Clinical spectrum in CADASIL family with a new mutation

Tomas Peisker^a, Libor Musil^a, Martin Hrebicek^c, Hana Vlaskova^c, Ilona Cihelkova^d, Ales Bartos^{a,b}

Background. Clinical presentation of CADASIL patients is variable due to the impact of other vascular risk factors and the type of a NOTCH3 mutation. This variability may impede the diagnosis of the disease.

Subjects and Methods. We report a comprehensive evaluation of several individuals in the CADASIL family whose member was identified to have the new mutation of NOTCH3 receptor on exon 6 (p. G296C). We performed genetic testing, clinical and neuropsychological examination, cerebral MRI, Doppler sonography of cerebral arteries, fundoscopic examination and fluorescent angiography in six family members to determine the corresponding clinical spectrum associated with the new mutation.

Results and Conclusion. The CADASIL mutation was detected in four individuals. Three of them were symptomatic, two having a history of stroke and one suffering from migraine. Although individuals had heterogeneous findings, the common feature included vascular changes that were present on cerebral and/or retinal arteries in all the mutation carriers even in one subject without clinical manifestation of the disease.

Key words: CADASIL, NOTCH3, stroke, migraine, fluorescent angiography, Doppler

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^aDepartment of Neurology, Third Faculty of Medicine, Charles University in Prague and University Hospital Kralovske Vinohrady, Prague, Czech Republic

^bPrague Psychiatric Center, Prague - Bohnice

^cInstitute of Hereditary and Metabolic Diseases, First Faculty of Medicine, Charles University in Prague

^dDepartment of Ophthalmology, Third Faculty of Medicine, Charles University in Prague and University Hospital Kralovske Vinohrady
Corresponding author: Tomas Peisker, e-mail: tomas.peisker@fnkv.cz

INTRODUCTION

Cerebral autosomal angiopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary form of cerebral microangiopathy manifested by migraine, recurrent strokes and progressive cognitive impairment. The NOTCH3 gene encodes a transmembrane receptor that is important for structure and function of vascular smooth muscle cells (VSMC). VSMC gradually undergo degeneration with mural fibrosis resulting in stenosis of the distal segment of medullary arteries. Cerebral vessels are thus transformed in "earthen pipe state" losing the ability to autoregulate and functionally becoming blood pressure dependent^{1,2}.

There is a large spectrum of CADASIL mutations. More than hundred different mutations in 18 exons have been already described^{1,3-5}. In European population exon 4 is the most frequently involved site of the NOTCH3 gene mutations, followed by exon 8 and 3 (ref.⁴). Genetic analysis remains the diagnostic gold standard of the disease but it is expensive and may be falsely negative, if only a cluster of most probably involved exons is examined.

Thus pre-genetic testing may be useful for selection of subjects with a high probability of the mutation though the phenotype is highly variable and may be modified by environmental factors. Different investigations are used to verify a suspicion of CADASIL. Cerebral magnetic resonance (MRI) is part of the diagnostic work-up for detection of vascular lesions. Immunohistochemical

examination of skin can display presence of NOTCH3 within the vessel wall and electron microscopy specific granular deposits between VSMC membrane and lamina externa. However, this requires invasive skin biopsy. Ophthalmologic evaluation and transcranial Doppler examination can reveal small vessel morphologic and functional changes^{6,7}. Neuropsychological examination is useful in assessment of cognitive deficits caused by subcortical lesions. Laboratory tests detect pro-coagulation conditions that may contribute to ischemic impairment.

PATIENTS AND METHODS

We offered comprehensive evaluation to the family members of a genetically confirmed CADASIL patient with new mutation on exon 6 - c.886G>T (p.G296C) (see the pedigree in Fig. 1) (ref.⁸). Six family members agreed with the examinations and signed an informed consent.

Genetic analysis with the examination of NOTCH3 gene mutation was carried out in the primary CADASIL suspected patient (subject KMs) by direct sequencing of PCR products, including exons 2-23 and adjacent introns. Genomic DNA was amplified using 11 pairs of specific PCR primers. PCR products were sequencing and subsequently analyzed on Alf-Express automated sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden). Other family members were tested for presence of the mutation p.G296C.

Fig. 1. Pedigree of the family. The arrow shows the patient with a genetically proven NOTH3 mutation. His sister had a genetic and pathological diagnosis of CADASIL, but she was not an object of our further exploration.

All of them had neurological and neuropsychological examinations. The tests included the Mattis dementia scale, clock drawing test, Mini mental state examination, verbal fluency test, Rey-Ostereich test, Stroop test and Zung depression scale. Routine thrombophilia work-up was completed to reveal associated prothrombotic vascular risk factors. This consisted of anticardiolipin antibodies, lupus anticoagulant, antinuclear antibodies, antithrombin III, Leiden mutation, prothrombin time, activated partial thromboplastin time and homocysteine level. Cerebral MRI using FLAIR sequence was carried out in four family members. Two subjects refused MRI (one because of pregnancy). Further standard duplex ultrasonographic examination of cervical arteries and transcranial doppler examination (TCD) were made to find out principal hemodynamic parameters (flow velocities, resistance and pulsatility indices) and to exclude stenotic

changes in any of the examined segments. Subsequently the cerebrovascular reserve (CVR) was measured by breath hold technique⁹. An ophthalmologic examination included assessment of visual acuity, contrast sensitivity, intraocular pressure, evaluation of the anterior eye segment, biomicroscopy of the fundus using optic coherence tomography (OCT) of macula and the nerve fibres layer and fluorescein angiography.

The project was approved by the local ethics committee.

RESULTS

The results of each investigated family member are summarized in Table 1.

Table 1. Results of clinical investigations and auxiliary tests in the family with the new CADASIL mutation (p.G296C).

Subject's initials	Mutation p.G296C	Age (years)	Sex	Vascular risk factors	Case history	Neurological finding	Fundo-scopy/ FAG	Cognitive domains affected	Thrombophilia	Sonography	Brain MR
KMs	+	53	m	hyper-lipidemia	stroke, mi-graine	left sided hemiparesis, cerebellar syndrom	retinal haemorrhage/ choroidal hypoperfusion	attention, visual and verbal memory, executive and visuospatial domains	MTHFR mutation	higher resistance, normal CVR	diffuse hyperintense lesions (incl. ant. T lobes)
SM	+	36	m	-	stroke	right sided hemiparesis	micro-aneurysms/ normal	none	MTHFR mutation	higher resistance, decrease of CVR	diffuse hyperintense lesions (incl. ant. T lobes)
KV	+	24	f	-	mi-graine	pyramidal sign	vesel exsudations/ not done	executive domain	-	normal resistance and CVR	not done
KMj	+	34	m	-	-	normal	normal	executive domain	-	normal resistance, decrease of CVR	not done
KS	-	50	m	-	-	normal	vesel tortuosity/ choroidal hypoperfusion	attention	-	normal resistance and CVR	normal
KMa	-	30	m	-	-	normal	not done	none	-	normal resistance and CVR	normal

MTHFR – methyl tetrahydrofolat reductase, FAG – fluorescein angiography, CVR – cerebrovascular reserve, ant. T lobe – anterior temporal lobe

Four individuals had mutation of the NOTCH3 gene. Three of them manifested with stroke and migraine. The clinical neurologic examination revealed abnormal findings in three affected members. No principal vascular risk factor was detected except for hyperlipidemia. A mutation of MTHFR (methyl tetrahydrofolate reductase) with hyperhomocysteinemia was the only concomitant prothrombotic factor in two individuals.

DISCUSSION

CADASIL is a genetically determined angiopathy affecting mainly small perforating arteries. Various factors make the diagnosis difficult. Firstly, the phenotypic manifestation is not uniform and may be dependent on the type of NOTCH 3 gene mutation and impact by other vascular risk factors. Secondly, the family history is not always available or may be negative in de novo mutations. Finally, genetic and histological evaluation may give falsely negative results.

The clinical manifestation of the disease is also variable in different cohorts¹⁰. Migraine is very frequent initial symptom in European CADASIL patients^{1,11} while intracerebral hemorrhage is more common in Asians especially in those with arterial hypertension³. In the presented family the clinical presentation and an abnormal neurological finding was present in all but one family members with the mutation.

All the affected members but one had decreased phonemic word fluency compared to age-related norms. In addition, the first identified patient, affected by a stroke, also suffered from general intellectual capacity impairment and visual and verbal memory deficits. In contrast, another CADASIL young subject with a history of stroke and ischemic lesions on brain MRI had normal cognitive functions.

T2-MRI hyperintense lesions in subcortical white matter appear regularly in CADASIL patients, but the list of differential diagnoses is large comprising other causes of vascular involvement and inflammatory diseases. A more specific sign is the affection of the external capsule. Lesions of the anterior temporal lobe seem to be very specific for CADASIL (ref.¹²) but their occurrence is not the rule, especially in the Asian CADASIL population. We found specific hyperintense lesions in the anterior temporal lobe and the external capsule in two members of the CADASIL family, who underwent MRI examination. Two other relatives without the NOTCH3 mutation had a normal finding on cerebral MRI.

None of the family members had a history of typical risk factors for microangiopathy such as hypertension or diabetes that might impact clinical manifestation¹³. Prothrombotic factors were also negative in all individuals except the MTHFR mutation with an elevated homocysteine level in two CADASIL positive subjects.

Vascular examination can detect changes of cerebral perfusion in CADASIL patients. CVR measured by MRI perfusion techniques appeared to be decreased within

Fig. 2. Cotton wool spot on a fundoscopy in a 24-year-old patient with the CADASIL mutation.

the areas of hyperintense lesions on T2 images¹⁴. A corresponding finding of reduced global vasoreactivity was shown in a study using TCD, where the degree of reduction reflected functional deficit⁶. Our measurements showed abnormal hemodynamic patterns in intracerebral arteries (higher resistance index and/or decreased CVR) in three of the CADASIL subjects.

Retinal ischemic changes may appear on the fundoscopic examination in CADASIL patients, especially nerve fiber loss and cotton wool spots. They are not very common and they do not seem to be related to the severity of the disease⁷. Retinal capillary blood flow, measured by laser Doppler flowmetry in one CADASIL patient, showed a mild reduction, which does not appear to be linked with ischemic changes¹⁵. In the presented family the changes related to microangiopathy (retinal hemorrhage, cotton wool spots, microaneurysms) were found in three of five subjects (Fig. 2), all with the CADASIL mutation. Fluorescein angiography revealed retinal hypoperfusion in two family members, of which one was not a mutation carrier.

Our observations in the family with the new mutation of NOTCH3 gene show different clinical manifestations and variable findings in auxiliary tests. It is noteworthy that no subject with the NOTCH3 mutation had completely normal findings on the tests including the subject KMj, with no clinical manifestation so far. Abnormal results of retinal vessels examination and Doppler investigation of cerebral arteries, especially in young subjects, may give rise to a suspicion of CADASIL.

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CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: *None declared.*

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