

# Neuropathological changes in essential tremor: 33 cases compared with 21 controls

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**Despite its being one of the most commonly observed neurological disorders, neuropathological studies of essential tremor (ET) are rare. There have been surprisingly few autopsy studies and even fewer case-control comparisons. The primary objective was to describe and quantify the pathological changes in 33 ET and 21 control brains. A secondary objective was to correlate clinical and pathological features. We examined autopsy tissue from the Essential Tremor Centralized Brain Repository. Eight (24.2%) of the 33 ET brains had Lewy bodies in the brainstem, mainly in the locus ceruleus. However, the majority of ET brains (25/33, 75.8%) had no Lewy bodies, but had pathological changes in the cerebellum. The mean number of Purkinje cells per 100× field was reduced in ET cases without Lewy bodies ( $6.6 \pm 2.4$  versus  $9.6 \pm 3.4$ ,  $P < 0.01$ ), and there were  $\sim 7\times$  more Purkinje cell torpedoes per section ( $12.6 \pm 7.9$  versus  $1.7 \pm 1.4$ ,  $P < 0.001$ ) compared to controls. ET cases without Lewy bodies also had degeneration of the dentate nucleus (two cases). Other findings in ET cases were Purkinje cell heterotopias and dendrite swellings. Lewy body ET cases were older than ET cases without Lewy bodies. Several trends were observed in ET cases without Lewy bodies, including a younger age of onset of tremor and higher proportions with gait difficulty and family history of ET. The pathological changes of ET seem to be heterogeneous and degenerative. The majority have cerebellar changes without Lewy bodies; a smaller proportion has brainstem Lewy bodies. The clinical differences between cases with versus without Lewy bodies require additional study.**

**Keywords:** essential tremor; cerebellum; Purkinje cell; Lewy body; neurodegeneration; locus ceruleus

**Abbreviations:** ET = essential tremor; GFAP = glial fibrillary acidic protein

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## Introduction

Despite its being one of the most prevalent neurological disorders (Louis *et al.*, 1998; Dogu *et al.*, 2003; Benito-León *et al.*, 2003, 2005), there has been little neuropathological investigation of essential tremor (ET). By 2003, there had only been 15 autopsy studies of ET (Frankl-Hochwart, 1903; Bergamasco, 1907; Hassler, 1939; Mylle and Van Bogaert, 1940, 1948; Herskovitz and Blackwood, 1969; Lapresle *et al.*, 1974; Rajput *et al.*, 1991; Boockvar *et al.*, 2000), and these had neither used control brains for

comparison nor systematically surveyed or quantified cerebellar pathology. In addition, most had not used immunohistochemical methods to evaluate brainstem Lewy bodies. In 2003, the Essential Tremor Centralized Brain Repository (ETCBBR) at Columbia University was established in order to collect and study ET brains (Louis *et al.*, 2005a). In a small autopsy series of 10 ET cases and 12 control brains that we published in 2006 (Louis *et al.*, 2006b), and an additional case published subsequently (Louis *et al.*, 2006a), heterogeneous pathological changes

seemed to be present in the ET brain; some cases had Lewy bodies relatively restricted to the locus ceruleus while others had no Lewy bodies but cerebellar degenerative changes. With our continued success at accruing ET brains, we now present a larger series of 33 ET brains and, importantly, compare them with 21 control brains. Our primary aims here are to: (i) describe and quantify the pathological changes in ET compared to control brains, (ii) provide a more accurate estimate of the proportions of ET cases with versus without Lewy bodies. A secondary aim is to begin to correlate clinical features with pathological findings to determine whether clinical features during life can be used to distinguish ET with versus without Lewy bodies.

## Methods

### Subjects

This study was conducted at the ETCBR, Columbia University. Autopsy tissue was available on 33 ET cases, including 22 (66.7%) not included in an earlier case series (Louis *et al.*, 2006b) or two case reports (Louis *et al.*, 2005b, 2006a). There were 21 control brains; 10 (47.6%) were not included in previous reports (Louis *et al.*, 2005b, 2006a, b), while one from a previous report was excluded from the present series because it met NIA-RI (The National Institute on Aging, 1997) criteria for Alzheimer's disease (AD). The 33 ET cases included 20 (60.6%) cases prospectively collected by the ETCBR beginning in 2003 and 13 (39.4%) cases banked between 1993 and 2002. Brains were well-characterized at the New York Brain Bank, including complete neuropathological assessment and determination of any pathological findings (see [nybb.hs.columbia.edu](http://nybb.hs.columbia.edu)). This consisted of a comprehensive neuropathological diagnostic assessment, including routine immunohistochemical staining for alpha-synuclein in the dorsal vagal nucleus, locus ceruleus and substantia nigra pars compacta, and for hyperphosphorylated tau and beta-amyloid. Brains received ratings of Braak stage (Braak and Braak, 1997), CERAD (Mirra, 1997) and NIA-RI (The National Institute on Aging, 1997) for Alzheimer's tangle and plaque pathologies.

The 13 ET cases banked prior to 2003 had been banked either at the New York Brain Bank (Columbia University Medical Center) or the Royal University Hospital (Saskatchewan, Canada), the Honolulu-Asia Aging Study, the University of Kansas Brain Bank, the Veterans Administration West Los Angeles Healthcare Center or Sunnybrook Health Sciences Centre (Toronto, Canada). In 12 of these 13 ET cases, the ET diagnosis had been assigned during life by the treating physician, and in 10 of these 12, this was a neurologist specializing in movement disorders. ETCBR neurologists obtained clinical records (office records from treating internists and neurologists) to acquire data about tremor type, distribution, severity, and duration, coexistence of other disorders and medications. Using these records, an ETCBR neurologist (blinded to autopsy data) who specialized in movement disorders (E.D.L.) confirmed their ET diagnoses after death using ETCBR diagnostic criteria, which were modelled after the criteria for ET proposed in the Consensus Statement of the Movement Disorder Society (Deuschl *et al.*, 1998). ETCBR criteria were as follows: (i) bilateral action tremor of the arms for 5 or more years with a diagnosis of ET during life, (ii) either head tremor or action tremor of at least one arm that was moderate or severe

(i.e. arm tremor resulted in difficulty with two or more activities of daily living or required medication) and (iii) action tremor was not the result of other movement disorders (e.g. dystonia, Parkinsonism, ataxic disorders), hyperthyroidism, other medical conditions or medications (Louis *et al.*, 2006b). One of the two ET cases from the Honolulu-Asia Aging Study had a clinical examination that included the motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn and Elton, 1987) and had a moderately severe action tremor; this individual fulfilled all clinical diagnostic criteria for ET, despite not having received this diagnosis during life. One of these 13 cases had ET for 65 years and then developed an additional clinical diagnosis of Parkinson's disease (PD) in the last 11 years of life. The remaining 12 cases had not received any additional neurological diagnoses (including Parkinsonism or ataxic disorders) during life.

The 20 prospectively collected ET cases were recruited through International Essential Tremor Foundation (IETF) newsletters beginning in 2003. IETF members who were interested in brain donation responded to quarterly advertisements about the brain bank in the IETF newsletter. Each of the 20 ET cases had been diagnosed with ET by their treating neurologist. To re-confirm these diagnoses, IETF members interested in brain donation were mailed standardized clinical questionnaires, which included questions on demographics, tremor characteristics, medications (including any that might cause cerebellar toxicity), ethanol intake, family history information and screening for PD and spinocerebellar ataxia. Based on written instructions, each ET case prepared a 30-min videotaped neurological examination. The videotaped examination included: (i) a full demonstration of tremor [assessments of postural arm tremor, rest tremor (while seated and while walking), kinetic tremor (while pouring, using a spoon, drinking, drawing spirals and performing finger-nose-finger maneuver with each hand), head tremor, chin tremor and voice tremor], (ii) an assessment of parkinsonism with items from the motor portion of the UPDRS (Fahn and Elton, 1987) [facial expression and blink frequency, speech, rest tremor evaluated while seated and while walking, rapid alternating movements (opening and closing each hand, pronating-supinating each hand and foot taps), rising from a chair, standing with arms at sides, and walking for 60 s] and (iii) an assessment of cerebellar signs with items taken from the International Cooperative Ataxia Rating Scale (Trouillas *et al.*, 1997) (as noted earlier: speech, three types of rapid alternating movements, finger-nose-finger maneuver, drawing spirals with each hand, sitting, stance and gait). With these questionnaires and the videotaped materials, each donor's diagnosis of ET was confirmed, using the ETCBR diagnostic criteria, by a brain bank neurologist who specialized in movement disorders and who was blinded to autopsy data (E.D.L.).

Nineteen of 21 control brains were obtained through the New York Brain Bank (Columbia University Medical Center). These were individuals who during life had been enrolled as normal elderly controls in the Alzheimer's Disease Research Center or the Washington Heights Inwood Columbia Aging Project, and during prospective serial neurological assessments, remained free of a diagnosis of AD, ET or PD during life, and did not have a neuropathological diagnosis (e.g. AD, PD) on autopsy evaluation. These 19 controls were selected for this study based on their age (i.e. they were frequency matched to ET cases based on age). One-to-one matching (i.e. 33:33) was not possible due to the relatively limited numbers of available control brains.

Later, two additional age-matched normal elderly controls were obtained from the Honolulu-Asia Aging Study to serve as controls for the two Asian ET cases from that study. During prospective serial neurological assessments, these had remained free of a diagnosis of AD, ET or PD during life and neither had a neuropathological diagnosis (e.g. AD, PD) on autopsy evaluation.

In one supplementary analysis, we included six additional control brains. These were not used for primary analyses because of their young age ( $57.3 \pm 10.0$  years).

Demographic and clinical information (from clinical records, treating physicians, family members and also directly from the patient in the 20 prospectively collected cases) were collected on all ET cases and controls. Heavy ethanol use was defined as consumption of an average of four or more standard drinks (15 ml of absolute ethanol) per day for a man, or three or more per day for a woman, at any point in their lives (Harasymiw and Bean, 2001). Lifetime exposure to medications that can produce cerebellar damage (e.g. lithium, diphenylhydantoin and others) was assessed. Family history of ET was defined as the presence of at least one first-degree relative with reported ET.

## Tissue processing

The cerebellum was one of our pre-designated primary regions of interest because in several clinical and neuroimaging studies, it has been implicated in the pathogenesis of ET (Will *et al.*, 1994; Deuschl *et al.*, 2000; Louis *et al.*, 2002) and our prior results indicated changes in this brain region in ET (Louis *et al.*, 2006a, b). In addition, pigmented brainstem nuclei (dorsal vagal nucleus, locus ceruleus and substantia nigra pars compacta) were of particular interest given our prior results (Louis *et al.*, 2005b, 2006b). Additional (secondary) regions of interest included the red nucleus, thalamus, inferior olivary nucleus, caudate, putamen, globus pallidum and motor cortex. Whenever possible, 17 standardized blocks were harvested from each brain and processed, and  $7 \mu\text{m}$  thick,  $20 \times 25 \text{ mm}^2$  paraffin sections were stained with Luxol fast blue/haematoxylin and eosin (LH&E) (Vonsattel *et al.*, 1995). In addition, selected sections were stained by the Bielschowsky method, and with mouse monoclonal antibodies to human glial fibrillary acidic protein (GFAP, clone GA5, Novocastra, Newcastle upon Tyne, UK), alpha-synuclein (clone KM51, Novocastra), phosphorylated tau (clone AT8, Research Diagnostics, Flanders, NJ) and beta-amyloid (clone 6F/3D, Dako, Carpinteria, CA). All tissues were examined microscopically by a senior neuropathologist blinded to clinical information including age and diagnosis. Post-mortem interval (PMI), the number of hours between death and placement of the brain in a cold room or upon ice) and brain weight (grams) were recorded for each brain.

## Microscopic examination

### Lewy bodies and Lewy neurites

LH&E-stained sections and alpha-synuclein-stained sections were used to assess Lewy bodies (Louis *et al.*, 2006b). Lewy neurites were assessed with alpha-synuclein-stained sections. Whenever possible, sections included three levels of midbrain showing the substantia nigra pars compacta, two to four levels of the pons showing the locus ceruleus, the medulla with dorsal vagal nucleus, hippocampus, cingulate gyrus, temporal cortex, prefrontal cortex and motor cortex (Louis *et al.*, 2006b). A semi-quantitative scale was used: 0 (absent or not discernable), + (rare or slight),

++ (moderate), +++ (severe). Test re-test reliability using this method was reasonable (for Lewy bodies, weighted kappa statistic = 0.80; for Lewy neurites, weighted kappa statistic = 0.91) (Louis *et al.*, 2006b).

### Purkinje cells

Using a  $20 \times 25 \text{ mm}^2$  LH&E-stained para-sagittal section of the cerebellar hemisphere that included portions of the cerebellar cortex, white matter and dentate nucleus ('standard cerebellar section'), Purkinje cells in five  $100\times$  fields were counted (Louis *et al.*, 2006b) and then averaged. In choosing the five  $100\times$  fields, a blinded assistant: (i) selected non-adjacent fields representing different regions of the section and (ii) selected fields that were between but not inclusive of the base of the fissure and the apex of the folium. A Purkinje cell was counted only when at least a portion of the nucleus was visible.

### Torpedoes

Using the standard cerebellar section, torpedoes (fusiform swellings of Purkinje cell axon) in the entire section were counted. For primary analyses, torpedoes were assessed using LH&E-stained sections ( $100\times$ ). Bielschowsky-stained sections ( $100\times$ ) were also analysed for separate counts.

### Bergmann astrogliosis

Using a GFAP-stained standard cerebellar section, GFAP-positive cells in the Purkinje layer were counted in five  $400\times$  fields (selected randomly by a blinded assistant) and then averaged.

### Cell loss and gliosis

In primary and secondary regions of interest, LH&E-stained and GFAP-labelled sections were examined for cell loss [0–4 (near complete or complete regional loss of cells)] and gliosis [0–3 (diffuse and heavy burden of gliotic cells)] (Louis *et al.*, 2006b).

## Data analyses

Characteristics of study groups were compared using Student's *t*-tests and chi-square tests. In several analyses, number of torpedoes per section or mean number of Purkinje cells per  $100\times$  field was stratified into quartiles. To test for associations between continuous variables, Pearson's correlation coefficient, *r*, was used. Linear regression analysis was performed to assess the association between number of torpedoes per section (dependent variable) and diagnosis (independent variable). This analysis was repeated with number of Purkinje cells per  $100\times$  field as the dependent variable. In adjusted linear regression analyses, we controlled for the effects of possible confounders (age, gender, race, PMI, brain weight, Braak stage and CERAD plaque score).

## Results

### Cases and controls

ET cases ( $N=33$ ) and controls ( $N=21$ ) were similar in age. The proportion of women was non-significantly higher in ET cases than controls (Table 1).

**Table 1** Clinical characteristics and pathological changes in ET cases and controls

	All ET (N = 33)	ET		Controls (N = 21)
		ET cases without Lewy bodies (N = 25)	ET cases with Lewy bodies (N = 8)	
<b>Clinical variables</b>				
Age in years	84.2 ± 7.6	82.9 ± 8.0	88.5 ± 4.3*	80.1 ± 9.5
Female gender	19 (57.6%)	15 (60.0%)	4 (50.0%)	8 (38.1%)
<b>Race</b>				
White	30 (90.9%)	24 (96.0%)	6 (75.0%)	14 (66.7%)
Hispanic	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (9.5%)
African-American	1 (3.0%)	1 (4.0%)	0 (0.0%)	2 (9.5%)
Other	2 (6.1%)	0 (0.0%)	2 (25.0%)	3 (14.3%)
Heavy ethanol use <sup>a</sup>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Exposure to cerebellar-toxic medication <sup>b</sup>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Autopsy variables</b>				
PMI in hours <sup>c</sup>	6.2 ± 3.3	5.7 ± 3.3	7.8 ± 3.3	7.3 ± 6.9
Brain weight in grams	1231 ± 137	1245 ± 137	1198 ± 144	1251 ± 120
<b>Microscopic neuropathology</b>				
Torpedoes (LH&E) <sup>d</sup>	10.5 ± 8.0***	12.6 ± 7.9***	3.9 ± 4.3	1.7 ± 1.4
Torpedoes (Bielschowsky) <sup>e</sup>	16.5 ± 14.2**	19.6 ± 14.5**	5.5 ± 4.8	3.3 ± 7.3
Purkinje cells (LH&E) <sup>f</sup>	7.2 ± 2.6**	6.6 ± 2.4**	9.2 ± 2.1	9.6 ± 3.4
Bergman glia <sup>g</sup>	10.9 ± 8.1	9.6 ± 6.6	14.6 ± 11.0	10.6 ± 5.9
Cerebellar dentate pathology <sup>h</sup>	2 (6.1%)	2 (8.0%)	0 (0.0%)	0 (0.0%)
CERAD plaque score	0.6 ± 0.8	0.4 ± 0.5	0.9 ± 1.1	0.3 ± 0.5
Braak stage	1.9 ± 1.3***	2.0 ± 1.3**	1.8 ± 1.3	0.8 ± 1.0
NIA-RIA Alzheimer's disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Values are mean ± SD or counts with percentages.

<sup>a</sup>Heavy ethanol use was defined as consumption of an average of four or more standard drinks (15 ml of absolute ethanol) per day for a man, or three or more per day for a woman, at any point in their lives (Harasymiw and Bean, 2001). <sup>b</sup>Lifetime exposure to medications that can produce cerebellar damage (e.g., lithium, diphenylhydantoin). <sup>c</sup>PMI = the number of hours between death and placement of the brain in a cold room or upon ice. <sup>d</sup>Using a 20 × 25 mm<sup>2</sup> LH&E-stained cerebellar section that included portions of the cerebellar cortex, white matter, and dentate nucleus ('standard section'), torpedoes in the entire section were counted. <sup>e</sup>Using the 'standard section' stained with Bielschowsky, torpedoes in the entire section were counted. <sup>f</sup>Purkinje cells in five randomly-selected 100× LH&E stained fields of the standard cerebellar section were counted. The average of these five counts is reported as the mean number of Purkinje cells per 100× field. <sup>g</sup>Using a GFAP-stained "standard section", the GFAP-positive cells in the Purkinje layer were counted in five random 400× fields. The average number of Bergmann cells was the value we used in our analyses. <sup>h</sup>Neuronal loss, microglial clusters and reduction in efferent fibres.

\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 when compared with controls.

### Neurodegenerative changes (Braak stage, CERAD plaque scores and Lewy bodies)

Mean CERAD plaque scores and mean Braak stages are shown (Table 1); patients with ET had slightly higher mean Braak stage than controls. Higher Braak stages, however, were uncommon (stages V/VI = 0 cases, 0 controls; stage IV = 4 cases, 1 control). Similarly, higher CERAD plaque scores were uncommon (C = 1 case and 0 controls, B = 1 case and 0 controls). None of the cases or controls met NIA-RI pathological criteria for AD (CERAD plaque score C and Braak stage V).

All ET brains had either brainstem Lewy bodies or cerebellar pathological changes. Eight (24.2%) of 33 ET brains had brainstem Lewy bodies, although with one exception (see later), none had a clinical diagnosis of PD. These eight brains included three prospectively collected and five collected before 2003. The remaining 25 (75.8%) of 33 ET brains had no Lewy bodies but had pathological

changes in the cerebellum. These 25 included 17 prospectively collected and 8 collected before 2003.

The distribution of Lewy bodies and Lewy neurites is shown in the eight ET cases with Lewy bodies (Table 2). Duration of ET in these cases ranged from 5 to 65 years (median = 19.5 years) and last examination before death ranged from 2 weeks to 2 years (median = 4 months); all except two were last examined within 6 months of death. In six cases, Lewy bodies were abundant in the locus ceruleus and either absent or infrequent in other brainstem structures. These six cases did not have Lewy bodies or Lewy neurites in other brain regions (hippocampus, cingulate gyrus, temporal cortex, prefrontal cortex and motor cortex). Two additional ET cases had more widespread brainstem Lewy bodies as well as Lewy bodies in other brain regions and they were assigned pathological diagnoses of PD. As they were included in our earlier report (Louis *et al.*, 2006b), they were also included in this



Four of 25 ET cases without Lewy bodies were in the lower two quartiles; three of these had large numbers of torpedoes on Bielschowsky-stained sections and the fourth had reduced number of Purkinje cells.

In our sample of 33 ET cases and 21 controls, number of torpedoes per LH&E-stained section was not correlated with age ( $r=0.04$ ,  $P=0.78$ ), PMI ( $r=-0.12$ ,  $P=0.46$ ), CERAD plaque score ( $r=0.02$ ,  $P=0.92$ ), Braak stage ( $r=0.02$ ,  $P=0.89$ ) or brain weight ( $r=0.03$ ,  $P=0.83$ ) and it did not differ by race. The number of torpedoes per LH&E-stained section differed marginally between men and women ( $5.3 \pm 7.0$  versus  $8.8 \pm 8.0$ ,  $t=1.69$ ,  $P=0.10$ ), with this difference reflecting the slightly higher proportion of women among ET cases and, in an analysis restricted to the 25 ET cases without Lewy bodies, the number of torpedoes per LH&E-stained section was similar in men and women ( $P=0.35$ ). In separate analyses for the 33 cases and 21 controls, the number of torpedoes per LH&E-stained section was not correlated with age [ $r=-0.20$ ,  $P=0.28$  (cases) and  $r=0.19$ ,  $P=0.40$  (controls)].

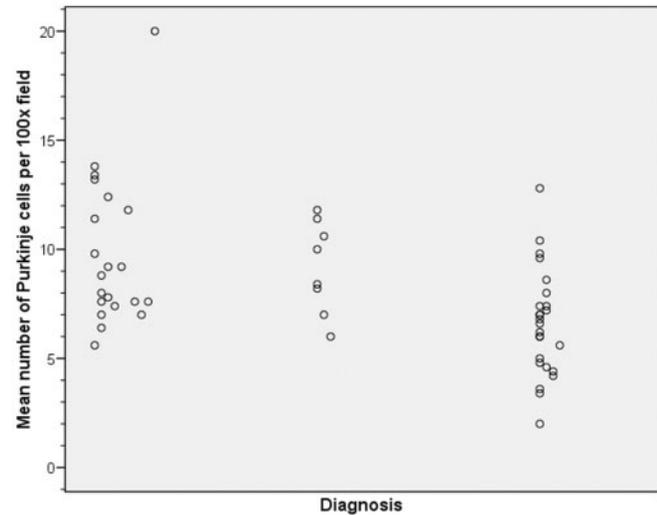
In an unadjusted linear regression analysis that compared ET cases without Lewy bodies ( $N=25$ ) versus controls ( $N=21$ ), the number of torpedoes per LH&E-stained section (dependent variable) was higher in ET cases without Lewy bodies than in controls ( $\beta=10.85$ ,  $P<0.001$ ). After adjusting in the same model for age, gender, race, PMI, brain weight, Braak stage and CERAD plaque score, the association remained robust ( $\beta=6.73$ ,  $P=0.002$ ).

In the six supplemental control brains, the number of torpedoes per LH&E-stained section was low ( $2.7 \pm 2.1$ , range = 0–6).

### Purkinje cell numbers

The mean number of Purkinje cells per 100× field was reduced by 25.0% in ET cases compared to controls, with this reduction being driven by the 31.3% mean reduction in ET cases without Lewy bodies (Table 1, Fig. 2). The case versus control difference persisted in analyses in which we: (i) excluded the two cases with more widespread brainstem Lewy bodies, (ii) stratified ET cases into those who were prospectively collected versus those collected prior to 2003, and (iii) stratified by gender (data not shown). The mean number of Purkinje cells per 100× field was also stratified into quartiles. Purkinje cell quartile differed by diagnosis ( $\chi^2=15.27$ ,  $P=0.02$ ); ET cases without Lewy bodies were five times more likely than controls to be in the lowest two quartiles than the highest two quartiles (OR = 5.2, 95% CI = 1.4–18.4,  $P=0.01$ ).

In our sample of 33 ET cases and 21 controls, higher number of torpedoes on LH&E was associated with lower number of Purkinje cells ( $r=-0.33$ ,  $P=0.016$ ). Mean number of Purkinje cells per 100× field was not associated with age ( $r=-0.17$ ,  $P=0.22$ ), PMI ( $r=0.23$ ,  $P=0.16$ ), Braak stage ( $r=-0.21$ ,  $P=0.14$ ), CERAD plaque score ( $r=-0.07$ ,



**Fig. 2** Mean number of Purkinje cells per 100× LH&E-stained field. Each brain is represented by a single data point. The mean number of Purkinje cells was reduced in ET cases without Lewy bodies (right) compared with controls (left). ET cases with Lewy bodies (middle) and controls (left) had a similar number of Purkinje cells.

$P=0.64$ ), brain weight ( $r=0.007$ ,  $P=0.96$ ), gender ( $8.5 \pm 3.5$  in men versus  $7.9 \pm 2.9$  in women,  $t=0.67$ ,  $P=0.51$ ) or race.

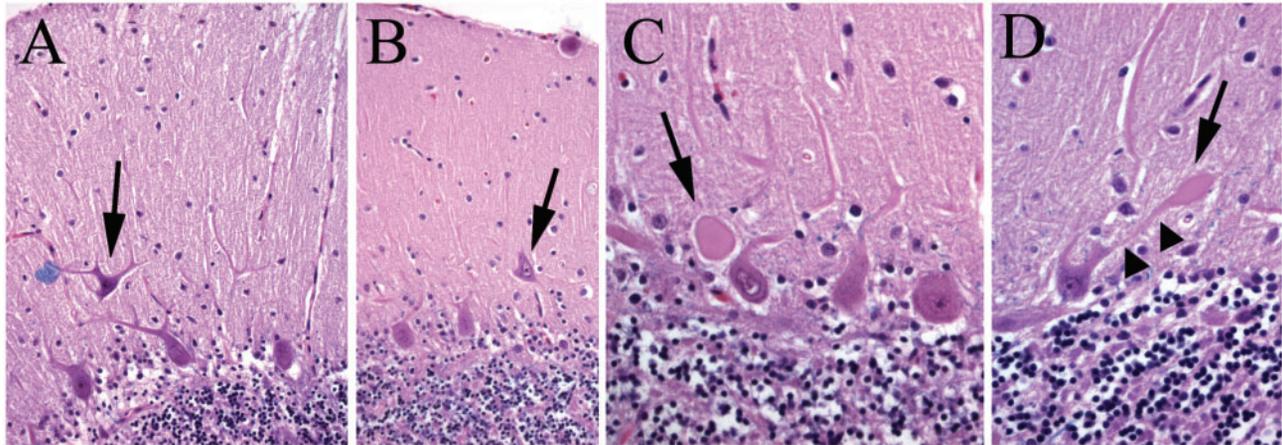
In an unadjusted linear regression analysis that compared ET cases without Lewy bodies ( $N=25$ ) versus controls ( $N=21$ ), the mean number of Purkinje cells per 100× field was lower in ET cases without Lewy bodies than in controls ( $\beta=3.19$ ,  $P=0.001$ ). After adjusting for age, gender, race, PMI, brain weight, Braak stage and CERAD plaque score, this remained significant ( $\beta=3.12$ ,  $P=0.005$ ).

### Other cerebellar changes

The mean number of Bergman glia per 400× field was similar in ET cases and controls (Table 1). Several other pathological changes were observed in ET cases without Lewy bodies. These included Purkinje cell heterotopias (cell bodies of Purkinje cells displaced upward into the molecular layer) and dendrite swellings (Fig. 3A–D). Tissue in each of the secondary regions of interest (red nucleus, thalamus, inferior olivary nucleus, caudate, putamen, globus pallidum and motor cortex) was examined and cell loss or gliosis were not evident.

### Clinical–pathological correlations

ET cases without Lewy bodies were compared to ET cases with Lewy bodies. The number of Lewy body cases was small ( $N=8$ ), which limited the power of these secondary analyses. Lewy body ET cases were, on average, 5.6 years older than ET cases without Lewy bodies (Table 3). Several other non-significant trends were observed in ET cases



**Fig. 3** Purkinje cell heterotopias and dendrite swellings in cerebellum from an ET case. LH&E-stained cerebellar cortical sections. In **A** and **B**, the cell body of an occasional Purkinje cell is displaced upward into the molecular layer (arrows) (200× magnification). In **C** and **D**, there are glassy, eosinophilic swellings in the molecular layer adjacent to Purkinje cell bodies (arrows). Connection to a Purkinje cell dendrite (arrowheads in **D**) clearly identifies this as a dendrite swelling (400× magnification).

without Lewy bodies: younger age of tremor onset, higher proportion with a family history of ET and higher proportion with gait difficulty (Table 3).

Within ET cases without Lewy bodies, there was no correlation between number of torpedoes per LH&E-stained section and age ( $r = -0.07$ ,  $P = 0.75$ ), gender (women =  $13.8 \pm 7.5$ , men =  $10.7 \pm 8.5$ ,  $t = 0.96$ ,  $P = 0.35$ ), presence of rest tremor (present =  $11.5 \pm 3.5$ , absent =  $14.2 \pm 7.5$ ,  $t = 0.47$ ,  $P = 0.66$ ) or family history of ET (present =  $9.3 \pm 6.8$ , absent =  $11.0 \pm 6.2$ ,  $t = 0.57$ ,  $P = 0.58$ ). In ET cases without Lewy bodies, higher number of torpedoes per LH&E-stained section, however, was associated with older age of tremor onset ( $r = 0.45$ ,  $P = 0.06$ ). Higher number of torpedoes was marginally associated with head tremor (head tremor present =  $11.4 \pm 6.5$ , head tremor absent =  $7.0 \pm 4.9$ ,  $t = 1.57$ ,  $P = 0.14$ ). Similar analyses did not find any correlations between mean number of Purkinje cells per 100× field and these clinical variables.

## Discussion

The view that ET is a disease with no identifiable brain pathology does not hold up to rigorous studies that use immunohistochemistry, quantitative assessments of cerebellar changes and control brains for comparison. In this and previous reports (Louis *et al.*, 2005b, 2006a, b), there are identifiable structural changes in the ET brain, with three-quarters of the cases exhibiting cerebellar degenerative changes and the remaining having brainstem Lewy bodies.

This and our previous work (Louis *et al.*, 2005b, 2006a, b) raise the possibility that there may be two different patterns of pathology: patients with degenerative changes in the cerebellum and patients with brainstem Lewy bodies and relatively preserved cerebellum. Two other reports,

**Table 3** Clinical-pathological correlations

	ET without Lewy bodies (N = 25)	ET with Lewy bodies (N = 8)
Age in years	82.9 ± 8.0*	88.5 ± 4.3
Female gender	15 (60.0%)	4 (50.0%)
Age of tremor onset in years	49.2 ± 22.4	58.1 ± 23.9
Tremor duration in years	33.9 ± 19.7	28.1 ± 24.0
Family history of ET	11/19 (57.9%)	3 (37.5%)
Head tremor	9/17 (52.9%)	5 (62.5%)
Rest tremor	3/17 (17.7%)	2 (25.0%)
Difficulty signing their name	16/17 (94.1%)	6/7 (85.7%)
Gait difficulty	10 (40.0%)	1 (12.5%)

Values are mean ± SD or counts with percentages.

For several variables, clinical data were incomplete.

Difficulty signing their name was by self-report.

Gait difficulty was defined as repeated unexplained falls or the need to use a cane or walker.

\* $P < 0.05$ .

each published in preliminary form, show similar results. Ross *et al.* (2004) compared 11 presumptive ET brains to 11 control brains and they noted that brainstem Lewy bodies and pale bodies were more common in ET cases than in controls and that ET cases had more changes in the cerebellum. Recently, in a series of 26 ET brains (no controls) (Shill *et al.*, 2007), pathological changes were heterogeneous, with the largest number of patients exhibiting degenerative changes in the cerebellum (including Purkinje cell loss), while others exhibited brainstem Lewy bodies and depletion of neurons in the locus ceruleus. In another uncontrolled series, two of the cases also exhibited mild Purkinje cell loss (Rajput *et al.*, 2004). In none of these series, however, was Purkinje cell loss or number of torpedoes systematically quantified.

The possibility that there is pathological heterogeneity in ET is consistent with the evidence of clinical heterogeneity (Jankovic, 2002). First, tremor is not limited to action tremor; rest tremor and intention tremor may occur in some patients (Deuschl *et al.*, 2000; Cohen *et al.*, 2003; Leegwater-Kim *et al.*, 2006). Second, changes in tandem gait and balance may occur in ET patients, in whom the gait has been described as ataxic (Singer *et al.*, 1994; Stolze *et al.*, 2001). Third, a variety of non-motor features have been observed (Findley, 2004; Louis *et al.*, 2005c), including specific personality traits (Chatterjee *et al.*, 2004; Lorenz *et al.*, 2006), anxiety (Tan *et al.*, 2005) and social phobia (Schneier *et al.*, 2001). Cognitive changes were documented in six studies (Gasparini *et al.*, 2001; Lombardi *et al.*, 2001; Vermilion *et al.*, 2001; Duane and Vermilion, 2002; Lacritz *et al.*, 2002; Benito *et al.*, 2006b) and dementia, in two (Benito-León *et al.*, 2006a; Bermejo-Pareja *et al.*, in press). Finally, response to pharmacotherapeutic agents is inconsistent across patients, with some benefiting while others do not (Zesiewicz *et al.*, 2005).

The ET patients without Lewy bodies had a marked increase in the number of torpedoes. On electron microscopy, these fusiform swellings consist of massive accumulations of disoriented neurofilaments, displacing normal neuronal structures (Lampert, 1967; Mizushima, 1976). Torpedoes occur in degenerating Purkinje cells; they also may be a feature of Purkinje cell recovery in response to injury (Blackwood, 1977; Kato and Hirano, 1985). They have been described in disease processes characterized by degeneration of cerebellar tissue, including cerebellar ataxias (Takahashi *et al.*, 1992), cerebellar damage from mercury toxicity (Hunter and Russell, 1954), and paraneoplastic cerebellar ataxia (Yaginuma *et al.*, 2000). In addition to the increased number of torpedoes in our ET cases without Lewy bodies, there seems to be a reduction in the number of Purkinje cells, indicating neuronal death. Two cases, one of which was reported previously (Louis *et al.*, 2006a), also demonstrated marked changes in the dentate nucleus (with neuronal loss, microglial clusters and reduction in efferent fibres). We have observed several other pathological changes in these ET cases, including the presence of Purkinje cell heterotopias and dendrite swellings. Purkinje cell heterotopias in the molecular layer have occasionally been reported in human cerebellar degenerative disorders (Nakamura *et al.*, 1999; Yang *et al.*, 2000; van Rootselaar *et al.*, 2004), most frequently in spinocerebellar atrophy type 6. The mechanism of the heterotopia and its relevance to neuronal degeneration remain to be elucidated. Whether more subtle pathological changes occur in the ET cerebellum is not yet clear and more detailed structural studies of Purkinje cell dendritic arborization are needed. That approximately three of four recently studied cases have mild cerebellar degenerative pathology is consistent with the abundance of clinical and neuroimaging evidence linking cerebellar dysfunction with ET (Dupuis *et al.*, 1989; Hallett and Dubinsky, 1993; Jenkins *et al.*, 1993;

Will *et al.*, 1994; Bucher *et al.*, 1997; Deuschl *et al.*, 2000; Koster *et al.*, 2002; Louis *et al.*, 2002; Helmchen *et al.*, 2003; Pagan *et al.*, 2003; Leegwater-Kim *et al.*, 2006).

A smaller proportion of ET cases had Lewy bodies in their locus ceruleus. The mechanism whereby this results in action tremor is not understood. The locus ceruleus is the principal source of central nervous system norepinephrine. Among its main efferent connections are those to Purkinje cells (Wang *et al.*, 1999). It has been suggested that these noradrenergic locus ceruleus-cerebellar connections are important for the normal function of Purkinje cells and their inhibitory output (Hoffer *et al.*, 1973; Moises and Woodward, 1980; Moises *et al.*, 1981). These connections may modulate, for example, Purkinje cell responses to climbing fibre afferent input (Moises *et al.*, 1981). Thus, while a well-known peripheral effect of catecholamines is the exacerbation of tremor, it is possible that catecholamines act on the central nervous system to suppress tremor.

The numbers of locus ceruleus Lewy bodies observed in ET is far in excess of that reported in normal ageing (Tomonaga, 1981; Lindboe and Hansen, 1998; Jellinger, 2003, 2004) and, indeed, far in excess of that observed in any of our 21 age-matched controls or six supplemental controls. Moreover, the pattern of Lewy bodies we have observed has not been described in individuals thought to have incipient PD, in whom Lewy body pathology in the dorsal vagal nucleus precedes and is often more marked than that found in the locus ceruleus (Braak *et al.*, 2003; Saito *et al.*, 2004). Hence, one cannot easily ascribe these findings to normal ageing or pre-clinical PD.

The spectrum of Lewy body disease includes entities other than PD (Hansen *et al.*, 1990; Perry *et al.*, 1990; Jackson *et al.*, 1994; Louis *et al.*, 1997; Heyman and Fillenbaum, 1999), such as diffuse Lewy body disease and AD with Lewy bodies, both of which are associated with dementia. With the description of Lewy bodies in the locus ceruleus in ET cases, Lewy body diseases would now also seem to include some cases of ET. A possible association between ET and PD has been discussed in the literature for some time (Geraghty *et al.*, 1985; Pahwa and Koller, 1993; Louis *et al.*, 2003b; Yahr *et al.*, 2003; Chaudhuri *et al.*, 2005; Ondo and Lai, 2005; Shahed and Jankovic, 2007). The development of PD has been described among ET patients, as has the co-occurrence of the two disorders in the same patient (Yahr *et al.*, 2003; Chaudhuri *et al.*, 2005). It is quite likely that the ET patients with the Lewy bodies are at increased risk of developing PD-ET, although this remains to be demonstrated (Louis *et al.*, in press).

In our ET cases with Lewy bodies, the main feature is the Lewy body, a microscopic abnormality associated with degenerative diseases like PD and diffuse Lewy body disease. The changes observed both in ET cases with and without Lewy bodies seem to be consistent with a degenerative process. Further support for this notion comes from epidemiological studies, which demonstrate that the incidence and prevalence of ET are associated with

age (Rajput *et al.*, 1984; Benito-León *et al.*, 2003; Dogu *et al.*, 2003) and the clinical observation that the disorder is often progressive (Critchley, 1949; Louis *et al.*, 2003a).

While CERAD scores were similar in cases and controls, patients with ET had a slightly higher mean Braak stage than controls. Braak stages IV, V and VI, however, were uncommon. Whether this greater burden of mild tangle pathology was indicative of an emerging dementia is not known. Of interest are the studies reporting greater cognitive impairment and an association between ET and dementia, with the majority of the demented cases having clinical diagnoses of AD (Benito-León *et al.*, 2006a, b; Bermejo-Pareja *et al.*, in press).

In a set of secondary analyses, we examined whether ET cases with Lewy bodies differed from their counterparts without Lewy bodies. The Lewy body cases were older and several additional trends were noted, although these will need to be assessed in future studies.

This study had limitations. First, approximately one-third of the brains we examined had been collected prior to 2003 and clinical information on these was less complete than in the two-thirds of brains that were collected prospectively. In particular, end of life clinical findings may have emerged yet not have been appreciated in some of these cases. Second, a stereological assessment of Purkinje cell number would have allowed for greater precision as well as a randomized assessment. Such a study is currently underway. This study, however, had several important strengths. This represents the largest series of ET brains reported to date. Most of the brains were collected prospectively. Importantly, we compared our ET brains to control brains, which has only been done once before in a preliminary study (Ross *et al.*, 2004). Brains were prepared in a standardized manner with detailed quantification of torpedoes and Purkinje cells. Furthermore, we routinely used immunohistochemistry to assess for Lewy bodies.

In summary, the pathological changes of ET seem to be heterogeneous and degenerative. Most cases have changes in the cerebellum; a smaller proportion has brainstem Lewy bodies. The clinical differences between cases with versus without Lewy bodies require additional study.

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