A Novel NOTCH3 Frameshift Deletion and Mitochondrial Abnormalities in a Patient With CADASIL

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Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which leads to strokes and dementia, is caused by single missense mutations or, in a few cases, small deletions in the NOTCH3 gene. These mutations result in a gain or a loss of 1 (or, rarely, 3) cysteine residue in 1 of 34 epidermal growth factor–like repeats in the extracellular amino-terminal region of NOTCH3.

Objective: To describe a patient with a novel NOTCH3 mutation in whom clinical and laboratory findings of mitochondrial abnormalities were associated with a diagnosis of CADASIL.

Patient: A patient with a history of migraines, repeated transient ischemic attacks, and generalized fatigue underwent muscle biopsy, brain magnetic resonance spectroscopic imaging, and screening of mitochondrial DNA and NOTCH3.

Results: Molecular genetic analysis showed a NOTCH3 mutation (the first documented frameshift deletion in a patient with CADASIL) in exon 4. Although the screening of mitochondrial DNA did not show mitochondrial mutations, findings from muscle biopsy and brain magnetic resonance spectroscopic imaging showed signs of mitochondrial impairment (ultrastructural mitochondrial abnormalities and increased parenchymal brain lactate, respectively).

Conclusions: A patient with CADASIL and a 5–base pair deletion leading to a frameshift mutation showed clinical and laboratory evidence of mitochondrial dysfunction. This adds to the previously reported hypothesis of a pathogenetic role of NOTCH3 or, less specifically, a microvascular pathologic effect on mitochondrial energy metabolism.

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult-onset, hereditary microvascular angiopathy leading to recurrent strokes, psychiatric changes, and premature dementia.1 In most (>95%) patients with CADASIL, the genetic defect is a missense point mutation in the extracellular domain of the NOTCH3 gene.2 These mutations result in a gain or a loss of 1 (or, rarely, 3) cysteine residue in 1 of 34 epidermal growth factor–like repeats in the extracellular amino-terminal region of NOTCH3. Small deletions causing loss of cysteine residues also have been reported.3,4 In these few patients, clinical manifestations and laboratory findings were similar to those of patients carrying point mutations.

Herein, we describe (1) a patient with CADASIL carrying a novel NOTCH3 deletion in exon 4 and (2) clinical and laboratory features suggesting an impairment of brain and muscle mitochondrial metabolism.

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graine. At age 48, she began to complain of severe fatigue. At that time, neurological examination showed mild left hemiparesis and fatigue after mild exertion, but no cognitive impairment (Mini-Mental State Examination score, 29 of 30).

Blood chemistry (including creatine kinase, lactic acid, pyruvic acid, and procoagulative factors), cerebrospinal fluid factors, electromyography, and evoked potentials showed no abnormalities. The findings from brain magnetic resonance imaging showed diffuse abnormal white matter (WM) signal in both hemispheres. The volume of interest for spectroscopy is shown in the transverse MRI. Proton MRI spectra from abnormal WM voxels (a-d) show decreases in the N-acetylaspartate–creatine ratio (NAA/Cr) and small increases in the resonance intensities of lactate (La). A spectrum of the brain WM of a healthy adult (e) is shown for comparison. In the latter, note the absence of La resonance intensity. Cho indicates choline.

Figure 1. Proton brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI) examination of a patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Conventional MRI shows diffuse abnormal white matter (WM) signal in both hemispheres. The volume of interest for spectroscopy is shown in the transverse MRI. Proton MRSI spectra from abnormal WM voxels (a-d) show decreases in the N-acetylaspartate–creatine ratio (NAA/Cr) and small increases in the resonance intensities of lactate (La). A spectrum of the brain WM of a healthy adult (e) is shown for comparison. In the latter, note the absence of La resonance intensity. Cho indicates choline.
and a small increase in the resonance intensities of lactate (which is, using this method, under detection limits in normal controls).

Findings from clinical and magnetic resonance spectroscopic imaging prompted us to perform muscle biopsy. Histologic examination results showed a slight variation in fiber size. With the oxidative reactions, there was increase of reactive granules in the subsarcolemma. Ultrastructural examination findings showed subsarcolemmal mitochondrial accumulation and evidence of abnormal mitochondria containing paracrystalline inclusions (Figure 2). Muscle respiratory chain enzyme activity was normal.

Total DNA was extracted from skeletal muscle, according to standard purification protocols, and investigated for common pathogenetic mitochondrial DNA (mtDNA) mutations and large-scale rearrangements by time-temperature gradient electrophoresis, denaturing gradient gel electrophoresis, and Southern blotting. These analyses failed to reveal any point mutations at transfer RNA Leu, Lys, Ser, and Ala or deletions.

Four years and 6 years later, the patient was reexamined for new transient ischemic attacks. The clinical picture and results of brain magnetic resonance imaging were unchanged.

Genomic DNA was isolated from whole blood according to standard procedures. Polymerase chain reaction was performed with primers specific for exons 3 and 4 of NOTCH3. The polymerase chain reaction products were sequenced, and a heterozygous 5–base pair (bp) deletion was identified in exon 4, leading to a frameshift and nonsense stretch of amino acids (Figure 3). Absence of the deletion in 6 clinically unaffected members of the family (and in 316 independent control chromosomes) was confirmed by sequencing exon 4.

Herein, we describe a patient with CADASIL and a novel 5-bp deletion leading to a frameshift (from amino acid 127 to amino acid 158) mutation with premature termination of translation (stop codon at amino acid 159). Although NOTCH3 deletions have occasionally been reported, to our knowledge, this is the first patient described in whom CADASIL is associated with a frameshift deletion.

In this patient, the classic clinical features of the disease were associated with severe fatigue. This symptom and results of muscle biopsy (mitochondrial accumulation and paracrystalline inclusions) and proton magnetic resonance spectroscopic imaging (increased brain lactate) suggest that mitochondrial dysfunction is relevant in this patient.

Muscle mitochondrial abnormalities have been reported in 3 independent families with CADASIL carrying different NOTCH3 point mutations. In 1 patient, a mtDNA mutation was also found.7 In our patient, the ultrastructural mitochondrial abnormalities were not associated with evidence of mitochondrial enzyme dysfunction or mtDNA mutation, indispensable conditions for a diagnosis of primary mitochondrial disorder.

Small increases in brain parenchymal lactate were seen in some patients with CADASIL in a previous study.9 Lactate is the end product of glycolysis and accumulates when oxidative metabolism is unable to meet energy requirements. However, lactate may also accumulate in the extracellular environment of necrotic tissue and where there is inflammation. Increases in lactate resonance intensities might therefore be due to impaired lactate removal after accumulation of foamy macrophages in pathologically active areas. We are unable to determine whether there is a metabolic defect that leads to lac-
tate accumulation or whether it is due to defective removal. However, because the slight increase in brain lactate was found in the absence of any clinically significant stroke or transient ischemic attack (often necessary to justify spectroscopic increases in lactate due to macrophage entrapment), it may be interpreted as due to a microvascular pathologic condition leading to local hypoperfusion and secondary mitochondrial damage.

Therefore, although our findings do not support a diagnosis of primary mitochondrial defect, a pathogenetic relationship between the NOTCH3 mutation and mitochondrial dysfunction has been hypothesized by analogy to that observed in Drosophila melanogaster, in which NOTCH3 lethal mutations impair respiratory chain enzyme activity. Alternatively, mitochondrial damage due to a microvascular pathologic condition leading to secondary impairment of energy metabolism should also be considered.

In conclusion, the evidence of a frameshift deletion of NOTCH3 broadens the mutational spectrum in CADASIL. Further studies are necessary to clarify whether this mutation can explain certain peculiar phenotypes of patients with CADASIL.

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