SUPPLEMENTAL MATERIAL

WHITE MATTER HYPERINTENSITIES ARE UNDER STRONG GENETIC INFLUENCE

Cover title: Heritability of white matter hyperintensities

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MRI parameters and quantitation of WMH

Both 3D T1-weighted scans and T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence scans were used for data analysis. The following protocol was used for T1-weighted MRI scans on the 1.5T scanners in all three centres: in-plane resolution 1×1 mm with slice thickness of 1.5 mm, contiguous slices, TR/TE/TI = 1530/3.24/780 ms, and flip angle = 8. FLAIR scans were acquired axially with the same acquisition parameters on the 1.5T scanners in all three centres, i.e. TR/TE/TI = 10000/120/2800 ms, with slice thickness 3.5 mm and in-plane resolution 0.898×0.898 mm^2. On the 3T scanner in centre 1, we had spatial resolution of 1×1×1 mm^3, TR/TE = 6.39/2.9 ms for T1-weighted scans, and TR/TE/TI = 10000/110/2800 ms, with slice thickness 3.5 mm and in-plane resolution 0.898×0.898 mm^2 for FLAIR scans. Intracranial volume (ICV), the sum of grey matter, white matter and cerebrospinal fluid was calculated using SPM8 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK. http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).

The contrast properties of FLAIR facilitate the possibility of automated segmentation and classification of WMH. The method has been previously described[1]. A parametric method[1] was adapted and applied to the initial WMH detection. The extracted candidate WMH clusters were further investigated using a non-parametric kNN rule and then classified into different brain regions and deep (DWMH), periventricular (PWMH), and false WMH clusters.

The automated classification of WMHs employed in this study was carried out in the native space of the T1-weighted images. Five pre-processing steps were taken to prepare the images for the analysis, as described previously [1]: (i) the FLAIR and T1 images of the same subject were co-registered using mutual information method [2]; (ii) segmentation [3] of T1-weighted images into three separate tissue components; (iii) removal of non-brain tissue from both T1-weighted and co-registered FLAIR images using the brain mask transformed from the average mask originally defined in the standard space by inverting the deformation matrix [4]; (iv) inverting the spatial normalization transformation to produce the brain masks and white matter probability maps in the native space for the WMH detection and non-brain tissue removal; (v) intensity correction [5] of both FLAIR and T1-weighted images after the removal of non-brain tissues. Some other smaller steps such as removal of the bright areas observed in the FLAIR sequence ventricles caused by choroid plexus and partial voluming were also carried out. SPM8 was used with Matlab R2013b (MathWorks, Natick, MA, U.S.A.) for these pre-processing steps.

References

1. Wen W, Sachdev P. The topography of white matter hyperintensities on brain MRI in healthy 60- to 64-year-old individuals. *NeuroImage*. 2004;22:144-154.
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5. Ashburner J, Friston KJ. Nonlinear spatial normalization using basis functions. *Hum Brain Mapp*. 1999;7:254-266.

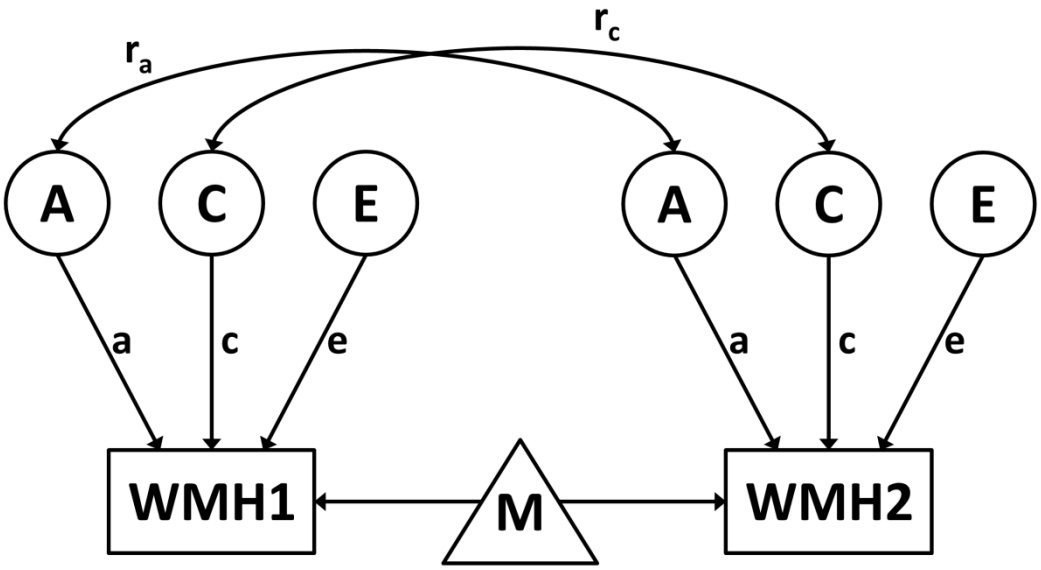

Figure I. Path diagram for the age and sex moderated ACE twin model. WMH of twin 1 (WMH1) and 2 (WMH2) are modelled as the function of the mean parameter (M) and the latent additive (A), shared environment (C) and environment (E) factors. The mean is further modelled as a function of the *k* covariates $M = \mu + \beta_1 X_1 + \cdots + \beta_k X_k$, where μ is the overall mean of the phenotypes and X_1, X_2,…,X_k are the k covariates (such as age, sex, scanners and ICV) and $\beta_1, \beta_2, \ldots, \beta_k$ are the regression parameters of the model. The path coefficients a, c and e are the estimated loadings of the latent factors, which are further decomposed as $a=a_0+a_1age+a_2sex$; $c=c_0+c_1age+c_2sex$; $e=e_0+e_1age+e_2sex$ to accommodate the moderating effects of age and sex. The parameter r_a (r_a=1 for MZ twin pairs and r_a=0.5 for DZ twin pairs) and r_c ($r_{c=1}$ for both MZ and DZ twin pairs) respectively denote the additive genetic and shared environmental correlations between the twin pairs.

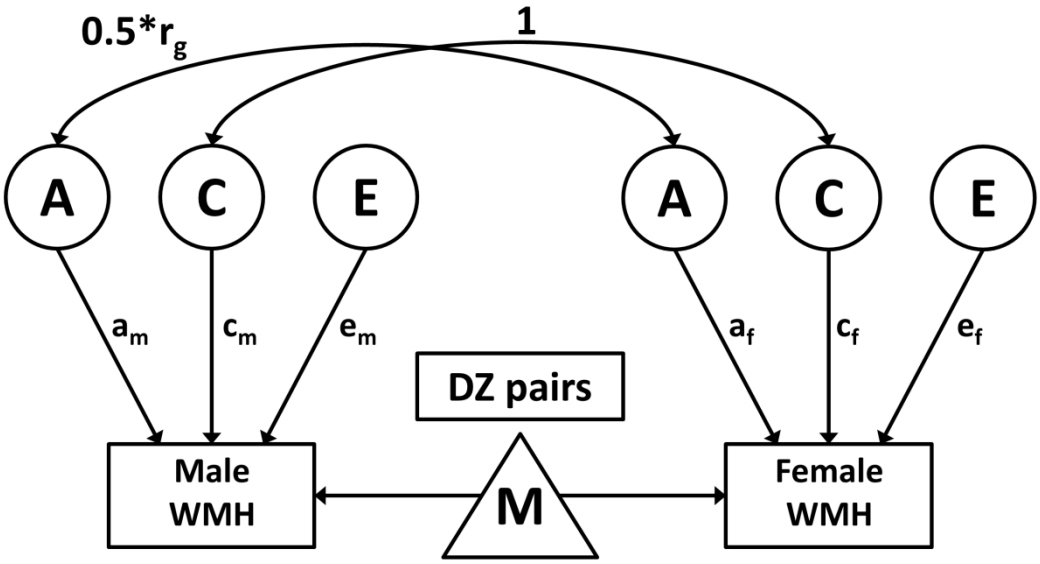

Figure II. The path diagram for the opposite sex DZ twin pairs in the general sex heterogeneity model. For opposite DZ twin pairs, the path coefficients for the male samples a_m, c_m, e_m are same as the path coefficients for the male MZ and DZ pairs. Similarly the path coefficients for the female samples a_f, c_f and e_f are also the same as the path coefficients for female MZ and DZ pairs. The correlation between the additive genetic components in opposite sex pairs is half of the genetic correlation between male-female genetic correlation r_g.

TABLE I. Sample characteristics by sex

Covariate	Female (N=213)	Male (N=107)	Stat	p
Age (years)	69.54 (4.75)	71.14 (5.08)	-2.72	0.005
Hypertension	136 (63.84%)	79 (73.83%)	2.78	0.017
BP systolic (mmHg)	135.66 (17.11)	142.40 (17.55)	-3.27	0.001
BP diastolic (mm Hg)	79.97 (9.77)	81.61 (10.26)	-1.37	0.174
BMI	27.33 (4.5)	27.69 (3.6)	-0.79	0.436
Homocysteine (µmol/L)	12.84 (3.52)	13.41 (3.16)	-1.48	0.141
Heart attack	8 (3.76%)	7 (6.54%)	0.69	0.131
Artrial fibrillation	7 (3.29%)	5 (4.67%)	0.09	0.416
Stroke	6 (2.81%)	5 (4.67%)	0.29	0.247

For continuous measures, means (SD) are presented. For categorical measures, N (%) is presented. T-tests for continuous variables and chi-square tests for all other measures were used to compare between the two sexes. All the p-values were obtained using 10000 permutations.

TABLE II. Phenotypic correlations across white matter hyperintensity (WMH) ROIs

	Total	Periventricular	Deep	Frontal	Temporal	Parietal	Occipital	Cerebellum	Brainstem
Total	1.00	0.96	0.94	0.83	0.72	0.92	0.69	0.40	0.39
Periventricular	0.96	1.00	0.85	0.76	0.64	0.85	0.58	0.30	0.25
Deep	0.94	0.85	1.00	0.85	0.76	0.95	0.76	0.43	0.42
Frontal	0.83	0.76	0.85	1.00	0.65	0.77	0.51	0.34	0.33
Temporal	0.72	0.64	0.76	0.65	1.00	0.68	0.57	0.41	0.43
Parietal	0.92	0.85	0.95	0.77	0.68	1.00	0.67	0.39	0.32
Occipital	0.69	0.58	0.76	0.51	0.57	0.67	1.00	0.48	0.53
Cerebellum	0.40	0.30	0.43	0.34	0.41	0.39	0.48	1.00	0.58
Brainstem	0.39	0.25	0.42	0.33	0.43	0.32	0.53	0.58	1.00

Pearson correlation coefficients are presented ignoring the relationship between the zygotic twin pairs.

TABLE III. Heritability of white matter hyperintensities (WMH) volumes in whole brain (total) and different brain regions: estimates and model summary

WMH ROI	ICC MZ (95 % CI)	ICC DZ (95 % CI)	A (95% CI)	C (95% CI)	E (95% CI)	P-AE	P-CE	P-E	Covariates
Total	0.78 (0.68,0.84)	0.39 (0.34,0.56)	0.77 (0.42,0.84)	0.01 (0.00,0.33)	0.22 (0.16,0.32)	0.97	<1E-04	<1E-16	111000000000
Periventricular	0.76 (0.66,0.83)	0.44 (0.34,0.60)	0.63 (0.29,0.83)	0.13 (0.00,0.43)	0.24 (0.17,0.34)	0.49	<1E-06	<1E-16	111000001100
Deep	0.78 (0.69,0.85)	0.39 (0.34,0.49)	0.78 (0.57,0.85)	0.00 (0.00,0.2)	0.22 (0.15,0.31)	1	0.014	<1E-16	111000000000
Frontal	0.63 (0.48,0.74)	0.36 (0.25,0.53)	0.53 (0.11,0.74)	0.10 (0.00,0.45)	0.37 (0.26,0.52)	0.63	0.026	<1E-10	111101000000
Temporal	0.66 (0.52,0.75)	0.44 (0.29,0.60)	0.44 (0.05,0.75)	0.22 (0.00,0.54)	0.34 (0.25,0.48)	0.27	<1E-04	<1E-12	111000001100
Parietal	0.71 (0.59,0.80)	0.35 (0.29,0.47)	0.71 (0.43,0.80)	0.00 (0.00,0.24)	0.29 (0.20,0.41)	1	<1E-07	<1E-13	111000000000
Occipital	0.79 (0.68,0.86)	0.39 (0.34,0.47)	0.79 (0.63,0.86)	0.00 (0.00,0.13)	0.21 (0.14,0.32)	1	<1E-07	<1E-16	101000000001
Cerebellum	0.51 (0.36,0.64)	0.47 (0.27,0.60)	0.07 (0.00,0.55)	0.43 (0.01,0.60)	0.49 (0.36,0.64)	0.04	0.738	<17E-9	101010010000
Brainstem	0.71 (0.60,0.80)	0.45 (0.31,0.62)	0.53 (0.16,0.79)	0.18 (0.00,0.52)	0.29 (0.20,0.40)	0.41	0.004	<1E-16	001000000000

Standardised additive genetic (A=heritability), shared environment (C) and unique environment (E) variance components (95% confidence intervals) of WMH for different ROIs obtained using ACE model. Missing values for the covariates were imputed using the multiple imputation procedure as implemented in the R-package "mice" (van Buuren S, Groothuis-Oudshoorn K: Mice: Multivariate Imputation by Chained Equations in R. J Stat Softw 2011;45:1-67).The columns P-AE, P-CE and P-E respectively denote the p-values from the likelihood ratio test comparing ACE model vs AE, CE and E models. P-CE is also the p-value for heritability because testing the component A=0 is equivalent to testing heritability is zero. Last column indicates the significance of covariates. Significance of the p-value ($p<0.05$) for any of the covariates age, sex, scanners, ICV, hypertension, systolic BP, diastolic BP, BMI, homocysteine, heart attack, atrial fibrillation and stroke in that order is indicated as a string; 1=significant; 0=not-significant.

TABLE IV. Heritability estimates under the sex heterogeneity model

WMH region	Female ICC MZ (95 % CI)	Female ICC DZ (95 % CI)	Male ICC MZ (95 % CI)	Male ICC DZ (95 % CI)	Male-Female ICC DZ (95 % CI)	Female h^2 (95% CI)	Male h^2 (95% CI)	Test of Homogeneity
Total (whole brain)	0.78 (0.66,0.86)	0.41 (0.34,0.54)	0.72 (0.52,0.83)	0.64 (0.28,0.81)	0.22 (0.00,0.40)	0.74 (0.46,0.85)	0.15 (0.00,0.81)	0.646
Periventricular	0.78 (0.65,0.85)	0.44 (0.35,0.58)	0.67 (0.46,0.81)	0.66 (0.28,0.80)	0.23 (0.00,0.43)	0.67 (0.37,0.84)	0.03 (0.00,0.72)	0.282
Deep	0.78 (0.66,0.86)	0.39 (0.33,0.48)	0.75 (0.54,0.86)	0.40 (0.27,0.72)	0.26 (0.00,0.39)	0.78 (0.59,0.86)	0.69 (0.04,0.86)	0.985
Frontal	0.66 (0.49,0.78)	0.35 (0.25,0.51)	0.56 (0.30,0.73)	0.50 (0.16,0.72)	0.18 (0.00,0.37)	0.62 (0.22,0.77)	0.11 (0.00,0.72)	0.468
Temporal	0.74 (0.60,0.82)	0.41 (0.31,0.59)	0.41 (0.12,0.64)	0.27 (0.07,0.55)	0.22 (0.00,0.38)	0.65 (0.24,0.82)	0.28 (0.00,0.64)	0.178
Parietal	0.72 (0.56,0.82)	0.36 (0.28,0.47)	0.66 (0.41,0.81)	0.41 (0.21,0.71)	0.24 (0.00,0.40)	0.72 (0.47,0.82)	0.50 (0.00,0.81)	0.975
Occipital	0.83 (0.72,0.89)	0.41 (0.36,0.50)	0.62 (0.37,0.77)	0.31 (0.19,0.49)	0.21 (0.08,0.29)	0.83 (0.65,0.89)	0.62 (0.22,0.77)	0.243
Cerebellum	0.54 (0.37,0.68)	0.53 (0.27,0.67)	0.47 (0.17,0.68)	0.35 (0.13,0.63)	0.19 (0.00,0.35)	0.01 (0.00,0.56)	0.24 (0.00,0.64)	0.059
Brainstem	0.75 (0.63,0.83)	0.47 (0.32,0.67)	0.52 (0.23,0.72)	0.29 (0.13,0.61)	0.17 (0.01,0.30)	0.56 (0.13,0.83)	0.46 (0.00,0.71)	0.156

Intra-class correlations (ICC), heritability (H^2) and 95% confidence intervals for WMH in different ROIs obtained using heterogeneity ACE model (age, scanner and ICV adjusted). The p-value from the likelihood ratio test of homogeneity (common variances and co-variances versus separate parameters for male and female samples) is also presented.

TABLE V. Heritability of white matter hyperintensities (WMH) as a function of age and sex

ROI	Age	Parameter Label	Lower Limit	Estimate	Upper Limit
Total volume	65	Female_H2	0.46	0.76	0.88
	70	Female_H2	0.49	0.78	0.86
	73	Female_H2	0.08	0.78	0.87
	75	Female_H2	0.01	0.78	0.88
	80	Female_H2	0.00	0.76	0.91
	83	Female_H2	0.00	0.74	0.93
	85	Female_H2	0.00	0.72	0.94
	65	Male_H2	0.00	0.16	0.87
	70	Male_H2	0.00	0.16	0.81
	73	Male_H2	0.00	0.16	0.79
	75	Male_H2	0.00	0.16	0.79
	80	Male_H2	0.00	0.17	0.80
	83	Male_H2	0.00	0.16	0.82
	85	Male_H2	0.00	0.16	0.84
Peri	65	Female_H2	0.36	0.68	0.87
	70	Female_H2	0.34	0.67	0.85
	73	Female_H2	0.18	0.67	0.86
	75	Female_H2	0.07	0.67	0.87
	80	Female_H2	0.00	0.66	0.91
	83	Female_H2	0.00	0.65	0.93
	85	Female_H2	0.00	0.65	0.94
	65	Male_H2	0.00	0.03	0.76
	70	Male_H2	0.00	0.01	0.72
	73	Male_H2	0.00	0.00	0.70
	75	Male_H2	0.00	0.00	0.69
	80	Male_H2	0.00	0.01	0.47
	83	Male_H2	0.00	0.02	0.57
	85	Male_H2	0.00	0.04	0.64
Deep	65	Female_H2	0.62	0.82	0.90
	70	Female_H2	0.42	0.74	0.87
	73	Female_H2	0.24	0.66	0.86
	75	Female_H2	0.12	0.59	0.86
	80	Female_H2	0.00	0.42	0.88
	83	Female_H2	0.00	0.32	0.89
	85	Female_H2	0.00	0.26	0.90
	65	Male_H2	0.02	0.50	0.91
	70	Male_H2	0.02	0.53	0.85
	73	Male_H2	0.01	0.53	0.82

	75	Male_H2	0.01	0.53	0.81
	80	Male_H2	0.00	0.48	0.81
	83	Male_H2	0.00	0.41	0.82
	85	Male_H2	0.00	0.35	0.83
Frontal	65	Female_H2	0.00	0.58	0.81
	70	Female_H2	0.00	0.17	0.80
	73	Female_H2	0.00	0.01	0.81
	75	Female_H2	0.00	0.01	0.35
	80	Female_H2	0.00	0.23	0.74
	83	Female_H2	0.00	0.35	0.85
	85	Female_H2	0.00	0.41	0.89
	65	Male_H2	0.11	0.70	0.88
	70	Male_H2	0.00	0.38	0.75
	73	Male_H2	0.00	0.13	0.70
	75	Male_H2	0.00	0.03	0.69
	80	Male_H2	0.00	0.07	0.58
	83	Male_H2	0.00	0.19	0.70
	85	Male_H2	0.00	0.27	0.76
Temporal	65	Female_H2	0.17	0.60	0.80
	70	Female_H2	0.15	0.56	0.82
	73	Female_H2	0.08	0.52	0.86
	75	Female_H2	0.03	0.49	0.88
	80	Female_H2	0.00	0.41	0.94
	83	Female_H2	0.00	0.36	0.96
	85	Female_H2	0.00	0.33	0.97
	65	Male_H2	0.00	0.33	0.66
	70	Male_H2	0.00	0.33	0.63
	73	Male_H2	0.00	0.32	0.65
	75	Male_H2	0.00	0.31	0.68
	80	Male_H2	0.00	0.28	0.78
	83	Male_H2	0.00	0.25	0.83
	85	Male_H2	0.00	0.23	0.86
Parietal	65	Female_H2	0.48	0.71	0.83
	70	Female_H2	0.35	0.70	0.82
	73	Female_H2	0.21	0.66	0.84
	75	Female_H2	0.12	0.62	0.85
	80	Female_H2	0.00	0.50	0.90
	83	Female_H2	0.00	0.42	0.93
	85	Female_H2	0.00	0.36	0.95
	65	Male_H2	0.00	0.27	0.79
	70	Male_H2	0.00	0.29	0.76
	73	Male_H2	0.00	0.31	0.76

	75	Male_H2	0.00	0.32	0.77
	80	Male_H2	0.00	0.35	0.82
	83	Male_H2	0.00	0.36	0.86
	85	Male_H2	0.00	0.35	0.89
Occipital	65	Female_H2	0.70	0.87	0.94
	70	Female_H2	0.50	0.82	0.89
	73	Female_H2	0.29	0.79	0.88
	75	Female_H2	0.18	0.77	0.88
	80	Female_H2	0.03	0.71	0.88
	83	Female_H2	0.00	0.68	0.88
	85	Female_H2	0.00	0.66	0.89
	65	Male_H2	0.33	0.74	0.90
	70	Male_H2	0.29	0.67	0.81
	73	Male_H2	0.14	0.63	0.78
	75	Male_H2	0.06	0.61	0.77
	80	Male_H2	0.00	0.55	0.77
	83	Male_H2	0.00	0.52	0.78
	85	Male_H2	0.00	0.50	0.79
Cerebellum	65	Female_H2	0.00	0.02	0.57
	70	Female_H2	0.00	0.11	0.60
	73	Female_H2	0.00	0.17	0.66
	75	Female_H2	0.00	0.22	0.70
	80	Female_H2	0.00	0.32	0.79
	83	Female_H2	0.00	0.38	0.83
	85	Female_H2	0.00	0.41	0.86
	65	Male_H2	0.00	0.50	0.83
	70	Male_H2	0.00	0.28	0.68
	73	Male_H2	0.00	0.16	0.62
	75	Male_H2	0.00	0.09	0.59
	80	Male_H2	0.00	0.00	0.55
	83	Male_H2	0.00	0.01	0.63
	85	Male_H2	0.00	0.03	0.69
Brainstem	65	Female_H2	0.00	0.32	0.82
	70	Female_H2	0.12	0.54	0.80
	73	Female_H2	0.26	0.65	0.83
	75	Female_H2	0.22	0.71	0.86
	80	Female_H2	0.32	0.78	0.93
	83	Female_H2	0.22	0.78	0.96
	85	Female_H2	0.16	0.77	0.97
	65	Male_H2	0.00	0.02	0.69
	70	Male_H2	0.00	0.12	0.61
	73	Male_H2	0.00	0.23	0.62

75	Male_H2	0.00	0.31	0.66
80	Male_H2	0.00	0.45	0.80
83	Male_H2	0.00	0.48	0.87
85	Male_H2	0.00	0.48	0.90

Heritability estimates of WMH and their 95% confidence intervals for male (Male_H2) and female (Female_H2) at different ages under the age and sex moderated ACE model (Figure I).

Supplemental Video I. A 3D movie of voxel-wise heritability of white matter hyperintensities using the binary AE model has been provided.