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Research article

Cerebellar pathology in childhood-onset vs. adult-onset essential tremor

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ABSTRACT

Although the incidence of ET increases with advancing age, the disease may begin at any age, including childhood. The question arises as to whether childhood-onset ET cases manifest the same sets of pathological changes in the cerebellum as those whose onset is during adult life. We quantified a broad range of postmortem features (Purkinje cell [PC] counts, PC axonal torpedoes, a host of associated axonal changes [PC axonal recurrent collateral count, PC thickened axonal profile count, PC axonal branching count], heterotopic PCs, and basket cell rating) in 60 ET cases (11 childhood-onset and 49 adult-onset) and 30 controls. Compared to controls, childhood-onset ET cases had lower PC counts, higher torpedo counts, higher heterotopic PC counts, higher basket cell plexus rating, and marginally higher PC axonal recurrent collateral counts. The median PC thickened axonal profile count and median PC axonal branching count were two to five times higher in childhood-onset ET than controls, but the differences did not reach statistical significance. Childhood-onset and adult-onset ET had similar PC counts, torpedo counts, heterotopic PC counts, basket cell plexus rating, PC axonal recurrent collateral counts, PC thickened axonal profile count and PC axonal branching count. In conclusion, we found that childhood-onset and adult-onset ET shared similar pathological changes in the cerebellum. The data suggest that pathological changes we have observed in the cerebellum in ET are a part of the pathophysiological cascade of events in both forms of the disease and that both groups seem to reach the same pathological endpoints at a similar age of death.

1. Introduction

Essential tremor (ET) is one of the most common movement disorders as well as the most common tremor disorder [1,2]. Despite its high prevalence, disease mechanisms are not completely understood [3,4]. Clinical [5–9] and neuroimaging [3,10–12] studies suggest that the cerebellum plays an important role in the generation of tremor in ET. Furthermore, in recent years we have observed a constellation of pathological changes in the ET cerebellum, present mainly in the cerebellar cortex and involving the Purkinje cell (PC) and its neighboring neuronal populations, and distinguishing ET from control brains. These changes include an increase in the number of torpedoes and associated PC axonal pathologies [13,14], an increase in heterotopic PCs [15], and abnormal basket cell axons with a dense and tangled appearance ("hairiness") surrounding the PC soma and elongated processes extending past the PC axon initial segment [16,17]. In addition to these changes we have reported PC loss [13,18], a finding that has been variably reproduced [19–21]. These pathological changes support the concept that the cerebellum is of mechanistic importance in ET [4,22,23]. They also support the concept that ET may be a neurodegenerative disease [4,22–24].

Although the incidence of ET increases with advancing age [25], the disease may begin at any age. Indeed, ET cases may arise during childhood [26–30], with one study reporting that 5.3% of cases began prior to age 20 years [25]. Studies indicate that the familial form of ET is enriched for earlier onset cases, many of which arise during childhood [29,31,32].

With the notion that ET could be degenerative [4,22–24], the question arises as to whether childhood-onset cases, whose disease duration is generally very long, are also degenerative. Whether they manifest the same sets of pathological changes as those whose onset is during adult life has yet to be examined. We capitalized on a large, prospectively-assembled collection of ET brains, including both childhood-onset and adult-onset forms, to investigate whether the postmortem changes in the cerebellum differ between childhood-onset and adult-onset cases.

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2. Methods

2.1. Brain repository, study subjects, sample size

ET brains were from the Essential Tremor Centralized Brain Repository (ETCBR), a joint effort between investigators at Yale and Columbia Universities [33,34]. ET diagnoses were all carefully assigned using three sequential methods, as described at length [14]. Briefly, the clinical diagnosis of ET was initially assigned by treating neurologists, and then 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 applied, as described [35]. 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 [33,34]. None of the ET cases had a history of (1) traumatic brain injury, (2) exposure to medications known to cause cerebellar damage, or (3) heavy ethanol use, as previously defined [33,34,36].

Childhood-onset ET was defined as ET whose age of onset was less than or equal to 18 years of age [26].

Most of the control brains were obtained from the New York Brain Bank (NYBB) (n = 21) and were from individuals followed at the Alzheimer disease (AD) Research Center or the Washington Heights Inwood Columbia Aging Project at Columbia University [33,34]. They had been followed prospectively with serial neurological examinations, and were clinically free of AD, ET, Parkinson's disease (PD), Lewy body dementia, or progressive supranuclear palsy (PSP). Nine control brains were from Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA) [33,34]. During life, all cases and controls signed informed consent approved by these University Ethics Boards.

These analyses were performed on a sample of 90 brains comprising a 2:1 age-match of 60 ET cases and 30 controls [33,34]. We performed a power analysis that utilized data from our previous publications on PC counts [13] and torpedo counts [7]. Our sample (11 childhood-onset ET cases, 49 adult-onset ET cases, and 30 controls) was powered at > 80% to detect differences between study groups (childhood-onset ET vs. controls, childhood-onset ET vs. adult-onset ET) of the magnitudes previously detected.

2.2. Neuropathological assessment

All ET and control brains had a complete neuropathological assessment at the NYBB and Harvard Brain Bank [33,34]. 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), Braak and Braak AD staging for neurofibrillary tangles [37,38], and Consortium to Establish a Registry for AD (CERAD) ratings for neuritic plaques [39]. We did not include any ET cases with either Lewy body pathology (α-synuclein staining) or PSP pathology [40].

2.3. Characterization of cerebellar pathology

A standard 3 × 20 × 25 mm parasagittal, formalin-fixed, tissue block was harvested from the neocerebellum; this block included cerebellar cortex, white matter and dentate nucleus [33,34]. A senior neuropathologist (P.L.F.), blinded to clinical information, counted torpedoes and heterotopic PCs (i.e., a PC whose cell body was completely surrounded by the molecular layer and that did not contact the granule layer) throughout a single Luxol fast blue Hematoxylin & Eosin (LH & E) stained 7-μm thick section from this block [15]. PCs were counted and averaged from 15 microscopic fields at 100 x magnification (LH & E) [33,34].

We have previously shown that examination of a single, standard section provides an adequate representation of the pathology within that sample block. Using a systematic uniform random (SUR) sampling approach, one of every five sections obtained from a series of 40 collected paraffin sections from the standard cerebellar block was stained with LH & E. We determined that there was little variation in torpedo and PC counts among sampled sections within this block in 11 ET and 9 control brains. The agreement between these counts was very high (for torpedo counts, intraclass correlation coefficient = 0.96, $p < 0.001$; for PC counts, intraclass correlation coefficient = 0.94, $p < 0.001$).

In addition, a single 7-μm thick paraffin section was stained by modified Bielschowsky silver technique and a semi-quantitative basket cell plexus rating scale was applied: 0 (few, or no discernible processes); 1 (sparse number of processes); 2 (moderate number of processes); and 3 (dense tangle of processes). In some instances, as described, the rater used intermediate values (0.5, 1.5, and 2.5) [17,33,34].

$Calbindin_{D28k}$ immunohistochemistry was performed in free-floating 100 μm thick, formalin-fixed vibratome sections of cerebellar cortex to visualize PC axonal morphology. The sections were heated at 37 °C for 10 min in 20 μg/ml Proteinase K (Roche Applied Science) in 10 mM Tris, 0.1 mM EDTA, pH 8, followed by 1% hydrogen peroxide in PBS for 30 min and serum blocking solution (10% normal goat serum, 1% IgG-free bovine serum albumin [Jackson Immunoresearch], 1% Triton™X-100, in PBS) for 1 h. Rabbit polyclonal anti-calbindin D28k (1:1000, Swant) was applied overnight at 4 °C in antibody diluent (1% IgG-free bovine serum albumin, 1% Triton™X-100 in PBS). Secondary antibody (1:200, 2 h, biotin-SP goat-anti-rabbit [Fisher Scientific]), followed by streptavidin-horseradish peroxidase (1:200, 1 h, AbD Serotec, for biotinlyated antibodies) was developed with 3,3′ diaminobenzidene chromogen solution (Dako). PC axonal morphology in 10 randomly-selected 100X images from three sections was quantified in each brain: axon recurrent collaterals (an axon with at least a 90° turn back towards the PC layer from its initial trajectory), thickened PC axonal profiles (an axon with at least double the width of other apparently normal axons), and PC axonal branching (any PC axon with at least one branch point; multiple bifurcations on the same axon were not separately counted). The raw counts of Purkinje cell axonal features were normalized to the total length of the Purkinje cell layer length [14,33,34].

2.4. Statistical analyses

We first compared clinical and pathological characteristics between ET cases and controls (Table 1), but our main analyses were to compare childhood-onset ET to controls and then childhood-onset ET to adult-onset ET (Table 2). Clinical characteristics such as gender were compared using chi-square tests. Age at death, total tremor score, and PC counts were normally distributed (Kolmogorov-Smirnov test p values > 0.05); thus, we compared groups using Student's *t*-tests. Age of tremor onset, duration of tremor, torpedo counts, heterotopic PC counts, basket cell plexus rating, PC axonal recurrent collateral counts, PC thickened axon counts, and PC axonal branching counts were not normally distributed (Kolmogorov-Smirnov test p values < 0.05). Therefore, we used Mann-Whitney tests. Data were analyzed in SPSS (version 24).

3. Results

The 60 ET cases and 30 controls were similar in age at death and gender (Table 1). When compared with controls, ET cases had lower PC counts, more torpedoes, more heterotopic PCs, a higher basket cell plexus rating, an increase in PC axonal collaterals, an increase in PC thickened axonal profiles, and an increase in PC axonal branching (Table 1).

We compared the clinical characteristics of cases with childhood-onset ET to controls (Table 2). There were no differences in age at death or gender (Table 2). Childhood-onset ET cases had lower PC counts, higher torpedo counts, higher heterotopic PC counts, higher basket cell plexus rating, and marginally higher (p = 0.059) PC axonal recurrent