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Climbing fiber-Purkinje cell synaptic pathology across essential tremor subtypes

Danielle Lee [a], Shi-Rui Gan [a, b], Phyllis L. Faust [c], Elan D. Louis [d, e, f], Sheng-Han Kuo [a, *]

[a] Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, NY, USA
[b] Department of Neurology and Institute of Neurology, First Affiliated Hospital, Fujian Medical University, Fuzhou, China
[c] Department of Pathology and Cell Biology, Columbia University Medical Center and the New York Presbyterian Hospital, New York, NY, USA
[d] Department of Neurology, Yale School of Medicine, Yale University, New Haven, CT, USA
[e] Department of Chronic Disease Epidemiology, Yale School of Public Health, Yale University, New Haven, CT, USA
[f] Center for Neuroepidemiology and Clinical Research, Yale School of Medicine, Yale University, New Haven, CT, USA

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ABSTRACT

Background: Essential tremor (ET) is heterogeneous in nature and cases may be subdivided based on clinical features. ET patients may thus be subdivided by age of onset, family history of tremor, and presence of head tremor. We recently described climbing fiber-Purkinje cell (CF-PC) synaptic abnormalities in ET; however, these CF pathological features have not been studied across different ET subtypes.

Objectives: To explore whether these CF-PC synaptic abnormalities differ across ET subtypes.

Methods: We studied two climbing fiber (CF-PC) synaptic pathologies (CF synaptic density and percentage of CFs in the parallel fiber [PF] territory) in the cerebella of 60 ET cases with a range of clinical presentations and 30 age-matched controls.

Results: Compared to controls, ET cases had lower CF synaptic density and a higher percentage of CFs in the PF territory. ET cases with tremor onset <50 years and tremor onset ≥ 50 years did not differ significantly with respect to CF synaptic density and percentage of CFs in the PF territory. Similar results were found when comparing familial *vs.* sporadic ET cases, and ET cases with head tremor *vs.* those without head tremor. Among all ET cases, lower CF synaptic density was associated with lower PC counts and higher torpedo counts. In addition, higher percentage of CFs in the PF territory was associated with lower PC counts and higher torpedo counts.

Conclusions: These findings support the notion that changes in the distribution of CF-PC synapses are broadly part of the neurodegenerative process in the ET cerebellum.

1. Introduction

Essential tremor (ET) is one of the most prevalent movement disorders among adults [1]. Clinical and neuroimaging evidence both suggest the importance of the cerebellum in tremor generation in ET [2,3]. In recent studies, we observed climbing fiber-Purkinje cell (CF-PC) synaptic abnormalities in the ET cerebellum; compared to controls, ET cases exhibited decreased CF synaptic density and an increased percentage of CFs extending into the parallel fiber (PF) territory [4–7]. These changes coexist with a host of other pathological changes in PCs in ET, such as a decrease in PC counts [8] and an increase in torpedoes and associated PC axonal pathologies [8,9], although PC loss is a finding that is variably reproduced [10–12]. The presence of such pathological changes in the ET cerebellum reinforces the notion that degeneration and reorganization of cerebellar structures may be important for disease progression in ET.

ET is also considered a heterogeneous disorder. Several clinical features may divide ET into subtypes. For example, early onset ET cases might differ in their rates of progression and their tremor-related brain oscillatory circuits when compared to late onset ET cases [13,14]. The presence of family history in ET might indicate an underlying genetic etiology [15,16] as well as possible differences in the ability to metabolize naturally-occurring tremorgenic

* Corresponding author. 650 West 168th Street, Room 305, New York, NY 10032, USA.
E-mail address: sk3295@columbia.edu (S.-H. Kuo).

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compounds [17]. In addition, ET cases with head tremor might have different degrees of cerebellar involvement than ET cases without head tremor, based on neuroimaging findings [18]. Collectively, these heterogeneous clinical features of ET could reflect diverse alterations in disease etiology, disease-associated changes in brain circuitry, and disease pathogenesis.

Although we recently described climbing fiber-Purkinje cell (CF-PC) synaptic abnormalities in ET [4–7], we have not explored whether these abnormalities differ across disease subtypes. Therefore, we now investigate CF-PC synaptic abnormalities across disease subtypes in ET: early *vs.* late onset ET, familial *vs.* sporadic ET, and ET cases with *vs.* without head tremor. We also examined the association of these more recently described CF-PC synaptic abnormalities with other described changes in ET (e.g., PC loss and higher torpedo counts), as this has yet to be studied.

2. Materials and methods

2.1. ET brains

ET brains were obtained from the Essential Tremor Centralized Brain Repository (ETCBR), a joint effort between investigators from Yale and Columbia Universities. Three sequential methods were used to carefully assign ET diagnoses, as described at length [9]. Briefly, the clinical diagnoses of ET was initially assigned by treating neurologists and then subsequently confirmed by an ETCBR study neurologist (EDL) using clinical questionnaires, review of medical records, and examination of Archimedes spirals. Third, a detailed, videotaped neurological examination was performed, and published diagnostic criteria were applied as described [19]. A total tremor score (range = 0–36) was assigned to each ET case based on the severity of postural and kinetic tremor (pouring, drinking, using spoon, drawing spirals, finger-nose-finger) on videotaped examination [19]. None of the ET cases had a history of traumatic brain injury, exposure to medications known to cause cerebellar damage, or heavy ethanol use as previously defined [20]. Head tremor was assessed as present or absent using clinical information and the videotaped neurological examination.

2.2. Control brains

Most control brains were obtained from the New York Brain Bank (NYBB, n = 21) and were from individuals followed at the Alzheimer's Disease (AD) Research Center or the Washington Heights Inwood Columbia Aging Project at Columbia University who were clinically free of Alzheimer's disease (AD), ET, Parkinson's disease (PD), Lewy body dementia, and progressive supranuclear palsy (PSP). Seven control brains were from the University of Miami Brain Endowment Bank (University of Miami Hospital, Miami, FL), and two were from the University of Maryland Brain and Tissue Bank (University of Maryland, Baltimore, MD), obtained through the NIH NeuroBioBank. During life, all study subjects signed informed consent approved by their respective University Ethics Boards.

2.3. Sample selection (cases and controls)

We selected ET cases for the current study based on age of onset. The mean age of onset for ET is most commonly reported to be between 45 and 55 years, with a bimodal distribution [21–23]. Of the ET cases in the ETCBR, there were 133 cases with clear documentation of age of tremor onset. This age of tremor onset distribution was bimodal, with a median at 50 years. Therefore, we chose the age of onset of 50 to divide the early vs. late onset ET cases. Using data from our previous publications on the PC pathology in subgroups of ET cases and controls [21,24,25], we determined that with each ET group and control group composed of 30 subjects, we would be powered at 90% to detect differences of the magnitude previously detected [21,24,25]. Accordingly, we randomly selected 30 early onset ET cases, 30 late onset ET cases, and 30 controls in the current study. Of the selected cases for this study, data from 14 of 30 controls and 15 of 60 ET cases were reported in prior publications [21,24,25].

2.4. Standard neuropathological assessment

All ET and control brains had a complete neuropathological assessment. Each brain had a standardized measurement of brain weight (grams), postmortem interval (PMI, hours between death and placement of brain in a cold room or upon ice). No ET cases with Lewy body or PSP pathology were included in this study.

A standard $3 \times 20 \times 25$ mm parasagittal, formalin-fixed tissue block was harvested from the neocerebellum, which included white matter and dentate nucleus [4,9]. A senior neuropathologist (PLF), blinded to clinical information, counted torpedoes throughout a single Luxol fast blue Hematoxylin & Eosin (LH&E) stained 7- μm section from this block [8]. PCs were also counted and averaged from 15 microscopic fields at 100 × magnification (LH&E) [8].

2.5. Cerebellar immunohistochemistry

Since CF terminals that form synapses with PCs express vesicular glutamate transporter type 2 (VGlut2, now known as SLC17A6), cerebellar tissues were stained for VGlut2, whose puncta in the molecular layer (ML) represent CF-PC synapses [5]. Seven-micrometer paraffin-embedded cerebellar sections were rehydrated and incubated in 3% hydrogen peroxide, followed by antigen retrieval in Tris-urea buffer (0.1 M Tris-base, 5% urea, pH 9.5) for 20 min at 95 °C [5]. A suppressive block of 10% normal goat serum and 0.5% bovine serum albumin was used prior to incubation with polyclonal rabbit anti-VGlut2 antibody (1:250) at 4 °C for 40 h, followed by incubation with biotinylated anti-rabbit IgG (1:100, Vector labs, Burlingame, CA, 15 μg/ml). Signals were amplified via avidin-biotin complex (Vector, Burlingame, CA), and sections were developed with 3,3′-diaminobenzidine chromagen solution (Dako). Images were acquired using bright field microscopy (Leica DFC7000T).

2.6. Assessment of CF synaptic density

We quantified CF synaptic density as previously described [4,5]. Image acquisition and quantification were performed by a trained rater (DL) who was blinded to the diagnoses of each subject. The rater was trained by a neurologist experienced in the study of CF pathology (SHK). The rater and the neurologist had an inter-rater reliability (Pearson's r) of 0.98, based on the quantification of CF synaptic density in 50 fields (10 fields for each of 5 ET cases).

Using a random digit generator, 10 fields in the cerebellar cortex were randomly selected for each subject, and a 400x image directly above the PC layer was acquired for each field. The total number of visualized VGlut2 puncta on proximal PC dendrites above the PC layer was quantified. In the same 400x field, the total visualized CF length was measured with NeuriteTracer (Neuron J), a plugin of Image J (NIH, Bethesda, MD). CF synaptic density was defined as the total number of visualized VGlut2 puncta, divided by the total CF length. Ten fields were quantified to obtain an average CF synaptic density for each subject.

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