Shifting the CARASIL Paradigm
Report of a Non-Asian Family and Literature Review

Inês Menezes Cordeiro, MD; Hipólito Nzwalo, MD; Francisca Sá, MD; Rita Bastos Ferreira BSc; Isabel Alonso, PhD; Luís Afonso, MD; Carlos Basílio, MD

Background and Purpose—Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a rare form of nonhypertensive cerebral small-vessel disease caused by mutations in the HTRA1 gene. CARASIL is characterized by early adulthood onset of subcortical infarcts, cognitive impairment, alopecia, and spondylosis. Until recently, this disorder was almost exclusively reported in the Asian population.

Methods—Description of the clinical, imaging, and genetic study of 2 siblings with CARASIL, with a brief comparative review of published non-Asian cases of the disease.

Results—Both patients exhibited the typical phenotype: cerebral small-vessel disease, spondylosis, and abnormal hair lost. Mutation screening was performed for NOTCH3 and HTRA1 genes. No mutations were found in NOTCH3. The study revealed the presence of a homozygous c.496C>T substitution in HTRA1 in both siblings.

Conclusion—This report highlights the need of considering this entity in the differential diagnosis of cerebral small-vessel disease in young patients, even in the non-Asian populations.

Key Words: CARASIL, HTRA1

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a familial autosomal recessive vascular disorder characterized by nonhypertensive cerebral small-vessel disease with early adulthood onset subcortical infarcts, progressive motor and cognitive impairment, alopecia, and spondylosis. CARASIL is a single-gene disorder caused by mutations in the HTRA1 gene. This gene encodes Htra serine peptidase/protease 1 (HTRA1), repressing the inhibition of TGF-β family signaling.

Intense arteriolosclerosis without granular osmiophilic materials, associated with fibrous intimal proliferation, hyaline degeneration of the media, thickening and splitting of the internal elastic lamina, and concentric narrowing of the lumen, are the histopathologic characteristic features.

CARASIL is extremely rare, with most cases reported in Asia, mainly Japan and China. Recent publications show that the prevalence of CARASIL in non-Asian populations may be higher than previously thought.

We report 2 white siblings of gypsy ethnicity with CARASIL caused by a novel HTRA1 mutation.

Methods
Description of the clinical, brain/spinal magnetic resonance imaging, and genetic study of 2 siblings with CARASIL. Mutation screening was performed in the proband for NOTCH3 and HTRA1 genes by PCR amplification followed by direct bidirectional sequencing of the entire coding regions and intronic flanking sequences.

In addition, a brief comparative review of all published non-Asian cases of CARASIL is discussed.

Results
A 45-year-old male Portuguese from gypsy ethnicity, with history of recurrent transient ischemic attacks, lacunar strokes, cervical, and lumbar pain over the past 12 years was admitted for acute psychosis. With the exception of cigarette smoking, no cardiovascular risk factors were present. During adolescence, he developed progressive alopecia with male-pattern baldness (Figure 1C). The examination disclosed severe cognitive impairment with dysexecutive syndrome, spatial and temporal disorientation, comprehension deficit, dysarthria, spastic paraparesis, hyperreflexia, and bilateral Babinski’s sign.

His youngest 32-year-old sister had, at the age of 28, a cerebellar vermis hemorrhage in the context of severe eclampsia (Figure 2A). A year after, she developed a spontaneous cerebellar hemorrhage. The angiographic study showed no vascular malformations. She developed mild depression and progressive difficulty in walking. Examination showed ataxic gait, spastic paraparesis, generalized hyperreflexia, and bilateral Babinski’s sign.
The complementary investigation, including extensive study for genetic and acquired thrombophilic disorders, was negative in both patients. The brain magnetic resonance imaging showed diffuse leukoencephalopathy involving periventricular and deep white matter with multiple lacunar infarcts in the deep white and gray matter of both brain hemispheres and brain stem in both siblings (Figures 1A and 2B). Spine magnetic resonance imaging showed multilevel degenerative changes causing moderate to severe stenosis of the cervical canal, more severe in the male patient (Figure 1B and 2C). At the last examination, the female patient presented complaints of hair lost, and some incipient baldness could be seen (Figure 2D).

Both patients have 2 healthy children. There was no history of consanguinity or any psychiatric or neurological illness in the rest of the large family (parents, grandparents, and 8 siblings).

No mutations were found in NOTCH3. The genetic study revealed the presence of a homozygous c.496C>T substitution in HTRA1 (Figure 3A) in both patients. This substitution replaces a highly conserved positively charged arginine by a neutral cysteine (p.R166C; Figure 3B) predicted to be probable damaging by different bioinformatic analysis softwares (PolyPhen-2, SIFT, and MutationTaster). Additionally, this mutation was not present in dbSNP131 or in the 1000 genomes database. The remaining family members, including the parents, refused or were not available to be studied clinically or genetically.

Discussion and Conclusion

Because the first report in 1976,\textsuperscript{4} there has been an increase in the number of reported cases, although almost exclusively in the Asian population. The first white case was reported in 2010 in a patient of Spanish ancestry.\textsuperscript{5} Since then, only 5 cases, including the present 2, have been published.

The characteristics of non-Asian patients are similar to those described in the Asian population (Table in the online-only Data Supplement).

Although being highly suggestive in its full bloom phenotype, the clinical characteristics of CARASIL are not necessary concensusaneous or present at the time of the diagnosis. Alopecia, for instance, one of the classical signs of CARASIL, maybe absent in some patients with genetic confirmation, especially females.\textsuperscript{2,9}

The signal changes in the anterior temporal lobes and the involvement of the external capsule, believed to be radiological markers of CADASIL, are frequently present in CARASIL patients.\textsuperscript{1,5–7,10} Extensive temporal lobe involvement was present in our male patient but not in the sibling (Table and Figures I and II in the online-only Data Supplement).

Although exceptionally described in association with CARASIL,\textsuperscript{9} occurrence of cerebral hemorrhage in other monogenic cerebral small-vessel disease, such as CADASIL\textsuperscript{11,12} and particularly in type IV collagen α-1 (COL4A1)–related diseases,\textsuperscript{13} is well recognized. Therefore, inclusion of cerebral hemorrhage in the clinical spectrum of CARASIL seems reasonable.

Until now, 9 different homozygous HTRA1 mutations were identified\textsuperscript{2–6} and 1 compound heterozygous patient has been reported.\textsuperscript{7} The p.R166C missense mutation described here for the first time is located in the trypsin-like serine protease domain. The prolytic activity of this serine protease has been shown to be reduced in the presence of

Despite the limitation that the remaining family members refused to be studied, we have strong arguments supporting the pathogenicity of this newly described missense mutation: (1) presence in 2 affected family members, (2) consistent bioinformatic analysis prediction of pathogenicity by several softwares, (3) absence of the mutation from normal variant databases, (4) location in the trypsin-like serine protease domain, and, most importantly, (5) a clinical phenotype highly compatible with mutations in *HTRA1*.

The fact that some of the typical CARASIL characteristics may not be present at the time that patients seek medical counseling, combined with a low level of suspicious in the non-Asian population, precludes or delays the diagnosis. Indeed, the long interval of time before the diagnosis in our patients confirms the notion of the under diagnosis of monogenic small-vessel disease in young patients, even in the non-Asian population.

In conclusion, this report highlights the need of considering CARASIL in the differential diagnosis of cerebral small-vessel disease in young patients, even in the non-Asian populations.

**Disclosures**

None.

**References**

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## Supplemental Table – Clinical and imaging characteristics of non-Asian patients with CARASIL

<table>
<thead>
<tr>
<th>Author</th>
<th>Age / Sex</th>
<th>Clinical history</th>
<th>Neurologic examination</th>
<th>Brain and spinal cord MRI</th>
<th>HTRA1 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Bianchi et al</td>
<td>29/F</td>
<td>Lumbar and cervical pain since the age of 14 years. Two isquemic strokes at 24 and 29 years of age, with left hemiparesis and dysarthria. No alopecia.</td>
<td>Ataxic gait, gaze-evoked nystagmus, dysmetria, hypoactive deep tendon reflexes at lower limbs. Normal cognitive status.</td>
<td>Brain MRI: diffuse leukoencephalopathy involving the anterior temporal lobe, genu of the corpus callosum, internal capsule, and left external capsule. Microbleeds in the pons, right thalamus, right temporal and frontal bilateral cortical and subcortical Spinal MRI: degenerative disc disease, predominantly in the lumbar region</td>
<td>Heterozygous mutations c.961G&gt;A in exon 4 (p.A321T) and c.126delG in exon 1 (p.E42Dfs*173)</td>
</tr>
<tr>
<td>3. Bayrakli et al</td>
<td>29/F</td>
<td>Back and neck pain, right-sided weakness and difficulty in walking for two years. Alopecia.</td>
<td>Right-sided hemiparesis, increased deep tendon reflexes upper and lower limbs. Extensor plantar responses and Hoffman reflexes were present bilaterally. Normal cognitive status.</td>
<td>Brain MRI: Bilateral diffuse white matter lesions involving the temporal poles. Multiple small infarcts in the brain stem, thalamus and periventricular white matter Spinal MRI: Degenerative spine findings at multiple levels</td>
<td>Homozygous c.1108 C&gt;T exon 6 (p.R370*)</td>
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<tr>
<td>Present cases</td>
<td>Case Number</td>
<td>Symptoms</td>
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<td>45/M</td>
<td>Alopecia before 18 yo. Recurrent AIT / lacunar strokes since the age of 31 yo. Cervical/ lumbar pain over the past 12 years, Acute psychosis in 2013</td>
<td>Dysarthria, spastic paraparesis, increased deep-tendon reflexes and bilateral extensor plantar responses. Severe cognitive impairment - dysexecutive syndrome, spatial and temporal disorientation, comprehension deficit.</td>
<td>Brain MRI: diffuse leukoencephalopathy involving periventricular and deep white matter, multiple lacunar infarcts in the deep white and gray matter (both hemispheres and brainstem, mainly pons); microbleeds: &lt;10 cerebelum and cortical Spine MRI - multilevel degenerative changes causing moderate to severe stenosis of the cervical canal.</td>
<td>Homozygous c.496C&gt;T (p. R166C)</td>
<td></td>
</tr>
<tr>
<td>F/32</td>
<td>Cerebellar vermis hemorrhage in the context of severe eclampsia at 28 yo. Spontaneous cerebellar hemorrhage at 29 yo</td>
<td>Ataxic gait, spastic paraparesis with hyperreflexia of upper and lower limbs and bilateral extensor plantar responses Depression Normal cognitive status</td>
<td>Brain MRI - diffuse leukoencephalopathy involving periventricular and deep white matter with fronto-parietal predominance; brainstem, mainly pons; microbleeds: &gt;20 infra and supratentorial (predominantly cortical) Spine MRI - degenerative disc disease with spinal compression at cervical level</td>
<td>Homozygous c.496C&gt;T (p. R166C)</td>
<td></td>
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</table>
Supplemental figures

**Figure I** - Proband: Temporal involvement in (A) axial FLAIR and (B) coronal T2.

**Figure II** - Probands' sister: Temporal involvement in (A) axial FLAIR and (B) coronal T2.
Supplemental references

