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Cerebellar Pathology in Early Onset and Late Onset Essential Tremor

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Abstract

Early onset and late onset essential tremor (ET) cases differ in several respects. Whether they differ with respect to cerebellar pathologic changes remains to be determined. We quantified a broad range of postmortem features (Purkinje cell (PC) counts, PC axonal torpedoes and

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Compliance with Ethical Standard

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associated axonal changes, heterotopic PCs, and hairy basket ratings) in 30 ET cases with age of tremor onset <50 years, 30 ET cases with age of tremor onset ≥ 50 years, and 30 controls (total $n = 90$). We also used two alternative age of onset cut-points (<40 vs. ≥ 40 years, and <60 vs. ≥ 60 years) to define early onset vs. late onset ET. We found that ET cases with tremor onset <50 years and tremor onset ≥ 50 years had similar PC counts (8.78 ± 1.70 vs. 8.86 ± 1.24 , $p = 0.839$), PC axonal torpedo counts (17.87 ± 18.27 [median = 13.00] vs. 12.90 ± 10.60 [median = 9.0], $p = 0.486$) and associated axonal pathology (all p values >0.05), heterotopic PC counts (9.90 ± 11.55 [median = 6.00] vs. 5.40 ± 5.10 [median = 3.50], $p = 0.092$), and hairy basket ratings (1.95 ± 0.62 [median = 2.00] vs. 2.05 ± 0.92 [median = 2.00], $p = 0.314$). When using the age of onset cut-points of 40 or 60 years, results were similar. Early onset and late onset ET cases share similar cerebellar postmortem features. These data do not support the notion that these age-of-onset related forms of ET represent distinct clinical-pathological entities.

Keywords

Essential tremor; Age of onset; Cerebellum; Neurodegenerative; Purkinje cell; Pathology

Introduction

Essential tremor (ET) is one of the most prevalent movement disorders [1]. Clinical and neuroimaging evidence suggests that the cerebellum plays an important role in tremor generation in ET [2–6]. We have reported a constellation of pathological changes in the ET cerebellum, including an increase in torpedoes and associated Purkinje cell (PC) axonal pathologies [7, 8], an increase in heterotopic PCs [9], 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 [10, 11]. In addition to these changes we have also reported PC loss [7, 12], a finding that has been variably reproduced [13–15]. These pathological changes further reinforce the notion that the cerebellum is of mechanistic importance in ET.

The age of tremor onset in ET is bimodal [16]. ET cases with an early age of tremor onset more commonly report a family history of tremor. By contrast, ET cases with a late age of tremor onset may have a faster rate of tremor progression and may differ from early onset cases in terms of their risk of dementia and mortality [16–19]. Therefore, it is possible that early onset ET and late onset ET represent different disease subtypes [16]. There are physiological data supporting this notion; for example, a recent electroencephalogram (EEG) coherence study suggested that early onset ET cases and late onset ET cases may differ with respect to brain circuitry [20]. Given the possibility that early onset ET and late onset ET could be pathophysiologically distinct, we capitalized on a large, prospectively assembled collection of ET brains, including both young and old onset, to investigate whether the postmortem changes in the cerebellum differ across age of onset groups.

Methods

Brain Repository, Study Subjects, and Neuropathological Assessment

ET brains were from the Essential Tremor Centralized Brain Repository (ETCBR), a joint effort between investigators at Yale and Columbia Universities. All ET diagnoses were carefully assigned using three sequential methods, as described in detail [8]. In brief, the clinical diagnosis of ET was initially assigned by treating neurologists, and then confirmed by an ETCBR study neurologist (EDL) using questionnaires, review of medical records, and review of Archimedes spirals. Finally, a detailed, videotaped, neurological examination was performed, and published diagnostic criteria applied, as described [21]. Total tremor scores (range =0–36) were assigned based on the severity of postural and kinetic (pouring, drinking, using spoon, drawing spirals, finger-nose-finger) tremor on examination. None of the ET cases had a history of traumatic brain injury, a history of exposure to medications known to cause cerebellar damage, or heavy ethanol use, as previously defined [8, 22].

The majority 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. They were 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). During life, all study subjects signed informed consent approved by these University Ethics Boards.

There were 133 ET cases in the ETCBR with clear documentation of age of tremor onset. This age of tremor onset distribution was bimodal, with a median at 50 years. We performed a power analysis that utilized data from our previous publications on PC counts [7], torpedo counts [7], and heterotopic PCs [9] in ET cases and controls. With a sample size of 14–26 in each group, we would be powered at 90 % to detect differences of the magnitude previously detected. Therefore, with 30 per group (30 early onset ET cases, 30 late onset ET cases, and 30 controls), power was >90 %. Based on age at death, and blinded to all other clinical information, we frequency-matched 30 in each of the two ET groups to the 30 available control subjects.

All ET and control brains had a complete neuropathological assessment at the NYBB and Harvard Brain Bank. Brains had standardized measurements 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 [23, 24], and Consortium to Establish a Registry for AD (CERAD) ratings for neuritic plaques [25]. We did not include ET cases with Lewy body pathology (α -synuclein staining) [7] or PSP pathology [26].

Characterization of Cerebellar Pathology

A standard $3 \times 20 \times 25$ mm parasagittal, formalin-fixed, tissue block was harvested from the neocerebellum; the block included the cerebellar cortex, white matter, and dentate nucleus [8]. A senior neuropathologist (P.L.F.) who was blinded to all clinical information counted

torpedoes and heterotopic PCs (PCs whose cell bodies were completely surrounded by the molecular layer and that did not contact the granule layer) throughout one entire LH&E 7- μ m thick section [9]. As described, PCs were counted and averaged from 15 microscopic fields at 100 $\times$ magnification (LH&E) [8].

In addition, 7- μ m thick paraffin sections were stained by modified Bielschowsky silver technique and a semi-quantitative hairy basket rating scale was applied in each section: 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) [10].

Calbindin_{D28k} immunohistochemistry was performed in free-floating 100 μ m 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 μ g/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 % TritonTMX-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 % TritonTMX-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 biotinylated antibodies) was developed with 3,3'-diaminobenzidine chromogen solution (Dako). As described, PC axonal morphology in ten randomly-selected 100 $\times$ images was quantified: axon recurrent collaterals (with at least a 90° turn back towards the PC layer from their initial trajectory), thickened PC axonal profiles (axons 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) [8].

Statistical Analyses

The mean age of onset is most commonly reported to be between 45 and 55 years in ET [27–33]; in the current sample it was just below 50. Therefore, we chose an age of onset of 50 years as the cut-point in our primary analysis. We first compared clinical and pathological characteristics between ET cases and controls, and then further compared these features in early onset ET cases (tremor onset <50 years old), late onset ET cases (tremor onset $\geq$ 50 years), and controls. Clinical characteristics such as gender and the presence of family history of tremor were compared using chi-square tests. Age at death and PC counts were normally distributed; thus we compared groups using analysis of variance followed by LSD post-hoc analyses. Torpedoes, heterotopic PCs, hairy basket rating, PC axonal recurrent collateral counts, PC thickened axon counts, and PC axonal branching counts were not normally distributed. Therefore, we used independent samples Kruskal-Wallis tests to compare among three groups, and we further compared the differences between two groups using Mann-Whitney tests. In addition to using the age of onset cut-point of 50 years old to define early vs. late onset ET cases, we performed additional analyses using the age of onset cut-points of 40 and 60 years old. We also performed a correlation analysis between disease duration, total tremor scores, and each pathological feature. For normally distributed

variables, we used Pearson's correlation coefficients; for non-normally distributed variables, we used Spearman's rank correlation coefficients. Data were analyzed in SPSS (v22).

Results

The 60 ET cases and 30 controls were similar in age at death and gender (Table 1). Consistent with previous reports [7–12], when compared with controls, ET cases had lower PC counts, more torpedoes, more heterotopic PCs, a higher hairy basket 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 ET cases with tremor onset before and after 50 years old (early onset and late onset) and controls. There were no differences in age at death and gender between early onset and late onset ET cases and controls. Early onset ET cases and late onset ET cases did not differ significantly in terms of their PC counts, torpedo counts, heterotopic PC counts, hairy basket ratings, PC axonal recurrent collateral counts, PC thickened axonal profile counts, and PC axonal branching counts (Table 2). When compared to controls, both ET groups had significantly lower PC counts, more torpedoes, higher hairy basket cell ratings, more PC axonal recurrent collaterals, and more PC axonal branching. Only early onset ET cases had more heterotopic PCs than controls. Late onset ET cases had more PC thickened axonal profiles than controls, with early onset ET cases having an intermediate value (Table 2).

Early onset ET cases had longer disease duration than late onset ET cases, which might be a confounding factor when comparing the pathological changes in these two groups. Therefore, we studied whether the disease duration correlated with each of the pathological features in ET. We found that disease duration did not correlate with any of the pathological features (Table 3). In addition, the total tremor scores did not correlate to a significant degree ($p < 0.05$) with pathological changes in ET (Table 3). Therefore, neither disease duration nor tremor severity would have been confounding factors.

We also explored alternative age of onset cut-points. First, we used the value 40 years to define early onset ET cases and late onset ET cases. Similarly, there were no differences in any of the pathological features assessed between ET cases with tremor onset <40 years old and ET cases with tremor onset ≥40 years old (Table 4). Second, we chose 60 years old as a second alternative age cut-point. We did not find any differences in cerebellar pathological features between ET cases with tremor onset <60 years old and ET cases with tremor onset ≥60 years old except for PC thickened axonal profiles: ET cases with tremor onset ≥60 years old had 64 % more PC thickened axonal profiles than ET cases with tremor onset <60 years old (Table 5).

We compared cerebellar pathological features in ET cases with and without head tremor. Interestingly, we found that ET cases with head tremor had lower PC counts than ET cases without head tremor; however, the two groups did not differ with respect to any of the other cerebellar pathological features (Table 6).

Discussion

In this study, we found that early onset ET cases and late onset ET cases shared similar pathological changes in cerebellum. The mean age of onset is most commonly reported to be between 45 and 55 years in ET [27–33]; therefore, we chose an age of onset of 50 years as the cut-point in our primary analysis. However, the mean age of ET onset has been reported to differ considerably across studies, with some studies reporting a much younger age of onset [28, 34–37] while other studies reported older ages of onset [16, 38, 39]. Therefore, in two secondary analyses, we employed the alternative age cut-points of 40 and 60 years. Our results were the same.

Several studies have reported that early onset and late onset ET cases might have different clinical characteristics. Early onset ET cases more commonly have a family history of tremor whereas late onset ET cases might have a faster rate of tremor progression and a higher risk of dementia [16–19]. Our study showed that both early onset ET cases and late onset ET cases had similar cerebellar pathological changes, suggesting that both groups reached the same pathological endpoints at a similar age of death.

Age of onset is one of the major determinants of disease features and disease progression in neurological disorders. For example, early onset PD cases have a better response to levodopa treatment, more motor fluctuations, and a slower disease progression when compared to late onset PD cases [40]. On the other hand, both early onset and late onset PD cases reached the clinical disease milestones such as dementia and hallucinations at a similar age and had similar extent of pathologic alterations, regardless the age of PD onset [40]. These findings suggest that clinical presentations and acquisition of pathologic changes are modulated by aging-related mechanisms in the PD brain. This concept could be applied to ET as well.

ET cases with head tremor have more cerebellar involvement than ET cases without head tremor based on the structural imaging findings [41] and physiological characteristics [42]. We compared cerebellar pathological features in ET cases with and without head tremor. Interestingly, we found that ET cases with head tremor had lower PC counts than ET cases without head tremor; however, the two groups did not differ with respect to any of the other cerebellar pathological features (Table 6).

Muthuraman et al. [20], studied EEG coherence in ET and concluded that early onset ET cases had cerebellar involvement whereas late onset ET cases had no cerebellar involvement. However, in our current study, we found that both early onset and late onset ET cases had similar degree of degenerative changes in the cerebellum, and this was significantly different from controls.

A limitation of the current study is that we did not study pathological features in other relevant brain areas that are known to be connected to the cerebellar cortex and have been postulated as part of oscillatory loops, such as the inferior olivary nucleus. Nonetheless, we did not observe any morphological alterations in the inferior olivary nucleus in our previous study between ET cases and controls [43]; therefore, it is unlikely that we will discover the differences in this brain region in early vs. late onset ET cases. Another limitation is that we mainly studied the axonal compartment in the cerebellar cortex. Other synaptic pathology in

the early vs. late onset ET cerebellum remains to be investigated [44, 45]. A third limitation is that we did not employ stereological methods for PC counts; nonetheless, we have previously validated PC counting with a random sampling approach [13]. A fourth limitation is that the disease duration is self-reported. Although age of onset is generally reported with high reliability by ET cases ($\rho = 0.85$, $p < 0.001$), in up to 20 % of cases, it is not. Furthermore, the validity of such reports is not known [46]. Our study had several strengths. First, we investigated a carefully diagnosed ET cohort. Second, we studied a broad range of pathological changes. Third, our sample size, of 90 brains, including 30 each with early and late onset ET, was considerable.

In conclusion, we did not find major pathological differences in the cerebellar cortex of early and late onset ET cases. These data do not support the notion that these age-of-onset related forms of ET represent distinct clinical-pathological entities.

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Table 1

Clinical and pathological features of controls and essential tremor cases

Variables	Controls	ET cases	<i>p</i> value
<i>n</i>	30	60	
Age at death (years)	85.23 ± 5.67	86.03 ± 6.63	0.573 ^a
Age of tremor onset (years)	NA	44.02 ± 21.60	NA
		Median = 47.50	
Duration of disease (years)	NA	42.02 ± 21.51	NA
		Median = 39.00	
Gender			0.226 ^b
Male	15 (50.0 %)	22 (36.7 %)	
Female	15 (50.0 %)	38 (63.3 %)	
History of tremor in family ^c			NA
No	NA	30 (52.6 %)	
Yes	NA	27 (47.4 %)	
Total tremor scores	NA	12.45 ± 3.19	NA
Purkinje cell counts	10.52 ± 1.49	8.82 ± 1.48	<0.001 ^a
Torpedo counts	3.90 ± 3.28	15.38 ± 15.02	<0.001 ^d
	Median = 3.00	Median = 12.00	
Heterotopic Purkinje cell counts	6.23 ± 11.45	7.65 ± 9.14	0.020 ^d
	Median = 2.00	Median = 4.50	
Hairy basket ratings	1.57 ± 0.61	2.00 ± 0.78	0.004 ^d
	Median = 1.50	Median = 2.00	
Purkinje cell axonal recurrent collateral counts	1.01 ± 1.16	1.88 ± 1.53	0.006 ^d
	Median = 0.60	Median = 1.42	
Purkinje cell thickened axonal profile counts	0.96 ± 1.60	1.76 ± 2.32	0.017 ^d
	Median = 0.55	Median = 1.09	
Purkinje cell axonal branching counts	0.09 ± 0.10	0.37 ± 0.40	<0.001 ^d
	Median = 0.07	Median = 0.27	

Values represent mean ± standard deviation or number (percentage), and for variables with non-normal distribution, the median is reported as well. Mean number of Purkinje cell counts (PCs) per 100× microscopic field, among 15 sampled fields

NA not applicable

^aIndependent samples *t* test

^bChi-square test

^cUnknown in three ET cases

^dIndependent samples Mann-Whitney U test

Table 2

Clinical and pathological features of controls and essential tremor cases grouped by age of tremor onset 50 years

Variables	Controls	ET cases		<i>p</i> value (all three groups)	<i>p</i> value		
		Age of tremor onset <50 years	Age of tremor onset 50 years		Controls vs. ET <50 years	Controls vs. ET 50 years	ET <50 years vs. ET 50 years
<i>n</i>	30	30	30				
Age at death (years)	85.23 ± 5.67	85.80 ± 7.45	86.27 ± 5.81	0.820 ^a	0.731 ^b	0.531 ^b	0.777 ^b
Age of tremor onset (years)	NA	25.47 ± 12.89	62.57 ± 8.44				< 0.001 ^c
Duration of disease (years)	NA	60.33 ± 13.42	23.70 ± 8.18				< 0.001 ^c
Gender				0.277 ^d			
Male	15 (50.0 %)	13 (43.3 %)	9 (30.0 %)				
Female	15 (50.0 %)	17 (56.7 %)	21 (70.0 %)				
History of tremor in family ^e							0.146 ^d
No	NA	18 (62.1 %)	12 (42.9 %)				
Yes	NA	11 (37.9 %)	16 (57.1 %)				
Total tremor scores	NA	13.01 ± 3.41	11.77 ± 2.86				0.205 ^c
Purkinje cell counts	10.52 ± 1.49	8.78 ± 1.70	8.86 ± 1.24	<0.001 ^a	< 0.001 ^b	< 0.001 ^b	0.839 ^b
Torpedo counts	3.90 ± 3.28	17.87 ± 18.27	12.90 ± 10.60	<0.001 ^f	< 0.001 ^g	< 0.001 ^g	0.486 ^g
	Median =3.00	Median =13.00	Median =9.00				
Heterotopic Purkinje cell counts	6.23 ± 11.45	9.90 ± 11.55	5.40 ± 5.10	0.021 ^f	0.009 ^g	0.155 ^g	0.092 ^g
	Median =2.00	Median =6.00	Median =3.50				
Hairy basket ratings	1.57 ± 0.61	1.95 ± 0.62	2.05 ± 0.92	0.012 ^f	0.022 ^g	0.008 ^g	0.314 ^g
	Median =1.50	Median =2.00	Median =2.00				
Purkinje cell axonal recurrent collateral counts	1.01 ± 1.16	1.99 ± 1.79	1.74 ± 1.17	0.022 ^f	0.016 ^g	0.021 ^g	0.772 ^g
	Median =0.60	Median =1.29	Median =1.73				
Purkinje cell thickened axonal profile counts	0.96 ± 1.60	1.59 ± 2.58	1.96 ± 2.01	0.018 ^e	0.133 ^f	0.006 ^f	0.107 ^f
	Median =0.55	Median =0.86	Median =1.47				
Purkinje cell axonal branching counts	0.09 ± 0.10	0.37 ± 0.49	0.37 ± 0.24	0.001 ^e	0.006 ^f	< 0.001 ^f	0.382 ^f
	Median =0.07	Median =0.24	Median =0.38				

Values represent mean ± standard deviation or number (percentage), and for variables with non-normal distribution, the median is reported as well. Mean number of Purkinje cell counts (PCs) per 100× microscopic field, among 15 sampled fields

NA not applicable

^aOne-way analysis of variance (ANOVA)

^bPost hoc multiple comparisons (LSD method) in one-way analysis of variance (ANOVA)

^cIndependent samples *t* test

^dChi-square test

^eUnknown in three ET cases

^fIndependent samples Kruskal-Wallis H test

^gIndependent samples Mann-Whitney U test

Table 3

Correlations of pathological characteristics with duration of disease and total tremor scores in ET cases

	<u>Disease duration</u>		<u>Total tremor scores</u>	
	<i>r</i> or <i>r_s</i>	<i>p</i> value	<i>r</i> or <i>r_s</i>	<i>p</i> value
Purkinje cell counts	0.075 ^b	0.570 ^b	-0.167 ^a	0.278 ^a
Torpedo counts	0.080 ^b	0.541 ^b	-0.038 ^b	0.807 ^b
Heterotopic Purkinje cell counts	0.209 ^b	0.110 ^b	-0.029 ^b	0.850 ^b
Hairy basket ratings	-0.049 ^b	0.708 ^b	-0.232 ^b	0.130 ^b
Purkinje cell axonal recurrent collateral counts	-0.094 ^b	0.575 ^b	0.186 ^b	0.326 ^b
Purkinje cell thickened axonal profile counts	-0.316 ^b	0.053 ^b	-0.340 ^b	0.066 ^b
Purkinje cell axonal branching counts	-0.238 ^b	0.169 ^b	-0.001 ^b	0.997 ^b

^a Pearson correlation analysis^b Spearman correlation analysis

Table 4

Clinical and pathological features of controls and essential tremor cases grouped by age of tremor onset 40 years

Variables	Controls	Essential tremor cases		<i>p</i> value	<i>p</i> value		
		Age of tremor onset <40 years	Age of tremor onset 40 years		Controls vs. <40 years	Controls vs. 40 years	<40 years vs. 40 years
<i>n</i>	30	23	37				
Age at death (years)	85.23 ± 5.67	84.61 ± 7.41	86.92 ± 6.03	0.332 ^a	0.721 ^b	0.279 ^b	0.171 ^b
Age of tremor onset (years)	NA	20.26 ± 9.82	58.78 ± 11.00				<0.001 ^c
Duration of disease (years)	NA	64.35 ± 12.23	28.14 ± 12.25				<0.001 ^c
Gender				0.458 ^d			
Male	15 (50.0 %)	9 (39.1 %)	13 (35.1 %)				
Female	15 (50.0 %)	14 (60.9 %)	24 (64.9 %)				
History of tremor in family ^e							0.306 ^d
No	NA	14 (60.9 %)	16 (47.1 %)				
Yes	NA	9 (39.1 %)	18 (52.9 %)				
Total tremor scores	NA	13.21 ± 2.92	11.81 ± 3.33				0.150 ^c
Purkinje cell counts	10.52 ± 1.49	8.69 ± 1.60	8.90 ± 1.41	<0.001 ^a	<0.001 ^b	<0.001 ^b	0.670 ^b
Torpedo counts	3.90 ± 3.28	18.17 ± 18.25	13.65 ± 12.58	<0.001 ^f	<0.001 ^g	<0.001 ^g	0.333 ^g
	Median =3.00	Median =14.00	Median =9.00				
Heterotopic Purkinje cell counts	6.23 ± 11.45	8.48 ± 11.14	7.14 ± 7.77	0.066 ^e	0.050 ^g	0.039 ^g	0.915 ^g
	Median =2.00	Median =4.00	Median =6.00				
Hairy basket ratings	1.57 ± 0.61	1.98 ± 0.67	2.01 ± 0.85	0.016 ^f	0.027 ^g	0.008 ^g	0.618 ^g
	Median =1.50	Median =2.00	Median =2.00				
Purkinje cell axonal recurrent collateral counts	1.01 ± 1.16	1.98 ± 1.65	1.81 ± 1.47	0.023 ^f	0.013 ^g	0.023 ^g	0.919 ^g
	Median =0.60	Median =1.18	Median =1.67				
Purkinje cell thickened axonal profile counts	0.96 ± 1.60	1.12 ± 1.13	2.22 ± 2.83	0.025 ^f	0.156 ^g	0.011 ^g	0.137 ^g
	Median =0.55	Median =0.80	Median =1.32				
Purkinje cell axonal branching counts	0.09 ± 0.10	0.29 ± 0.25	0.44 ± 0.49	0.001 ^f	0.015 ^g	<0.001 ^g	0.403 ^g
	Median =0.07	Median =0.27	Median =0.30				

Values represent mean ± standard deviation or number (percentage), and for variables with non-normal distribution, the median is reported as well Mean number of Purkinje cell counts (PCs) per 100× microscopic field, among 15 sampled fields

NA not applicable

^aOne-way analysis of variance (ANOVA)

^bPost hoc multiple comparisons (LSD method) in one-way analysis of variance (ANOVA)

^cIndependent samples *t* test

^dChi-square test

^eUnknown in three ET cases

^fIndependent samples Kruskal-Wallis H test

^gIndependent samples Mann-Whitney U test

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Table 5

Clinical and pathological features of controls and essential tremor cases grouped by age of tremor onset 60 years

Variables	Controls	Essential tremor cases		<i>p</i> value	<i>p</i> value		
		Age of tremor onset <60 years	Age of tremor onset 60 years		Controls vs. <60 years	Controls vs. 60 years	<60 years vs. 60 years
<i>n</i>	30	41	19				
Age at death (years)	85.23 ± 5.67	85.17 ± 7.13	87.89 ± 5.09	0.256 ^a	0.967 ^b	0.152 ^b	0.122 ^b
Age of tremor onset (years)	NA	33.34 ± 17.18	67.05 ± 7.31				<0.001 ^c
Duration of disease (years)	NA	51.83 ± 18.58	20.84 ± 7.53				<0.001 ^c
Gender				0.259 ^d			
Male	15 (50.0 %)	17 (41.5 %)	5 (26.3 %)				
Female	15 (50.0 %)	24 (58.5 %)	14 (73.7 %)				
History of tremor in family ^e							0.976 ^d
No	NA	21 (52.5 %)	9 (52.9 %)				
Yes	NA	19 (47.5 %)	8 (47.1 %)				
Total tremor scores	NA	12.63 ± 3.34	11.96 ± 2.83				0.539 ^c
Purkinje cell counts	10.52 ± 1.49	8.85 ± 1.51	8.77 ± 1.44	<0.001 ^a	<0.001 ^b	<0.001 ^b	0.799 ^b
Torpedo counts	3.90 ± 3.28	16.80 ± 17.08	12.32 ± 8.75	<0.001 ^f	<0.001 ^g	<0.001 ^g	0.627 ^g
	Median =3.00	Median =13.00	Median =9.00				
Heterotopic Purkinje cell counts	6.23 ± 11.45	8.51 ± 10.21	5.79 ± 6.04	0.033 ^f	0.011 ^g	0.268 ^g	0.216 ^g
	Median =2.00	Median =6.00	Median =3.00				
Hairy basket ratings	1.57 ± 0.61	2.01 ± 0.70	1.97 ± 0.95	0.017 ^f	0.005 ^g	0.055 ^f	0.858 ^g
	Median =1.50	Median =2.00	Median =2.00				
Purkinje cell axonal recurrent collateral counts	1.01 ± 1.16	1.87 ± 1.64	1.89 ± 1.31	0.022 ^f	0.012 ^f	0.033 ^f	0.653 ^g
	Median =0.60	Median =1.20	Median =1.85				
Purkinje cell thickened axonal profile counts	0.96 ± 1.60	1.46 ± 2.34	2.39 ± 2.23	0.005 ^f	0.127 ^g	0.002 ^g	0.018 ^g
	Median =0.55	Median =0.80	Median =1.59				
Purkinje cell axonal branching counts	0.09 ± 0.10	0.36 ± 0.45	0.40 ± 0.26	0.001 ^e	0.002 ^g	0.001 ^g	0.299 ^g
	Median =0.07	Median =0.25	Median =0.49				

Values represent mean ± standard deviation or number (percentage), and for variables with non-normal distribution, the median is reported as well. Mean number of Purkinje cell counts (PCs) per 100× microscopic field, among 15 sampled fields

NA not applicable

^aOne-way analysis of variance (ANOVA)

^bPost hoc multiple comparisons (LSD method) in one-way analysis of variance (ANOVA)

^cIndependent samples *t* test

^dChi-square test

^eUnknown in three ET cases

^fIndependent samples Kruskal-Wallis H test

^gIndependent samples Mann-Whitney U test

Table 6

Clinical and pathological features of essential tremor cases grouped by head tremor

Variables	Controls	ET cases ^a		<i>p</i> value (all three groups)	<i>p</i> value		
		With head tremor	Without head tremor		Controls vs. ET with head tremor	Controls vs. ET without head tremor	ET with head tremor vs. ET without head tremor
<i>n</i>	30	40	19				
Age at death (years)	85.23 ± 5.67	85.53 ± 6.34	87.16 ± 7.42	0.731 ^b	0.850 ^c	0.306 ^c	0.360 ^c
Age of tremor onset (years)	NA	42.58 ± 23.39	45.84 ± 17.50				0.385 ^d
Duration of disease (years)	NA	42.95 ± 23.69	41.32 ± 16.32				0.759 ^d
Gender				0.133 ^e			
Male	15 (50.0 %)	12 (30.0 %)	10 (52.6 %)				
Female	15 (50.0 %)	28 (70.0 %)	9 (47.4 %)				
History of tremor in family ^d							0.260 ^e
No	NA	18 (47.4 %)	12 (63.2 %)				
Yes	NA	20 (52.6 %)	7 (36.8 %)				
Total tremor scores	NA	25.58 ± 5.47	23.27 ± 8.20				0.364 ^d
Purkinje cell counts	10.52 ± 1.49	8.30 ± 1.92	9.34 ± 1.46	<0.001 ^b	<0.001 ^c	0.019 ^c	0.030 ^c
Torpedo counts	3.90 ± 3.28	17.43 ± 17.31	11.79 ± 7.57	<0.001 ^g	<0.001 ^h	<0.001 ^h	0.445 ^h
	Median =3.00	Median =13.00	Median =9.00				
Heterotopic Purkinje cell counts	6.23 ± 11.45	7.23 ± 9.01	8.84 ± 9.70	0.035 ^g	0.066 ^h	0.016 ^h	0.261 ^h
	Median =2.00	Median =4.00	Median =6.00				
Hairy basket ratings	1.57 ± 0.61	2.03 ± 0.85	1.95 ± 0.66	0.015 ^g	0.006 ^h	0.054 ^h	0.450 ^h
	Median =1.50	Median =2.00	Median =2.00				
Purkinje cell axonal recurrent collateral counts	1.01 ± 1.16	1.81 ± 1.38	2.15 ± 2.10	0.023 ^g	0.008 ^h	0.093 ^h	0.930 ^h
	Median =0.60	Median =1.32	Median =1.67				
Purkinje cell thickened axonal profile counts	0.96 ± 1.60	1.75 ± 2.52	1.76 ± 1.47	0.050 ^g	0.026 ^h	0.093 ^h	0.515 ^h
	Median =0.55	Median =1.09	Median =1.29				
Purkinje cell axonal branching counts	0.09 ± 0.10	0.38 ± 0.43	0.32 ± 0.19	0.001 ^g	0.001 ^h	0.008 ^h	0.815 ^h
	Median =0.07	Median =0.27	Median =0.36				

Values represent mean ± standard deviation or number (percentage), and for variables with non-normal distribution, the median is reported as well. Mean number of Purkinje cell counts (PCs) per 100× microscopic field, among 15 sampled fields

NA not applicable

^aMissing head tremor information in one ET case

^bOne-way analysis of variance (ANOVA)

^cPost hoc multiple comparisons (LSD method) in one-way analysis of variance (ANOVA)

^dIndependent samples *t* test

^eUnknown in three ET cases

^fChi-square test

^gIndependent samples Kruskal-Wallis H test

^hIndependent samples Mann-Whitney U test