Magnetic resonance imaging characteristics of CADASIL

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Cerebral microbleeds in CADASIL

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Abstract

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary arteriopathy leading to recurrent cerebral infarcts and dementia. Intracerebral haemorrhage (ICH) has been described sporadically in patients with CADASIL, suggesting that the affected arteries in CADASIL are not bleed-prone. However, the presence of cerebral microbleeds, which often remain undetected on conventional magnetic resonance (MR) imaging, has not been determined in CADASIL. Purpose was to determine whether cerebral vessels in patients with CADASIL are prone to microbleeding.

T2*-weighted gradient echo MR imaging, which is highly sensitive for visualizing microbleeds, was performed in patients with CADASIL and their family members (n=63). Known risk factors for ICH were determined for all individuals. On an exploratory basis, the presence of cerebral microbleeds was correlated with demographic variables, vascular risk factors, disease progression, ischemic MR lesions, and genotype.

Cerebral microbleeds were present in 31% of symptomatic CADASIL mutation carriers, predominantly in the thalamus. Vascular risk factors such as hypertension did not account for the microbleeds in these patients. Factors associated with microbleeds were age (P=0.008), Rankin disability score (P=0.017), antiplatelet use (P=0.025), number of lacunae on MR images (P=0.009), and the Arg153Cys *NOTCH3* mutation (P=0.017). After correction for age, only the Arg153Cys mutation remained significantly associated with the presence of microbleeds.

Patients with CADASIL have an age-related increased risk of intracerebral microbleeds. This implies that they may have an increased risk for ICH, which should be taken into account in CADASIL diagnosis and patient management.

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary small-vessel disease caused by mutations in the *NOTCH3* gene on chromosome 19[19]. Clinically, the disease is characterized by recurrent cerebral infarcts and dementia, and patients may also experience migraine or affective disorders. Magnetic resonance (MR) imaging reveals extensive cerebral white matter lesions and subcortical infarcts. On pathologic examination, there are marked changes in the small cerebral arteries with thickening of the media, loss of vascular smooth muscle cells, and accumulation of extracellular nonamyloid, noncholesterol, electron-dense granular material[51].

Primary intracerebral haemorrhage (pICH) has rarely been described in CADASIL, in contrast to other small-vessel diseases associated with white matter changes and infarcts, including hypertensive lipofibrohyalinosis and cerebral amyloid angiopathy (CAA)[52-54]. This discrepancy could indicate that CADASIL vessels, though thickened, are not fragile and thus do not lead to pICH or that CADASIL patients with pICH remain unrecognized. In the last 5 years, a strong association has been found between ischemic small-vessel disease and the presence of cerebral microbleeds detected on T2*-weighted gradient echo MR images[54-57]. This is a highly sensitive technique to detect haemosiderin deposits and thus remnants of intracerebral microbleeds, which are clearly visualized as small areas of signal loss[56]. Microbleeds in a randomly selected elderly population occur in 6.4% of individuals and are related to other indicators of small-vessel disease, whereas up to 26% of patients with a history of ischemic stroke have microbleeds[55,58]. Determining the presence of microbleeds in patients with CADASIL could answer the question of whether intracerebral extravasation of blood occurs in CADASIL arteriopathy. If so, this may implicate an increased risk of pICH in patients with CADASIL.

We sought to determine whether cerebral microbleeds occur in individuals with a confirmed *NOTCH3* mutation and, if so, whether the presence of microbleeds is associated with other patient characteristics such as severity of concomitant MR lesions, vascular risk factors, and genotype.

Materials and methods

Subjects

Members of 15 families with CADASIL were asked to participate in a clinical and genetic study on CADASIL. For all families, the respective *NOTCH3* mutation had previously been determined in at least one clinically affected member. Symptomatic as well as asymptomatic family members were included in the

study. Participants were considered symptomatic if a clinical diagnosis of CADASIL had been made by a physician before the study, based on a history of unexplained neurologic deficits or cognitive decline and white matter abnormalities with or without lacunar lesions on MR imaging. Participants were considered asymptomatic if they had never consulted a physician with symptoms compatible with CADASIL. Migraine was not included in this definition. Control subjects consisted of asymptomatic family members without a *NOTCH3* mutation. Disability at the time of the study was measured semiquantitatively with the Rankin scale. Sixty-three individuals participated in the study between August 1999 and July 2000. Only individuals cognitively capable of giving informed consent, and who did so, were included. The study was approved by the Medical Ethics Committee of the Leiden University Medical Centre.

MR imaging

MR examinations were performed on a 1.5 T MR system (Philips Medical Systems, Best, the Netherlands). T1-weighted spin echo (SE; slice thickness 6 mm with a 0.6 mm interslice gap [6.0/0.6 mm], repetition time [TR] 600 ms, echo time [TE] 20 ms, matrix of 256 x 205), T2-weighted turbo SE (TSE; 3.0/0.0 mm, TR/TE 3000/120 ms, 256 x 205), proton density-weighted (3.0/0.0 mm, 3000/27 ms, 256 x 205), and fluid-attenuated inversion recovery (FLAIR; 3.0/0.0 mm, TR/TE 8000/100 ms, 256 x 192) images were obtained in the axial plane. To specifically detect cerebral microbleeds, T2*-weighted gradient echo planar imaging was performed (6.0/0.6 mm, TR/TE 2598/48 ms, 256 x 192), echo-planar imaging factor, 25).

Microbleeds were defined as focal areas of signal loss on T2-weighted TSE images that increased in size on the T2*-weighted gradient echo planar images ("blooming effect"). In this way, microbleeds were differentiated from areas of signal loss based on vascular flow void. Areas of symmetric hypointensity in the basal ganglia likely to represent calcification or nonhaemorrhagic iron deposits were disregarded. Finally, to differentiate microbleeds from other intra-axial lesions with a haemorrhagic component, only areas of signal loss that were not locally associated with other abnormalities were counted as microbleeds. The location, number, and size of microbleeds were recorded. White matter lesion load was graded according to the signal hyperintensity rating scale of Scheltens et al on T2-weighted TSE, proton density-weighted, and FLAIR images[59]. Lacunar lesions were defined as focal areas of hyperintense lesions on T2-weighted TSE images, with corresponding hypointense lesions on T1-weighted SE images. The signal intensity of these lesions corresponded with

that of the cerebrospinal fluid . The number, location, and size of lacunar lesions were recorded. All MR images were reviewed by the same neuroradiologist, blinded to genetic and clinical data.

Mutation analysis

NOTCH3 mutation carriership was determined in all 63 individuals by direct sequencing analysis, according to previously described techniques[22]. Previously unreported *NOTCH3* mutations were considered disease causative when 1) there was a change in the number of cysteine residues, typical for CADASIL mutations; 2) the mutations did not occur in 100 control chromosomes; and 3) the mutation clustered in affected family members.

To assess whether families with the same mutation had a common ancestor, haplotype analysis was performed with four polymorphic microsatellite markers (D19S929, D19S841, D19S1153, D19S917) located close to the *NOTCH3* gene.

Risk factors

Vascular comorbidity including hypertension, smoking, diabetes mellitus, history of myocardial infarction, and hypercholesterolemia was recorded in all subjects, with definitions similar to those in comparable studies, as follows[55,58,60].

1. Hypertension: at least two blood pressure measurements of ≥150/95 mm Hg in the medical records of the preceding 5 years or two such measurements on the day of examination or current antihypertensive drug therapy;
2. Smoker: a person currently smoking at least one cigarette, cigar, or pipe per day for at least 1 year or who has done so in the past;
3. Diabetes mellitus: current use of oral antidiabetics or insulin;
4. Hypercholesterolemia: a cholesterol measurement of ≥7 mmol/L in the medical records of the preceding 5 years or current use of cholesterol-lowering drugs; and
5. Myocardial infarction: a history of myocardial infarction in the medical records.

All individuals filled in a structured questionnaire concerning vascular risk factors. In addition, an extensive oral medical history, including medication use, was taken on the day of examination. All affected patients were accompanied by a clinically unaffected family member or spouse who, when necessary, supplied additional medical history. Questionnaires and history were validated with information from medical records supplied by the participants' general practitioners and medical specialists.

Statistical analysis

Statistical analysis was performed with SPSS-10 (SPSS, Inc., Chicago, IL). An exploratory analysis was performed to detect any association between the presence of cerebral microbleeds and demographic variables, vascular risk factors, concomitant MR abnormalities, and genotype. The association between microbleeds and the patient variables was studied in the setting of logistic regression. First, all univariate P values were computed, followed by the computation of P values after correction for age. A significance threshold of $P<0.05$ was maintained.

Results

Table 1 gives an overview of the studied variables in carriers of *NOTCH3* mutations with and without microbleeds.

Table 1 Characteristics of NOTCH3 mutation carriers with and without microbleeds

Characteristic	Microbleeds, n=10	No microbleeds, n=30	P (p*)
Age, y (range)	53.4 (43-58)	43.4 (21-59)	≤0.008
Male, n (%)	5 (50)	14 (47)	≤0.85
Hypertension , n (%)	0	3 (10)	≤0.3
Smoking, n (%)	6 (60)	19 (63)	≤0.85
Hypercholesterolemia, n (%)	3 (30)	12 (40)	≤0.6
Diabetes mellitus, n (%)	0	3 (10)	≤0.3
Myocardial infarction, n (%)	1 (10)	5 (17)	≤0.7
Antiplatelet use, n(%)	10 (100)	19 (63)	≤0.3 (0.025)
Antiplatelet use, mean y (range)	6.5 (1-10)	2.7 (0-12)	≤0.2 (0.003)
Mean antiplatelet dose, mg/d (range)	93.2 (38-240)	53.1 (0-300)	≤0.8 (0.07)
Dipyridamol, n (%)	4 (40)	4 (13)	≤0.5 (0.07)
Mean white matter lesion load (range)	53.8 (37-65)	41,4 (4-64)	≤0.9 (0.053)
Mean no. of lacunes (range)	34.1 (5-66)	16.9 (0-52)	≤0.3 (0.009)
Mean Rankin score (range)	2.8 (0-5)	1.5 (0-4)	≤0.6 (0.017)

p = P value before correction for age*

Mutation analysis and subjects

The 15 participating families had 11 different *NOTCH3* mutations. Some families therefore shared the same genotype. Three mutations were not previously described in the literature (table 2). Per family, the number of participants varied from only the index patient to 10 members. The total number of participants was 63, but one participant had an incomplete MR study because of claustrophobia and was therefore excluded from the analysis. The mean ± SD age of the remaining 62 participants was 43.8 ± 11.5 years (range 21 to 67 years).

Forty participants had a mutation in the *NOTCH3* gene, and 22 did not. Thirty-two mutation carriers were symptomatic, with a wide range of severity from one TIA to multiple strokes and cognitive decline. Eight mutation carriers were asymptomatic. The mean age of the 40 mutation carriers was 45.9 ± 10.5 years (range 21 to 59 years) and of the 22 non mutation carriers 40.0 ± 12.7 years (range 22 to 67 years).

Microbleeds

Ten of the 40 participants (25%) with a *NOTCH3* mutation had microbleeds. These 10 individuals were all clinically symptomatic. Thus, almost a third (31%) of the 32 symptomatic *NOTCH3* mutation carriers had microbleeds. None of the eight asymptomatic mutation carriers and none of the 22 individuals without a *NOTCH3* mutation had microbleeds.

Eighty-seven focal areas of signal loss were detected on T2*-weighted gradient echo images. Most of these were smaller than 5 mm in diameter (92%); the largest was 10 mm. These foci all resembled microbleeds in their homogeneous shape and distribution. Microbleeds were located predominantly in the thalamus (table 3; figure 1). Of the cerebral lobes, the occipital lobe had the most microbleeds (nine) compared with five microbleeds in the temporal lobe, two in the parietal lobe, and one in the frontal lobe. Mutation carriers with microbleeds were significantly older than mutation carriers without microbleeds, and microbleeds were present equally in men and women (table 1). All microbleeds occurred in patients with five different mutations. Six of the mutations, therefore, were not associated with microbleeds. Of the 10 patients with microbleeds, six had an Arg153Cys mutation. These six patients originated from two families: four individuals from one family and two from the other. Haplotype analysis showed that these families were unrelated. The Arg153Cys mutation is associated with microbleeds before (P=0.017) as well as after (P=0.037) correction for age.

Table 2 NOTCH3 mutations in study population

Genotype, exon	Subjects with microbleeds n=10	Total mutated n=40
Arg153Cys, 4	6	12
Arg141Cys, 4	1	9
Cys446Phe, *8	1	1
Arg207cys, 4	1	2
Arg544Cys, 11	1	1
Cys1015Arg, 19	0	3
Arg133Cys, 4	0	2
Arg182Cys, 4	0	4
Arg1076Cys,*20	0	3
Cys144Phe, 4	0	1
Cys162Trp, *4	0	2

** previously unreported NOTCH3 mutation*

Table 3 Cerebral microbleed distribution in CADASIL

Cerebral structure	No. of subjects with microbleeds in structure	Total no. of microbleeds, sum (range)
Subcortical white matter	5	23 (0-15)
Thalamus	8	53 (0-18)
Basal ganglia	2	2 (0-1)
Cerebellum	2	5 (0-4)
Brainstem	3	4 (0-2)
Cortex	0	0

Concomitant cerebral MR abnormalities

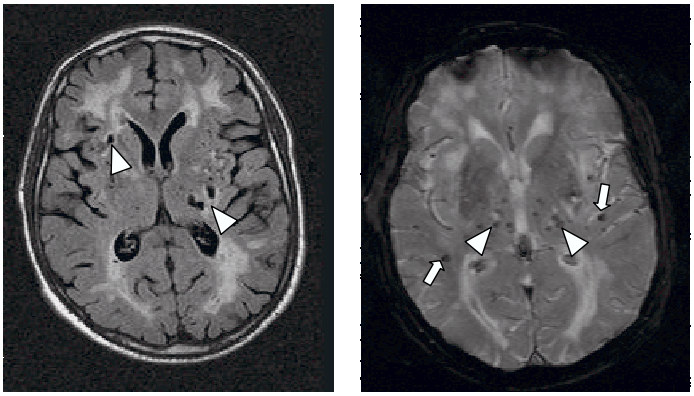
The MR imaging results described here pertain only to the carriers of *NOTCH3* mutations (n=40). Mutation carriers all had white matter abnormalities on MR imaging. Individuals with microbleeds did not have a significantly higher white matter lesion load than those without microbleeds but did have a significantly higher lacunar lesion count (table 1). Those with microbleeds had more lacunae in the temporal lobe (P=0.025), occipital lobe (P=0.015), and thalamus (P<0.001). After correction for age, the difference in mean total lacunar lesion count disappeared.

Vascular risk factors

Vascular risk factors (hypertension, smoking, hypercholesterolemia, diabetes mellitus, and history of myocardial infarction) did not occur significantly more in mutation carriers with microbleeds than in those without microbleeds (table 1). Hypertension and diabetes mellitus were not present in any of the individuals with microbleeds. Those with microbleeds had used antiplatelet drugs more (P=0.025) and longer (P=0.003) than those without microbleeds. These associations, however, disappeared after correction for age. One patient with microbleeds had a history of long-term anticoagulant use (>10 years). A patient without microbleeds had started using anticoagulants just before the study, and one had a history of short-term anticoagulant use.

Rankin score

An association was found between Rankin score and microbleeds (P=0.017). After correction for age, however, this was no longer present.



a) b)

Figure1 MR images at the level of the basal ganglia in a 55 year old patient with CADASIL. FLAIR image (a) shows periventricular and deep white matter hyperintensities and lacunes in the thalamus and basal ganglia (arrows). T2-weighted gradient echo planar image (b) shows numerous circumscript hypodense lesions from previous microbleeds, located in the thalamus (arrowhead) as well as deep and subcortical white matter (white arrow).*

Discussion

The main finding of the current study is that 31% of symptomatic *NOTCH3* mutation carriers had evidence of cerebral microbleeds on MR imaging. We postulate that the microbleeds are directly related to CADASIL arteriopathy, as known risk factors for cerebral bleeding, such as hypertension, were absent. CAA is a post-mortem diagnosis and therefore could not be excluded as a cause for the microbleeds. However, it is unlikely to have played a role, owing to the relatively young age of our study population and the mainly

thalamic location of the microbleeds, both of which are atypical for CAA[61]. Hereby, the borders of CADASIL disease expression are expanded beyond that of a purely ischemic cerebral disease, to include cerebral bleeding. A relevant clinical implication is that patients with CADASIL may be at increased risk of pICH, which has been shown to be associated with the presence of microbleeds[54,56,57,62].

In this CADASIL population, the Arg153Cys mutation is an independent risk factor for microbleeds. Although confirmation of this finding is warranted because of the small sample size and the exploratory nature of this study, it supports the possibility that various *NOTCH3* mutations may lead to differences in severity or pattern of disease expression, as has been suggested by other authors[63,64].

Microbleeds were found to be age related, and their presence may thus reflect disease progression in CADASIL. This is also suggested by the fact that microbleeds occurred in areas with a large number of lacunae. Possibly, the more damaged the intracerebral vessels become (reflected in increased number of lacunae), the more bleed-prone they tend to get. However, the total number of lacunae and other MR and clinical data reflecting disease progression, such as the Rankin scale and white matter lesion load, were higher in patients with microbleeds, but not within the range of significance after correction for age (table 1).

ICH has so far rarely been reported in patients with CADASIL. Several explanations could account for this. First, when pICH occurs, the underlying CADASIL may be obscured and thus remain undiagnosed, especially because pICH is classically considered not to form part of the CADASIL disease spectrum. A second explanation could be that true pICH in CADASIL is provoked only when additional risk factors are present. Interestingly, of the two CADASIL patients with pICH reported in the literature, one died as a consequence of this at age 30, 6 months after starting anticoagulant therapy[1,7]. In our study, previous anticoagulant use was recorded in only one patient with microbleeds. Therefore, whether anticoagulants play a role in CADASIL ICH, as has also been found in other small-vessel disease, remains to be determined[65]. We did find that patients with microbleeds used antiplatelet drugs more and longer than those without microbleeds. However, the significance of this finding was lost after correction for age, implying that antiplatelet drug therapy probably does not play a role in increased risk for cerebral bleeding in CADASIL. To avoid missing the diagnosis and to further delineate the disease spectrum, CADASIL should be considered in the diagnostic workup of (normotensive) patients presenting with pICH and concomitant white matter abnormalities on MR imaging.