SUPPLEMENTAL MATERIAL

Innovative MRI markers of hereditary Cerebral Amyloid Angiopathy at 7 Tesla

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Supplemental Methods

Participants

This prospective cohort study was conducted between 2012 and 2014. Subjects were selected via the HCHWA-D patient association in Katwijk, the Netherlands, (http://www.hchwa-d.nl) 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. Both symptomatic and pre-symptomatic 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 (i.e. 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 receiving a negative genetic test. In this study only subjects who underwent 7T MRI were included. In total 55 participants were scanned: 26 were DNA-proven HCHWA-D mutation carriers of whom 15 were symptomatic, and 11 were pre-symptomatic. The mean age of the symptomatic subjects was 55 years, and 53% of them were women. The mean age of the pre-symptomatic subjects was 35 years, and 82% of them were women. In total 29 controls participated with a mean age of 45 years, of whom 59% were women. Demographics, vascular risk-factors, blood pressure and MMSE score were obtained at enrollment. The ethics committee of our institution approved the study, and written informed consent was obtained from all subjects.

MRI

Image acquisition

MRI was performed on a whole body human 7T MR-system (Philips Healthcare, Best, The Netherlands) using a quadrature transmit and 32-channel receive head coil (Nova Medical, Wilmington, MA, USA). Participants were scanned using a 2D flow-compensated transverse T_2^*-weighed gradient echo scan, with a total imaging duration of 20 minutes. Imaging parameters were: 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 – resulting in an in-plane spatial resolution of 0.24 x 0.24 mm^2. The bandwidth per pixel was 46 Hz, corresponding to a readout length of approximately 22 ms. The frequency and phase encoding directions were along the anterior-posterior and right-left axes, respectively. Shimming up to third order was performed using an image based shimming approach.[1]

Image analysis

Image analysis was performed on the T_2^*-weighed gradient echo scan. The following conventional markers were scored: ICH, MBs, SAH and superficial siderosis as previously described.[2] Based on prior visual inspection of the images by an experienced neuroradiologist (MvB (25 years of experience in neuroradiology)), the following changes were scored: intragyral hemorrhaging and specific cortical changes. For the detection of these potential markers the T_2^*-weighed gradient echo images were scored independently by two readers (EK (1 year of experience in neuroradiology)) and EvE (4 years of experience in neuroradiology)) blinded to clinical diagnosis. In the case of non-concordant scores a

consensus reading was performed with a third experienced reader (SvR (10 years of experience in neuroradiology)).

Intragyral hemorrhaging is a subcategory of intracerebral hemorrhaging and is defined based on its location within the brain, namely as a parenchymal hemorrhage restricted to the subcortical white matter of an individual gyrus. By carefully investigating the surrounding MRI slices around the intragyral hemorrhage, it was checked if the hemorrhage was restricted to the subcortical white matter to avoid confusion with blooming superficial siderosis which would be presented as linear residues of blood in the superficial layers of the cortex. Examples are shown in figure 1. A 'striped' cortical pattern was found and is defined as linear hypointense stripes perpendicular to the cortex. Examples of this pattern are shown in figure 2.

Statistics

Where appropriate, data are expressed as mean and standard deviation. Significance level was set at 0.05 and all tests of significance were two-tailed. Demographic characteristics were analyzed using a Kruskal-Wallis test for the 3-group comparisons followed by a Mann-Whitney U-test for MMSE score and age; to be able to adjust for age and sex for comparison of blood-pressure measurements a general linear model, univariate analysis was performed; and for prevalence of cardiovascular risk factors and the differences in sex a chi-square test was used.

For each marker prevalence, univariate general linear modelling was used, adjusted for age and sex for the 3-group comparisons followed by an univariate general linear model adjusted for age and sex for post-hoc 2-group comparisons. R2 (% of variance explained) was reported so that the reader can interpret how well the model fits. P-values in the tables were FDR corrected. There is incomplete data for intragyral hemorrhages and a striped cortex sign for the comparison of the pre-symptomatic group versus controls because these features do not occur in both groups, so the model cannot be fit (also no significant results) (supplementary table II). For all dichotome features the interobserver variability (kappa value) was calculated and the grading of interobserver agreement was performed according to the recommendations of Landis and Koch.[3] All statistical analyses were performed with the Statistical Package of Social Sciences (SPSS, version 20.0; SPSS, Chicago, Ill).

Supplemental Tables

Table I; Demographics and characteristics

	Symptomatic carriers (n=15)	Pre-symptomatic carriers (n=11)	Controls (n=29)	p-value
Mean Age (years) (SD)	55.1 (5.2)	34.6 (12.6)	44.7 (14.0)	0.005[§]
Sex (male/female)	7/8	2/9	12/17	0.398
Mean systolic blood Pressure (SD)	144.2 (19.8)	122.5 (11.4)	130.0 (26.8)	0.645
Mean diastolic blood Pressure(SD)	89.1 (10.4)	79.8 (9.6)	80.6 (10.4)	0.398
Mean arterial pressure (SD)	107.5 (12.4)	94.0 (8.6)	97.1 (15.1)	0.448
Hypertension (%)	40 (6/15)	0 (0/11)	17 (5/29)	0.090
Hyperlipidemia (%)	33 (5/15)	0 (0/11)	7 (2/29)	0.053
Diabetes mellitus (%)	7 (1/15)	9 (1/11)	0 (0/29)	0.398
Cardiovascular disease (%)	7 (1/15)	0 (0/11)	0 (0/29)	0.398
Mean MMSE* score (SD)	26.7 (3.8)	29.7 (0.6)	29.4 (0.7)	$p < 0.0001^{‡§}$

* MMSE= mini mental state examination
† p-values were FDR corrected
Significant differences indicated by: ‡, symptomatic vs. controls, P<0.05; §, symptomatic vs. pre-symptomatic, P<0.05

Table II; Results of univariate general linear modelling for each MRI marker for detection of hereditary CAA on T_2^*-w 7T MRI.

	R squared 3 groups comparison	p-value *	R squared sympt vs. controls	p-value *	R squared pre-sympt vs. controls	p-value *	R squared sympt vs. pre-sympt	p-value *
Innovative Features								
Intragyral hemorrhage %	0.718	p < 0.0001	0.710	p < 0.0001	-	-	0.665	0.162
Striped cortex sign %	0.396	p < 0.0001	0.377	p < 0.0001	-	-	0.286	0.176
Classic Markers								
Lobar microbleeds %	0.772	p < 0.0001	0.849	p < 0.0001	0.214	0.275	0.794	0.008
Superficial siderosis %	0.844	p < 0.0001	0.911	p < 0.0001	0.106	0.291	0.752	0.008
ICH %	0.923	p < 0.0001	1.000	p < 0.0001	0.106	0.291	0.864	p < 0.0001
SAH %	0.313	0.001	0.406	p < 0.0001	0.237	0.249	0.160	0.900

* p-values were FDR corrected

Supplemental References

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