Preparing for CADASIL therapy

Gravesteijn, G.

Chapter 4

Long-term disease progression in CADASIL: an 18-year follow-up study

Gido Gravesteijn
Remco J. Hack
Anna M. van Opstal
Bastian J. van Eijsden
Maurice Overzier
Joseph F. Arboleda-Velasquez
Huub A.M. Middelkoop
Mar D.M. Rodriguez Girondo
Charlotte E. Teunissen
Annemieke Aartsma-Rus
Jeroen van de Grond
Julie W. Rutten*
Saskia A.J. Lesnik Oberstein*

Submitted

Abstract

Objective

To gain more insight into long-term disease progression and survival in CADASIL patients, we performed a prospective clinical and neuroimaging 18-year follow-up study, including cerebral hemodynamics and blood biomarkers.

Methods

Forty-one CADASIL patients and 21 unaffected family members were characterised at baseline, including MRI, functional independence and neuropsychological testing. Cerebral hemodynamics were measured using PC-MRA and functional MRI. At 18-year follow-up, 15 patients and 9 controls re-visited our hospital for the same battery of tests. Blood levels of Neurofilament-Light-chain (NfL) were measured using single molecule array. HTRA1, Endostatin, IGF-BP1 and TGF-ß were quantified using ELISA.

Results

Half of the patients (22/41) were still alive after 18 years. Lacune load and disability at baseline were significantly associated with survival. Eight of the 15 patients who were able to participate in the follow-up study still had no significant disability, executive dysfunction or global cognitive impairment after 18 years (age range 40-63 years). At baseline and follow-up these relatively stable patients had significantly less lacunes than the severely affected patients. Of the tested blood biomarkers, only serum NfL levels were associated with disease severity at both time points, but its 18-year increase did not significantly differ between patients and controls. Patients and controls did not differ in longitudinal hemodynamic changes.

Conclusion

CADASIL patients can remain clinically relatively stable over almost two decades. Disease severity, disease progression and survival are most strongly associated with lacune load. NfL was the only blood biomarker associated with disease severity at both time points.

Introduction

CADASIL is the most prevalent monogenic cerebral small vessel disease and is caused by *NOTCH3* mutations.[1] These mutations lead to aggregation of the NOTCH3 ectodomain ($NOTCH3^{ECD}$) in the vessel wall of small arteries, which is associated with impaired cerebrohemodynamics, leading to recurrent stroke and vascular cognitive impairment.[2–5] Disease severity is increasingly recognized to be highly variable in CADASIL, partially attributable to modifiers such as mutation position[6] and classical cardiovascular risk factors.[7,8] There are only a few prospective studies in CADASIL cohorts, of which the longest is a seven year follow-up.[9–14]

Here, we performed a prospective 18-year follow-up study in CADASIL patients and unaffected family members to gain more insight into long-term disease progression and survival. Longitudinal assessment included functional independence, a neuropsychological test battery and structural neuroimaging, as well as cerebral hemodynamics and blood withdrawal for the analysis of potential blood biomarkers, including Neurofilament Light-chain (NfL).[15–18]

Methods

Cohort

In 2000, 41 genetically confirmed CADASIL patients and 22 unaffected family members were recruited from the Dutch CADASIL registry and enrolled in the baseline study.[19] The participants came from 15 families, of which 10 families had 2 or more participants in the study. *NOTCH3* sequence analysis showed that most of the patients had mutations in exon 4 (n=32), while the other nine patients had a mutation in exon 8 (n=1), exon 11 (n=1), exon 19 (n=4) or exon 20 (n=3).

Study procedure

A full medical history was taken, blood was withdrawn and neuropsychological tests and brain MRI were performed.[19] In 2018, survival data were obtained from the Dutch population registry from all participants. All living patients were re-invited for the 18-year follow-up study for the same battery of tests. Medical information was collected for patients who could not participate on-site and for those who were deceased. Cause of death could be obtained from seven deceased patients. Patients not participating in the follow-up study were considered to have had a stroke when patients were deceased during the follow-up or when a stroke was reported by a neurologist. Relevant disease progression was

defined as an increased modified Ranking Scale score, but not counting the increase from stage 0 to 1. Both studies were approved by the medical ethics committee of the Leiden University Medical Center (P80.89; P17.170). Written informed consent of each participant was obtained at each visit.

Neuroimaging

MRI at baseline was performed on a 1.5 Tesla MR system, as described previously.[12,20] At 18-year follow-up, MRI was performed on a whole body magnetic resonance system with a 3 Tesla field strength (Philips Medical Systems, Best, Netherlands). Neuroimaging follow-up was available for 13/15 patients: one did not undergo MRI due to severe claustrophobia and one had a contra-indication for MRI. We obtained three dimensional T1-weighted images (echo time [TE] 4.6ms, repetition time [TR] 9ms, Flip 8°, field of view [FOV] = 224 × 177 × 168mm, scan duration ~5mins), T2-weighted images (TE/TR/Flip: 80ms/4.2s/90°, 40 slices, FOV 224 × 180 × 144mm, slice thickness 3.6mm, matrix size 448 × 320), fluid-attenuated inversion recovery (TE/TR/Flip angle: 125ms/11.0s/90°, slices 25, FOV 252 × 179.76 × 250mm, matrix size 224 × 224, scan duration 293s) and susceptibility-weighted images (TE/TR/Flip angle: 31ms/45ms/13°, 125 slices, matrix size: 250 × 175 × 112mm voxel size: 0.78 × 0.78 × 0.8mm). Phase-contrast quantitative flow scans (PC-MRA) were acquired using 2 localizer angiograms in the sagittal and coronal planes (TR=11ms; TE 7·5ms; flip angle=10°; slice thickness=5mm; field of view 150 × 103mm; voxel size 1.17 × 1.17mm; velocity sensitivity=200cm/s, 20 signal averages). The visually stimulated blood-oxygen-level-dependent (BOLD) fMRI scans were acquired with a TE/TR: 31ms/1499ms, FOV 220 × 75 × 220mm, matrix 80 × 80mm, slices 25, 130 dynamics, scan duration 201sec. Lacunar infarcts of presumed vascular origin (lacunes) were re-counted for the baseline scans and counted for the follow-up scans using the definition of the STRIVE criteria.[21] Volumetric analyses of MR images at baseline were described previously[12,20] and volumetric analysis of MR images at 18-year follow-up was performed using FMRIB Software Library (FSL) version 5.0.8 (Analysis Group, FMRIB, Oxford, UK).[22] Brain parenchymal fraction (BPF) was calculated at each time-point by dividing brain parenchymal volume over intracranial volume. Absolute white matter hyperintensity (WMH) volume was analysed at baseline as described previously[13] and for 18-year follow-up, FSL was used to quantify WMH automatically as described before.[23] WMH volume was normalized to brain parenchymal volume in order to obtain normalized WMH volume (WMH_V). In order to correct for potential differences in WMH_V quantification methods at baseline and follow-up, WMH were also quantified on MRI scans made in a 7-year follow-up study of the same cohort,[12,20] using similar methods to the baseline scans,[12,20] as well as using FSL similar to the 18-year follow-up scans.[23] Baseline WMH_V were then normalized to the quantification method used for the 7- and 18-year follow-up studies, using mixed model linear regression with random intercepts, with quantification method and time point as

variables. WMH were also classified semi-quantitatively using the Fazekas scale for deep white matter hyperintensities.[24]

Neuropsychological and functional ability tests

Disability was assessed using the modified Rankin Scale (mRS). Global cognition was assessed using the Cambridge Cognitive Examination (CAMCOG) and Mini–Mental State Examination (MMSE).[25,26] Global memory was determined using the Wechsler Memory Scale.[27] Executive function was assessed using the symbol substitution test of the Wechsler Adult Intelligence Scale (WAIS; Pearson, London, UK) and the Trail Making Test part A (TMT-A) and part B (TMT- B).[28]

CVR and BOLD procedure

At 18-year follow-up, blood-oxygen-level dependent (BOLD) fMRI studies were performed in patients participating in the 18-year follow-up study, as well as in seven additional CADASIL patients and 10 additional healthy controls in order to increase sample size for the BOLD analyses (extended cohort). A visual stimulus, consisting of 16 blocks of an 8 Hz flashing radial black and white checkerboard pattern was shown for 20 seconds, alternated with 28 seconds of grey screen, as used in an earlier study in cerebral amyloid angiopathy.[29] BOLD fMRI scans were analysed for BOLD amplitude, time to peak and time to baseline as described before.[29,30]

At baseline and follow-up, cerebrovascular reactivity (CVR) measurements were performed using a gradient-echo phase-contrast technique (PC-MRA) before and after administration of 14 mg/kg acetazolamide, as described previously.[31] At 18-year follow-up, cerebral blood flow was measured in the internal carotid and basilar arteries using the Dicom viewing software Osirix with region of interest measurement tools. In addition to the patients and controls participating in the 18-year follow-up study, seven additional CADASIL patients and 10 additional healthy controls were enrolled in order to increase sample size (extended cohort). Flow was measured for basilar artery and both internal carotid arteries separately. Total cerebral blood flow was calculated as the sum of the flow of all three vessels. CVR was defined as $(CBF_{challenge} - CBF_{resting}) / CBF_{resting} \times 100\%$.

Blood biomarkers

Serum and plasma samples were taken at baseline and 18-year follow-up, centrifuged at 2750*g*, aliquoted and stored at -80°C until analysis. Commercially available enzyme-linked immunosorbent assays (ELISA) were used to quantify levels of Endostatin (DNST0, R&D Systems, USA; plasma diluted 1/50), HTRA1 (SEL604Hu, American Research Products, USA; plasma diluted 1/50), IGF-BP1 (DGB100, R&D; plasma diluted 1/2.5) and TGF-β (DB100B, R&D; serum diluted 1/80), according to manufacturer's instructions. All

standard curves had r^2 >0.99. The previously reported home-brew $NOTCH3^{ECD}$ ELISA[17] was adapted for determining $NOTCH3^{ECD}$ levels in human samples, by using 4 µL horse radish peroxidase-streptavidin complex in 100 µL volume per well and by using a 1/20 dilution of plasma samples. Serum NfL levels at baseline were previously determined.[16] Only NfL and Endostatin levels were measured in 18-year follow-up samples, as the other blood biomarkers showed no differences between patients and controls at baseline. In baseline and follow-up samples, the levels of serum NfL were measured as previously reported,[16] and the levels of Endostatin were measured using ELISA (DNST0, R&D Systems).

Statistics

Independent samples *t*-test and independent samples Mann-Whitney U test were used to compare baseline differences between the whole cohort and the cohort that completed the 18-year follow-up study, to compare baseline differences in 18-year change between patients and controls, and to compare patients with mRS≥2 versus patients with mRS≤1. The median age at first stroke was calculated using Kaplan Meier stroke-free survival analysis. Kaplan Meier analysis was also used to analyse 18-year overall survival with log-rank using the factors lacunes (0; 1-10; >10), stroke (yes/no), and mRS (0-2; 3-5). Age-corrected *P*-values for survival were calculated using Cox regression models. The difference in age-dependent increase in disease measures and biomarkers between patients and controls was modelled with linear mixed models with random slopes (which showed lower AIC values than random intercepts) with correction for sex. Non-normally distributed data were transformed for this analysis to obtain plausible normal distributions: WMH_V was square root transformed; CAMCOG and MMSE were quadratic transformed; TMT-A and -B were $\log_{10}$ transformed; microbleed count was $\log_{10}(1+x)$ transformed; and NfL was natural log transformed. For exploring which vascular risk factors at baseline were associated with changes in disease measures over 18-years (stroke count, lacune count, mRS, CAMCOG), linear regression models were used with correction for age. Differences in cerebral hemodynamics and blood biomarkers were tested using ANCOVA with correction for age and Bonferroni correction for multiple testing. Associations with NfL levels in patients were performed using simple linear regression and forward linear regression with covariates lacunes, WMH_V, BPF, mRS, CAMCOG and age. All statistical analyses were performed in IBM SPSS Statistics version 25.0. All statistical tests were two-sided and the threshold for statistical significance was 0.05.

Data availability

With the permission of the authors and their institutions, all data used for analysis will be shared after ethics approval if requested by other investigators for reasonable purposes of replicating procedures and results.

Table 4.1: Patient characteristics at baseline and at 18-year follow-up

18-year follow-up data of the whole cohort			
	Baseline	18-year follow-up	*P*-value[d]
N	41	37[a]	
Age, y (mean, SD)	45.8 (10.4)	-[b]	
Female	22 (54%)	20 (54%)	
History of stroke	23 (56%)	29 (78%)[c]	0.03*
mRS			
score 0-2	30 (73%)	13 (35%)	
score 3-5	11 (27%)	5 (14%)	
score 6 (deceased)	-	19 (51%)	
Patients who visited the study site after 18 years			
	Baseline	18-year follow-up	*P*-value[d]
N	15	15	
Age, y (mean, SD)	39.6 (8.9)†[e]	57.6 (8.8)[e]	
Female	9 (60%)	9 (60%)	
History of stroke	4 (27%)	9 (60%)	0.06
Disability			
score 0-2	15 (100%)‡	12 (80%)	0.25
score 3-5	0	3 (20%)	
Neuroimaging measures (N)	13[f]	13[f]	
Lacunes	0 (0 – 7)‡	4 (0 – 16)	0.008**
yes	6 (46%)	11 (85%)	0.06
no	7 (54%)	2 (15%)	
WMH_V (‰)	13.76 (0.63 – 55.22)†	39.7 (19.3 – 92.1)	<0.001***
BPF (%)	83.3 (3.2)	78.1 (3.5)	n/a[g]
Microbleeds	0 (0 – 0)†	3 (0 – 37)	0.046*
yes	0 (0%)	8 (62%)	0.008**
no	13 (100%)	5 (38%)	
Cognitive measures (N)	15	15	
CAMCOG	96.3 (4.1)†	89.6 (7.4)	0.03*
MMSE	28.1 (1.5)	28.7 (1.2)	0.19
WMS	69.3 (11.0)	62.6 (11.8)	0.47
WAIS substitution	53.7 (12.1)	45.6 (18.2)	0.54
TMT-A (s)	33 (10 – 48)	37 (12 – 156)	0.19
TMT-B (s)	64 (36 – 140)	76 (43 – 383)	0.48

Summary statistics are shown as count (%), mean (SD) or mean (range).

†,‡ For the indicated measures, the fifteen patients participating at 18-year follow-up were significantly younger and less severely affected at baseline († $P < 0.05$, ‡ $P < 0.01$).

[a] Four patients were lost to follow-up.

[b] No average age could be calculated since part of the patients were decreased at follow-up.

[c] All patients deceased at follow-up due to CADASIL were considered to have had a stroke.

[d] *P*-value represents the change over 18 years of the respective variable in patients, compared to controls. Significance level of 0.05, 0.01 and 0.001 is indicated by asterisks (*, **, ***, respectively). For dichotomous variables, *P*-value represents McNemar test for the difference in proportion at baseline and follow-up.

[e] The age range was 22 – 51 years at baseline and 40 – 70 years at follow-up.

[f] For two patients neuroimaging was not available due to claustrophobia and an MRI contra-indication.

[g] Due to technical differences between baseline and follow-up, BPF at the two time points could not be compared.

Results

18-year disease progression

Of the 41 patients who participated in the baseline study, 19 (46%) were deceased at the time of the 18-year follow-up study. The mean age at death of these 19 patients was 62.4±7.1 years, the overall mean survival estimate was 66.5 years (95%-CI 63.7–69.3 years). One patient was deceased due to stroke, and the majority of the other patients was deceased due to secondary complications of CADASIL, including swallowing difficulties and pneumonia. Mortality was associated with the following variables at baseline: age (P=0.005), prior stroke (P=0.02), presence of lacunes (P<0.001) and disability (P<0.001)(Figure 4.1). Patients without any disability (mRS 0) at baseline had a 85% (11/13) survival rate. Of the 22 patients who were still alive, fifteen visited the study site after 18 years for a complete assessment including MRI and neuropsychological testing. Three additional patients could not visit the study site, but did give consent for medical record retrieval and hetero-anamnesis via their spouse. Four patients were completely lost to follow-up.

During the 18-year follow-up, 29/37 (78%) patients had at least one incident or recurrent stroke (Table 4.1). Of these 29 patients, 23 (79%) had prior stroke at baseline. Three patients who had prior stroke at baseline did not have recurrent stroke during the 18-year follow-up. The mean age at first stroke was 49.5 years (95%-CI: 45.5–53.5 years, range 31–73 years). In our cohort, the total stroke incidence was 5.5 per 100 person years.

In the fifteen patients and nine controls from the baseline study who visited the study site after 18 years, the mean follow-up time was 18.1±0.3 years. Compared to all patients, the 15 patients for whom 18-year follow-up data were available were younger (39.6±8.9 versus 45.8±10.4 years, P=0.044) and less severely affected at baseline (mRS 0.5±0.6 versus 1.6±1.5, P=0.006). Eight of the fifteen patients remained clinically relatively stable over 18 years: they had no relevant disability (mRS≤1) and also no significant cognitive impairment at 18-year follow-up (Table 4.2). Compared to the seven patients who showed disease progression over 18-years (increase of 1 unit on the mRS, except going from mRS 0 to 1) and who had significant disability at follow-up (mRS≥2), the eight patients with mRS≤1, differed in baseline characteristics only with respect to age and lacune count (Table 4.2). Three of the 8 patients who remained stable had an incident stroke during follow-up, whereas incident or recurrent stroke occurred in 6/7 patients who progressed. WMH_V increase was similar between the patients that remained stable and those who progressed. Patients who progressed showed a stronger deterioration on CAMCOG and WAIS compared those who remained stable, but not on the other cognitive measures (Table 4.2). For the whole group of 15 patients, the only cognitive test that showed deterioration compared to controls

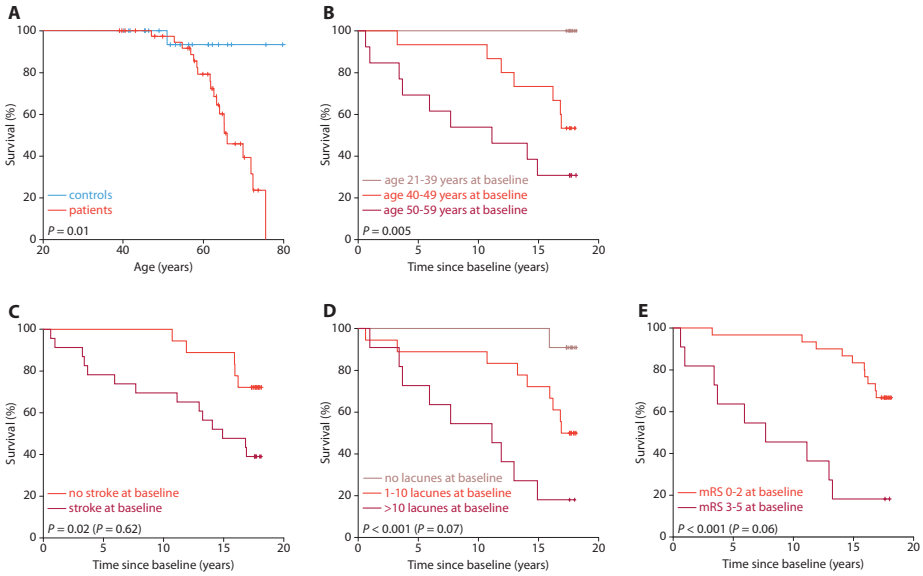

Figure 4.1: Association between disease severity at baseline and 18-year survival

(A) Overall mean survival of CADASIL patients was 66.5 years (95%-CI 63.7–69.3 years). (B) Age at baseline was associated with survival (log-rank *P*=0.005). (C) Prior stroke at baseline was associated with a higher mortality before correction for age (HR 3.1, *P*=0.02; after correction for age: HR 1.31, *P*=0.62). Median survival time for patients with prior stroke at baseline was 14.9 years (95%-CI: 9.3–20.5). (D) The presence of lacunes at baseline was associated with a higher mortality (log-rank *P*<0.001, age-corrected log-rank *P*=0.07)(>10 lacunes HR 21.6, *P*=0.004, age-corrected HR 6.9, *P*=0.07; and 1-10 lacunes HR 6.9, *P*=0.07, age-corrected HR 3.1, *P*=0.28, compared to patients without lacunes). Median survival time for patients with >10 lacunes at baseline was 11.1 years (95%-CI: 4.7–17.6). (E) Patients with mRS ≥ 3 at baseline had a median survival time of 7.7 years (95%-CI: 0–15.7) with a HR of 5.8 (*P* < 0.001, age-corrected HR 2.7, *P*=0.05), compared to patients with mRS ≤ 2 at baseline (log-rank *P*<0.001, age-corrected log-rank *P*=0.06). *P*-values in figures represent log-rank test for differences between survival curves, and *P*-values in brackets represent age-corrected *P*-values.

was the CAMCOG (Table 4.1, Figure 4.2). There was no association between 18-year disease progression and vascular risk factors (smoking, hypertension, hyperlipidaemia, Diabetes Mellitus type II and ApoE4 genotype).

There were some striking differences in disease progression, even in patients who were the same age at baseline. This is clearly illustrated by two siblings in their forties at baseline who were two years apart in age. One sibling was already more severely affected at baseline and had a rapid disease progression, while the other sibling was mildly affected at baseline and had hardly progressed at 18-year follow-up (Figure 4.3).

Table 4.2: Disease progression in the subset of patients studied at 18-year follow-up

	At baseline			Delta over 18-years		
	mRS 0-1 at FU18	mRS ≥ 2 at FU18	P-value	mRS 0-1 at FU18	mRS ≥ 2 at FU18	P-value for difference in 18-year delta between groups
Age	34.8 (22.1–45.7)	46.0 (38.9–51.7)	0.009**	-	-	-
Stroke count	0 (0–0)	1 (0–3)	0.07	0 (0–1)	1 (0–3)	0.04*
Lacune count	0 (0–1)	1 (0–7)	0.02*	2 (0–9)	6.5 (1–12)	0.07
BPF (%)	82.9 (4.1)	83.7 (2.0)	0.69	n/a[a]	n/a[a]	n/a[a]
WMH_V (‰)	8.5 (0.6–21.6)	29.9 (4.0–55.2)	0.07	27.5 (15.1–36.2)	23.9 (15.2–38.9)	0.95
Microbleed count	0 (0–0)	0 (0–0)	0.99	0 (0–11)	5 (0–37)	0.14
mRS	0 (0–1)	1 (0–2)	0.05	0 (0–1)	2 (1–2)	0.001**
CAMCOG	98 (93–100)	96 (87–101)	0.46	-4 (-7–3)	-8 (-16 – -5)	0.004**
MMSE	28 (25–30)	28 (26–29)	0.99	1.1 (1.3)	0 (1.6)	0.17
WMS	71.5 (9.9)	66.8 (12.4)	0.43	-4.0 (8.2)	-8.2 (6.1)	0.31
WAIS-substitution	58.5 (10.0)	48.1 (12.5)	0.10	-1.5 (7.3)	-18.3 (9.9)	0.003**
TMT-A (s)	26 (10–39)	37 (30–48)	0.05	2.5 (-17–19)	22 (-17–108)	0.19
TMT-B (s)	60 (36–140)	77 (44–102)	0.23	10 (-13–27)	67.5 (-42–315)	0.14
NfL (pg/mL)	6.1 (2.2–16.2)	7.7 (6.8–22.2)	0.07	4.4 (-0.3–5.5)	5.2 (0–33.5)	0.28
CVR	69.1 (20.4)	52.7 (16.2)[b]	0.34	-3.14 (36.1)	-0.45 (n/a)[c]	n/a
Resting CBF	642 (120)	548 (118)[d]	0.18	-52 (170)	-220 (100)[e]	0.11

Depending on whether normal distribution could be assumed, data is shown as mean (SD) or median (range). Group were based on mRS at follow-up, with patients with mRS 0-1 (n=7 for neuroimaging measures, n=8 for other measures unless otherwise stated) and patients with $mRS_{\geq 2}$ (n=6 for neuroimaging measures, n=7 for other measures unless otherwise stated).

[a] Due to technical differences between baseline and follow-up, BPF at the two time points could not be compared.

[b,c,d,e] Not all patients consented to acetazolamide challenge, resulting in a group size of n=2, n=1, n=6, n=4, respectively.

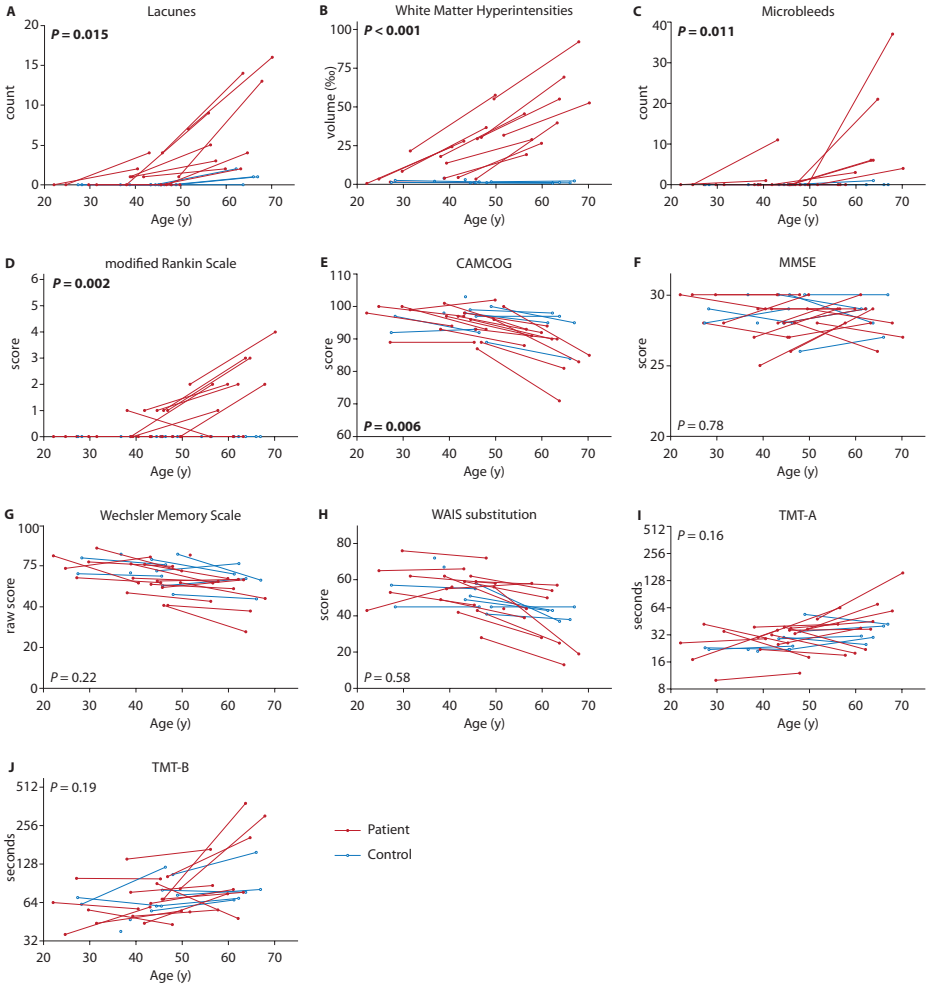

Figure 4.2: Changes in disease measures over 18 years in CADASIL patients versus controls

Graphs showing the age-dependent difference between patients and controls for neuroimaging measures, disability and cognitive measures. Each line represents patient (red) or control (blue), with measurements at baseline and 18-year follow-up. (A) Lacunes mainly start to appear on MRI from the age of 40 onwards, although some patients do not have lacunes at the age of 60 years. Three controls had lacunes on MRI. (B,C) WMH_V and microbleed count significantly increase in patients compared to controls. (D-J) Only disability (mRS) and global cognition measured by CAMCOG show a steeper age-dependent deterioration in patients than in controls, whilst other measures (for global cognition [MMSE], global memory [WMS] and executive function [WAIS substitution, TMT-A, TMT-B]) do not. *P*-values represent the difference in age-dependent change between patients and controls. Graphs with the *P*-value located in the upper corner represent a higher-is-worse covariate.

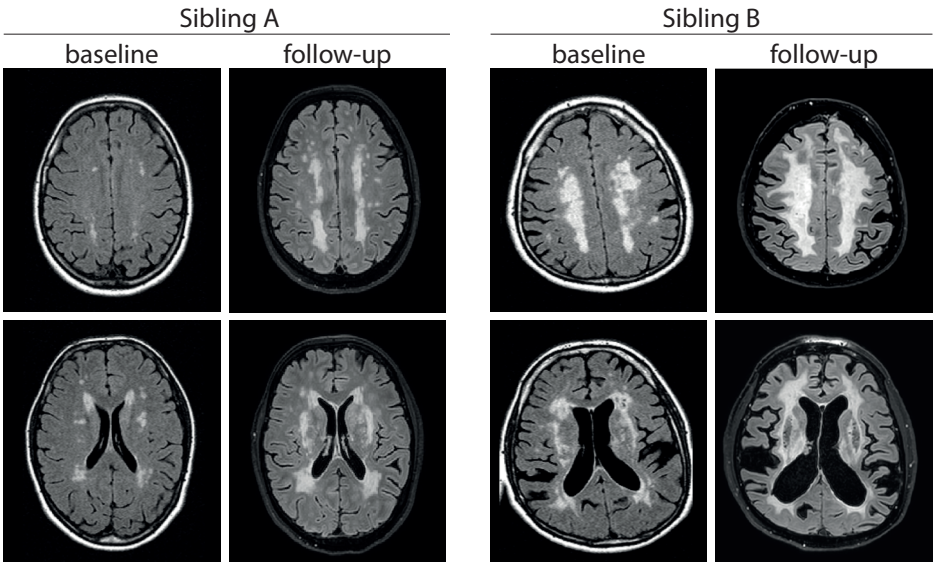

Figure 4.3: Differences in disease severity and disease progression between two siblings
(A) FLAIR MRI images of sibling A (aged between 40 and 45 years), with only white matter hyperintensities (Fazekas grade II) and no lacunes. Over 18 years, WMH increased to Fazekas grade III, but there were still no lacunes or microbleeds. Clinically, there was no significant decline in cognitive function (CAMCOG). (B) Sibling B, differing 2 years in age from sibling A, was already much more severely affected at baseline with Fazekas grade III, brain atrophy, and 4 lacunes. There was significant progression at follow-up, both clinically and on MRI. Neither sibling had a history of hypertension, hyperlipidemia or diabetes. Sibling A was an ex-smoker (6 pack years).

Cerebral hemodynamics

Next, we assessed functional neuroimaging measures. Age-corrected resting CBF and CVR did not differ between patients and controls participating at follow-up (Figure 4.4a,d). Longitudinal analysis of CBF and CVR did also not show a steeper age-dependent decrease in patients than in controls (Figure 4.4b,e). CBF at baseline was associated with survival, but not after correction for age (Figure 4.4c), while CVR at baseline was not associated with survival (Figure 4.4f).

In order to increase sample size, seven additional patients (mean age 56 years (range 50 – 66 years), 4/7 had a history of stroke, 5/7 had lacunes, and 4/7 had mRS≥2) and ten additional controls (mean age 56 years (range 46 – 64 years), 0/10 had history of stroke, 1/10 had lacunes, and 0/10 had mRS≥2) were enrolled for the CBF and CVR analyses, as well as for BOLD analysis of the vascular reactivity analysis of the visual cortex. The level of disability was included in the analyses (mRS≤1 versus mRS≥2). Only patients with mRS≥2 had a significant lower resting cerebral blood flow (CBF) than controls (Figure 4.4g). No differences were found in CVR, BOLD amplitude, BOLD time-to-peak and BOLD time-to-baseline (Figure 4.4h-j).

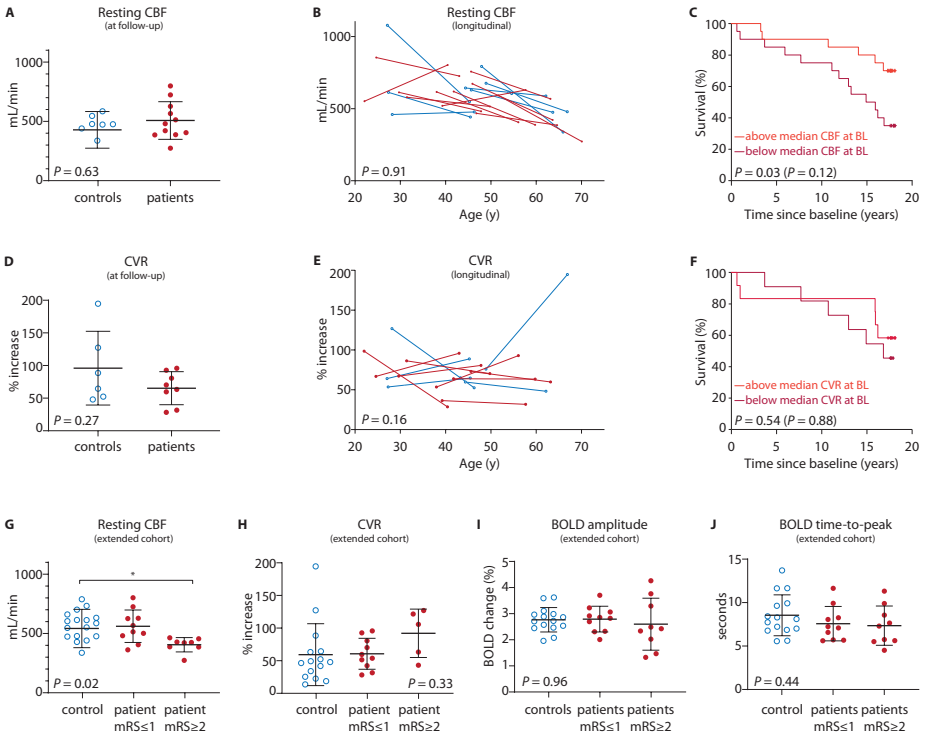

Figure 4.4: Cerebral hemodynamics

(A) At follow-up, resting CBF was similar in patients and controls. (B) Longitudinal analysis of resting CBF in the whole brain did also not show statistical differences between patients and controls. Lines connect measurements of individuals at baseline and follow-up. (C) Above median CBF at baseline was associated with higher survival (P=0.03, age-corrected P=0.12). (D) Whole brain CVR upon acetazolamide challenge was similar in patients and controls at follow-up. (E) Longitudinal CVR analysis showing no statistical differences between patients and controls. (F) CVR at baseline was not associated with long-term survival. (G-J) In order to increase sample size at follow-up, seven additional CADASIL patients and ten additional controls were recruited. Together with the participants of the follow-up study, they are called the 'extended cohort'. (G) Resting CBF was lower in the patients with disability compared to controls, but not in patients without disability. (H) CVR showed no difference between patients with and without disability in the extended follow-up cohort. (I,J) BOLD response of the occipital cortex was measured during a visual stimulus, showing no difference between patients and controls in BOLD amplitude, time-to-peak and time-to-baseline (latter not shown). All analyses are corrected for age unless otherwise stated.

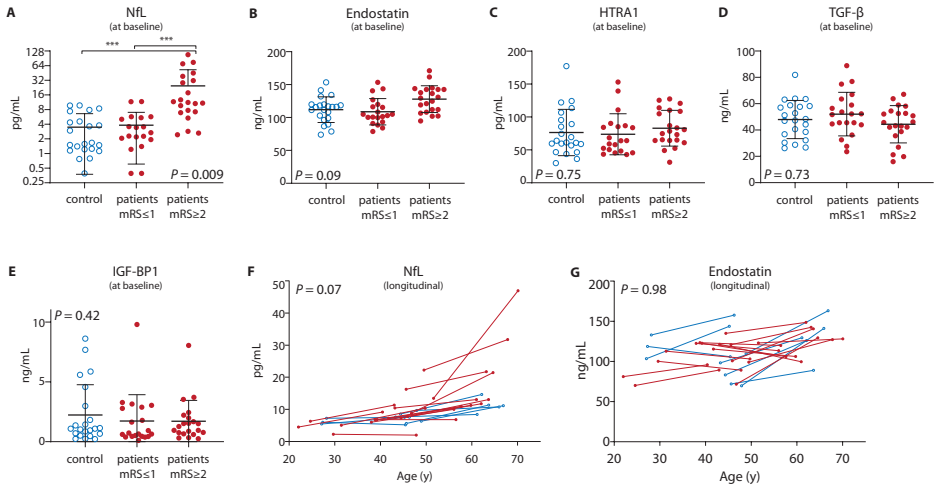

Figure 4.5: Blood biomarkers

(A,F) At baseline, blood levels of NfL were significantly elevated in patients with mRS≥2 (median 11.22 pg/mL, range 2.43 – 107.7 pg/mL) compared to patients with mRS≤1 (2.68 pg/mL, 0.39 – 11.67 pg/mL) and controls (1.62 pg/mL, range 0.39 – 9.73 pg/mL, *P*=0.009). Serum NfL levels show a steeper age-dependent increase in patients than in controls, although this effect did not reach significance (*P*=0.07). The six highest values all belong to patients with lacunes. (B-E) Five potential blood biomarkers were tested in blood withdrawn at baseline, showing no differences between patients and controls, nor in patients with and without disability. (B,G) As there seemed to be slightly higher Endostatin levels in patients with mRS≥2 (mean 110.5 ng/mL ± SD 15.4 ng/mL) than in patients with mRS≤1 (91.6 ng/mL ± 20.5 ng/mL) and controls (98.1 ng/mL ± 20.4 ng/mL, *P*=0.09), we assessed Endostatin levels longitudinally, showing no differences between patients and controls (*P*=0.98). All analyses are corrected for age.

Blood biomarkers

Of the potential serum biomarkers that were tested in the baseline samples (HTRA1, IGF-BP1, TGF-ß, Endostatin and NfL), only serum NfL levels were elevated in patients with mRS≥2 (Figure 4.5a-e). Serum NfL was associated with lacune count at baseline and at 18-year follow- up. The 18-year changes in serum NfL levels also correlated with the increase in lacune count before correction for age (St.ß 0.63, *P*=0.02). NfL showed a tendency to increase in patients compared to age-matched to controls (*P*=0.07, Figure 4.5f); there were four patients who had a strong increase in serum NfL. Three of these four patients had a high increase in lacune count (range 4 – 12). However, there were also four patients with an increased lacune count (range 4 - 10) who had NfL levels similar to controls. We were not able to optimize the homebrew NOTCH3^{ECD} ELISA in order to get consistent data. Endostatin levels were not significantly higher in patients with mRS≥2 after correction for age (*P*=0.09, Figure 4.5b). Longitudinal profiles of Endostatin at baseline and follow-up did not differ between patients and controls (*P*=0.98, Figure 4.5g).

Discussion

Here, we describe an 18-year follow-up study in a cohort of CADASIL patients, thereby providing insight into long-term disease progression and survival. In addition, we assessed cerebral hemodynamics longitudinally and we tested candidate blood biomarkers.

Such a prospective long follow-up study has never been performed in CADASIL patients and gives new insights into the differences in long-term disease progression. Most remarkably, we found that a subset of patients remained clinically relatively stable and were still only mildly affected after 18 years, even those who were already in their forties at baseline. In line with this, patients without lacunes or disability (mRS 0) at baseline had high long-term survival rates, namely more than 80% over 18 years. On the other hand, patients with moderate-severe disability (mRS 3-5) had estimated median survival rates of 7.7 years. Secondary complications to CADASIL are the major cause of death in CADASIL patients, such as pneumonia and generalized weakness.[32]

In line with previous studies, cognitive impairment and disability at 18-year follow-up was associated with lacune count at baseline.[8,10,11,33] Although age is strongly associated with disease progression, there is still a notable variation even between patients of the same age, with some patients remaining stroke-free until their sixties, while others were already deceased in their fifties. These findings underline that disease modifiers play an important role in CADASIL.[7,8,34–36] In this study we found no association between disease progression and classical vascular risk factors, which may be attributable to the relatively small sample size. This study was also too small to take the effect of the *NOTCH3* mutation position into account.[6] Larger cohort studies are needed to further determine the association between long-term disease progression and both known and novel genetic and environmental modifiers.[6–8,34–36] Development of CADASIL disease prediction models, incorporating these modifiers, is imperative for tailored prognosis for CADASIL patients.

In the subset of patients who were able to participate in the 18-year follow-up study, there was a significant increase in lacune count, WMH_V, and a decrease in global cognition (CAMCOG). Several cross-sectional studies have reported that executive dysfunction is one the earliest domains of cognitive deterioration in CADASIL patients, even preceding first stroke.[37–39] In this long-term follow-up, we found no significant difference in deterioration on executive function profiles in patients compared to controls.

Cerebral hemodynamics were studied at baseline and 18-year follow-up, as cerebral blood flow and cerebrovascular reactivity have been shown to be reduced in most studies.[4,5,15,40–47] CBF at baseline[15] and follow-up was reduced in patients with more advanced disease

compared to controls, but remarkably, there was no difference in CBF changes over time between patients and controls. This may be partially due to the fact that patients able to participate in the 18-year follow-up study were inherently less severely affected and therefore may have had a better preservation of cerebral hemodynamics. We could not determine changes in CBF specifically in the subcortex or in WMH,[5,15,40,41] due to technical limitations inherent to the study set-up using 18-year old data. Cerebrovascular reactivity (CVR) changes over time also did not significantly differ between patients and controls and, moreover, we also found no association between CVR and disease severity at baseline[15] or follow-up. Previous studies on CVR in CADASIL show contradictory findings, with reduction of CVR in some studies[4,41,47] and no reduction in others.[15,42,44,45] In line with previous studies, we also did not find a reduction in BOLD signal between patients and controls.[42,45] Studies in which CVR reduction was not found, including the current study, may be due to small sample sizes and techniques used, as CVR and CBF changes have been detected using region-specific analyses or by assessing temporal hemodynamics.[40,46,47]

Of the potential blood biomarkers which were previously described in a pre-clinical study[17,18], none showed differences in blood levels between patients and controls, including levels of Endostatin, which was found to be enriched in CADASIL vessels.[48] However, ELISA might not be sensitive enough to detect small differences between patients and controls with our relatively small sample size. Using a more sensitive single molecule analysis approach, we and others previously showed that NfL blood levels correlate with disease severity in CADASIL.[16,49] The fact that we could longitudinally study blood biomarker levels only in the milder subset of 15 patients who participated at follow-up might explain why we found no statistically significant differences in serum NfL level changes over 18 years between patients and controls. Nevertheless, in patients, 18-year increase in NfL levels was correlated with increase in lacune count. However, not all patients with an increase in lacune count had high NfL levels, likely explained by NfL levels rising upon axonal damage after a recent stroke and returning to normal in approximately a year.[16,49,50]

The strength of our study is the very long follow-up time of patients and controls, but the long follow-up time also has inherent limitations e.g. new consensus guidelines and technical equipment. Differences between baseline and follow-up in measuring WMH_V were overcome by using a statistical correction approach, and lacunes were re-counted using STRIVE criteria. Differences in BPF measurements could not be overcome, so this could only be assessed cross-sectionally at both time points.

In conclusion, although survival rate of our cohort of CADASIL patients was 54% over 18 years, here we show that a subset of CADASIL patients remain remarkably stable over the course of 18 years.

Appendix

Acknowledgments

The authors thank all the CADASIL patients and their family members who participated in the study. We acknowledge Merve Atli, Lesse Tieman, Hella Janssen, Annick den Hartog and Yvette Knaap for conducting the neuropsychological assessments.

Funding

This work was funded by the Netherlands Brain Foundation (HA2016-02-03; BG2015-2).

Contribution

Study design and conceptualization by BJvE, JvdG, JWR and SAJLO. Data acquisition by GG, RJH, AMvO, BJvE, MO, JFAV, HAMM, CET, JvdG, JWR and SAJLO. Data analysis by GG, AMvO, MDMRG, JWR and SAJLO. Data interpretation by GG, AMvO, MDMRG, AAR, JRW and SAJLO. Manuscript drafted by GG and SAJLO. Manuscript edited and/or reviewed by all authors.

Disclosures

GG was funded by the Netherlands Brain Foundation (HA2016-02-03; BG2015-2). Part of the experiments were made possible by the Dutch Alzheimer Society and the Leiden University Fund. NOTCH3 antisense therapies have been patented by the Leiden University Medical Center. As co-inventors on this patent AAR and SAJLO are entitled to a share of potential royalties. RJH was funded by Dutch National Institutes of Health (ZonMW, reference 91717325). JFAV is funded by NS110048 grant from the United States National Institute on Aging and the National Institutes of Neurological Disorders and Stroke. CET is supported by the European Commission (Marie Curie International Training Network, JPND), Health Holland, the Dutch Research Council (ZonMW), The Weston Brain Institute, Alzheimer Netherlands. CET has a collaboration contract with ADx Neurosciences, performed contract research or received grants from Probiodrug, AC Immune, Biogen-Esai, CogRx, Toyama, Janssen prevention center, Boehringer, AxonNeurosciences, Fujirebio, EIP farma, PeopleBio, Roche. AAR reports being employed by LUMC which has patents on exon skipping technology, some of which has been licensed to BioMarin and subsequently sublicensed to Sarepta. As co-inventor of some of these patents AAR is entitled to a share of royalties. AAR further discloses being ad hoc consultant for PTC Therapeutics, Sarepta Therapeutics, CRISPR Therapeutics, Summit PLC, Alpha Anomeric, BioMarin Pharmaceuticals Inc., Eisai, Astra Zeneca, Santhera, Audentes, Global Guidepoint and GLG consultancy, Grunenthal, Wave and BioClinica, having been a member of the Duchenne Network Steering Committee (BioMarin) and being a member of the scientific advisory boards of ProQR, hybridize therapeutics, silence therapeutics, Sarepta therapeutics and Philae Pharmaceuticals. Remuneration for these activities is paid to LUMC. LUMC also received speaker honoraria from PTC Therapeutics and BioMarin Pharmaceuticals and funding for contract research from Italpharmaco and Alpha Anomeric. AMvO, BJvE, MO, HAM, MDMRG and JvdG report no disclosures.

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