Notch Signaling and CADASIL

Sung-Chun Tang[1,2], Jiann-Shing Jeng[1], Ming-Jen Lee[1], and Ping-Keung Yip[3]

Abstract- Notch signaling plays an essential role in vascular development and human vascular diseases. In adults, mutations of the Notch3 gene cause a hereditary vascular degenerative disease known as cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL). CADASIL is characterized by recurrent strokes and cognitive impairment. Over the past decade, the number of CADASIL patients increased significantly with improvements in genetic testing and other diagnostic tools, but the true prevalence of CADASIL is still underestimated, especially in Asia. Basic studies suggest that Notch3 is essential for the development and survival of the vascular smooth muscle cells, but the mechanisms by which Notch3 mutations become pathogenic are still unclear. This article reviews the clinical features and possible pathogenesis of CADASIL. Efforts to improve the diagnostic accuracy and define the role of Notch3 mutation in brain damage and clinical presentations of CADASIL should be continued.

Key Words: CADASIL, Dementia, Genetic analysis, Notch, Stroke

Acta Neurol Taiwan 2009;18:81-90

INTRODUCTION

The Notch proteins encompass a family of type I transmembrane receptors. Four Notch members (Notch1-4) are found in mammalian species[1-3]. The primary protein of Notch is first constitutively cleaved by a furin-like convertase (S1) and the cleavage products assemble into a heterodimer that inserts and is expressed in the cell membrane[1-3]. Activation of Notch requires ligands that can broadly be divided into two groups, Delta and Jagged[4,5]. Binding of ligands results in two proteolytic cleavages of Notch: the first is an extracellular domain and the second is a transmembrane domain. The latter is accomplished by presenilin-1 and the gamma-secretase enzyme complex, and results in the release of Notch intracellular domain[1-2]. The Notch intracellular domain then translocates to the nucleus, interacts with several transcriptional factors (such as CBF, SU(H), and Lag-2), which convert it to a transcriptional activator[6]. The (Hairy/Enhancer of Split) HES and HES-related genes are the direct targets of Notch signaling.

Notch and its ligands participate in an evolutionarily conserved signaling pathway that functions to modulate

From the [1]Stroke Center & Department of Neurology, National Taiwan University Hospital, Taiwan; [2]Department of Neurology, National Taiwan University Hospital, Yun-Lin Branch, Yun-Lin, Taiwan; [3]Department of Neurology, Cardinal Tien Hospital, Taipei, Taiwan.
Received February 17, 2009.
Revised and Accepted February 20, 2009.

Reprint requests and correspondence to: Jiann-Shing Jeng, MD, PhD, Department of Neurology, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei 100, Taiwan.
E-mail: jsjeng@ntu.edu.tw

cell-fate decisions of a variety of cell types[6,7]. In the vascular system, Notch1 and Notch4 are predominant in the endothelium, whereas Notch1 and Notch3 are present in smooth muscle cells[3,8]. Previous studies have shown that changes in Notch signaling lead to abnormal vascular development at multiple stages and to various degrees[3,8,9]. The importance of Notch genes in the development of the vascular system is indicated by severe cardiovascular anomalies in patients with Alagille syndrome[10,11]. In adults, mutations of Notch3 gene cause a severe vascular degenerative disease known as cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL)[12,13]. In this review, we describe the clinical features of CADASIL and discuss recent studies regarding the functional role of Notch3 mutation and the pathogenesis of CADASIL.

CLINICAL MANIFESTATIONS OF CADASIL

CADASIL is clinically characterized by recurrent ischemic strokes, migraine, and cognitive impairment. It is the most common cause of single gene disorders causing ischemic stroke[14]. CADASIL is an autosomal dominant inherited disorder with high penetration and varying expression. The defective gene, Notch3, is located on the chromosome 19[15]. CADASIL occurs worldwide and has been reported in many race-ethnicities. So far, most of the CADASIL patients have been found in Caucasian families, especially in Europe[16-18]; while in Asia, the numbers of reported cases as well as spectrum of mutations are relatively low[19]. This may indicate an underestimation of the CADASIL prevalence in Asians population.

Migraine

The onset age of migraine in CADASIL varies greatly. Migraine may begin early during childhood or adolescence, but most commonly during the third decade. It is significantly younger in women compared with men and occurred a mean of 15 years prior to stroke onset. Unlike in general population, migraine in CADASIL patients is usually accompanied by aura, which is often atypical, long lasting, and severe. The aura can be a visual disturbance, and be followed by sensory or motor abnormality or brief confusion[20-22]. Sometimes, it is difficult to distinguish between prolonged aura and the new onset of ischemic stroke in CADASIL patients, thus careful history taking, neurological examination, and neuroimage work-up are required. Previous studies showed 22-64% of CADASIL patients have migraine[16-18], therefore, a certain proportion of patients would be expected not to have a history of headache throughout their disease course. For the treatment of severe migraine attacks in CADASIL patients, there were two case reports showing that acetazolamide could effectively block the acute attacks of migraine[23,24]; further study with a larger number of cases should be performed to validate its efficacy.

Stroke

Ischemic stroke is the most frequent and the key feature found in about 85% of symptomatic CADASIL patients. The mean age at onset is around 45 years (range 30-70 years)[16-18]. Most of the ischemic stroke patients with CADASIL present as lacunar syndromes (pure motor stroke, pure sensory stroke, sensorimotor paralysis, ataxic hemiparesis, or dysarthria-clumsy hand)[25]. The infarcts occur mainly in the subcortical white matter and some in the basal ganglion, brainstem, or spinal cord. It may occur silently and most studies suggest that lesions of strokes are associated with long-term disability and cognitive decline[26-28].

Ischemic stroke involving the territory of large arteries has occasionally been reported. One study using conventional or magnetic resonance angiography (MRA) in 13 patients showed that 38% of patients had stenosis at the middle cerebral, vertebral, or internal carotid arteries[29]. Whether these observations were coincidental or suggested involvement of large vessels in CADASIL is still a matter of debate. By the way, it is mentionable that conventional angiography should be avoided in highly suspected cases because contrast agents have been reported to frequently impair consciousness or induce acute focal neurological deficit in CADASIL patients[30].

In addition to ischemic stroke, intracerebral hemorrhage (ICH) has been described in patients with

CADASIL[31]. By MRI or post-mortem examinations, cerebral microbleeds were found in 31-69% of patients with CADASIL[32,33]. This implies that CADASIL patients may have an increased risk for ICH, especially while under antithrombotic therapy. One recent study showed cerebral microbleeds were independently associated with increased modified Rankin scores; and the severities of microbleeds were significantly related to high blood pressure and the level of hemoglobin A1c. Therefore, modulation of blood pressure and glucose levels might affect the outcome of CADASIL patients[34].

Cognitive decline, dementia and psychiatric symptoms

Cognitive decline, dementia and psychiatric symptoms are frequent features in CADASIL[16-18]. There is considerable a variation in the age at onset and incidence of dementia in CADASIL patients depending on the diagnostic criteria and study design. In general, cognitive decline is observed in at least 50% of all CADASIL patients, and about 80% of CADASIL patients aged over 65 years are demented[16-18]. Cognitive decline can be observed during the early course of CADASIL, but significant cognitive impairment is rarely observed prior to the age 35 years[35]. The course of cognitive decline often follows a stepwise pattern and is frequently accompanied by gait disturbance, incontinence, and pseudobulbar palsy. Other than that, psychiatric symptoms, such as mood disorder, especially depression or schizophrenia, are common features and be described in more than 20% of CADASIL patients[16-19].

One study showed that working memory and executive function is already impaired in the pre-stroke phase[36]. These findings implied that early involvement of cognitive function in CADASIL patients may be due to the disturbance of cerebral blood flow rather than to the cumulative effect of ischemic strokes. In the post-stroke phase, studies showed that the performance of Mini-Mental State Examination has significant correlations with the total volume of infarct lesion load but not white matter hyperintensities or microbleeds on brain MRI[26,37]. Other studies showed that MRI diffusion tensor imaging also correlated well with cognitive dysfunction as well as disease progression[38-40]. However, detailed neuropsychological assessment is still being suggested in the assessment of cognitive function in CADASIL patients individually since one article containing three CADASIL patients failed to see any relationship between cognitive performance and parameters from either structural or functional image studies[41,42].

Other features

In addition to central nervous system (CNS) symptoms, there are sporadic reports of involvement of the heart[36], renal system[43], neuromuscular system[44,45], retina[46,47] or hearing[48], but the incidence and the clinical relevance of symptoms other than CNS are still controversial. For example, one cardiovascular survey concluded that Notch3 mutation carriers may be at increased risk of early myocardial infarction[44], while another report failed to find electrocardiographic evidence for increased myocardial ischemia or arrhythmias in CADASIL patients[49]. Overall, why the major symptoms of CADASIL are limited to the CNS even though degeneration of small blood vessels is present systemically remains unclear.

Clinical course and long-term prognosis

The clinical course of CADASIL is variable even within single family. Some gene carriers remain asymptomatic throughout their life while others are severely disabled at an early age. One study of 127 patients from 65 families with 17 different mutations showed a correlation between phenotype and some modulating factors but not between phenotype and Notch3 genotypes[50]. Smoking appears to increase the risk of stroke and high homocysteine levels that are associated with increased risk of migraine. Similar results were reported by a study of 80 CADASIL subjects with a clinical follow-up of 2 years[51]. It also showed a large, inter-subject variability in the duration and outcome of the clinical course. They may deteriorate rapidly, remain stable, or even have some improvement. For the long-term prognosis, one study including 411 CADASIL patients in Germany found that the expected life year was shortened, with more significant in men than women (age at death, 64.6

years and 70.7 years, respectively)[28]. Pneumonia was the most frequent cause of death in CADASIL patients, followed by sudden unexpected death and asphyxia[28]. The high rate of sudden unexpected death may be partially explained by the observation of higher percentage of autonomic derangement, representing as heart rate variability in CADASIL patients than in normal subjects[52]. However, whether they can have benefit from prophylaxis with anti-arrhythmic agents to prevent life-threatening arrhythmia is unknown.

DIAGNOSTIC STRATEGIES

Brain MRI

Brain MRI is the first line diagnostic tool for suspected CADASIL patients. It usually demonstrates confluent white matter changes over the peri-ventricular areas (leukoaraiosis) and lacunar infarcts on T2-weighted or FLAIR images[53]. MRI is usually abnormal in asymptomatic patients, and a previous report showed that all CADASIL patients have abnormalities on MRI by the age of 35 years[35]. A study containing 112 CADASIL patients from 64 families showed that lesion loads increased progressively with age and were maximal in the frontal, parietal, and anterior temporal cortexes and the external capsule; intermediate in the pons; and relatively low in the corpus callosum, caudate globus pallidus, cerebellum, midbrain, and medulla[54]. Another MRI study of 40 CADASIL patients in different age groups[55] showed that hyperintense lesions in the anterior temporal lobe and subcortical lacunar lesions were the only abnormalities in patients aged 20-30 years, while lacunar infarcts and more areas of hyperintensity involving the external capsule, basal ganglion, and brainstem were present in 75% of patients aged 30-40 years. Microbleeds occurred in 19% of patients aged 41-50 years and 47% of patients older than fifty years old[55].

For diagnostic purposes, the MRI finding that distinguishes between CADASIL and other diseases with subcortical ischemia is abnormal signals in anterior temporal pole and external capsule. One study showed that involvement of anterior temporal pole had high sensitivity (89%) and specificity (86%), whereas external capsule involvement had high sensitivity (93%) but low specificity (45%) in diagnosis of CADASIL[18].

Skin biopsy

Vascular changes in CADASIL are seen in small- to medium-sized arteries and in some veins of almost all organs including skin, so the skin biopsy was developed as a diagnostic tool in CADASIL. The hallmark finding on tissue specimen is granular osmiophilic material (GOM), which is seen adjacent to the basement membrane of the smooth muscle cells of arterioles on electron microscopy[56]. The composition of GOM is not well known and one study showed that Notch3 ectodomain might be the major component[59]. The strategy that detects GOM from skin biopsy can diagnose CADASIL with 100% specificity but variable sensitivity (45-90%) in different studies[18,56-58]. Recent studies showed that staining the skin samples with the monoclonal anti-Notch3 antibody could detect abnormal Notch3 expressing in the walls of arterioles and hence increased the diagnostic validity with more than 95% diagnostic sensitivity and 100% specificity[60,61].

Genetic analysis

The Notch3 gene includes 33 exons, and Notch3 mutations in CADASIL affect highly conserved cysteine residues within the epidermal growth factor-like repeat domain, which is the extracellular part of the receptor[15]. The mutational spectrum of this gene in CADASIL includes missense mutations (about 95%), splice site mutations, and small in-frame deletions[62]. To date, more than 100 different mutations have been reported from all over the world, which reveals a large genetic heterogeneity[50,62].

One study including 48 British families showed that most mutations were located in exon 4, followed by exons 3, 5 and 6, and 8, 18, and 22[18]. Another study from a Dutch DNA diagnostic laboratory (44 Dutch and 22 foreign families) also found the mutation rate was highest in exon 4, but followed by exon 11, 5, 6, and 19[63]. Thus, it is suggested that the exons 3~6 should be screened first, and then exons 11, 18~23[18,63,64]. Clinically suspected patients who do not show a mutation in these

exons should be screened in batches. So far the genetic screening for Notch3 mutations has provided the most definitive diagnosis with around 95% sensitivity and 100% specificity.

PATHOGENESIS

At least three mechanisms mediate the pathogenic effects of Notch3 mutation in CADASIL: loss of receptor function, gain of function, and neomorphic processes (e.g., toxic). Mutations of Notch3 characteristically lead to an epidermal growth factor-like repeat domain (six repeats in normal) to an odd number of cysteine residues (either five or seven) through gain or loss of a residue[15]. No mutations leading to three cysteine residues or not involving a cysteine residue have ever been reported[65,66]. Though the pattern of cysteine mutations is fixed, the mechanism by which mutated Notch3 receptors lead to degeneration of vascular smooth muscle cells and clinical symptoms in CADASIL is still uncertain.

Pathologic findings

CADASIL is a non-atherosclerotic, amyloid negative, angiopathy involving small vessels of the brain and other organs[67,68]. In brain, histological sections show that the walls of small- and medium-sized leptomeningeal arteries are markedly thickened. Accumulations of GOM and Notch3 ectodomains are found near vascular smooth muscle cells (VSMC) of cerebral arteries, veins, and recently found in capillaries within or near the basal lamina[69].

One study showed widespread neuronal apoptosis in the cerebral cortex by immunostaining the activated caspase3 protein. It suggested that programmed neuronal death may be a common feature in CADASIL and associated with cortical atrophy[70]. Other studies showed that choline acyltransferase activities in the frontal and temporal neocortices were significantly reduced, indicating the impairment of cholinergic neurons in CADASIL patients[71,72]. Since neuron doesn't express Notch3 receptor, the early neuronal loss in CADASIL might be secondary to cerebral ischemia or infarction induced by vascular occlusion. These findings also imply the potential of anti-apoptotic or cholinomimetic agents in treating CADASIL patients.

By the way, although vasculopathy and GOM deposition are seen in most internal organs, pathological changes other than vasculopathy on those organs or associated symptoms are usually mild. For example, vasculopathy and GOM deposition in coronary arteries very rarely results in myocardial infarction[16,17]. Therefore, other unknown mechanisms should be responsible for the stroke and related CNS symptoms.

Animal models

Gene targeting procedures were used to introduce a deletion allele of Notch3 and thereby create Notch3 knockout mice. The mutant mice were viable and fertile. However, adult Notch3-/- mice exhibited marked arterial defects[73]. Although not deficient in VSMC, mutant mice had arteries with thinner VSMC layers than were present in wild-type arteries and impaired cerebral blood flow and cerebrovascular resistance in response to angiotensin II or phenylephrine injections. These findings indicated that Notch3 is required for the structural and functional integrity of arteries, particularly smaller-diameter arteries.

Notch3 transgenic mice that express full-length human Notch3 carrying the Arg90Cys mutation have been generated[74]. This transgenic mouse had two hallmarks of CADASIL angiopathy (GOM deposits and Notch3 accumulation within both the cerebral and peripheral arteries) but no prominent brain parenchyma damage. Time course analysis revealed anchorage of disrupted normal VSMC to adjacent extracellular matrix and cells, and VSMC cytoskeleton degeneration at 10 months of age, and Notch3 and GOM accumulation at around 14-16 months of age. These findings suggested early disruption of anchorage of the VSMC rather than Notch3 accumulation may be the key event initiating a cascade of events leading to VSMC degeneration.

On further study using two potent vasodilator stimuli (acetaxolamide and hypercapnia) and monitoring cerebral blood flow by laser Doppler flowmetry, this transgenic mice model showed impaired cerebral vasoreactivity as early as age 10 months[75]. Another study using tail

artery segments from 10-11 month-old mice also showed that flow and pressure is selectively impaired[76]. These findings support the hypothesis that early vascular abnormality may arise from VSMC dysfunction rather than loss of VSMC since the characteristic vascular pathology in these transgenic mice does not appear before age 10 months. However, although these transgenic mice have the characteristic vascular pathology, they don't exhibit brain parenchyma damage or strokes and the reasons remain unclear.

In vitro assays

Notch3 signaling was shown to be a critical determinant of VSMC survival and vascular structure in vitro[77]. Activation of Notch3 could provide the cytoprotection by directly increasing the expressional level of c-Flip, a caspase inhibitor, in response to the proapoptotic Fas ligand stimulation or indirectly modulates the c-Flip activity through the ERK/MAPK pathway in a cross-talk fashion[78].

Several studies have been done to determine whether mutations of Notch3 result in change of receptor functions[79-82]. One study generated a stable cell line expressing a murine Notch3 R142C mutation. As compared with the wild type, the mutant cell line responded normally to Notch3 ligand stimulation, but expressed lower proportion of the Notch3 site 1-cleaved configuration, thus the amount of Notch3 receptor at the cell surface was reduced[80]. This change would ultimately increase the number of intracellular aggregates of Notch3 and result in accumulation or slow transport in the secretary process. Interestingly, in a study that tested three different mutation sites in A7r5 VSMC, cells carrying two mutations located outside the ligand binding domain (R133C and C183R) responded normally to Jagged 1 induced signaling, but cells with the mutation within the ligand binding domain (C455R) had markedly reduced signaling activity[81]. In addition, all three mutant molecules showed altered S1 cleavage/receptor configuration and maturation. Similarly, another study confirmed that the mutation of C428S, which is located within the ligand binding domain, alters ligand binding as well as signal transduction[82].

In summary, Notch3 is essential for the normal development of the VSMC layer in vivo and survival of VAMC in vitro. R90C transgenic mice showed that expression of Notch3 mutation could lead to GOM and Notch3 accumulation. Cell lines carrying certain types of Notch3 mutations may alter the ability of receptor expression, ligand binding, or signaling transduction. However, two major key questions (how Notch3 mutations lead to phenotypic presentation of CADASIL, and why the clinical manifestations restricted mostly in the CNS) remain to be clarified.

CADASIL IN TAIWAN

The CADASIL had not been reported in Taiwan until year 2005. The diagnosis of that CADASIL family was confirmed by the characteristic electro microscopic findings on skin biopsy and the presence of a Notch3 mutation at exon 6 (R332C) in the proband patient[19]. The clinically affected members shared common manifestations of recurrent strokes, dementia and depressive disorders but no migraine. Another study reported 5 CADASIL patients from different families carrying Notch3 mutations (two were in exon4 and the other three were in exon6, 11 and 18, respectively) one year later. Among them, only one patient had positive skin biopsy results and none had migraine history.

Considering the estimated prevalence of CADASIL in Scotland is approximately 1 in 50,000 adults; though this estimate cannot be generalized worldwide, there should be more CADASIL families than currently reported in Taiwan. Besides, the low sensitivity of skin biopsy (33.3%) and lack of migraine history in this case series may suggest the possibility of racial-ethnic differences in spectrums of phenotype or genotype of CADASIL. Further research should be conducted to clarify this issue. These findings also emphasize the importance of genetic screening of the Notch3 with a phenotype suggesting CADASIL.

PERSPECTIVE

Clearly, Notch signaling plays an important role in

vascular development and in human vascular diseases. Over the past few years, the number of CADASIL patients has increased significantly in response to advances in technology (neurovascular imaging, molecular genetic confirmation techniques, and ultrastructural methods for assessing pathological change). However, the true prevalence of CADASIL is still underestimated especially in areas outside Europe. In addition, though basic research has identified various functional roles of Notch3 in vascular smooth cells in vitro and animal models in vivo, much remains to be elucidated. Studies should continue to explore the molecular basis of Notch3 mutation leading to brain damage and clinical manifestations of CADASIL.

ACKNOWLEDGEMENTS

This work was supported by the National Taiwan University Hospital Yun-Lin Branch research grant NTUHYL 96. No 24.

REFERENCES

1. Fleming RJ. Structural conservation of Notch receptors and ligands. Semin Cell Dev Biol 1998;9:599-607.
2. Yoon K, Gaiano N. Notch signaling in the mammalian central nervous system: insights from mouse mutants. Nat Neurosci 2005;8:1411.
3. Shawber CJ, Kitajewski J. Notch function in the vasculature: insights from zebrafish, mouse and man. Bioessays 2004;26:225-34.
4. Harper JA, Yuan JS, Tan JB, et al. Notch signaling in development and disease. Clin Genet 2003;64:461-72.
5. Lasky JL, Wu H. Notch signaling, brain development, and human disease. Pediatr Res 2005;57:104-9.
6. Struhl G, Adachi A. Nuclear access and action of notch in vivo. Cell 1998;93:649-60.
7. Fortini ME, Artavanis-Tsakonas S. The suppressor of hairless protein participates in notch receptor signaling. Cell 1994;79:273-82.
8. Karsan A. The role of notch in modeling and maintaining the vasculature. Can J Physiol Pharmacol 2005;83:14-23.
9. Hofmann JJ, Iruela-Arispe ML. Notch signaling in blood vessels: who is talking to whom about what? Circ Res 2007;100:1556-68.
10. McDaniell R, Warthen DM, Sanchez-Lara PA, et al. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. Am J Hum Genet 2006;79:169-73.
11. Kamath BM, Spinner NB, Emerick KM, et al. Vascular anomalies in Alagille syndrome: a significant cause of morbidity and mortality. Circulation 2004;109:1354-8.
12. Kalimo H, Ruchoux MM, Viitanen M, et al. CADASIL: a common form of hereditary arteriopathy causing brain infarcts and dementia. Brain Pathol 2002;12:371-84.
13. Hassan A, Markus HS. Genetics and ischaemic stroke. Brain 2000;123:1784-812.
14. Razvi SS, Bone I. Single gene disorders causing ischaemic stroke. J Neurol 2006;253:685-700.
15. Joutel A, Corpechot C, Ducros A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996;383:707-10.
16. Chabriat H, Vahedi K, Iba-Zizen MT, et al. Clinical spectrum of CADASIL: a study of 7 families. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Lancet 1995;346:934-9.
17. Dichgans M, Mayer M, Uttner I, et al. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. Ann Neurol 1998;44:731-9.
18. Markus HS, Martin RJ, Simpson MA, et al. Diagnostic strategies in CADASIL. Neurology 2002;59:1134-8.
19. Tang SC, Lee MJ, Jeng JS, et al. Arg332Cys mutation of NOTCH3 gene in the first known Taiwanese family with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. J Neurol Sci 2005; 228:125-8.
20. Verin M, Rolland Y, Landgraf F, et al. New phenotype of the cerebral autosomal dominant arteriopathy mapped to chromosome 19: migraine as the prominent clinical feature. J Neurol Neurosurg Psychiatry 1995;59:579-85.
21. Ceroni M, Poloni TE, Tonietti S, et al. Migraine with aura and white matter abnormalities: Notch3 mutation. Neurology 2000;54:1869-71.
22. Gladstone JP, Dodick DW. Migraine and cerebral white matter lesions: when to suspect cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoen-

cephalopathy (CADASIL). Neurologist 2005;11:19-29.
23. Weller M, Dichgans J, Klockgether T. Acetazolamide-responsive migraine in CADASIL. Neurology 1998;50: 1505.
24. Forteza AM, Brozman B, Rabinstein AA, et al. Acetazolamide for the treatment of migraine with aura in CADASIL. Neurology 2001;57:2144-5.
25. Dichgans M. Genetics of ischaemic stroke. Lancet Neurol 2007;6:149-61.
26. Liem MK, van der Grond J, Haan J, et al. Lacunar infarcts are the main correlate with cognitive dysfunction in CADASIL. Stroke 2007;38:923-8.
27. Viswanathan A, Gschwendtner A, Guichard JP, et al. Lacunar lesions are independently associated with disability and cognitive impairment in CADASIL. Neurology 2007;69:172-9.
28. Opherk C, Peters N, Herzog J, et al. Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. Brain 2004;127:2533-9.
29. Choi EJ, Choi CG, Kim JS. Large cerebral artery involvement in CADASIL. Neurology 2005;65:1322-4.
30. Dichgans M, Petersen D. Angiographic complications in CADASIL. Lancet 1997;349:776-7.
31. Choi JC, Kang SY, Kang JH, et al. Intracerebral hemorrhages in CADASIL. Neurology 2006;67:2042-4.
32. Dichgans M, Holtmannspotter M, Herzog J, et al. Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. Stroke 2002;33:67-71.
33. Lesnik Oberstein SA, van den Boom R, van Buchem MA, et al. Cerebral microbleeds in CADASIL. Neurology 2001;57:1066-70.
34. Viswanathan A, Guichard JP, Gschwendtner A, et al. Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. Brain 2006;129:2375-83.
35. Lesnik Oberstein SA, van den Boom R, Middelkoop HA, et al. Incipient CADASIL. Arch Neurol 2003;60:707-12.
36. Amberla K, Wäljas M, Tuominen S, et al. Insidious cognitive decline in CADASIL. Stroke 2004;35:1598-602.
37. Dichgans M, Filippi M, Bruning R, et al. Quantitative MRI in CADASIL: correlation with disability and cognitive performance. Neurology 1999;52:1361-7.
38. Chabriat H, Pappata S, Poupon C, et al. Clinical severity in CADASIL related to ultrastructural damage in white matter: in vivo study with diffusion tensor MRI. Stroke 1999;30:2637-43.
39. O'Sullivan M, Singhal S, Charlton R, et al. Diffusion tensor imaging of thalamus correlates with cognition in CADASIL without dementia. Neurology 2004;62:702-7.
40. Holtmannspotter M, Peters N, Opherk C, et al. Diffusion magnetic resonance histograms as a surrogate marker and predictor of disease progression in CADASIL: a two-year follow-up study. Stroke 2005;36:2559-65.
41. Scheid R, Preul C, Lincke T, et al. Correlation of cognitive status, MRI- and SPECT-imaging in CADASIL patients. Eur J Neurol 2006;13:363-70.
42. Lesnik Oberstein SA, Jukema JW, Van Duinen SG, et al. Myocardial infarction in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Medicine (Baltimore) 2003;82:251-6.
43. Kusaba T, Hatta T, Kimura T, et al. Renal involvement in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Clin Nephrol 2007;67:182-7.
44. Schroder JM, Zuchner S, Dichgans M, et al. Peripheral nerve and skeletal muscle involvement in CADASIL. Acta Neuropathol 2005;110:587-99.
45. Sicurelli F, Dotti MT, De Stefano N, et al. Peripheral neuropathy in CADASIL. J Neurol 2005;252:1206-9.
46. Haritoglou C, Rudolph G, Hoops JP, et al. Retinal vascular abnormalities in CADASIL. Neurology 2004;62:1202-5.
47. Rufa A, Dotti MT, Frezzotti P, et al. Hemodynamic evaluation of the optic nerve head in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Arch Neurol 2004;61:1230-3.
48. Phillips JS, King JA, Chandran S, et al. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) presenting with sudden sensorineural hearing loss. J Laryngol Otol 2005;119:148-51.
49. Cumurciuc R, Henry P, Gobron C, et al. 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 2006;37:1100-2.
50. Singhal S, Bevan S, Barrick T, et al. The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. Brain 2004;127:2031-8.

51. Peters N, Herzog J, Opherk C, et al. A two-year clinical follow-up study in 80 CADASIL subjects: progression patterns and implications for clinical trials. Stroke 2004;35: 1603-8.
52. Rufa A, Guideri F, Acampa M, et al. Cardiac autonomic nervous system and risk of arrhythmias in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Stroke 2007;38:276-80.
53. O'Sullivan M, Jarosz JM, Martin RJ, et al. MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. Neurology 2001;56:628-34.
54. Singhal S, Rich P, Markus HS. The spatial distribution of MR imaging abnormalities in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy and their relationship to age and clinical features. AJNR Am J Neuroradiol 2005;26:2481-7.
55. van den Boom R, Lesnik Oberstein SA, Ferrari MD, et al. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR imaging findings at different ages--3rd-6th decades. Radiology 2003;229:683-90.
56. Ebke M, Dichgans M, Bergmann M, et al. CADASIL: skin biopsy allows diagnosis in early stages. Acta Neurol Scand 1997;95:351-7.
57. Mayer M, Straube A, Bruening R, et al. Muscle and skin biopsies are a sensitive diagnostic tool in the diagnosis of CADASIL. J Neurol 1999;246:526-32.
58. Rubio A, Rifkin D, Powers JM, et al. Phenotypic variability of CADASIL and novel morphologic findings. Acta Neuropathol 1997;94:247-54.
59. Ishiko A, Shimizu A, Nagata E, et al. Notch3 ectodomain is a major component of granular osmiophilic material (GOM) in CADASIL. Acta Neuropathol 2006;112:333-9.
60. Joutel A, Favrole P, Labauge P, et al. Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. Lancet 2001;358:2049-51.
61. Malandrini A, Gaudiano C, Gambelli S, et al. Diagnostic value of ultrastructural skin biopsy studies in CADASIL. Neurology 2007;68:1430-2.
62. Federico A, Bianchi S, Dotti MT. The spectrum of mutations for CADASIL diagnosis. Neurol Sci 2005;26:117-24.
63. Oberstein SA. Diagnostic strategies in CADASIL. Neurology 2003;60:2020.
64. Joutel A, Vahedi K, Corpechot C, et al. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. Lancet 1997;350:1511-5.
65. Dichgans M, Herzog J, Gasser T. NOTCH3 mutation involving three cysteine residues in a family with typical CADASIL. Neurology 2001;57:1714-7.
66. Kim Y, Choi EJ, Choi CG, et al. Characteristics of CADASIL in Korea: a novel cysteine-sparing Notch3 mutation. Neurology 2006;66:1511-6.
67. Ruchoux MM, Guerouaou D, Vandenhaute B, et al. Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Acta Neuropathol 1995; 89:500-12.
68. Miao Q, Paloneva T, Tuominen S, et al. 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;14:358-64.
69. Rafalowska J, Fidzianska A, Dziewulska D, et al. CADASIL or CADVaSIL? Neuropathology 2004;24:16-20.
70. Viswanathan A, Gray F, Bousser MG, et al. Cortical neuronal apoptosis in CADASIL. Stroke 2006;37:2690-5.
71. Keverne JS, Low WC, Ziabreva I, et al. Cholinergic neuronal deficits in CADASIL. Stroke 2007;38:188-91.
72. Mesulam M, Siddique T, Cohen B. Cholinergic denervation in a pure multi-infarct state: observations on CADASIL. Neurology 2003;60:1183-5.
73. Krebs LT, Xue Y, Norton CR, et al. Characterization of Notch3-deficient mice: normal embryonic development and absence of genetic interactions with a Notch1 mutation. Genesis 2003;37:139-43.
74. Ruchoux MM, Domenga V, Brulin P, et al. Transgenic mice expressing mutant Notch3 develop vascular alterations characteristic of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Am J Pathol 2003;162:329-42.
75. Lacombe P, Oligo C, Domenga V, et al. Impaired cerebral vasoreactivity in a transgenic mouse model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy arteriopathy. Stroke 2005;36: 1053-8.
76. Dubroca C, Lacombe P, Domenga V, et al. Impaired vascular mechanotransduction in a transgenic mouse model of CADASIL arteriopathy. Stroke 2005;36:113-7.

77. Domenga V, Fardoux P, Lacombe P, et al. Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. Genes Dev 2004;18:2730-5.
78. Wang W, Prince CZ, Mou Y, et al. Notch3 signaling in vascular smooth muscle cells induces c-FLIP expression via ERK/MAPK activation. Resistance to Fas ligand-induced apoptosis. J Biol Chem 2002;277:21723-9.
79. Haritunians T, Boulter J, Hicks C, et al. CADASIL Notch3 mutant proteins localize to the cell surface and bind ligand. Circ Res 2002;90:506-8.
80. Karlstrom H, Beatus P, Dannaeus K, et al. A CADASIL-mutated Notch 3 receptor exhibits impaired intracellular trafficking and maturation but normal ligand-induced signaling. Proc Natl Acad Sci USA 2002;99:17119-24.
81. Peters N, Opherk C, Zacherle S, et al. CADASIL-associated Notch3 mutations have differential effects both on ligand binding and ligand-induced Notch3 receptor signaling through RBP-Jk. Exp Cell Res 2004;299:454-64.
82. Joutel A, Monet M, Domenga V, et al. 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 2004; 74:338-47.