C455R *notch3* mutation in a Colombian CADASIL kindred with early onset of stroke

Abstract—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the *notch3* epidermal growth factor–like repeats. A Colombian kindred carries a novel C455R mutation located in the predicted ligand-binding domain. Stroke occurred in the patients at an unusually early age (median age: 31 years) in comparison to the more frequent onset in the fourth decade of life in other CADASIL populations, including a second Colombian kindred with an R1031C mutation.

NEUROLOGY 2002;59:277–279

J.F. Arboleda–Velasquez, MD*; F. Lopera, MD*; E. Lopez, MSc; M.P. Frosch, MD, PhD; D. Sepulveda–Falla; J.E. Gutierrez, MD; S. Vargas, MD; M. Medina, PhD; C. Martinez de Arrieta, PhD; R.V. Lebo, PhD; S.A. Slaugenhaupt, PhD; R.A. Betensky, PhD; A. Villegas, MD, MSc; M. Arcos–Burgos, MD, PhD; D. Rivera; J.C. Restrepo; and K.S. Kosik, MD

Many patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) experience subcortical ischemic events leading to a stepwise decline and eventually to dementia. CADASIL is caused by highly penetrant dominant mutations in the *notch3* gene, which encodes a transmembrane receptor that contains 34 epidermal growth factor (EGF)–like sequences repeated in tandem in its extracellular domain. All of the mutations result in altered disulfide binding within the repeats by changing the number of cysteines to an odd number.[1]

Methods. *Patient recruitment.* All patients and their relatives underwent comprehensive neurologic and neuropsychological evaluation by a neurologist and a neuropsychologist of the Neurology Service of the University of Antioquia. The diagnosis of dementia was assigned according to *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* criteria. The neuropsychological test battery contains Spanish-language versions of instruments used by the Consortium to Establish a Registry for AD. All individuals signed the informed consent agreement and were 18 years or older.

MRI methods. Brain MRI examinations were acquired in a 1.5 Tesla system (Philips ACS-NT). Two neuroradiologists interpreted the MRI studies by consensus. Scheltens' rating scale was used to quantify the size and number of hyperintensities in T_2-weighted MRI as: 0 (absent), 1 (<3 mm; $n < 5$), 2 (<3 mm; $n > 5$), 3 (4 to 10 mm; $n < 5$), 4 (4 to 10 mm; $n > 5$), 5 (>10 mm; $n > 1$), or 6 (confluent).[2] A global scale of MRI abnormalities was calculated using Cabanis–Iba Zizen score: 1 (normal), 2 (Virchow–Robin spaces dilatation), 3 (symmetric, nodular, isolated areas of increased signal), 4 (larger areas of increased signal in the external capsule and in the white matter of temporal lobes), 5 (bihemispheric confluent areas of increased white matter signal), and 6 (cerebellar and brainstem involvement).[3]

Mutation analysis. DNA from peripheral blood leukocytes of proband was amplified and each of the 33 exons including the intron–exon boundaries was sequenced.[4] Relatives of the proband were screened by gel-based length multiplex single base extension.

Statistics. Exact Wilcoxon tests were used to compare continuous measurements. To properly adjust for right-censored ages of onset, the Kaplan–Meier curve was used to calculate median ages of onset, and exact (permutation) log rank tests were used to compare ages of onset. Ages of evaluation were used as the right censoring times for ages of onset. It was assumed that the ages of onset among family members were independent given mutation status.

Results. The proband was a 47-year-old woman, evaluated 1 year earlier for a stroke. She had a history of migraine with aura and a previous stroke at age 24. The proband's family is originally from Amaga (southern part of the state of Antioquia). A T-C substitution at position 1441 (T1441C) in exon 8 of *notch3* was found in the proband patient. The patient was heterozygous for this mutation, which was not found in 50 unrelated Colombian control subjects. This mutation is predicted to change the fourth cysteine to an arginine (C455R) in EGF-like repeat 11. The T1441C mutation was present in six relatives of the proband patient.

All seven individuals (median age: 42 years, age range: 27 to 67 years; table) with the C455R mutation had stroke at the median age of 31 years (range: 19 to 40 years). All of the subjects had migraine with aura beginning in childhood (range: 5 to 16 years). In every patient, the first ischemic event presented as a stroke without preceding transient ischemic attacks. At the time of this study, four

*These authors contributed equally to this research.

From the Center for Neurological Diseases (Drs. Arboleda–Velasquez, Medina, Martinez de Arrieta, and Villegas), Brigham and Women's Hospital–Harvard Medical School; Pathology Department (Dr. Frosch) and Neurology Department (Dr. Slaugenhaupt), Massachusetts General Hospital–Harvard Medical School; Department of Biostatistics (Dr. Betensky), Harvard School of Public Health, Boston, MA; Wilson Laboratory (Dr. Lebo), George Washington University, Washington, DC; Grupo de Neurociencias de Antioquia (Drs. Lopera, Gutierrez, and Vargas, E. Lopez, D. Sepulveda–Falla, and J. Restrepo), and Grupo de Genetica de Poblaiciones, Mutacarciogenesis y Epidemiologica Genetica (Dr. Arcos–Burgos and D. Rivera), Universidad de Antioquia, Medellin, Colombia.

Supported by an award from the American Heart Association. J.A.–V. was supported in part by a "Guillermo Velasquez Tangarife" scholarship, CONAVI, and ACES. The clinical work in Colombia was supported by "Programa de Sostenibilidad" 01-03 (CODI), Universidad de Antioquia," and COLCIENCIAS project number 1115-04–11919.

Received December 12, 2001. Accepted in final form April 6, 2002.

Address correspondence and reprint requests to Dr. Kenneth Kosik, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Boston, MA 02115; e-mail: kosik@cnd.bwh.harvard.edu

Table *Clinical and MRI features of CADASIL in patients with the C455R mutation*

Sex/Age, y	Age at onset of migraine, y	Age at onset of stroke, y	Schelten's scores						C-Iba Z
			PWM	SWM	BG	IT	In	CC	
W/27	6	25	6	24	4	4	6	1	6
M/36	7	31	6	21	0	0	1	1	5
M/40	5	19	6	24	2	3	6	2	6
M/42	7	33	6	24	6	3	6	1	6
W/47	8	24	6	24	6	4	6	1	6
W/61	16	40	6	24	9	3	6	2	6
W/67	15	34	6	24	6	2	6	2	6

In SWM, individual Scheltens' scores from frontal, parietal, occipital, and temporal lobes were summed. In BG, individual scores from caudate and lenticular nucleus as well as thalamus and capsules were summed.

PWM = periventricular white matter; SWM = subcortical white matter; BG = basal ganglia and capsules; IT = infratentorial structures: brain stem and cerebellum; In = insula; CC = corpus callosum; C-Iba Z = Cabanis–Iba Zizen stage.

affected individuals had survived a mean of 20.2 years after the first stroke. Interestingly, all of these patients had relatively well-preserved cognitive and functional status more than two decades after the onset of strokes. MRI hyperintensities were confluent and spread throughout the cerebral lobes in most patients. Alterations of the corpus callosum and in the insula were a hallmark of the MRI in all the patients (see the table).

Discussion. We have described a family with CADASIL with early onset of stroke and the novel mutation, C455R, in notch3. In previous studies, the mean age at onset of stroke or transient ischemic attacks in patients with CADASIL was 41.2 years (45 patients with stroke[5]), 45 years (50 families with CADASIL[1]), and 46.1 years (72 patients with biopsy-proven CADASIL[6]). The age at onset of stroke in individuals with the C455R mutation preceded by more than one decade the onset of stroke in other populations.

We previously reported on the clinical characterization of another large Colombian family with CADASIL that carries an R1031C mutation in notch3.[7] This population constitutes an appropriate sample for comparison with the family that carries the C455R mutation because they share a similar environment and were studied by the same researches using standardized clinical protocols. Furthermore, the ages of evaluation of the two families were not significantly different ($p = 0.95$). Briefly, the clinical features of CADASIL in 19 carriers of the R1031C mutation (median age: 48 years, age range: 19 to 68 years) included stroke (5 individuals, median age at onset: 53), vascular dementia (6 individuals, median age at onset: 50), migraine, and hearing loss. Twenty-one percent of the individuals with the mutation were asymptomatic. The ages at the time of the first symptomatic stroke differed between the two families ($p < 0.0001$): the age at onset in the five individuals with the R1031C mutation did not overlap with the seven individuals with the C455R mutation, and the median age at the time of the first stroke was 22 years earlier in those with the C455R mutation. Corresponding to this earlier onset, young members of this family had severe MRI abnormalities throughout the cerebral lobes (see the table).

Surprisingly, the frequency of strokes for patients with the C455R mutation was similar to those with the R1031C mutation, but those with the C455R mutation did not become demented even after many years. Four patients in this family had their last stroke approximately 20 years earlier and since that time have remained autonomous and with relatively good cognition. In contrast, patients in the R1031C kindred, which is a much larger family with a more typical age at onset, had a high frequency of dementia by age 50. A strong risk factor for vascular dementia is age, and indeed the high frequency of dementia among patients with the R1031C mutation suggests that the age at which the insult occurs contributes strongly to cognitive decline. The median ages of first stroke in these two kindreds were 53 (R1031C carriers) and 31 (C455R) years, suggesting that brain changes that increase the risk for dementia in CADASIL occur as early as the fourth decade of life. Further studies in a larger number of patients are necessary to establish if late onset of stroke is a risk factor for vascular dementia in CADASIL.

There is interfamilial and intrafamilial variability of the age at onset of stroke, age at onset of migraine, and the degree of disability in patients with CADASIL.[6] However, certain peculiarities of the C455R mutation suggest a correlation of this genotype with early onset of stroke in this Colombian family. The C455R mutation abolishes the fourth cysteine residue at EGF-like repeat 11 and could affect the interaction between notch3 and its ligands. This repeat in notch3 corresponds to repeat 12 of the Drosophila notch protein (figure). Interestingly, only the 11th and 12th EGF-like repeats of Drosophila notch protein are necessary and sufficient for the interaction of the receptor with its ligands Delta and Serrate.[8] Recently, it was reported that Delta1 bind-

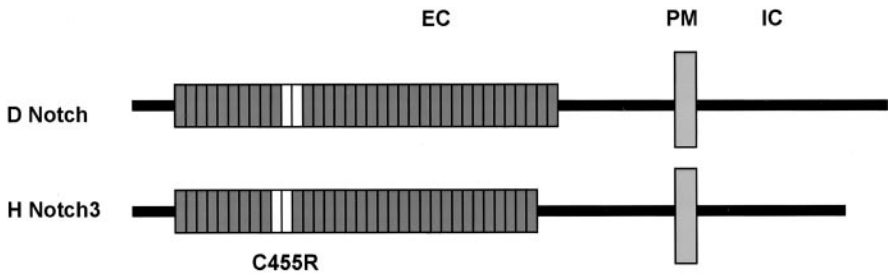

Figure. Comparison of Drosophila Notch (D notch) and human notch3 (H notch3). The sequence of Neurogenic Locus notch Protein (accession number P07207) was used in the Blast analysis. EC = extracellular domain; IC = intracellular domain; PM = plasma membrane. Boxes represent epidermal growth factor (EGF)-like repeats in notch proteins (36 in D notch and 34 in H notch3). The positions of EGF repeats 11–12 (ligand binding domain) or 10–11 are indicated with white boxes in D notch and H notch3. EGF repeats 11 and 12 of D notch match EGF repeat 10 and 11 of H notch3 with 62% identity and 76% similarity.

ing is not affected in 293T cells transfected with a rat notch3 construct with specific CADASIL-like mutations.[9] However, none of the mutations studied was in the predicted ligand-binding domain and it remains to be established if notch3 signaling is normal with CADASIL mutations.

The only previous report of a genotype–phenotype correlation in CADASIL comes from a family with a splice site mutation in the *notch3* gene associated with a peculiar phenotype that included a low frequency of stroke, high prevalence of migraine with aura, and the occurrence of episodic coma in some patients.[10] Although unrecognized epigenetic factors may have contributed to the early onset of the CADASIL phenotype in the Colombian family, we have ruled out homozygosity, concomitant *notch3* mutations, or cardiovascular disease in individuals with the C455R mutation. We suggest that the site of this mutation modifies the CADASIL phenotype by causing an earlier age at onset.

Acknowledgment

The authors thank the members of the "Club Rotario Medellin–Occidente" (Rotary Club West–Medellin), the medical and administrative team of Hospital San Juan de Dios (Santa Fe de Antioquia, Colombia), the patients, and especially Mrs. Flor who helped make this work possible.

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