Original Article
Anxiety disorder as the sole clinical manifestation of a new CADASIL mutation associated with occasional GOM deposits

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Abstract: Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common hereditary disease of small cerebral vessels. The defective gene involved is *NOTCH3*. The main clinical manifestations of CADASIL include recurrent transient ischemic attacks, stroke, cognitive impairment, epilepsy, and migraine. Psychiatric symptoms, especially mood disturbances, are also described in CADASIL patients. Aims of the study: Our aim was to understand if CADASIL may be diagnosed in patients with typical white-matter MRI abnormalities but with anxiety disorder as the sole clinical manifestation. Methods: A 76-year-old woman who suffered from anxiety was recruited. Genetic analysis for *NOTCH3* gene mutations and morphological examination by electron microscopy of a skin biopsy for the detection of deposits of granular osmiophilic material (GOM) in small and medium sized arteries were performed. Results: The patient was diagnosed with CADASIL, leading to discovery of a new heterozygous missense mutation (p.C875R) in *NOTCH3* gene. Despite being a common mutation, it has never to our knowledge been described in exon 17 of *NOTCH3* gene. Interestingly, it is associated with occasional GOM deposits. Conclusions: Since CADASIL is often underdiagnosed, it is important that psychiatrists consider this potential differential diagnosis in patients with typical white-matter MRI abnormalities.

Keywords: CADASIL, *NOTCH3* gene, heterozygous missense mutation, anxiety, GOM

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common hereditary disease of small cerebral vessels. The defective gene involved, *NOTCH3*, localizes to chromosome 19p13.1 [1, 2]. CADASIL is diagnosed by genetic analysis as well as via a morphological approach, through detection of deposits of granular osmiophilic material (GOM) in small and medium sized arteries [3-6].

The main clinical manifestations of CADASIL include recurrent transient ischemic attacks, stroke, cognitive impairment, epilepsy, and migraine. Psychiatric disturbances are also frequent [7], and psychiatric symptoms, especially mood disturbances, are described in about 25% of patients [8].

We describe how a patient who suffered from anxiety disorder was eventually diagnosed with CADASIL, leading to discovery of a new heterozygous missense mutation (p.C875R) in *NOTCH3* gene. Despite being a common mutation, involving a cysteine residue, it has never to our knowledge been described in exon 17 of *NOTCH3* gene. Interestingly, it is associated with occasional GOM deposits in skin biopsies.

Case report

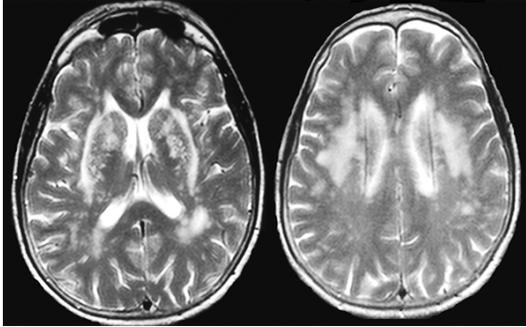A 76-year-old woman came to our attention for distal lower limb dysesthesia and awkward gait.

Figure 1. Axial scans showing severe leukoencephalopathy with bilateral areas of increased signal intensity involving the external capsule on T2-weighted images.

Her medical history included hypothyroidism, arterial hypertension since 60 years of age, primary hypercholesterolemia with mixed dyslipidemia, ocular myopathy likely due to endocrine dysfunction diagnosed at age 64, a traumatic brain injury due to a syncope at age 66, and myocardial infarction (MI) at age 69, which led to implantation of a pacemaker. The patient had been suffering from anxiety disorder since she was about 30. She had no history of alcohol drinking and was not a smoker.

The patient's family history was negative for neurological or psychiatric disorders. Her mother died of MI aged 74 years, her father of lung cancer, aged 77, and her brother of liver cirrhosis, aged 56. Her 43-year-old only son suffered from headache and panic disorder, whereas her sister, who was then 65, did not suffer from any significant medical condition.

The admission neurological examination showed mild psychomotor slowing and distal (superficial and deep) hypoesthesia with weak osteotendinous reflexes in the lower limbs. Her gait was characterized by enlarged basis and worsened on eye closing.

Blood tests showed megaloblastic anemia (red blood count = 3,150,000/l; mean corpuscular volume = 112.1 fl), hyperhomocysteinemia (> 50 µmol/l), and low B12 level (100 pg/ml; normal > 160 pg/ml).

Cerebrospinal fluid was normal.

Esophago-gastro-duodenoscopy disclosed diffuse atrophy of the gastric mucosa without esophageal or duodenal lesions, and a small

erosion affecting the antral mucosa. A gastric biopsy documented mild chronic gastritis of the antrum and body. The search for *Helicobacter pylori* was negative.

Electromyography and electroneurography showed a chronic, bilateral focal lesion of L5-S1. Posterior tibial nerve stimulation yielded abnormal somatosensory evoked potentials with delayed P37.

The patient was diagnosed with myelopathy due to vitamin B12 deficiency and was prescribed vitamin B12 and folate supplementation.

A brain CT scan (due to her pacemaker) documented marked, diffuse leukoencephalopathy due to chronic vasculopathy, bilateral lesions in the temporoinsular and frontopolar regions, basal ganglia, external capsule, and periventricular white matter, and diffuse cortico-subcortical atrophy. The findings were consistent with her earlier MRI scans - taken at age 64 during the workup for ocular myopathy and at age 66 after her traumatic brain injury - which showed severe leukoencephalopathy with bilateral areas of increased signal intensity on T2-weighted images in the external capsule and anterior temporal lobes (**Figure 1**).

Epiaortic echocardiography, ECG and EEG findings were unremarkable. The neuropsychological assessment found normal general cognitive abilities and normal age- and education-adjusted scores on the Mini Mental State Examination and selective difficulties on visuospatial and visuoconstructional tests (immediate and delayed copying of geometric shapes and of the Rey complex figure). Despite her clinically evident psychomotor slowing, the patient achieved normal age- and education-adjusted scores on standard neuropsychological tests tapping short- and long-term memory, abstract-reasoning, executive functions and praxic skills.

The brain abnormalities found on CT and on her previous MRI scans, her anxiety disorder since age 30, and the selective neuropsychological defects suggested a diagnosis of CADASIL despite the negative family history [7-9].

The syndrome is diagnosed by a dual approach: genetic analysis for *NOTCH3* gene mutations [1, 2] and morphological examination for GOM
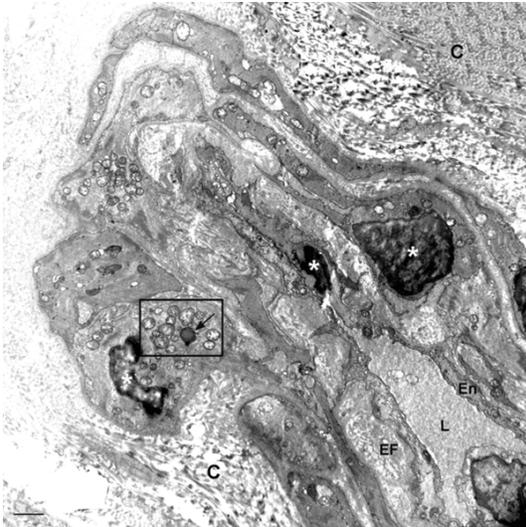
Figure 2. Portion of an artery showing degenerating smooth muscle cells with pyknotic nuclei (asterisks) and large mitochondria clusters (boxed area). Arrow indicates a lipofuscin granule. C, collagen; EF, elastic fiber; En, endothelium; L, lumen of the artery. Scale bar, 1 μm.
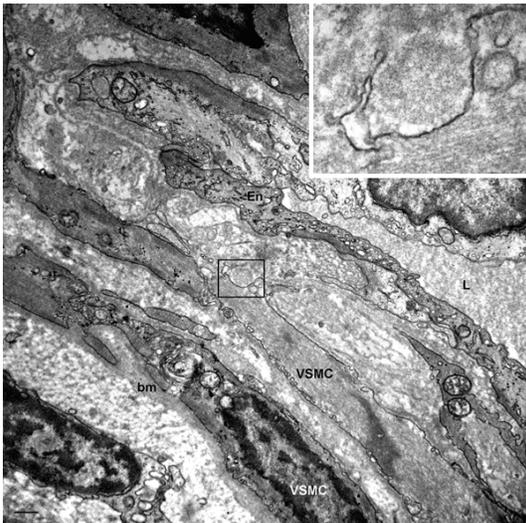

Figure 3. A GOM deposit with low electron-density in the indentation of a vascular smooth muscle cell (boxed area). Inset: enlargement of the boxed area. bm, basal membrane; En, endothelium; L, lumen of the artery; VSMC, vascular smooth muscle cell. Scale bar, 300 nm; inset, 100 nm.

deposits in small and medium-sized arteries [3-6].

This study was conducted in accordance with the Helsinki Declaration as revised in 2000. The patient gave her written informed consent

for genetic analysis and collection of a skin biopsy. Genomic DNA, isolated from peripheral blood leukocytes, was analyzed by PCR and direct *NOTCH3* gene sequencing, as described previously [10]. The analysis disclosed a heterozygous nucleotide substitution in exon 17 (c.2623T > C) of the *NOTCH3* gene, resulting in a new missense mutation involving a cysteine residue at codon 875 (p.C875R), and affecting epidermal growth factor (EGF) repeat 22. Analysis with the PolyPhen-2 and SIFT bioinformatics softwares predicted the mutation as possibly damaging (scores of 1 and 0, respectively). The patient's son showed no mutation and did not suffer from CADASIL.

The skin biopsy was processed as described previously [4] and examined with a CM10 transmission electron microscope (Philips, Eindhoven, The Netherlands). Four ultrathin sections were subjected to ultrastructural examination. Most small arteries had a normal morphology apart from widespread endothelial activation and some small irregularities on the surface of vascular smooth muscle cells (VSMCs). Very few arteries exhibited abnormalities such as VSMC degeneration (pyknotic nuclei, clusters of swollen mitochondria, and lipofuscin granules) or a thickened, multilayered basal lamina (**Figure 2**). In such arteries, occasional slightly electron-dense deposits reminiscent of/consistent with GOM were detected in VSMC indentations (**Figure 3**). The electron-lucent halo around the deposits helped their identification as GOM (manuscript under review). Most capillaries also showed endothelial activation with partial occlusion of the lumen and a multilayered basal lamina.

The patient was discharged home with a prescription of benzodiazepine, vitamin B12, and folic acid supplementation. Two months later her blood counts had returned to normal and her neurological examination showed only weak lower limb osteotendinous reflexes, consistent with her bilateral L5-S1 radiculopathy.

Discussion

The patient described here had myelopathy due to vitamin B12 deficiency, as suggested by her dysesthesia, gait disturbances with impaired position and vibration sense, altered somatosensory evoked potentials, megaloblastic anemia, low B12 levels, and atrophic gastri-

tis. Clinical improvement after treatment with vitamin B12 confirmed the diagnosis.

Vitamin B12 deficiency is associated with several neurological disorders, neuropsychological disturbances (mainly involving long-term memory) and neuropsychiatric abnormalities [11]. These patients may also exhibit white matter lesions; it has also been reported that severe periventricular lesions are related to low vitamin B12 levels, whereas deep lesions fail to show this correlation [12-14].

The age of our patient, her vascular risk factors and the negative family history were consistent with sporadic small cerebral vessel disease [15]. However, she had anxiety disorder since she was about 30 years old and severe leuko-encephalopathy with bilateral subcortical lesions involving the external capsule and the anterior temporal lobes dating back to at least age 64. These features are often described in CADASIL patients [9]. Although the clinical expression of the disease is mainly neurological, CADASIL is characterized by psychiatric disturbances [7, 8]. Anxiety disorder was the sole clinical manifestation that could be ascribed to CADASIL in this patient. Since selective memory and visuospatial disorders have been reported in non-demented CADASIL patients [16], the neuropsychological picture of this patient was consistent with the syndrome, despite her negative family history.

Genetic analysis showed a new heterozygous mutation in exon 17 of the *NOTCH3* gene, a C to T transition at nucleotide 2623 (c.2623T > C) that caused a missense mutation at codon 875 (p.C875R). The mutation is a typical missense mutation causing CADASIL through alteration of the number of cysteine residues within an EGF repeat of *NOTCH3* protein. Notably, p.C875R involved for the first time the loss of a cysteine residue, from six to five, in EGF repeat 22.

CADASIL generally arises in mid-adult age and has a progressively disabling course [17], although some patients show slow progression or later clinical onset (> 60 years) [18, 19]. Our patient had a history of cardiac disease (ischemic MI, leading to implantation of a pacemaker), which had killed her mother. In a small case series of 23 CADASIL patients without severe dementia, Cumurciuc et al. [20] found no ECG

evidence for MI or ischemia, but the technique may be not powerful enough to detect moderate or mild myocardial ischemia. Indeed, a high frequency of MI (10 of 41 patients) has been described in a series of Dutch CADASIL patients, in whom myocardial tissue pathology revealed typical CADASIL arteriopathic changes of the coronary microvasculature [21]. Moreover, Raghu et al. [22] described a case of acute myocardial infarction (AMI) in a 30-year-old woman with CADASIL. These data suggest that ischemic heart disease may be part of its clinical manifestations [21]. Since CADASIL affects small and medium sized arteries [4, 16, 22, 23], it is reasonable to surmise that ischemic stroke and AMI are both symptoms of the syndrome. Coronary vessels can be affected by the typical pathological CADASIL changes [24], and patients with *NOTCH3* mutation may well be at increased risk of early AMI. This means that CADASIL disease involves structures beyond the central nervous system, including at least the heart.

Our findings therefore support the notion that CADASIL is a systemic vascular disease and that it may be a cause of heart damage, since heart and brain lesions likely share a common pathogenesis in CADASIL. However, ischemic cardiopathy is rare [17], and the discrepancy between heart and brain involvement might be related to differences in the structure and function of blood vessels. Even though further studies are required to gain further insights into the pathogenic mechanism of MI in *NOTCH3* mutation carriers, it is conceivable that some CADASIL mutations and/or families have a more frequent association with heart disease.

The small number and modest electron-density of the GOM deposits detected - notwithstanding the ultrastructural examination of more than five arteries of the skin specimen as suggested by Markus et al. [25] -seem to be another peculiarity of the present case. Indeed, electron microscopy is held to be highly specific and sensitive for CADASIL diagnosis [3, 4]. However, considering the patient's age (76 years), the ultrastructural features of the biopsy sample are in line with the observations of Brulin et al. [26], who reported that GOM deposits are most numerous around 50 years of age, whereas in elderly patients they are sparse and difficult to identify. Accordingly, Lewandowska et al. [27] described a genetically confirmed 84-year-old

CADASIL patient without typical clinical features of the disease and with rare GOM deposits that were difficult to identify because they were less osmiophilic and granular. Therefore, though affected by a different mutation, our patient closely resembled the one described by Lewandowska et al. [27].

The patient's son was not found to have CADASIL mutations, but it was impossible to study other close relatives. Nonetheless, the case of our patient confirms that diffuse leukoencephalopathy involving the external capsule bilaterally and the anterior temporal lobes on MRI is highly suggestive for CADASIL, even when the clinical picture is not typical and the family history is negative for the neurological or psychiatric disorders usually associated with CADASIL. The lack of typical CADASIL symptoms among the patient's close relatives supports the hypothesis that the novel p.C875R mutation, the first to be described in exon 17, exerts a very limited effect on disease expression. However, it cannot be ruled out that a *de novo* mutation occurred in our patient as a result of a mutation in a gamete of one of the parents or in the developing fetus [28, 29].

Since CADASIL is often underdiagnosed, particularly in psychiatric patients [30], it is important for psychiatrists to be aware of this potential differential diagnosis in patients with typical white-matter MRI abnormalities.

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Disclosure of conflict of interest

None.

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