Early visual function impairment in CADASIL

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Abstract—The authors carried out genetic analyses and visual electrophysiologic evaluations in six asymptomatic sons and daughters of patients with symptomatic cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Three subjects showed Notch3 Cys146Tyr missense mutation and a dysfunction of the outer, middle, and innermost retinal layers, with normal neural conduction in postretinal visual pathways, whereas in the remaining subjects without genetic mutations, no electrophysiologic abnormalities were found. An early vascular retinal impairment in CADASIL may precede the onset of clinical manifestations.

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a disease caused by mutations of the *Notch3* gene, located on chromosome 19,[1] and is characterized by a nonatherosclerotic, nonamyloid cerebral angiopathy mainly affecting the small arteries penetrating the white matter.[2]

It was recently observed that symptomatic patients with CADASIL showed a dysfunction in the outer, middle, and innermost retinal layers, with a possible concomitant functional sparing of postretinal visual structures, as revealed by abnormal electroretinographic responses evoked by flash (electroretinogram [ERG], oscillatory potentials [OP]) or patterned (pattern ERG [PERG]) stimuli, with delayed visual evoked potentials (VEP) but a normal index of neural conduction in postretinal visual pathways (retino-cortical time [RCT]).[3]

We carried out an electrophysiologic evaluation of visual function in asymptomatic fourth-generation members of the same CADASIL family that has been studied previously.[3] The relationship between visual function and genetic findings was analyzed.

Materials and methods. Six of seven subjects (four men and two women: IV-1, IV-2, IV-3, IV-5, IV-6, IV-7, mean age 28.8 ± 5.2 years) gave their informed consent to be enrolled in this study; IV-4 did not.

None of the subjects enrolled showed symptoms or signs of CNS involvement. Extensive laboratory investigations failed to demonstrate any known risk factors for vascular diseases.

Brain MRI was performed in all subjects using a 1.5 T machine (Philips Intera Master). The protocol included axial and coronal spin-echo proton density and T2-weighted images (repetition time [TR]/echo time [TE] 2295/20,90), axial fluid-attenuated inversion recovery (FLAIR) images (TR/TE/inversion time 6000/100/2000), and sagittal and axial T1-weighted images (TR/TE 582/15).

Electrophysiologic visual examinations including ERG, OP, PERG, and VEP were performed in all subjects enrolled in the study and in 14 age-matched controls according to methods described elsewhere.[3]

Molecular analyses were performed on the six subjects enrolled as well as on their parents, as previously described.[4,5]

Detection of the G437A substitution in the *Notch3* gene was performed by dye-terminator direct sequencing (377-AB Automatic Sequencer) of amplified products from genomic DNA samples of the above-mentioned subjects.

The research followed the tenets of the Declaration of Helsinki and the study was approved by the local ethics committee.

Results. Subjects III-2, III-4, and III-5, parents of IV-1 and IV-2, IV-3, and IV-5 to IV-7, shared the abnormal haplotype and the G437A substitution (Cys146Tyr), as did three of the children (IV-3, IV-6, and IV-7).

In Subjects IV-3, IV-6, and IV-7, who were positive for the CADASIL gene mutation, MRI showed small T2/hyperintense foci in the periventricular white matter, in two cases combined with small bilateral T2/hyperintense areas in the subcortical white matter. MRI results were normal in Subjects IV-1, IV-2, and IV-5.

In all subjects, ERG a- and b-wave implicit times, VEP N75-P100 amplitudes, and RCT (difference between VEP P100 and PERG P50 implicit times) were within our normal limits. ERG a- and b-wave amplitudes were reduced in Subjects IV-3 and IV-6 and normal in subjects IV-1, IV-2, IV-5, and IV-7. Subjects IV-1, IV-2, and IV-5 showed OP amplitudes; PERG N35, P50, and VEP P100 implicit times; and PERG N35-P50 and P50-N95 amplitudes within normal limits. In Subjects IV-3, IV-6, and IV-7, we observed reduced OP amplitudes; delayed PERG N35, P50, and VEP P100 implicit times; and reduced PERG N35-P50 and P50-N95 amplitudes.

The main electrophysiologic data are shown in the table and examples of ERG, OP, PERG, and VEP recordings are shown in the figure.

Discussion. Several mutations have been described in CADASIL, mainly located in the 5' end of the *Notch3* gene, corresponding to the predicted epi-

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Table *Main electrophysiologic findings in subjects with CADASIL*

Patient/sex/age, y	ERG b-wave amplitude, μV	OP amplitude, μV	PERG P50 implicit time, ms	PERG P50-N95 amplitude, μV	VEP P100 implicit time, ms
IV-1/M/20					
RE	150.6	120.7	53.6	1.9	109.2
LE	138.8	118.6	54.6	1.8	108.7
IV-2/M/34					
RE	124.9	115.5	53.2	2.0	110.8
LE	131.6	123.4	54.8	1.8	111.1
IV-5/M/29					
RE	125.1	116.4	56.4	1.8	109.4
LE	146.9	133.1	51.9	1.7	107.3
IV-3/F/33					
RE	70.7*	55.3*	62.8*	1.1*	115.7*
LE	75.5*	58.4*	63.4*	1.3*	118.2*
IV-6/M/28					
RE	87.8*	87.6*	63.2*	1.2*	119.4*
LE	89.8*	63.3*	63.6*	1.4*	118.7*
IV-7/M/24					
RE	142.2	82.6*	62.4*	1.4*	113.4*
LE	138.2	49.7*	63.5*	1.2*	115.0*
Controls, n = 14					
Mean	125.7	115.1	52.0	1.96	103.8
1 SD	11.2	6.83	1.67	0.19	2.51
NL	92.1	94.6	57.01	1.58	111.3

* Abnormal value when compared with our normal limits (ERG b-wave, OP, amplitudes: mean values—3 SD of controls; PERG P50 implicit times, VEP P100 implicit time: mean values + 3 SD of controls; PERG P50-N95 amplitudes: mean values—2 SD of controls).

CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; ERG = flash electroretinogram; OP = oscillatory potentials (sum of O1 + O2 + O3 + O4 amplitudes); PERG = pattern electroretinogram; VEP = visual evoked potentials; RE = right eye; LE = left eye; NL = normal limits.

dermal growth factor–like extracellular domain.[6] In this CADASIL family, the Cys146Tyr mutation found in affected subjects was also present in all asymptomatic subjects carrying the haplotype at risk.

The main result of our study is represented by the finding that subjects carrying the Cys146Tyr mutation in the *Notch3* gene without signs or symptoms of CNS involvement (IV-3, IV-6, IV-7) show an impairment of visual function in the presence of normal visual acuity. In fact, in those subjects with the *Notch3* gene mutation, we found impaired ERG, OP, PERG, and VEP responses.

The ERG response is related to the activity of the outer (preganglionic cells) retinal layer whereas OP are likely related to the middle (amacrine cells) retinal layer.[3] The abnormal ERG and OP responses found in our subjects suggest the presence of a dysfunction of the outer and middle retinal layers, even in the absence of functional or clinical visual symptoms (all subjects tested had visual acuity of 20/20). OP amplitudes are considered electrophysiologic indicators of retinal blood supply.[7] Therefore, the reduced OP amplitudes found in our patients could be ascribed to the involvement of retinal vessels similar to the well-documented involvement of brain arteries.[2]

It is known that PERG reflect the bioelectric activity of ganglion cells and their fibers, as demonstrated in animal models[8] and in humans.[9] Nevertheless, the presence of a concomitant impairment of the outer retinal layers (reduced ERG and OP responses) leads us to believe that an involvement of preganglionic cells in the abnormal PERG responses cannot be excluded.

These asymptomatic CADASIL subjects showed delayed visual cortical responses (longer VEP P100 implicit times) associated with abnormal PERG responses and normal RCT.[3]

Because VEP responses reflect both the bioelectrical activity of the retina (evaluated by ERG, OP, and PERG responses) and the neural conduction in postretinal visual pathways (RCT index), the presence of abnormal ERG, OP, and PERG results with nor-

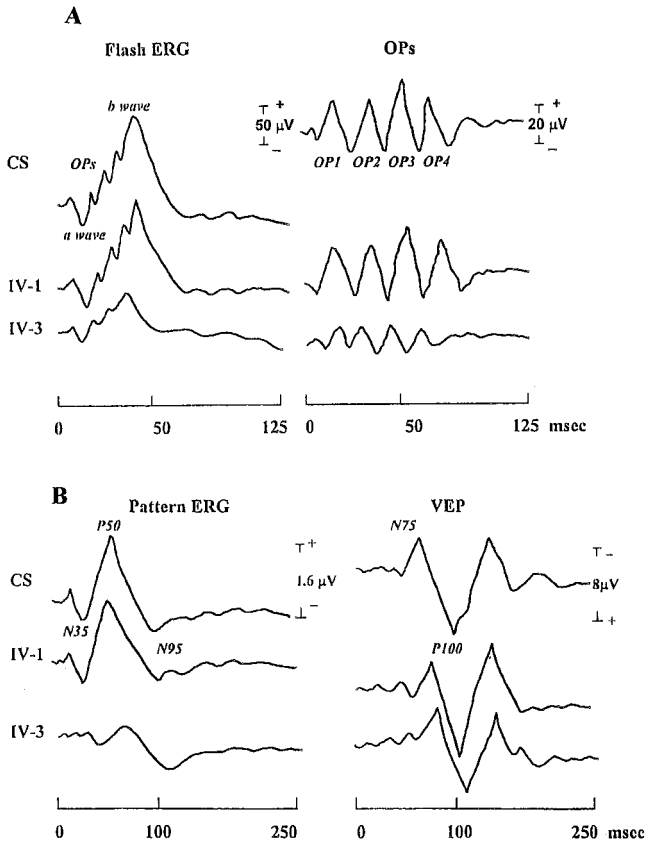

Figure. (A) Electroretinogram (ERG) and oscillatory potential (OP) recordings in the right eye of one control subject (CS) and in the right eye of Subjects IV-1 and IV-3. Subject IV-1 showed normal ERG and OP responses, whereas Subject IV-3 showed a reduction in ERG b-wave and OP amplitudes. (B) Simultaneous recordings of visual evoked potentials (VEP) and pattern ERG (PERG) in the right eye of one control subject (CS) and in the right eye of Subjects IV-1 and IV-3. Subject IV-1 showed normal PERG and VEP responses, whereas Subject IV-3 showed a delay in PERG N35 and P50 implicit times and in VEP P100 implicit times and a reduction in PERG amplitudes, but normal VEP amplitudes.

mal RCT leads us to believe that the delayed VEP responses are mainly ascribable to a retinal dysfunction, with normal postretinal neural conduction.

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