Association between herpes simplex virus infection and Alzheimer's disease biomarkers: analysis within the MAPT trial

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Supplementary files

a. Supplementary file 1 – Methodological details

Anti-HSV serology

The presence of anti-HSV-1 immunoglobulin G (IgG) in the blood was assessed using the LIAISON® HSV1 Type Specific IgG kit (Diasorin – Italy – chemiluminescence immunoassay technology). The presence of anti-HSV-1/2 immunoglobulin M (IgM), reflecting either a primary infection or a recent reactivation, was assessed using the LIAISON® HSV1/2 IgM kit. As recommended by the manufacturers, i) an index value ≥1.10 was considered to indicate the presence of IgG (or IgM), ii) an index value < 0.9 was considered to indicate the absence of IgG (or IgM) and iii) no conclusion could be made for index values between 0.9 and 1.10. In the latter case, a second serology was performed to verify the result.

Of the 271 participants who underwent an amyloid PET scan in the MAPT trial, 183 participants had an available plasma sample at baseline for performing anti-HSV serologies. One subject was further excluded from the analysis because he was negative for anti-HSV-1 IgG but positive for anti-HSV-1/2 IgM (reflecting either measurement errors or a primary infection, which is relatively unlikely given his age).

Notably, the low prevalence of IgM-positive participants in the sample (1.1% - n=2) prevented the study of this marker.

Amyloid PET scans

Details regarding the inclusion criteria of the amyloid PET ancillary study and acquisition parameters were described previously [1–3]. Briefly, the ancillary study started in July 2010 (after obtaining funding) and was proposed to participants enrolled in the ten

centers close to one of the five nuclear medicine departments offering amyloid PET scans (Bordeaux, Limoges, Montpellier, Nice and Toulouse). The protocol was approved by the French Ethical Committee located in Toulouse in December 2007 and included participants who signed an additional consent form. [18F] Florbetapir (AV45) PET scans were performed on five different hybrid PET-CT scanners, including one PET CT 690 (GE Healthcare; Cleveland, OH), one Discovery RX VCT (General Electric; Fairfield, CT), two True Point HiRez (Siemens Medical Solutions; Malvern, PA), and one Biograph 4 Emission Duo LSO (Siemens Medical Solutions). All scanners were operated in 3D detection mode. PET sinograms were reconstructed with a 3D iterative algorithm, with corrections for randomness, scatter, photon attenuation and decay, which produced images with an isotropic voxel of 2 × 2 × 2 mm and a spatial resolution of approximately 5-mm full-width at a half-maximum at the center of the field of view. The acquisition data were processed using the standard package delivered with each acquisition system. Data acquisition began 50 min after injection of a mean of 4 MBq/kg weight of [18F] florbetapir. In each subject, 10-min or 15-min frames were acquired to ensure movement-free image acquisition. For the semiautomated quantitative analysis, the 18F-AV45 PET images were coregistered to the 18F-AV45 template provided by Avid Radiopharmaceuticals. A quality control based on a semiquantification process was also provided by Avid.

Plasma biomarkers

The plasma Aβ42/40 ratio and NfL concentration were measured in blood samples taken at the 12-month follow-up (on the same day as the cognitive tests) and stored in EDTA-coated tubes.

The plasma Aβ42/40 ratio was measured using immunoprecipitation and mass spectrometry, as previously described [4,5]. Prior to immunoprecipitation, analytical internal standards were determined by spiking samples with a known quantity of 12C15N-Aβ40 and 12C15N-Aβ42.

Aβ42 and Aβ40 isoforms were then simultaneously immunoprecipitated from 0.45 mL of plasma via a monoclonal anti-Aβ mid-domain antibody (HJ5.1, anti-Aβ13-28) conjugated to M-270 Epoxy Dynabeads (Invitrogen, Waltham, Massachusetts, USA). Protein digestion into peptides was performed using LysN endoprotease (Pierce, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Liquid chromatography–mass spectrometry was performed as described previously [6]. An Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher, Waltham, MA) interfaced with an M-class nanoAcquity chromatography system (Waters Corporation, Milford, Massachusetts, USA) was used to analyze plasma for targeted parallel reaction monitoring. For the analysis of Aβ isoforms, the precursor and product ion pairs were chosen as previously described [7,8]. Derived integrated peak areas were analyzed using the Skyline software package [9]. Aβ42 and Aβ40 concentrations (in pg/ml) were calculated by integrated peak area ratios to known concentrations of the internal standards. The plasma Aβ42/40 ratio was then determined by dividing Aβ42 by Aβ40.

The plasma NfL concentration (pg/ml) was measured by an electrochemiluminescence-based assay using the R-PLEX human neurofilament L antibody set (Meso Scale Discovery, F217X-3) with MSD Gold 96-well Small Spot SA SECTOR plates (L45A-1) [4,10]. Samples were diluted twice in Diluent 12 (R50JA-3) and assayed in duplicate following the manufacturer's instructions. The mean value of the duplicate assay measurements was used for analyses.

The plasma p-tau181 concentration (pg/ml) was measured in blood samples obtained at baseline and at 36 months of follow-up. The assays were carried out in the Clinical Neurochemistry Laboratory, University of Gothenburg (Mölndal, Sweden) following an in-house Simoa method previously described in detail [11]. Briefly, the AT270 mouse monoclonal antibody (MN1050, Invitrogen), which recognizes the tau sequence phosphorylated specifically at threonine 181, was coupled to paramagnetic beads (103207; Quanterix) and used for capture. Conjugated to biotin (A3959; Thermo Scientific), the anti-tau mouse monoclonal antibody tau12 (806502, BioLegend), which binds the N-terminal epitope 6-18 on

human tau protein, was used as a detector. Full-length recombinant tau-441 phosphorylated by glycogen synthase kinase 3β (TO8–50FN, SignalChem) was used as a calibrator.

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b. Supplementary table S1: Association between HSV-1 serostatus and cortical amyloid load. Univariate, sensitivity and stratified models.

	Univariate analysis n= 165			Sensitivity analysis[1] n= 165			Interact. APOE4	Among *APOE4* carriers[2] n= 43			Among *APOE4* non carriers[2] n= 122		
Cortical-to-cerebellar SUVR	Beta	Std	P value	Beta	Std	P value	P value	Beta	Std	P value	Beta	Std	P value
Anti-HSV-1 IgG													
Positive (vs negative)	- 0.08	0.04	0.08	- 0.07	0.04	0.08	0.08	- 0.21	0.08	0.01	- 0.03	0.05	0.45
Anti-HSV-1 IgG level													
1st tercile (vs negative)	- 0.03	0.05	0.50	- 0.04	0.05	0.43							
2nd tercile (vs negative)	- 0.09	0.05	0.06	- 0.10	0.05	0.03							
3rd tercile (vs negative)	- 0.10	0.05	0.04	- 0.08	0.04	0.07							
Cortical amyloid load ≥ 1.17	OR	95% CI	P value	aOR	95% CI	P value	P value	Beta	Std	P value	Beta	Std	P value
Anti-HSV-1 IgG													
Positive (vs negative)	0.55	0.22-1.39	0.21	0.51	0.18-1.42	0.20							
Anti-HSV-1 IgG level													
1st tercile (vs negative)	0.72	0.25-2.05	0.53	0.64	0.20-2.04	0.45							
2nd tercile (vs negative)	0.65	0.23-1.87	0.43	0.57	0.17-1.89	0.36							
3rd tercile (vs negative)	0.40	0.14-1.10	0.07	0.41	0.14-1.25	0.12							

[1] Adjusted for age, sex, *APOE4* genotype, education, history of hypertension, diabetes or dyslipidemia at baseline, randomization arm and time from randomisation to PET scan

[2] Adjusted for age, sex and education. In these models, due to the small sample sizes (particularly among *APOE4* carriers), we did not consider the levels of IgG in terciles. Analyses using the binary variable "cortical amyloid load ≥ 1.17" were not performed due to the absence of uninfected participants with a cortical amyloid load ≥ 1.17 among *APOE4* carriers.

Abbreviations: AD, Alzheimer's disease; aOR, adjusted odds ratio; CI, confidence interval; HSV-1, herpes simplex virus 1; IgG, immunoglobulin G; OR, odds ratio; std, standard deviation; SUVR, standard uptake value ratio.

c. Supplementary table S2: Association between HSV-1 serostatus and cortical amyloid load in several brain areas. Multivariate and stratified models.

SUVR in several brain areas	Multivariate analysis[1] n= 165				Among APOE4 carriers[2] n= 43			Among APOE4 non carriers[2] n= 122		
	Beta	Std	P value	Interaction APOE4	Beta	Std	P value	Beta	Std	P value
Anterior cingulate cortex										
Anti-HSV-1 IgG										
Positive (vs negative)	-0.06	0.05	0.22	**0.006**	**-0.32**	**0.10**	**0.004**	0.02	0.06	0.68
Anti-HSV-1 IgG level										
1st tercile (vs negative)	0.003	0.06	0.96							
2nd tercile (vs negative)	-0.09	0.06	0.13							
3rd tercile (vs negative)	-0.09	0.06	0.09							
Posterior cingulate cortex										
Anti-HSV-1 IgG										
Positive (vs negative)	**-0.09**	**0.04**	**0.04**	0.32	-0.17	0.10	0.08	-0.06	0.05	0.20
Anti-HSV-1 IgG level										
1st tercile (vs negative)	-0.05	0.05	0.31							
2nd tercile (vs negative)	**-0.11**	**0.05**	**0.02**							
3rd tercile (vs negative)	**-0.10**	**0.05**	**0.04**							
Precuneus										
Anti-HSV-1 IgG										
Positive (vs negative)	-0.10	0.06	0.08	0.10	**-0.28**	**0.12**	**0.03**	-0.04	0.06	0.50
Anti-HSV-1 IgG level										
1st tercile (vs negative)	-0.06	0.06	0.37							
2nd tercile (vs negative)	**-0.13**	**0.06**	**0.04**							
3rd tercile (vs negative)	-0.10	0.06	0.09							
Temporal cortex										
Anti-HSV-1 IgG										
Positive (vs negative)	**-0.09**	**0.04**	**0.02**	0.15	**-0.20**	**0.07**	**0.01**	-0.05	0.04	0.23
Anti-HSV-1 IgG level										
1st tercile (vs negative)	-0.05	0.04	0.26							
2nd tercile (vs negative)	**-0.11**	**0.04**	**0.01**							
3rd tercile (vs negative)	**-0.10**	**0.04**	**0.02**							
Medial occipito-frontal cortex										
Anti-HSV-1 IgG										
Positive (vs negative)	-0.06	0.04	0.17	**0.03**	**-0.23**	**0.08**	**0.008**	-0.002	0.05	0.97
Anti-HSV-1 IgG level										
1st tercile (vs negative)	-0.01	0.05	0.91							
2nd tercile (vs negative)	-0.09	0.05	0.07							
3rd tercile (vs negative)	-0.08	0.05	0.09							
Medial orbito-frontal cortex										
Anti-HSV-1 IgG										
Positive (vs negative)	-0.05	0.03	0.09	0.63	-0.10	0.06	0.09	-0.04	0.04	0.24
Anti-HSV-1 IgG level										
1st tercile (vs negative)	-0.04	0.04	0.33							
2nd tercile (vs negative)	-0.06	0.04	0.09							
3rd tercile (vs negative)	-0.06	0.04	0.07							
Fronto-parietal cortex										
Anti-HSV-1 IgG										
Positive (vs negative)	-0.08	0.04	0.07	0.08	**-0.22**	**0.08**	**0.01**	-0.03	0.05	0.52
Anti-HSV-1 IgG level										
1st tercile (vs negative)	-0.05	0.05	0.35							
2nd tercile (vs negative)	**-0.10**	**0.05**	**0.03**							
3rd tercile (vs negative)	-0.08	0.05	0.09							

[1]Adjusted for age, sex, APOE4 genotype and education.

[2] Adjusted for age, sex and education. In these models, due to the small sample sizes (particularly among APOE4 carriers), we did not consider the levels of IgG in terciles.

uptake value ratio

d. Supplementary table S3: Association between HSV-1 serostatus and plasma Aβ42/40 ratio or NfL. Univariate, sensitivity and stratified models.

	Univariate analysis n=150			Sensitivity analysis[1] n=142			Interaction APOE4	Among *APOE4* carriers[2] n= 41			Among *APOE4* non carriers[2] n= 109		
Plasma Aβ42/40 ratio	Beta	Std	P value	Beta	Std	P value	P value	Beta	Std	P value	Beta	Std	P value
Anti-HSV-1 IgG Positive (vs negative)	0.003	0.003	0.43	0.001	0.003	0.75	0.64	0.002	0.006	0.67	0.004	0.004	0.36
Anti-HSV-1 IgG level													
1st tercile (vs negative)	0.007	0.004	0.05	0.006	0.004	0.11							
2nd tercile (vs negative)	0.001	0.004	0.87	0.0004	0.004	0.91							
3rd tercile (vs negative)	0.001	0.004	0.89	- 0.002	0.003	0.60							
Plasma NfL[3]	Beta	Std	P value	Beta	Std	P value	P value	Beta	Std	P value	Beta	Std	P value
Anti-HSV-1 IgG Positive (vs negative)	0.004	0.09	0.96	0.03	0.09	0.70	0.72	0.06	0.19	0.76	0.009	0.10	0.94
Anti-HSV-1 IgG level													
1st tercile (vs negative)	0.05	0.11	0.64	0.08	0.10	0.42							
2nd tercile (vs negative)	- 0.04	0.11	0.70	0.02	0.11	0.84							
3rd tercile (vs negative)	0.002	0.10	0.99	0.01	0.10	0.93							

[1] Adjusted for age, sex, *APOE4* genotype, education, history of hypertension, diabetes or dyslipidemia at baseline, randomisation arms and serum creatinine levels at 12 months

[2] Adjusted for age, sex and education. In these models, due to the small sample sizes (particularly among *APOE4* carriers), we did not consider the levels of IgG in terciles.

[3] in pg/ml, log-transformed

Abbreviations: AD, Alzheimer's disease; HSV-1, herpes simplex virus 1; IgG, immunoglobulin G; NfL, neurofilament light chain; std, standard deviation.

e. <u>Supplementary table S4: Association between HSV-1 serostatus and plasma p-tau181. Univariate, sensitivity and stratified models.</u>

	Univariate analysis n= 123			Sensitivity analysis[1] n= 110 or 118			Interaction APOE4	Multivariate analysis among *APOE4* carriers[2] n= 33			Multivariate analysis among *APOE4* non carriers[2] n= 90		
Plasma p-tau181 at baseline[3]	Beta	Std	P value	Beta	Std	P value	P value	Beta	Std	P value	Beta	Std	P value
Anti-HSV-1 IgG Positive (vs negative)	- 0.004	0.12	0.97	0.004	0.12	0.98	0.81	- 0.15	0.22	0.50	- 0.03	0.14	0.83
Anti-HSV-1 IgG level													
1st tercile (vs negative)	0.02	0.13	0.85	0.03	0.14	0.82							
2nd tercile (vs negative)	- 0.08	0.13	0.53	- 0.13	0.14	0.37							
3rd tercile (vs negative)	0.04	0.13	0.78	0.07	0.14	0.60							
Plasma p-tau181 at 36 months[3]	Beta	Std	P value	Beta	Std	P value	P value	Beta	Std	P value	Beta	Std	P value
Anti-HSV-1 IgG Positive (vs negative)	-0.06	0.14	0.66	- 0.09	0.15	0.54	0.65	- 0.06	0.21	0.77	- 0.12	0.18	0.49
Anti-HSV-1 IgG level													
1st tercile (vs negative)	0.04	0.15	0.82	- 0.04	0.17	0.80							
2nd tercile (vs negative)	- 0.11	0.16	0.49	- 0.13	0.17	0.47							
3rd tercile (vs negative)	- 0.10	0.15	0.50	- 0.10	0.16	0.52							

[1] Adjusted for age, sex, *APOE4* genotype, education, history of hypertension, diabetes or dyslipidemia at baseline and serum creatinine levels at the time of measurement + randomisation arms for p-tau181 at 36 months. Numbers for p-tau181 at baseline and at 36 months, respectively.
[2] Adjusted for age, sex and education. In these models, due to the small sample sizes (particularly among *APOE4* carriers), we did not consider the levels of IgG in terciles.
[3] in pg/ml, log-transformed
Abbreviations: AD, Alzheimer's disease; HSV-1, herpes simplex virus 1; IgG, immunoglobulin G; ptau-181, phosphorylated tau 181; std, standard deviation