Tracing tremor: Neural correlates of essential tremor and its treatment
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Citation for published version (APA):

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Chapter 7

Decreased cerebellar fiber density in cortical myoclonic tremor but not in essential tremor

Accepted as

Decreased cerebellar fiber density in cortical myoclonic tremor but not in essential tremor.

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Abstract

Background
Pathophysiology of tremor generation remains uncertain in ‘familial cortical myoclonic tremor with epilepsy’ (FCMTE) and essential tremor (ET). In both disorders, imaging and pathological studies suggest involvement of the cerebellum and its projection areas. MR diffusion tensor imaging (DTI) allows estimation of white matter tissue composition, and therefore is well suited to quantify structural changes in vivo.

Objective
To compare cerebellar fiber density between FCMTE and ET patients and healthy controls.

Methods
Seven FCMTE patients, eight ET patients, and five healthy controls were studied. Cerebellum was annotated based on Fractional Anisotropy (FA)- and Mean Diffusivity-volumes. Mean cerebellar FA values were computed as well as mean cerebellar volume. Group statistics included one way ANOVAs and post hoc independent T-tests.

Results
Mean FA of the cerebellar region for FCMTE was 0.242 (SD = 0.012), for ET 0.259 (SD = 0.0115), and for controls 0.262 (SD = 0.0146). There was a significant group effect for FA (F(2) = 4.9, p = 0.02). No difference in mean cerebellar volume was found. Post hoc independent T-tests revealed significantly decreased mean FA in FCMTE patients compared to controls (t[10] = 2.5, p = 0.03) and ET patients (t[13] = 2.9, p = 0.01), while there was no difference in mean FA between ET patients and controls (t[11] < 1.0).

Conclusion
This study indicates for the first time microstructural damage of the cerebellar white matter in FCMTE in vivo. These results ascertain a role of the cerebellum in ‘cortical tremor’.
Introduction

The neuronal circuits involved in tremor remain uncertain in most tremor types. Both ‘familial cortical myoclonic tremor with epilepsy’ (FCMTE) and essential tremor (ET) are characterized by distal tremulous movements. Although FCMTE and ET can be differentiated based on clinical and electrophysiological features, imaging and pathological studies suggest common involvement of the cerebellum in both disorders, especially Purkinje cell changes.\textsuperscript{205,219}

Since FCMTE was first reported in 1984, it has been described in over 60 pedigrees, mainly in Japan and Europe, also under different names including BAFME and FAME.\textsuperscript{84,220} FCMTE is an autosomal dominant condition characterized by distal tremulous myoclonus, epilepsy, and signs of cortical hyperexcitability.\textsuperscript{204} Seizures usually manifest some time after the onset of the distal tremulous movements. These tremulous movements, in fact not real ‘tremor’, but myoclonus mimicking tremor, can easily be misinterpreted as ET.\textsuperscript{84,204} Neurophysiological measures can differentiate FCMTE from ET. FCMTE patients show short irregular EMG bursts, signs of cortical hyperexcitability including a giant somatosensory evoked potential, and strong cortico- and intermuscular coherence in the 8- to 30-Hertz range while ET patients show weak coherence around tremor frequency.\textsuperscript{101} Anti-epileptic drugs (AEDs), such as valproic acid, benzodiazepines, carbamazepine, phenytoin and levetiracetam, reduce the number of seizures and diminish the tremulous movements.\textsuperscript{84} Clinically the syndrome, with a relatively mild course, can be differentiated from the progressive myoclonus epilepsies. A gene has not been identified yet and linkage analysis showed different loci in different pedigrees, or showed exclusion of linkage to known loci.\textsuperscript{206} In FCMTE, cortical functional changes and cerebellar signs were described in a Dutch pedigree.\textsuperscript{101,110,205,216} Post-mortem studies in FCMTE patients showed severe loss of Purkinje cells and abnormal Purkinje cell morphology with somal sprouting and loss of the dendritic tree.\textsuperscript{205,216} In ET, two different kinds of pathological changes are found. In one study, around 75% of ET cases showed axonal and dendritic swelling of Purkinje cells throughout the cerebellum.\textsuperscript{221,222} In addition, several other Purkinje cell abnormalities have been described.\textsuperscript{219,223} In around 25%, no cerebellar abnormalities were observed, but instead, Lewy bodies were found in the brainstem.\textsuperscript{32} Functional studies in both FCMTE and ET point towards changes in cerebello-thalamo-cortical pathways.\textsuperscript{105,205}

MR diffusion tensor imaging (DTI) allows estimation of white matter tissue composition in living tissue.\textsuperscript{224} In white matter, water molecules show strong anisotropic displacement...
because of the shape of white matter fibers. Fractional anisotropy (FA), depicting the normalized standard deviation of diffusivities, has gained widespread acceptance as a sensitive indicator to quantify white matter composition. It is a scalar quantity between zero and one that describes the anisotropy i.e. the directionality of a diffusion process, where zero depicts isotropic diffusion, and one depicts complete linear diffusion. Microstructural damage of white matter can be quantified in neurodegenerative diseases by using FA. Fiber tracking of white matter can be performed on the same data, and is used to assess fiber density. In this study we compare cerebellar FA, measured with DTI, between FCMTE, ET, and healthy controls. In FCMTE, microstructural white matter changes have been observed in pathology studies. In ET, studies showed contradicting results with respect to white matter involvement. We hypothesize that FA values in the cerebellum are decreased in FCMTE, and show minor changes in ET compared to healthy controls.

Materials and Methods
Seven FCMTE patients from one family (4 men; mean age 48 y, range 19-56, Table 1), reported before, with bilateral myoclonic tremor and diagnosed according to previously described criteria participated. The oldest four FCMTE patients had a history of epileptic attacks and used various AEDs (Table 1). Eight patients (5 men; mean age 39 y, range 20-69, Table 1) with ET according to the diagnostic criteria of the Tremor Investigation Group were included. All ET patients had bilateral postural arm tremor. Two ET patients were on propranolol medication. Five healthy age-matched controls (3 men; mean age 43 y, range 31-63) were included, and had no history of neurological or psychiatric conditions. Written, informed consent was obtained from all participants. The study was approved by the local medical-ethical board and was performed in accordance with the Declaration of Helsinki.

Table 1. Patient characteristics
Abbreviations: CBZ = carbamazepine, EEG = electroencephalography, g-SEP = giant somatosensory evoked potential, GTCS = generalized tonic-clonic seizures, nd = no neurophysiologic testing performed, OCB = oxcarbazepine, PhB = Phenobarbital, VPA = valproic acid, ± = few seizures per lifetime, good control with medication, + = present/few seizures per year on medication.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Age at onset (yrs)</th>
<th>Age at onset tremor (yrs)</th>
<th>Seizure frequency</th>
<th>Medication</th>
<th>Neurophysiologic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 FCMTE</td>
<td>F</td>
<td>19</td>
<td>12</td>
<td>-</td>
<td>-</td>
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<td>Normal EEG, positive C-reflex</td>
</tr>
<tr>
<td>2 FCMTE</td>
<td>F</td>
<td>31</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Normal EEG, g-SEP, positive C-reflex</td>
</tr>
<tr>
<td>3 FCMTE</td>
<td>M</td>
<td>33</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Normal EEG, g-SEP, positive C-reflex</td>
</tr>
<tr>
<td>4 FCMTE</td>
<td>M</td>
<td>43</td>
<td>19</td>
<td>20</td>
<td>+</td>
<td>PhB, VPA, CBZ</td>
<td>nd</td>
</tr>
<tr>
<td>5 FCMTE</td>
<td>F</td>
<td>45</td>
<td>38</td>
<td>42</td>
<td>±</td>
<td>VPA, CZP</td>
<td>Irregular EEG, g-SEP, positive C-reflex</td>
</tr>
<tr>
<td>6 FCMTE</td>
<td>M</td>
<td>46</td>
<td>12</td>
<td>31</td>
<td>±</td>
<td>VPA, CZP</td>
<td>nd</td>
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<tr>
<td>7 FCMTE</td>
<td>M</td>
<td>56</td>
<td>30</td>
<td>43</td>
<td>+</td>
<td>OCB, CZP</td>
<td>Spike-wave complexes on EEG, positive C-reflex</td>
</tr>
<tr>
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<td>&lt;18</td>
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<td>-</td>
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<td>nd</td>
</tr>
<tr>
<td>2 ET</td>
<td>F</td>
<td>25</td>
<td>&lt;18</td>
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<td>nd</td>
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<tr>
<td>3 ET</td>
<td>F</td>
<td>43</td>
<td>&lt;18</td>
<td>-</td>
<td>-</td>
<td>Propranolol</td>
<td>nd</td>
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<tr>
<td>4 ET</td>
<td>M</td>
<td>49</td>
<td>&lt;18</td>
<td>-</td>
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<td>nd</td>
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<tr>
<td>5 ET</td>
<td>M</td>
<td>59</td>
<td>&lt;18</td>
<td>-</td>
<td>-</td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>6 ET</td>
<td>M</td>
<td>61</td>
<td>46</td>
<td>-</td>
<td>--</td>
<td></td>
<td>nd</td>
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<tr>
<td>7 ET</td>
<td>M</td>
<td>61</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Propranolol</td>
<td>nd</td>
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<tr>
<td>8 ET</td>
<td>M</td>
<td>69</td>
<td>62</td>
<td>-</td>
<td>-</td>
<td>CBZ</td>
<td>nd</td>
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<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>nd</td>
</tr>
<tr>
<td>2 Control</td>
<td>F</td>
<td>32</td>
<td>-</td>
<td>-</td>
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<td>nd</td>
</tr>
<tr>
<td>3 Control</td>
<td>M</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>4 Control</td>
<td>M</td>
<td>53</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>nd</td>
</tr>
<tr>
<td>5 Control</td>
<td>M</td>
<td>63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Atenolol</td>
<td>nd</td>
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DTI-data were acquired on a Philips Intera 3.0 Tesla MRI scanner (Philips Medical Systems, Best, The Netherlands) by means of a spin-echo EPI sequence. The diffusion weighting was along 15 directions (TE 83 msec, TR 7897 msec, b=700 s/mm², FOV 230 mm, scan matrix 96 x 95, image matrix 128 x 128, slice thickness 2.5 mm, voxelsize was 1.8 x 1.8 x 2.5 mm). Eddy current induced morphing was corrected for by an affine registration in the phase direction.

AFvR annotated the cerebellum based on FA-and Mean Diffusivity-volumes using 3D-Slicer (http://www.slicer.org). Tensor fields were computed, and the FA and principal eigenvector were derived (http://teem.sf.net). Mean FA values in the cerebellum were computed as well as mean cerebellar volume after normalization of the cerebellar volume with respect to the total intracranial volume (B0 threshold). Group differences were assessed using SPSS 15 (Chigaco, IL) by one way ANOVAs and post hoc independent two-sided T-tests.

For illustration purposes, fiber tracking was performed with in-house developed software. Three boxes were positioned interactively, in such a way that only cranial fibers passing through the cerebellum were visualized. Fibers passing through the corpus callosum were considered false positives and were therefore excluded.

Results

On visual inspection, fiber tracking of the cerebellar outflow tracts showed no particular differences between individual FCMTE- and ET-patients and controls (Figure 1).

Mean FA was decreased in FCMTE patients. Mean FA of the cerebellar region for FCMTE was 0.242 (SD = 0.012), for ET 0.259 (SD = 0.0115), and for controls 0.262 (SD = 0.0146; Figure 2). There was a significant group effect for FA (F(2) = 4.9, p = 0.02). Post hoc independent T-tests revealed significantly decreased mean FA in FCMTE patients compared to controls (t[10] = 2.5, p = 0.03) and ET patients (t[13] = 2.9, p = 0.01), while there was no difference in mean FA between controls and ET patients (t[11] < 1.0). No differences were observed between FCMTE patients using AEDs and patients not using AEDs (mean FA AED users 0.237 [SD = 0.0110], mean FA non AED users 0.248 [SD = 0.0113], t[5] < 1). There was no linear relationship between FA and age in ET (r = 0.237, p = 0.573), controls (r = 0.722, p = 0.169) and FCMTE (r = 0.737, p = 0.69).

Mean cerebellar volume (relative to intra-cranial volume) for FCMTE was 0.11 (SD = 0.008), for ET 0.12 (SD = 0.014), and for controls 0.11 (SD = 0.010; Figure 2), with no significant group effect. There was no significant correlation between FA values and cerebellar volume for each group (FCMTE r(5) = 0.642, p = 0.12, ET r(6) = 0.234, p =
A post-hoc power calculation showed that a sample size of 8 subjects in the ET group and 5 subjects in the control group had a power of 99% to detect a difference in FA means of 0.052 (20% FA difference), assuming that the common standard deviation is 0.013 using a two group t-test with a 0.05 two-sided significance level.

In the ET group, there was no significance between ET cases with short (less than 20 years, n=4, mean 0.267 SD ± 0.0166) or long disease duration (more than 20 years, n=4, mean 0.256 SD ± 0.0116, p = 0.306 (two-sided t-test)).

**Figure 1. Fiber tracking of the cerebellar outflow tracts.** Selected cranial fibers, passing through the cerebellum, for one control, ET- and FCMTE-patient (left to right). Except for reduced cerebellar FA, compatible with white matter degeneration in the FCMTE patients, no differences in outflow tracts are seen between patients and control subjects.
FA of the cerebellum was reduced in FCMTE patients compared to ET patients and healthy controls, without differences in cerebellar volume. To our knowledge, this is the first study indicating microstructural damage of the cerebellar white matter in cortical tremor patients using diffusion tensor imaging. The clinical picture of FCMTE includes distal tremulous myoclonus, epilepsy, and signs of cortical hyperexcitability. In 1921, Hunt already pointed out the association between cortical myoclonus and cerebellar changes, which has also been described in celiac disease. Cortical myoclonus has been hypothesized to be a result of primary cerebellar pathology by dysfunction of the cerebello-thalamo-cortical loop. Purkinje cell abnormalities would lead to reduced inhibitory cerebellar output to the sensorimotor and frontal cortices via the thalamus. Subsequently, this would result in cortical myoclonus and epileptic attacks. An ¹H-MR spectroscopy study in benign adult familial myoclonic epilepsy, a condition similar to FCMTE, revealed neurochemical changes in the cerebellum indicating cerebellar dysfunction reflected by changes in the chemical and functional nature of cell membranes. An fMRI study in FCMTE patients showed, also in line with this hypothesis, decreased cerebellar activations and bilateral activations of motor areas.
during unilateral tremulous movements. An alternative hypothesis for the cortical hyperexcitability in combination with cerebellar Purkinje cell changes is that they are both caused by a common factor, leading to cortical functional changes on the one hand, and degenerative cerebellar changes on the other. It has been suggested that FCMTE, like other idiopathic epilepsies with autosomal dominant inheritance, is a channelopathy.

Axonal degeneration and microglial activation have been shown to correlate with low FA values. One of the FCMTE patients included in the current study deceased (patient 5, Table 1). This patient showed neuropathological changes including diffuse microglial activation in white matter identical to findings in two relatives of the included FCMTE patients. It is likely that low FA values are to be attributed to microglial activation in FCMTE as part of the disease. Supporting this hypothesis, Striano et al. found abnormal choline concentrations in the cerebellum in similar patients, indicating gliosis.

Another possible explanation of the changes seen in FCMTE is the use of AEDs. However, FA values of patients using AEDs overlapped with FA values of patients not using AEDs. Furthermore, the decreased FA values are not likely to be the result of seizure mediated cellular damage. There is no known relationship between decreased FA values and seizure mediated cellular damage. Also, most FCMTE subjects studied here did not suffer from chronic seizures (Table 1). There is however a possible interaction of age on FA values in FCMTE, which supports the view of FCMTE being a neurodegenerative disorder.

In neurodegenerative epilepsy disorders including the progressive myoclonus epilepsies, no studies investigating cerebellar FA values have been reported in literature. In patients with idiopathic generalized epilepsy however, decreased FA values in the cerebellum have been reported compared to healthy controls. Furthermore, in Juvenile Myoclonus Epilepsy, decreased FA values are observed throughout the cortex, compared to patients with idiopathic generalized epilepsy and healthy controls. Unfortunately, this study did not look at FA differences in the cerebellum due to methodological problems.

In the ET group, FA did not differ from FA in controls. These findings are partly in line with the study by Martinelli and colleagues showing normal apparent diffusion coefficients in the cerebellum in ET patients. Recently, a DTI study in twenty-five ET cases showed reduced FA in the dentate nucleus and the superior cerebellar peduncle compared to controls and patients with Parkinson’s disease. Moreover, reduced FA in ET has been described in the bilateral cerebellum, the inferior cerebellar peduncle and red nucleus, which underlines the pathophysiological role of the cerebellum and its projections for tremor generation in ET. These studies all provide convincing evidence
for structural changes in ET as do a number of pathology studies.\textsuperscript{32,219,221–223} In the present study, looking for microstructural damage of cerebellar white matter and FA changes particularly in the outflow tracts of the cerebellum in FCMTE, we compared the entire cerebellum of FCMTE, ET and controls.

**Limitations**

In this study, we did not observe significant structural changes in ET compared to controls. However, because of our relatively small sample size and the fact that we compared mean FA of the whole cerebellum, our findings cannot exclude the existence of more subtle neurodegenerative changes in specific regions of the cerebellum in ET. The fact that ET is a heterogeneous disorder\textsuperscript{219} might explain the variable FA values in the ET group. A post-hoc power calculation showed that the sample size of our study was sufficient to detect a 20% difference in FA means between the ET and control group.

As previously mentioned, Nicoletti et al. reported reduced FA in ET in the dentate nucleus and the superior cerebellar peduncle compared to controls.\textsuperscript{181} However, they did not find an association between FA values and disease severity. They did find a difference in dentate nucleus FA values between ET cases with a short (less than 20 years) or long disease duration (more than 20 years). This difference was not observed in MD values of the dentate nuclei and FA and MD values of superior cerebellar peduncle. In our patient group, there was no significant difference between ET cases with short or long disease duration.

**Conclusion**

This study indicates for the first time microstructural damage of the cerebellar white matter in FCMTE in vivo. In ET, no differences were found compared to controls. In literature, the tremulous movements in FCMTE are believed to originate from the sensorimotor cortex. Cerebellar white matter damage can potentially alter the cortico-cerebello-thalamo-cortical loop, and thereby increase tonic facilitation of the motor cortex, which suggests a role of the cerebellum in cortical tremor. This is in line with previous studies that showed dysfunction of the cerebello-thalamo-cortical loop.