Clinical/Scientific Notes

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LATE-ONSET RESPIRATORY FAILURE DUE TO *TK2* MUTATIONS CAUSING MULTIPLE mtDNA DELETIONS

Mutations in nuclear genes involved in the maintenance of mitochondrial DNA (mtDNA) are associated with an extensive spectrum of clinical phenotypes, manifesting as either mtDNA depletion syndromes or multiple mtDNA deletion disorders.[1]

Mutations in thymidine kinase 2 (*TK2*; GenBank accession number NM_004614.4) have been reported to cause an early fatal myopathic mtDNA depletion syndrome,[2] culminating in respiratory failure with or without encephalopathy,[3] adult-onset indolent myopathy,[4] or progressive external ophthalmoplegia (PEO) associated with multiple mtDNA deletions.[1] We describe a 74-year-old woman who presented with ptosis, a limb-girdle muscular dystrophy–like phenotype, and progressive respiratory failure due to recessive *TK2* mutations and multiple mtDNA deletions in muscle.

Methods. ***Case report.*** A 74-year-old woman with sensorineural hearing loss (SNHL) presented with progressive muscle weakness and rapidly progressive respiratory failure, decompensated following iatrogenic oxygen administration. She improved clinically with withdrawal of high-dose oxygen therapy and was subsequently discharged for domiciliary ventilation. She had presented 2 years previously for surgical correction of ptosis but had no other medical history of note. Her parents lived to old age and she has an 82-year-old clinically unaffected brother.

On examination, she had mild ptosis, subtle PEO, facial weakness, marked wasting of proximal musculature, including sternocleidomastoids, and scapular winging, and neck flexion and extension were weak (figure, A). Hip flexion (Medical Research Council [MRC] grade 2) and dorsiflexion were weak (MRC grade 4) with relative preservation of hip extensors, knee extension, and plantar flexion. Tendon reflexes were intact and she was not ataxic. Standard histopathologic analysis of a muscle biopsy was performed. DNA was extracted from muscle homogenate and individual muscle fibers and subjected to quantitative and qualitative mtDNA analysis. Candidate mtDNA maintenance disorder genes (*POLG*, *POLG2*, *SLC25A4*, *PEO1*, and *RRM2B*) were excluded, prompting screening of the entire coding region of the *TK2* gene. Sequence variants were cross-referenced against dbSNP (build 135) and variants of unknown pathologic significance investigated using in silico methodologies. Total RNA was derived from patient fibroblasts and reverse transcribed to cDNA using a *TK2*-specific primer (details available on request). Allele-specific primers incorporating and exploiting 2 in cis single nucleotide polymorphisms prompted allele dropout to establish phase. To investigate the pathogenicity of the novel p.Lys194Asn variant, site-directed mutagenesis was employed to engineer the mutation in the human TK2 protein, and express this and wild-type human TK2 in *Escherichia coli*. Purification of the protein and determination of enzyme activity was performed as previously described[5] (see appendix e-1 on the *Neurology*® Web site at www.neurology.org).

Results. Muscle biopsy analysis revealed evidence of fiber diameter variation and internal nuclei and fat replacement, in addition to 20% COX-deficient fibers and subsarcolemmal mitochondrial accumulation (figure, B). Long-range PCR revealed multiple mtDNA deletions, while mtDNA copy number assessment showed increased (185% of control values) mtDNA levels. Individual COX-deficient and COX-positive muscle fibers confirmed comparable mtDNA copy number (figure, D) but clonally expanded *MTND4* gene deletions in the majority of COX-deficient muscle fibers (figure, E), diagnostic of a multiple mtDNA deletion disorder.[6]

Sequencing of the *TK2* gene revealed 2 novel heterozygous variants: a nonsense mutation (c.103C>T, predicting p.Gln35*) and a missense variant (c.582 G>T, predicting p.Lys194Asn). Analysis of fibroblast-derived patient cDNA confirmed recessive inheritance of the 2 novel *TK2* variants (figure, C) while we were not able to detect either of these changes in the patient's clinically unaffected brother.

Direct measurement of recombinant p.Lys194Asn TK2 activity confirmed a marked reduction in the V_{max} relative to the wild-type sample while the K_m of the p.Lys194Asn enzyme did not differ from that of the wild-type enzyme (figure, F).

Discussion. We report a very late presentation of respiratory failure in a 74-year-old woman with *TK2* gene mutations associated with late-onset myopathy and minimal PEO. To date, 54 patients

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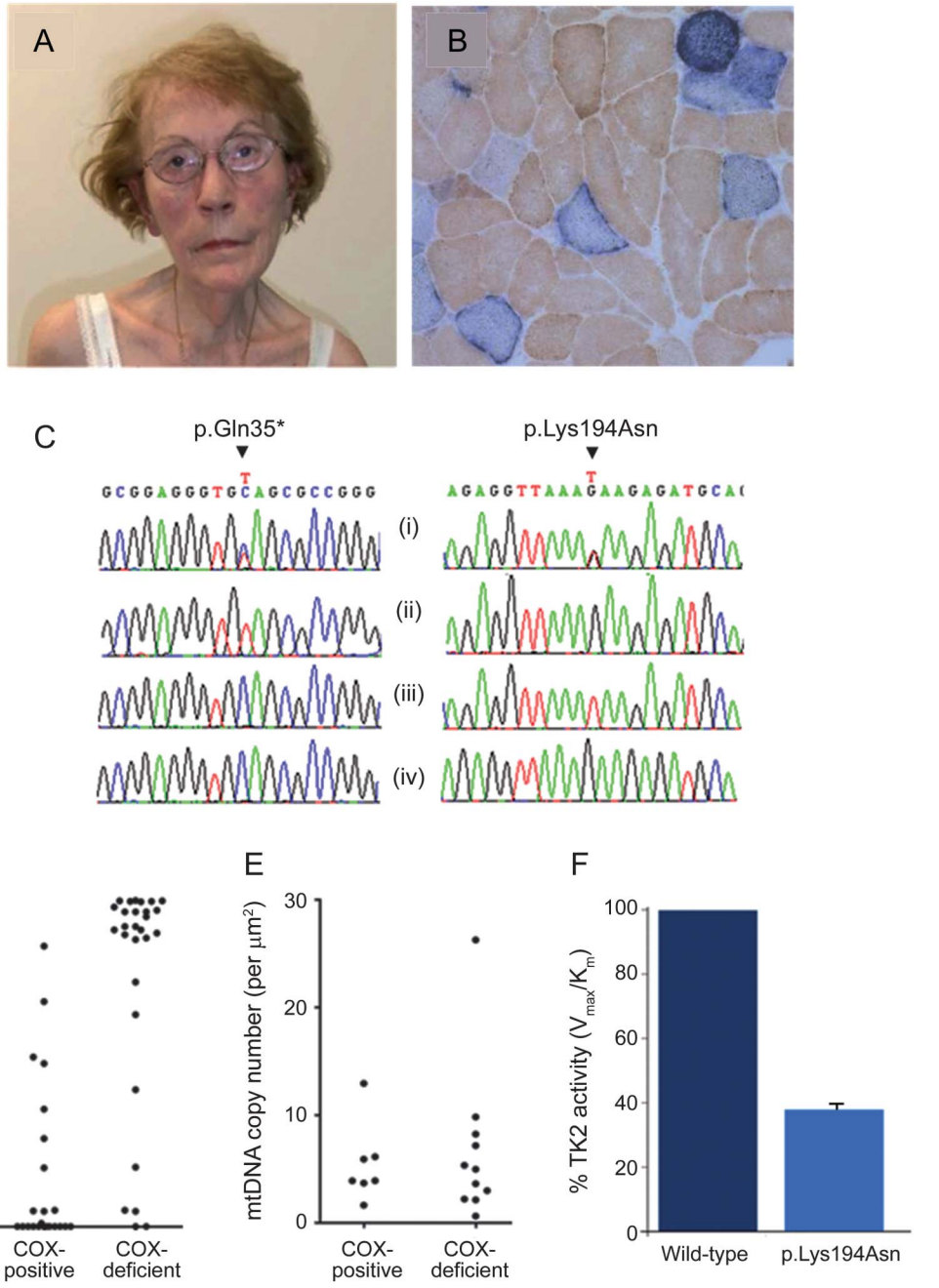
Figure Clinical and molecular genetic characterization



(A) Clinical presentation includes mild ptosis despite previous corrective surgery, subtle progressive external ophthalmoplegia, and facial weakness. The patient has marked wasting of proximal musculature, including the sternocleidomastoids. Neck flexion and extension were weak, resulting in head drop. (B) Sequential COX-SDH histochemistry demonstrates focal COX deficiency affecting a number of fibers. (C) [i] gDNA *TK2* sequencing reveals 2 novel heterozygous variants—c.103C>T (p.Gln35*) and c.582G>T (p.Lys194Asn); [ii and iii] allele-specific cDNA analysis confirms both variants are allelic; [iv] wild-type reference sequence. (D) Assessment of individual COX-positive and COX-deficient fibers reveals high levels of clonally expanded *MTND4* deletion in the majority of COX-deficient fibers, while only 2 COX-positive fibers have mitochondrial DNA (mtDNA) deletions quantified at levels of >60% mutation load. This profile in individual fibers is consistent with a diagnosis of multiple mtDNA deletions. (E) Assessment of mtDNA copy number in individual COX-positive and COX-deficient fibers shows no evidence of quantitative mtDNA copy number loss, as comparable mtDNA levels are noted in both groups of fibers. (F) Biochemical assessment of TK2 activity in the *Escherichia coli* model reveals a marked decrease in activity due to the p.Lys194Asn substitution (38 ± 2% of controls; n = 4), confirming pathogenicity.

have been described in the literature, presenting with either mtDNA depletion or multiple mtDNA deletion disorders due to recessive *TK2* mutations (table e-1). SNHL has been described and facial weakness,[1,4,7] ptosis, and eye movement abnormalities are not infrequent.[1,7] Overt respiratory muscle weakness or failure is almost universally reported.

The p.Gln35* nonsense mutation is assumed to be subject to nonsense-mediated decay of the corresponding mRNA. Pathogenicity of the p.Lys194Asn mutation is supported by functional evidence from the recombinant mutant enzyme that shows the same affinity for its substrate but a clear reduction in V_{max}, confirming a decreased efficacy of the mutant TK2 protein. The observed elevation in muscle mtDNA copy number suggests a compensatory mechanism that could account for the late onset of our patient's clinical symptoms, particularly given 1 mutation represents a null allele.

The demonstration of recessive *TK2* mutations highlights that mtDNA maintenance disorders should be considered in cases of late-onset myopathy where respiratory failure is prominent, irrespective of age.

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