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

Identification of a Novel 15–Base Pair NOTCH3 Duplication

Soo Jung Lee, MS; He Meng, MD, PhD; Omar Elmadhoun; Mila Blaivas, MD, PhD; Michael Mei-Hwa Wang, MD, PhD

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.

Arch Neurol. 2011;68(12):1584-1586
African American patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Magnetic resonance imaging of the patient demonstrates severe white matter disease, involving the anterior temporal lobes (A) and the external capsule (B). Vessels from the patient’s clinically affected sister showed granular periodic acid–Schiff reactivity in degenerating arteries (C). Electron microscopic examination of the proband demonstrated degenerative changes of small dermal vessels that exhibited granular osmiophilic material (in the center of D) characteristic of patients with CADASIL. Frontal cortex homogenates from this patient were examined by Western blotting using a NOTCH3-specific monoclonal antibody (Abnova, M01) (E). NOTCH3 bands at greater than 250 and 210 kDa were detected in this patient (E; lane 3). We detected 50% NOTCH3 levels in normal control brain (E; lanes 1 and 4). Frontal cortex homogenates from a known patient with CADASIL having a C117Y NOTCH3 mutation showed a 210-kDa band (E; lane 2) that comigrated with the 210-kDa protein from this patient. F, Genetic analysis was performed on DNA extracted from the brain of the proband. Sequence of exon 7 DNA purified from an agarose gel (F; top gel) revealed overlapping sequences, suggesting 2 populations of DNA (F; top sequence; derived from DNA in lane 4). Indeed, polyacrylamide gel electrophoresis (F; bottom gel) resolved 2 bands amplified from exon 7 (Figure, F; bottom panel, lane 4) from the proband. Amplified wild-type exon 7 is shown for comparison (F; lanes 2 and 3). Lane 1 is a negative control polymerase chain reaction without DNA template. The patient’s clinically affected sister also exhibited the duplication within exon 7 (F, lane 5). Sequencing revealed a 15–base-pair duplication in the larger band (F, center sequence, antisense primer used). The lower band demonstrated wild-type sequence (F, bottom sequence). The predicted amino acid sequence of the upper band includes an insertion of 5 amino acids, including a cysteine (F).

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’-GCCTTTTGGGAGAGACGAGGAA-3’ and antisense: 5’-CCCTTCTCTCCCTCCTTC-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).

COMMENT

To our knowledge, our investigation is the first pathologically examined of an African American individual with CADASIL. Several features should be noted. First, the family includes individuals with fulminant 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 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 genotype.

Third, to our knowledge, this is the first description of an African American patient with CADASIL, and supports the common assertion 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.

Accepted for Publication: May 6, 2011.

Correspondence: Michael Mei-Hwa Wang, MD, PhD, Department of Neurology, University of Michigan, 7629 Medical Science Bldg II 5622, 1137 Catherine St, Ann Arbor, MI 48109-5626 (micwang@umich.edu).


Financial Disclosure: None reported.

Funding/Support: This study was supported in part by grants NS052681, NS054724, and NS062816 from the National Institutes of Health (Dr Wang). The Alzheimer’s Disease Research Center is supported by grant P50-AG08671 and supplied control tissues for studies.

Additional Contributions: Jimo Borjigin, PhD, provided helpful discussions and Andrew Lieberman, MD, PhD, of the Michigan Alzheimer’s Disease Research Center, provided patient samples. Nancy McAnsh of the University of Michigan Comprehensive Cancer Center provided excellent technical support for experiments. We thank the patients and families who have donated precious tissue resources that were used in this study.

REFERENCES