epilepsy with generalized and other seizures in adolescence. *Epilepsia* 42, 317–320.
- Bartenstein, P.A., Duncan, J.S., Prevett, M.C., Cunningham, V.J., Fish, D.R., Jones, A.K., Luthra, S.K., Sawle, G.V., Brooks, D.J., 1993. Investigation of the opioid system in absence seizures with positron emission tomography. *J. Neurol. Neurosurg. Psychiatry* 56, 1295–1302.
- Commission on Classification and Terminology of the International League Against Epilepsy, 1981. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* 22, 489–501.
- Commission on Classification and Terminology of the International League Against Epilepsy, 1989. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 30, 389–399.
- Delgado-Escueta, A.V., Medina, M.T., Serratos, J.M., Castroviejo, I.P., Gee, M.N., Weissbecker, K., Westling, B.W., Fong, C.Y., Alonso, M.E., et al., 1999. Mapping and positional cloning of common idiopathic generalized epilepsies: juvenile myoclonus epilepsy and childhood absence epilepsy. *Adv. Neurol.* 79, 351–374.
- Devinsky, O., Gershengorn, J., Brown, E., Perrine, K., Vazquez, B., Luciano, D., 1997. Frontal functions in juvenile myoclonic epilepsy. *Neuropsychiatry Neuropsychol. Behav. Neurol.* 10, 243–246.
- Durner, M., Keddache, M.A., Tomasini, L., Shinnar, S., Resor, S.R., Cohen, J., et al., 2001. Genome scan of idiopathic generalized epilepsy: evidence for major susceptibility gene and modifying genes influencing the seizure type. *Ann. Neurol.* 49, 328–335.
- Engel, J.M., 1989. *Seizures and Epilepsy*. F.A. Davis, Philadelphia.
- Ernst, T., Kreis, R., Ross, B.D., 1993. Absolute quantification of water and metabolites in the human brain: I. Compartments and water. *J. Magn. Reson.* B102, 1–8.
- Gastaut, H., 1970. Clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* 11, 102–113.
- Gloor, P., 1978. Generalized epilepsy with bilateral synchronous spike and wave discharge. New findings concerning its physiological mechanisms. *Electroencephalogr. Clin. Neurophysiol., Suppl.* 34, 245–249.
- Gloor, P., Metrakos, J., Metrakos, K., Andermann, E., van Gelder, N., 1982. Neurophysiological, genetic and biochemical nature of the epileptic diathesis. *Electroencephalogr. Clin. Neurophysiol., Suppl.* 35, 45–56.
- Helms, G., 1999. Analysis of 1.5 Tesla proton MR spectra of human brain

- using LCModel and an imported basis set. *Magn. Reson. Imaging* 17, 1211–1218.
- Helms, G., 2000. A precise and user-independent quantification technique for regional comparison of single volume proton MR spectroscopy of the human brain. *NMR Biomed.* 13, 398–406.
- Helms, G., Piringer, A., 2001. Restoration of motion-related signal loss and line-shape deterioration of proton MR spectra using the residual water as intrinsic reference. *Magn. Reson. Med.* 46, 395–400.
- Janz, D., 1997. The idiopathic generalized epilepsies of adolescence with childhood and juvenile age of onset. *Epilepsia* 38, 4–11.
- Janz, D., Christian, W., 1957. Impulsiv–Petit mal. *Dtsch. Z. Nervenheilk* 176, 348–386.
- Klisch, J., Juengling, F., Spreer, J., Koch, D., Thiel, T., Büchert, M., Arnold, S., Feuerhake, F., Schumacher, M., 2001. Lhermitte–Duclos disease: assessment with MR imaging, positron emission tomography, single-photon emission CT, and Mr spectroscopy. *Am. J. Neurorad.* 22, 824–830.
- Koepp, M.J., Richardson, M.P., Brooks, D.J., Cunningham, V.J., Duncan, J.S., 1997. Central benzodiazepine/gamma–aminobutyric acid A receptors in idiopathic generalized epilepsy: an [<sup>11</sup>C]flumazenil positron emission tomography study. *Epilepsia* 38, 1089–1097.
- McLean, M.A., Woermann, F.G., Barker, G.J., Duncan, J.S., 2000. Quantitative analysis of short time 1H-MRSI of cerebral gray and white matter. *Magn. Reson. Med.* 44, 401–411.
- Meencke, H.J., Janz, D., 1984. Neuropathological findings in primary generalized epilepsy: a study of eight cases. *Epilepsia* 25, 8–21.
- Meencke, H.J., Veith, G., 1992. Migration disturbances in epilepsy. *Epilepsy Res., Suppl.* 9, 31–39 (Discussion 39–40).
- Michaelis, T., Merboldt, K.D., Bruhn, H., Hanicke, W., Frahm, J., 1993. Absolute concentrations of metabolites in the adult human brain in vivo: quantification of localized proton MR spectra. *Radiology* 187, 219–227.
- Nevander, G., Ingvar, M., Auer, R., Siesjö, B.K., 1985. Status epilepticus in well-oxygenated rats causes neuronal necrosis. *Ann. Neurol.* 18, 281–290.
- Pouwels, P.J., Frahm, J., 1998. Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. *Magn. Reson. Med.* 39, 53–60.
- Provencher, S.W., 1993. Estimation of metabolite concentrations from localized in vivo proton NMR Spectra. *Magn. Reson. Med.* 30, 672–679.
- Reitan, R.M., 1986. *The Trail Making Test: Manual for Administration and Scoring*. Reitan Neuropsychology Laboratory, Tuscon, AR, pp. 1–50.
- Reutens, D.C., Berkovic, S.F., 1995. Idiopathic generalized epilepsy of adolescence: are the syndromes clinically distinct? *Neurology* 45, 1469–1476.
- Ross, B.D., Jacobson, S., Villamil, F., Korula, J., Kreis, R., Ernst, T., Shonk, T., Moats, R.A., 1994. Subclinical hepatic encephalopathy: proton MR spectroscopic abnormalities. *Radiology* 193, 457–463.
- Santiago-Rodriguez, E., Harmony, T., Fernandez-Bouzas, A., Hernandez-Balderas, A., Martinez-Lopez, M., Graef, A., et al., 2002. Source analysis of polyspike and wave complexes in juvenile myoclonic epilepsy. *Seizure* 11, 320–324.
- Savic, I., Seitz, R.J., Pauli, S., 1998. Brain distortions in patients with primarily generalized tonic–clonic seizures. *Epilepsia* 39, 364–370.
- Savic, I., Lekvall, A., Greitz, D., Helms, G., 2000. MR spectroscopy shows reduced frontal lobe concentrations of *N*-acetyl aspartate in patients with juvenile myoclonic epilepsy. *Epilepsia* 41, 290–296.
- Suzuki, T., Morita, R., Sugimoto, Y., Sugawara, T., Bai, D., Alonso, M., et al., 2002. Identification and mutational analysis of candidate genes for juvenile myoclonic epilepsy on 6p11–p12: LRRRC1, GCLC, KIAA0057 and CLIC5. *Epilepsy Res.* 50, 265.
- Swartz, B.E., Simpkins, F., Halgren, E., Mandelkern, M., Brown, C., Krisdakumtom, T., Gee, M., 1996. Visual working memory in primary generalized epilepsy: an 18FDG-PET study. *Neurology* 47, 1203–1212.
- Tedeschi, G., Bertolino, A., Righini, A., Campbell, G., Raman, R., Duyn, J.H., et al., 1995. Brain regional distribution pattern of metabolite signal intensities in young adults by proton magnetic resonance spectroscopic imaging. *Neurology* 45, 1384–1391.
- Unterberger, I., Trinka, E., Luef, G., Bauer, G., 2001. Idiopathic generalized epilepsies with pure grand mal: clinical data and genetics. *Epilepsy Res.* 44, 19–25.
- Velasco, M., Velasco, F., Velasco, A.L., Lujan, M., Vazquez del Mercado, J., 1989. Epileptiform EEG activities of the centromedian thalamic nuclei in patients with intractable partial motor, complex partial, and generalized seizures. *Epilepsia* 30, 295–306.
- Woermann, F.G., Sisodiya, S.M., Free, S.L., Duncan, J.S., 1998. Quantitative MRI in patients with idiopathic generalized epilepsy. Evidence of widespread cerebral structural changes. *Brain* 121, 1661–1667.
- Woermann, F.G., Free, S.L., Koepp, M.J., Sisodiya, S.M., Duncan, J.S., 1999. Abnormal cerebral structure in juvenile myoclonic epilepsy demonstrated with voxel-based analysis of MRI. *Brain* 122 (Pt 11), 2101–2108.