Rare Causes of genetic stroke (CADASIL, CARASIL) as a clinical and research model of Vascular Dementia

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CADASIL

• Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy
• Adult onset hereditary autosomal dominant disorder
• Recurrent ischemic attacks (TIAs) and strokes, migraine, seizures and dementia
CADASIL/History

• 1955 L van Bogaert, Livre joubilaire prof. Pamboukis. Hellin iatr: Encephalopatie sous-corticale progressive (Biswanger) a evolution rapide chez deux soeurs

CADASIL

• The gene, corresponding to Notch3 gene, has been localized on chr 19: a transmembrane protein involved in cell specification during development

• De novo mutations have been reported
BACKGROUND CADASIL/MRI

The white matter abnormalities are strongly suggestive, but often undistinguishable from other neurologic disorders (MS, SCVD, LD).

Background

Conventional MRI in CADASIL

De Stefano et al AAN 2000 S39.005
Temporal pole hyperintensity is a radiologic marker of CADASIL. Involvement of the external capsule and corpus callosum are also characteristic findings that may help to distinguish the disease.
**CADASIL/MRI**
Ischemic lacunae and cerebral microhemorrhages

- Evidence of characteristic granular osmyophilic material (GOM) within the basal membrane of brain vascular smooth muscle cells;
- These vascular changes were also later reported in nerve, striated muscle and skin.
**CADASIL/Neuropathology**

WM Pallor and ischemic lacunae

Frontotemporal section

Frontal section

*Courtesy of Prof A Malandrini, Siena*

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**CADASIL/typical pathological findings**

WM Artery: thickening of the Wall with GOM in the media

Basal lamina thickening

Numerosi GOM, degenerazione VSMC

*Malandrini et al, Acta Neuropathol 1996*
CADASIL/ brain lesion pathogenesis

1. Small vessel stenosis

2. VSMCs Degeneration $\rightarrow$ Changes in cerebral autoregulation
   ↓  ↓  ↓
   • Cerebral hypoperfusion / stroke

CADASIL

Clinical heterogeneity
**The phenotypic spectrum of CADASIL: clinical findings in 102 cases**


- TIA or stroke in 71% of cases
- Cognitive deficit in 48% of cases
- Dementia (28%) accompanied by gait disturbances (90%), urinary incontinence (86%) and pseudobulbar palsy (52%)
- History of migraine (38%) (mean age at onset 26 years) classified as migraine with aura in 87%
- Psychiatric disturbances (30%)
- Epileptic seizures (10%)

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**Phenotypic spectrum of CADASIL**


- 55% of the patients older than age 60 were unable to walk without assistance
- 14% in this age group exhibited no disability at all
- Medial survival times of 64 years (males) and 69 years (females).
- Marked intrafamilial variation
The differential diagnosis of CADASIL is broad

- Multiple sclerosis
- Cerebral vasculitis
- Cerebral amyloid angiopathy
- Binswanger’s disease
- Alzheimer’s disease
- HERNS (Jen J et al, Neurology 1997)
- MELAS
- Leukoencephalopathy of undetermined cause

De novo mutation in the Notch3 gene causing CADASIL

Joutel A et al
Ann Neurol 2000; 47:388-91
CADASIL mutation screening in the Neurometabolic Unit, University of Siena (2000-2013)

Selected patients: more than 1100 subjects with leucoencephalopathy, variable clinical spectrum, not constant familiarity (more than 100 families)

CADASIL: 208 patients

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Number</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Notch3 positivity media age 55.85±15.3</td>
<td>208 (106 families)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>104</td>
<td>50%</td>
</tr>
<tr>
<td>Female</td>
<td>104</td>
<td>50%</td>
</tr>
<tr>
<td>Symtomatic</td>
<td>174</td>
<td>84%</td>
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<tr>
<td>Asymtomatic</td>
<td>34</td>
<td>16%</td>
</tr>
<tr>
<td>Familiarity</td>
<td>94/97</td>
<td>97%</td>
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</tbody>
</table>

- TIA/Stroke: 64%
- Emicrania/Emicrania con aura: 16%
- Epilessia: 4%
- Deterioramento cognitivo: 35%
- Disturbi psichiatrici (depressione/ ansia/ psicosi): 13%

Vertigini: 3%
Altro: 2%

The prevalence of CADASIL in the west of Scotland

Razvi et al JNNP 2005

Predicted prevalence of Notch3 mts carriers 4.15/100.000
Wide Phenotypic Variability

Additional genetic or acquired factors?

CADASIL/ Family Ter...

- III-3: 47 y migraine with aura, TIAs, depression, leukoencephalopathy
- III-4 and 5: 43 y, mild migraine, leukoencephalopathy. Monozygotic twins. Different severity.
- III-4 married. The wife underwent prenatal diagnosis (affected), decided to continue the pregnancy
- *II-6: 68y, pseudobulbar palsy, dementia, leukoencephalopathy
- II-5: 75y, mild depression, leukoencephalopathy
- II-1 & 2 died in the adolescence with severe neurological impairment
- I-1; died at 56y, dementia

Exon 20 mutation
Arg@1076→Cys©

*II-6, Previous diagnosis of ortochromatic LD
CADASIL in monozygotic twins


Different clinical phenotypes in monozygotic CADASIL twins with a novel NOTCH3 mutation.

Mykkänen K, Junna M, Amberla K, Bronge L, Kääriäinen H, Pöyhönen M, Kalimo H, Viitanen M.

**Peculiar Clinical Features**

- Early onset with stroke (1 pt, 30y)
- Confusion episodes (1 pt, onset 23y)

- Ischemic optic neuropathy (visual loss) as presenting symptom (2 pts, 15 and 31y)
- Peripheral neuropathy (2 unrelated cases)

- MRI: Cerebral microbleeds in 3/14 cases (21%)

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**CADASIL/ Pro..Gi..62y**

- Father died after stroke (52y); a 27y-old son with transient acute visual loss at 15y, mitral valvulopathy, stroke. A 35y-old son asymptomatic, with slight WM changes
- 30y: ischemic optic neuropathy
- TIAs and stroke
- Pseudobulbar palsy
- MRI: leukoencephalopathy
- Exon 3 mutation Arg®110→Cys®

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Typical pathological changes of CADASIL in the optic nerve.

Rufa A, Malandrini A, Dotti MT, Berti G, Salvadori C and Federico A

Normal structure. Diffuse myelin fiber pallor and rarefaction

Arterial vessels with thickened walls, GOMs and PAS positive material in tunica media. Positivity with anti-amyloid antibodies

Retinal nerve fiber layer thinning in **CADASIL**: an optical coherence tomography and MRI study.

**Rufa** A, Pretegiani E, Frezzotti P, De Stefano N, Cevenini G, Dotti MT, Federico A.

*Cerebrovasc Dis.* 2011;31(1):77-82.

**CADASIL/Fab...Ro... 42y**

- Related parents; a 40y-old asymptomatic sister with leukoencephalopathy
- Since age 23y repeated episodes of confusion and mild memory impairment
- MRI: leukoencephalopathy
- Peripheral sensorymotor neuropathy
- Screening for leukodistrophies: negative
- Exon 4 **homozygous** mutation Cys ©183→Ser(S)
Homozygosity and severity of phenotypic presentation in a CADASIL family.


CADASIL/Tor..Gg..69y

• Family history: apparently negative (a brother died for suicide?)

• 63y: severe depression
• progressive dementia, ataxia
• Hypertension
• MRI: leukoencephalopathy
• **Peripheral neuropathy**
• Leukodystrophies screening: negative

• Exon 11 mutation Arg\(\rightarrow\)Cys\(\rightarrow\)Cys©
Peripheral neuropathy in CADASIL

Sicurelli F, Dotti MT, De Stefano N, Malandrini A, Mondelli M, Bianchi S, Federico A.

CADASIL/Di P... Ma... 55y

- Cases of stroke and progressive dementia and leukoencephalopathy on the maternal side
- Thyroid dysfunction
- Repeted episodes of migraine with aura and confusion
- Stroke at age 43 and several episodes of aphasia
- Severe fatigue for mild effort
CADASIL/Di..P...Ma...55y

Screening for mtDNA mutations: neg
Deletion of 5-bp in exon 4 leading to a frameship (aa 127 to 158)

Muscle biopsy: presence of GOM
Mitochondrial dysfunction in CADASIL

- Finnila S et al, J Mol Med 2001 (myopathy with RRF, heteroplasmic mtDNA mutation) coincidental relationship?
- De La Pena P et al, Neurology 2001 (complex I activity, COX neg fibers, RRF) epiphenomenon or OXPHOS defect linked to the pathophysiology of the disease?
- Malandrini A et al, Neurology 2002 (paracrystalline mit inclusions, asymptomatic cores; mtDNA and RYR1 gene mutations excluded) Notch3 gene may influence mitochondrial metabolism

Notch3 gene mutations and mitochondrial function impairment:

1) Notch lethal mutations affected the activity of the mitochondrial respiratory chain enzymes (Thorig et al., 1981).
2) CADASIL patients exhibited clinical phenotype of a mitochondrial pathology (De la Pena et al., 2001; Finnila et al., 2001; Dotti et al., 2006).
3) Mutations in the Notch gene in Drosophila lead to decreased activity of complex I and V of the mitochondrial respiratory chain (De la Pena et al., 2001).

Possible increase of oxidative stress which could result in high levels of apoptosis in cells from CADASIL patients.
CADASIL
clinical variability

- Genotype/phenotype correlation questioned
- Additional genetic or acquired factors?

Variabilità nell’età d’esordio e nel fenotipo clinico
At the age of 79, episode of dysarthria, right arm hyposteny in a smoker with hypertension. Three years later, good health; slight executive capacities changes.

Systemic blood pressure profile in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.


- BP profile correlated with cognitive decline but not with MRI lesions
- Abnormalities in BP profile could be related to impaired central or peripheral mechanism controlling BP variations
**Cardiac autonomic nervous system and risk of arrhythmias in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).**


Stroke. 2007 Feb;38(2):276-80

We found a statistically significant reduction in all frequency domain parameters of heart rate variability associated with a higher low frequency/high frequency ratio for CADASIL patients with respect to normal subjects. These data are consistent with autonomic derangement and suggests that CADASIL patients may be at risk for life-threatening arrhythmias. This could at least in part explain their higher recurrence of sudden unexpected death and should be taken into account in planning therapy.


- Large RLS was diagnosed in 47% of patients. No significant clinical or MRI differences were found between patients with and without RLS.
- This may not be a coincidence, but can be rather related to the role of the Notch receptor family in the development of cardiovascular system.
Increased QT variability in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

Piccirillo G, Magrì D, Mitra M, Rufa A, Zicari E, Stromillo ML, De Stefano N, Dotti MT.

Molecular genetic diagnosis

- A cluster of mutations around exon 3 and 4 originally reported (Joutel et al Lancet 1997)
- Limited scanning of these exons currently suggested for the diagnosis in 70-80% of cases

![Diagram of Notch3 gene](image)

First report of a pathogenic mutation on exon 24 of the NOTCH3 gene in a CADASIL family.
Valenti R, Bianchi S, Pescini F, D'Eramo C, Inzitari D, Dotti MT, Pantoni L.
J Neurol. 2011 Sep;258(9):1632-6

- We report the first missense mutation involving exon 24 and causing CADASIL in a 64-year-old man
- This report underlines that when CADASIL is suspected the genetic analysis should be performed on all the NOTCH3 exons coding for EGF-like repeats including exon 24 and confirms that CADASIL may have heterogeneous phenotypes.
**CADASIL with cord involvement associated with a novel and atypical NOTCH3 mutation.**

**Bentley P, Wang T, Malik O, Nicholas R, Ban M, Sawcer S, Sharma P.**


- Three members of a family presented with CADASIL caused by a novel NOTCH3 missense mutation, C212Y. Two daughters of the proband also manifested a distinctive pattern of cord lesions confined to the posteroentral zone, cerebral lesions showing both a demyelinating and a typical CADASIL topography, positive antinuclear antibodies and intrathecally derived oligoclonal bands. The mutation occurred in exon 4—that is, outside the Notch3 ligand-binding domain—yet unusually for this location impaired Notch function as assessed by Jagged1 signal transduction. The C212Y mutation did not occur in 100 separate MS cases.

- This is the first description of an inherited pattern of cord lesions in association with CADASIL. The fact that certain features of dysregulated immunity also occurred, in association with a novel and atypical loss-of-function NOTCH3 mutation, supports evidence for functional interactions of Notch3 with the immune system, in addition to its vascular support role.

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**Schizophrenia in a patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL)**

Lagas PA and Juvonen V

*Nord J Psychiat 2001, 55: 41-42*

**CADASIL presenting as bipolar disorder**

Kumar SK, Mahr G

*Psychosomatics 1997; 38: 393-8*
Exon 10 mutation and CADASIL
High frequency of exon 10 mutations in the NOTCH3 gene in Italian CADASIL families: phenotypic peculiarities.

• Out of more than 50 CADASIL unrelated families, 7 had mutations in exon 10 (more than 10%).
• Mood disturbances and psychiatric symptoms are relevant

Exon 10 mts Phenotype

Psychiatric disorders: Depression, Bipolar disorders
anxiety
(even at the onset)

Peripheral vascular system involvement (deep venous thrombosis,
atrial thrombosis, skin microvascular changes,
ischemic optic neuropathy)
First deep intronic mutation in the NOTCH3 gene in a family with late-onset CADASIL.


Neurobiol Aging. 2013 Sep;34(9):2234

Apathy: a major symptom in CADASIL.


Among 132 patients, 54 (41%) were apathetic. Apathy is common in CADASIL, in association with cognitive impairment, global functional disability and severe neuropsychiatric symptoms and can occur separately from depression
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome mutations increase susceptibility to spreading depression.
Eikermann-Haerter K, Yuzawa I, Dilekoz E, Joutel A, Moskowitz MA, Ayata C.

- Migraine with aura is often the first manifestation of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome (CADASIL), a disorder caused by NOTCH3 gene mutations expressed predominantly in vascular smooth muscle. Here, we report that cortical spreading depression (CSD), the electrophysiological substrate of migraine aura, is enhanced in mice expressing a vascular Notch 3 CADASIL mutation (R90C) or a Notch 3 knockout mutation. The phenotype was stronger in Notch 3 knockout mice, implicating both loss of function and neomorphic mutations in its pathogenesis. Our results link vascular smooth muscle Notch 3 mutations to enhanced spreading depression susceptibility, implicating the neurovascular unit in the development of migraine aura.

Apathy is related to cortex morphology in CADASIL. A sulcal-based morphometry study.

- Complete MRI datasets of high quality were available in 119 patients. Depth of the posterior cingulate sulcus exhibited the strongest association with apathy in fully adjusted models (right: p value = 0.0006; left: p value = 0.004). Depth and width of cortical sulci in mediofrontal and orbitofrontal areas were independently associated with apathy. By contrast, cortical thickness was not.
- Cortical morphology in mediofrontal and orbitofrontal areas, by contrast to cortical thickness, is strongly and independently associated with apathy. These results suggest that apathy is related to a reduction of cortical surface rather than of cortical thickness secondary to lesion accumulation in CADASIL.
The pathological process occurring in CADASIL leads to damage of WM and neocortex much before the evidence of clinical symptoms. At this preclinical stage, this seems to take place prevalently in the frontal brain region.

TIW MRI of the SIENAX output for neocortex assessment of a HC (A) and a preclinical CADASIL (B). Note the pronounced difference in neocortical volume between the HC and the preclinical CADASIL subject in the frontal and parietal regions.

MR in Cadasil

- WM lesions are evident before symptoms
- They are more evident in functionally important areas as ant and sup corona radiata
- The involvement of such lesions linearly coincide with increase of disability.
Perspectives on treatment

Cholinergic neuronal deficits in CADASIL.

- ChAT activities were significantly reduced by 60% to 70% in frontal and temporal cortices of CADASIL cases, as were ChAT and P75(NTR) immunoreactivities in the nucleus basalis.
- These findings suggest cholinergic neuronal impairment in CADASIL and implicate cholinomimetic therapy for subcortical vascular dementias.
Donepezil had no effect on the primary endpoint, the V-ADAS-cog score. Improvements were noted on several measures of executive function.

Lancet Neurol 2008

- The effect of donepezil on executive tests emphasises an involvement of cholinergic deficits in executive dysfunction.
- One of the major networks implicated in executive control is the dorsolateral prefrontal-subcortical circuit.
- The prefrontal cortex is densely innervated by cholinergic projections from the nucleus basalis of Meynert.
- Histopathological studies have shown a loss of cortical and subcortical cholinergic fibres in CADASIL pts and in patients with sporadic SVD.
- Thus, there is an anatomical and pathophysiological basis to explain the beneficial effects of cholinesterase inhibitors in this disease.

Lancet Neurol 2008
ENDOTHELIAL FUNCTION (EF) AND PLASMA THIOLS IN PATIENT WITH CADASIL

- We designed a study to assess whether endothelial function may be improved by BH4 administration in 60 CADASIL patients enrolled at 5 Italian centres.
- Preliminary results show that CADASIL patients have a lower endothelium-dependent vasodilatation (PAT index) than healthy controls with/without conventional cardiovascular risk factors (RF).
- Thiol profile showed significantly increased cysteinylglycine (37±7 vs 32±9 mmol/l, P=0.02) and decreased glutathione (5.5±2 vs 7.7±3 mmol/l, P=0.02) concentrations in CADASIL patients vs healthy controls.
- These findings underscore the importance of specific assessment of Endothelial function and redox state and accurate management of Risk factors in CADASIL patients.

Bipolar Disord. 2009 May;11(3):256-69.
Valproate activates the Notch3/c-FLIP signaling cascade: a strategy to attenuate white matter hyperintensities in bipolar disorder in late life?

(A) Valproate (VPA) promotes vascular smooth muscle cell (VSMC) survival under Fas-ligand (FasL) challenge

These results raise the intriguing possibility that VPA may be a novel therapeutic agent for the treatment of CADASIL and related disorders.
**Clin Nucl Med. 2011 Feb;36(2):158-9.**

**Assessment of cerebral hemodynamics to acetazolamide using brain perfusion SPECT in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.**

Park SA, Yang CY, Choi SS, Kim WH.

- Reduced CBF was dramatically improved after administration of ACZ on Tc-99m ECD brain perfusion SPECT in a CADASIL patient.

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**Acetazolamide improves cerebral hemodynamics in CADASIL.**

Huang L, Yang Q, Zhang L, Chen X, Huang Q, Wang H.

CADASIL patients (n=16) were treated with ACZ (250 mg) daily for 24 weeks. The mean blood flow velocity (MFV) of the middle cerebral artery (MCA) and CO(2)-induced cerebrovascular reactivity (CVR) were tested using transcranial Doppler sonography (TCD) before and after treatment.

- After ACZ treatment, the MFV in the MCA was significantly greater at rest (57.68+/−12.7 cm/s versus 67.12+/−9.4 cm/s; P=0.001). Additionally, the CO(2)-induced vasoreactivity increased significantly (13.17+/−6.9% versus 20.69+/−8.2%; P=0.004), and the pulsatility index (PI) decreased significantly (0.82+/−0.1 versus 0.73+/−0.08; P=0.001). The relative ACZ-induced enhancement of CO(2) vasoreactivity was not correlated with pretreatment MFV (SRCC=0.122; P=0.659).

- The present study provides the first evidence that ACZ therapy can increase CBF and CVR in CADASIL patients.
Is the oxidant/antioxidant status altered in CADASIL patients?


The altered aggregation of proteins in non-native conformation is associated with endoplasmic reticulum derangements, mitochondrial dysfunction and excessive production of reactive oxygen species. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare hereditary systemic vasculopathy, caused by NOTCH3 mutations within the receptor extracellular domain, that lead to abnormal accumulation of the mutated protein in the vascular wall. NOTCH3 misfolding could cause free radicals increase also in CADASIL. Aim of the study was to verify whether CADASIL patients have increased oxidative stress compared to unrelated healthy controls. We enrolled 15 CADASIL patients and 16 gender- and age-matched healthy controls with comparable cardiovascular risk factor. Blood and plasma reduced and total aminothiols (homocysteine, cysteine, glutathione, cysteinylglycine) were measured by HPLC and plasma 3-nitrotyrosine by ELISA. Only plasma reduced cysteine (Pr-Cys) and blood reduced glutathione (Br-GSH) concentrations differed between groups: in CADASIL patients Br-GSH levels were higher (p = 0.019) and Pr-Cys lower (p = 0.010) than in controls. No correlation was found between Br-GSH and Pr-Cys either in CADASIL patients (rho 0.25, P = 0.36) or in controls (rho -0.15, P = 0.44). Conversely, 3-nitrotyrosine values were similar in CADASIL and healthy subjects (p = 0.82).

The high levels of antioxidant molecules and low levels of oxidant mediators found in our CADASIL population might either be expression of an effective protective action against free radical formation at an early stage of clinical symptoms or they could suggest that oxidative stress is not directly involved in the pathogenesis of CADASIL.

Take home messages and conclusions
Migrain and cerebral white matter lesions: when to suspect CADASIL

- One or more of recurrent subcortical ischemic stroke (especially before age 60 and in the absence of vascular risk factors)
- Migraine (especially with aura, including atypical and prolonged auras)
- Early cognitive decline or subcortical dementia
- Bilateral multifocal T2/FLAIR hyperintensities in the deep white matter and periventricular white matter with lesions involving temporal pole, external capsule, basal ganglia and/or pons
- Autosomal dominant inheritance of migraine, early stroke and dementia

CADASIL
Clinical suspicion

- Symptoms may be not only limited to CNS (eyes, peripheral nerve, heart)
- Evidence of subclinic ophthalmologic signs
- Autosomal dominant inheritance, but de novo mutations may be present
- Abnormalities in blood pressure (non deepers)
- No a priori exclusion in presence of ATS risk factors (Hyperhomocysteine or others) in absence of MRI abnormalities of temporal lobe
- Atypical clinical manifestations
- Utility of a national registry
Friedrich Nietzsche's disease consisted of migraine, psychiatric disturbances, cognitive decline with dementia, and stroke. Despite the prevalent opinion that neurosyphilis caused Nietzsche's illness, there is lack of evidence to support this diagnosis. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) accounts for all the signs and symptoms of Nietzsche's illness.
Other Brain Small Vessel Disorders

CARASIL

Cerebral autosomal-recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) patients are typically normotensive and have alopecia with onset in their teen years, spondylosis with onset in their 20 s and 30 s, stroke beginning in their 30 s, and dementia with onset in their 30 s to 50 s.

Linkage analysis has shown mutations in the HtrA serine protease 1 (HTRA1) gene have been shown to cause CARASIL. Patients with mutations tend to have protein products with low protease activity that is not able to repress signaling by the transforming growth factor-β family (TGF-beta).
BACKGROUND:
- Rare hereditary cerebral vascular disease
- It was considered to be geographically confined to Asian regions, mainly Japan and China, until Mendioroz et al. reported the first Caucasian patient

CLINICAL PICTURE:
- Onset: II decade, with alopecia
- **Neurological**: TIA/strokes before 40 y. Step-like or chronic-progressive pyramidal and extrapyramidal symptoms, pseudobulbar palsy, and cognitive deterioration.
- **Extraneurological**: arthropathy, lumbago, spondylosis deformans and disc herniation
- Cases without alopecia have been described
- Prognosis: poor

ALOPECIA (25-30 y, 90% of cases)  SPONDYLOSIS
MRI

(A-D) T2-weighted: multiple small infarcts in the bilateral external capsule, corpus callosum, centrum semiovale, periventricular white matter and brain stem, as well as hyperintensity of the white matter of the bilateral temporal pole.

(E) Cervical MRI: multiple level cervical disc herniations, degeneration of vertebral bodies and nodular thickening of the posterior longitudinal ligament.

similar to CADASIL: temporal lobe, external capsule involvement, confluent infratentorial WM lesions
CASE : F, 28 Y

- 29 year-old female of Romanian nationality
- no consanguineous parents
- Family history: mute

- Chronic lumbar and cervical pain since 14-yr-old
- First ischemic stroke with left hemiparesis and dysarthria at 24-yr-old; a second similar stroke occurred at 29-yr-old

**Neurological examination:** ataxic gait, gaze-evoked nystagmus, dysmetria, weak bone-tendon reflexes and no alopecia. Cognitive status was normal.

**Brain and spinal cord MRI:** T1-weighted (1), T2-weighted (2-4), FLAIR (3): diffuse confluent leukoencephalopathy, U-fibers relatively spared, and alterations extend to the anterior temporal lobes, genu of the corpus callosum, internal capsules, and left external capsule (similar to CADASIL). Microbleeds evident in the pons, right thalamus, right temporal and bilateral cortico-subcortical frontal regions. Brain atrophy. Disc degeneration disease, more in lumbar tract.
- CADASIL, MELAS, Fabry's disease, rheumatological diseases, vasculitis were excluded.

- Skin and muscle biopsy were negative (in particular no GOMs).

- Exons 1-9 of HTR1A gene were sequenced: two novel heterozygous mutations have been identified: the c.961G>A in exon 4 and a 6 deletion (c.126delG) in exon 1. The missense mutation in exon 4 results in substitution of a highly conserved alanine residue with threonine (p.Ala321Thr). This mutation is predicted to be pathogenic by PolyPhen 2, SIFT and Mutation Taster software. The deletion in exon 1 causes a frame shift, altering the amino acid sequence from position 42 (p.Glu42fs), and introduces a stop codon at position 214.

<table>
<thead>
<tr>
<th>MUTATIONS</th>
<th>MUTATION TESTER</th>
<th>SIFT</th>
<th>POLYPHEN</th>
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<td>c.961G&gt;A → p.Ala321Thr</td>
<td>DISEASE CAUSING</td>
<td>DAMAGING</td>
<td>PROBABLY DAMAGING</td>
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<tr>
<td>c.126delG</td>
<td>DISEASE CAUSING</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- Japanese patients
- Japanese patient without alopecia
- Spanish patient
- Our patient
A novel mutation in the HTRA1 gene causes **CARASIL** without alopecia.


MRI and genetic analysis was performed in both parents:

- The father, 61 years, had negative history for neurological and cardiovascular diseases. Neurological examination was normal. *HTR1A* gene analysis showed an heterozygous missense mutation in exon 4.

- The mother, 60 years, reported mild hypertension since the age of 54 years, and was undergoing treatment. No other vascular risk factors were evident. *HTR1A* gene analysis showed a G deletion in exon 1.

Proband parents' MRI

Larger involvement in the proband’s mother brain tissue, including bilateral deep white matter, left external capsule, and subtle signal alterations in the pons bilaterally. No involvement of the anterior temporal lobes in both parents.
Family pedigree indicating mutation status for HTRA1 (N= normal genotype; arrow=index case); black symbol indicates CARASIL phenotype, gray symbols indicate leukoencephalopathy

Schematic representation showing domain organization and location of the mutations of the HTRA1 protein. Previously reported pathogenic mutations are represented below the protein, the two novel mutations above the protein. Abbreviations: SP, signal peptide domain; IGFBP, insulin growth factor-binding protein; PDZ, postsynaptic density protein.

Multiple protein alignment showing highly conserved serine protease domain across a broad range of different HTRA1 orthologous proteins. The missense substitution of threonine (T) for alanine (A) in codon 321 is shown (#). Consensus sequences were obtained from the Uniprot database (http://www.uniprot.org/). Symbols below the sequence alignment indicate when residues across species encode for identical (*), conserved (:), or semiconserved (.) amino acids.
DIFFERENTIAL DIAGNOSIS

- CADASIL
- Binswanger disease
- MELAS
- chronic progressive multiple sclerosis

THERAPHY

No effective treatment. Primary treatments include genetic counseling, supportive care, and medications for treating dementia and secondary prevention of ischemic stroke. The effects of antiplatelet agents and anticoagulants are unclear.

Main clinical and biological findings in CADASIL and CARASIL

<table>
<thead>
<tr>
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<th>CADASIL</th>
<th>CARASIL</th>
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<tbody>
<tr>
<td>Onset (years)</td>
<td>40–50</td>
<td>20–30</td>
</tr>
<tr>
<td>Clinical features</td>
<td>Migraine, TIA/strokes, psychiatric disorders, cognitive impairment</td>
<td>Cerebrovascular disturbances and strokes (gait and cognitive deficits)</td>
</tr>
<tr>
<td>Additional signs</td>
<td>–</td>
<td>Arthropathy, lumbago, spondylosis deformans, disc herniation and alopecia in some cases</td>
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<tr>
<td>Inheritance</td>
<td>Autosomal dominant</td>
<td>Autosomal recessive</td>
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<tr>
<td>Cerebral MRI</td>
<td>Involvement of temporal lobe and/or externe capsules</td>
<td>White matter lesions in the periventricular and deep white matter, with sparing of U-fibres.</td>
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<tr>
<td>Gene</td>
<td>NOTCH3 (chromosome 19q12)</td>
<td>HTRA1 (chromosome 10q26)</td>
</tr>
<tr>
<td>GOMs</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>
Conclusions:

- The second case in Caucasian population

- The first report of compound heterozygosity with two new mutations of the HTRA1 gene causing CARASIL in a patient with unrelated parents

- Two new mutations never described before

- Absence of alopecia and other extraneurological signs in the proband

- Temporal lobe involvement, such as in CADASIL

- Leukoencephalopathy in both heterozygous parents, both asymptomatic

Two novel HTRA1 mutations in a European CARASIL patient.


Neurology. 2014 Feb 5. [Epub ahead of print]
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Onset</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADASIL</td>
<td>NOTCH3 (autosomal dominant)</td>
<td>IV-V decades</td>
<td>Progressive dementia, mood disorders, migraine, recurrent subcortical cerebral infarctions. AT MRI, leucoencephalopathy, mainly in temporal poles.</td>
</tr>
<tr>
<td>CARASIL</td>
<td>HTRA1 (autosomal recessive)</td>
<td>II-III decades</td>
<td>Mood changes, pseudobulbar palsy, cognitive dysfunction, scalp alopecia in the teen, acute mid to lower back pain.</td>
</tr>
<tr>
<td>COL4A1</td>
<td>COL4A1 (autosomal dominant)</td>
<td>IV-V decades</td>
<td>Ischemic stroke, intracerebral haemorrhage, retinal arteriolar tortuosity, cataracts, glaucoma, anterior segment dysgenesis of the eye (Axenfeld-Rieger anomaly), muscle cramps, Raynaud phenomena, kidney defects.</td>
</tr>
<tr>
<td>RVCL</td>
<td>TRECX1 (autosomal dominant)</td>
<td>IV-V decades</td>
<td>Retinal vasculopathy, TIA, strokes, cognitive dysfunction, headaches, personality disorders, Raynaud’s phenomena, liver and kidney dysfunction.</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>GALA (X-linked) Deficiency enzyme α-galactosidase (α-Gal A)</td>
<td>Childhood</td>
<td>Classic form: acroparesthesias, angiokeratomas, hypohidrosis, characteristic corneal and lenticular opacities, proteinuria, peripheral neuropathy, TIA and stroke; heart disturbances and cardiomyopathy. Heterozygous females: milder symptoms, later onset</td>
</tr>
</tbody>
</table>

“The study of rare diseases: butterfly collecting or an entrée to understanding common conditions?

K. Talbot
Pract. Neurol. 7: 210-211, 2007

“Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces after working apart from the beathen path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discoveries of the usual law of nature by careful investigation of causes of rarer forms of diseases. For it has been found, in almost all things, that what they contain of useful or applicable is hardly perceived under we are deprived of them or they become deranged in some way”.

William Harvey, 1647