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Letter to the Editor

A new Italian family with *HTRA1* mutation associated with autosomal-dominant variant of CARASIL: Are we pointing towards a disease spectrum?

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1. Introduction

Cerebral small vessel disease (SVD) is recognized to be the leading cause of vascular dementia. Although the most cases are sporadic, familial monogenic causes have been identified. Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), a recessive form of heritable SVD, is caused by a mutation in the HtrA serine peptidase 1 (*HTRA1*) gene, and characterized by progressive neurological deterioration along with early baldness and severe spondylosis [1]. Recently, emerging heterozygous mutations of *HTRA1* are evidenced as rare causes of SVD [2,3]. We present an Italian family with an heterozygous mutation in the *HTRA1* gene that presented protean clinical, neuroradiological, neurosonological and neuropsychological features.

2. Case reports

2.1. Patient 1

A 43-year old Caucasian man, with a history of vitiligo, autoimmune hyperthyroidism and mild cigarette smoking, reported a two-year history of progressive memory deficits, emotional lability, speech impairment and recurrent “tingling and numbness” involving his right limbs. Upon neurological examination, he showed mild right sided hemiparesis with brisk tendon reflexes, left facial nerve weakness, slight left limb ataxia and slurred speech. Neuropsychological assessment showed impairment in executive functions, attention, verbal short-term memory and calculation ability. No baldness or receding hairline was evidenced. His spine showed physiological cervical and lumbar lordosis, with no signs of spondylosis; his spinal MRI was unremarkable.

Brain MRI showed diffuse and confluent deep periventricular white matter alterations sparing U fibers and multiple lacunar lesions in brainstem, external capsule, corpus callosum and basal ganglia, bilaterally. Temporal lobes were only minimally affected. Small microbleeds were detectable in the frontal lobes and punctuate areas of restricted diffusion, consistent with recent ischemic lesions, involved deep frontal white matter bilaterally. Intracranial MR-angiography was unremarkable (Fig. 1A–F). Echo-Color Doppler of the cervical vessels and Transcranial color-coded Doppler were unremarkable. Contrast Transcranial Doppler excluded a right-to-left shunt. Vasomotor reactivity was tested with breath holding maneuver, showing bilateral impairment (breath-holding index = 0.60 bilaterally) [4]. Routine laboratory investigations, including autoimmune and thrombophilic screening as well as analysis of cerebrospinal fluid, were unremarkable. Alfa-galactosidase activity was normal. Cardiological assessment with echocardiography was within normal limits.

Extensive genetic testing was carried out: the search for mutations in NOTCH3, COL4A1, COL4A2 genes turned out negative. Molecular analysis of *HTRA1* gene, performed by PCR amplification followed by direct sequencing of the entire coding regions and intronic flanking sequences, showed a heterozygous nucleotidic substitution (c.496C > T) in exon 2 resulting in a missense mutation (p.Arg166Cys), previously described in a homozygous and heterozygous form [5,6]. In silico analysis with Polyphen-2, SIFT and Mutation Taster software classified this mutation respectively as “damaging”, “possibly damaging” and “disease causing”. This mutation was not previously reported in databases dbSNP, the 1000 Genomes Projects, ExAc database, Exome Variant Server, NHLBI Exome Sequencing. Consequently, a familiar analysis was performed.

2.2. Patient 2

The patient's mother was a 70-year old Caucasian woman with an history of vitiligo, lack of cardiovascular risk factors, an unremarkable neurological examination and no evidence of hair thinning or history of back pain. Neuropsychological assessment showed impairment in visuospatial and executive functions, verbal fluency and recall. Neurosonological examination of cervical and intracranial vessels was normal. Brain MRI showed diffuse white matter alterations involving deep periventricular white matter and external capsule, sparing the U fibers and temporal lobes. Minute lacunar lesions involved deep white matter, basal ganglia and thalami. DWI sequences were negative for recent lesions and no hemosiderin deposits were detected; intracranial MR-angiography was unremarkable. An intramedullary cavernous angioma was found at cervical MRI (Fig. 1G–I). A genetic analysis showed the same *HTRA1* mutation as the proband.

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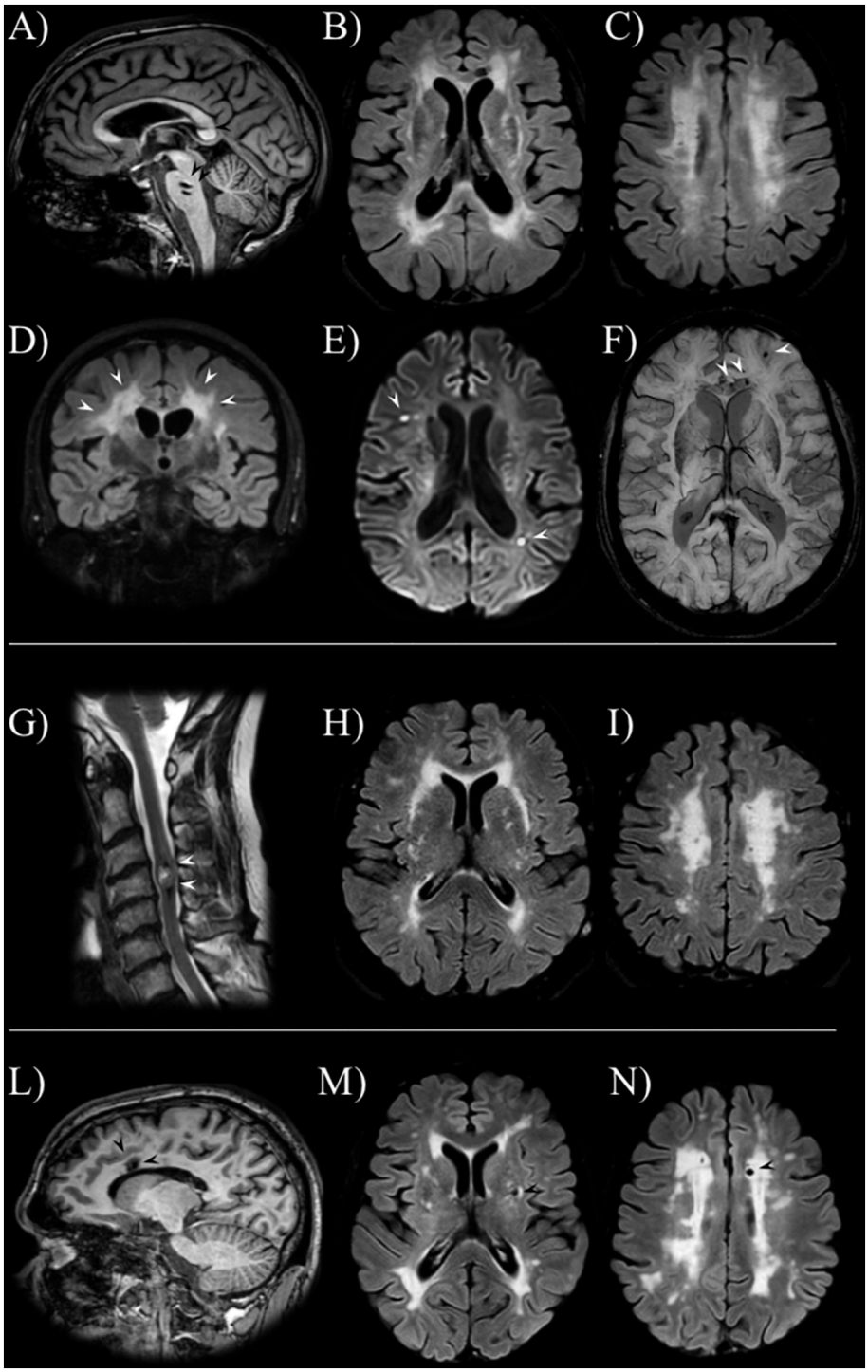

Fig. 1. MRI of the proband (A-F), his mother (G-I) and his brother (L-N).
A) Sagittal T1 image: lacunar strokes in the pons and in the splenium of the corpus callosum (arrowheads).
B—C) FLAIR axial images at the level of the basal ganglia: diffuse white matter involvement with sparing of the U fibers.
D) FLAIR coronal image: prevalent involvement of the cranial white matter with relative sparing of the inferior external capsule and temporale poles (arrowheads).
E) Diffusion weighted axial image: two small acute ischemic foci (arrowheads).
F) Susceptibility weighted axial image: small hypointense hemosiderinic foci in the left frontal cortex and in the left portion of the genu of the corpus callosum (arrowheads).
G) Midsagittal T2 image at C4 level: intramedullary cavernous angioma (arrowheads).
H—I) FLAIR axial images: diffuse white matter involvement with sparing of the U fibers.
L) Left parasagittal T1 image: lacunar stroke in the anterior corpus callosum (arrowheads);
M-N) FLAIR axial images: diffuse white matter involvement with sparing of the U fibers and scattered lacunar strokes (arrowheads).

2.3. Patient 3

The proband's brother, a 47-year-old man with an unremarkable past medical history and lack of cardiovascular risk factors, admitted slight memory deficits occurring in the last two years. Neurological examination was unremarkable. There was no evidence of baldness nor receding hairline. Neuropsychological assessment disclosed a mild impairment in visuospatial and executive functions, and verbal recall. Neurosonological examination of cervical and intracranial arteries was normal; vasomotor reactivity was impaired bilaterally (breath holding index = 0.66), just like the proband. Routine laboratory tests were unremarkable. Brain MRI showed diffuse and confluent deep white matter alterations with sparing of U fibers, and multiple lacunar lesions involving the external capsule, corpus callosum and left putamen. Temporal lobes were minimally affected. A recent ischemic lesion involving the deep left frontal white matter (Fig. 1L–N) was evidenced at DWI. No microbleeds were detected. A genetic analysis disclosed the same *HTRA1* mutation as the proband.

3. Case discussion

HTRA1 mutation is an emerging cause of genetically-determined cerebral SVD. Originally discovered in Japanese patients, homozygous *HTRA1* mutations determine a distinct clinical phenotype characterized

by early baldness, disc herniation and spondylosis with chronic back pain and progressive severe neurological deterioration [7]. Typically, brain MRI shows diffuse and confluent white matter alterations, lacunar infarcts, and microbleeds represented by hemosiderin deposits [8]. In the late stage of the disease, lesions in cerebral peduncles and middle cerebellar peduncles (Arc sign) can be detected [9].

Extracranial circulation and Willis Circle are typically unremarkable [10]. Brain neuropathology reveals small artery disease with fibrous thickening of the intima, fragmentation of internal elastic lamina, and degeneration of smooth muscle cells in the media, without basophilic granulation (as in CADASIL) [11].

Recently, observations pointed out also a pathogenic role of *HTRA1* heterozygous mutations [2,3] which differ from "traditional" CARASIL by a later onset of stroke and cognitive decline, and by the absence of extra-neurological symptoms, such as spondylosis and hair loss, suggesting a disease phenotype confined to the central nervous system [8].

In our three cases with CARASIL-type diffuse cerebral white matter alterations, an heterozygous mutation (c.496C > T) of *HTRA1* gene was detected. This substitution replaces a highly conserved positively charged arginine by a neutral cysteine at codon 166 (p.Arg166Cys) located in the interdomain region, a linker region close to the *HTRA1* protease domain, predicted to be deleterious by three in silico tools (PolyPhen-2, SIFT, and MutationTaster).

This mutation has been previously described in homozygosity in two white siblings of gypsy ethnicity with CARASIL [5]. Recently, the same mutation p.Arg166Cys has also been reported in the first Greek heterozygous *HTRA1* patient [6].

In addition, a different heterozygous *HTRA1* missense variant at the same residue (c.497G > T,p.Arg166Leu) was identified in all affected members of a French family. The p.Arg166Leu variant was predicted to be pathogenic by bioinformatic analysis and above all showed in vitro a dramatic reduction of protease activity [2].

As regards to the pathogenic mechanism of heterozygous *HTRA1* mutations, Nozaki et al. [12] suggested a dominant-negative effect. *HTRA1* is a serine protease involved into quality control of cellular proteins. Wild-type *HTRA1* protein forms trimers, mutant *HTRA1* observed in manifesting heterozygotes might cause a deficient *HTRA1* trimer-associated activation cascade, or might impair the assembly of stable trimers. The arginine at codon 166, involved in our mutation, is close to a stacking site crucial for trimerization.

Taken together, these data strongly supports the pathogenic role of the heterozygous p.Arg166Cys mutation in our family.

In our three cases with CARASIL-type diffuse cerebral white matter alterations, an heterozygous mutation of *HTRA1* gene was detected, which seems to have a variable clinical expression within the same family: overt cognitive impairment and pyramidal signs in Patient-1, moderate cognitive impairment in Patient-2 and subtle cognitive alterations in Patient-3. Typical age-dependent neurological deterioration described in homozygous CARASIL was not respected: the proband's mother reached senior age with only mild signs of neurological involvement, while the proband had already a severe neurological deterioration at 43 years old. Upon neuroradiological assessment, Patient-1 expressed both supra- and infratentorial involvement while Patient-2 and 3 only supratentorial; hemosiderin deposits were only detected in Patient-1, possibly being related to a more severe degree of involvement of small intracranial arteries.

The presence of acute DWI-positive ischemic asymptomatic lesions in Patients-1 and 3 is an intriguing observation, likely underlining the tumultuous evolution of brain involvement.

In Patient-2 an asymptomatic intramedullary cervical cavernous angioma was found: more observations are needed to determine if it represents a further feature of our specific *HTRA1* mutation or simply an anecdotal observation, since studies on spinal cord in *HTRA1* patients are lacking.

In Patient-1 and 3, intracranial neurosonological assessment showed an impaired vasomotor reactivity: this could be the expression of arteriolar and capillary stiffness with loss of normal reactivity to vasodilatatory stimuli. This functional anomaly was evidenced both in mild (Patient-3) and advanced disease stages (Patient-1). More observations are needed to assess if vasomotor reactivity impairment might represent an early (even preclinical) hallmark of disease.

Respect to previous observations [2,3] of other *HTRA1* heterozygously mutated patients (at different loci), we found an earlier age of onset (around 40 years old for patient 1 and 3, undetermined for paucisymptomatic patient 2), and lack of psychiatric symptoms and negative history for migraine with or without aura.

Significant differences can be also found respect to recently described patients with the same *HTRA1* p.Arg166Cys mutation [5,6]. The only homozygous family so far reported (two siblings) was clinically characterized by early alopecia, lumbar back pain, recurrent ischemic attacks and psychiatric symptoms since early age. The heterozygous patient had an history of migraine with aura, hear loss and early baldness, and a brain MRI characterized by diffuse white matter hyperintensities consistently involving the temporal poles bilaterally. Our patients are significant for a different clinical expression (of notice, complete lack of extraneurological signs; absence of psychiatric symptoms or migraine) respect to previous reports, with a variable clinical severity within the same family. The presence of modifying factors is an intriguing possibility to justify a protean clinical and radiological manifestation of the same mutation.

4. Conclusion

HTRA1 gene is involved in cerebral small arteries structure and functioning. Its mutations cause a wide range of symptoms and radiological changes. While homozygous mutations seem to be associated with a younger-onset disease and systemic manifestations (alopecia, spondylosis), heterozygous mutations show a more variable age of onset with an exquisite neurological involvement. We have documented an Italian family with an heterozygous *HTRA1* mutation with a different clinical expression, a different degree of neuroradiological involvement and an impaired vasomotor reactivity since the early stages.

Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosure

The authors have no conflicts of interest.

Approval and consent

Approval and consent statements are not required by the local regulations of our local ethic committee being retrospective observational reports, and the patients being unidentifiable.

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