Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is the most common heritable cause of stroke and vascular dementia in adults. Clinical and neuroimaging features resemble those of sporadic small-artery disease, although patients with CADASIL have an earlier age at onset of stroke events, an increased frequency of migraine with aura, and a slightly variable pattern of ischaemic white-matter lesions on brain MRI. NOTCH3 (Notch homolog 3), the gene involved in CADASIL, encodes a transmembrane receptor primarily expressed in systemic arterial smooth-muscle cells. Pathogenetic mutations alter the number of cysteine residues in the extracellular domain of NOTCH3, which accumulates in small arteries of affected individuals. Functional and imaging studies in cultured cells, genetically engineered mice, and patients with CADASIL have all provided insights into the molecular and vascular mechanisms underlying this disease. A recent multicentre trial in patients with cognitive impairment emphasises the feasibility of randomised trials in patients with CADASIL. In this Review, we summarise the current understanding of CADASIL, a devastating disorder that also serves as a model for the more common forms of subcortical ischaemic strokes and pure vascular dementia.

Introduction
CADASIL is the acronym for cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy suggested in 1993 to designate and characterise a hereditary disease of small cerebral arteries that affects middle-aged adults and leads to disability and dementia. CADASIL was possibly first described by van Bogaert in 1955 as “Binswanger’s disease with a rapid course in two sisters”. Before 1993, six additional families with similar patterns of presentation were reported under various terms. In 1976, one of us (M-GB) saw a 50-year-old man with a lacunar infarct and extensive leucoencephalopathy. The tentative diagnosis was Binswanger’s disease, but the absence of hypertension was atypical and led us to undertake a systematic study of his family. The data were reported under three different names until the relevant gene on chromosome 19 could be mapped. Linkage studies in other families enabled further refinement of this genetic interval and identification of the mutated gene as NOTCH3 (Notch homolog 3).

Since then, CADASIL has been reported in more than 500 families worldwide, but its overall prevalence is unknown. A small study from Scotland, UK, provided an estimate of 4-15 cases per 100,000. However, the actual prevalence could be much higher because sporadic cases occur. CADASIL has been reported to account for 2% of cases of lacunar stroke with leukoaraisis in patients younger than 65 years and for 11% of cases in those younger than 50 years.

In this Review, we present the main clinical, neuroimaging, pathological, and therapeutic features of CADASIL, and discuss the molecular, genetics, and pathophysiological features of this disorder.

Clinical presentation
Although the clinical presentation of CADASIL varies substantially between and within families, this disease is essentially characterised by five main symptoms—migraine with aura, subcortical ischaemic events, mood disturbances, apathy, and cognitive impairment. These symptoms vary in frequency with age and duration of disease.

Migraine with aura
20–40% of patients with CADASIL have migraine with aura, a proportion that is five times greater than in the general population. By contrast, migraine without aura has the same frequency in patients with CADASIL and the general population. When present, migraine with aura is usually the first symptom, with an average age at onset of 30 years (range from 6 to 48 years of age; mean age in women is 26 years; mean age in men is 36 years). In one study, an early age of onset correlated with a high serum concentration of homocysteine. Most attacks are typical with visual or sensory aura symptoms lasting 20–30 min followed by a headache lasting a few hours; however, 50% of patients also have atypical attacks with basilar, hemiplegic, or prolonged aura, and some patients have very severe attacks with confusion, fever, meningitis, or coma. The frequency of attacks varies widely, and triggering factors are the same as those typical for migraine. In some families, migraine with aura is the prominent symptom of CADASIL.

Subcortical ischaemic events
Transient ischaemic attacks and ischaemic strokes are the most frequent manifestations in CADASIL, occurring in 60–85% of patients with an estimated incidence of 10–4 per 100 patient-years. These events occur at a mean age of 49 years (range from 20 to 70 years of age), in most cases in the absence of conventional vascular risk factors. However, in one series, hypertension was present in 20% of patients and the risk factors of high cholesterol concentrations and smoking were present in 50%, with an association between current smoking and earlier stroke onset. Ischaemic events are almost invariably subcortical and present in 67% of patients as lacunar syndromes (eg, pure motor or sensory
deficit, ataxic hemiparesis, sensory-motor deficit, dysarthria–clumsy hand syndrome). Most patients have two to five recurrent strokes over several years, progressively leading to gait difficulties, urinary urgency with or without incontinence, and pseudobulbar palsy.\textsuperscript{12,23,34}

**Mood disturbances and apathy**

Mood disturbances are present in 20\% of patients with CADASIL and generally present as severe depressive episodes. These episodes sometimes alternate with manic episodes that could be mistaken for bipolar mood disorder until the typical CADASIL abnormalities are seen on MRI.\textsuperscript{11,22–24,31} Apathy, characterised by absence of motivation associated with decreased voluntary behaviour, has been recognised as a major clinical manifestation that is present in about 40\% of patients, and that is independent from depression.\textsuperscript{25}

**Cognitive impairment and dementia**

Cognitive impairment is the second most frequent clinical manifestation of CADASIL. The earliest sign in most cases is impairment in executive function and processing speed, detectable with dedicated tests such as the Wisconsin card-sorting and the trail-making tests.\textsuperscript{26,37} Executive dysfunction was present in all individuals aged 35–50 years in a series of 42 symptomatic patients,\textsuperscript{26} and is commonly associated with alterations in attention and memory.\textsuperscript{36,38} Cognitive decline becomes more extensive with ageing, with a progressive appearance of alterations in instrumental activities, verbal or visual memory, language, reasoning, and visuospatial abilities.\textsuperscript{38} There is, however, some preservation of recognition and semantic memory, and severe aphasia, apraxia, or agnosia is rare.\textsuperscript{36,39} Although cognitive decline is progressive and isolated in up to 10\% of patients, it most commonly worsens with recurrent strokes and, in the years preceding death, dementia is invariably associated with motor impairment, gait disturbances, and, later, pseudobulbar palsy.\textsuperscript{22,33,34}

**Other clinical manifestations**

Other clinical manifestations are uncommon in CADASIL and include seizures in 5–10\% of patients,\textsuperscript{13,22–24} intracerebral haemorrhages reported in a few cases (mostly in hypertensive patients),\textsuperscript{7,40} and, even more rarely, territorial infarcts (possibly coincidental),\textsuperscript{3} deafness,\textsuperscript{39} and parkinsonism.\textsuperscript{41} One feature of CADASIL is the absence of clinical manifestations indicative of organs other than the brain. An apparent exception is myocardial infarction reported in ten of 41 Dutch patients,\textsuperscript{33} although this feature was not reported in previous larger series or in a case–control study.\textsuperscript{20,35,40}

The temporal profile of clinical manifestations is shown in figure 1. Although each of the five main manifestations can appear in isolation, they mostly occur in succession. Migraine with aura first starts at around 30 years, ischaemic events and mood disturbances between 40 and 60 years, and dementia between 50 and 60 years.\textsuperscript{22,23,37} Patients have difficulties in walking at around 60 years, are bedridden at around 65 years, and have a life expectancy of about 65 years in men and 71 years in women.\textsuperscript{31} A few cases differ widely from this typical pattern with a very rapid or very slow progression or with a late age (>60 years) of clinical onset.\textsuperscript{14,46} Overall, CADASIL is a severe disease affecting young or middle-aged adults leading to a dramatic terminal stage within a mean of 25 years, when patients are fully bedridden and mute, and have dementia.

**Neuroimaging and other investigations**

Subcortical infarcts and leucoencephalopathy are best detected by use of MRI. Their presence is crucial for the diagnosis of CADASIL, particularly in patients with misleading presentations such as epilepsy, depression, hemiplegic migraine, progressive cognitive decline, or psychiatric manifestations.

**MRI**

Except for very rare cases of early migraine with aura and normal MRI at onset,\textsuperscript{33} MRI changes precede the onset of other symptoms by 10–15 years. These MRI changes appear at a mean age of 30 years, increase with age, and are present in all individuals carrying the mutation after the age of 35 years.\textsuperscript{12,35–37} The earliest and most frequent abnormalities are areas of increased signal on T2-weighted imaging or fluid-attenuated inversion recovery (figure 2). First appearing as punctiform or nodular, predominating in periventricular areas and in the centrum semiovale, these abnormalities later become more diffuse, mostly symmetrical, and mostly occur in the external capsule and the anterior part of the temporal lobes—a location highly suggestive of CADASIL (figure 3).\textsuperscript{35–37,49} The basal
ganglia and thalamus are also affected (a crucial difference from multiple sclerosis, a frequent mimic of CADASIL), as, on occasion, are the brainstem and corpus callosum.

Lacunar infarcts of variable shape, size, and number appear on T1-weighted imaging as punctiform or larger areas of decreased signal. These infarcts essentially occur within the same areas as T2 changes but occur later in life (figure 2). Diffusion-weighted MRI can show small areas of increased signal, suggestive of recent, sometimes multiple, infarcts. Other magnetic resonance findings include dilated perivascular spaces, sometimes with a typical “état criblé” (or status cribrosum) predominating in the basal ganglia, and microbleeds detected on gradient echo images (T2*) in 25–69% of patients; the frequency of microbleeds increases with age, blood pressure, haemoglobin A1c concentration, and extent of leuencephalopathy (figure 2).

Other magnetic resonance techniques have no diagnostic value in practice but are useful to study the clinical significance of MRI lesions. In the thalamus and areas of abnormal, but also of normal, white matter, use of diffusion tensor imaging can show an increase in water diffusion, which is better correlated with severity of executive dysfunction and clinical disability than are T2 hyperintensities. Follow-up studies of whole-brain diffusion have shown detectable changes over 1 or 2 years that correlated with clinical worsening, which suggests that diffusion histograms could be used as a predictor of disease progression and as a surrogate marker in future treatment trials. Measures of brain volume have shown brain atrophy, the extent of which is correlated with cognitive and disability scales. Brain atrophy progresses three times more rapidly in patients with CADASIL than in normal ageing and is independently associated with the mean apparent diffusion coefficient and the volume of lacunar lesions.
Other investigations
Apart from mutational screening and skin biopsy, which are used to confirm the diagnosis (see below), other investigations are not helpful for this purpose, although could be useful to exclude other disorders. The results of examination of cerebrospinal fluid, usual blood tests, electromyography, ultrasound studies, electrocardiography, and conventional spinal cord MRI are normal in most patients. Echocardiography results are also generally normal, although a high frequency of patent foramen ovale (47%) has been reported in an Italian series. Conventional cerebral angiography occasionally shows intracranial stenosis, but should not be undertaken because of a high rate of complications. Although patients with CADASIL have no ocular symptoms, various retinal abnormalities are common, such as arteriolar sheathing and narrowing, nerve fibre loss, and cotton-wool spots.

Pathology, genetics, and pathogenesis
Pathology
Macroscopic examination of the brain shows changes typical of chronic small-artery diseases of the brain: diffuse myelin pallor and rarefaction of the hemispheric white matter predominating in periventricular areas and centrum semiovale; lacunar infarcts located in white matter and basal ganglia; and dilated Virchow-Robin spaces. In the cortex, which was thought to be unaffected,
there is widespread neuronal apoptosis (particularly in layers three and five) that is more extensive in the presence of a large subcortical ischaemic lesion load (figure 4).\(^7\)

Microscopic and ultrastructural investigations (figure 5) show a specific arteriopathy affecting mainly the small penetrating cerebral and leptomeningeal arteries. This arteriopathy is characterised by a thickening of the arterial wall leading to lumen stenosis,\(^7\) a largely normal endothelium, the presence of a non-amyloid granular osmiophilic material within the media extending into the adventitia, and prominent morphological alterations of smooth-muscle cells.\(^15,73,75,76\) These cells can eventually disappear from the vessel wall. The granular osmiophilic material, a specific ultrastructural feature of CADASIL, is extracellular, located close to the cell surface of smooth-muscle cells, but it can also be occasionally found in capillaries (figure 5).

Although clinical manifestations are only cerebral, arteriopathy is also present in other organs, such as the spleen, liver, kidneys, muscle, aorta, and skin.\(^7\) The presence of granular osmiophilic material on electron-microscopic study of skin biopsy samples thus indicates a diagnosis of CADASIL,\(^7,77,79,81\) but the sensitivity of this test is variable.\(^84\) Immunostaining of skin samples with a NOTCH3 monoclonal antibody, which can reveal the accumulation of NOTCH3 protein in the vessel wall, is highly sensitive (85–95%) and specific (95–100%).\(^80,83\)

Molecular genetics

CADASIL is an autosomal dominant disease caused by mutations in NOTCH3. This gene encodes a single-pass transmembrane receptor of 2321 amino acids with an extracellular domain containing 34 epidermal growth factor repeats (EGFR), each including six cysteine residues, three Notch/Lin12 repeats, a single transmembrane domain, and an intracellular domain (containing seven ankyrin repeats;\(^18,84\) figure 6). More than 150 mutations have been reported in at least 50 pedigrees. NOTCH3 has 33 exons but all CADASIL mutations occur in exons 2–24, which encode the 34 EGFR, with strong clustering in exons 3 and 4, which encode EGFR 2–5 (>40% of mutations in >70% of families occur in these exons). Over 95% of mutations are missense mutations; others are small in-frame deletions or splice-site mutations.\(^15–49\) All mutations lead to an odd number of cysteine residues within a given EGFR (figure 6).\(^7,86,88–91\) De novo mutations have been reported but their exact frequency is unknown.\(^15,21,23\) Two homozygous patients have so far been described.\(^19,92\)

Genetic testing is the gold standard for the diagnosis of CADASIL. Screening of the 23 exons that encode the 34 EGFR has 100% specificity when a mutation leading to an odd number of cysteine residues within an EGFR is detected, and the sensitivity is close to 100%.\(^49,54,61,93\) Ultrastructural examination of a skin biopsy should be restricted to two rare situations: a negative molecular test (screening of the 23 exons) in a patient with clinical and MRI features highly suggestive of CADASIL; and the identification of a sequence variant of unknown significance not involving a cysteine residue.

Genetic testing is indicated if the patient has a characteristic clinical syndrome in combination with characteristic neuroimaging features or a positive family history, particularly if there is no history of hypertension. The need is more debatable if a patient without a family history has only migraine with aura and a few hypersignals on T2-weighted imaging. Unless there is a specific request from the patient, genetic testing is not done at our institution in such cases for the following reasons: white-matter abnormalities are common in migraine
General features of CADASIL

(partially migraine with aura); up to 30 years can elapse in CADASIL between the onset of migraine with aura and the first stroke or onset of cognitive decline; and there is no treatment for CADASIL at present. In asymptomatic adult relatives of patients with CADASIL, genetic testing raises the same psychological and ethical concerns as in other adult-onset neurological autosomal dominant disorders leading to dementia and premature death, such as Huntington’s disease. Screening has no benefit for asymptomatic children and is therefore not indicated. In the experience of the authors of this Review, requests for prenatal testing are rare.

Mechanisms underlying symptoms

Various studies with single photon emission computed tomography, PET, or MRI bolus tracking methods have shown an early decrease in cerebral blood flow and metabolism, which suggests chronic subcortical ischaemia. Compromised cerebral haemodynamics probably arises from both structural and functional changes in brain arteries. Autopsy studies in patients with CADASIL have shown stenosis of arterioles in the white matter, but not in the basal ganglia, another common site of lacunar infarcts. Additionally, vasoreactivity of small penetrating arteries is likely to be compromised by both vascular fibrosis and degeneration of smooth-muscle cells. Moreover, functional studies have indicated a blunted increase in cerebral blood flow response to carbon dioxide inhalation or acetazolamide infusion in patients with CADASIL. Reactivity is also altered in the skin microcirculation, with a delayed post-occlusive hyperaemia response.

Vascular alterations in CADASIL have been partly modelled in transgenic mice, in which the regulatory sequences of transgelin (TAGLN; SM22α) drive the expression of a human NOTCH3 protein with an archetypal CADASIL mutation (Arg90Cys) in smooth-muscle cells. These mice have impaired cerebral blood flow autoregulation and increased myogenic tone before changes on MRI are detectable and generally starts long before the first ischaemic events. Furthermore, infarcts are subcortical and migraine with aura is not seen in chronic hypertension-related small-artery diseases of the brain, which suggests a specific mechanism for migraine with aura in CADASIL. Studies of cortical spreading depression in transgenic mice with CADASIL might help to elucidate how mutations in NOTCH3 decrease the threshold for this event. Because CADASIL-linked migraine with aura is one of the best examples of symptomatic migraine, an understanding of this symptom might also prove crucial for migraine in general.

NOTCH3 function and association with disease pathogenesis

The Notch signalling pathway has a central role in the development of most vertebrate organs, with pleiotropic effects depending on dose and cellular context. Expression studies of late embryos and adult tissues of mice and human beings have shown that Notch3/NOTCH3 is predominantly expressed in vascular smooth-muscle cells, preferentially in small arteries. Genetically engineered mice without Notch3 have prominent structural defects of small arteries because of impaired differentiation and maturation of arterial smooth-muscle cells. Additionally, Notch3-null mice have strongly defective autoregulation of cerebral blood flow and vascular myogenic tone. However, total loss of Notch3 does not cause CADASIL pathology.

As with all NOTCH receptors, full-length NOTCH3 is initially synthesised as a single polypeptide chain, which subsequently undergoes constitutive proteolytic processing. NOTCH3 functions at the cell surface as a heterodimer composed of its extracellular domain (NOTCH3) non-covalently attached to the membrane-tethered intracellular domain (NOTCH3). Ligand binding initiates a series of proteolytic cleavages that release the NOTCH intracellular domain, which then translocates to the nucleus. Here, the NOTCH intracellular domain interacts with the transcription factor RBPJκ and co-activators and activates the transcription of target genes.

CADASIL mutations cause gradual accumulation of NOTCH3, without associated accumulation of NOTCH3. NOTCH3 forms microscopic aggregates around vascular smooth-muscle cells and pericytes of brain arteries and capillaries, in close proximity to deposits of granular osmiophilic material. However, whether NOTCH3 is part of the granular osmiophilic material is much debated.

(Continued)
Recent work strongly suggests that CADASIL mutations act through neomorphic (gain of novel function) mechanisms rather than compromised canonical NOTCH3 function. Reporter gene assays in cultured cells have shown that most CADASIL-associated NOTCH3 mutant alleles can activate RBP-Jκ transcription at wild-type levels. Moreover, genetic studies in a mouse model of CADASIL expressing a representative mutation in EGFR2 (Arg90Cys) indicated that the mutant receptor retains normal function in brain arteries despite accumulation of Notch3ECD95. By contrast, some naturally occurring mutations in the ligand-binding domain (EGFR 10 and 11) are predicted to result in a loss of functional Notch3 receptor. The in vivo relevance and functional importance of this observation with regard to clinical disease expression remain to be investigated. The current data indicate that the change in the number of cysteine residues, and not the effect of the mutation on signalling, is the common denominator in CADASIL. At present, the hypothesis is that gain of novel function for the mutant protein, which could arise from novel protein–protein interactions, is a likely mechanism for the CADASIL mutations. Thus, the unpaired cysteine residues within mutant NOTCH3 receptors might titrate key factors for viability and function of vascular smooth-muscle cells within the deposits of granular osmiophilic material; however, other mechanisms have been suggested.

**Treatment**

At present, there is no treatment of proven efficacy for CADASIL, either for the disease or for the main symptoms. Treatment is thus entirely pragmatic.

Migraine with aura rarely requires prophylactic treatment as the frequency of attacks is low in most patients. If required, the usual prophylactic drugs such as antiepileptic drugs or β blockers can be used. According to anecdotal reports, acetazolamide has been found to be effective. For acute treatment, we avoid vasoconstrictors such as ergot derivatives and triptans, and we prefer conventional analgesics and non-steroidal anti-inflammatory drugs.

Prevention of ischaemic attacks is based on the usual preventive measures for non-cardioembolic ischaemic stroke: use of antiplatelet drugs rather than anticoagulants (because of the increased risk of intracerebral haemorrhage) and treatment of vascular risk factors. Antihypertensive drugs are used when there is hypertension, although the putative risk of making the chronic hypoperfusion worse is not known. In patients with hypercholesterolaemia, we use statins because of their well-established preventive effects in arterial diseases and because data from animals indicate that these drugs increase cerebral blood flow.

The only randomised controlled trial to be done in CADASIL tested the efficacy of donepezil in patients with cognitive impairment. Inclusion criteria included a mini-mental state examination score of 10–27 or a trail-making test part B time score that is at least 1.5 SD below the mean, after adjustment for age and education. 168 patients were included and the follow-up was 18 weeks. Donepezil showed no effect on the primary endpoint (the cognitive subscale of the vascular Alzheimer’s disease assessment scale), whereas improvements were found on several measures of executive functions. However, the clinical relevance of this finding is not clear. Although essentially negative, this study is important because it shows the feasibility of multicentre trials in CADASIL and because it has implications for the design of future trials in subcortical vascular cognitive impairment. The limitations of the global cognitive scales originally designed for Alzheimer’s disease are highlighted, as is the need to use executive function tests as outcome measures, particularly those that measure processing speed such as the trail-making test.

The wide variability in the natural history of CADASIL hampers the design of any preventive trial. 602 patients would be needed in an interventional 2-year study with an assumed treatment effect of 40% and stroke occurrence as the outcome measure. Thus, the use of quantitative MRI measures as surrogate markers or for stratification could be necessary in future trials.

Rehabilitation, physiotherapy, psychological support, and nursing care are important in this severe chronic, debilitating disease, as well as genetic counselling, particularly for asymptomatic members at risk of carrying the mutation.

**Conclusions and future directions**

CADASIL has gained great interest as a model for the more common forms of ischaemic cerebral small-artery diseases and subcortical ischaemic vascular dementia. The clinical presentation, profile of neuropsychological deficits, and neuroimaging abnormalities of CADASIL closely resemble those of sporadic small-artery diseases with subcortical ischaemic vascular dementia. The main difference is, however, the absence in CADASIL of Alzheimer’s-type pathological changes that are common in elderly patients with sporadic small-artery diseases. CADASIL is thus a model of pure subcortical ischaemic vascular dementia, which was the rationale for a proof-of-concept trial in CADASIL that tested the efficacy of donepezil in subcortical ischaemic vascular dementia and that might also help to refine the criteria for vascular dementia.

Evidence is accumulating of other autosomal dominant small-artery diseases that closely resemble CADASIL but are unlinked to NOTCH3—as is the case for both the Swedish family reported by Sourander and Walinder and a large Portuguese and French family previously reported by Vererreault and colleagues. These families showed no granular osmiophilic material in small arteries, thus indicating distinct arteriopathies.
The genetic defects underlying these disorders remain to be identified but there are probably more diseases that meet the operational criteria of CADASIL (ie, a cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy). These disorders could be labelled consecutively in accordance with the order of gene identification (ie, CADASIL type 2 and 3).

CADASIL is now recognised as the most common cause of inherited stroke and vascular cognitive impairment in adults. The discovery of mutations in a cell-surface receptor on vascular smooth-muscle cells has facilitated targeted studies in various biological systems. Degenerative and functional abnormalities of small cerebral arteries play a central part in this progressive disorder. The mechanisms underlying stroke, migraine with aura, and vascular cognitive impairment are far from being understood. However, initial studies in patients with CADASIL and transgenic mice hold promise that these questions might eventually be answered. Meanwhile, efforts to investigate novel treatment strategies in randomised controlled trials should continue. Such trials are feasible and could provide insights beyond CADASIL, a disease that is a genetic variant of stroke and vascular dementia.

Contributors
HC, AJ, and MD contributed equally to the acquisition of all the necessary data for this Review and to the writing of the paper. ET-L and M-GB contributed to the idea and drafting of the Review.

Conflicts of interest
We have no conflicts of interest.

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