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# MORPHOMETRIC STUDY OF CEREBRAL ARTERIOLES IN CADASIL

by

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# ABSTRACT

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**MORPHOMETRIC STUDY OF CEREBRAL ARTERIOLES IN CADASIL**

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common hereditary small vessel disease (SVD) leading to vascular dementia. The cause of the disease is mutations in *NOTCH3* gene located at chromosome 19p13.1. The gene defect results in accumulation of granular osmiophilic material and extracellular domain of NOTCH3 at vascular smooth muscle cells (VSMCs) with subsequent degeneration of VSMCs. This arteriopathy leads to white matter (WM) rarefaction and multiple lacunar infarctions in both WM and deep grey matter (GM) visible in magnetic resonance imaging.

This thesis is focused on the quantitative morphometric analysis of the stenosis and fibrosis in arterioles of the frontal cerebral WM, cortical GM and deep GM (lenticular nucleus (LN), i.e. putamen and globus pallidus). It was performed by assessing four indicators of arteriolar stenosis and fibrosis: (1) diameter of arteriolar lumen, (2) thickness of arteriolar wall, (3) external diameter of arterioles and (4) sclerotic index. These parameters were assessed (a) in 5 elderly CADASIL patients with the mean age of onset 47 years and of death 63 years, (b) in a 32-year-old young CADASIL patient with the first ischemic episode at the age of 29 years and (c) a very old CADASIL patient aged 95 years, who suffered the first stroke at the age of 71 years. These measurements were compared with age-matched controls without stroke, dementia, hypertension, and cerebral amyloid angiopathy.

Morphometric analyses disclosed that in all age groups of CADASIL patients compared to corresponding controls there was significant narrowing of arteriolar lumen (stenosis) and fibrotic thickening of the walls (fibrosis) in the WM arterioles, although the significance of stenosis in the very old patient was marginal. In the LN arterioles there was only significant fibrosis without stenosis. These results suggest that the ischemic lesions and lacunar infarcts in the cerebral WM are mainly attributable to the stenosis of arterioles, whereas those in the LN are probably mainly due to hemodynamic changes of the cerebral blood flow.

In conclusion: The SVD of CADASIL is characterized by narrowing of lumina and fibrotic thickening of walls predominantly in the cerebral WM arterioles. On the other hand, in the LN the ischemic lesions and lacunar infarcts are most probably hemodynamic due to impaired autoregulation caused by the rigidity of fibrotic arterioles. The pathological cerebral arteriolar alterations begin to develop already at a relatively young age but the onset may be delayed to a remarkably old age. This underlines the well known great variability in the clinical picture of CADASIL. The very late onset of CADASIL may cause its underdiagnosis, because the strokes are common in the elderly and are attributed to common risk factors.

**Key words:** CADASIL, cerebral arterioles, morphometry, stenosis, fibrosis, sclerotic index, ageing.

# YHTEENVETO

Qing Miao

**CADASIL-POTILAIDEN AIVOVERISUONTEN MORFOMETRINEN TUTKIMUS**

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) on tavallisin perinnöllinen pienten suonten tauti joka johtaa vaskulaariseen dementiaan. CADASILin aiheuttaa mutaatiot *NOTCH3* geenissä, joka sijaitsee kromosomissa 19p13.1. Geenivirhe johtaa ns. granulaarisen osmiofiilisen materiaalin ja NOTCH3-proteiin solunulkoisen osan kertymiseen verisuonten sileiden lihassolujen pinnalle sekä tätä seuraavaan verisuonten sileiden lihassolujen degeneroitumiseen. Tämä arteriopatia johtaa aivojen valkean aineen harventumiseen ja lukuisiin pieniin (lakunaarisiin) infarkteihin sekä aivojen valkean että syvän harmaan aineen alueilla. Nämä muutokset ovat nähtävissä magneettikuvauksessa.

Tässä väitöskirjatyössä analysoidaan CADASIL-potilaiden aivojen [otsalohkon valkean aineen, aivokuoren harmaan aineen sekä syvän harmaan aineen (nucleus lenticulariksen, eli putamenin ja globus palliduksen)] arteriolien ahtautumista(stenoosia) ja sidekudostumista (fibroosia) kvantitatiivisen morfometrian menetelmin. Analyysi tehdään mittaamalla neljä arteriolien ahtautumisen ja sidekudostumisen indikaattoria: (1) arteriolien lumenin sisäläpimitta, (2) arteriolien seinämän paksuus, (3) arteriolien ulkoläpimitta ja (4) ns. skleroottinen indeksi. Nämä parametrit määritettiin (a) viidellä iäkkäällä CADASIL-potilaalla, joiden keskimääräinen sairastumisikä oli 47 vuotta ja kuolinikä 63 vuotta. (b) 32-vuotiaalla potilaalla, jolla ensimmäinen aivoverenkiertohäiriö oli 29 vuoden iässä ja (c) hyvin iäkkäällä, eli 95-vuotiaana kuolleella CADASIL-potilaalla, joka sai ensimmäisen aivohalvauksensa vasta 71-vuotiaana.

Morfometriset tutkimukset osoittivat, että kaikissa ikäryhmissä CADASIL-potilaiden aivojen valkean aineen arteriolien lumen oli kaventunut (stenoosi) ja seinämä paksumpi (fibroosi) kuin verrokkien suonissa, joskin hyvin iäkkään potilaan stenoosi-aste oli melko niukka. Sen sijaan lentiformis-tumakkeen alueella suonten seinämät olivat paksuuntuneita, mutta suonet eivät olleet kaventuneet.

Yhteenveto: Pienten suonten taudille CADASILille on tyypillistä pienten valtimoiden ahtautuminen (stenoosi) lumenin kapeutumisen ja seinämän fibroottisen paksuuntumisen vuoksi ensisijaisesti aivojen valkean aineen alueella. Toisaalta syvän harmaan aineen alueella (lentiformis-tumake) iskeemiset vauriot ja lakunaariset infarktit todennäköisimmin ovat luonteeltaan hemodynaamisia, johtuen fibroottisten arteriolien jäykistymisen myötä tapahtuvasta aivoverenkierron autoregulaation pettämisestä. CADASIL-potilailla aivojen arteriolien patologiset muutokset alkavat kehittyä jo varsin nuorella iällä, mutta joillakin yksilöillä niiden alkaminen voi viivästyä huomattavankin korkeaan ikään, mikä vastaa CADASILin hyvin tunnettua kliinisen kuvan erittäin suurta vaihtelua.

**Avainsanat:** CADASIL, aivojen arteriolit, morfometria, stenoosi, fibroosi, skleroottinen indeksi, ikääntyminen.

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| BBB | Blood brain barrier |
| CAA | Cerebral amyloid angiopathy |
| CADASIL | Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy |
| CBF | Cerebral blood flow |
| CBV | Cerebral blood volume |
| ECD | Extracellular domain |
| EGF | Epidermal growth factor |
| EM | Electron microscopy |
| GM | Grey matter |
| GOM | Granular osmiophilic material |
| H&E | Hematoxylin and eosin |
| LN | Lenticular nucleus |
| MCA | Middle cerebral artery |
| MRI | Magnetic resonance imaging |
| N3ECD | Extracellular domain of NOTCH3 |
| NICD | NOTCH3 intracellular domain |
| PBHs | Parenchymal brain hemorhages |
| PDGF | Platelet-derived growth factor |
| PET | Positron emission tomography |
| SD | Standard deviation |
| SI | Sclerotic index |
| SPECT | Single photon emission computed tomography |
| SVD | Small vessel disease |
| α-SMA | α-smooth muscle actin |
| T2w | T2-weighted |
| TACE | Tumor necrosis factor-alpha converting enzyme |
| TIA | Transient ischemic attack |
| VD | Vascular dementia |
| VEGF | Vascular endothelial growth factor |
| VSMCs | Vascular smooth muscle cells |
| WM | White matter |
| WMLs | White matter lesions |

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by their Roman numerals:

**I** **Miao Q**, Paloneva T, Tuominen S, Poyhonen M, Tuisku S, Viitanen M, Kalimo H. Fibrosis and stenosis of the long penetrating cerebral arteries: the cause of the white matter pathology in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Brain Pathol* 2004; 14: 358-364.

**II** **Miao Q**, Paloneva T, Tuisku S, Roine S, Poyhonen M, Viitanen M, Kalimo H. Arterioles of the lenticular nucleus in CADASIL. *Stroke* 2006; 37: 2242-2247.

**III** **Miao Q**, Kalimo H, Bogdanovic N, Kostulas K, Börjesson-Hanson A, Viitanen M. Cerebral arteriolar pathology in a 32-year-old patient with CADASIL. *Neuropathol Appl Neurobiol* 2006; 32: 455-458.

**IV** **Miao Q**, Tuisku S, Kalimo H, Viitanen M. CADASIL with very late onset and prolonged course. Manuscript.

# 1. INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common hereditary vascular dementia (VD) caused by cerebral small vessel disease (SVD). The underlying mutated gene is *NOTCH3* (Joutel et al. 1996), which is predominantly expressed in vascular smooth muscle cells (VSMCs) and pericytes (Joutel et al. 2000a, Prakash et al. 2002). Consequently, VSMCs in small (arterioles) and medium sized arteries throughout the body degenerate (Ruchoux and Maurage 1997). The major pathological findings in CADASIL include the depositions of granular osmiophilic material (GOM) (Baudrimont et al. 1993) and mutated extracellular domain of *NOTCH3* (N3ECD) (Joutel et al. 2000a) on and/or close to degenerating VSMCs, cerebral white matter (WM) lesions (WMLs) and lacunar infarcts in the cerebral WM and basal ganglia (Kalimo et al. 2008). Clinical symptoms in CADASIL are migraneous headache with aura, recurrent subcortical ischemic strokes, cognitive impairment progressing to dementia and psychiatric disorders (Chabriat et al. 1995a). CADASIL usually exhibits early characteristic magnetic resonance imaging (MRI) hyperintensities (Chabriat et al. 1998), which are very helpful in making the clinical diagnosis. The definite diagnosis can be established either by molecular genetic verification of a pathogenic mutation in *NOTCH3* gene or by demonstration of GOM or N3ECD in a skin biopsy (Joutel et al. 2001, Tikka et al. 2009).

Until year 2004, most studies concerning the arteriolar stenosis and fibrosis due to degeneration of VSMCs in CADASIL were mainly qualitative. In one semiquatitative study it was reported that there was rarely severe stenosis in small subcortical medullary arteries (Okeda et al. 2002). Brulin and others (Brulin et al. 2002) did quantitative analysis of cerebral capillaries in CADASIL by using electron microscopy (EM), where they did not find significant sclerotic index (SI) difference compared to controls. Thus, it is of a great interest for us to know if there are really quantitative morphometric changes of arteriolar stenosis and fibrosis – and if so, to what extent – so that we can explain the occurrence of lacunar infarcts and WMLs more reasonably in CADASIL. At same time, since the onset of the disease and natural course vary greatly in CADASIL (Kalimo et al. 2008), we want to follow the same quantitative morphometric data of the arteriolar stenosis and fibrosis in CADASIL patients of different ages so that we can trace the development of arteriopathy from young age to very old age. We believe that this kind of quantitative analysis will be of a great help to understand how these morphometric changes in the arterioles lead to cerebral lacunar infarcts and WMLs associated with CADASIL.

# 2. REVIEW OF THE LITERATURE

## 2.1. Cerebral small vessel disease and vascular dementia

Cerebral SVD is a heterogeneous group of diseases with similar risk factors, characteristic phenotypes, pathology and prognosis (Munoz 2003, Lammie 2005, Ferrer 2010, Román et al. 2002, Yamamoto et al. 2011). SVD affects small cerebral penetrating arteries or arterioles, causing characteristic pathogenic structural and functional alterations in each SVD entity. Since the penetrating arteries usually do not have distal collateralization the stenosis or occlusion of affected vessels causes ischemic injury (Menon and Kelley 2009, Ogata et al. 2011). Characterized by their etiological diversity, cerebral SVD may share several common pathological lesions: atherosclerosis, lipohyalinosis (fibrinoid necrosis), microaneurysms and hyaline arteriolosclerosis (Lammie 2002, Munoz 2003, Lammie 2005, Pantoni 2010, Pantoni and Simoni 2003). SVD is one of the causes of dementia, which (all forms together) is found in about 7% of general population older than 65 years of age, and 30% of people older than 80 (Bowler 2005, Hofman et al. 1991, Rocca et al. 1991a, Rocca et al. 1991b, Lobo et al. 2000). VD is the second most frequent dementing disorders after Alzheimer's disease, being the cause of about 20% of all demented patients (Knopman 2007, O'Brien et al. 2003, Román et al. 2004). Of VDs, SVD accounts for about 50% of patients (Munoz 2003, Chui 2007, Román et al. 2002). The other major causes of VDs include infarcts in strategic cerebral regions, large vessel disease with multiple cortical/subcortical infarcts (classic multiple infarct dementia), hypoperfusion syndrome and cerebral micro- and macrohaemorrhages (Wallin et al. 2003, Román et al. 2004).

## 2.2. Stroke and brain infarctions

The original definition of stroke by World Health Organization (WHO) is: abrupt onset of neurological deficit of cerebrovascular cause that persists beyond 24 hours (WHO 1978). Approximately 80% of stroke patients are ischemic in origin; the remaining 20% are non-traumatic haemorrhagic (Kalimo et al. 2002a). Ischemic stroke can be classified on the basis of etiology into five diagnostic subgroups: 1) large artery atherosclerosis, 2) cardioembolism, 3) SVD, 4) stroke of other determined etiology and 5) stroke of undetermined etiology (Adams et al. 1993, Goldstein et al. 2001). SVD mainly causes focal ischemic strokes, which account for about a quarter of all ischemic strokes (Sudlow and Warlow 1997, Sacco et al. 2006, Bejot et al. 2008). Lacunar infarcts are small cavitated ischemic infarcts of less than 15 mm in diameter. They are typically located in the cerebral WM or deep grey matter (GM), as well as in the brain stem. Some lacunar infarcts are clinically silent (Bamford et al. 1991). Lacunar infarcts are mainly due to intrinsic cerebral arteriolar abnormality, which include atherosclerosis, lipohyalinosis, microaneurysms and hyaline arteriolosclerosis (Lammie 2005, Wardlaw

2005). Non-traumatic intracranial haemorrhage is located either intraparenchymally or in the subarachnoid space (Kalimo et al. 2002a).

## 2.3. CADASIL

### 2.3.1. History

CADASIL is an autosomal dominant SVD and also considered to be the most common form of familial VD. In 1977 Sourander and Walinder described a disorder which they called hereditary multi-infarct dementia in a Swedish family (Sourander and Walinder 1977). The disorder was originally considered the first described CADASIL. However, it has been established that this disease is not CADASIL (Low et al. 2007). It appears retrospectively that van Bogaert and others probably described the first CADASIL patient in 1955 as a familial form of subcortical arteriosclerotic encephalopathy of Binswanger's (van Bogaert 1955, Kalimo et al. 2008). Since the 1970s, several European research groups have reported some patients with hereditary cerebrovascular disorders that mainly involve the cerebral WM and exhibit similar clinical and pathological findings. The names of the diseases which they described are chronic familial vascular encephalopathy (Stevens et al. 1977), familiäre zerebrale Gefässerkrankung or familiäre zerebrale arteriosklerose (Colmant 1980, Gerhard 1980), familial subcortical dementia with arteriopathic leukoencephalopathy (Davous and Fallet-Bianco 1991), autosomal dominant syndrome with stroke like episodes and leukoencephalopathy (Bousser et al. 1988, Tournier-Lasserve et al. 1991), familial disorder with subcortical ischemic strokes, dementia and leukoencephalopathy (Mas et al. 1992) and familial Binswanger's syndrome (Salvi et al. 1992, Gutierrez-Molina et al. 1994). The first CADASIL family in Finland was described in 1987 as hereditary multi-infarct dementia (Sonninen and Savontaus 1987). The present acronym of the disease was given in 1993 when the disease was found to be linked to chromosome 19 (Tournier-Lasserve et al. 1993). In 1996 the defective gene was found to be *NOTCH3* located at chromosome 19p13.1, which encodes a transmembrane receptor protein NOTCH3 (Joutel et al. 1996).

### 2.3.2. Epidemiology

CADASIL occurs in many different ethnic groups. It is estimated that at least over 500 CADASIL families exist worldwide (Kalimo and Kalaria 2005, Chabriat et al. 2009) and yet it is certainly still markedly underdiagnosed. Most of the patients are European Caucasians. In Germany there are alone over 200 genetically confirmed families (Opherk et al. 2004, Kalimo and Kalaria 2005) and more than 100 in France (Kalimo et al. 2002b) and the United Kingdom (Kalimo et al. 2002b, Adib-Samii et al. 2010). In Finland, there are 21 genetically confirmed CADASIL families with more than 100 patients. The precise prevalence and incidence are not known yet. In the United Kingdom, the prevalence has been estimated to be at least 1 per 100 000 (Markus et al. 2002). In Scotland, the

prevalence is about 4.15 per 100 000 (Razvi et al. 2005). The prevalence in Finland is estimated to be at least 4 per 100 000 (Kalimo et al. 2008). CADASIL has been reported to account for 2% of patients with lacunar infarcts and leukoaraiosis younger than 65 years of age (1 out of 48) and for 11% of those patients younger than 50 years of age (1 out of 9) (Dong et al. 2003), or 6.6% (2 out of 30) of non-hypertensive and non-diabetic patients with lacunar infarcts younger than 65 years of age (Cocho et al. 2011).

### 2.3.3. Clinical features

Women and men are equally affected. The overall course of CADASIL is highly variable between families, within same families, or even between monozygotic twins (Mykkänen et al. 2009). In general, the type of gene mutations do not influence the onset, clinical features and course of the disease (Singhal et al. 2004), but younger age of onset for stroke, immobilization and death has been reported to associate with mutation of p.C174Y (Opherk et al. 2004) and younger age of onset for stroke with mutation of p.C455R (Arboleda-Velasquez et al. 2002). The mutation of p.R153C is also reported to be associated with presence of microhaemorrhages (microbleeds) in brain (Lesnik Oberstein et al. 2001). The major symptoms of CADASIL are migraneous headache with aura, transient ischemic attack (TIA) or stroke, cognitive impairment progressing to dementia and psychiatric disorders (Chabriat et al. 1995a, Dichgans et al. 1998, Desmond et al. 1999, Kalimo et al. 2008). These symptoms vary according to age, severity and duration of disease. Although each of the four main symptoms can appear individually, they most often occur in succession. The age of onset of CADASIL varies greatly, which in fact depends on the criterion used for the onset of the disease. Because migraine is common as an independent disease, the age of onset is usually given on the basis of the first ischemic attack. However, it is difficult to distinguish between TIA and very severe and long lasting migraneous aura. In general, migraine with aura starts at about 30 years of age, TIA or stroke or psychiatric disorder appears between 40 and 60 years of age, and dementia manifests between 50 and 60 years of age. Patients begin to have walking disturbance at about 60 years of age and become bedridden at about 65 years of age (Chabriat et al. 1995a, Dichgans et al. 1998, Desmond et al.1999, Opherk et al. 2004, Chabriat et al. 2009). Mean age of death is about 60 years in early studies (Chabriat et al. 1995a, Dichgans et al. 1998, Desmond et al. 1999) and 68 years (about 65 years for men and 71 years for women) in a newer study (Opherk et al. 2004). After the onset, CADASIL progresses slowly with aggravation associated with TIA or stroke episodes and leads to death within a mean of 23 years (range from onset to death: 3 to 43 years) (Opherk et al. 2004, Kalimo et al. 2008). In addition to this general rule, there are some exceptions with a very rapid or very slow progression or with a late clinical onset age with minimal symptoms (after 60 years of age) (Opherk et al. 2004, Mourad et al. 2006, Lee et al. 2009).

Recurrent headache occurs in up to 60% of the patients and 20-40% of them are most often in the form of migraine with aura. When present, migraine with aura is usually the

first symptom. The onset varies from 6 to 54 years of age and an average age is about 30 years (mean age in women is 26 years and mean age in men is 36 years) (Chabriat et al. 1995a, Chabriat et al. 1995b, Dichgans et al. 1998, Desmond et al. 1999, Vahedi et al. 2004, Kalimo et al. 2008, Chabriat et al. 2009). The aura usually involves the visual and sensory systems, lasting 20-30 minutes followed by a headache lasting several hours. The half of patients has often atypical manifestations such as basilar, hemiplegic and long lasting aura. Some patients have exceptionally severe presentations with confusion, fever, meningitis and even coma (Feuerhake et al. 2002, Schon et al. 2003). On the other hand, the severity of migraine may also decrease, or migraine becomes less frequent or even disappears after the first ischemic attack (Dichgans et al. 1998). In women the migraine can be aggravated in late pregnancy or puerperium (Roine et al. 2005). The triggering factors of migraine with aura are the same as those for migraine (Vahedi et al. 2004). In some CADASIL families, migraine with aura is the prominent symptom (Chabriat et al. 1995b, Verin et al. 1995, Vahedi et al. 2004).

The key symptom of CADASIL is TIA or stroke which exists in about 60-85% of symptomatic patients. The estimated incidence is about 10.4 per 100 patient-years (Peters et al. 2004a). The onset of TIA or stroke generally occurs in midlife, most frequently around 40 to 60 years of age (at a mean age of 49 years), but it can vary from 25 to 70 years of age (Chabriat et al. 1995a, Dichgans et al. 1998, Desmond et al. 1999, Kalimo et al. 2008, Adib-Samii et al. 2010). The ischemic episodes most often manifest as lacunar syndrome (i.e. pure motor or sensory deficit, ataxic hemiparesis, sensory-motor deficit and dysarthria clumsy hand syndrome). Most patients have two to five recurrent strokes over several years and progressively lead to gait disturbance, urinary urgency with or without incontinence, locomotion difficulty and pseudobulbar palsy in the late stage of the disease. Ischemic infarcts may also develop silently. In 5 to 20% of CADASIL patients subcortical dementia develops without identified episode of stroke (Mellies et al. 1998, O'Sullivan et al. 2003). Although all infarcts occur in subcortical region, i.e. in the cerebral WM and basal ganglia, they may also occur in the brain stem and rarely in the spinal cord (Chabriat et al. 1999b). Big parenchymal brain hemorhages (PBHs) are uncommon in CADASIL, although it has been reported that 25% of symptomatic CADASIL patients have PBHs (Choi et al. 2006), but this high percentage may be related to the ethnicity of the patients in the study.

Cognitive impairment progressing to dementia is the second most common clinical manifestation in CADASIL, which exists in about 60% of symptomatic patients (Dichgans et al. 1998, Desmond et al. 1999). Cognitive impairment usually occurs between 50 and 60 years of age (Kalimo et al. 2008, Chabriat et al. 2009). The earliest and most prominent manifestation in most patients is impairment in executive function, which can in some CADASIL patients change in the very early phase of the disease even before TIA or stroke (Amberla et al. 2004). Executive dysfunction is reported to be present in all symptomatic patients aged 35-50 years and is commonly associated with alterations in attention and memory (Dichgans 2009, Taillia et al. 1998, Peters

et al. 2005a, Buffon et al. 2006, Charlton et al. 2006). Cognitive impairment becomes more extensive with ageing. Although cognitive impairment is progressive, it most commonly worsens with recurrent strokes (Amberla et al. 2004). The increasing severity of cognitive impairment gradually leads to a subcortical type of dementia, which is manifest in 80% of the patients by the age of 65. The dementia fulfils the suggested criteria for a subcortical ischemic VD (Erkinjuntti 2003). Dementia is often associated with motor impairment, gait disturbances, urinary incontinence and pseudobulbar palsy (Dichgans et al. 1998, Opherk et al. 2004, Peters et al. 2004a). However, episodic memory is preserved untill relatively late in the course and severe aphasia, apraxia and agnosia are rare (Amberla et al. 2004, Peters et al. 2005a, Buffon et al. 2006, List et al. 2011).

Psychiatric disorders occur in about 20-40% of CADASIL patients (Chabriat et al. 1995a, Dichgans et al. 1998, Desmond et al. 1999, Valenti et al. 2008). Depression is the most common manifestation. Other manifestations include manic episode, panic disorder, hallucination and delusion. One recent paper reports that apathy is also a major symptom independent from depression, which is present in about 40% of patients (Reyes et al. 2009). In one large French family psychiatric disorders are common, which is considered to represent a variant phenotype of CADASIL (Verin et al. 1995). Schizophrenia is rare in CADASIL patients (Lagas and Juvonen 2001). In addition, agitation, aggression, dysthymia and emotional lability have been described (Chabriat and Bousser 2007, Valenti et al. 2008).

There are also some other uncommon symptoms in CADASIL. Seizures exist in about 10% of patients (Velizarova et al. 2011). Deafness (Tournier-Lasserve et al. 1991), Parkinsonism (van Gerpen et al. 2003, Wegner et al. 2007, Valenti et al. 2011, our own unpublished observations), strokes due to large cerebral artery stenosis and territorial infarcts (Choi et al. 2005), and progressive ataxia and spastic paraparesis (Vedeler and Bindoff 2011) are also reported. Uncommon symptoms may also be due to another coexistent disease, which cannot be diagnosed with the clinical examinations *in vivo* but requires an autopsy, e.g. multiple system atrophy (Rice et al. 2011).

A systemic disease, such as myocardial infarction, exists in some Dutch patients (Lesnik Oberstein et al. 2003a), although it is controversial (Cumurciuc et al. 2006). However, some other studies confirm that CADASIL patients may be at risk for life-threatening arrhythmias (Rufa et al. 2007, Piccirillo et al. 2008). The mitochondrial pathology and mitochondrial DNA mutations are also reported (Finsterer 2007, Finnilä et al. 2001). Renal insufficiency has also been described in CADASIL (Kusaba et al. 2007, Guerrot et al. 2008).

### 2.3.4. Risk factors

The risk factors for CADASIL are largely same as those classical vascular risk factors, such as smoking, high serum cholesterol and hypertension, although the latter is

uncommon in CADASIL patients (Singhal et al. 2004, Sternic et al. 2009, Adib-Samii et al. 2010).

### 2.3.5. MRI

Subcortical infarcts and WMLs can be detected by MRI. Except for very early migraine with aura (Golomb et al. 2004), abnormal MRI signals precede usually the onset of other symptoms by 10-15 years. These MRI signals may be detectable already before 20 years of age, the mean age for their appearance being 30. They increase with age or clinical severity and are present in all individuals carrying the mutations over age of 35 (Bousser et al. 1994, Chabriat et al. 1998, Dichgans et al. 1999). The earliest and most frequent abnormalities are hyperintensities on T2-weighted (T2w) imaging or fluid-attenuated inversion recovery, which first appears as punctiform or nodular in the periventricular areas and centrum semiovale. Later MRI signals characteristically become more diffuse and symmetrical in the anterior part of temporal lobes and external capsule (Auer et al. 2001, O'Sullivan 2001, Markus et al. 2002) (Figure 1A). Basal ganglia, thalamus and even brainstem and corpus callosum are also affected (Chabriat et al. 1999b, Jacqmin et al. 2010, Bentley et al. 2011). In symptomatic CADASIL patients who have experienced strokes, lacunar infarcts (or lesions) can be detected on T1-weighted imaging as punctiform or larger areas of hypointensities (Chabriat et al. 1998, van den Boom et al. 2002, van den Boom et al. 2003a, Herve et al. 2009). Diffusion-weighted MRI can also show small areas of hyperintensities, suggestive of recent or multiple infarcts (Gobron et al. 2006, Saito et al. 2011). Diffusion tensor MRI can also reveal microstructural change with enlargement of the extracellular space due to vasogenic edema possibly associated with myelin and axonal damage in normal appearing WM outside the T2w hyperintensities (Chabriat et al. 1999a).

MRI can also detect microhaemorrhages corresponding to small hypointensities, which are present in 25-69% of the patients on T2w gradient echo MRI. Microhaemorrhages occur mainly in the cortical or deep GM and to a lesser extent in cerebral WM. The frequency of microhaemorrhages increases while age, blood pressure, haemoglobin $A_{1c}$ concentration and extent of WMLs increase (Lesnik Oberstein et al. 2001, Dichgans et al. 2002, van den Boom et al. 2003a, Viswanathan et al. 2006, Viswanathan and Chabriat 2006). They are more common in patients with anticoagulant therapy (Lesnik Oberstein et al. 2001). The significance of microhaemorrhages for the pathogenesis of CADASIL is still uncertain, since they are clinically silent and most common in the relatively spared GM, 82% being outside the area of T2w hyperintensities or ischemic lesions. Thus, microhaemorrhages are considered to be largely an independent manifestation of the underlying arteriopathy and may predict an increased risk of PBHs (Lesnik Oberstein et al. 2001, Dichgans et al. 2002).

### 2.3.6. Hemodynamics

Several studies have demonstrated reduced cerebral blood flow (CBF) in CADASIL patients by using several functional imaging methods such as single photon emission computed tomography (SPECT) (Mellies et al. 1998), positron emission tomography (PET) (Chabriat et al. 1995c, Tuominen et al. 2004) and MRI bolus tracking method (Chabriat et al. 2000). Already at the presymptomatic stage the CBF in the WM becomes reduced and oxygen extraction fraction becomes increased. The reduction in CBF becomes more obvious beyond the age of 30 years. At the symptomatic stages, CBF in the GM begins to reduce. In the dementia stage cerebral metabolic rate of oxygen and glucose start to reduce, indicating the tissue loss (Chabriat et al. 1995c, Tuominen et al. 2004). Reduced cerebral blood volume (CBV) (Chabriat et al. 2000, Bruening et al. 2001) is also reported within areas of T2w hyperintensities in the WM and is more severe in the demented than in the nondemented patients. Prolonged arteriovenous cerebral transit time in both disabled and nondisabled CADASIL patients has been demonstrated by using Doppler sonography (Liebetrau et al. 2002). In addition, the hemodynamic reserve is also reduced in CADASIL patients, since the acetazolamide induced increase in CBF and CBV is lower in T2w hyperintensity areas (Chabriat et al. 2000). In the systemic vasculature, the impaired hemodynamic reserve in arterioles of the forearm of CADASIL patients leads to reductions in both basal and stimulated blood flow (Stenborg et al. 2007). The reduced retinal capillary blood flow is also reported in CADASIL (Harju et al. 2004).

### 2.3.7. Major pathological features

Although symptoms of CADASIL are almost exclusively neurological, vascular lesions are present in the small and medium sized arteries of almost all organs (Ruchoux et al. 1994, Ruchoux et al. 1995, Schröder et al. 1995). These lesions are present in the arterioles supplying the peripheral nerves, skin, muscle and viscera. The skin biopsy shows marked arteriolar damages which include the abnormal endothelium, alterations of the basal lamina and degeneration of VSMCs (Brulin et al. 2002). EM also shows that GOM is deposited near degenerating VSMCs (Ruchoux et al. 1994, Mayer et al. 1999) (Figure 1E), which appears to begin already before 20 years of age (Tuominen et al. 2001), even at the age of 13 (Okumura et al. 2007). GOM is also present in degenerating VSMCs of biopsies from muscle or peripheral nerve (Ruchoux et al. 1995, Schröder et al. 1995). N3ECD also accumulates on the degenerating VSMCs (Joutel et al. 2001). N3ECD is a major component of GOM (Ishiko et al. 2006). Other components of GOM include clusterin and collagen 18α1/endostatin (Arboleda-Velasquez et al. 2011). In a human foetus with a *NOTCH3* mutation vessel wall was reported to be normal (Saskia et al. 2008).

In the brain the lacunar infarcts are present in the cerebral WM, deep GM, as well as brain stem. The WMLs (partial or total loss of axons, myelin rarefaction that spares U fibers, astrogliosis, dilatated perivascular spaces, diffuse vacuolization of the cerebral WM and

reactive gliosis) are widespread, whereas the cortical GM is largely preserved well. On the other hand, cortical infarcts (Paquet et al. 2010, Jouvent et al. 2011) and stenosis of large arteries (Gong et al. 2010) have also been reported. Histopathology of cerebral WM arterioles shows non-atherosclerotic and non-amyloid arteriolosclerotic changes mainly involving the tunica media of the arterioles. On hematoxylin and eosin (H&E) staining granular basophilic materials accumulate in the degenerating tunica media (Figure 1D), which are also positive for periodic acid Schiff (Ruchoux and Maurage 1997) and immunohistoreactive for N3ECD (Joutel et al. 2000a) (Figure 1B) and various types of collagen and laminin. Degeneration of VSMCs is obvious in immunohistostaining for α-smooth muscle actin (α-SMA) (Figure 1C). EM studies also reveal the presence of GOM in cerebral arterioles (Baudrimont et al. 1993).

So far, there are over 100 CADASIL patients whose brains have been examined in autopsy studies reported world wide (Table 1). Of these patients, 21 patients have been studied quantitatively in the morphometry of cerebral arterioles, including six patients in the studies **I-III** of this thesis.

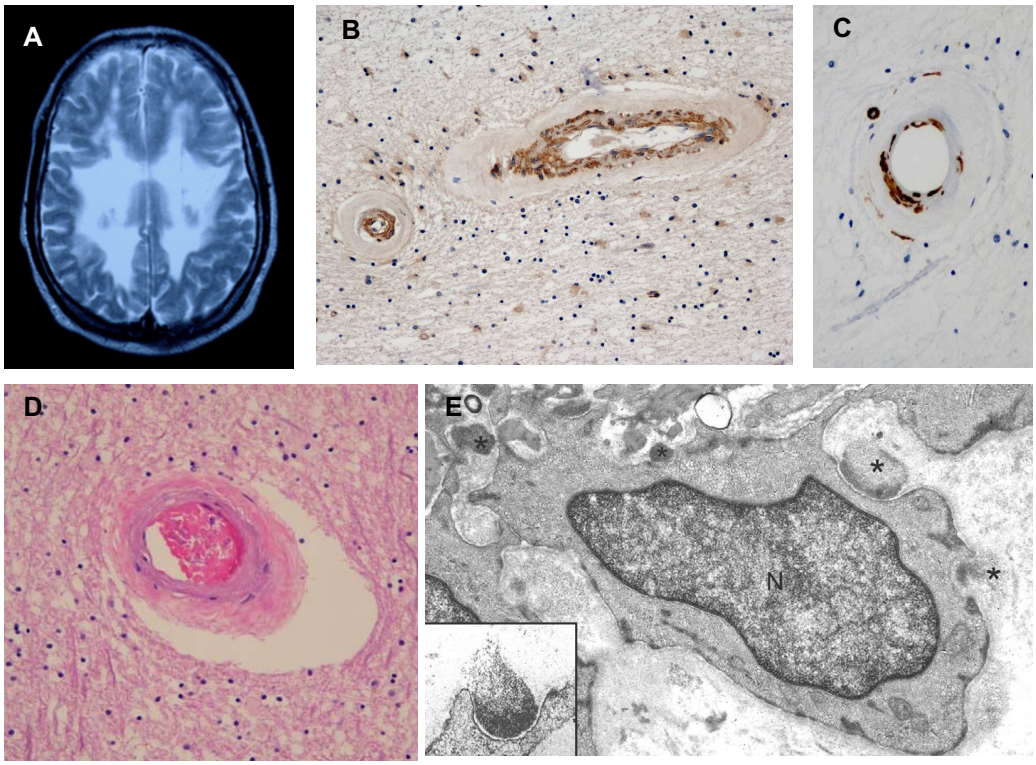



**Figure 1.** A, T2w-MRI confluent hyperintensity in a moderately demented CADASIL patient; B, Immunohistostaining for NOTCH3 extracellular domain (N3ECD) in cerebral white matter (WM) arterioles; C, Immunohistostaining for α-smooth muscle actin in cerebral WM arterioles; D, H&E staining of cerebral WM arterioles; E, Electron microscopy of a dermal artery: deposition of granular osmiophilic material (GOM *) in indentations (notches) of degenerating smooth muscle cells and between these cells. (N = nucleus)

**Table 1.** Some Information About CADASIL Patients Involved in Cerebral Autopsy Studies Reported Since 1977

| Patients | Authors | Year | Name of journal | Sex | mutation types | Age of onset | Age of death | Duration of disease in years | Investigation coverage | | Other information |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | parenchyma | vessels | |
| 1 | Stevens et al. | 1977 | Lancet 1:1364-5 | | | 39-57 | 56-65 | | yes | yes | |
| 2 | Sonninen et al. | 1987 | Eur Neurol 27:209-15 | F | | 55 | 56 | 1 | yes | | |
| 3 | Davous et al. | 1991 | Rev Neurol (Paris) 147:376-84 | M | | 47 | 54 | 7 | yes | yes | |
| 4 | Berthier et al. | 1992 | Rev Neurol (Paris) 148:146-9 | M | | 34 | 44 | 10 | yes | yes | |
| 5 | Baudrimont et al. | 1993 | Stroke 24:122-5 | F | | 52 | 59 | 7 | yes | yes | |
| 6 | Gray et al. | 1994 | Neuropathol Appl Neurobiol 20:22-30 | M | | 40 | 49 | 9 | yes | yes | |
| 7 | Gutiérrez-Molina et al. | 1994 | Acta Neuropathol 87:98-105 | F | | 46 | 55 | 9 | yes | yes | |
| 8 | Clair et al. | 1995 | J Med Genet 32:57-60 | F | | | | | yes | yes | |
| 9 | Clair et al. | 1995 | J Med Genet 32:57-60 | M | | | | | yes | yes | |
| 10 | Ragno et al. | 1995 | Ann Neurol 38:231-6 | F | | 44 | 44 | 1 | yes | yes | |
| 11 | Jung et al. | 1995 | J Neurol Neurosurg Psychiatry 59:138-43 | M | | | 59 | | yes | yes | |
| 12 | Ruchoux et al. | 1995 | Acta Neuropathol 89:500-12 | | | | 54 | | | yes | |
| 13 | Bergmann et al. | 1996 | Acta Neuropathol 92:341-50 | M | | 25 | 48 | 23 | yes | yes | |
| 14 | Malandrini et al. | 1996 | Acta Neuropathol 92:115-22 | F | | 33 | 41 | 8 | yes | yes | |
| 15 | Hedera et al. | 1997 | J Neurol Sci 146:27-33 | M | | 54 | 67 | 13 | yes | yes | |
| 16 | Hedera et al. | 1997 | J Neurol Sci 146:27-33 | F | | 58 | 68 | 10 | yes | yes | |
| 17 | Hedera et al. | 1997 | J Neurol Sci 146:27-33 | M | | 43 | 57 | 14 | yes | yes | |
| 18 | Hedera et al. | 1997 | J Neurol Sci 146:27-33 | M | | 51 | 59 | 8 | yes | yes | |
| 19 | Rubio et al. | 1997 | Acta Neuropathol 94:247-54 | F | | 54 | 62 | 8 | yes | yes | |
| 20 | Nishio et al. | 1997 | Rinsho Shinkeigaku 37:910-6 | | | 63 | 75 | 12 | yes | yes | |
| 21 | Caronti et al. | 1998 | Acta Neurol Scand 98:259-67 | M | | | 49 | | | yes | |
| 22 | Marrero Falcon et al. | 1999 | Neurologia 14:275-82 | | | | | | | | |
| 23 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R153C | | | | yes | yes | |
| 24 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R153C | | | | yes | yes | |
| 25 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R169C | | | | yes | yes | |
| 26 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R90C | | | | yes | yes | |
| 27 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R182C | | | | yes | yes | |
| 28 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R141C | | | | yes | yes | |
| 29 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | R110C | | | | yes | yes | |
| 30 | Joutel et al. | 2000 | J. Clin. Invest 105:597-605 | | Deletion | | | | yes | yes | |
| 31 | Binzer et al. | 2000 | Ugeskr Laeger 162:1739-42 | | R133C | | | | | | |
| 32 | Mikol et al. | 2001 | Rev Neurol (Paris) 157:655-67 | | | 59 | 67 | 8 | yes | yes | |
| 33 | Mikol et al. | 2001 | Rev Neurol (Paris) 157:655-67 | | | | 63 | | yes | yes | |

| Patients | Authors | Year | Name of journal | Sex | mutation types | Age of onset | Age of death | Duration of disease in years | Investigation coverage parenchyma | vessels | Other information |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | Okeda et al. | 2002 | Stroke 33:2565-9 | M | | 10 | 75 | 65 | yes | yes | Quantitative study |
| 35 | Brulin et al. | 2002 | Acta Neuropathol 104:241-8 | | | | 57 | | yes | yes | Quantitative study |
| 36 | Brulin et al. | 2002 | Acta Neuropathol 104:241-8 | | | | | | yes | yes | Quantitative study |
| 37 | Brulin et al. | 2002 | Acta Neuropathol 104:241-8 | | | | | | yes | yes | Quantitative study |
| 38 | Brulin et al. | 2002 | Acta Neuropathol 104:241-8 | | | | | | yes | yes | Quantitative study |
| 39 | Brulin et al. | 2002 | Acta Neuropathol 104:241-8 | | | | 59 | | yes | yes | Quantitative study |
| 40 | van den Boom et al. | 2002 | Radiology 224:791-6 | | | | | | yes | yes | |
| 41 | Ferrer et al. | 2002 | Neuroscience Letters 321:21-4 | F | | | 65 | | yes | yes | |
| 42 | Ferrer et al. | 2002 | Neuroscience Letters 321:21-4 | M | | | 68 | | yes | yes | |
| 43 | Dichgans et al. | 2002 | Stroke 33:67-71 | F | R110C | | 28 | | yes | yes | |
| 44 | Dichgans et al. | 2002 | Stroke 33:67-71 | M | R117F | | | | yes | yes | |
| 45 | Dichgans et al. | 2002 | Stroke 33:67-71 | M | R133C | | | | yes | yes | |
| 46 | Dichgans et al. | 2002 | Stroke 33:67-71 | M | C174Y | | | | yes | yes | |
| 47 | Dichgans et al. | 2002 | Stroke 33:67-71 | M | C185R | | | | yes | yes | |
| 48 | Dichgans et al. | 2002 | Stroke 33:67-71 | M | | | | | yes | yes | |
| 49 | Dichgans et al. | 2002 | Stroke 33:67-71 | F | | | 64 | | yes | yes | |
| 50 | Mesulam et al. | 2003 | Neurology 60:1183-5 | M | | 30 | 36 | 6 | yes | | |
| 51 | Posada et al. | 2003 | Neurologia 18:229-33 | | R110C | | | | | | |
| 52 | Thijs et al. | 2003 | J Neurol Neurosurg Psychiatry 74:790-2 | M | R169C | 54 | 64 | 10 | yes | yes | |
| 53 | Rafalowska et al. | 2003 | Acta Neuropathol 106:569-74 | M | | 45 | 51 | 6 | yes | yes | |
| 54 | Rafalowska et al. | 2003 | Acta Neuropathol 106:569-74 | F | | 45 | 52 | 7 | yes | yes | |
| 55 | Rafalowska et al. | 2003 | Acta Neuropathol 106:569-74 | M | | 32 | 38 | 6 | yes | yes | |
| 56 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | R133C | 38 | | | yes | yes | |
| 57 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | R133C | 51 | | | yes | yes | |
| 58 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | R90C | 37 | | | yes | yes | |
| 59 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 47 | | | yes | yes | |
| 60 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | R213K | 63 | | | yes | yes | |

| Patients | Authors | Year | Name of journal | Sex | mutation types | Age of onset | Age of death | Duration of disease in years | Investigation coverage | | Other information |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | parenchyma | vessels | |
| 61 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 59 | | | yes | yes | |
| 62 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 34 | | | yes | yes | |
| 63 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 40 | | | yes | yes | |
| 64 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | | | 54 | | | yes | yes | |
| 65 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | | 39 | | | yes | yes | |
| 66 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | | 33 | | | yes | yes | |
| 67 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 42 | | | yes | yes | |
| 68 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 46 | | | yes | yes | |
| 69 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | R133C | 42 | | | yes | yes | |
| 70 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | R133C | | | | yes | yes | |
| 71 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | | | 38 | | | yes | yes | |
| 72 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | C174F | 52 | | | yes | yes | |
| 73 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | R169C | 45 | | | yes | yes | |
| 74 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 48 | | | yes | yes | |
| 75 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 50 | | | yes | yes | |
| 76 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | C174R | 50 | | | yes | yes | |
| 77 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | | 41 | | | yes | yes | |
| 78 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 61 | | | yes | yes | |
| 79 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 35 | | | yes | yes | |
| 80 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | M | | 63 | | | yes | yes | |
| 81 | Santa et al. | 2003 | J Neurol Sci 212:79-84 | F | | 40 | | | yes | yes | |
| 82 | Brulin et al. | 2003 | Neuropathol Appl Neurobiol 29:400-10 | M | | | 57 | | | yes | |
| 83 | Brulin et al. | 2003 | Neuropathol Appl Neurobiol 29:400-10 | M | | | 64 | | | yes | |
| 84 | Brulin et al. | 2003 | Neuropathol Appl Neurobiol 29:400-10 | F | | | 43 | | | yes | |
| 85 | Brulin et al. | 2003 | Neuropathol Appl Neurobiol 29:400-10 | F | | | 57 | | | yes | |
| 86 | Brulin et al. | 2003 | Neuropathol Appl Neurobiol 29:400-10 | F | | | 59 | | | yes | |
| 87 | Miao et al. | 2004 | Brian pathol 14:358-64 | M | R133C | | 63 | | yes | yes | Quantitative study |

| Patients | Authors | Year | Name of journal | Sex | mutation types | Age of onset | Age of death | Duration of disease in years | Investigation coverage parenchyma | vessels | Other information |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 88 | Miao et al. | 2004 | Brian pathol 14:358-64 | M | R133C | | 68 | | yes | yes | Quantitative study |
| 89 | Miao et al. | 2004 | Brian pathol 14:358-64 | F | R133C | | 63 | | yes | yes | Quantitative study |
| 90 | Miao et al. | 2004 | Brian pathol 14:358-64 | F | R133C | | 63 | | yes | yes | Quantitative study |
| 91 | Miao et al. | 2006 | Neuropathol Appl Neurobiol 32:455-8 | M | C174R | | 32 | | yes | yes | Quantitative study |
| 92 | Miao et al. | 2006 | Stroke 37:2242-7 | M | R133C | | 60 | | yes | yes | Quantitative study |
| 93 | Gray et al. | 2007 | J Neuropathol Exp Neurol 66:597-607 | M | R110C | | 52 | | yes | yes | Apoptosis study |
| 94 | Gray et al. | 2007 | J Neuropathol Exp Neurol 66:597-607 | F | R110C | | 70 | | yes | yes | Apoptosis study |
| 95 | Gray et al. | 2007 | J Neuropathol Exp Neurol 66:597-607 | M | exon 4, nt 583, CGCTGC | | 65 | | yes | yes | Apoptosis study |
| 96 | Gray et al. | 2007 | J Neuropathol Exp Neurol 66:597-607 | F | exon 3, nt 291, TGGTGT | | 69 | | yes | yes | Apoptosis study |
| 97 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | F | R153C | 36 | 44 | 8 | yes | yes | Quantitative study |
| 98 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | M | R141C | 42 | 52 | 10 | yes | yes | Quantitative study |
| 99 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | F | R133C | 47 | 53 | 6 | yes | yes | Quantitative study |
| 100 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | M | R558C | 44 | 55 | 11 | yes | yes | Quantitative study |
| 101 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | M | R169C | 47 | 59 | 12 | yes | yes | Quantitative study |
| 102 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | M | R169C | 51 | 61 | 10 | yes | yes | Quantitative study |
| 103 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | M | R141C | 53 | 63 | 10 | yes | yes | Quantitative study |
| 104 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | M | R141C | 52 | 65 | 13 | yes | yes | Quantitative study |
| 105 | Yamamoto et al. | 2009 | Stroke 40:2004-11 | F | R133C | 50 | 68 | 18 | yes | yes | Quantitative study |
| 106 | Battisti et al. | 2009 | Clin Neuropathol 28:358-61 | | | | | | | | Apoptosis study |
| 107 | Paquet et al. | 2010 | Acta Neuropathol 120:813-20 | M | R141C | | 53 | | yes | yes | |
| 108 | Jouvent et al. | 2011 | Stroke 42:e27-30 | M | | 50 | 53 | 3 | yes | yes | |
| 109 | Rice et al. | 2011 | Parkinsonism Relat Disord 17:390-2 | F | C466Y | 52 | 59 | 7 | yes | yes | |

### 2.3.8. Genetics

After the disease locus is mapped to chromosome 19 (Tournier-Lasserve et al. 1993), the mutation of *NOTCH3* gene is identified as the cause of the disease (Joutel et al. 1996). *NOTCH3* is located at chromosome 19p13.1 and it encodes a transmembrane receptor protein, which functions as a transcription factor. All mutations in CADASIL are located in epidermal growth factor (EGF) repeat domains with a strong cluster at the N-terminal part of the protein (Joutel et al. 1997). Most of the mutations (almost 60%) are located within exons 3, 4, 5 and 8, which encode the first five EGF repeats (Kalimo et al. 2008, Tikka et al. 2009). The mutational spectrum includes missense point mutations (Joutel et al. 1997), small in-frame deletions, some of which are splice site mutations with the loss of cysteine residues, as well as duplications (Dichgans et al. 2000, Joutel et al. 2000b, Dichgans et al. 2001, Tikka et al. 2009). The majority of mutations (over 95%) are missense point mutations in exons 2-24. So far, there are over 200 different point mutations reported all over the world (Tikka et al. 2009, Junna et al. our own unpublished data). There are seven in-frame deletions and two duplications (Tikka et al. 2009). Mutations show a highly stereotyped nature: most mutations involve either loss or gain of a conserved cysteine residue in one of the 34 EGF repeats. As a result, six cysteine residues within EGF repeat domains change into either five or seven cysteine residues. In small in-frame deletions the loss of either one or three cysteine residues occurs and thereby it also causes a change from an even to odd number of cysteines in the deleted EGF repeat (Kalimo et al. 2008). Two *de novo* mutations of p.R182C and p.C128G have been reported (Joutel et al. 2000c, Coto et al. 2006). Two CADASIL patients have been diagnosed to be homozygous: one for p.R133C mutation (Tuominen et al. 2001) and another for p.R578C (Liem et al. 2008). Seven CADASIL patients with non-cysteine mutations have been also reported (Tikka et al. 2009). A non-cysteine mutation of p.L1515P has been shown to cause a non-CADASIL SVD without accumulation of N3ECD or GOM on the VSMCs (Fouillade et al. 2008).

### 2.3.9. NOTCH3 signalling

NOTCH3 is a member of NOTCH family with four mammalian homologues (Gridley 2001, Artavanis-Tsakonas et al. 1999, Hansson et al. 2004). NOTCH molecules are highly conserved in organisms ranging from nematodes to man (Beatus and Lendahl 1998, Artavanis-Tsakonas et al. 1999). NOTCH pathway regulates stem-cell renewal, proliferation, differentiation and apoptosis during organogeneses, including vasculogenesis and formation of VSMCs (Lendahl 1998, Beatus and Lendahl 1998, Lasky and Wu 2005, Roca and Adams 2007). The human *NOTCH3* gene has 33 exons and encodes a NOTCH3 receptor protein of 2321 amino acids with a single transmembrane domain. The NOTCH3 protein is predominantly expressed in VSMCs and pericytes in the vasculature in both human adults and rodents (Joutel et al. 2000a, Prakash et al. 2002). The extracellular N-terminal part of NOTCH3 contains 34 tandemly arranged EGF repeats and three NOTCH/lin-12 repeats. The recognition site for the ligand is at

EGF repeats 10 and 11. All EGF repeats contain six conserved cysteine residues that form three disulfide bonds. The intracellular side of NOTCH3 has six ankyrin repeats (Lardelli et al. 1995, Joutel et al. 1996).

The three members (DLL1, DLL3 and DLL4) of Delta-like family and two members (JAG1 and JAG2) of Serrate family Jagged constitute the ligands for NOTCH3 receptors (Gridley 2007, Gray et al. 1999, Shimizu et al. 2000). The molecular structure of Delta and Jagged is similar to that of NOTCH3. VSMCs express both the ligand and the receptor on the cell surface, but the signal transduction occurs only between neighbouring cells (Parks et al. 2000, Nichols et al. 2007). NOTCH3 are cleaved three times before functioning. NOTCH3 is first transcribed as a single polypeptide chain and then cleaved by a furin-like convertase in the Golgi apparatus (S1 cleavage). The two cleaved products are held together by calcium and then it is transported and inserted into the plasma membrane (Blaumueller et al. 1997). The ligand binding induces the second cleavage (S2 cleavage) by tumor necrosis factor-alpha converting enzyme (TACE) at the site located 12 amino acids external to the intramembranous domain (Brou et al. 2000). The N3ECD with the ligand is then released, transported to the ligand expressing cell and endocytosed (Parks et al. 2000, Nichols et al. 2007). Finally, NOTCH3 transmembrane-intracellular domain within the plasma membrane is cleaved (S3 cleavage) by γ-secretase (Mumm et al. 2000). S3 cleavage releases the NOTCH3 intracellular domain (NICD), which then enters the nucleus. In the nucleus it has two pathways: (1) it forms a complex with the CBF-1/RBP-Jκ protein and displaces a histone deacetylase co-repressor complex. Then, the NICD-CBF-1/RBP-Jκ complex recruits an activation complex and leads to the transcriptional activation of NOTCH3 target genes including IκB. (2) it activates the Deltex-mediated pathway and/or inhibits integrin activation (Kopan 2002, Morrow et al. 2008).

NOTCH3 signalling pathway has been focus of intensive research in recent years for the molecular pathogenesis of CADASIL. Some studies suggest that CADASIL mutations do not affect normal NOTCH3 receptor function. It has been reported that a mutant NOTCH3 receptor expressed in a human cell line has normal cell maturation, expression and binding capacity (Haritunians et al. 2002). Karlström and others have demonstrated that the mutant receptor can also be activated by the ligand by using cell lines that express the murine NOTCH3 protein corresponding to a mutant form of p.R141C in CADASIL (Karlström et al. 2002). Similar receptor maturation also occurs irrespective of mutation site (Peters et al. 2004b). Low and others also report that CADASIL-associated mutations do not alter NOTCH3 receptor processing and activation (Low et al. 2006). On the other hand, several studies have shown that distinct CADASIL mutations have apparently different effects on NOTCH3 signaling, especially, such mutations are within or near the ligand binding domain (Joutel et al. 2004, Peters et al. 2004b). At the same time, other lines of evidence have also emerged, for example, it has been reported that CADASIL-associated mutations significantly enhance higher order multimerization of N3ECD compared with wild-type (Opherk et al. 2009, Duering et al. 2011). Takahashi and others reports that mutants of NOTCH3 are prone to aggregation and being retained in the endoplasmic reticulum. This

prolonged retention of mutant NOTCH3 aggregates decreases cell growth (Takahashi et al. 2010). The similar results are also reported by Karlström and others (Karlström et al. 2002). As discussed in the section of Genetics, a non-cysteine mutation of p.L1515P causes a constitutively active NOTCH3 signaling, which exceeds the wild type activity approximately by ten-fold (Fouillade et al. 2008). Recently, Arboleda-Velasquez and others report that some NOTCH3 mutations in CADASIL are associated with hypomorphic NOTCH3 functions (Arboleda-Velasquez et al. 2011).

### 2.3.10. Animal models

Several NOTCH3 mutant mouse models have been developed. Some reproduce CADASIL-like arteriopathy and WMLs, thus, providing some insights into the role of NOTCH3 in the pathogenesis (Ayata 2010).

#### *2.3.10.1. Knock-out mice*

NOTCH3 knock-out mice are viable and fertile. These mice exhibit marked arterial defects such as thinner VSMCs layers and impaired cerebrovascular resistance (Krebs et al. 2003, Domenga et al. 2004). However, they do not have deposits of GOM. Although NOTCH3 knock-out mice do not develop any parenchymal pathology resembling CADASIL, they show increased sensitivity when challenged by induced focal cerebral ischemia (Arboleda-Velasquez et al. 2008).

#### *2.3.10.2. Mutant mice*

So far, three mutant mouse models expressing CADASIL mutations (p.R90C, p.R169C and p.C428S) have been developed (Ruchoux et al. 2003, Monet et al. 2009, Joutel et al. 2010). These transgenic models show age-dependent ultrastructural abnormalities in cerebral and systemic arterioles characteristic for CADASIL, including GOM and N3ECD deposits. Mutant mice also show abnormal vasomotor responses (Dubroca et al. 2005, Lacombe et al. 2005, Joutel et al. 2010). However, cerebral parenchymal CADASIL-like lesions are absent in the p.R90C and p.C428S mutants. There are WMLs, but not lacunar infarcts, in the p.R169C mutant (Joutel et al. 2010). Surprisingly, p.R142C knock-in mice do not develop any arteriolar, parenchyma or behaviour abnormalities (Lundkvist et al. 2005).

### 2.3.11. Mechanism and pathogenesis

#### *2.3.11.1. Molecular pathogenesis*

*Loss of function or gain of function or dominant negative effect or new function*

Many studies have attempted to clarify the property of NOTCH3 signalling, but results are contradictory. Although NOTCH3 signalling is dosage sensitive, some evidence argues

against loss of function in CADASIL. First, only few specific mutations within or near the ligand binding domain exhibits a significant reduction of transcriptional activity in CBF-1/RBP-Jκ signalling pathway, whereas majority of CADASIL mutations do not interfere with the membrane trafficking of the receptor or downstream signalling (Joutel et al. 2004, Peters et al. 2004b). This normality in NOTCH3 signalling is also compatible with late onset of the disease. Second, the clinical picture of the patients with mutations within or near the ligand binding domain is similar to that of the patients with mutations outside of the ligand binding domain. Third, so is the clinical picture of the homozygous to heterozygous CADASIL patients (Tuominen et al. 2001). Fourth, NOTCH3 knock-out mice do not develop the characteristic tissue or arteriolar changes (e.g. deposits of GOM) observed in CADASIL and display vasomotor abnormalities that are opposite to those observed in CADASIL mutant mice (Krebs et al. 2003). Fifth, the conservation of *NOTCH3* genome sequences among different species and different *NOTCH* genes does not support loss of function (Donahue and Kosik 2004). Last, transgenic expression of the p.R90C mutant NOTCH3 on knock-out background also reproduces GOM and NOTCH3 deposits (Monet et al. 2007). Thus, loss of function might not be the cause of CADASIL. However, a recent study suggests the loss of function in CADASIL (Arboleda-Velasquez et al. 2011). On the other hand, some evidence against gain of function also appears, since CADASIL mutations do not increase downstream NOTCH3 signal transduction (Monet et al. 2007). Interestingly, however, a new mutation indeed has been reported in a patient with cerebral SVD lacking GOM deposits and N3ECD accumulation. This mutation is located in the juxtamembranous region of NOTCH3, which constitutes the heterodimerisation domain. The mutation destabilises the metal ion bridge leading to a ligand-independent, constitutively active receptor (Fouillade et al. 2008). Thus, it seems that gain of function leads to a cerebral SVD different from CADASIL. The dominant negative effect is also studied with mixed results. Transgenic expression of p.C428S mutant NOTCH3 on a heterozygous knock-out background inhibits downstream signalling by the normal NOTCH3 copy (Monet-Lepretre et al. 2009). However, similar experiments using the p.R90C mutant do not reveal a dominant negative effect (Monet et al. 2007). On the other hand, the accumulation of GOM and NOTCH3 might suggest a neomorphic function (gain of a novel, pathological function). However, it is not known whether GOM or NOTCH3 aggregates are causally related to vascular dysfunction, leukoaraiosis and lacunar infarcts, or whether they are just purely biomarkers.

*Misfolding*

All CADASIL mutations lead to an uneven number of cysteine residues in the mutated EGF repeat, therefore, sulphur bridges do not form. Although NOTCH3 three-dimensional structure is altered in CADASIL, the mutated receptor is still transported to the cell surface as a misfolded protein (Joutel et al. 2000a, Karlström et al. 2002, Joutel et al. 2004, Peters et al. 2004b, Sayeed and Ng 2005). Consequently, VSMCs at the same time become oxidatively stressed and up-regulated in proteasomal degradation.

These stresses due to the protein misfolding and degradation may lead to degeneration of VSMCs (Ihalainen et al. 2007).

*Disturbed endocytosis of the N3ECD*

In CADASIL the accumulated NOTCH3 contains only its extracellular domain (ECD): N3ECD (Joutel et al. 2000a). Normally N3ECD would be transported and endocytosed with the ligand to the signal-donor cell (Parks et al. 2000, Nichols et al. 2007). The ligand ECD binding to the NOTCH3 ECD is assumed to place mechanical strain on the NOTCH3 protein, which then can expose the cleavage site for TACE to carry out S2 cleavage (Wang and Struhl 2005). Abnormal ligand binding would lead to disturbance of mechanical strain, and subsequent inhibition of S2 cleavage and endocytosis of N3ECD (Wang and Struhl 2004).

*Different NOTCH3 pathway*

Since NOTCH3 receptors can carry out their functions independently from CBF-1/RBP-Jκ pathway (Shawber et al. 1996, Brennan et al. 1999, Martinez et al. 2002, Hu et al. 2003), alternative pathways may play a role in the pathogenesis. However, since the NOTCH3 canonical signalling is still active in CADASIL, this possibility remains to be confirmed.

*Crosstalks with other signalling pathways*

It has been reported that α-SMA is a direct target of canonical NOTCH3 pathway (Noseda et al. 2006). The expression of constitutively active NOTCH3 in cultured VSMCs leads to increased actin stress fibers and steady-state levels of polymerised actin (Domenga et al. 2004). In human CADASIL VSMCs, the ability of these cells to contract is impaired due to abnormal expression of several proteins interacting with actin cytoskeleton (Ihalainen et al. 2007).

Platelet-derived growth factor (PDGF) signalling, esp. PDGF-β/PDGFRβ signalling, is important in vascular development and homeostasis of blood vessels, since defects in this pathway cause hemorrhage, microaneurysms and VSMCs hypoplasia (Betsholtz 2004). PDGF stimulation has been reported to inhibit NOTCH3 expression and signalling (Marmur et al. 1992, Campos et al. 2002, Wang et al. 2002a). PDGFR-β has been also demonstrated to be an immediate downstream target gene of NOTCH3 activation (Jin et al. 2008).

*2.3.11.2. Functional pathogenesis*

*Endothelial hypothesis*

Ultrastructural and functional abnormalities of endothelial cells have been reported in CADASIL mutant mice and patients and therefore endothelial cells may play a role in

the pathogenesis of lacunar infarcts (Ruchoux et al. 1998, Ruchoux et al. 2003, Stenborg et al. 2007, Peters et al. 2008, Rufa et al. 2008).

*Vascular endothelial growth factor hypothesis*

The lesion of VSMCs may lead to decreased secretion of vascular endothelial growth factor (VEGF), which is a potent permeability factor (Ferrarra et al. 1991, Yonekura et al. 1999). In turn, loss of VEGF leads to a decrease in permeability and subsequent ischemic lesions (Brulin et al. 2002).

*Earthen pipe hypothesis*

The diffuse degeneration of VSMCs is widespread in penetrating arteries. These arteries might be transformed into the so-called earthen pipe state, in which neither dilatation nor constriction is possible. Therefore, the resistance arterioles are likely to be an ineffective auto-regulatory organ and brain becomes vulnerable to the injurious effects, as well as to low blood pressure and low CBF (Okeda et al. 2002).

*Stenosis and thrombosis hypothesis*

The destruction of VSMCs leads to secondary fibrosis, resulting in thickening of the walls and narrowing of the lumen of cerebral arteries. These vascular changes then lead to either thrombosis or obliteration of affected arteries and consequently to lacunar infarcts. The final cause of the lacunar infarcts is most likely thrombosis in the affected small arteries, since fibrin degradation products have been detected in the plasma of CADASIL patients with recent infarcts (Kalimo et al. 1999, Kalimo et al. 2002b, Kalimo et al. 2008).

### 2.3.12. Diagnosis

The diagnostic criteria for CADASIL have been proposed (Davous 1998): possible, probable and definite. These criteria are based on the information on age of onset, clinical symptoms, vascular risk factors, heredity and MRI imaging. The definite confirmation of CADASIL can only be made by combining this information with presence of mutations in NOTCH3 and pathological findings of GOM and N3ECD deposits.

#### *2.3.12.1. Brain MRI*

MRI is the first diagnostic tool to use for suspected CADASIL patients. Although MRI is not specific diagnostic examination for CADASIL, it is highly useful. Hyperintensity in temporal pole has high sensitivity (89%) and specificity (86%), while the hyperintensity in external capsule has high sensitivity (93%), but low specificity (45%) (O'Sullivan et al. 2001, Markus et al. 2002, Singhal et al. 2005, Yamamoto et al. 2009, Pantoni et al. 2010).

#### *2.3.12.2. Skin biopsy*

Physicians should verify the characteristic MRI findings with EM or immunohistochemical examination of skin biopsy. The positive GOM deposits in the arterioles from skin biopsy can diagnose CADASIL with 100% specificity, but variable sensitivity (45-90%) (Ruchoux et al. 1994, Ruchoux et al. 1995, Ebke et al. 1997, Mayer et al. 1999, Markus et al. 2002). One recent study reports that the sensitivity of presence of GOM in the arterial walls in representative skin biopsies appears to be 100% (Tikka et al. 2009). Immunohistochemical detection for N3ECD is another method for the diagnosis with high sensitivity (96%) and specificity (100%) (Joutel et al. 2001), although false positive results have been reported (Lesnik Oberstein et al. 2003b).

#### *2.3.12.3. Genetic testing*

Genetic testing for *NOTCH3* mutations is the most accurate diagnosis. The testing should be initially focused on the exons where mutations are most frequent (Joutel et al. 1997, Peters et al. 2005b, Tikka et al. 2009). More than 80% of mutations have been reported to be located within exons 2-6, 8, 11, 18 and 19 (Kalimo et al. 2008, Tikka et al. 2009). Screening of the 23 exons that encode the 34 EGF repeats (exons 2-24) has almost 100% sensitivity and 100% specificity (Joutel et al. 2001, Valenti et al. 2011).

### 2.3.13. Treatment

At present, there is no effective treatment for CADASIL. However, secondary prevention can be considered. For example, antihypertensive drugs can be used for hypertension and statins can be used for hypercholesterolemia (Endres et al. 1998). Antiepileptic drugs or β-blockers or acetazolamide can be used for migraine with aura (Forteza et al. 2001). Cholinesterase inhibitors (Dichgans et al. 2008) can be used to alleviate cholinergic deficits in CADASIL patients (Mesulam et al. 2003, Keverne et al. 2007, Manganelli et al. 2008). Increased blood viscosity should be avoided, i.e. the patients should not become dehydrated (M. Viitanen, personal communications, 2010). Anticoagulant therapy and all drugs causing vasoconstriction are not recommended for CADASIL patients (Kalimo et al. 2008). The conventional analgesics and nonsteroidal anti-inflammatory drugs are usually recommended. In addition to medicines, rehabilitation procedures, physiotherapy, nursing care and psychological support are also important (Herve and Chabriat 2010).

# 3. AIMS OF THE PRESENT STUDY

The purpose of this study was:

1. To analyze the alterations of following morphometric parameters: (1) diameter of arteriolar lumen (stenosis), (2) thickness of arteriolar wall (fibrosis), (3) external diameter of arterioles and (4) SI of cerebral arterioles in cerebral WM and cortical GM, as well as in deep GM (lenticular nucleus (LN)) in CADASIL patients of different ages.

2. To compare the parameters above with those in age-matched healthy controls.

3. To critically evaluate the previously published, somewhat controversial results on the morphometry of cerebral arteries in CADASIL.

4. To analyse whether the morphometric alterations and ageing contribute to understanding the pathogenesis of CADASIL.

# 4. PATIENTS AND CONTROLS

## 4.1. Patients

Four deceased elderly CADASIL patients (2 women and 2 men, mean age 64.3 ± 2.5 years, range 63-68 years of age, from three different Finnish families, with genetically confirmed C475T (p.R133C) *NOTCH3* mutation) were available for the study of lobar areas (frontal cerebral WM and cortical GM) of elderly CADASIL patients (**I**).

Five deceased elderly CADASIL patients (2 women and 3 men, mean age 63.4 ± 2.9 years, range 60-68 years of age, from four different Finnish families, with molecular genetically confirmed C475T (p.R133C) *NOTCH3* mutation) and same deceased 32-year-old man in the study **III** were available for the study of LN (putamen and globus pallidus) (**II**).

One deceased 32-year-old Swedish CADASIL patient (man) with genetically confirmed T598C (p.C174R) *NOTCH3* mutation was available for the study of lobar areas of young CADASIL patient (**III**).

One deceased 95-year-old Finnish CADASIL patient (woman) with genetically confirmed C475T (p.R133C) *NOTCH3* mutation was available for the study of lobar areas of the very old CADASIL patient (**IV**).

All CADASIL patients involved in the thesis are listed in the Table 2.

## 4.2. Controls

Six different groups were used as controls in the quantitative morphometric study: (1) five deceased persons (2 women and 3 men, mean age 61.4 ± 6.2 years, range 57-72 years of age) with a history of another type of cerebrovascular disease as cerebrovascular lobar controls for the elderly CADASIL patients (**I**); (2) four deceased persons (4 men, mean age 59.8 ± 4.5 years, range 56-66 years of age) without cerebrovascular diseases as non-cerebrovascular lobar controls for the elderly CADASIL patients (**I**) (this control group was used in this thesis as lobar controls for the elderly CADASIL patients); (3) seven deceased elderly persons (3 women and 4 men, mean age 64.3 ± 7.1 years, range 56-73 years of age) without cerebrovascular diseases served as LN controls for the elderly CADASIL patients (**II**); (4) four deceased young persons (2 women and 2 men, mean age 29.3 ± 2.5 years, range 26-32 years of age) without cerebrovascular diseases served as LN controls for the young CADASIL patient (**II**); (5) three deceased young persons (1 woman and 2 men, mean age 28.3 ± 2.1, range 26-30 years of age) without cerebrovascular diseases served as lobar controls for the young CADASIL patient (**III**); (6) six deceased very old persons (2 woman and 4 men, mean age 95.17 ± 1.17, range 94-97 years of age) without dementia, hypertension, diabetes and cerebral amyloid

angiopathy (CAA) served as lobar controls for the very old CADASIL patient (**IV**). The controls in (2) and (5) are also used for comparison in the study IV.

All controls without cerebrovascular diseases involved in the thesis are listed in the Table 2.

**Table 2.** The CADASIL Patients and Controls Involved in the Study I-IV

| CADASIL patients | Controls | Gender | Mutation | Study | | | | Age of onset | Age at death |
|---|---|---|---|---|---|---|---|---|---|
| | | M/F | | I | II | III | IV | | |
| No1 | | M | R133C | + | + | | | 39 | 63 |
| No2 | | M | R133C | + | + | | | 48 | 68 |
| No3 | | F | R133C | + | + | | | 52 | 63 |
| No4 | | F | R133C | + | + | | | 52 | 63 |
| No5 | | M | R133C | | + | | | 46 | 60 |
| No6 | | M | C174R | | + | + | | 29 | 32 |
| No7 | | F | R133C | | | | + | 71 | 95 |
| | No1# | M | | + | | | | | 62 |
| | No2# | F | | + | | | | | 58 |
| | No3# | M | | + | | | | | 57 |
| | No4# | F | | + | | | | | 72 |
| | No5# | M | | + | | | | | 58 |
| | No6* | M | | + | | | | | 57 |
| | No7* | M | | + | | | | | 56 |
| | No8* | M | | + | | | | | 60 |
| | No9* | M | | + | | | | | 66 |
| | No10¤ | F | | | + | | | | 58 |
| | No11¤ | M | | | + | | | | 56 |
| | No12¤ | M | | | + | | | | 64 |
| | No13¤ | F | | | + | | | | 58 |
| | No14¤ | F | | | + | | | | 71 |
| | No15¤ | M | | | + | | | | 70 |
| | No16¤ | M | | | + | | | | 73 |
| | No17♣ | M | | | + | | | | 29 |
| | No18♣ | F | | | + | | | | 32 |
| | No19♣ | F | | | + | | | | 26 |
| | No20♣ | M | | | + | | | | 30 |
| | No21♥ | M | | | | + | | | 29 |
| | No22♥ | M | | | | + | | | 30 |
| | No23♥ | F | | | | + | | | 26 |
| | No24♦ | F | | | | | + | | 95 |
| | No25♦ | M | | | | | + | | 96 |
| | No26♦ | M | | | | | + | | 95 |
| | No27♦ | F | | | | | + | | 98 |
| | No28♦ | F | | | | | + | | 94 |
| | No29♦ | F | | | | | + | | 95 |

#: Controls with a history of another type of cerebrovascular disease as cerebrovascular lobar controls for the elderly CADASIL patients

*: Controls without cerebrovascular diseases as non-cerebrovascular lobar controls for the elderly CADASIL patients

¤: Controls without cerebrovascular diseases served as LN controls for the elderly CADASIL patients

♣: Controls without cerebrovascular diseases served as LN controls for the young CADASIL patient

♥: Controls without cerebrovascular diseases served as lobar controls for the young CADASIL patient

♦: Controls without dementia, hypertention, diabetes and cerebral amyloid angiopathy served as lobar controls for the very old CADASIL patient

# 5. METHODS

## 5.1. Pathological study

### 5.1.1. Histology and immunohistochemistry

The brains were fixed in 4% phosphate buffered formaldehyde solution and samples were selected according to the protocol of the Laboratory of Neuropathology of the University of Turku and were embedded in paraffin according to the routine methodology of the laboratory. Representative samples of the frontal lobe and LN were selected. Sections (5 μm thickness) covering frontal cortical GM and the underlying cerebral WM, or basal ganglia including LN were cut and stained with H&E. Additional sections were cut and stained by immunoperoxidase methods for detection of α-SMA (monoclonal mouse antibody, Sigma, St. Louis, USA), collagen I (polyclonal rabbit antibody, Biotrend, Cologne, Germany), N3ECD (clone 1E4, monoclonal mouse antibody, a kind gift from Dr. A Joutel, Faculté de Médecine Lariboisière, Paris), fibrinogen (Dako, Glostrup, Denmark) and fibronectin (Dako, Glostrup, Denmark). The bound primary antibodies were visualized by using appropriate peroxidase-labelled secondary antibodies (Vector Laboratories, Burlingame, CA, USA). Diaminobenzidine was used as the chromogen and hematoxylin as the counterstain (**I**-**IV**).

### 5.1.2. Electron microscopy

Samples were taken from the frontal cerebral WM and cortical GM of the CADASIL brains, which had been routinely fixed in 4% phosphate buffered formaldehyde. After post-fixation in 3% phosphate buffered glutaraldehyde the samples were postosmicated, dehydrated and embedded in epon. Semi-thin sections were stained with toluidine blue. Regions of interest were selected for cutting thin sections, which were contrasted with uranyl acetate and lead citrate and then examined in a JEOL JEM 1200 electron microscope (**I**, **III**).

## 5.2. Quantitative morphometric study

**Study I:** In the analysis of the cerebral WM and cortical GM of the elderly CADASIL patients the analysis was carried out in three different ways on H&E stained sections.

In the first analysis the sections (5 μm thickness) were cut conventionally perpendicular to the surface of the brain. All transversely cut arterioles in the cerebral WM and cortical GM with external diameter of arteriole ≥ 30 μm and with maximum of 300 μm (Toole 1990) were examined from each patient and control in one representative section of the frontal lobe extending from the surface of the cortex deep into the cerebral WM. Since these sections were cut perpendicular to the brain surface and parallel to the penetrating arterioles, virtually most of the measured arterioles seen as cross sections were branches

of the primary penetrating arteries. Images of selected regions of the sections were captured at an objective magnification of ×10 by using a video camera attached to a light microscope. The diameter of arteriolar lumen and external diameter of arteriole were then measured using the tailor-made software for on screen measurements (Pit Oy, Turku, Finland). In arterioles with elliptical profiles the lesser diameter, i.e. the longest diameter perpendicular to the maximum axis, was measured. The external diameter of arteriole was measured at the same orientation and corresponded to the diameter spanning external boundaries of the connective tissue. The SI was defined and calculated as 1 – (diameter of arteriolar lumen / external diameter of arteriole) (Furuta et al. 1991, Lammie et al. 1997).

The second analysis was performed for measuring diameters of the primary penetrating arteries, which run perpendicular to the brain surface. One thousand serial sections (each 5 μm thick) were cut from a block of frontal lobe of one elderly CADASIL patient parallel to the surface of the brain, i.e. transversely to the primary penetrating arterioles. Every fifth section was stained and examined. Then, three penetrating arterioles were traced and reconstructed from the cerebral WM down to deep cerebral WM. The diameter of arteriolar lumen and external diameter of arteriole were measured and the SI was calculated in the same way as in the first analysis.

The third analysis was performed for longitudinal tracing and measuring selected primary penetrating arteries along their length from the surface of the brain into the cerebral WM, although the measurement of the diameters in this analysis is less exact than in transverse sections. One thousand serial sections (each 5 μm thick) were cut from a block of frontal lobe of one elderly CADASIL patient similarly as in the first analysis, i.e. perpendicular to the surface of the brain. Every fifth section was stained and examined light microscopically. Six primary penetrating arterioles were traced from the site of penetration at the cortical surface to their distal portion in deep cerebral WM, where they had decreased to a diameter of 20-30 μm. The diameter of arteriolar lumen and external diameter of arteriole were measured and the SI was calculated in the same way as in the first analysis.

**Study II:** In the study of the LN of the young and elderly CADASIL patients the method employed was same as that of the first analysis in the study I, except that the upper limit of the diameter of arteriolar lumen of transversely cut arterioles examined in the cerebral WM, cortical GM and LN was set to be 50 μm (selection based on the fact that the majority of arterioles have luminal diameters of 10-45 μm (Edvinsson et al. 1993) or below 70 μm (Toole 1990) and arterioles of this size are decisive for the small vessel ischemia). The four indicators of arteriolar stenosis and fibrosis (diameter of arteriolar lumen, thickness of arteriolar wall, external diameter of arterioles and SI) were measured and calculated. The thickness of arteriolar wall was defined as (external diameter of arteriole – diameter of arteriolar lumen) / 2.

**Study III:** In the analysis of the cerebral WM and cortical GM of the young CADASIL patient the method employed was same as that of the first analysis in the study I.

**Study IV:** In the study of very old CADASIL patient the method employed was same as that used in the study II.

**Thesis:** The method employed for the calculation and comparison of four indicators of arteriolar stenosis and fibrosis among the patients and controls was same as that in the study II.

## 5.3. Statistical analysis

In the quantitative morphometric study Student's t-test (**I**) or one-way ANOVA (**II-IV**) was used. The distributions of diameter of arteriolar lumen were compared using Mann-Whitney test (**I, III**). One-way ANOVA was used in this thesis. Data are presented as means ± SD. Values of $p < 0.01$ were considered statistically significant.

# 6. RESULTS AND DISCUSSION

## 6.1. The elderly CADASIL patients (Study I and II)

The cerebral WM was spongy with lacunar infarcts and associated loss of myelinated nerve fibers. Basophilic granular material had accumulated in the tunica media of cerebral WM arterioles and α-SMA staining was markedly irregular. Adventitia immunostained intensely for collagen I. A strong immunopositivity for N3ECD was seen in the degenerating tunica media, being the most prominent in the cerebral WM arterioles. In the elderly CADASIL patients the cortical GM appeared relatively well preserved (**I**).

EM showed the characteristic GOM in the arterioles of both cerebral WM and cortical GM in the elderly CADASIL patients. There was no substantial difference in the appearance and distribution of GOM between the cerebral WM and cortical GM arterioles. However, it remains to be investigated, whether there is no substantial difference either in the smooth muscle fibers between the cerebral WM and cortical GM arterioles (**I**).

The histopathological and immunohistochemical findings in the LN arterioles of the patients were similar to those in the cerebral WM arterioles of the patients except for the lesser stenosis (see below for quantitative details). Immunoreactivity for fibrinogen or fibronectin, as a marker of blood brain barrier (BBB) leakage, was very uncommon around arterioles with N3ECD positive walls. Such immunoreactivity in the parenchyma was seen almost only in the areas close to definite lacunar infarcts (**II**).

The four indicators of arteriolar stenosis and fibrosis, (1) diameter of arteriolar lumen, (2) thickness of arteriolar wall, (3) external diameter of arterioles and (4) SI, as well as the numbers of arterioles measured in the cerebral WM, cortical GM and LN of the patients and two control groups (for lobar and LN respectively) are given in the Table 3 and Table 4.

In the cerebral WM arterioles all four indicators of the patients were significantly different from those of the controls ($p < 0.01$). For example, the SI of the cerebral WM arterioles was over two times greater than that of the controls (0.74 vs. 0.35, $p < 0.01$), being attributable to the markedly narrowed lumen and thickened wall. In the LN arterioles, only the thickness of the arteriolar wall and SI were significantly greater than those of the controls (both $p < 0.01$), indicating that CADASIL also causes remarkable fibrosis in the LN arterioles, but there is no significant stenosis (Table 4) (Figure 2). However, in the cortical GM arterioles the only significant difference in the above mentioned four indicators between the patients vs. the controls was in the SI (0.55 vs. 0.48, $p < 0.01$). This was due to only slightly narrowed lumen and thickened wall of arterioles in the patients.

**Table 3.** Diameter of Arteriolar Lumen, Thickness of Arteriolar Wall, External Diameter of Arterioles and Sclerotic Index of Cerebral White Matter, Cortical Grey Matter and Lenticular Nucleus in CADASIL Patients and Controls

| | Diameter of Arteriolar Lumen (µm) | | | Thickness of Arteriolar Wall (µm) | | | External Diameter of Arterioles (µm) | | | Sclerotic Index | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WM | GM | LN | WM | GM | LN | WM | GM | LN | WM | GM | LN |
| Elderly CADASIL | 14.80 ± 10.45 | 17.32 ± 6.84 | 19.82 ± 9.25 | 20.49 ± 8.84 | 10.60 ± 3.82 | 15.26 ± 8.79 | 55.78 ± 23.35 | 38.52 ± 10.14 | 50.33 ± 22.30 | 0.74 ± 0.13 | 0.55 ± 0.13 | 0.60 ± 0.13 |
| (N = 5) | (n = 1056) | (n = 171) | (n = 486) | (n = 1056) | (n = 171) | (n = 486) | (n = 1056) | (n = 171) | (n = 486) | (n = 1056) | (n = 171) | (n = 486) |
| Elderly lobar controls | 28.29 ± 7.45 | 19.58 ± 7.43 | | 7.56 ± 3.12 | 8.70 ± 2.23 | | 43.41 ± 10.78 | 36.97 ± 8.01 | | 0.35 ± 0.08 | 0.48 ± 0.12 | |
| (N = 4) | (n = 49) | (n = 75) | | (n = 49) | (n = 75) | | (n = 49) | (n = 75) | | (n = 49) | (n = 75) | |
| Elderly LN controls | | | 21.49 ± 10.08 | | | 12.68 ± 5.02 | | | 46.85 ± 15.88 | | | 0.55 ± 0.12 |
| (N = 7) | | | (n = 309) | | | (n = 309) | | | (n = 309) | | | (n = 309) |
| Young CADASIL | 18.46 ± 10.90 | 17.21 ± 7.01 | 21.61 ± 10.25 | 15.73 ± 6.19 | 10.28 ± 2.43 | 13.67 ± 4.09 | 49.93 ± 20.30 | 37.77 ± 8.59 | 48.96 ± 15.39 | 0.64 ± 0.11 | 0.55 ± 0.11 | 0.57 ± 0.10 |
| (N = 1) | (n = 61) | (n = 69) | (n = 35) | (n = 61) | (n = 69) | (n = 35) | (n = 61) | (n = 69) | (n = 35) | (n = 61) | (n = 69) | (n = 35) |
| Young lobar controls | 26.09 ± 8.59 | 22.12 ± 6.47 | | 9.13 ± 2.95 | 7.44 ± 1.59 | | 44.36 ± 11.33 | 37.00 ± 7.13 | | 0.42 ± 0.10 | 0.41 ± 0.08 | |
| (N = 3) | (n = 52) | (n = 82) | | (n = 52) | (n = 82) | | (n = 52) | (n = 82) | | (n = 52) | (n = 82) | |
| Young LN controls | | | 25.34 ± 9.02 | | | 9.13 ± 2.47 | | | 43.61 ± 10.69 | | | 0.43 ± 0.10 |
| (N = 4) | | | (n = 134) | | | (n = 134) | | | (n = 134) | | | (n = 134) |
| Very old CADASIL | 17.71 ± 9.24 | 20.02 ± 7.69 | | 23.72 ± 11.28 | 12.75 ± 4.03 | | 65.15 ± 27.41 | 45.51 ± 12.26 | | 0.72 ± 0.10 | 0.56 ± 0.10 | |
| (N = 1) | (n =79) | (n =69) | | (n =79) | (n =69) | | (n =79) | (n =69) | | (n =79) | (n =69) | |
| Lobar controls | 20.84 ± 9.94 | 16.73 ± 7.50 | | 12.24 ± 2.77 | 10.09 ± 1.82 | | 45.33 ± 13.03 | 36.91 ± 9.03 | | 0.56 ± 0.11 | 0.56 ± 0.09 | |
| (N = 6) | (n = 165) | (n = 38) | | (n = 165) | (n = 38) | | (n = 165) | (n = 38) | | (n = 165) | (n = 38) | |

WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; N: number of patients analysed; n: number of arterioles measured

Brulin and others (Brulin et al. 2002) analysed 20 brain capillaries (not arterioles) in five CADASIL patients and reported that there was no significant difference in the SI of cerebral WM capillaries between CADASIL vs. controls, or in the SI of capillaries between the cerebral WM vs. cortical GM. The conclusion they have drawn is probably due to wrong mathematical calculations (**I**). On the other hand, Okeda and others (Okeda et al. 2002) examined relatively large medullary arteries, and found segmental fibrous or hyalinous thickening, but stenosis or occlusion was absent.

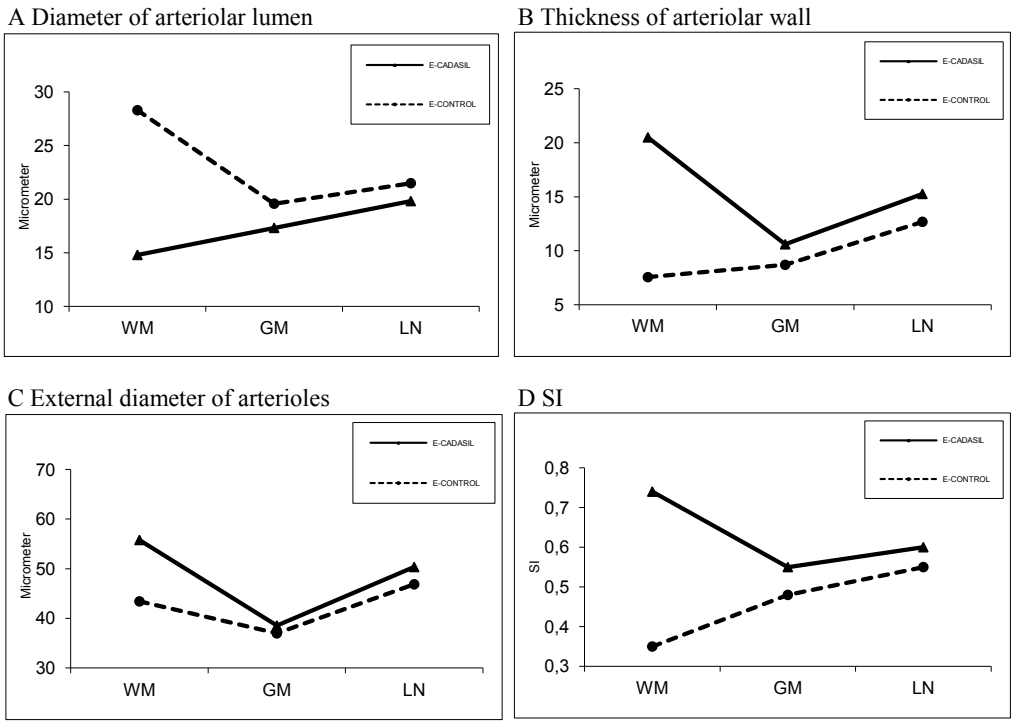
These two studies were in discrepancy with our observations in all our autopsy analyses. In our elderly CADASIL patients all four indicators in cerebral WM arterioles showed that the cerebral WM arterioles had significantly ($p < 0.01$) narrower lumen and thicker wall than those in the GM and LN arterioles. On the other hand, the LN arterioles had significant larger lumen and thicker walls than those in the cortical GM arterioles (Table 4) (Figure 2).

It is important to point out that the sharp increase in the SI of the cerebral WM arterioles (i.e. narrowing of the lumen and thickening of the wall) did not occur until the diameter of arteriolar lumen had decreased to 20-30 μm and the external diameter of arterioles to 100-130 μm (**I**). Furthermore, the arterioles in CADASIL are not dilated, but display a variable degree of luminal stenosis, despite a substantial loss of smooth muscle fibres which, in turn, would reduce their contractility. This indicates that the degenerative changes in the media develop slowly, and concomitantly with the perivascular fibrosis, which may protect against dilatation of the weakened vessel wall and occurrence of microbleeds, which

**Table 4.** Diameter of Arteriolar Lumen, Thickness of Arteriolar Wall, External Diameter of Arterioles and Sclerotic Index of Cerebral White Matter, Cortical Grey Matter and Lenticular Nucleus in Elderly CADASIL Patients and Controls

| | Diameter of Arteriolar Lumen (μm) | | | Thickness of Arteriolar Wall (μm) | | | External Diameter of Arterioles (μm) | | | Sclerotic Index | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WM | GM | LN | WM | GM | LN | WM | GM | LN | WM | GM | LN |
| Elderly CADASIL (N = 5) | 14.80 ± 10.45 [d] (n = 1056) | 17.32 ± 6.84 (n = 171) | 19.82 ± 9.25 [e f] (n = 486) | 20.49 ± 8.84 [d] (n = 1056) | 10.60 ± 3.82 (n = 171) | 15.26 ± 8.79 [e f] (n = 486) | 55.78 ± 23.35 [d] (n = 1056) | 38.52 ± 10.14 (n = 171) | 50.33 ± 22.30 [e f] (n = 486) | 0.74 ± 0.13 [d] (n = 1056) | 0.55 ± 0.13 (n = 171) | 0.60 ± 0.13 [e f] (n = 486) |
| Elderly lobar controls (N = 4) | 28.29 ± 7.45 [b] (n = 49) | 19.58 ± 7.43 (n = 75) | | 7.56 ± 3.12 [b] (n = 49) | 8.70 ± 2.23 (n = 75) | | 43.41 ± 10.78 [b] (n = 49) | 36.97 ± 8.01 (n = 75) | | 0.35 ± 0.08 [b] (n = 49) | 0.48 ± 0.12 [a] (n = 75) | |
| Elderly LN controls (N = 7) | | | 21.49 ± 10.08 (n = 309) | | | 12.68 ± 5.02 [c] (n = 309) | | | 46.85 ± 15.88 (n = 309) | | | 0.55 ± 0.12 [c] (n = 309) |

WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; N: number of patients analysed; n: number of arterioles measured
One-way ANOVA, $p < 0.01$: a: patient's GM vs. corresponding control's GM; b: patient's WM vs. corresponding control's WM;
c: patient's LN vs. corresponding control's LN; d: patient's GM vs. patient's WM; e: patient's GM vs. patient's LN; f: patient's WM vs. patient's LN




WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; SI: sclerotic index; E: elderly

**Figure 2.** A, The diameter of arteriolar lumen ; B, thickness of arteriolar wall; C, external diameter of arterioles and D, SI of the WM, GM and LN in the elderly CADASIL patients

occur almost exclusively in GM (Dichgans et al. 2002), where the fibrous thickening is markedly lesser than in WM. These results suggest that the arterioles in the cerebral WM and cortical GM respond to the pathological process differentially. This view is supported by the notion in NOTCH3 knock-out mice that there appear to be differences in genuine molecular phenotypic characteristics and distinct physiological properties between brain and aorta VSMCs (Arboleda-Velasquez et al. 2008). However, it remains to be investigated, whether the changed environment and decreased blood flow in the damaged WM might secondarily add to the perivascular fibrosis and stenosis in WM arterioles. All these findings underline the fact that CADASIL is above all a disease of cerebral WM arterioles and, thus, it is the cerebral WM that suffers the brunt of the disease, whereas the cerebral cortex is relatively better preserved.

The presence of stenosis and fibrosis in the cerebral WM arterioles agree very well with MRI changes, which was recently shown as an association between temporal pole WM MRI hyperintensities and increased SI in CADASIL (Yamamoto et al. 2009). On the other hand, these structural changes also agree with several hemodynamic studies. Chabriat (Chabriat et al. 2000) and others (Bruening et al. 2001) reported a decreased CBF or regional CBV in the cerebral WM of symptomatic CADASIL patients, but

not in the cortical areas by using the MRI bolus tracking method. The same French group (Chabriat et al. 2000) and others (Pfefferkorn et al. 2001) also reported that the response of arteries to acetazolamide and $CO_2$ was markedly decreased, which was most likely due to reduced compliance of the fibrotic arterioles. Similarly Liem and others also reported that decreased cerebrovascular reactivity was correlated with the disease progression as indicated by increasing WM hyperintensities in MRI (Liem et al. 2009). On the other hand, lomerizine, a diphenylmethylpiperazine calcium channel blocker, an oral antimigraine drug, which can selectively increase CBF, could improve cerebral hypoperfusion and cognitive decline in CADASIL patients (Mizuno et al. 2009). The stenosis of these arterioles is also in accordance with the findings of the prolonged cerebral transit time which indicates slower velocity of CBF (Liebetrau et al. 2002). Similarly, middle cerebral artery (MCA) mean flow velocity and total baseline CBF as measured by phase contrast MRI were reported to be decreased (Pfefferkorn et al. 2001, van den Boom et al. 2003b). Our own PET studies also demonstrated a decreased CBF in the cerebral WM already at an early stage of the disease (beyond 30 years of age) (Tuominen et al. 2004).

The mechanism by which the changes in the lobar arterioles translate into the lacunar infarcts and WMLs in CADASIL can be speculated. When the stenosis progresses beyond certain thresholds and the systolic pressure decreases, "incipient" cerebral ischemia ensues and finally lacunar infarcts develop. The degeneration of VSMCs and fibrotic thickening of the arteriolar walls may lead to stiff arterioles (i.e. cerebral autoregulation may be disturbed), which in turn may further aggravate the insults. Indeed, the WMLs due to chronic cerebral hypoperfusion can be induced experimentally in the rat brain by bilateral clipping of the carotid arteries (Wakita et al. 1994). A progressive reduction of the arterial pressure has been shown to produce a cessation of CBF in the centrum semiovale in the primate brain while the cortical GM is perfused (Symon et al. 1973). Furthermore, the low systemic blood pressure profile has also been observed in CADASIL patients and this may disturb cerebral hemodynamics (Rufa et al. 2005).

Remarkably in the LN arterioles we also demonstrated that even though there was significant fibrosis (increased thickness of arteriolar wall), there was no stenosis, but instead the luminal diameter was only slightly smaller than in the controls (Table 4) (Figure 2). This finding was supported by Liem and others who by using a high-field in vivo MRI detected no differences in luminal diameters of lenticulostriate arteries between CADASIL patients and controls (Liem et al. 2010). The lack of stenosis was also reflected in the lower SI values of the LN arterioles than in the cerebral WM arterioles. This probably suggests that CBF is not significantly reduced in the LN. Indeed, Chabriat and others reported no difference in the CBF of basal ganglia between the patients and controls (Chabriat et al. 2000). However, in our PET study a moderately reduced CBF in the putamen was found in one homozygous (52 years of age) and another heterozygous (53 year of age) patient. However, the unavoidably lower age of the controls (41.4 years of age) may have influenced this result (Tuominen et al. 2001). Similarly, the markedly

older age of CADASIL patients as compared to that of the controls (43 vs. 27 years of age) in a SPECT study could explain the reduced CBF in the basal ganglia (Mellies et al. 1998). Thus, the basic flow values alone probably do not explain the appearance of lacunar infarcts in the LN.

The number of lacunar infarcts identified in basal ganglia by MRI is only 10% less than in cerebral WM (van den Boom 2003a), although the diameter of the arteriolar lumen in the LN arterioles was significantly greater than that in the cerebral WM arterioles, where the majority of lacunar infarcts occur. Furthermore, the diameter of the arteriolar lumen in the LN arterioles was even greater than that in the cortical GM arterioles. Therefore, it is paradoxical that multiple lacunar infarcts occur in the LN but not in the cortical GM. These findings imply that the pathogenesis of lacunar infarcts in the LN (deep GM) is different from that in the cerebral WM where definite stenosis exists. Indeed, Uehara and others reported that the risk factors and thus most likely causes of silent cerebral infarcts between the cerebral WM vs. basal ganglia were different (Uehara et al. 1999). Lacunar infarcts in the cerebral WM are considered to be most comonly due to intrinsic small vessel diseases in the penetrating arteries, a situation prevailing in CADASIL, whereas the lesions in basal ganglia appear to be more often associated with atherosclerosis, emboli and carotid artery stenosis (Wardlaw 2005, Uehara et al. 1999). However, the studies on lacunar infarcts have all too infrequently distinguished between the cerebral WM and deep GM lesions.

At present there is no definite evidence for thrombotic or thromboembolic aetiology of lacunar infarcts in CADASIL patients. Stenosis in the large cerebral arteries has been reported in 5 out of 13 (38%) elderly (mean age 55.0 ± 9.3 years, range 40-69 years of age) CADASIL patients (Choi et al. 2005), however, such stenoses are uncommon in this age group and may well be related to CADASIL. Besides, it has been recently reported that no significant association exists between the severity of carotid atherosclerosis and occurrence of stroke in CADASIL (Mawet et al. 2011). Whether stenosis in the large cerebral arteries has an important role in the development of lacunar infarcts has been a controversial issue (Devuyst et al. 2003). More often such stenoses would preferentially cause larger infarcts, as occurred in the 32-year-old CADASIL patient, whom we will discuss later. The risk of cardiac embolic brain infarcts due to arrhythmias in CADASIL patients is controversial (Cumurciuc et al. 2006, Rufa et al. 2007).

We suggest that in the elderly CADASIL patients the major cause of lacunar infarcts in the LN is hemodynamic due to the loss of autoregulation resulting from the VSMC degeneration and fibrosis and consequent rigidity of arterioles (Okeda et al. 2002). Indeed, the skin microvascular reactivity in CADASIL patients was found to be changed (Gobron et al. 2007). The reduced responses to vasodilator stimuli examined in humans (Chabriat et al. 2000, Liebetrau et al. 2002) as well as in a transgenic mouse model of CADASIL (Lacombe et al. 2005) also support this hypothesis. The higher metabolic demand for oxygen in the basal ganglia may further aggravate the ischemic insult

(Ganong 2003). The low systemic blood pressure profile observed in CADASIL patients may further disturb cerebral hemodynamics (Rufa et al. 2005). However, Singhal and Markus reported no changes in cerebrovascular reactivity and dynamic autoregulation in non-demented CADASIL patients. The reason for this may be either the early stage of the disease and/or insensitivity of transcranial Doppler (Singhal and Markus 2005).

A new hypothesis for the pathogenesis of lacunar infarctions was presented by Wardlaw (Wardlaw 2005). She proposed that the increased permeability of the BBB with chronic leakage of toxic substances through the walls of penetrating arterioles could be a decisive factor for lacunar stroke. The extensive T2w MRI hyperintensities in CADASIL patients reflect edema, which was also verified by the increased diffusivity in diffusion tensor MRI (Molko et al. 2001). However, this edema is widely distributed and difficult to specifically associate with lacunar infarctions. Furthermore, our immunostaining did not disclose major leakage of large plasma proteins through the thickened, N3ECD immunopositive walls of most penetrating arterioles in either the cerebral WM or LN. Thus, the mechanism proposed by Wardlaw is an unlikely explanation for the lacunar infarcts in CADASIL.

Microhaemorrhages have also been reported in about 25% to 69% CADASIL patients (Lesnik Oberstein et al. 2001, Dichgans et al. 2002, van den Boom et al. 2003a). These microhaemorrhages are most often located in the cortical GM, basal ganglia and are outside the T2w MRI hyperintensities of the cerebral WM (Lesnik Oberstein et al. 2001, Dichgans et al. 2002, Viswanathan and Chabriat 2006, Viswanathan et al. 2006). Cerebral microhaemorrhages have been reported to predict an increased risk of PBHs, but not that of the lacunar infarcts (Lesnik Oberstein et al. 2001, Dichgans et al. 2002, Choi et al. 2006). The significance of the microhaemorrhage in CADASIL is not clearly known yet.

## 6.2. The young CADASIL patient (Study II and III)

In the 32-year-old CADASIL patient infrequent small fatty streaks were detected in aorta as well as in coronary and carotid arteries. The left, moderately edematous hemisphere weighed 860 g and a tentorial herniation was observed. The parenchyma in the left MCA territory appeared infarcted and this was verified in coronal sections through the left hemisphere. Atherosclerosis was seen only in the left MCA and a recent thrombus was detected approximately one centimetre from its origin. Histologically the wall of the left MCA was thickened with a focal atherosclerotic plaque. At that site an old mural thrombus caused stenosis of the lumen and a recent thrombus occluded the remaining lumen. There were also a few older subcortical lacunar infarcts (**III**).

The histopathological and immunohistochemical findings of arterioles (degeneration of VSMCs as well as accumulation of N3ECD and collagen I) in the frontal lobar area and LN of the patient were similar to those in the elderly CADASIL brains, though

somewhat less prominent. EM of frontal samples also revealed a massive deposition of GOM between the degenerating VSMCs in the walls of cerebral WM arterioles (**II, III**).

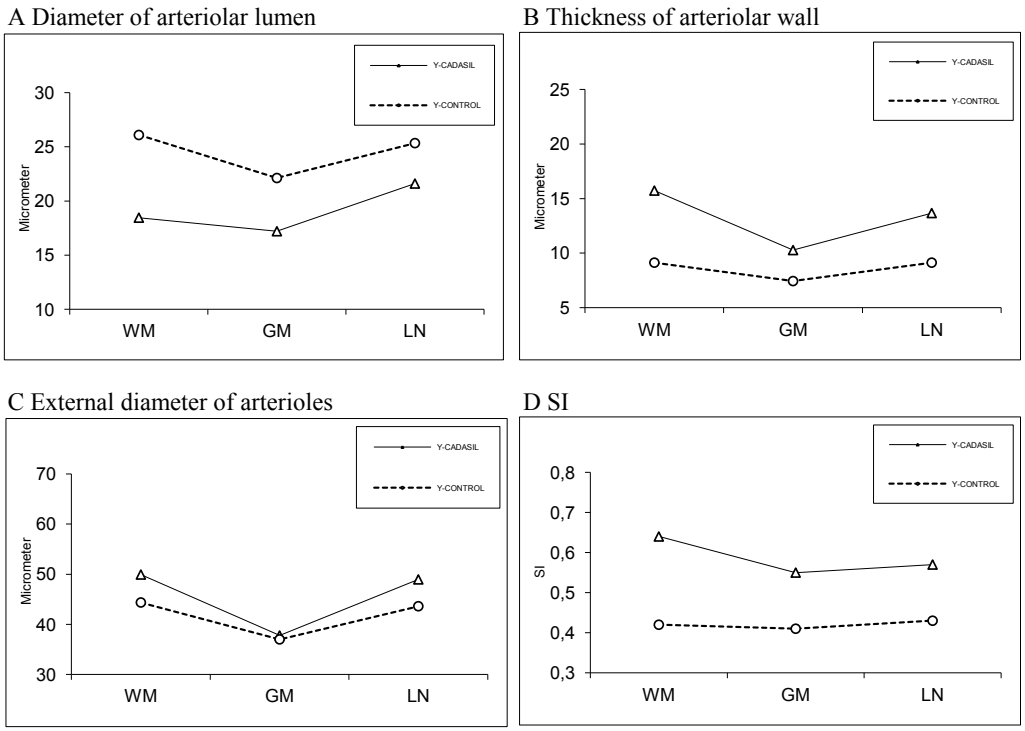
The four indicators of arteriolar stenosis and fibrosis and the numbers of arterioles measured in the cerebral WM, cortical GM and LN of the patient and two control groups (for lobar and LN regions) are given in Table 3 and Table 5.

In this patient already at this young age significant narrowing of arteriolar lumen, thickening of arteriolar wall and consequently increase of SI were recorded in both cerebral WM and cortical GM arterioles ($p < 0.01$) compared to that of the age-matched controls. This difference persisted even when compared to the elderly controls without cerebrovascular diseases (mean age: 59.8 years) and controls with a history of another type of cerebrovascular disease (mean age: 61.4 years) (**III**). This is remarkable, since higher age is known to be associated with an increased SI of cerebral penetrating arterioles (Table 3). The thickness of arteriolar wall, external diameter of arterioles and SI were significantly greater in the cerebral WM arterioles than in the cortical GM arterioles, whereas the diameter of arteriolar lumen was still larger in the WM arterioles than in the GM arterioles as in the age-matched controls, although its size had decreased to almost the same as in the GM arterioles (Table 5) (Figure 3).

These findings in the WM arterioles suggest that although already at this early stage of the disease there was narrowing and fibrosis, these had not encroached on the lumen and decreased CBF to such an extent that major lesions/symptoms had occurred. This is analogous to arteriosclerosis, in which the disease in the arteriolar wall is usually advanced before the CBF is compromised to such an extent that symptoms arise. Another thing worth noting is that the mean diameter of arteriolar lumen in the cortical GM arterioles was also markedly decreased compared to that in the controls, but typically of CADASIL no ischemic lesions or infarcts actually existed yet.

In the LN, only the thickness of the arteriolar wall and SI were significantly greater in the patient than in the controls (both $p < 0.01$). Compared to the cerebral WM and cortical GM arterioles the changes in LN arterioles were similar as in the elderly CADASIL patients. These findings indicate that – similarly as in the elderly patients – CADASIL causes remarkable fibrosis in the LN arterioles already at such a young age, but the arterioles do not become stenosed (Table 5) (Figure 3). In our PET study on the young CADASIL patients (mean age: 32.8 years) we found that CBF in the putamen was actually greater than that in the cerebral cortex and even slightly higher in the patients than that in the controls (Tuominen et al. 2004). However, according to the T2w MRI records of the patient there were only ischemic lesions, but no infarcts in the LN. Therefore, the reason for the ischemic lesions could also be hemodynamic, as discussed and proposed in the LN of the elderly CADSIL patients.

How early the arteriolar changes appear in CADASIL is poorly known. The reported earliest ages of onset of the symptoms are 6 years for migraine with aura (Vahedi et al.




WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; SI: sclerotic index; Y: young

**Figure 3.** A, The diameter of arteriolar lumen ; B, thickness of arteriolar wall; C, external diameter of arterioles and D, SI of the WM, GM and LN in the young CADASIL patient

2004), 8 years for deficits in executive functions (Hartley et al. 2010) and 11 years for the first ever stroke (Granild-Jensen et al. 2009). Other researchers have also reported MRI changes in patients aged between 18 and 20 years (Opherk et al. 2004). One report by using magnetic resonance angiography already showed the calibre variation of the anterior cerebral arteries and the left MCA at the age of 11 (Granild-Jensen et al. 2009). Among Finnish p.R133C CADASIL patients we have identified deposits of GOM near VSMCs in dermal arteries of one 18-year-old patient (Tuominen et al. 2001) and narrowing of retinal arterioles in this patient and in another 19-year-old patient (Harju et al. 2004). These two patients also had characteristic periventricular and anterior temporal lobe WM hyperintensities in T2w MRI (T. Kurki, personal communications, 2005). In our previous PET study on the young CADASIL patients (mean age 32.8, range 19-42 years of age) CBF in the cerebral WM did not begin to decrease until after the age of 30 (Tuominen et al. 2004). Thus, our result of incipient stenosis at the age of 32 corresponds well to the hemodynamic analyses. The youngest reported patient subjected to post-mortem analysis died at the age of 28 (Dichgans et al. 2002). Like our patient, this patient had already GOM deposits. Considering the fact that many young patients, even the patients in their childhood as discussed above, have had already WM hyperintensities in T2w MRI or ischemic attacks, the stenosis and fibrosis of arterioles could be possible.

Our young patient died of a massive infarction in the territory of left MCA due to a fresh thrombus superimposed on a focal atherosclerotic plaque with an older mural thrombus in proximal MCA. This was unexpected, as the patient had experienced only a mild TIA prior to this lethal episode. The patient did not have any common vascular risk factors, thus, we do not have any obvious cause for the MCA atherosclerotic plaque, which furthermore was focal, the rest of the arteriolar tree being only minimally affected. In general, large infarctions are rare in CADASIL although stenosis has recently been reported in large cerebral arteries in 5 out of 13 (38%) elderly CADASIL patients (Choi et al. 2005). The patient's p.C174R mutation is relatively rare, having been reported in only two families, without any specific features (Santa et al. 2003, Opherk et al. 2004). Interestingly, however, another mutation p.C174Y of the same codon is associated with a lower age of onset for stroke, immobilization and death (Opherk et al. 2004). Yet, mutations in this codon 174 are not associated with large infarcts. Thus, the ultimate reason for the premature atherosclerosis and thrombosis in the patient remains open.

## 6.3. The very old CADASIL patient (Study IV)

The patient is a woman, who was born in 1911. She had suffered from migraine with aura. At the age of 66 she developed psychotic symptoms of short duration. The first cerebral infarction was diagnosed at the age of 71 with impaired speech, memory problems and decreased muscle strength in upper extremities. She remained ambulatory until the age of 85, whereafter she was bedridden, dysarthric and demented until death at the age of 95. Her son had had recurrent strokes since the age of 35 years and died at the age of 63 years. He received the diagnosis of CADASIL on the basis of genetic demonstration of p.R133C mutation in NOTCH3. Therefore, the patient was also examined for this mutation, which was verified as the cause of her disease.

**Table 5.** Diameter of Arteriolar Lumen, Thickness of Arteriolar Wall, External Diameter of Arterioles and Sclerotic Index of Cerebral White Matter, Cortical Grey Matter and Lenticular Nucleus in Young CADASIL Patient and Controls

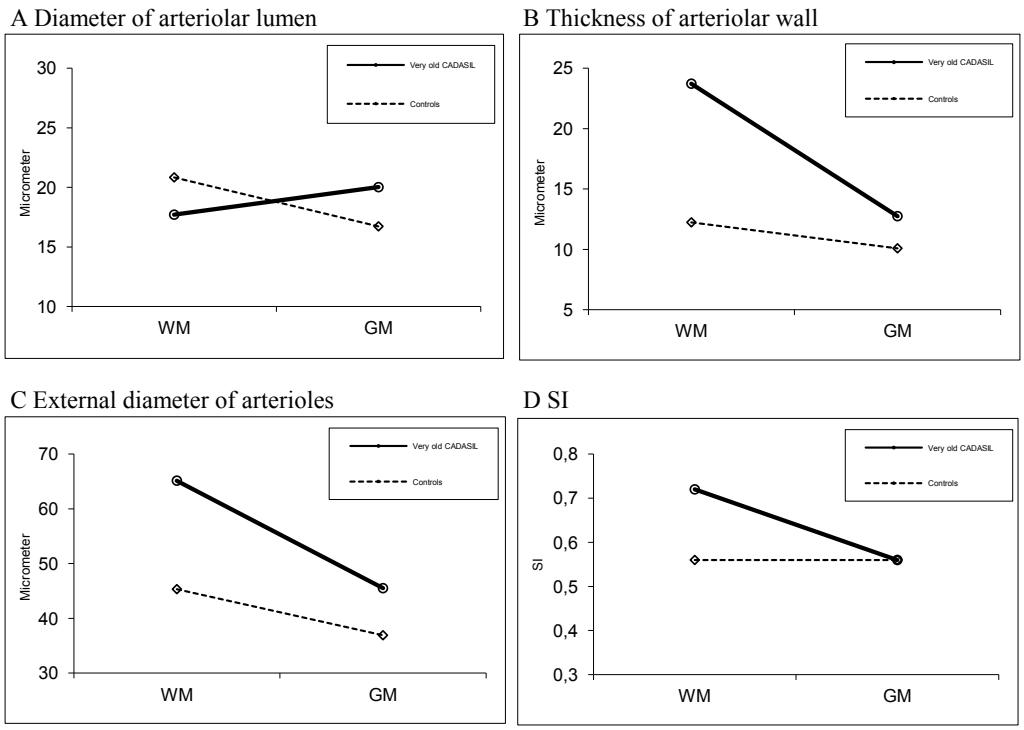
| | Diameter of Arteriolar Lumen (µm) | | | Thickness of Arteriolar Wall (µm) | | | External Diameter of Arterioles (µm) | | | Sclerotic Index | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WM | GM | LN | WM | GM | LN | WM | GM | LN | WM | GM | LN |
| Young CADASIL (N = 1) | 18.46 ± 10.90 (n = 61) | 17.21 ± 7.01 (n = 69) | 21.61 ± 10.25 (n = 35) | 15.73 ± 6.19 [d] (n = 61) | 10.28 ± 2.43 (n = 69) | 13.67 ± 4.09 (n = 35) | 49.93 ± 20.30 [d] (n = 61) | 37.77 ± 8.59 (n = 69) | 48.96 ± 15.39 [e] (n = 35) | 0.64 ± 0.11 [d] (n = 61) | 0.55 ± 0.11 (n = 69) | 0.57 ± 0.10 [f] (n = 35) |
| Young lobar controls (N = 3) | 26.09 ± 8.59 [b] (n = 52) | 22.12 ± 6.47 [a] (n = 82) | | 9.13 ± 2.95 [b] (n = 52) | 7.44 ± 1.59 [a] (n = 82) | | 44.36 ± 11.33 (n = 52) | 37.00 ± 7.13 (n = 82) | | 0.42 ± 0.10 [b] (n = 52) | 0.41 ± 0.08 [a] (n = 82) | |
| Young LN controls (N = 4) | | | 25.34 ± 9.02 (n = 134) | | | 9.13 ± 2.47 [c] (n = 134) | | | 43.61 ± 10.69 (n = 134) | | | 0.43 ± 0.10 [c] (n = 134) |

WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; N: number of patients analysed; n: number of arterioles measured
One-way ANOVA, $p < 0.01$: a: patient's GM vs. corresponding control's GM; b: patient's WM vs. corresponding control's WM;
c: patient's LN vs. corresponding control's LN; d: patient's GM vs. patient's WM; e: patient's GM vs. patient's LN; f: patient's WM vs. patient's LN

In the very old patient histopathology and immunohistochemistry for N3ECD, α-SMA and collagen I in the tunica media or adventitia of WM arterioles were by and large similar to those in both elderly and young CADASIL patients. Immunopositivity for N3ECD was finely granular and more discrete than in elderly CADASIL patients, while the number of α-SMA positive VSMCs did not appear to be lesser. This gave an impression that the degree of pathology was less severe than in the elderly CADASIL patients, which could be one explanation the late onset and long course of this patient.

The four indicators of arteriolar stenosis and fibrosis and the numbers of arterioles measured in the cerebral WM and cortical GM of the patient and controls are given in Table 3 and Table 6.

In the very old patient's WM arterioles the diameter of arteriolar lumen was almost significantly smaller than that in the age-matched controls ($p$ = 0.011), while the thickness of arteriolar wall, external diameter of arterioles and SI were significantly ($p <$ 0.01) greater than those in the age-matched controls. In the GM arterioles the diameter of arteriolar lumen ($p$ = 0.07) and thickness of the arteriolar wall ($p$ = 0.01) were greater than those in the controls, and because of the significantly larger external diameter of arterioles the SI value was the similar as in the controls ($p$ = 0.89) (Table 6) (Figure 4).




WM: cerebral white matter; GM: cortical grey matter; SI: sclerotic index

**Figure 4.** A, The diameter of arteriolar lumen ; B, thickness of arteriolar wall; C, external diameter of arterioles and D, SI of the WM and GM in the very old CADASIL patient, and controls

Before we reported this patient, the oldest patient, whose post-mortem brain pathology has been reported, was 75 years old. He had suffered from migraine since the age of 10 and began to develop motor and psychiatric symptoms at the age of 55 (Okeda et al. 2002). There is also a report on an 86-year-old man with CADASIL (p.R544C) who was doing well with minimal symptoms (Lee et al. 2009). Our very old CADASIL patient, with death at the age of 95 and with 29-year-long clinical course, is the oldest CADASIL patient so far reported (Kalimo et al. 2008). The patient's first ever stroke did not occur until at the age of 71, which is a common age for "general" strokes. Establishing correct diagnosis was further confused by the preceding psychotic symptoms. Most likely she would not have been examined genetically at all, if her son had not suffered from a "typical" CADASIL. These patients underline the marked variance in the clinical picture even in the same family and show the importance to consider CADASIL even among aged persons with leukoaraiosis and/or lacunar infarctions (Dong et al. 2003).

The stenosis in the patient's WM arterioles was less severe than in the elderly CADASIL patients. It was unexpected, since the patient was approximately 30 years older at death than the elderly CADASIL patients. If it is considered that the patient had 29-year-long clinical course and the elderly CADASIL patients had only average 15-year-long clinical course, this less stenosis was more striking. Considering the fact that the patient had later onset and relatively longer course of the disease than the elderly CADASIL patients carrying the same mutation, it is probable that in the patient the pathogenic effect of the mutation is milder or she has some protective genetic and/or epigenetic factors, which have delayed

**Table 6.** Diameter of Arteriolar Lumen, Thickness of Arteriolar Wall, External Diameter of Arterioles and Sclerotic Index of Cerebral White Matter and Cortical Grey in Very Old CADASIL Patient, Controls and Elderly CADASIL Patients

| | Diameter of Arteriolar Lumen (µm) | | Thickness of Arteriolar Wall (µm) | | External Diameter of Arterioles (µm) | | Sclerotic Index | |
|---|---|---|---|---|---|---|---|---|
| | WM | GM | WM | GM | WM | GM | WM | GM |
| Very old CADASIL (N = 1) | 17.71 ± 9.24 [e] (n = 79) | 20.02 ± 7.69 (n =69) | 23.72 ± 11.28 [b c e] (n = 79) | 12.75 ± 4.03 [d] (n =69) | 65.15 ± 27.41 [b c e] (n = 79) | 45.51 ± 12.26 [a d] (n =69) | 0.72 ± 0.10 [b c] (n = 79) | 0.56 ± 0.10 (n =69) |
| Age -matched Controls (N = 6) | 20.84 ± 9.94 (n = 165) | 16.73 ± 7.50 (n = 38) | 12.24 ± 2.77 (n = 165) | 10.09 ± 1.82 (n = 38) | 45.33 ± 13.03 [f] (n = 165) | 36.91 ± 9.03 (n = 38) | 0.56 ± 0.11 (n = 165) | 0.56 ± 0.09 (n = 38) |
| Elderly CADASIL (N = 5) | 14.80 ± 10.45 (n = 1056) | 17.32 ± 6.84 (n = 171) | 20.49 ± 8.84 (n = 1056) | 10.60 ± 3.82 (n = 171) | 55.78 ± 23.35 (n = 1056) | 38.52 ± 10.14 (n = 171) | 0.74 ± 0.13 (n = 1056) | 0.55 ± 0.13 (n = 171) |
| Elderly Controls (N = 4) | 28.29 ± 7.45 [g i] (n = 49) | 19.58 ± 7.43 (n = 75) | 7.56 ± 3.12 [i] (n = 49) | 8.70 ± 2.23 (n = 75) | 43.41 ± 10.78 (n = 49) | 36.97 ± 8.01 (n = 75) | 0.35 ± 0.08 [g i] (n = 49) | 0.48 ± 0.12 [h] (n = 75) |
| Young controls (N = 3) | 26.09 ± 8.59 (n = 52) | 22.12 ± 6.47 (n = 82) | 9.13 ± 2.95 (n = 52) | 7.44 ± 1.59 (n = 82) | 44.36 ± 11.33 (n = 52) | 37.00 ± 7.13 (n = 82) | 0.42 ± 0.10 (n = 52) | 0.41 ± 0.08 (n = 82) |

WM: white matter; GM: grey matter; N: number of patients analysed; n: number of arterioles measured One-way ANOVA, $p < 0.01$: a: patient's GM vs. age-matched controls' GM; b: patient's WM vs. age-matched controls' WM; c: patient's GM vs. patient's WM; d: patient's GM vs. elderly CADASIL patients' GM; e: patient's WM vs. elderly CADASIL patients' WM; f: age-matched controls' GM vs. WM; g: elderly controls' GM vs. WM; h: age-matched controls' GM vs. elderly controls' GM; i: age-matched controls' WM vs. elderly controls' WM

the pathological changes in the vascular walls. Whatever the mechanism is, the preservation of the WM arteriolar luminal size nearly comparable to that of the age-matched controls was most probably a beneficial factor.

In the patient the arteriolar lumina were narrower in the WM than in the GM. This relationship together with the marked increase (almost two times) in the fibrosis/thickness and consequent rigidity of the walls may aggravate the severity of the stenosis in the WM arterioles and explains the vulnerability of WM in CADASIL. On the contrary, in the elderly controls the mean luminal diameter of arterioles in the WM was significantly larger than in the GM (Table 6) (Figure 4). However, in the WM of age-matched controls the lumina of arterioles narrowed and the fibrosis/thickness of the walls increased more than in the GM (Table 6) (Figure 4), which may well contribute to the increase in WM MRI changes. These age-related changes contribute only to a minor degree to the prominent alterations in CADASIL.

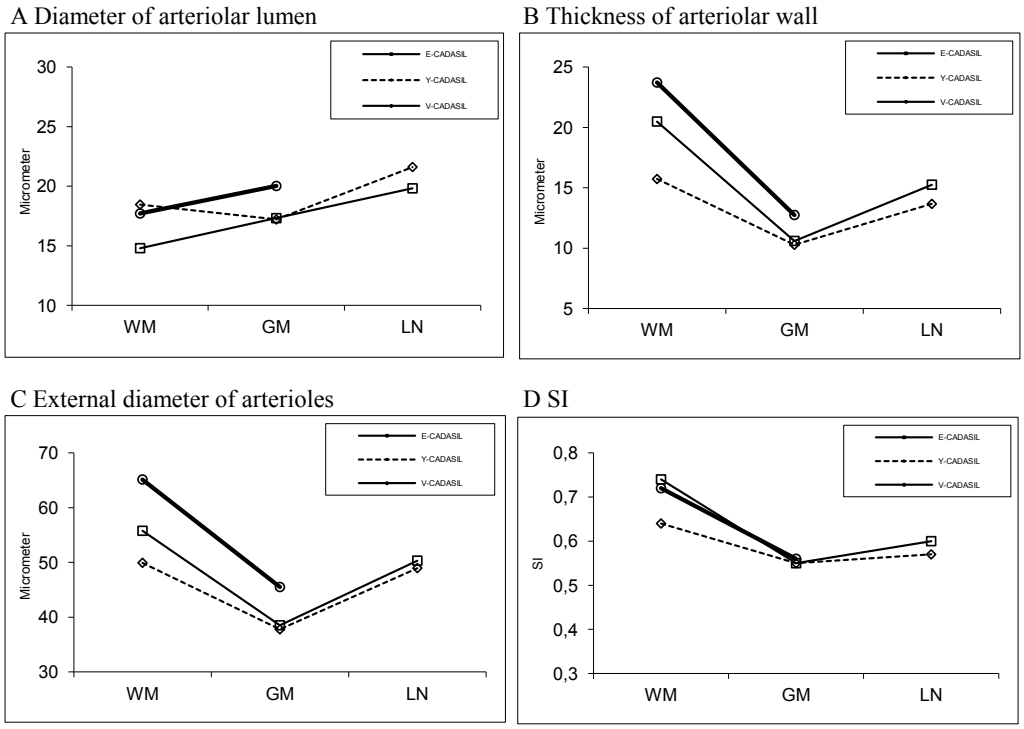
## 6.4. Ageing and CADASIL

In the cerebral WM arterioles all four indicators were more severely altered in the elderly and very old patients than in the young one. Our results indicate that marked progression of the disease with age occurs in the cerebral WM arterioles, where the lumina become markedly narrowed and walls thickened (Table 3) (Table 7) (Figure 5). Remarkably when the young CADASIL patient were compared with the elderly CADASIL patients, we observed that in the cortical GM and LN arterioles all four indicators were similar, indicating that the duration of the disease (patients'age) do not have a significant or minor effect on the progression in either cortical or deep GM arterioles. These results underline the basic difference between arterioles in different brain

**Table 7.** Diameter of Arteriolar Lumen, Thickness of Arteriolar Wall, External Diameter of Arterioles and Sclerotic Index of Cerebral White Matter, Cortical Grey Matter and Lenticular Nucleus in Elderly, Young and Very Old CADASIL Patients

| | Diameter of Arteriolar Lumen (µm) | | | Thickness of Arteriolar Wall (µm) | | | External Diameter of Arterioles (µm) | | | Sclerotic Index | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WM | GM | LN | WM | GM | LN | WM | GM | LN | WM | GM | LN |
| Elderly CADASIL (N = 5) | 14.80 ± 10.45 (n = 1056) | 17.32 ± 6.84 (n = 171) | 19.82 ± 9.25 (n = 486) | 20.49 ± 8.84 (n = 1056) | 10.60 ± 3.82 (n = 171) | 15.26 ± 8.79 (n = 486) | 55.78 ± 23.35 (n = 1056) | 38.52 ± 10.14 (n = 171) | 50.33 ± 22.30 (n = 486) | 0.74 ± 0.13 (n = 1056) | 0.55 ± 0.13 (n = 171) | 0.60 ± 0.13 (n = 486) |
| Young CADASIL (N = 1) | 18.46 ± 10.90 [b] (n = 61) | 17.21 ± 7.01 (n = 69) | 21.61 ± 10.25 (n = 35) | 15.73 ± 6.19 [b] (n = 61) | 10.28 ± 2.43 (n = 69) | 13.67 ± 4.09 (n = 35) | 49.93 ± 20.30 [b] (n = 61) | 37.77 ± 8.59 (n = 69) | 48.96 ± 15.39 (n = 35) | 0.64 ± 0.11 [b] (n = 61) | 0.55 ± 0.11 (n = 69) | 0.57 ± 0.10 (n = 35) |
| Very old CADASIL (N = 1) | 17.71 ± 9.24 [e] (n =79) | 20.02 ± 7.69 (n =69) | | 23.72 ± 11.28 [e,g] (n =79) | 12.75 ± 4.03 [d,f] (n =69) | | 65.15 ± 27.41 [e,g] (n =79) | 45.51 ± 12.26 [d,f] (n =69) | | 0.72 ± 0.10 [g] (n =79) | 0.56 ± 0.10 (n =69) | |

WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; N: number of patients analysed; n: number of arterioles measured
One-way ANOVA, $p < 0.01$: a: elderly CADASIL's GM vs. young CADASIL's GM; b: elderly CADASIL's WM vs. young CADASIL's WM;
c: elderly CADASIL's LN vs. young CADASIL's LN; d: elderly CADASIL's GM vs. very old CADASIL's GM;
e: elderly CADASIL's WM vs. very old CADASIL's WM; f: young CADASIL's GM vs. very old CADASIL's GM;
g: young CADASIL's WM vs. very old CADASIL's WM;




WM: cerebral white matter; GM: cortical grey matter; LN: lenticular nucleus; SI: sclerotic index; E: elderly; Y: young; V: very old

**Figure 5.** A, The diameter of arteriolar lumen ; B, thickness of arteriolar wall; C, external diameter of arterioles and D, SI of the WM, GM and LN in the elderly CADASIL patients, young CADSIL patient and very old CADASIL patient

regions, which is also seen in the opposite pattern of distribution of CAA, which afflicts arteries in the GM, but not those in the WM.

Furthermore, our results (Table 3) showed that the diameter of arteriolar lumen in the three groups of lobar controls was larger in the WM arterioles than in the GM arterioles. This difference was already narrowed in the young CADASIL patient, while in the elderly and very old patients the progression of the disease had reversed this difference, i.e. the WM arterioles were clearly narrower. As pointed out already above, the very old patient was somewhat exceptional, since the stenosis and fibrosis were not proportional to the age of the patient/duration of the disease, i.e. this patient may have had factors which had a beneficial effect.

## 6.5. Limitations of the study

Our results are mainly derived from post-mortem pathological studies. The *in vivo* situation may be different and thus the basis for the presumed pathogenetic mechanisms

(disturbances of CBF, hemodynamics, increased resistance and rigidity of arterioles) is indirect. Secondly, we only analysed arterioles from the frontal lobe and LN. The arterioles in other topographic regions remain to be analysed. Thirdly, in our study the number of patients is low, and besides there is only one young and one very old CADASIL patient, but the availability of CADASIL autopsies is unavoidably limited. Besides, the number of controls and their age distribution are also limited, but we attempted to match their ages as closely as possible and exclude cases with diseases causing overt cerebrovascular effects. Fourthly, we have only assessed the basic morphometric parameters, while using additional specific immunohistochemical staining could provide more detailed information of the nature of the vascular alterations.

# 7. SUMMARY AND CONCLUSIONS

Our study has shown that in CADASIL:

(1) There is both stenosis and fibrosis in cerebral WM arterioles, whereas there is only fibrosis in deep GM (arterioles in LN). In cortical GM there is neither significant stenosis nor fibrosis of arterioles. Thus, different vascular beds within the brain are differentially affected by CADASIL.

(2) These findings are different from changes which occur during ageing where the arteriolar lumen in the WM despite lesser narrowing remains wider than in the GM.

(3) The previous controversial results appear to be due to measuring cerebral arteries of larger caliber, in which the pathology is not obvious, or there have been some errors in mathematical calculations.

(4) There are two different pathogenetic mechanisms for the ischemic lesions. (a) In cerebral WM the lacunar infarcts are due to stenosis of rigid fibrotic arterioles. (b) In deep GM the infarcts are hemodynamic due to loss of cerebral vasoregulation in the rigid arterioles. (c) In cortical GM neither stenosis nor fibrosis is severe enough to cause infarctions.

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# REFERENCES


Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 24: 35-41.

Adib-Samii P, Brice G, Martin RJ, Markus HS (2010) Clinical spectrum of CADASIL and the effect of cardiovascular risk factors on phenotype: study in 200 consecutively recruited individuals. *Stroke* 41: 630-4.

Amberla K, Waljas M, Tuominen S, Almkvist O, Poyhonen M, Tuisku S, Kalimo H, Viitanen M (2004) Insidious cognitive decline in CADASIL. *Stroke* 35: 1598-602.

Arboleda-Velasquez JF, Lopera F, Lopez E, Frosch MP, Sepulveda-Falla D, Gutierrez JE, Vargas S, Medina M, Martinez De Arrieta C, Lebo RV, Slaugenhaupt SA, Betensky RA, Villegas A, Arcos-Burgos M, Rivera D, Restrepo JC, Kosik KS (2002) C455R notch3 mutation in a Colombian CADASIL kindred with early onset of stroke. *Neurology* 59: 277-9.

Arboleda-Velasquez JF, Manent J, Lee JH, Tikka S, Ospina C, Vanderburg CR, Frosch MP, Rodríguez-Falcón M, Villen J, Gygi S, Lopera F, Kalimo H, Moskowitz MA, Ayata C, Louvi A, Artavanis-Tsakonas S (2011) PNAS Plus: Hypomorphic Notch 3 alleles link Notch signaling to ischemic cerebral small-vessel disease. *Proc Natl Acad Sci U S A* 108: E128-35.

Arboleda-Velasquez JF, Zhou Z, Shin HK, Louvi A, Kim HH, Savitz SI, Liao JK, Salomone S, Ayata C, Moskowitz MA, Artavanis-Tsakonas S (2008) Linking notch signaling to ischemic stroke. *Proc Natl Acad Sci U S A* 105: 4856-61.

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signalling: cell fate control and signal integration in development. *Science* 284: 770-6.

Auer DP, Pütz B, Gössl C, Elbel G, Gasser T, Dichgans M (2001) Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. *Radiology* 218: 443-51.

Ayata C (2010) CADASIL: experimental insights from animal models. *Stroke* 41 (10 Suppl): S129-34.

Bamford J, Sandercock P, Dennis M, Burn J, Warlow C (1991) Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet* 337: 1521-6.

Baudrimont M, Dubas F, Joutel A, Tournier-Lasserve E, Bousser MG (1993) Autosomal dominant leukoencephalopathy and subcortical ischemic stroke. A clinicopathological study. *Stroke* 24: 122-5.

Beatus P, Lendahl U (1998) Notch and neurogenesis. *J Neurosci Res* 54: 125-36.

Bejot Y, Catteau A, Caillier M, Rouaud O, Durier J, Marie C, Di Carlo A, Osseby GV, Moreau T, Giroud M (2008) Trends in incidence, risk factors, and survival in symptomatic lacunar stroke in Dijon, France, from 1989 to 2006: a population-based study. *Stroke* 39: 1945-51.

Bentley P, Wang T, Malik O, Nicholas R, Ban M, Sawcer S, Sharma P (2011) CADASIL with cord involvement associated with a novel and atypical NOTCH3 mutation. *J Neurol Neurosurg Psychiatry* 82: 855-60.

Betsholtz C (2004) Insight into the physiological functions of PDGF through genetic studies in mice. *Cytokine Growth Factor Rev* 15: 215-28.

Blaumueller CM, Qi H, Zagouras P, Artavanis-Tsakonas S (1997) Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. *Cell* 90: 281-91.

Bousser MG, Tournier-Lasserve E (1994) Summary of the proceedings of the First International Workshop on CADASIL. Paris, May 19-21, 1993. *Stroke* 25: 704-7.

Bousser MG, Tournier-Lasserve E, Aylward R, Tourbah A, Dormont D, Romero N, Cabanis E (1988) Recurrent stroke in a family with diffuse white-matter abnormalities and muscular lipidosis. A new mitochondrial cytopathy. *J Neurol* 235: 54-5.

Bowler JV (2005) Vascular cognitive impairment. *J Neurol Neurosurg Psychiatry* 76 (Suppl 5): v35-44.

Brennan K, Tateson R, Lieber T, Couso JP, Zecchini V, Arias AM (1999) The abruptex mutations of notch disrupt the establishment of proneural clusters in Drosophila. *Dev Biol* 216: 230-42.

Brou C, Logeat F, Gupta N, Bessia C, LeBail O, Doedens JR, Cumano A, Roux P, Black RA, Israël A (2000) A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell* 5: 207-16.

Bruening R, Dichgans M, Berchtenbreiter C, Yousry T, Seelos KC, Wu RH, Mayer M, Brix G, Reiser M (2001) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: decrease in regional cerebral blood volume in hyperintense subcortical lesions inversely correlates with disability and cognitive performance. *Am J Neuroradiol* 22: 1268-74.

Brulin P, Godfraind C, Leteurtre E, Ruchoux MM (2002) Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. *Acta Neuropathol (Berl)*104: 241-8.

Buffon F, Porcher R, Hernandez K, Kurtz A, Pointeau S, Vahedi K, Bousser MG, Chabriat H (2006) Cognitive profile in CADASIL. *J Neurol Neurosurg Psychiatry* 77: 175-80.

Campos AH, Wang W, Pollman MJ, Gibbons GH (2002) Determinants of Notch-3 receptor expression and signaling in vascular smooth muscle cells: implications in cell-cycle regulation. *Circ Res* 91: 999-1006.

Chabriat H, Bousser MG (2007) Neuropsychiatric manifestations in CADASIL. *Dialogues Clin Neurosci* 9: 199-208.

Chabriat H, Bousser MG, Pappata S (1995c) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: a positron emission tomography study in two affected family members. *Stroke* 26: 1729-30.

Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser MG (2009) CADASIL. *Lancet Neurol* 8: 643-53.

Chabriat H, Levy C, Taillia H, Iba-Zizen MT, Vahedi K, Joutel A, Tournier-Lasserve E, Bousser MG (1998) Patterns of MRI lesions in CADASIL. *Neurology* 51: 452-7.

Chabriat H, Mrissa R, Levy C, Vahedi K, Taillia H, Iba-Zizen MT, Joutel A, Tournier-Lasserve E, Bousser MG (1999b) Brain stem MRI signal abnormalities in CADASIL. *Stroke* 30: 457-9.

Chabriat H, Pappata S, Ostergaard L, Clark CA, Pachot-Clouard M, Vahedi K, Jobert A, Le Bihan D, Bousser MG (2000) Cerebral hemodynamics in CADASIL before and after acetazolamide challenge assessed with MRI bolus tracking. *Stroke* 31: 1904-12.

Chabriat H, Pappata S, Poupon C, Clark CA, Vahedi K, Poupon F, Mangin JF, Pachot-Clouard M, Jobert A, Le Bihan D, Bousser MG (1999a) Clinical severity in CADASIL related to ultrastructural damage in white matter: *in vivo* study with diffusion tensor MRI. *Stroke* 30: 2637-43.

Chabriat H, Tournier-Lasserve E, Vahedi K, Leys D, Joutel A, Nibbio A, Escaillas JP, Iba-Zizen MT, Bracard S, Tehindrazanarivelo A (1995b) Autosomal dominant migraine with MRI white-matter abnormalities mapping to the CADASIL locus. *Neurology* 45: 1086-91.

Chabriat H, Vahedi K, Iba-Zizen MT, Joutel A, Nibbio A, Nagy TG, Krebs MO, Julien J, Dubois B, Ducrocq X, Levasseur M, Homeyer P, Mas JL, Lyon-Caen O, Tournier-Lasserve E, Bousser MG (1995a) Clinical spectrum of CADASIL: a study of 7 families. *Lancet* 346: 934-9.

Charlton RA, Morris RG, Nitkunan A, Markus HS (2006) The cognitive profiles of CADASIL and sporadic small vessel disease. *Neurology* 66: 1523-6.

Choi EJ, Choi CG, Kim JS (2005) Large cerebral artery involvement in CADASIL. *Neurology* 65: 1322-4.

Choi JC, Kang SY, Kang JH, Park JK (2006) Intracerebral haemorrhages in CADASIL. *Neurology* 67: 2042-4.

Chui HC (2007) Subcortical ischemic vascular dementia. *Neurol Clin* 25: 717-40.

Cocho D, Martí-Fàbregas J, Baiget M, Gallardo E, Rio E, Arboix A, Ruscalleda J, Martí-Vilalta JL (2011) Diagnosis of CADASIL disease in normotensive and non-diabetics with lacunar infarct. *Neurologia* 26: 325-30.

Colmant H (1980) Familiäre zerebrale Gefäberkrankung. *Zbl Allg Path Bd* 124: 163.

Coto E, Menéndez M, Navarro R, García-Castro M, Alvarez V (2006) A new de novo Notch3 mutation causing CADASIL. *Eur J Neurol* 13: 628-31.

Cumurciuc R, Henry P, Gobron C, Vicaut E, Bousser MG, Chabriat H, Vahedi K (2006) Electrocardiogram in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy patients without any clinical evidence of coronary artery disease: a case-control study. *Stroke* 37: 1100-2.

Davous P (1998) CADASIL: a review with proposed diagnostic criteria. *Eur J Neurol* 5: 219-33.

Davous P, Fallet-Bianco C (1991) Familial subcortical dementia with arteriopathic leukoencephalopathy. A clinico-pathological case. *Rev Neurol* 147: 376-84.

Desmond DW, Moroney JT, Lynch T, Chan S, Chin SS, Mohr JP (1999) The natural history of CADASIL: a pooled analysis of previously published cases. *Stroke* 30: 1230-3.

Devuyst G, Bogousslavsky J (2003) Editorial comment: The fall and rise of lacunar infarction with carotid stenosis. *Stroke* 34: 1409-11.

Dichgans M (2009) Cognition in CADASIL. *Stroke* 40 (Suppl 3): S45-7.

Dichgans M, Filippi M, Brüning R, Iannucci G, Berchtenbreiter C, Minicucci L, Uttner I, Crispin A, Ludwig H, Gasser T, Yousry TA (1999) Quantitative MRI in CADASIL: correlation with disability and cognitive performance. *Neurology* 52: 1361-7.

Dichgans M, Herzog J, Gasser T (2001) Notch3 mutation involving three cysteine residues in a family with typical CADASIL. *Neurology* 57: 1714-7.

Dichgans M, Holtmannspotter M, Herzog J, Peters N, Bergmann M, Yousry TA (2002) Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. *Stroke* 33: 67-71.

Dichgans M, Ludwig H, Muller-Hocker J, Messerschmidt A, Gasser T (2000) Small in-frame deletions and missense mutations in CADASIL: 3D models predict misfolding of Notch 3 EGF-like repeat domains. *Eur J Hum Genet* 4: 280-5.

Dichgans M, Markus HS, Salloway S, Verkkoniemi A, Moline M, Wang Q, Posner H, Chabriat HS (2008) Donepezil in patients with subcortical vascular cognitive impairment: a randomised double-blind trial in CADASIL. *Lancet Neurol* 7: 310-8.

Dichgans M, Mayer M, Uttner I, Bruning R, Muller-Hocker J, Rungger G, Ebke M, Klockgether T, Gasser T (1998) The phenotypic spectrum of CADASIL: Clinical findings in 102 cases. *Ann Neurol* 44: 731-9.

Domenga V, Fardoux P, Lacombe P, Monet M, Maciazek J, Krebs LT, Klonjkowski B, Berrou E, Mericskay M, Li Z, Tournier-Lasserve E, Gridley T, Joutel A (2004) Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. *Genes Dev* 18: 2730-5.

Donahue CP, Kosik KS (2004) Distribution pattern of Notch3 mutations suggests a gain-of-function mechanism for CADASIL. *Genomics* 83: 59-65.

Dong Y, Hassan A, Zhang Z, Huber D, Dalageorgou C, Markus HS (2003) Yield of screening for CADASIL mutations in lacunar stroke and leukoaraiosis. *Stroke* 34: 203-5.

Dubroca C, Lacombe P, Domenga V, Maciazek J, Levy B, Tournier-Lasserve E, Joutel A, Henrion D (2005) Impaired vascular mechanotransduction in a transgenic mouse model of cadasil arteriopathy. *Stroke* 36: 113-7.

Duering M, Karpinska A, Rosner S, Hopfner F, Zechmeister M, Peters N, Kremmer E, Haffner C, Giese A, Dichgans M, Opherk C (2011) Co-aggregate formation of CADASIL-mutant NOTCH3: a single-particle analysis. *Hum Mol Genet* 20: 3256-65.

Ebke M, Dichgans M, Bergmann M (1997) CADASIL: skin biopsy allows diagnosis in early stages. *Acta Neurol Scand* 95: 351-7.

Edvinsson L, Mackenzie ET, McCulloch J (1993) The blood vessel wall: Endothelial and smooth muscle cells. In: *Cerebral Blood Flow and Metabolism*. New York: Raven Press: 40-56.

Endres M, Laufs U, Huang Z (1998) Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 95: 8880-5.

Erkinjuntti T (2003) Subcortical ischemic vascular disease and dementia. *Int Psychogeriatr* 15 (Suppl 1): S23-6.

Ferrarra N, Winer J, Burton T (1991) Aortic smooth muscle cells express and secrete vascular endothelial growth factor. *Growth Factors* 5: 141-8.

Ferrer I (2010) Cognitive impairment of vascular origin: neuropathology of cognitive impairment of vascular origin. *J Neurol Sci* 299: 139-49.

Feuerhake F, Volk B, Ostertag CB, Jungling FD, Kassubek J, Orszagh M, Dichgans M (2002) Reversible coma with raised intracranial pressure: an unusual clinical manifestation of CADASIL. *Acta Neuropathol* 103: 188-92.

Finnilä S, Tuisku S, Herva R, Majamaa K (2001) A novel mitochondrial DNA mutation and a mutation in the Notch3 gene in a patient with myopathy and CADASIL. *J Mol Med* 79: 641-7.

Finsterer J (2007) Neuromuscular implications in CADASIL. *Cerebrovasc Dis* 24: 401-4.

Forteza AM, Brozman B, Rabinstein AA, Romano JG, Bradley WG (2001) Acetazolamide for the treatment of migraine with aura in CADASIL. *Neurology* 57: 2144-5.

Fouillade C, Chabriat H, Riant F, Mine M, Arnoud M, Magy L, Bousser MG, Tournier-Lasserve E, Joutel A (2008) Activating NOTCH3 mutation in a patient with small-vessel-disease of the brain. *Hum Mutat* 29: 452.

Furuta A, Ishii N, Nishihara Y, Horie A (1991) Medullary arteries in aging and dementia. *Stroke* 22: 442-6.

Ganong WF (2003) Circulation through special regions. In: *Review of Medical Physiology*. New York: McGraw-Hill: 614-23.

Gerhard (1980) Familiäre zerebrale arteriosklerose. *Zbl Allg Path Bd* 124: 163.

Gobron C, Vahedi K, Vicaut E, Stucker O, Laemmel E, Baudry N, Bousser MG, Chabriat H (2007) Characteristic features of in vivo skin microvascular reactivity in CADASIL. *J Cereb Blood Flow Metab* 27: 250-7.

Gobron C, Viswanathan A, Bousser MG, Chabriat H (2006) Multiple simultaneous cerebral infarctions in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Cerebrovasc Dis* 22: 445-6.

Goldstein LB, Jones MR, Matchar DB, Edwards LJ, Hoff J, Chilukuri V, Armstrong SB, Horner RD (2001) Improving the reliability of stroke subgroup classification using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria. *Stroke* 32: 1091-8.

Golomb MR, Sokol DK, Walsh LE, Christensen CK, Garg BP (2004) Recurrent hemiplegia, normal MRI, and NOTCH3 mutation in a 14-year-old: is this early CADASIL? *Neurology* 62: 2331-2.

Gong M, Rueschendorf F, Marx P, Schulz H, Kraft HG, Huebner N, Koennecke HC (2010) Clinical and genetic features in a family with CADASIL and high lipoprotein (a) values. *J Neurol* 257: 1240-5.

Granild-Jensen J, Jensen UB, Schwartz M, Hansen US (2009) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy resulting in stroke in an 11-year-old male. *Dev Med Child Neurol* 51: 754-7.

Gray GE, Mann RS, Mitsiadis E, Henrique D, Carcangiu ML, Banks A, Leiman J, Ward D, Ish-Horowitz D, Artavanis-Tsakonas S (1999) Human ligands of the Notch receptor. *Am J Pathol* 154: 785-94.

Gridley T (2001) Notch signaling during vascular development. *Proc Natl Acad Sci U S A* 98: 5377-8.

Gridley T (2007) Notch signaling in vascular development and physiology. *Development* 134: 2709-18.

Guerrot D, François A, Boffa JJ, Boulos N, Hanoy M, Legallicier B, Triquenot-Bagan A, Guyant-Marechal L, Laquerriere A, Freguin-Bouilland C, Ronco P, Godin M (2008) Nephroangiosclerosis in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: is NOTCH3 mutation the common culprit? *Am J Kidney Dis* 52: 340-5.

Gutierrez-Molina M, Caminero Rodriguez A, Martinez Garcia C, Arpa Gutierrez J, Morales Bastos C, Amer G (1994) Small arterial granular degeneration in familial Binswanger's syndrome. *Acta Neuropathol (Berl)* 87: 98-105.

Hansson EM, Lendahl U, Chapman G (2004) Notch signaling in development and disease. *Semin Cancer Biol* 14: 320-8.

Haritunians T, Boulter J, Hicks C, Buhrman J, DiSibio G, Shawber C, Weinmaster G, Nofziger D, Schanen C (2002) CADASIL Notch3 mutant proteins localize to the cell surface and bind ligand. *Circ Res* 90: 506-8.

Harju M, Tuominen S, Summanen P, Viitanen M, Poyhonen M, Nikoskelainen E, Kalimo H, Kivela T (2004) Scanning laser Doppler flowmetry shows reduced retinal capillary blood flow in CADASIL. *Stroke* 35: 2449-52.

Hartley J, Westmacott R, Decker J, Shroff M, Yoon G (2010) Childhood-onset CADASIL: clinical, imaging, and neurocognitive features. *J Child Neurol* 25: 623-7.

Hervé D, Chabriat H (2010) CADASIL. *J Geriatr Psychiatry Neurol* 23: 269-76.

Hervé D, Godin O, Dufouil C, Viswanathan A, Jouvent E, Pachaï C, Guichard JP, Bousser MG, Dichgans M, Chabriat H (2009) Three-dimensional MRI analysis of individual volume of Lacunes in CADASIL. *Stroke* 40: 124-8.

Hofman A, Rocca WA, Brayne C (1991) The prevalence of dementia in Europe: a collaborative study of 1980-1990 findings-Eurodem Prevalence Research Group. *Intern J Epidemiol* 20: 736-48.

Hu QD, Ang BT, Karsak M, Hu WP, Cui XY, Duka T, Takeda Y, Chia W, Sankar N, Ng YK, Ling EA, Maciag T, Small D, Trifonova R, Kopan R, Okano H, Nakafuku M, Chiba S, Hirai H, Aster JC, Schachner M, Pallen CJ, Watanabe K, Xiao ZC (2003) F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. *Cell* 115: 163-75.

Ihalainen S, Soliymani R, Iivanainen E, Mykkänen K, Sainio A, Pöyhönen M, Elenius K, Järveläinen H, Viitanen M, Kalimo H, Baumann M (2007) Proteome analysis of cultivated vascular smooth muscle cells from a CADASIL patient. *Mol Med* 13: 305-14.

Ishiko A, Shimizu A, Nagata E, Takahashi K, Tabira T, Suzuki N (2006) Notch3 ectodomain is a major component of granular osmiophilic material (GOM) in CADASIL. *Acta Neuropathol (Berl)* 112: 333-9.

Jacqmin M, Herve D, Viswanathan A, Guichard JP, During M, Dichgans M, Chabriat H (2010) Confluent thalamic hyperintensities in CADASIL. *Cerebrovasc Dis* 30: 308-13.

Jin S, Hansson EM, Tikka S, Lanner F, Sahlgren C, Farnebo F, Baumann M, Kalimo H, Lendahl U (2008) Notch signaling regulates platelet-derived growth factor receptor-beta expression in vascular smooth muscle cells. *Circ Res* 102: 1483-91.

Joutel A, Andreux F, Gaulis S, Domenga V, Cecillon M, Battail N, Piga N, Chapon F, Godfrain C, Tournier-Lasserve E (2000a) The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J Clin Invest* 105: 597-605.

Joutel A, Chabriat H, Vahedi K, Domenga V, Vayssiere C, Ruchoux MM, Lucas C, Leys D, Bousser MG, Tournier-Lasserve E (2000b) Splice site mutation causing a seven amino acid Notch3 in-frame deletion in CADASIL. *Neurology* 54: 1874-5.

Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E (1996) Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* 383: 707-10.

Joutel A, Dodick DD, Parisi JE, Cecillon M, Tournier-Lasserve E, Bousser MG (2000c) De novo mutation in the Notch3 gene causing CADASIL. *Ann Neurol* 47: 388-91.

Joutel A, Favrole P, Labauge P, Chabriat H, Lescoat C, Andreux F, Domenga V, Cecillon M, Vahedi K, Ducros A, Cave-Riant F, Bousser MG, Tournier-Lasserve E (2001) Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. *Lancet* 358: 2049-51.

Joutel A, Monet M, Domenga V, Riant F, Tournier-Lasserve E (2004) Pathogenic mutations associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy differently affect Jagged1 binding and Notch3 activity via the RBP/JK signaling Pathway. *Am J Hum Genet* 74: 338-47.

Joutel A, Monet-Lepretre M, Gosele C, Baron-Menguy C, Hammes A, Schmidt S, Lemaire-Carrette B, Domenga V, Schedl A, Lacombe P, Hubner N (2010) Cerebrovascular dysfunction and microcirculation rarefaction precede white matter lesions in a mouse genetic model of cerebral ischemic small vessel disease. *J Clin Invest* 120: 433-45.

Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssiere C, Cruaud C, Maciazek J, Weissenbach J, Bousser MG, Bach JF, Tournier-Lasserve E (1997) Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet* 350: 1511-5.

Jouvent E, Poupon C, Gray F, Paquet C, Mangin JF, Le Bihan D, Chabriat H (2011) Intracortical infarcts in small vessel disease: a combined 7-T postmortem MRI and neuropathological case study in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Stroke* 42: e27-30.

Kalimo H, Kalaria RN (2005) Hereditary forms of vascular dementia. In: Kalimo H, eds, *Pathology & Genetics: Cerebrovascular Diseases*. Basel: ISN Neuropath Press: 324-34.

Kalimo H, Kaste M, Haltia M (2002a) Vascular diseases. In: Graham DI, Lantos PL, eds, *Greenfield's Neuropathology*. London: Arnold: 281-356.

Kalimo H, Miao Q, Tikka S, Mykkänen K, Junna M, Roine S, Viitanen M, Pöyhönen M, Baumann M (2008) CADASIL: the most common hereditary vascular subcortical dementia. *Future Neurology* 3: 683-704.

Kalimo H, Ruchoux MM, Viitanen M, Kalaria RN (2002b) CADASIL: a common form of hereditary arteriopathy causing brain infarcts and dementia. *Brain Pathol* 12: 371-84.

Kalimo H, Viitanen M, Amberla K, Juvonen V, Marttila R, Poyhonen M, Rinne JO, Savontaus M, Tuisku S, Winblad B (1999) CADASIL: hereditary disease of arteries causing brain infarcts and dementia. *Neuropathol Appl Neurobiol* 25: 257-65.

Karlström H, Beatus P, Dannaeus K, Chapman G, Lendahl U, Lundkvist J (2002) A CADASIL-mutated Notch 3 receptor exhibits impaired intracellular trafficking and maturation but normal ligand-induced signaling. *Proc Natl Acad Sci U S A* 99: 17119-24.

Keverne JS, Low WC, Ziabreva I, Court JA, Oakley AE, Kalaria RN (2007) Cholinergic neuronal deficits in CADASIL. *Stroke* 38: 188-91.

Knopman DS (2007) Cerebrovascular disease and dementia. *Br J Radiol* 80 (Spec 2): S121-7.

Kopan R (2002) Notch: a membrane-bound transcription factor. *J Cell Sci* 115: 1095-7.

Krebs LT, Xue Y, Norton CR (2003) Characterization of Notch3-deficient mice: normal embryonic development and absence of genetic interactions with a Notch1 mutation. *Genesis* 37: 139-43.

Kusaba T, Hatta T, Kimura T, Sonomura K, Tanda S, Kishimoto N, Kameyama H, Okigaki M, Mori Y, Ishigami N, Mizuno T, Nakagawa M, Matsubara H (2007) Renal involvement in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Clin Nephrol* 67: 182-7.

Lacombe P, Oligo C, Domenga V, Tournier-Lasserve E, Joutel A (2005) Impaired cerebral vasoreactivity in a transgenic mouse model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy arteriopathy. *Stroke* 36: 1053-8.

Lagas PA, Juvonen V (2001) Schizophrenia in a patient with cerebral autosomally dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL disease). *Nord J Psychiatry* 55: 41-2.

Lammie GA (2002) Hypertensive cerebral small vessel disease and stroke. *Brain Pathol* 12: 358-70.

Lammie GA (2005) Small vessel disease. In: Kalimo H, eds, *Pathology & Genetics: Cerebrovascular Diseases*. Basel: ISN Neuropath Press: 85-91.

Lammie GA, Brannan F, Slattery J, Warlow C (1997) Nonhypertensive cerebral small-vessel disease. An autopsy study. *Stroke* 28: 2222-9.

Lardelli M, Williams R, Lendahl U (1995) Notch-related genes in animal development. *Int J Dev Biol* 39: 769-80.

Lasky JL, Wu H (2005) Notch signaling, brain development, and human disease. *Pediatr Res* 57 (5 Pt 2): 104R-109R.

Lee YC, Yang AH, Soong BW (2009) The remarkably variable expressivity of CADASIL: report of a minimally symptomatic man at an advanced age. *J Neurol* 256: 1026-7.

Lendahl U (1998) A growing family of Notch ligands. *Bioessays* 20: 103-7.

Lesnik Oberstein SA, Jukema JW, van Duinen SG, Macfarlane PW, van Houwelingen HC, Breuning MH, Ferrari MD, Haan J (2003a) Myocardial infarction in cerebral autosomal dominant arteriopathy with subcortical infarcts

and leukoencephalopathy (CADASIL). *Medicine (Baltimore)* 82: 251-6.

Lesnik Oberstein SA, van den Boom R, van Buchem MA, van Houwelingen HC, Bakker E, Vollebregt E, Ferrari MD, Breuning MH, Haan J; Dutch CADASIL Research Group (2001) Cerebral microbleeds in CADASIL. *Neurology* 57: 1066-70.

Lesnik Oberstein SA, van Duinen SG, van den Boom R, Maat-Schieman ML, van Buchem MA, van Houwelingen HC, Hegeman-Kleinn IM, Ferrari MD, Breuning MH, Haan J (2003b) Evaluation of diagnostic NOTCH3 immunostaining in CADASIL. *Acta Neuropathol* 106: 107-11.

Liebetrau M, Herzog J, Kloss CU, Hamann GF, Dichgans M (2002) Prolonged cerebral transit time in CADASIL: a transcranial ultrasound study. *Stroke* 33: 509-12.

Liem MK, Lesnik Oberstein SA, Haan J, Boom R, Ferrari MD, Buchem MA, Grond J (2009) Cerebrovascular reactivity is a main determinant of white matter hyperintensity progression in CADASIL. *Am J Neuroradiol* 30: 1244-7.

Liem MK, Lesnik Oberstein SA, Vollebregt MJ, Middelkoop HA, van der Grond J, Helderman-van den Enden AT (2008) Homozygosity for a NOTCH3 mutation in a 65-year-old CADASIL patient with mild symptoms: a family report. *J Neurol* 255: 1978-80.

Liem MK, van der Grond J, Versluis MJ, Haan J, Webb AG, Ferrari MD, van Buchem MA, Lesnik Oberstein SA (2010) Lenticulostriate arterial lumina are normal in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy: a high-field in vivo MRI study. *Stroke* 41: 2812-6.

List J, Duning T, Meinzer M, Kürten J, Schirmacher A, Deppe M, Evers S, Young P, Flöel A (2011) Enhanced rapid-onset cortical plasticity in CADASIL as a possible mechanism of preserved cognition. *Cereb Cortex*: Epub ahead of print.

Lobo A, Launer LJ, Fratiglioni L (2000) Prevalence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts-Neurologic Diseases in the Elderly Research Group. *Neurology* 54 (11 Suppl 5): S4-9.

Low WC, Junna M, Borjesson-Hanson A, Morris CM, Moss TH, Stevens DL, St Clair D, Mizuno T, Zhang WW, Mykkanen K, Wahlstrom J, Andersen O, Kalimo H, Viitanen M, Kalaria RN (2007) Hereditary multi-infarct dementia of the Swedish type is a novel disorder different from NOTCH3 causing CADASIL. *Brain* 130: 357-67.

Low WC, Santa Y, Takahashi K, Tabira T, Kalaria RN (2006) CADASIL-causing mutations do not alter Notch3 receptor processing and activation. *Neuroreport* 17: 945-9.

Lundkvist J, Zhu S, Hansson EM, Schweinhardt P, Miao Q, Beatus P, Dannaeus K, Karlstrom H, Johansson CB, Viitanen M, Rozell B, Spenger C, Mohammed A, Kalimo H, Lendahl U (2005) Mice carrying a r142c notch 3 knock-in mutation do not develop a cadasil-like phenotype. *Genesis* 41: 13-22.

Manganelli F, Ragno M, Cacchiò G, Iodice V, Trojano L, Silvaggio F, Scarcella M, Grazioli M, Santoro L, Perretti A (2008) Motor cortex cholinergic dysfunction in CADASIL: a transcranial magnetic demonstration. *Clin Neurophysiol* 119: 351-5.

Markus HS, Martin RJ, Simpson MA, Dong YB, Ali N, Crosby AH, Powell JF (2002) Diagnostic strategies in CADASIL. *Neurology* 59: 1134-8.

Marmur JD, Poon M, Rossikhina M, Taubman MB (1992) Induction of PDGF-responsive genes in vascular smooth muscle. Implications for the early response to vessel injury. *Circulation* 86: 53-60.

Martinez Arias A, Zecchini V, Brennan K (2002) CSL-independent Notch signalling: a checkpoint in cell fate decisions during development? *Curr Opin Genet Dev* 12: 524-33.

Mas JL, Dilouya A, de Recondo J (1992) A familial disorder with subcortical ischemic strokes, dementia, and leukoencephalopathy. *Neurology* 42: 1015-9.

Mawet J, Vahedi K, Aout M, Vicaut E, Duering M, Touboul PJ, Dichgans M, Chabriat H (2011) Carotid atherosclerotic markers in CADASIL. *Cerebrovasc* 31: 246-52.

Mayer M, Straube A, Bruening R, Uttner I, Pongratz D, Gasser T, Dichgans M, Muller-Hocker J (1999) Muscle and skin biopsies are a sensitive diagnostic tool in the diagnosis of CADASIL. *J Neurol* 246: 526-32.

Mellies JK, Baumer T, Muller JA, Tournier-Lasserve E, Chabriat H, Knobloch O, Hackeloer HJ, Goebel HH, Wetzig L, Haller P (1998) SPECT study

of a German CADASIL family. A phenotype with migraine and progressive dementia only. *Neurology* 50: 1715-21.

Menon U, Kelley RE (2009) Subcortical ischemic cerebrovascular dementia. *Int Rev Neurobiol* 84: 21-33.

Mesulam M, Siddique T, Cohen B (2003) Cholinergic denervation in a pure multi-infarct state: observations on CADASIL. *Neurology* 60: 1183-5.

Mizuno T, Kondo M, Ishigami N, Tamura A, Itsukage M, Koizumi H, Isayama R, Hosomi A, Nagakane Y, Tokuda T, Sugimoto E, Ushijima Y, Nakagawa M (2009) Cognitive impairment and cerebral hypoperfusion in a CADASIL patient improved during administration of lomerizine. *Clin Neuropharmacol* 32: 113-6.

Molko N, Pappata S, Mangin JF, Poupon C, Vahedi K, Jobert A, LeBihan D, Bousser MG, Chabriat H (2001) Diffusion tensor imaging study of subcortical gray matter in CADASIL. *Stroke* 32: 2049-54.

Monet M, Domenga V, Lemaire B, Souilhol C, Langa F, Babinet C, Gridley T, Tournier-Lasserve E, Cohen-Tannoudji M, Joutel A (2007) The archetypal r90c cadasil-notch3 mutation retains notch3 function in vivo. *Hum Mol Genet* 16: 982-92.

Monet-Lepretre M, Bardot B, Lemaire B, Domenga V, Godin O, Dichgans M, Tournier-Lasserve E, Cohen-Tannoudji M, Chabriat H, Joutel A (2009) Distinct phenotypic and functional features of CADASIL mutations in the Notch3 ligand binding domain. *Brain* 132: 1601-12.

Morrow D, Guha S, Sweeney C, Birney Y, Walshe T, O'Brien C, Walls D, Redmond EM, Cahill PA (2008) Notch and vascular smooth muscle cell phenotype. *Circ Res* 103: 1370-82.

Mourad A, Levasseur M, Bousser MG, Chabriat H (2006) CADASIL with minimal symptoms after 60 years. *Rev Neurol (Paris)* 162: 827-31.

Mumm JS, Schroeter EH, Saxena MT, Griesemer A, Tian X, Pan DJ, Ray WJ, Kopan R (2000) A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. *Mol Cell* 5: 197-206.

Munoz DG (2003) Small vessel disease: neuropathology. *Int Psychogeriatr* 15 (Suppl 1): S67-9.

Mykkänen K, Junna M, Amberla K, Bronge L, Kääriäinen H, Pöyhönen M, Kalimo H, Viitanen M (2009) Different clinical phenotypes in monozygotic CADASIL twins with a novel NOTCH3 mutation. *Stroke* 40: 2215-8.

Mykkänen K, Savontaus ML, Juvonen V, Sistonen P, Tuisku S, Tuominen S, Penttinen M, Lundkvist J, Viitanen M, Kalimo H, Poyhonen M (2004) Detection of the founder effect in Finnish CADASIL families. *Eur J Hum Genet* 12: 813-9.

Nichols JT, Miyamoto A, Olsen SL, D'Souza B, Yao C, Weinmaster G (2007) DSL ligand endocytosis physically dissociates Notch1 heterodimers before activating proteolysis can occur. *J Cell Biol* 176: 445-58.

Noseda M, Fu Y, Niessen K, Wong F, Chang L, McLean G, Karsan A (2006) Smooth Muscle alpha-actin is a direct target of Notch/CSL. *Circ Res* 98: 1468-70.

O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, Bowler JV, Ballard C, DeCarli C, Gorelick PB, Rockwood K, Burns A, Gauthier S, DeKosky ST (2003) Vascular cognitive impairment. *Lancet Neurol* 2: 89-98.

Ogata J, Yamanishi H, Ishibashi-Ueda H (2011) Review: role of cerebral vessels in ischaemic injury of the brain. *Neuropathol Appl Neurobiol* 37: 40-55.

Oide T, Nakayama H, Yanagawa S, Ito N, Ikeda S, Arima K (2008) Extensive loss of arterial medial smooth muscle cells and mural extracellular matrix in cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). *Neuropathology* 28: 132-42.

Okeda R, Arima K, Kawai M (2002) Arterial changes in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in relation to pathogenesis of diffuse myelin loss of cerebral white matter: examination of cerebral medullary arteries by reconstruction of serial sections of an autopsy case. *Stroke* 33: 2565-9.

Okumura K, Tsuru T, Aizaki K, Akikusa B (2007) A case of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), diagnosed in the age of puberty. *No To Hattatsu* 39: 49-53.

Opherk C, Peters N, Herzog J, Luedtke R, Dichgans M (2004) Long-term prognosis and causes of

death in CADASIL: a retrospective study in 411 patients. *Brain* 127: 2533-9.

Opherk C, Duering M, Peters N, Karpinska A, Rosner S, Schneider E, Bader B, Giese A, Dichgans M (2009) CADASIL mutations enhance spontaneous multimerization of NOTCH3. *Hum Mol Genet* 18: 2761-7.

O'Sullivan M, Jarosz JM, Martin RJ, Deasy N, Powell JF, Markus HS (2001) MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. *Neurology* 56: 628-34.

O'Sullivan M, Rich PM, Barrick TR, Clark CA, Markus HS (2003) Frequency of subclinical lacunar infarcts in ischemic leukoaraiosis and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *AJNR Am J Neuroradiol* 24: 1348-54.

Pantoni L (2010) Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 9: 689-701.

Pantoni L, Pescini F, Nannucci S, Sarti C, Bianchi S, Dotti MT, Federico A, Inzitari D (2010) Comparison of clinical, familial, and MRI features of CADASIL and NOTCH3-negative patients. *Neurology* 74:57-63.

Pantoni L, Simoni M (2003) Pathophysiology of cerebral small vessels in vascular cognitive impairment. *Int Psychogeriatr* 15 (Suppl 1): S59-65.

Paquet C, Jouvent E, Mine M, Vital A, Hugon J, Chabriat H, Gray F (2010) A cortical form of CADASIL with cerebral Aβ amyloidosis. *Acta Neuropathol* 120: 813-20.

Parks AL, Klueg KM, Stout JR, Muskavitch MA (2000) Ligand endocytosis drives receptor dissociation and activation in the Notch pathway. *Development* 127: 1373-85.

Peters N, Freilinger T, Opherk C, Pfefferkorn T, Dichgans M (2008) Enhanced l-arginine-induced vasoreactivity suggests endothelial dysfunction in CADASIL. *J Neurol* 255: 1203-8.

Peters N, Herzog J, Opherk C, Dichgans M (2004a) A two-year clinical follow-up study in 80 CADASIL subjects: progression patterns and implications for clinical trials. *Stroke* 35: 1603-8.

Peters N, Opherk C, Bergmann T, Castro M, Herzog J, Dichgans M (2005b) Spectrum of mutations in biopsy-proven CADASIL: implications for diagnostic strategies. *Arch Neurol* 62: 1091-4.

Peters N, Opherk C, Danek A, Ballard C, Herzog J, Dichgans M (2005a) The pattern of cognitive performance in CADASIL: a monogenic condition leading to subcortical ischemic vascular dementia. *Am J Psychiatry* 162: 2078-85.

Peters N, Opherk C, Zacherle S, Capell A, Gempel P, Dichgans M (2004b) CADASIL-associated Notch3 mutations have differential effects both on ligand binding and ligand-induced Notch3 receptor signaling through RBP-Jk. *Exp Cell Res* 299: 454-64.

Pfefferkorn T, von Stuckrad-Barre S, Herzog J, Gasser T, Hamann GF, Dichgans M (2001) Reduced cerebrovascular $CO_2$ reactivity in CADASIL: A transcranial Doppler sonography study. *Stroke* 32: 17-21.

Piccirillo G, Magrì D, Mitra M, Rufa A, Zicari E, Stromillo ML, De Stefano N, Dotti MT (2008) Increased QT variability in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Eur J Neurol* 15: 1216-21.

Prakash N, Hansson E, Betsholtz C, Mitsiadis T, Lendahl U (2002) Mouse Notch 3 expression in the pre- and postnatal brain: relationship to the stroke and dementia syndrome CADASIL. *Exp Cell Res* 278: 31-44.

Razvi SS, Davidson R, Bone I, Muir KW (2005) The prevalence of cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) in the west of Scotland. *J Neurol Neurosurg Psychiatry* 76: 739-41.

Reyes S, Viswanathan A, Godin O, Dufouil C, Benisty S, Hernandez K, Kurtz A, Jouvent E, O'Sullivan M, Czernecki V, Bousser MG, Dichgans M, Chabriat H (2009) Apathy: a major symptom in CADASIL. *Neurology* 72: 905-10.

Rice CM, McGuone D, Kurian KM, Love S, Renowden SA, Giffin NJ (2011) Autopsy-confirmed, co-existent CADASIL and multiple system atrophy. *Parkinsonism Relat Discord* 17: 390-2.

Roca C, Adams RH (2007) Regulation of vascular morphogenesis by Notch signaling. *Genes Dev* 21: 2511-24.

Rocca WA, Hofman A, Brayne C (1991a) Frequency and distribution of Alzheimer's disease in Europe:

a collaborative study of 1980–1990 prevalence findings: the EURODEM-Prevalence Research Group. *Ann Neurol* **30**: 381-90.

Rocca WA, Hofman A, Brayne C, Breteler MM, Clarke M, Copeland JR, Dartigues JF, Engedal K, Hagnell O, Heeren TJ, Jonker C, Lindesay J, Lobo A, Mann AH, Mölsä PK, Morgan K, O'Connor DW, Droux ADS, Sulkava R, Kay DWK, Amaducci L; EURODEM-Prevalence Research Group (1991b) The prevalence of vascular dementia in Europe: facts and fragments from 1980-1990 studies. *Ann Neurol* 30: 817-24.

Roine S, Pöyhönen M, Timonen S, Tuisku S, Marttila R, Sulkava R, Kalimo H, Viitanen M (2005) Neurologic symptoms are common during gestation and puerperium in CADASIL. *Neurology* 64: 1441-3.

Román GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC (2002) Subcortical ischaemic vascular dementia. *Lancet Neurol* 1: 426-36.

Román GC, Sachdev P, Royall DR, Bullock RA, Orgogozo JM, López-Pousa S, Arizaga R, Wallin A (2004) Vascular cognitive disorder: a new diagnostic category updating vascular cognitive impairment and vascular dementia. *J Neurol Sci* 226: 81-7.

Ruchoux MM, Chabriat H, Bousser MG, Baudrimont M, Tournier-Lasserve E (1994) Presence of ultrastructural arterial lesions in muscle and skin vessels of patients with CADASIL. *Stroke* 25: 2291-2.

Ruchoux MM, Chabriat H, Bousser MG, Baudrimont M, Tournier-Lasserve E (1995) Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Acta Neuropathol (Berl)* 89: 500-12.

Ruchoux MM, Domenga V, Brulin P, Maciazek J, Limol S, Tournier-Lasserve E, Joutel A (2003) Transgenic mice expressing mutant Notch3 develop vascular alterations characteristic of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Am J Pathol* 162: 329-42.

Ruchoux MM, Maurage CA (1997) CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *J Neuropathol Exp Neurol* 56: 947-64.

Ruchoux MM, Maurage CA (1998) Endothelial changes in muscle and skin biopsies in patients with CADASIL. *Neuropathol Appl Neurobiol* 24: 60-5.

Rufa A, Blardi P, De Lalla A, Cevenini G, De Stefano N, Zicari E, Auteri A, Federico A, Dotti MT (2008) Plasma levels of asymmetric dimethylarginine in cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy. *Cerebrovasc Dis* 26: 636-40.

Rufa A, Dotti MT, Franchi M, Stromillo ML, Cevenini G, Bianchi S, De Stefano N, Federico A (2005) Systemic blood pressure profile in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Stroke* 36: 2554-8.

Rufa A, Guideri F, Acampa M, Cevenini G, Bianchi S, De Stefano N, Stromillo ML, Federico A, Dotti MT (2007) Cardiac autonomic nervous system and risk of arrhythmias in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Stroke* 38: 276-80.

Sacco S, Marini C, Totaro R, Russo T, Cerone D, Carolei A (2006) A population-based study of the incidence and prognosis of lacunar stroke. *Neurology* 66: 1335-8.

Saito S, Ozaki A, Takahashi M, Ito H, Matsumoto S, Tomimoto H (2011) Clustering of multifocal cerebral infarctions in CADASIL: a case report. *J Neurol* 258: 325-7.

Salvi F, Michelucci R, Plasmati R, Parmeggiani L, Zonari P, Mascalchi M, Tassinari CA (1992) Slowly progressive familial dementia with recurrent strokes and white matter hypodensities on CT scan. *Ital J Neurol Sci* 13: 135-40.

Santa Y, Uyama E, Chui de H, Arima M, Kotorii S, Takahashi K, Tabira T (2003) Genetic, clinical and pathological studies of CADASIL in Japan: a partial contribution of Notch3 mutations and implications of smooth muscle cell degeneration for the pathogenesis. *J Neurol Sci* 212: 79-84.

Saskia AJ, Lesnik Oberstein SA, Maat-Schieman ML, Boon EM, Haan J, Breuning MH, van Duinen SG (2008) No vessel wall abnormalities in a human foetus with a NOTCH3 mutation. *Acta Neuropathol* 115: 369-70.

Sayeed A, Ng DT (2005) Search and destroy: ER quality control and ER-associated protein degradation. *Crit Rev Biochem Mol Biol* 40: 75-91.

Schon F, Martin RJ, Prevett M, Clough C, Enevoldson TP, Markus HS (2003) "CADASIL coma": an underdiagnosed acute encephalopathy. *J Neurol Neurosurg Psychiatry* 74: 249-52.

Schröder JM, Sellhaus B, Jorg J (1995) Identification of the characteristic vascular changes in a sural biopsy of a case with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Acta Neuropathol (Berl)* 89: 116-21.

Shawber C, Nofziger D, Hsieh JJ, Lindsell C, Bogler O, Hayward D, Weinmaster G (1996) Notch signaling inhibits muscle cell differentiation through a CBF1-independent pathway. *Development* 122: 3765-73.

Shimizu K, Chiba S, Saito T, Kumano K, Hirai H (2000) Physical interaction of Delta1, Jagged1, and Jagged2 with Notch1 and Notch3 receptors. *Biochem Biophys Res Commun* 276: 385-9.

Singhal S, Bevan S, Barrick T, Rich P, Markus HS (2004) The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. *Brain* 127: 2031-8.

Singhal S, Markus HS (2005) Cerebrovascular reactivity and dynamic autoregulation in nondemented patients with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). *J Neurol* 252: 163-7.

Singhal S, Rich P, Markus HS (2005) The spatial distribution of MR imaging abnormalities in cerebral autosomal leukoencephalopathy and their relationships to age and clinical features. *AJNR Am J Neuroradiol* 26: 2481-7.

Sonninen V, Savontaus ML (1987) Hereditary multi-infarct dementia. *Eur Neurol* 27: 209-15.

Sourander P, Walinder J (1977) Hereditary multi-infarct dementia. Morphological and clinical studies of a new disease. *Acta Neuropathol (Berl)* 39: 247-54.

Stenborg A, Kalimo H, Viitanen M, Terent A, Lind L (2007) Impaired endothelial function of forearm resistance arteries in CADASIL patients. *Stroke* 38: 2692-7.

Sternic N, Pavlovic A, Miljic P, Bajcetic M, Lackovic M, Lackovic V (2009) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy with severe factor XII deficiency. *Neurol India* 57: 657-9.

Stevens DL, Hewlett RH, Brownell B (1977) Chronic familial vascular encephalopathy. *Lancet* 1: 1364-5.

Sudlow CLM, Warlow CP (1997) Comparable studies of the incidence of stroke and its pathological types. Results from an international collaboration. *Stroke* 28: 491-9.

Symon L, Pasztor E, Dorsch NW, Branston NM (1973) Physiological responses of local areas of the cerebral circulation in experimental primates determined by the method of hydrogen clearance. *Stroke* 4: 632-42.

Taillia H, Chabriat H, Kuetz A, Verin M, Levy C, Vahedi K, Tournier-Lasserve E, Bousser MG (1998) Cognitive alterations in non-demented CADASIL patients. *Cerebrovasc Dis* 8: 97-101.

Takahashi K, Adachi K, Yoshizaki K, Kunimoto S, Kalaria RN, Watanabe A (2010) Mutations in NOTCH3 cause the formation and retention of aggregates in the endoplasmic reticulum, leading to impaired cell proliferation. *Hum Mol Genet* 19: 79-89.

Tikka S, Mykkänen K, Ruchoux MM, Bergholm R, Junna M, Pöyhönen M, Yki-Järvinen H, Joutel A, Viitanen M, Baumann M, Kalimo H (2009) Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. *Brain* 132: 933-9.

Toole JF (1990) Microcirculatory and hematologic disorder affecting the nervous system. In: Toole JF, *Cerebrovascular Disorders*. New York: Raven Press: 421-37.

Tournier-Lasserve E, Iba-Zizen MT, Romero N, Bousser MG (1991) Autosomal dominant syndrome with strokelike episodes and leukoencephalopathy. *Stroke* 22: 1297-302.

Tournier-Lasserve E, Joutel A, Melki J, Weissenbach J, Lathrop GM, Chabriat H, Mas JL, Cabanis EA, Baudrimont M, Maciazek J, Bach MA, Bousser MG (1993) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. *Nat Genet* 3: 256-9.

Tuominen S, Juvonen V, Amberla K, Jolma T, Rinne JO, Tuisku S, Kurki T, Marttila R, Poyhonen M, Savontaus ML, Viitanen M, Kalimo H (2001) Phenotype of a homozygous CADASIL patient in comparison to 9 age-matched heterozygous patients with the same R133C Notch3 mutation. *Stroke* 32: 1767-74.

Tuominen S, Miao Q, Kurki T, Tuisku S, Poyhonen M, Kalimo H, Viitanen M, Sipila HT, Bergman J, Rinne JO (2004) Positron emission tomography examination of cerebral blood flow and glucose metabolism in young CADASIL patients. *Stroke* 35: 1063-7.

Uehara T, Tabuchi M, Mori E (1999) Risk factors for silent cerebral infarcts in subcortical white matter and basal ganglia. *Stroke* 30: 378-82.

Vahedi K, Chabriat H, Levy C, Joutel A, Tournier-Lasserve E, Bousser MG (2004) Migraine with aura and brain magnetic resonance imaging abnormalities in patients with CADASIL. *Arch Neurol* 61: 1237-40.

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

Valenti R, Poggesi A, Pescini F, Inzitari D, Pantoni L (2008) Psychiatric disturbances in CADASIL: a brief review. *Acta Neurol Scand* 118: 291-5.

van Bogaert L (1955) Encéphalopathie sous-corticale progressive (Binswanger) à évolution rapide chez deux soeurs. *Med Hellen* 24: 961-72.

van den Boom R, Lesnik Oberstein SA, Ferrari MD, Haan J, van Buchem MA (2003a) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR imaging findings at different ages--3rd-6th decades. *Radiology* 229: 683-90.

van den Boom R, Lesnik Oberstein SA, Spilt A, Behloul F, Ferrari MD, Haan J, Westendorp RG, Van Buchem MA (2003b) Cerebral hemodynamics and white matter hyperintensities in CADASIL. *J Cereb Blood Flow Metab* 23: 599-604.

van den Boom R, Lesnik Oberstein SA, van Duinen SG, Bornebroek M, Ferrari MD, Haan J, van Buchem MA (2002) Subcortical lacunar lesions: an MR imaging finding in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Radiology* 224: 791-6.

van Gerpen JA, Ahlskog JE, Petty GW (2003) Progressive supranuclear palsy phenotype secondary to CADASIL. *Parkinsonism Relat Disord* 9: 367-9.

Vedeler C, Bindoff L (2011) A family with atypical CADASIL. *J Neurol*: Epub ahead of print.

Velizarova R, Mourand I, Serafini A, Crespel A, Gelisse P (2011) Focal epilepsy as first symptom in CADASIL. *Seizure* 20: 502-4.

Verin M, Rolland Y, Landgraf F, Chabriat H, Bompais B, Michel A, Vahedi K, Martinet JP, Tournier-Lasserve E, Lemaitre MH, Edan G (1995) New phenotype of the cerebral autosomal dominat arteriopathy mapped to chromosome 19: migraine as the prominent clinical feature. *J Neurol Neurosurg Psychiatry* 59: 579-85.

Viswanathan A, Chabriat H (2006) Cerebral microhemorrhage. *Stroke* 37: 550-5.

Viswanathan A, Guichard JP, Gschwendtner A, Buffon F, Cumurcuic R, Boutron C, Vicaut E, Holtmannspotter M, Pachai C, Bousser MG, Dichgans M, Chabriat H (2006) Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. *Brain* 129: 2375-83.

Wakita H, Tomimoto H, Akiguchi I, Kimura J (1994) Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. *Acta Neuropathol (Berl)* 87: 484-92.

Wallin A, Milos V, Sjogren M, Pantoni L, Erkinjuntti T (2003) Classification and subtypes of vascular dementia. *Int Psychogeriatr* 15 (Suppl 1): S27-37.

Wang W, Campos AH, Prince CZ, Mou Y, Pollman MJ (2002a) Coordinate Notch3-hairy-related transcription factor pathway regulation in response to arterial injury. Mediator role of platelet-derived growth factor and ERK. *J Biol Chem* 277: 23165-71.

Wang W, Struhl G (2004) Drosophila Epsin mediates a select endocytic pathway that DSL ligands must enter to activate Notch. *Development* 131: 5367-80.

Wang W, Struhl G (2005) Distinct roles for Mind bomb, Neuralized and Epsin in mediating DSL endocytosis and signaling in Drosophila. *Development* 132: 2883-94.

Wardlaw JM (2005) What causes lacunar stroke? *J Neurol Neurosurg Psychiatry* 76: 617-9.

Waterston JA, Brown MM, Butler P, Swash M (1990) Small deep cerebral infarcts associated with occlusive internal carotid artery disease. A hemodynamic phenomenon? *Arch Neurol* 47: 953-7.

Wegner F, Strecker K, Schwarz J, Wagner A, Heinritz W, Sommerer F, Thal DR, Schneider JP, Kendziorra K, Sabri O (2007) Vascular parkinsonism in a CADASIL case with an intact nigrostriatal dopaminergic system. *J Neurol* 254: 1743-5.

World Health Organisation (1978) *Cerebrovascular Disorders (Offset Publications)*. Geneva: World Health Organization. ISBN 9241700432.

Yamamoto Y, Craggs L, Baumann M, Kalimo H, Kalaria RN (2011) Review: molecular genetics and pathology of hereditary small vessel diseases of the brain. *Neuropathol Appl Neurobiol* 37: 94-113.

Yamamoto Y, Ihara M, Tham C, Low RW, Slade JY, Moss T, Oakley AE, Polvikoski T, Kalaria RN (2009) Neuropathological correlates of temporal pole white matter hyperintensities in CADASIL. *Stroke* 40: 2004-11.

Yonekura M, Sakurai S, Liu X, Migita M, Wang M, Yamagishi SI, Nomura M, Abedin MD, Unoki M, Yamamoto Y, Yamamoto H (1999) Placental growth factor and vascular endothelial growth factor B and C expression in microvascular endothelial cells and pericytes. Implication in autocrine and paracrine regulation of angiogenesis. *J Biol Chem* 274: 35172-8.