Supplementary Material

The Biological Substrate of the Motoric Cognitive Risk Syndrome: A Pilot Study Using Amyloid-/Tau-PET and MR Imaging

Gait speed reference values

Reference values obtained from a local cohort of healthy older adults, free from neurological conditions, assessed using the same gait protocol, and subdivided into two age classes (< or ≥ 75) and gender:

- Men, <75 years old: 7 subjects included, with a mean age of 71.2±2.4 years, a mean walking speed of 1.24±0.13 m/s. Education level was set as I (<9 years), II (9-12 years), or III (>12 years): 0/4/3 subjects had an I/II/III education level, respectively.
- Women, <75 years old: 21 subjects included, with a mean age of 70.9±2.5, a mean walking speed of 1.27±0.11; 4/5/12 subjects had an I/II/III education level, respectively.
- Men, ≥75 years old: 5 subjects included, with a mean age of 78.8±3.2, a mean walking speed of 1.04±0.09; 0/0/5 subjects had an I/II/III education level, respectively.
- Women, ≥75 years old: 12 subjects included, with a mean age of 80.4±2.7, a mean walking speed of 1.21±0.14; 3/5/7 subjects had an I/II/III education level, respectively.

PET acquisition and preprocessing

Amyloid PET imaging was performed using ^{18}F-Florbetabir (for 5 patients, images acquired 50 min after injection of 200 MBq of radiotracer, 3 x 5-min frames) or ^{18}F-Flumetamol (for 15 patients, images acquired 90 min after injection of 150 MBq of radiotracer, 4 x 5-min frames). Tau-PET images were acquired using ^{18}F-Flortaucipir (^{18}F-AV1451), synthesized at the Center of Radiopharmaceutical Sciences ETH-PSI-USZ in Zurich, Switzerland under license from the IP owner (Avid/Lilly), 75 min after injection of 180 MBq of radiotracer (6 x 5-min frames).

Data were processed using Statistical Parametric Mapping 12 (SPM12) software. Briefly, data were corrected for motion, frames realigned, averaged over time, and co-registered to each subject's T1-weighted MRI sequence. Next, structural T1-weighted images were transformed to the Montreal Neurological Institute (MNI) atlas space, and the resulting transformations used to warp PET images into MNI space, following established and previously published analysis pipelines.

MR imaging acquisition

Subjects underwent MRI on a Siemens MAGNETOM Skyra 3T. The protocol included: i) a T1-weighted MPRAGE (0.9 mm isotropic voxel size, repetition time (TR) 1930 ms, echo time (TE) 2.4 ms, flip angle 8 deg); ii) diffusion weighted imaging acquired with a b value of 1000 s/mm^2 at each of 30 non-collinear diffusion directions (voxel size: 1.0 x 1.0 x 2.0, TR 10000 ms, TE 71 ms, 2 shells: b 0/1000 s/mm^2); iii) Fluid-attenuated inversion recovery (voxel size: 0.5 x 0.5 x 1.0, TR 5000 ms, TE 386 ms).

Further details on MRI imaging processing

White matter hyperintensities (WMH)

Lesions were segmented from FLAIR sequences using the lesion prediction algorithm as implemented in the Lesion Segmentation Toolbox (SPM) [1]. The segmentation was visually inspected and manually corrected when required. Additionally, we performed a visual rating of WMH, using the ARWMC scale [2] and the lesion volume was automatically computed during Freesurfer segmentation. The WMH volume assessed by SPM highly correlated with the total ARWMC score (rho=0.78, $p<0.001$), and to the lesion volume computed by Freesurfer (rho=0.97, $p<0.001$).

Volumetric measures

The quality of the automatic segmentation and normalization was visually checked, and volumetric measures were derived. Lateral ventricular volume and the estimated total intracranial volume were obtained by the aseg.stats file. Values of cortical thickness were obtained using the Mindboggle-101 dataset (Desikan-Killiany-Tourville cortical labeling protocol) [3].

DTI processing

Data were processed using FMRIB's Diffusion Toolbox (part of FSL). First, they were corrected for motion and eddy currents artifacts and then standard DTI metrics of fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity were computed. The respective regions of interest (ROI) for each tract were extracted from the JHU DTI based white matter atlas (ICBM-DTI-81 white-matter labels) [4], including the genu, body and splenium for the CC and the pyramids, cerebral peduncle and posterior limbs of the internal capsule for the CST. Masks were thresholded at 0.9, co-registered to the diffusion images of each subject using a combination of linear and non-linear registration (FLIRT

https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT and FNIRT
https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT [5]). Each mask was visually inspected after registration and manually corrected if necessary (in 2 patients).

Supplementary Table 1. Thickness of cortical regions involved in MCR [6,7] and DTI parameters (FA, MD, AxD, and RD) for other relevant white matter tracts and differences between MCR+ and MCR- patients.

	All patients (n=20)	**MCR+ (n=8)**	**MCR– (n=12)**	**p**	**Effect size**
	Cortical Thickness				
Entorhinal	3.31 (0.41)	3.32 (0.32)	3.25 (0.29)	0.511	0.16
Inferior Parietal	2.43 (0.17)	2.45 (0.12)	2.41 (0.27)	0.244	0.28
Inferior Temporal	2.79 (0.25)	2.78 (0.36)	2.79 (0.20)	0.758	0.07
Lateral Orbito-frontal	2.65 (0.20)	2.61 (0.25)	2.67 (0.16)	0.884	0.04
Medial Orbito-frontal	2.38 (0.14)	2.39 (0.10)	2.37 (0.19)	0.632	0.11
Middle Temporal	2.74 (0.23)	2.75 (0.22)	2.74 (0.25)	0.382	0.21
Parahippocampal	2.63 (0.47)	2.75 (0.50)	2.53 (0.35)	0.406	0.20
Paracentral	2.34 (0.19)	2.43 (0.27)	2.33 (0.19)	0.268	0.27
Pars Opercularis	2.52 (0.15)	2.52 (0.09)	2.52 (0.22)	0.727	0.08
Pars Orbitalis	2.60 (0.24)	2.55 (0.21)	2.66 (0.30)	0.702	0.09
Pars Triangularis	2.35 (0.20)	2.29 (0.22)	2.38 (0.17)	0.708	0.09
Precentral	2.44 (0.19)	2.44 (0.24)	2.44 (0.17)	0.755	0.08
Precuneus	2.32 (0.17)	2.36 (0.14)	2.26 (0.13)	0.052	0.49
Superior Frontal	2.56 (0.23)	2.59 (0.24)	2.55 (0.19)	0.372	0.22
Supramarginal	2.48 (0.17)	2.50 (0.15)	2.45 (0.17)	0.705	0.09
Insula	2.91 (0.20)	2.88 (0.19)	2.95 (0.30)	0.185	0.32
	FA				
Middle cerebellar peduncle	0.46 (0.02)	0.46 (0.02)	0.47 (0.03)	0.807	0.05
Pontine crossing tract	0.42 (0.02)	0.42 (0.02)	0.42 (0.03)	0.596	0.13
Anterior Limb of the Internal Capsule	0.47 (0.02)	0.47 (0.03)	0.47 (0.01)	0.606	0.12
Anterior Corona Radiata	0.38 (0.04)	0.38 (0.04)	0.38 (0.04)	0.509	0.15
Posterior Corona Radiata	0.44 (0.04)	0.44 (0.02)	0.43 (0.04)	0.675	0.10
Inferior Longitudinal fasciculus	0.45 (0.04)	0.45 (0.02)	0.46 (0.05)	0.429	0.18
Cingulate gyrus	0.44 (0.03)	0.44 (0.01)	0.44 (0.04)	0.924	0.03
Superior longitudinal fasciculus	0.44 (0.03)	0.45 (0.02)	0.44 (0.03)	0.388	0.21
Uncinate fasciculus	0.40 (0.05)	0.40 (0.05)	0.41 (0.05)	0.698	0.10
	MD				
Middle cerebellar peduncle	0.91 (0.07)	0.91 (0.04)	0.91 (0.08)	0.658	0.01
Pontine crossing tract	0.68 (0.04)	0.68 (0.05)	0.69 (0.03)	0.931	0.02
Anterior Limb of the Internal Capsule	0.77 (0.06)	0.77 (0.02)	0.76 (0.07)	0.130	0.37
Anterior Corona Radiata	0.88 (0.12)	0.92 (0.09)	0.88 (0.13)	0.129	0.36
Posterior Corona Radiata	0.98 (0.19)	1.00 (0.15)	0.92 (0.21)	0.183	0.31
Inferior Longitudinal fasciculus	0.96 (0.15)	0.98 (0.10)	0.94 (0.16)	0.077	0.43
Cingulate gyrus	0.76 (0.04)	0.77 (0.05)	0.75 (0.04)	0.227	0.30
Superior longitudinal fasciculus	0.74 (0.05)	0.74 (0.06)	0.74 (0.05)	0.334	0.23
Uncinate fasciculus	0.82 (0.04)	0.82 (0.04)	0.82 (0.05)	0.893	0.03
	AxD				
Middle cerebellar peduncle	1.39 (0.09)	1.40 (0.07)	1.38 (0.11)	0.456	0.18
Pontine crossing tract	1.03 (0.09)	1.03 (0.10)	1.02 (0.87)	0.434	0.19
Anterior Limb of the Internal Capsule	1.23 (0.07)	1.25 (0.04)	1.21 (0.08)	0.022	0.59
Anterior Corona Radiata	1.26 (0.10)	1.29 (0.06)	1.25 (0.14)	0.200	0.31
Posterior Corona Radiata	1.40 (0.24)	1.46 (0.22)	1.34 (0.26)	0.252	0.27
Inferior Longitudinal fasciculus	1.47 (0.16)	1.50 (0.13)	1.44 (0.15)	0.130	0.37
Cingulate gyrus	1.19 (0.05)	1.19 (0.04)	1.18 (0.08)	0.694	0.10
Superior longitudinal fasciculus	1.15 (0.08)	1.15 (0.09)	1.13 (0.08)	0.127	0.40
Uncinate fasciculus	1.20 (0.08)	1.19 (0.05)	1.21 (0.08)	0.532	0.16

	RD				
Middle cerebellar peduncle	0.65 (0.07)	0.65 (0.05)	0.65 (0.07)	0.581	0.13
Pontine crossing tract	0.53 (0.04)	0.52 (0.04)	0.54 (0.02)	0.827	0.05
Anterior Limb of the Internal Capsule	0.54 (0.05)	0.55 (0.03)	0.54 (0.06)	0.391	0.20
Anterior Corona Radiata	0.70 (0.10)	0.72 (0.89)	0.70 (0.16)	0.207	0.29
Posterior Corona Radiata	0.75 (0.14)	0.76 (0.12)	0.69 (0.16)	0.128	0.36
Inferior Longitudinal fasciculus	0.69 (0.11)	0.71 (0.09)	0.69 (0.13)	0.148	0.34
Cingulate gyrus	0.56 (0.05)	0.56 (0.04)	0.55 (0.04)	0.409	0.20
Superior longitudinal fasciculus	0.55 (0.04)	0.54 (0.04)	0.55 (0.05)	0.617	0.12
Uncinate fasciculus	0.63 (0.06)	0.63 (0.04)	0.62 (0.07)	0.803	0.06

MCR, motoric cognitive risk; FA, fractional anisotropy; MD, mean diffusivity; AxD, axial diffusivity; RD, radial diffusivity. To notice that: i) regarding cortical thickness, the only trend to significance involved the precunes, with larger thickness found in MCR+, in line with what observed by [7], ii) regarding DTI parameters, the only significant difference involved the AxD of the anterior limb of the internal capsule, another white matter tract adjacent to lateral ventricles.

REFERENCES

[1] Schmidt P (2016) Bayesian inference for structured additive regression models for large-scale problems with applications to medical imaging. Dissertation, LMU München: Faculty of Mathematics, Computer Science and Statistics: Chapter 6.1.

[2] Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjögren M, Wallin A, Ader H, Leys D, Pantoni L, Pasquier F, Erkinjuntti T, Scheltens P; European Task Force on Age-Related White Matter Changes (2001) A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke* **32**, 1318–1322.

[3] Klein A, Tourville J (2012) 101 labeled brain images and a consistent human cortical labeling protocol. *Front Neurosci* **6**, 171.

[4] Mori S, Wakana S, van Zijl PCM, Nagae-Poetscher L (2005) *MRI Atlas of Human White Matter, 1st Ed*. Elsevier Science

[5] Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* **17**, 825–841.

[6] Blumen HM, Schwartz E, Allali G, Beauchet O, Callisaya M, Doi T, Shimada H, Srikanth V, Verghese J (2021) Cortical thickness, volume, and surface area in the motoric cognitive risk syndrome. *J Alzheimers Dis* **81**, 651–665.

[7] Blumen HM, Allali G, Beauchet O, Lipton RB, Verghese J (2019) A gray matter volume covariance network associated with the motoric cognitive risk syndrome: A multicohort MRI study. *J Gerontol A Biol Sci Med Sci* **74**, 884–899.