ONLINE SUPPLEMENT

Early MRI and cognitive markers of hereditary cerebral amyloid angiopathy

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Supplemental Methods

Participants

Subjects were selected via the HCHWA-D patient association in Katwijk (the Netherlands) and the outpatient clinic of the Department of Neurology of the Leiden University Medical Center based on DNA analysis for confirmation of codon 693 mutation in the amyloid-β precursor protein (AβPP) gene. Twenty-seven DNA-proven HCHWA-D mutation carriers were included in the present study. These subjects had a mean age of 45.9 years (25^{th} -75^{th} percentile 35 to 55 years). Seventeen of them were female (mean age 44.4 years, 25^{th} -75^{th} percentile 33 to 53 years) and ten male (mean age of 48.4 years, 25^{th} -75^{th} percentile 34 to 58 years). Both symptomatic (n=15) and pre-symptomatic (n=12) mutation carriers were included. Subjects were considered symptomatic when they had experienced signs of the disease reported to a general practitioner. Control subjects were recruited from individuals at risk for HCHWA-D (one of the parents has HCHWA-D) but who tested genetically negative and from subject spouses, family or friends, who also underwent genetic testing for inclusion. All controls were ascertained to be both stroke-free as well as negative genetic tested. Thirty-three controls were included in the present study. These subjects had a mean age of 45.6 years (25^{th} -75^{th} percentile 34 to 57 years). Twenty of them were female (mean age 41.9 years, 25^{th} -75^{th} percentile 32 to 52 years) and thirteen male (mean age of 51.3 years, 25^{th} - 75^{th} percentile 41 to 61 years). The ethics committee of our institution approved the study, and written informed consent was obtained from all subjects.

MRI

Image acquisition

All participants underwent a 7T and 3T MRI scan. 7T MRI was performed on a whole body human MRI system (Philips Healthcare, Best, the Netherlands) using a quadrature transmit and 32-channel receive head coil. 2D flow-compensated transverse T_2^*-weighted gradient-echo scan was performed with repetition time (TR)/echo time (TE) 794/25 ms, flip angle 45°, slice thickness 1.0 mm with a 0.1 mm interslice gap, 50 slices and coverage of 10 cm, 240 x 180 x 22 mm field of view (FOV), 1000 x 1024 matrix size – spatial resolution of 0.24 x 0.24 mm^2 ~ scan duration 20 minutes.[1] 3D magnetization prepared sagittal fluid attenuated inversion recovery (FLAIR) sequence was performed with TR/TE/inversion time (TI) 8000/300/2200 ms, flip angle 100°, slice thickness 1 mm, with 1 mm^3 isotropic voxels, scan duration ~ 8 minutes.[2] At 3T FLAIR scans were performed with: TR/TE: 11.0s/125ms, flip angle 90°, slices 25, FOV 252x179.76x250mm, matrix size 224x224, scan duration ~ 5 minutes. 3D T1-weighted images were acquired with: TR/TE 9/4.6ms, flip angle 8°, FOV = 224×177×168mm, scan duration ~5 minutes. T2-weighted images were acquired with: TR/TE: 4.2s/ 80ms, flip angle 90°, 40 slices, FOV 224x180x144 mm, slice thickness 3.6 mm, matrix size 448x320 and scan duration ~3 minutes.

Image analysis

To be as sensitive and precise as possible for all lesions scored in this study, two different field strengths (7T and 3T) and the best validated methods were used per lesion. The detection of hemorrhagic lesions (microbleeds, ICHs, convexity SAHs and superficial siderosis) was evaluated at 7T T_2^*-weighted sequences as this field strength and sequence is

most sensitive for these lesions. Lobar microbleeds (location as described by the Boston criteria[3]) were defined and scored as previously described.[4] Only remote, resorbed ICHs were observed and they were defined as parenchymal defects with evidence of hemosiderin in their wall. The number and presence of convexity SAHs was assessed described as a subarachnoid bleeding localized to the convexities of the brain.[5] The presence of superficial siderosis was assessed described as linear residues of blood in the superficial layers of the cortex.[6] The number and presence of cortical microinfarcts were scored on 7T FLAIR images as previously described, as these lesions are visualized best using this field strength and sequence.[7] Since counting microinfarcts is a relatively new technique, these lesions were scored by two independent experienced raters and interrater reliability was calculated. WMHs are defined and analysed using 3T FLAIR images as described earlier using a semi-automated and validated method.[8] DPVS were evaluated and assessed in the basal ganglia (BG) and centrum semiovale (CSO) on 3T T2-weighted and T1-weighted images. DPVS are defined and rated according to a 4-point semi-quantitative score.[9] Presence of lacunar infarcts was assessed using 3T T2-weighted and FLAIR images as described earlier.[10]

Cognitive and neuropsychiatric function

A battery of neuropsychological tests measuring global cognitive functioning (Mini mental state examination (MMSE)[11]), memory (Wechsler Memory Scale (WMS)[12] and Hopkins Verbal Learning Test (HVLT)[13]), psychomotor speed (Trailmaking test (TMT) part A), executive function (TMT part B (cognitive flexibility)[14], Digit symbol substitution test (DSST) of the WAIS III[15] and Clock drawing), and language (letter and animal naming (letter and category fluency)[16] and Boston naming test (BNT)[17]) were performed. Also neuropsychiatric tests were performed measuring apathy (Apathy scale of Starkstein)[18], anxiety (hospital anxiety and depression scale (HADS)[19]) and depression (HADS).

Statistics

Mann-Whitney U-testing was used to assess differences in age between groups, univariate general linear modeling analysis was used to assess differences in blood pressure measurements between groups, adjusted for age and sex, and chi-square tests were used to assess differences in sex, educational level and percentage cardiovascular risk factors between groups. For counting microinfarcts, the interobserver variability (kappa value) was calculated and the grading of interobserver agreement was performed according to the recommendations of Landis and Koch.[20] Univariate general linear modeling analysis was used to assess the association between the prevalence and median of MRI markers (for MRI markers (except cortical microinfarcts) natural log transformation was used because of a non-normal distribution) and mutation status, adjusted for age and sex. Univariate general linear modeling analysis was also used to assess the association between cognitive and neuropsychiatric tests and mutation status, adjusted for age, sex and. All statistical analyses were performed with the Statistical Package of Social Sciences (SPSS, version 20.0; SPSS, Chicago, Ill).

Supplemental Tables

Table I: Baseline characteristics of pre-symptomatic and symptomatic mutation carriers versus controls

	Controls (SD; 25th-75th percentile) (N=33)	Pre-symptomatic carriers (SD; 25th-75th percentile) (N=12))	Symptomatic carriers (SD; 25th-75th percentile) (N=15)
Age (years)	45.6 (14.1; 34–57)	34.3 (12.1; 23–47)*	55.1 (5.2; 51–60)*
Sex (male/female)	13/20	3/9	7/8
Education (median)	Associate's degree	Bachelor's degree	Associate's degree
Systolic blood pressure	130.6 (26.1; 109 – 142)	125.2 (14.4; 112 – 133)	144.2 (19.8; 129 – 168)
Diastolic blood pressure	81.5 (12.2;72 – 87)	80.4 (9.4; 73 – 87)	89.1 (10.4; 78 – 96)
Mean arterial pressure	97.6 (15.9; 86 – 107)	95.3 (9.4; 88 – 102)	107.5 (12.4; 95 – 117)
Pulse pressure	49.1 (18.4; 36 – 60)	44.8 (13.2; 33 – 50)	55.1 (14.7; 43 – 69)
Hypertension (%)	18.2	0	40
Hyperlipidemia (%)	6.1	0	33.3*
Diabetes Mellitus (%)	0	8.3	6.7
Cardiovascular disease (%)	0	0	6.7

Supplemental References

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