Clinical Pathologic Conference

Progressive Parkinsonism, Balance Difficulties, and Supranuclear Gaze Palsy

A 76-year-old man came to our service with a 4-year history of a progressive parkinsonian syndrome. The patient’s medical history was notable only for hypertension, which was reported to be pharmacologically controlled. Regarding family history, his father had died of a stroke when he was 56 years old and his sister had recurrent strokes in her 40s. His neurological symptoms started with slowness of gait, subtle apathy, and depression, with further symptom of memory difficulties. This slowly progressed and 3 years after symptom onset, he started having occasional unexplained falls. Therefore, he was admitted to another hospital, where clinical examination revealed moderate parkinsonism with facial hypomimia, shuffling gait, and both bradykinesia and rigidity of all limbs (right > left), along with a supranuclear upgaze palsy. There were no reported pyramidal or cerebellar signs. A diagnosis of possible progressive supranuclear palsy (PSP) was made, and he started treatment with levodopa, titrating to 400 mg daily, which proved unsuccessful. After 10 months, he was referred to our clinic for a second opinion.

Collateral history was taken from caregivers as the patient was considerably bradyphrenic and inaccurate with responses. His caregivers dated his symptoms to 4 years prior to presentation, when the patient began to slow down and have an unsteady gait. Given that the symptoms were slowly progressive, with only a mild impact on day-to-day functioning, he did not seek medical advice until he began falling 3 years after onset. Further symptoms developed including a mild dysphagia for solid foods and occasional urinary incontinence. On examination, he showed a severe parkinsonian syndrome featuring bradykinesia, rigidity (axial > appendicular), and positive pull-test finding. Moreover, there was an upgaze supranuclear palsy and slow saccades on vertical plane. Magnetic resonance imaging was performed that revealed significant basal ganglia lesions and white matter hyperintensities, including periventricular regions and both frontal and temporal subcortical areas, along with moderate widespread atrophy and ventricular enlargement. Here, we reveal the pathological diagnosis and discuss the approach to the clinical data.

Laboratory and Imaging Studies

Laboratory study findings showed a normal blood cell count. Serum electrolytes, renal and liver function, coagulation studies, lipid profile, and glucose level findings were within normal range. Both electrocardiogram and echocardiogram findings showed mild abnormalities related to his hypertension. Magnetic resonance imaging (MRI) of the brain revealed significant basal ganglia lesions and white matter hyperintensities, including periventricular regions and both frontal and temporal subcortical areas, along with moderate widespread atrophy and ventricular enlargement (Figure 1). Iodine I 123-radiolabeled fluoropropyl 2-carbomethoxy-3-(4-iodophenyl) tropane with single-photon emission computed tomography imaging disclosed normal nigrostriatal pathway.

Clinical Discussion

The salient clinical findings of this case were an akinetic-rigid parkinsonian syndrome along with supranuclear gaze palsy and neuropsychiatric symptoms of likely subcortical type (ie, bradyphrenia, impairment of executive function, recall abnormalities, depression, and apathy). Ocular movements in older healthy subjects may also show to some extent a limitation on vertical plane. However, it is never accompanied by abnormalities of vertical saccades, which instead are often seen in patients with true supranuclear gaze palsy.
nuclear palsy. Given the further evidence of gait and balance difficulties in our patient, these features fit well with a phenotypic label of PSP-like syndrome. When approaching a patient with a PSP-like syndrome, a broad differential diagnosis needs to be considered.

Sporadic PSP was first described in association with a tau pathology, and its clinical hallmarks include vertical gaze supranuclear palsy and balance difficulties with backward falls. However, a PSP-like phenotype has been less frequently described in association with other neurodegenerative conditions. The initial clinical features in our case were also compatible with parkinsonism associated with Lewy-body pathology, and the L-dopa dosage was too low to exclude dopaminergic responsiveness. Nevertheless, virtually all these conditions share a common pattern of iodine I 123–radiolabeled fluoropropyl 2-carbomethoxy-3-(4-iodophenyl) tropane with single-photon emission computed tomography imaging, which is characterized by degeneration of the nigrostriatal pathway, which was not the case here.

Normal pressure hydrocephalus (NPH) may present with a PSP-like phenotype, and it should be considered when unsteadiness of gait, urinary incontinence, and cognitive decline (ie, the classical clinical triad of NPH) occur in the same patient. However, it may be argued that clinical onset in our patient was not typical for NPH in that there was no clear precipitant and the progression was slow for NPH. Moreover, MRI almost universally provides striking clues for NPH, leading to the correct diagnosis. In our case, MRI showed widespread white matter changes that allowed us to narrow the differential diagnosis to adult-onset leukoencephalopathies.

A diagnosis of a PSP-parkinsonism due to Binswanger leukoaraiosis would have been a reasonable diagnosis at the time of the first neurological examination and MRI, although a higher vascular risk factor burden may have been expected. However, the family history of relatively young-onset cerebrovascular disease in 2 first-degree relatives cannot be ignored and raised the possibility of an autosomal dominant leukoencephalopathy presenting with a PSP-like clinical picture. Although there was no history of migraine in the patient or affected family members, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) needs to be excluded by genetic testing for mutations in the NOTCH3 gene. These patients sometimes have early temporal lobe involvement on MRI as occurred in our case. Cardinal clinical manifestations of CADASIL include migraine with aura, transient ischemic attacks or fixed focal neurologic deficits caused by brain infarction, and cognitive decline of subcortical type. However, uncommon presentations have been reported, and Van Gerpen and colleagues described a case of PSP secondary to CADASIL. Hereditary diffuse leukoencephalopathy with spheroids is also a possibil-

Figure 1. Magnetic Resonance Image Findings

Axial fluid-attenuated inversion recovery images show areas of encephalomalacia within the basal ganglia and cerebral white matter including periventricular regions and both frontal and temporal subcortical areas.
ity because it is characterized by memory decline, depression, executive dysfunction, pyramidal signs, and atypical parkinsonism. Genetic testing for a mutation of the colony stimulating factor 1 receptor gene is needed to confirm the diagnosis. Alexander disease was less likely here because most cases occur as de-novo mutations and to have 2 affected adult cases would be very rare. The most frequent symptoms in adult-onset Alexander disease are related to bulbar dysfunction (ie, dysarthria, dysphagia, and dysphonia) and pyramidal and cerebellar involvement, but it has rarely been reported to present as PSP. These patients may have an associated palatal tremor and exhibit striking atrophy of the medulla oblongata and cervical spine on MRI, even in the absence of white matter changes when onset is in adulthood. Definitive diagnosis hinges on the finding of a gliarial fibrillary acidic protein mutation on genetic testing. Another rare cause of adult-onset leukodystrophy is owing to a duplication of the lamina protein, Lamin B1.

**Neuropathology**

DNA was extracted from whole-blood samples using standard procedures. Blood and DNA samples were conserved and are available on request for further studies, according to the informed consent signed.

DNA from patients was amplified using primers, provided by Martin Dichgans, MD (Ludwig Maximilians University, Munich, Germany), designed to amplify the 2-23 exons of the NOTCH3 gene including the intron-exon boundaries. The polymerase chain reactions were performed using AmpliTaq Gold DNA polymerase (Applied Biosystems). Direct sequence was performed on an automated sequencing system (Applied Biosystems 3730 DNA Analyzer) using the BigDye Terminator Cycle Sequencing Kit Version 1.1 (Applied Biosystems). Sequencing of exons 3, 4, 6, and 8 was done first; screening of the 18 remaining exons encoding the epidermal growth factor (EGF) repeats was pursued until a mutation creating or deleting a cysteine residue was identified.

DNA analysis of the proband revealed the novel heterozygous NOTCH3 mutation c.307 C/T (Figure 2); the nucleotide position of mutation refers to the mRNA sequence (NM_000435). The variant is located in the EGF-like 2 region of exon 3 and causes the substitution of arginine with a stop codon at position 103 of the protein (p.R103X).

The formation of such premature stop codon results in the production of a truncated protein product lacking in part of exon 3 and all the subsequent exons (4/33); therefore, it is characterized by the absence of all EGF-like repeat domains except EGF-like 1.

The mutation was also found in the patient’s affected sister, whereas it was absent in another 3 unaffected relatives. It has not been found on screening 200 normal control chromosomes; moreover, it is not reported in different online genetic databases of control individuals such as the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), National Institutes of Health Heart, Lung and Blood Institute Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), and the 1000 Genome Project (http://www.1000genomes.org/).

**Summary**

The final diagnosis was CADASIL. Our case provided many interesting points to be noted. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy is an autosomal dominant hereditary cerebral small-vessel disease caused by mutations in the NOTCH3 gene. Clinical manifestations of CADASIL usually appear in early/middle adulthood, but late-onset cases (after age 60 years) have been reported. The CADASIL phenotype most commonly includes recurrent headache of migraine pattern, focal deficits secondary to brain infarction (more rarely bleeding), and, in later
stages, progressive neuropsychiatric disorders leading to a frank dementia. However, gait problems, urinary incontinence and pseudodobular symptoms and signs are also extremely frequent. Moreover, clinical heterogeneity has also been reported within the same families and this was also the case here. The patient’s father and sister had clinical features consistent with a classic presentation of CADASIL, while the patient presented with an almost pure parkinsonian syndrome resembling PSP. Interestingly, it has been recently unveiled that parkinsonism may be a common unrecognized manifestation of CADASIL. To our knowledge, to date, there has been only one report of a PSP-like phenotype in CADASIL. Thus, our case supported the evidence that parkinsonism may not be an uncommon feature of CADASIL. Therefore, we would suggest the genetic testing for mutations in the NOTCH3 gene in all cases of parkinsonism in which the MRI is consistent with a leukoencephalopathy. Neuroradiological abnormalities are often already detected at symptom onset and are almost universally present in symptomatic patients with CADASIL. Genetic testing for NOTCH3 should be pursued even in the absence of a clear family history. In fact, nearly 30% of patients with CADASIL apparently have a documented negative family history. This is likely related to a restricted search only for early cerebrovascular disease in the family, while CADASIL may also develop later in life and feature uncommon presentations including epileptic syndromes and complex and acute encephalopathies.

The NOTCH3 gene encodes a single-pass transmembrane protein, which is predominantly expressed in vascular smooth muscle cells. Mutations in NOTCH3 typically affect the extracellular domain within 1 of the 34 EGF-like repeat domains. Each EGF-like repeat domain contains a highly conserved number of 6 cysteine residues, which seem to stabilize the domain by the formation of disulfide bonds. Virtually all CADASIL mutations hitherto described result in an odd number of cysteine residues, thus leaving one residue unpaired and leading to a multimerization of the mutated protein with a gain of function effect on vascular smooth muscle cells. Still, the mechanisms and determinants of NOTCH3 multimerization are poorly understood. Intriguingly, certain naturally occurring mutations are predicted to result, by contrast, in a loss of functional NOTCH3 receptor. Monet-Leprêtre and colleagues demonstrated that a representative mutation lying in EGF-like repeat 10 (C428S, accounting for 4% of all CADASIL mutations) abrogates NOTCH3 function in vivo and antagonizes the function of the wild-type NOTCH3 allele. Furthermore, it has been shown that mutations leading to NOTCH3 overexpression dominantly suppress Notch signaling rather than increase it, and this is consistent with a dominant negative effect.

Conclusions

We reported here a new mutation in the NOTCH3 gene, which leads to a truncated protein lacking part of exon 3 and all the subsequent exons (4/33). It caused the loss of almost all of the 34 EGF-like repeats with all the cysteine residues they contain and is therefore likely to act through a dominant negative effect. We acknowledge that, being a new mutation, pathological confirmation through skin or brain biopsy would have been useful. Unfortunately, we do not have pathology in our case, but a number of pieces of evidence (ie, family history, MRI findings, and the segregation of the mutation with the disease) suggest that such a mutation is very likely to be pathogenic.

The intimate pathogenetic mechanisms of CADASIL have not yet been fully understood, and our case supports the evidence that Notch function inhibition in vascular smooth muscle cells can be functionally significant.