Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy Affecting an African American Man

Identification of a Novel 15–Base Pair NOTCH3 Duplication

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Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the best characterized genetic cause of vascular dementia and stroke and has been extensively reported in European and Asian populations.

Objective: To report the pathological and genetic analysis of CADASIL in an African American man with a 15–base pair NOTCH3 duplication.

Design: Case report.

Setting: University hospital.

Patient: A 78-year-old man with dementia, recurrent strokes, a family history of similar neurological disease, and white matter abnormalities seen on brain magnetic resonance imaging.

Main Outcome Measures: Brain pathology and genetic analysis of NOTCH3.

Results: The patient's brain showed widespread arteriopathy in large and small arteries. Using electron microscopy, granular osmiophilic material typical of CADASIL was identified abutting the plasma membrane of smooth muscle cells. Brain extracts contained elevated NOTCH3 protein levels. Sequencing of the NOTCH3 gene revealed a novel 15–base pair heterozygous duplication in exon 7, which is predicted to direct expression of a protein that contains 5 extra amino acids, including a cysteine residue.

Conclusions: To our knowledge, this is the first reported pathological and genetic analysis of an African American patient with CADASIL. The mutation in NOTCH3 is the longest duplication within this gene yet reported.

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the NOTCH3 gene that create or delete a cysteine residue. Most disease-related changes are point mutations; however, several uncommon mutations of CADASIL have been described that are predicted to cause small in-frame insertions, in-frame deletions, and frame-shift deletions. CADASIL has been described in multiple races and has been reported in many countries, with the vast majority of cases being from Europe and Asia. Although the disease is thought to occur in all racial groups, African American patients have yet to be described. In this article, we characterize an African American man with CADASIL and identify a novel 15–base pair duplication within the NOTCH3 gene.

The proband was an African American man who died at age 78 years. He was geographically separated from his family for most of his life. In his 60s, he returned to his family to live his last decade in a nursing home, after suffering cognitive difficulties that rendered him incapable of caring for himself. During the same period, he suffered multiple strokes. He did not have a history of migraine headaches. He was described by family members as having frequent uncontrolled laughing or crying. During the workup for one of his strokes, magnetic resonance imaging was performed that revealed significant white matter hyperintensities, including anterior temporal (Figure, A) and external capsule (Figure, B) involvement.

Several family members had recurrent strokes and cognitive impairment. The patient's mother had multiple strokes beginning in her 30s, but lived into her 80s and died after slowly progressive cognitive decline. A
brother experienced early-onset recurrent strokes and was diagnosed as having vascular dementia. He also had a pseudobulbar affect. After a 5-year course of ischemic strokes, he died in a nursing home at the age of 46 years. Another brother experienced a similar course, with recurrent strokes, dementia, and death at the age of 49 years. A sister experienced similar ischemic events and vascular dementia, and died at age 49 years. This sister was seen at the University of Michigan Hospital owing to failure to thrive. She had a Mini-Mental Status Examination score of 4/30 and was unable to recount major historical events or the name of the president; her examination was also notable for left-sided hemiparesis with hyperreflexia and an ataxic gait. The clinical diagnosis, at that time, was central nervous system vasculitis, and a brain biopsy was performed that showed a noninflammatory cerebral small vessel arteriopathy with extensive wall thickening and granular periodic acid–Schiff deposits in the media (Figure, C). The biopsy finding was highly suggestive of CADASIL, but genetic testing was not pursued. A family member suspected that the proband may also have had CADASIL and requested that we study his brain at autopsy.

Postmortem examination of the proband’s cerebral cortex revealed vascular abnormalities similar to those previously described in CADASIL, including striking arteriosclerosis of the meningeal vessels with frequent balloon cells in the degenerating media. Penetrating vessels of the gray and white matter showed marked thickening and loss of vascular media. Electron microscopic examination of small arteries showed granular osmiophilic material abutting smooth muscle cell membranes (Figure, D).

NOTCH3 protein levels have been reported to be increased in 3 European patients with CADASIL. Therefore, we examined NOTCH3 protein levels by Western blot analysis, which demonstrated increased expression and abnormal mobility in brain homogenates (Figure, E; proband’s sample is in lane 3 and contains a band of increased molecular weight). In an unrelated patient with CADASIL, the level of brain NOTCH3 was similarly increased, but the upper band was faint (Figure, E; lane 2); NOTCH3 levels were detectable but substantially lower in control subjects (Figure, E; lanes 1 and 4). The relative band intensities, normalized to tubulin, were 2-fold higher in CADASIL samples compared with controls (both bands were summed for the proband with CADASIL).

DNA sequence analysis of the proband was performed for all NOTCH3 ectodomain coding exons, using primers described by Smith et al. Exon 7 primers were sense: 5′-GGCTTTTGCGAGAGAGGA-3′ and antisense: 5′-CCCTCTCCTCCTGGGTTTC-3′. Three heterozygous small nucleotide polymorphisms that do not involve cys-
teine residues were identified: R680H, A1020P, and V1183M. Analysis of exon 7 DNA purified from agarose gels revealed partially superimposed sequences (Figure, F; top gel and top tracing). On analysis with high percentage polyacrylamide gels (Figure, F; bottom gel; lane 4), the amplification products from the patient migrated as a doublet, whereas wild-type DNA ran as a single band (Figure, F; bottom gel; lanes 2 and 3). Sequence analysis of individual bands revealed that this pattern was a result of the presence of 2 independent sequences (Figure, F; center and bottom tracings). One sequence represented a wild-type exon, while the second represented a novel duplication of 15 bp of sequence at coding position c.1057-1071 (c.1057_1071dup). This sequence is predicted to result in insertion of 5 amino acids at positions 353 to 357, including a cysteine (C355; Figure, G). Amplification of exon 7 from DNA extracted from a tissue block of the proband’s clinically affected sister also resulted in this novel doublet (Figure, F; bottom gel; lane 5).

To our knowledge, our investigation is the first pathological examination of an African American individual with CADASIL. Several features should be noted. First, the family includes individuals with fulminating courses of disease, who apparently were asymptomatic in their early 40s but suffered very rapid decline and died before the age of 50 years. On the other hand, the proband and his mother lived to advanced ages and appeared to have slow, indolent courses. This case underscores the broad spectrum of presentations of the disease, even within the same family, and serves as a reminder that CADASIL could remain unrecognized because of phenotypic variability.

Second, we report a novel mutation in a patient with CADASIL, which resulted in duplication of 5 amino acids within NOTCH3 and is predicted to encode an ectodomain with an odd number of cysteines. This mutation has not been reported in large sequencing studies of patients with CADASIL described in public databases (http://www.dbsnp.gov). Others have reported smaller insertions, but most cases of CADASIL are point mutations. The case expands the spectrum of potential mutations that lead to disease, while emphasizing that cysteine-affecting mutations play a key role in CADASIL pathogenesis. Three NOTCH3 polymorphisms identified in this proband do not involve cysteines and are thus unlikely that they are the cause of CADASIL in this patient. From a technical perspective, the case illustrates that caution must be taken in interpretation of sequencing data since small duplications like the one illustrated in Figure, F could be missed if insufficient overlap between sequences from opposite orientations of an exon is used to establish genotypy.

Third, to our knowledge, this is the first description of an African American patient with CADASIL, and supports the common observation that this disease can occur in all races. In some ethnic groups, specific mutations account for the predominance of CADASIL. Testing whether this duplication within NOTCH3 is unique to or, alternatively, characteristic of African American patients awaits identification of other African American families with CADASIL. Despite the novel features of this case, the radiological and pathological features associated with this patient strongly resemble those of European and Asian patients with CADASIL that have been examined before, including the characteristic pattern of white matter changes on magnetic resonance imaging, accumulation of NOTCH3 ectodomain in brain lysates (which heretofore has only been examined in a small number of patients in France), and the pathognomonic presence of granular osmiophilic material in the vasculature.

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