Supplemental material

White matter microstructural damage in SVD is associated with MoCA but not with MMSE performances: VMCI-Tuscany Study

Methodology and study protocol of the VMCI-Tuscany Study
The Vascular Mild Cognitive Impairment (VMCI) Tuscany Study is a multicenter, prospective, observational study, carried out in the Tuscany region of Italy, and aimed at estimating the role of a large set of clinical, cognitive, neuroimaging, and biological markers of SVD as independent predictors of the transition from MCI to dementia. According to the study protocol, at baseline, each enrolled patient undergoes an extensive clinical, functional and neuropsychological assessment, an MRI examination, and the collection of blood samples.

MRI assessment

MRI protocol
Patients were examined on a 1.5 T system (Intera, Philips Medical System, Best, The Netherlands) with 33 mT/m gradients capability and a head coil with SENSE technology. The examination protocol was previously described and included a sagittal T1 sequence, an axial FLAIR sequence, and an axial single-shot echo planar imaging sequence for DTI (diffusion sensitizing gradients applied along 15 non-collinear directions using b value of 0 (b0 image) and 1000 s/mm²) [1].

Conventional MRI features
For the purpose of the present investigation the following features were evaluated: a) lacunar infarcts visible on MRI were defined as hypointense lesions on T1 imaging with corresponding hyperintense lesion on FLAIR images with a diameter <20 mm; lacunar infarcts were classified as absent, 1-3, and >3; b) deep WMH on FLAIR graded according to the modified Fazekas scale [2]. A score of 2 (moderate) was attributed to beginning confluent lesions and a score of 3 (severe) to large confluent lesions [2]; d) global cortical atrophy using the scale of Pasquier et al. that assesses sulci opening of sulci and narrowing of gyri [3]. Scores 0–3 represent absent, mild, moderate, and severe cortical atrophy, respectively; e) medial temporal lobe atrophy (MTA) assessed by means of the Scheltens' scale [4]. This scale assesses coronal T1-weighted images acquired parallel to the brainstem. Scores 0–4 indicate progressive medial temporal volume loss. The above MRI features were visually evaluated in all patients by one experienced neurologist (AP).

Patients with incidental non-lacunar infarcts in the cerebral cortex, cerebellum, or brainstem were excluded to avoid a possible confounding effect on DTI analysis and also to have a more homogenous patients sample.

DTI and analysis of microstructural damage of cerebral WM
Diffusion-weighted images were corrected for head motion and eddy current distortions using FDT (FMRIB’s Diffusion Toolbox 2.0), part of FSL 5.0.2 [5] after which brain tissue was segmented using BET, also part of FSL [6]. The b-matrix was reoriented by applying the rotational part of the affine transformation employed in the eddy-correction step [7]. A tensor model was fitted to the raw data using a constrained nonlinear least squares procedure implemented in the software package CAMINO, and residual non-positive definite tensors (in isolated regions where the nonlinear algorithm failed to converge, mainly located at the edge of the brain) were removed by tensor interpolation in the log-euclidean domain [8]. Mean diffusivity (MD) and fractional anisotropy (FA) maps were then computed from the estimated tensor field. The segmentation method employed to obtain cerebral WM masks was previously detailed [9]. Briefly, WM segmentation on T1-weighted images was carried out using FAST 4, part of FSL [10]. To reduce partial volume effects, a preliminary WM mask was obtained by retaining only those
voxels which had a tissue class probability equal to or above 0.75. In order to select identical cerebral regions across subjects, a standard space (MNI152 average normal brain) WM mask was mapped onto each subject's native space and multiplied by each subject's WM mask (Figure 1). Resulting WM masks were successively mapped onto native diffusion space by applying intra-subject affine transformations (12 degrees of freedom) between the T1 and b0 images [11] in order to compute WM-wide MD and FA statistics for each subject. The inter-subject agreement of our WM segmentation was assessed by using inverse transformations to map single subject WM masks to standard space, averaging and calculating descriptive statistics of the resultant agreement image. The inter-subject agreement was substantial (median 87%, mode: 97%, s.d. 19%),

While several metrics can be used to evaluate the MD and FA properties of brain tissue [12], in the present study we employed median values of MD and FA to characterize the microstructural properties of cerebral WM.

**Statistical analysis**
Descriptive analyses were used to briefly characterize the baseline sample in terms of demographic, clinical and neuroimaging features. Bivariate statistical analyses (independent samples t test, ANOVA, Pearson’s r) were used to exclude statistical significant differences between the group composed by the excluded patients for DTI data unavailability and presence of non-lacunar infarcts and the final sample (data not shown). The considered variables were age, education, gender, MoCA, MMSE, lacunar infarcts, WMH, global cortical atrophy, MTA, and median value of MD and FA of the cerebral WM.

Bivariate statistical analyses (independent samples t test, ANOVA, Pearson’s r) were used to evaluate the association of demographic and neuroimaging variables, with MoCA and MMSE total score. The considered variables were: demographic characteristics (age, education level, and gender), conventional MRI features (lacunar infarcts, WM hyperintensities, global cortical atrophy, MTA), DTI-derived features (median value of MD and FA of the cerebral WM), and MoCA and MMSE total score.

To evaluate which test was more strongly associated with DTI-derived indices we performed a model of partial correlation analysis adjusting for demographic variables (age, education level, and gender) and conventional MRI features (lacunar infarcts, WMH, global cortical atrophy, MTA). In order to evaluate if psychometrical structure of MoCA influenced the possible association with DTI parameters, correlation analysis (Spearman’s Rho and biserial point r_{bp}) were performed for each single subtest (visuoexecutive, naming, digit span, attention, calculation, language, verbal fluency, abstraction, recall, and orientation). All data analyses were performed using SPSS 20.

**Definition of the final sample included in this study**
From December 2011 to March 2013, 104 patients were enrolled in the VMCI-Tuscany Study in the Florence center, where the MRI protocol included also DTI evaluation. For the purposes of the present study, 7 patients were excluded because DTI imaging was not available for technical reasons, 21 patients were excluded for the presence of non-lacunar infarcts in the cerebral cortex, cerebellum or brainstem. No statistically significant differences between excluded and final sample patients were found in terms of age, education, gender, MoCA, MMSE, and MRI features.
Supplemental references:


Supplemental table I. Association between each MoCA subtest and DTI parameters

<table>
<thead>
<tr>
<th>MoCA subtest</th>
<th>Median MD cerebral WM</th>
<th>Median FA cerebral WM</th>
</tr>
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<tbody>
<tr>
<td>Visuoeexecutive</td>
<td>-0.372*, p=0.001</td>
<td>0.279*, p=0.015</td>
</tr>
<tr>
<td>Naming</td>
<td>-0.148*, p=0.206</td>
<td>0.026*, p=0.825</td>
</tr>
<tr>
<td>Digit Span</td>
<td>-0.255*, p=0.028</td>
<td>0.185*, p=0.114</td>
</tr>
<tr>
<td>Attention</td>
<td>-0.259§, p=0.026</td>
<td>0.147§, p=0.212</td>
</tr>
<tr>
<td>Calculation</td>
<td>-0.077*, p=0.514</td>
<td>0.061*, p=0.608</td>
</tr>
<tr>
<td>Language</td>
<td>-0.141*, p=0.232</td>
<td>0.156*, p=0.185</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>-0.095§, p=0.421</td>
<td>0.030§, p=0.797</td>
</tr>
<tr>
<td>Abstraction</td>
<td>-0.239*, p=0.040</td>
<td>0.139*, p=0.237</td>
</tr>
<tr>
<td>Recall</td>
<td>-0.241*, p=0.039</td>
<td>0.057*, p=0.628</td>
</tr>
<tr>
<td>Orientation</td>
<td>-0.138*, p=0.238</td>
<td>0.001*, p=0.997</td>
</tr>
</tbody>
</table>

MD: mean diffusivity
FA: fractional anisotropy
* Spearman rho
§ biserial point $r_{bp}$
Appendix. List of participating centers and personnel in the VMCI-Tuscany.

University of Florence: (Coordinating Center): Domenico Inzitari (Study coordinator), Rosanna Abbate, Maria Boddi, Francesca Cesari, Laura Cioli, Mirella Coppo, Alessandra Del Bene, Stefano Diciotti, Andrea Ginestrioni, Bettì Giusti, Anna Maria Gori, Sandro Marini, Mario Mascalchi, Serena Nannucci, Leonardo Pantoni, Marco Pasi, Francesca Pescini, Anna Poggesi, Giovanni Pracucci, Emilia Salvadori, Raffaella Valentí.

University of Pisa: Ubaldo Bonuccelli, Paolo Cecchi, Alberto Chiti, Mirco Cosottini, Giovanni Orlandi, Cristina Pagni, Gabriele Siciliano, Gloria Tognoni.

University of Siena: Antonio Federico, Nicola De Stefano, Ilaria Di Donato, Maria Teresa Dotti, Patrizia Formichi, Claudia Gambetti, Antonio Giorgio, Francesca Rossi, Laura Stromillo, Enza Zicari.

Tuscany Region: Arezzo (Paolo Zolo, Alessandro Tiezzi); Empoli (Elisabetta Bertini, Stefania Brotini, Leonello Guidi, Maria Lombardi, Stefania Mugnai, Antonella Notarelli); Florence (Laura Bracco, Massimo Cadelo, Renzo Cisbani, Luciano Gabbani, Guido Gori, Lorella Lambertucci, Luca Massacesi, Enrico Mossello, Marco Paganini, Maristella Piccininini, Francesco Pinto, Claudia Pozzi, Sandro Sorbi, Gaetano Zaccara); Grosseto (Tiziano Borgogni, Mario Mancuso, Roberto Marconi); Lucca (Monica Mazzoni, Marco Vista); Livorno (Giuseppe Meucci, Giovanna Bellini); Massa Carrara (Luciano Gabrielli); Pisa (Cristina Frittelli, Renato Galli, Gianna Gambaccini); Pistoia (Stefano Bartolini, Carlo Biagini, Veronica Caleri, Paola Vanni); Prato (Donatella Calvani, Carla Giorgi, Stefano Magnolfi, Pasquale Palumbo, Carlo Valente); Siena (Alessandro Rossi, Rossana Tassi, Stefania Boschi); Viareggio (Filippo Baldacci).