

Cognitive aspects of MELAS and CARASAL

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ABSTRACT

Monogenic diseases, although rare, should be always considered in the diagnostic work up of vascular dementia (VaD), particularly in patients with early onset and a familial history of dementia or cerebrovascular disease. They include, other than CADASIL, Fabry disease, Col4A1-A2 related disorders, which are well recognized causes of VaD, other heritable diseases such as mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and cathepsin-A related arteriopathy strokes and leukoencephalopathy (CARASAL). MELAS, caused by mtDNA (80% of adult cases m.3243A>G mutations) and more rarely POLG1 mutations, has minimum prevalence of 3.5/100,000. CARASAL, which is caused by mutations in the CTSA gene, has been described in about 19 patients so far. In both these two disorders cognitive features have not been fully explored and are described only in case series or families. This review paper is aimed at providing an update on the clinical manifestations, with particular focus on cognitive aspects, but also neuroradiological and genetic features of these less frequent monogenic diseases associated with VaD.

Introduction

A number of heritable conditions as cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Fabry disease, COL4A1/A2-related disorders and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) have been identified as causes of vascular dementia (VaD). In addition to these extensively studied conditions, other monogenic disorders such as mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and cathepsin-A related arteriopathy strokes and leukoencephalopathy (CARASAL) have been associated with clinical evidence of cognitive impairment [1]. However, in these diseases cognitive aspects have not been fully investigated.

The aim of this review is to provide an update on the clinical, neuroradiological and genetic features of these less frequent monogenic

diseases associated with VaD, as MELAS and CARASAL, pointing out on cognitive aspects. This work should help in making aware clinicians in considering these disorders as possible causes of VaD. The identification of these rare genetic causes of VaD is important to implement adequate management measures, including presymptomatic testing, genetic counselling and, if available, therapy.

Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)

Inherited mitochondrial diseases are complex neurogenetic and neurometabolic conditions that present several clinical, diagnostic, and treatment challenges for physicians. Since mitochondria are essential organelles in all human cells, mitochondrial disease can affect all organs. However, involvement of the nervous system may be dominant. These

Abbreviations: CARASAL, cathepsin-A related arteriopathy strokes and leukoencephalopathy; COL4A1/A2, gene encoding type IV collagen alpha 1–2 chain; cSVD, cerebral small vessel disease; EAN, European academy of neurology; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; MRI, magnetic resonance imaging; OXPHOS, oxidative phosphorylation system; SLEs, stroke-like episodes; TIA, transitory ischemic attack; VaD, vascular dementia; VCI, vascular cognitive impairment; WMHs, white matter hyperintensities.

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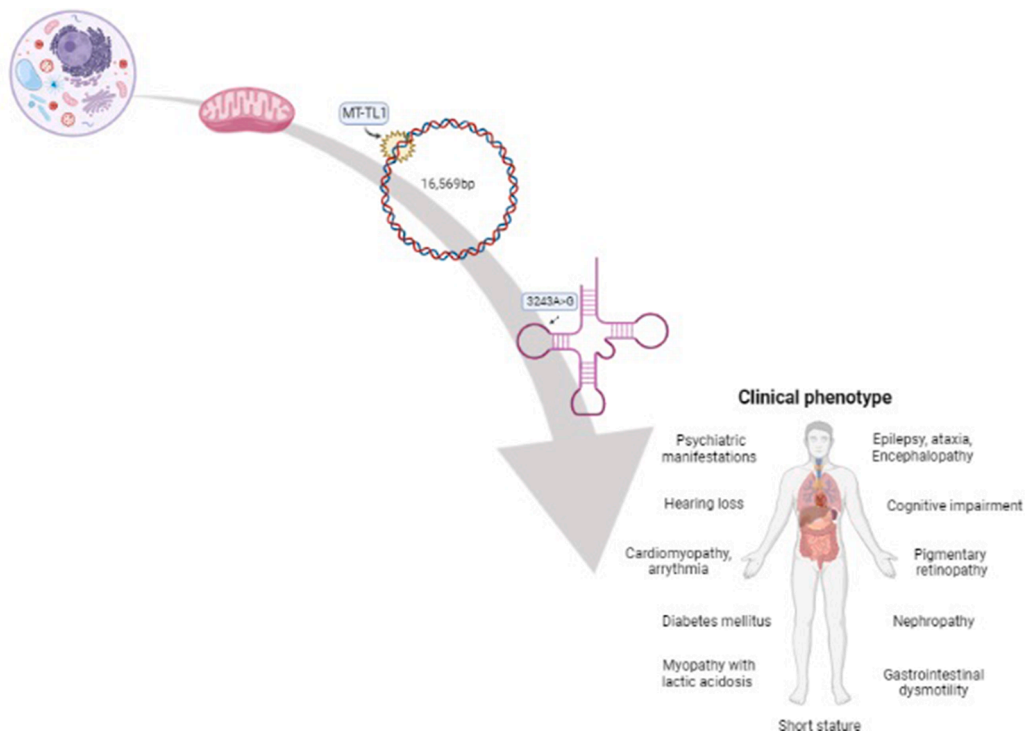


Fig. 1. Pathogenic mechanism of MELAS.

diseases are caused by mutations either in the mitochondrial DNA (mtDNA) or in the nuclear DNA [2].

The mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a rare, mitochondrial, multisystem disorder characterized by: (a) stroke-like episodes, typically before age 40 years, (b) encephalopathy, characterized by a different combination of seizures, ataxia, cognitive impairment leading to dementia and (c) evidence of myopathy with lactic acidosis and/or ragged-red fibers, which are the accumulation of red-staining material in the muscle subsarcolemmal and intermyofibrillar regions at Modified Gomori trichome stains [2,3].

Genetic aspects

Recent studies showed that mitochondrial disease are the most

prevalent groups of inherited neurological disorders, affecting up to 1 in 4000–5000 adults [4]. It is estimated that mutations in the mtDNA are responsible for 15–30% of childhood-onset and over 50% of adult-onset cases of mitochondrial diseases [4–6]. As far as the common m.3243A>G mutation is concerned, the prevalence of affected individuals ranges from 0.95/100 000 to 16.3/100 000.

Most of the reported cases of MELAS had an mtDNA point mutation, although a MELAS-like phenotype has been described in patients carrying mutations in the *POLG* gene. About 80% of MELAS patients harbor the m.3243A>G mutation in the *MT-TL1* gene that encodes tRNA^{Leu(UUR)} in the mitochondrial genome, although more than 30 additional mtDNA mutations have been associated with MELAS (listed on www.mitomap.org).

The m.3243A>G pathogenic variant affects mitochondrial protein

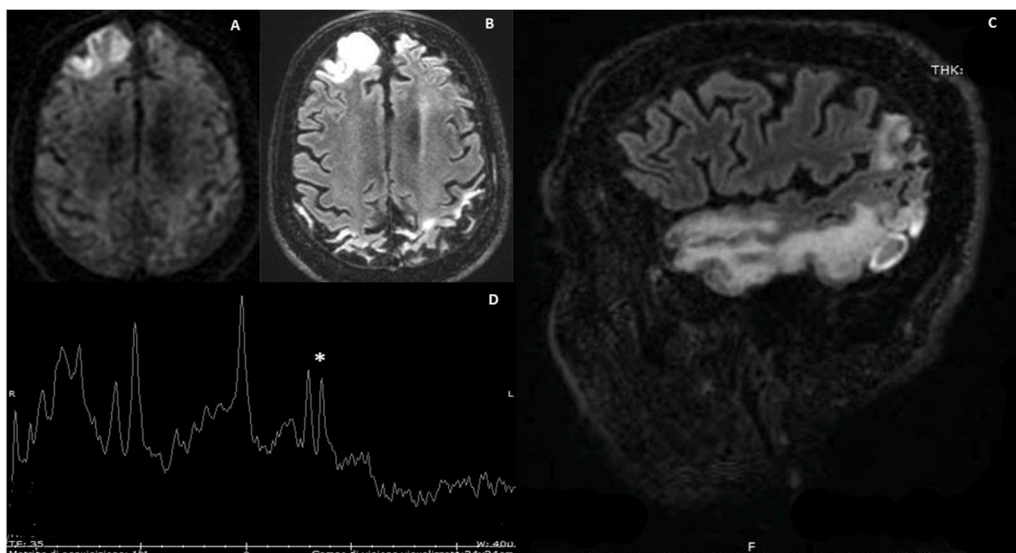


Fig. 2. MELAS Neuroimaging
Cerebral MRI of a 14-year-old male with MELAS carrying the variant m.3243A>G showing a stroke-like lesion in the frontal and occipito-temporal regions, bilaterally. DWI and FLAIR (A,B) sequences show an acute stroke-like lesion in the right frontal lobe (red arrow), with an older bigger stroke-like lesion in the right temporal lobe (red arrow) (C). The asterisk (D) shows lactate peak at the proton spectroscopy [3].

synthesis by reducing amino acid incorporation efficiency in the translation of the 13 mtDNA-encoded proteins of respiratory chain [7–9]. The accumulation of mutated cellular mtDNA copies disrupts the physiological function of the Oxidative Phosphorylation System and results in an impaired mitochondrial energy production. Thus, the dysfunctional mitochondria are unable to generate sufficient ATP to supply the energy needs of various organs, particularly those with high energy demand [10]. Usually, not all mtDNA copies are mutated and there is a variation in mutation load between different tissues of one patient and between one tissue in one patient in time, conditioning disease severity and symptoms. In this case, the mutation is called heteroplasmic.

Maternal relatives are likely to harbor the m.3243A>G mutation. However, very few families have more than one member with full MELAS syndrome; more often, oligosymptomatic maternally-related individuals manifest only with some of the features of MELAS [3]. m.3243A>G heteroplasmy levels in blood, urine and muscle are strongly associated with disease burden and progression, especially when age-corrected blood m.3243A>G heteroplasmy is considered [11]. Nevertheless, additional factors such as mtDNA copy number and nuclear factors may also influence disease severity and, thus, explain the m.3243A>G phenotype spectrum [3,11].

Clinical presentation

Stroke-like episodes (SLEs) are recognized feature of MELAS. A mitochondrial SLE is a subacute, evolving brain syndrome in genetically determined mitochondrial disease, which can be present at any age with neurological symptoms typically associated with cortical/subcortical MRI changes and electroencephalogram (EEG) abnormalities [2,3]. Clinical features in SLE are polymorphic, with acute-subacute onset, and include migraine, focal neurological deficits, altered consciousness, hallucinations, focal or generalized seizures, non-convulsive status epilepticus, drowsiness, vomiting, and visual field defects. Moreover, during the disease course, MELAS patients usually manifest additional signs of CNS involvement. They include myoclonus, ataxia, episodic coma, optic nerve atrophy, basal ganglia calcifications, psychiatric manifestation, and cognitive impairment [2,3,12], (Fig. 1). These features are associated with cortical and sub-cortical signal abnormalities not confined to vascular territories [2], (Fig. 2).

Cognitive aspects

Cognitive impairment and dementia are frequently described in patients with MELAS syndrome. However, neuropsychological deficits of patients with MELAS have been investigated only in single cases or small cohorts. Sartor et al. [13] found an increasing deterioration of phasic alertness, tactile functions and the discrimination of tone pitch and rhythm in one patient. Neargarder et al. [14] reported neuropsychological profiles of two MELAS cases and found global patterns of deterioration in executive function, attention, language, memory, visuospatial, and motor functioning. A distinctive pattern of cognitive deficits, with impairment in visual-constructive skills, attention, and executive functions was described in ten MELAS patients with the m.3243A>G variant [15]. Although cognitive performances and clinical symptoms were found to correlate with the total SLE lesion load, degenerative and metabolic cerebral pathways are probably also involved [15].

Beside SLEs and other CNS manifestations, the most common features reported in the majority of patients with MELAS include exercise intolerance, limb weakness and wasting, short stature, hearing loss, diabetes, cardiomyopathy and arrhythmias, pigmentary retinopathy gastrointestinal dysmotility, and nephropathy [3,16]. While the current management strategy for mitochondrial disease focuses on supplementation with L-arginine and taurine to prevent SLEs and surveillance for multisystem involvement and symptomatic treatment, new attempts are in process to find better therapeutic options, including repurposing

current drugs, use of novel small molecules, and gene therapies [16].

Neuroimaging features

SLEs lesions are frequently located in occipito-temporal regions not involving a classic major vascular territory and, in the acute stage, may present as hyperintensity on T2/FLAIR, DWI, and ADC, suggesting a vasogenic edema, although some patients may present with a cytotoxic edema [2]. Hyper-perfusion of the stroke-like lesions was also documented. The underlying mechanism of SLEs remains unclear, and several hypotheses have attempted to elucidate the pathophysiology based on laboratory, clinical, radiological and neuropathological observations. Specifically there are 3 main hypotheses which are the most intriguing scenarios: (i) the vascular hypothesis; (ii) the hypothesis of a link between nitric oxide deficiency, low plasma levels of arginine and citrulline and SLEs; and (iii) the hypothesis of neuronal hyperexcitability, suggesting that SLEs are mediated by ictal activity [2].

Cathepsin-A related arteriopathy with strokes and leukoencephalopathy (CARASAL)

Cathepsin-A related arteriopathy with strokes and leukoencephalopathy (CARASAL) is a rare hereditary adult-onset, cerebral small vessel disease (cSVD), which is caused by mutations in the CTSA gene, located on chromosome 20q13.12 [1]. CARASAL was firstly described in five French patients with leukoencephalopathy in 2012, without genetic confirmation [17], and later in further 13 Dutch patients [18] and a single British patient [19].

Genetic aspects

The pathophysiological mechanism underlying CARASAL is still unclear. CTSA encodes for the serine carboxypeptidase cathepsin-A, a member of the peptidase S10 family, that associates with the lysosomal enzymes beta-galactosidase and neuraminidase, promoting their stabilization [18–20]. Another function of cathepsin-A is the degradation of endothelin-1, which has probably a role in the development of white matter hyperintensities (WMHs) through inhibition of oligodendrocyte progenitor maturation [18]. In fact, a strong immune-reactivity of endothelin-1 was found in white matter astrocytes of CARASAL patients, correlating with increased numbers of premyelinating oligodendrocyte progenitors [18]. Thus, it has been speculated that increased endothelin-1 levels might lead to a diffuse leukoencephalopathy [18].

So far, the single point mutation c.973C>T; p.R325C has been reported in 14 of the 19 CARASAL patients (in 13 patients from the Dutch families and in the British patient described by Lynch et al.) [18,19,21]. An autosomal dominant trait of transmission has been seen in the pedigree of the affected families [17,18]. Instead, recessive mutations in the CTSA gene have been shown to cause galactosialidosis [21].

Clinical presentation

CARASAL manifests clinically not only with central nervous system but also with systemic symptoms. However, as only 19 patients have been diagnosed with this disease so far, the whole phenotypic spectrum of the disease has not yet fully identified [17–19].

The phenotype is dominated by ischemic and haemorrhagic strokes between the third and fifth decade, slow and late cognitive deterioration, and therapy-resistant hypertension [18]. Headache, migraine, and gait disturbance were also reported in CARASAL patients. Of these, migraine is the only manifestation reported in all described patients [17–19]. Most patients have positive history of stroke, intracerebral haemorrhage or TIAs. Cognitive impairment and dementia as well as movement disorder, dysarthria, dysphagia, tinnitus, impaired REM sleep, restless legs, dry mouth and dry eyes (sicca syndrome), muscle cramps, gait disturbance and depression have been also described

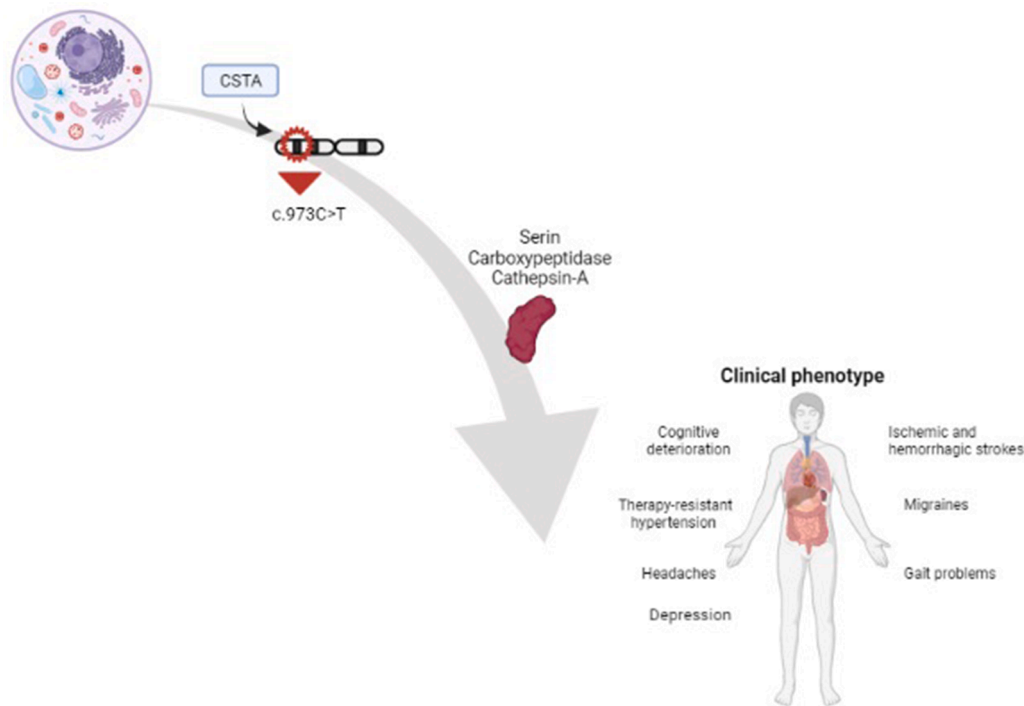


Fig. 3. Pathophysiological features of CARASAL.

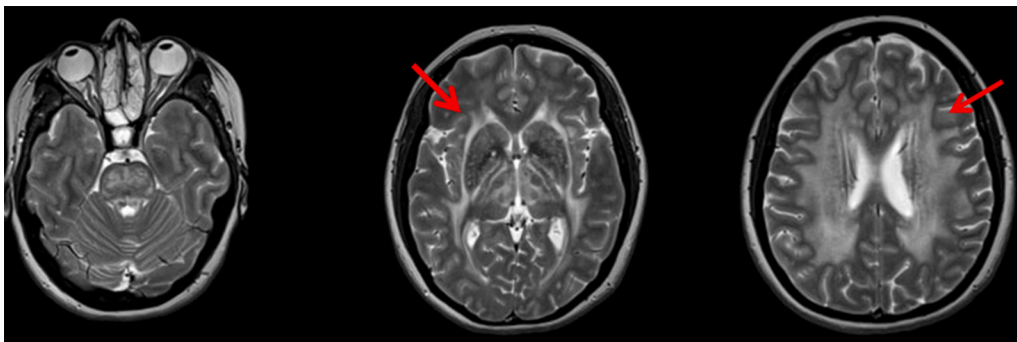


Fig. 4. CARASAL neuroimaging. Cerebral MRI T2 axial images show the lack of the anterior temporal lobe involvement (left image), an extensive involvement of internal and external capsules (red arrow), basal ganglia and thalami (middle image) and periventricular and deep white matter (red arrow) (right).

Images have been provided with the courtesy of Professor J. Finsterer and reproduced with permission from Finsterer et al. [21].

[17–19] (Fig. 3).

As reported in the EAN guidelines on monogenic cerebral small-vessel disease published in 2020 [2], clinical manifestations can be absent or mild despite the extensive leukoencephalopathy. According to these guidelines, CARASAL should be considered in the differential diagnoses of CADASIL, CARASIL and early-onset cSVDs. Thus a CSTA gene analysis should be considered in patients with an unusual severe leukoencephalopathy, a positive family history of stroke, and absence of *HTRA1*, *NOTCH3* and *COL4A1/A2* gene mutations [18]. There are insufficient data to recommend any specific treatment in CARASAL and there is no evidence that thrombolysis, antithrombotic treatment or anticoagulation is indicated [2]. Gene therapy has been reported as promising in few cell models but clinical translation is far [21].

Cognitive aspects

Late and slow cognition deterioration is a feature of CARASAL [21]. Bugiani et al. described mild cognitive impairment and late cognitive deterioration in most patients [18].

Cognitive slowing was also observed also by Lynch et al. in their British patient [19]. This patient had a history of depression and, at age of 42 years, she developed mild cognitive symptoms consisting of

episodic memory and facial recognition deficits and mild behavioral changes with some dis-inhibition. The Mini-Mental State Examination was 27/30 and performance IQ was 99 [19].

Cognitive dysfunction was also reported in the series from Herve et al. [17]. Particularly, an executive function failure was observed in 2/5 patients. Interestingly, the post-mortem diagnosis of Alzheimer disease (corresponding of stage IV of Braak & Braak) was made in one of these patients after microscopic examination of the brain [17].

Neuroimaging features

Cerebral MRI of CARASAL patients shows the typical patterns of cSVDs related abnormalities (Fig. 4), including a leukoencephalopathy with disproportionate severity compared to the clinical picture [12]. Fluid Attenuated Inversion Recovery (FLAIR) images reveal a patchy involvement of the frontoparietal periventricular and of the deep white matter in the early stage, which become diffuse over time, with an extensive subcortical and brainstem hyperintensity.

The white matter hyperintensities (WMHs) are seen in the pyramidal tracts, tegmental tracts, middle and superior cerebellar peduncles [18]. Differently from CADASIL, the temporal white matter is relatively spared and the U-fibers are not affected [1,17,18]. Conversely, the grey

Table 1
MELAS and CARASAL features in comparison with other heritable SVDs associated with VaD.

DISEASE	MELAS	CARASAL	CADASIL	Fabry Disease	RVCL	COL4A1	CARASIL
OMIM	#540,000	#6,011,015	#125,310	#301,500	#192,315	# 120,130	#60,142
Pattern of inheritance	Mitochondrial	AD	AD	X-linked recessive	AD	AD	AD
Gene	MT-TL1	CTSA	NOTCH3	GLA	TREX1	COL4A1	HTRA1
Locus	m.3243A>G	20q13.12	19p13	Xq22	3p21.3	13q34	10q25
Gene product	tRNA Leu (UUR)	Cathepsin-A	Notch 3 receptor	Alfa-galactosidase	TREX1 3'exonuclease	Type IV collagen α1	HTRA1 serine peptidase
CLINICAL FEATURES							
<i>Stroke</i>							
Age at onset (yrs)	2–40	26–65	20–70	33–46 (M) 40- 52 (F)	40–50	14–49	20–40
<i>Stroke subtype</i>							
Small vessel disease	+	+	+	+	+	+	+
Large vessel disease	+	-	-	+	-	-	-
Cardioembolic	-	-	-	+	-	-	-
Haemorrhagic	-	+	Rare	Rare	+/-	+	Rare
<i>Other neurological manifestations</i>							
Cognitive impairment	+	+	+	+	+	+	+
Psychiatric disturbance	+	+	+	+	+	+	+
Migraine with/without aura	+	+	+	-	+	+	+
Seizures	+	+	+	+/-	+	+	+
<i>Extra-neurological manifestations</i>							
Neuropathy	+	-	-	++	+/-	+	-
Myopathy	+	+/-	-	-	+	+	+
Renal disease	+	-	-	+	+	+	-
Skin involvement	-	-	-	+	-	-	-
Ocular involvement	+/-	+	-	+	+	+	+
		Dry eyes		Cornea verticillata	Retinopathy	Cataracts, Retinopathy	Retinopathy
Gastrointestinal involvement	+	-	-	+	+/-	+	-
Cardiac involvement	+	-	-	+	-	+	-
Others	Lactic Acidosis Diabetes Hearing loss	Disphagia Facial pain Dry mouth Hypertension Diabetes Hyperacusia	-	Pain (acroparhesthesia) Hypoacusia Angiokeratoma Small fiber neuropathy Autonomic disorders	Raynaud phenomena Hepatopathy	Poroencephaly, Prenatal bleeding Infantile hemiparesis	Alopecia Spondylosis deformans Acute lumbago
NEURADIOLOGICAL FEATURES							
White matter lesions	+	+	+	+	+	+	+
Lacunar lesions	+	+	-	+	+/-	-	-
Cortical-subcortical lesions							
ICH	-	-	Rare	Rare	-	+	+
Microbleeds	-	-	+	+/-	+	+	+
Aneurysms	-	-	-	+	-	+	-
Peculiar findings	Involving multiple vascular regions. Common parieto-occipital and parieto-temporal involvement	U-fibers sparing Brainstem involvement gray matter involvement	Temporal lobes and external capsules involvement	Pulvinar hyperintensities on T1-weighted images	Subcortical contrast-enhancing lesions with surrounding oedema	Porencephaly, ICH	U-fibers sparing Late basal ganglia involvement
PATHOLOGICAL FINDINGS							
	Lactic acidosis (in both blood and CSF) with lactate peak on 1H-MR spectroscopy. Ragged red fibers with normal COX		Granular Osmiophilic material (GOM) surrounding vascular smooth muscle cells	Typical cytoplasmatic Gb3inclusions in vascular endothelial cells and smooth muscle cells (zebra bodies)	Multilaminated vasculare basement membranes	Interruption and thickening of basement membrane	Degeneration in vascular smooth muscle cells

(continued on next page)

Table 1 (continued)

DISEASE	MELAS	CARASAL	CADASIL	Fabry Disease	RVCL	COL4A1	CARASIL
	activity on muscle biopsy.						
DIAGNOSIS							
	Genetic testing m.3243A>G in urine or at least two different tissues	Genetic testing	Genetic testing Skin biopsy to demonstrate GOM	Genetic testing Low a-galactosidase level in plasma and leucocytes (male) Plasmatic LysoGb3 dosage	Genetic testing	Genetic testing	Genetic testing
THERAPY							
	No specific Coenzyme Q10, riboflavin, L-carnitine, alpha lipoic acid, creatine monohydrate	No specific Conventional therapies	No specific Conventional Therapies	Algasidasi-alfa Algasigasi-beta Migalastat Conventional therapies	No specific Conventional therapies	No specific Conventional therapies	No specific Conventional therapies

matter, including the thalamus, the basal ganglia, and the right dentate nucleus can be involved [1,17,18].

Discussion

Heritable causes, although rare, should be always considered in differential diagnosis of VaD since their lifelong sequelae and psychological consequences have a significant impact to the lives of patients and their families. Therefore, their prompt diagnosis is important to implement adequate management measures and, if available, therapies [1,22].

However, the clinical heterogeneity of VaD, due also to the contribution of overlapping traits of other dementias (e.g. AD), often makes the diagnosis and the identification of a heritable trait very difficult.

Because of their multiorgan involvement, a detailed physical examination and a thorough medical history are highly recommended for a correct diagnosis. A careful neuroimaging investigation and the evaluation of other specific neurological and non-neurological examinations (i.e. dermatologic, ophthalmologic, rheumatologic, cardiologic) should be also performed. The familial history collection with a specific focus on cognitive impairment is also important [17,19], particularly in young patients with early disease onset [1].

Recently, some 'red-flag' features useful to clinicians for the diagnosis of monogenic cSVD, including CARASAL and MELAS, even in the presence of conventional vascular risk factors, have been proposed by experts in the field [12]. These 'red-flags' include positive family history, young age at onset, consanguinity, suggestive extracerebral/systemic features and clinical and neuroimaging characteristics of a specific monogenic disease [12]. However, despite the attempt of developing diagnostic criteria, clear guidelines on the diagnostic work up of these disorders are still lacking. Due to the rarity of the conditions, evidence supporting specific treatment strategies are not available.

Cognitive impairment progressing to dementia has been described in most of monogenic SVDs. Of these, CADASIL is by far the most common and studied condition followed by Fabry disease, COL4A1/2-related disorders and, finally, CARASIL [23]. CARASIL is a relatively new cause of cSVD and cognitive impairment. Although the phenotypic spectrum and the pathophysiological mechanisms of CARASAL have not been entirely encountered yet, an adulthood onset cognitive decline with impairment mostly in executive function has been described in almost all cases in association with WMHs [21]. Based on available literature, CARASAL should be suspected in adult patients with cognitive impairment and cSVD, with a familial history of stroke or cognitive impairment and a negative result at genetic testing for CADASIL and CARASIL.

MELAS is characterized by a more specific clinical phenotype.

Although SLEs cannot be strictly classified as cSVD, MELAS is sometimes included among cerebral angiopathies and have to be considered in the differential diagnosis of cSVD. Among MELAS patients, cognitive impairment has been investigated only in small cohorts and includes deterioration in executive function, attention, language, memory, visuospatial, and motor functioning. The numbers of SLE lesions and the degenerative/metabolic cerebral changes on cerebral imaging seem to explain the patient cognitive profile [15]. Although the neuroimaging pattern of MELAS is usually typical (i.e. multifocal infarct-like cortical areas that do not conform to any known vascular territory; lesions that often occur in the occipital or parietal lobes with eventual involvement of the cerebellum, cerebral cortex, basal ganglia, and thalamus), MELAS is also usually taken into account as a differential diagnosis of CADASIL [24,25]. However, the screening of m.3243A>G has been reported to be of limited value in CADASIL-like patients [26].

In conclusion, although rare, CARASAL and MELAS should be always considered in the differential diagnosis of patients with early onset VaD. For this reason, neurologists should become familiar with the complete clinical feature of these disorders to properly investigate and therefore, diagnose them. Table 1 resumes the clinical, genetic and neuroimaging features of CARASAL and MELAS in comparison to the other monogenic disorders associated with VaD. Since the clinical phenotype and the pathogenesis of these disorders are still largely unknown, we hope that the increasing application of rapid and cost effective extensive gene sequencing strategies as next generation sequencing (NGS) technologies will increase the identification of these patients and, then, will clarify the unknown aspects of these diseases.

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