Clinical/Scientific Notes

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CLIMBING FIBER SYNAPTIC CHANGES CORRELATE WITH CLINICAL FEATURES IN ESSENTIAL TREMOR

Changes in the Purkinje cells (PCs) and climbing fibers (CFs) have been postulated to be involved in essential tremor (ET) disease pathogenesis.[1] PCs receive 2 excitatory inputs: CFs and parallel fibers (PFs). CFs form synapses predominantly on the thick, proximal PC dendrites, whereas PFs form synapses on the thin, distal PC dendritic branchlets. CF-PC and PF-PC innervation territories on PC dendritic arbors are tightly regulated for proper PC function. We recently reported more CF-PC synapses on the thin, distal PC dendritic branchlets in ET cases than controls,[2] and this pathologic feature was associated with tremor severity in a small sample of 8 ET cases. We now expand the ET case sample nearly fivefold (37 cases) and assess the association between abnormal CF-PC connections and a wider range of clinical features. The overarching goal was to begin to mark out clinical characteristics that track with pathologic features in ET.

Methods. Thirty-seven ET brains were obtained from the New York Brain Bank. During life, severity of action tremor in the arms and hands was rated (total tremor scores [TTS] [range 0–36]) and the presence of rest tremor and head and voice tremors was noted.[3] All cases consented to brain donation and pathology studies. We performed dual immunofluorescence of vesicular glutamate transporter type 2 (VGlut2) and calbindin to visualize CF-PC synapses in 7-μm-thick paraffin cerebellar cortical sections. We used random digits to choose PC dendritic trees for image acquisition. Different from our previous study using random field selections of a given PC dendritic arbor,[2] we acquired serial images (Leica TSC SP2 microscope) to reconstruct the dendritic arbors from the PC layer to the pial surface (figure, A). In Image J (NIH, imagej.nih.gov), we calculated the percentage of CF-PC synapses on PC dendrites <1 μm thickness (%CFPC1). Therefore, we quantified the average %CFPC1 in 5 PCs in each ET case. A priori, the primary clinical variable of interest was the TTS. We further assessed additional clinical variables of interest: sex, age at tremor onset, age at death, presence of voice tremor, head tremor, and rest tremor. For these additional comparisons, we set the significance level at 0.0083 (i.e., 0.05/6) after Bonferroni adjustment.

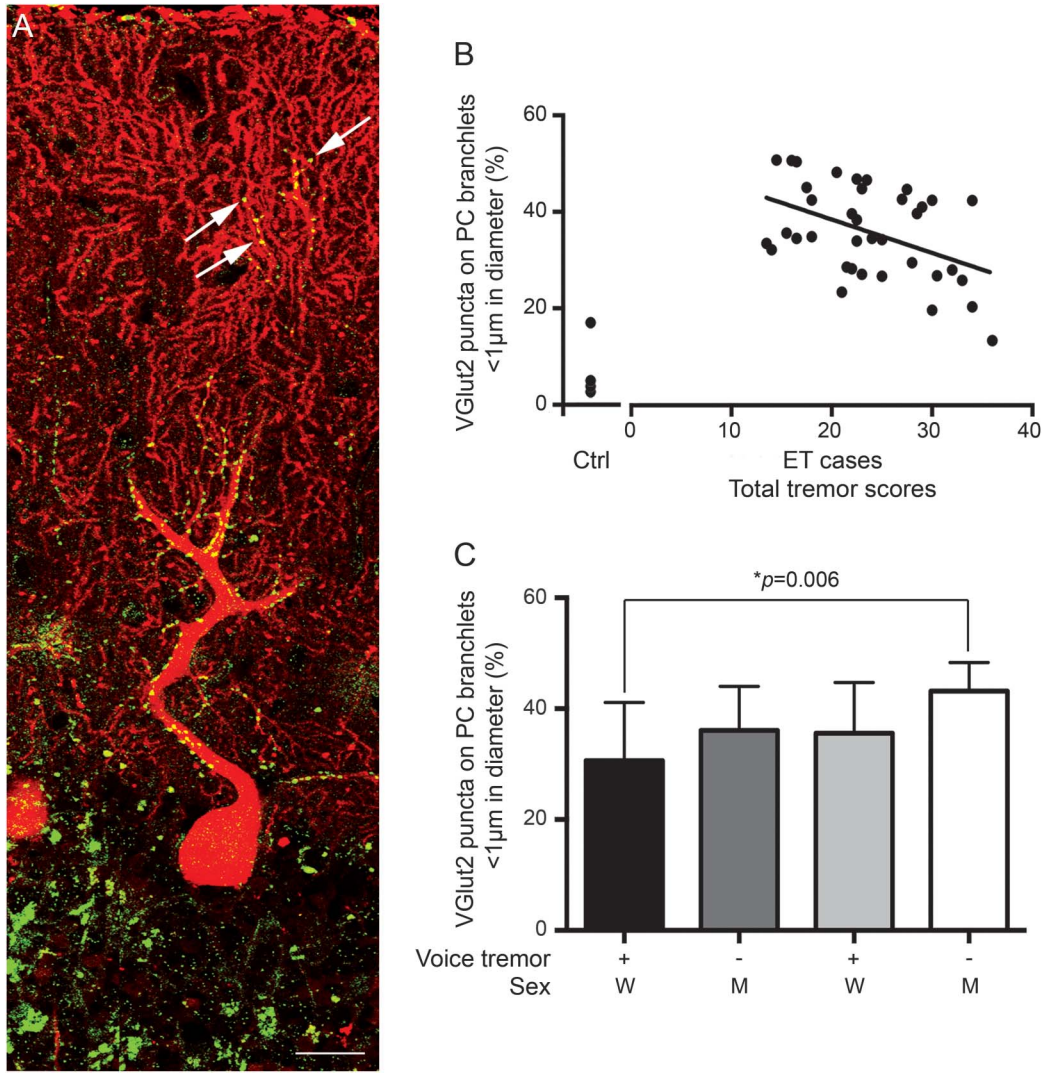
Results. There were 37 ET cases (25 women) with mean ± SD age at onset 42.1 ± 22.6 years, age at death 86.8 ± 7.1 years, and TTS 23.7 ± 6.3. Of these, 58.3% had head tremor and 38.9% had voice tremor. There was a robust, inverse correlation between TTS and %CFPC1 ($r = -0.45$, $p = 0.005$) (figure, B). The %CFPC1 was not related to the age at tremor onset ($r = -0.04$, $p = 0.79$) or age at death ($r = 0.02$, $p = 0.90$). The %CFPC1 was lower in ET cases with vs without voice tremor (31.8 ± 10.0% vs 38.3 ± 8.7%, $p = 0.045$) and was lower in women than men (33.4 ± 9.9%, vs 41.4 ± 6.2%, $p = 0.02$), although the differences were not statistically significant after Bonferroni adjustment. The greatest difference was observed between women with voice tremor vs men without voice tremor (30.6 ± 10.5% vs 43.2 ± 5.1%, $p = 0.006$) (figure, C). %CFPC1 was not associated with presence of head tremor or rest tremor ($p = 0.34$ and 0.13, respectively).

Discussion. With an expanded sample size, we showed a robust association between CF-PC synaptic pathology and tremor severity in ET cases, and uncovered additional sources of clinical-pathologic heterogeneity.

In mild ET, we observed increased CF-PC synaptic connections on the thin PC dendrites, which should have been receiving inputs from PFs. The abnormal CF-PC connections could lead to disturbed PC physiology, contributing to abnormal oscillation networks in ET. Alternatively, the abnormal CF-PC synaptic connections could represent a compensatory change, resulting from longstanding rhythmic firing of CFs onto PCs. In ET cases, a loss of PC dendritic arborization and spines has been observed.[4] With disease progression, it is possible that distal pruning of PC dendritic spines could lead to decreased distal distribution of CF-PC synapses, thereby increasing cerebellar dysfunction in ET. These possibilities deserve further exploration.

Both sex and voice tremor seemed to track with the extent of abnormal CF-PC connections in ET.

Figure Clinicopathologic correlations of climbing fiber–Purkinje cell synaptic pathology and tremor



(A) Dual immunofluorescence with anti-VGlut2 (Alexa 488, green) and anti-calbindin D_{28k} antibody (Alexa 594, red) of a cerebellar section in an essential tremor (ET) case. Each Purkinje cell (PC) dendritic arbor was imaged, from the PC layers to pial surface, and reconstructed in Image J. VGlut2 puncta followed the climbing fibers and were distributed over proximal, thick PC dendrites and occasionally VGlut2 puncta localized over the distal, thin PC branchlets (arrows). Scale bar: 25 μm. (B) The percentage of VGlut2 puncta on PC branchlets <1 μm inversely correlated with the total tremor scores in ET cases. We also included data on 4 controls, collected previously.[2] (C) Men without voice tremor had the highest percentage of VGlut2 puncta on PC branchlets <1 μm whereas women with voice tremor had the lowest percentage of VGlut2 puncta on PC branchlets <1 μm. M = men; W = women.

Prior work in ET has shown that both sex and cranial tremors seem to be associated with certain clinical differences.[5,6] The present study highlights the notion that ET may represent not one but several clinical-pathologic entities, and future studies to link specific clinical features with identifiable pathologic characteristics are critically important.

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SEVERE TK2 ENZYME ACTIVITY DEFICIENCY IN PATIENTS WITH MILD FORMS OF MYOPATHY

Thymidine kinase 2 (TK2) is a mitochondrial enzyme participating in the salvage of deoxyribonucleotides needed for mitochondrial DNA (mtDNA) replication. TK2 catalyzes the first and rate-limiting step of the deoxypyrimidine salvage pathway. Mutations in *TK2* were typically associated with a severe myopathic form of mtDNA depletion syndrome (MDS) characterized by a dramatic decrease in mtDNA copy number in muscle that manifests during infancy and leads to the early death of most patients.[1] Recently, several patients have been diagnosed with a late-onset or slow-progressing form of the disease manifesting as a milder myopathy with mtDNA multiple deletions.[2–5] Here we describe 7 adult cases presenting with a mild myopathy compatible with a relatively normal life for decades and associated with multiple mtDNA deletions and no marked depletion in skeletal muscle. TK2 activity was drastically reduced in cultured fibroblasts of 2 of these patients, suggesting that redundant or complementary biochemical mechanisms could bypass the defect in some individuals, in contrast with severely affected infantile patients.

Results. We report 7 patients (P1–P7) diagnosed in their adulthood, between the ages of 16 (P6) and 55 (P2), with different forms of mitochondrial myopathy associated with multiple mtDNA deletions. Phenotypic presentation varied from mild myopathic signatures, such as ptosis and myalgia, to progressive marked weakness and respiratory dysfunction (see table 1 for more information on patients in our study and table e-1 on the *Neurology*® Web site at Neurology.org for a review of previously described cases of TK2 mild myopathies). Histochemical or biochemical evidence of mitochondrial dysfunction is summarized in table 1 and figure e-1 (see also e-Methods). Long-range PCR analysis revealed multiple mtDNA deletions in muscle from all subjects (figure e-2). After genetic analysis, previously reported pathogenic mutations in *TK2* were identified in all patients (table 1).[2] Analysis of muscle mtDNA did not reveal drastic reductions of mtDNA copy number (table 1), in contrast to what is observed in typical infantile patients with *TK2* mutations.[1]

We measured TK2 activity in fibroblasts from 2 of the patients and found severe reductions in both (3% and 6% residual activity, as compared with age-matched healthy controls). Similar reductions were observed in fibroblasts from pediatric patients with severe myopathic MDS that were analyzed in parallel; however, one of these patients conserved 40% of activity (figure e-2).

Discussion. Next-generation sequencing has revealed TK2 deficiency as a prevalent defect leading not only to pediatric forms of severe myopathy but also to milder forms of the disease with later onset or slower progression. The number of milder myopathy cases recently diagnosed (7 previously reported plus 7 described here) suggests that pathogenic mutations in *TK2* could have been largely missed in the past. Therefore, *TK2* mutations should be investigated in patients with myopathy associated with either mtDNA depletion or multiple deletions, independently of age at onset. Importantly, p.K202del and p.T108M mutations seem particularly frequent in the Spanish population.

Severe pediatric myopathic forms are associated with a dramatic reduction in mtDNA levels. In milder cases, mtDNA deletions are the predominant molecular defects and mtDNA copy number is always above 30% of the normal value, appearing higher the later the age at onset and the milder the disease progression (table 1 and table e-1).

All patients in this study have *TK2* mutations reported earlier in typical pediatric cases of severe

Supplemental data at Neurology.org