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LETTER TO THE EDITOR

Identification of a novel *NOTCH3* mutation in an Italian family affected by a mild form of CADASIL

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Dear Editor,

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare hereditary vascular disease characterized by five main neurological manifestations: migraine with aura, recurrent subcortical ischemic infarctions, mood disturbances, apathy, and cognitive impairment, usually in absence of vascular risk factors. Subcortical signal abnormalities on magnetic resonance imaging (MRI) are the hallmarks of the pathology, detectable as early as 20 and always present after 35 years of age in affected people.

Diagnosis of CADASIL is confirmed through the identification of mutations in the *NOTCH3* gene (exons 2/24) affecting the number of cysteine residues. The disorder is inherited in an autosomal dominant fashion with wide expressivity within families [1].

Here, we describe an Italian family with a new variant identified in exon 6 of *NOTCH3* gene and affected by a CADASIL form characterized by a mild symptomatology.

A 41-year-old female patient presented to the emergency room because of severe pulsating right-sided headache that disappeared in 48 h after painkilling therapy. Seven years before, she reported a similar attack characterized by moderate continuous diffuse pressing headache for about 1 month. Later, she referred with a monthly frequency of a tension-type headache attack lasting about 12 hours, responsive to painkilling therapy. She had neither history of hypertension or smoking, nor a family history for stroke, dementia, or headache; the 68-year-old father and the 62-year-old mother were healthy, as well as her two younger sisters.

Clinical, neurological, and neuropsychological examinations on the proband were normal. Brain CT showed multiple areas of hypodensity in the cerebral bilateral white matter. Brain MRI showed in long TR-weighted images multiple hyperintensities with a nearly symmetrical involvement in the periventricular and subcortical white matter, more evident in the subcortical anterior temporal white matter and in the external capsule (Fig. 1a, b); GRE-T2-weighted images showed one microbleed in the right pallidus nucleus (Fig. 1c).

A diagnosis of CADASIL was suspected and the molecular analysis of exons 2/24 of the *NOTCH3* gene was performed. The genetic test was offered during genetic counseling and after the signing of the informed consent approved by the local Ethics Committee.

Direct Sanger sequencing was performed on both strands on an automated 3730 DNA Analyzer (Applied Biosystems). DNA sequences were analyzed by Sequencing Analysis V5.2 and SeqScape V2.5 softwares (Applied Biosystems).

Direct sequencing identified in exon 6 the presence in the heterozygous state of the variant c.971_973-delinsGTGCCACACCTGTGGCAG (p.Phe324_His325-delinsCysAlaThrProValAlaAsp) (Fig. 2) which causes a gain of a cysteine residue in the EGF-like 8 domain of the protein and the insertion of other six amino acid residues. The presence of the mutation in the proband was confirmed on a second DNA sample.

The variant is novel, since it is not reported in various public genetic databases (Human Genome Mutation Database, Leiden Open Variation Database, ExAC browser), and follows the stereotypical nature of most *NOTCH3* causative mutations.

Silvana Penco died before publication of this work was completed.

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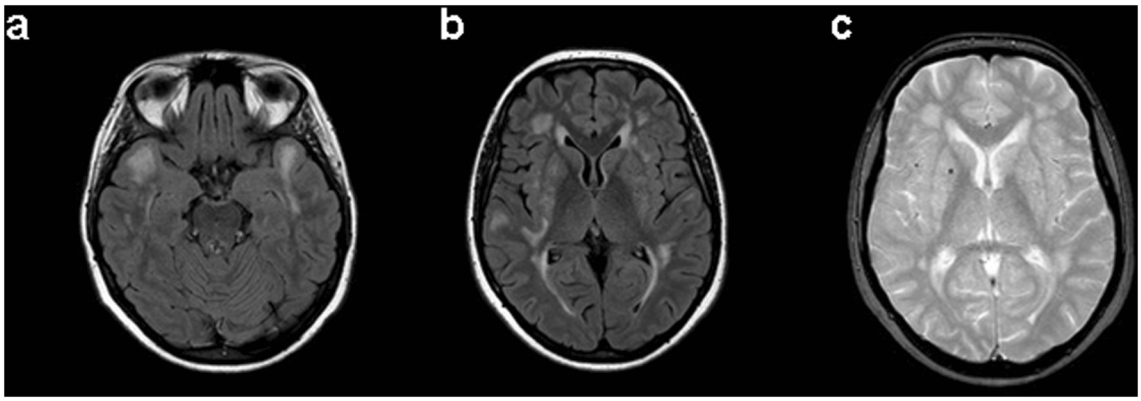

Fig. 1 Brain MRI images of the proband. **a**, **b** Axial long TR weighted images: multiple symmetrical hyperintensities in the periventricular and subcortical white matter, more evident in the subcortical anterior temporal white matter and in the external capsule. **c** GRE-T2-weighted image: microbleed in the right pallidus nucleus

In order to confirm the pathogenetic role of the identified variant, we evaluated its segregation within the family, identifying it in the proband's father. His brain MRI showed in long TR sequences isolated and confluent white matter hyperintensities bilaterally, symmetrical, extended to external capsules, and temporal lobes (Fig. 3a, b, c).

The two proband's sister decided not to undergo the genetic test.

After 2 years of follow-up, the patient complained episodes of tension-type headache, usually of 12 h' duration, 4/5 times a month and responsive to painkiller therapy. Brain MRI resulted unchanged.

Migraine with aura, reported in 20–40% of the CADASIL patients, is often the first clinical manifestation, with onset around age 30. However, the ischemic events (transient ischemic attacks and stroke) are the clinical events most frequently reported (60–85% of patients), usually during the fourth/fifth decade; such episodes generally recur, on average two to five events during a patient's lifetime. The fully developed clinical picture, including psychiatric disorders and subcortical dementia, usually occurs in 70% of patients between 50 and 60 years of age. Other less common clinical manifestations are seizures (5–10%), intracerebral hemorrhage (ICH), deafness, and parkinsonism [2].

MRI is a fundamental tool in evaluation of suspected CADASIL. In particular, T2-weighted (T2w) and FLAIR sequence with high resolution and sensitivity are the most important methods of diagnostic imaging. Usually changes seen in MRI precede the onset of symptoms of 10–15 years. First changes displayed as punctiform or nodular changes, localized mainly in the periventricular regions and centrum semiovale, are present at a mean age of 30 years; later, they become widespread, symmetric, involve the external capsule and extend to the white matter of the anterior temporal lobes.

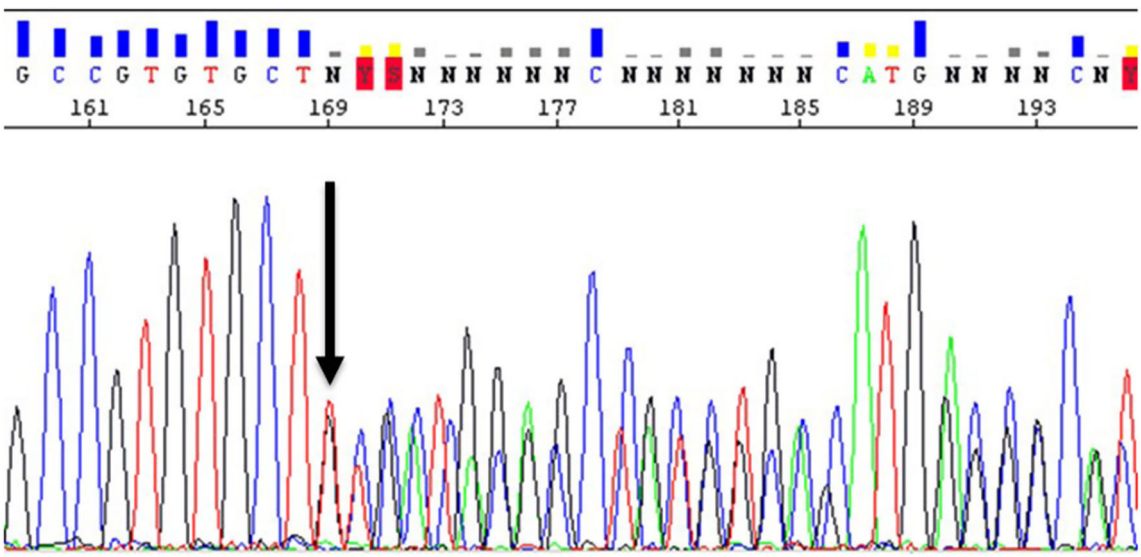

Fig. 2 Sanger sequencing of *NOTCH3* exon 6 with forward primer shows the presence of c.971_973delinsGTGCCACACCTGTGGCAG variant in the heterozygous state (mRNA reference sequence: NM_000435)

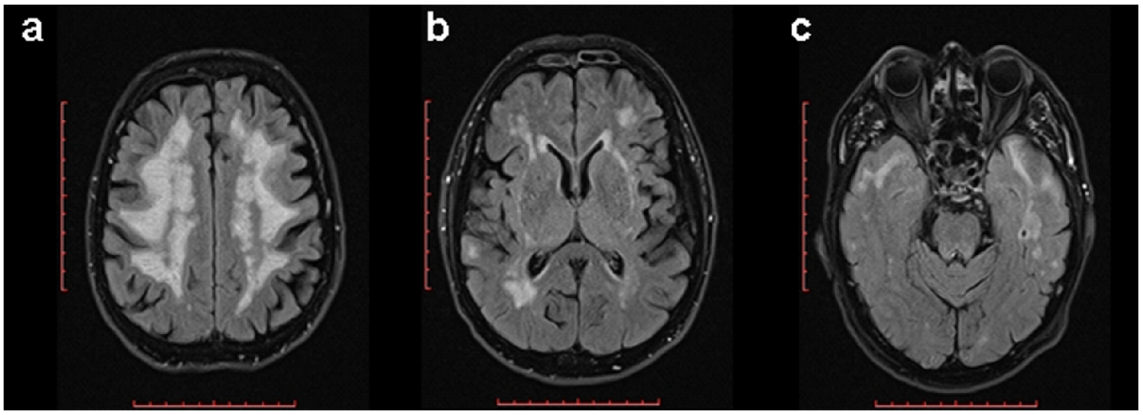

Fig. 3 Brain MRI images of the proband's father. **a** Axial long TR sequences: bilateral, symmetrical, isolated, and confluent white matter hyperintensities extended to **b** external capsules and **c** temporal lobes

Anterior temporal pole changes have been shown to have a high sensitivity and specificity for the disease (approximatively 90%); external capsule changes also have a high sensitivity (approximatively 90%) but a lower specificity (approximately 50%). After 35 years, all subjects with diagnosis of CADASIL have an abnormal MRI.

However, MRI findings alone are not sufficient for diagnosis. Electron microscopy of skin biopsies for the detection of the accumulation of granular osmiophilic material (GOM) around the smooth muscle cells of blood vessel was the first diagnostic method proposed, but was found not sufficiently sensitive, although highly specific. The "gold standard" for the diagnosis is the genetic test focalized on the molecular characterization of exons 2/24 encoding for the 34 EGFR domains of the *NOTCH3* gene.

To date, at least 230 distinct causative mutations leading to an odd number of cysteine residues within an EGFR have been identified [3]. Even among carriers of the same mutation, the expression of the disease is variable in terms of age of onset, variety of symptoms, disease severity, and progression [3, 4].

The vast variety of presentations of CADASIL can confer challenges in recognition and diagnosis of the disease. In our case the young proband manifested an atypical clinical pattern characterized by only infrequent episodic tension-type headache (code 2.1) and probable migraine without aura (code 1.5.1), according to the classification of the international society of headaches criteria (ICHD-2013) [5]. Brain MRI findings were essential for CADASIL suspicion confirmed by the genetic test subsequently.

The proband's father, carrier of the same mutation, presented a characteristic CADASIL brain MRI, but he was asymptomatic.

These results confirmed that the genetic screening of *NOTCH3* gene is an optimal approach in CADASIL diagnosis, also in patients with no convincing evidence for a classical phenotype, and underlies the need for analyzing the entire region encoding the 34 EGF-like repeats (exons 2/24) to avoid misdiagnosis.

It is probable that the novel mutation here identified might be associated with an atypical CADASIL phenotype characterized by a mild picture. Further studies are needed to confirm our hypothesis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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