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1. Hallett M. Tremor: pathophysiology. Parkinsonism Relat Disord 2014;20:S118–S122.
2. Lin CY, Louis ED, Faust PL, Koeppen AH, Vonsattel JP, Kuo SH. Abnormal climbing fibre-Purkinje cell synaptic connections in the essential tremor cerebellum. Brain 2014;137:3149–3159.
3. Louis ED, Ottman R, Clark LN. Clinical classification of borderline cases in the family study of essential tremor: an analysis of phenotypic features. Tremor Other Hyperkinet Mov 2014;4:220.
4. Louis ED, Lee M, Babij R, et al. Reduced Purkinje cell dendritic arborization and loss of dendritic spines in essential tremor. Brain 2014;137:3142–3148.
5. Louis ED, Rios E, Rao AK. Tandem gait performance in essential tremor: clinical correlates and association with midline tremors. Mov Disord 2010;25:1633–1638.
6. Louis ED. When do essential tremor patients develop head tremor? Influences of age and duration and evidence of a biological clock. Neuroepidemiology 2013;41:110–115.

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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

Table 1 Genetic and phenotypic description of mild myopathy TK2-deficient patients in the present study

Patient Id[a]	Mutation[b]	mtDNA levels[c]	Main clinical features	Histochemical findings[d]	Respiratory chain activities[e]	Onset[f]; progression	TK2 activity[g]
P1	p.K202del; p.T108M	166%	Myalgias, exertional rhabdomyolysis, congenital cardiopathy, gastric lymphoma, brain venous thrombosis	10% COX− and 2% RRF, nuclear internalization, necrotic fibers	Normal	NA	NA
P2	p.K202del	61%	Ptosis, dysphonia, dysphagia, limb-girdle muscle weakness	11% COX− and 10% RRF, atrophy of type 2 fibers, necrotic fibers	CIII deficiency	Adulthood (30 y)	NA
P3	p.K202del	80%	Ptosis without ophthalmoplegia, bilateral and peripheral facial paresis	Abundant COX−, RRF, isolated atrophic fibers	CI deficiency; increased CS	Adulthood (36 y)	3%
P4	p.K202del	183%	Limb-girdle muscular weakness, dysphagia and dysarthria, severe ptosis, mild ophthalmoplegia, facial diplegia	Few COX−, RRF	NA	Adulthood (50 y)	6%
P5	p.T108M	39%	Dysphagia, muscle weakness, respiratory dysfunction	40% COX−, RRFs, nuclear internalization, necrotic fibers, dispersed rimmed vacuoles, endomysial fibrosis	Normal; increased CS	Adulthood (21 y)	NA
P6	p.T108M	57%	Exercise difficulties, muscle weakness, myopathy, pelvic and scapular limb-girdle atrophy	50% of COX−, abundant RRF, occasional small angulated fibers, nuclear internalization, sparse inflammatory infiltrates	Normal	Infancy (4 y); slow	NA
P7	p.R192K; p.T108M	42%	Myalgias, exertional rhabdomyolysis, muscle weakness, hypertrophic myocardiopathy, myopathy	80% of COX−, RRF, nuclear internalization, necrotic fibers	NA	Adolescence (13 y); slow	NA

Abbreviations: COX = cytochrome *c* oxidase; CS = citrate synthase; mtDNA = mitochondrial DNA; NA = information not available; RRF = ragged red fibers; TK2 = thymidine kinase 2.
[a] Patient identification.
[b] Mutation according to RefSeq NP_004605.4.
[c] Residual levels of mtDNA in muscle biopsies as quantified by real-time PCR. Results are shown as percentage of control samples.
[d] Histochemical findings on muscle biopsies.
[e] Respiratory chain complexes activity on muscle biopsies.
[f] Onset of the clinical manifestations is indicated in years when available.
[g] TK2 activity expressed as percentage of control samples.

myopathy.[6,7] This is also the case for some previously reported mild myopathy cases.[2] Importantly, a significant level of residual TK2 activity had been documented in some of these patients (table e-1). Here, we have measured TK2 activity in fibroblasts from severely affected and mild myopathic patients, in some cases sharing the same mutation (figure e-2 and e-Methods). We observed no correlation between residual TK2 activity and the age at onset or severity. For example, adult patients P3, P4, and previously reported Pb (table 1 and table e-1) showed reduced TK2 activities, at the level of that observed for severely affected infantile patients (P8, P9, and P11, e-Methods), while P10 (another infant with typical MDS, e-Methods) had only a partial reduction. Although additional studies would be needed to determine the effect of specific mutations on different substrate kinetics, our observations indicate that different individuals may tolerate TK2 deficiency to a different extent, and suggest that interindividual variations in other metabolic steps involved in mtDNA replication may account for these differences. However, excluding the potential contribution of other factors will require larger investigations. Similarly, this marked clinical variability is observed in patients with mutations in *DGUOK* and *RRM2B*, also involved in dNTP metabolism. Genetic variations affecting the interconnected networks responsible for dNTP homeostasis and mtDNA replication should thus be a target for further research.

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1. Chanprasert S, Wang J, Weng SW, et al. Molecular and clinical characterization of the myopathic form of mitochondrial DNA depletion syndrome caused by mutations in the thymidine kinase (TK2) gene. Mol Genet Metab 2013;110:153–161.
2. Alston CL, Schaefer AM, Raman P, et al. Late-onset respiratory failure due to TK2 mutations causing multiple mtDNA deletions. Neurology 2013;81:2051–2053.
3. Behin A, Jardel C, Claeys KG, et al. Adult cases of mitochondrial DNA depletion due to TK2 defect: an expanding spectrum. Neurology 2012;78:644–648.
4. Paradas C, Gutierrez Rios P, Rivas E, Carbonell P, Hirano M, Dimauro S. TK2 mutation presenting as indolent myopathy. Neurology 2013;80:504–506.
5. Tyynismaa H, Sun R, Ahola-Erkkila S, et al. Thymidine kinase 2 mutations in autosomal recessive progressive external ophthalmoplegia with multiple mitochondrial DNA deletions. Hum Mol Genet 2012;21:66–75.
6. Mancuso M, Filosto M, Bonilla E, et al. Mitochondrial myopathy of childhood associated with mitochondrial DNA depletion and a homozygous mutation (T77M) in the TK2 gene. Arch Neurol 2003;60:1007–1009.
7. Vila MR, Segovia-Silvestre T, Gamez J, et al. Reversion of mtDNA depletion in a patient with TK2 deficiency. Neurology 2003;60:1203–1205.