Dissemination in time and space in presymptomatic granulin mutation carriers: a GENFI spatial chronnectome study


*Stroke Unit, Azienda Socio Sanitaria Territoriale Spedali Civili Brescia, Brescia, Italy
bCentre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
cTri-institutional Center for Translational Research in Neuroimaging and Data Science (TREND), Georgia State University, Georgia Institute of Technology, Emory University, Atlanta, Georgia, USA
dDepartments of Psychology and Computer Science, Georgia State University, Atlanta, USA
eDepartment of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, USA
fNeuropsychology Unit, Azienda Socio Sanitaria Territoriale Spedali Civili Brescia, Brescia, Italy
gNeuroradiology Unit, University of Brescia, Brescia, Italy
hLaboratory Unit, Azienda Socio Sanitaria Territoriale Spedali Civili Brescia, Brescia, Italy
iDepartment of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
jDepartment of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands
kAlzheimer’s disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, University of Barcelona, Barcelona, Spain
lCognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain
mClinique Interdisciplinaire de Memoire, Department des Sciences Neurologiques, CHU de Quebec, and Faculte de Medecine, Universite Laval, Quebec, Canada
nCenter for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden
oDepartment of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany
pFondazione Ca’ Granda, IRCCS Ospedale Policlinico, Milan, Italy
qUniversity of Milan, Centro Dino Ferrari, Milan, Italy
rDepartment of Clinical Neurosciences, University of Cambridge, Cambridge, UK
sSunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
tTanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada
uDepartment of Clinical Neurological Sciences, University of Western Ontario, London, Ontario Canada
vLaboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium
wNeurology Service, University Hospitals Leuven, Leuven, Belgium
xLeuven Brain Institute, KU Leuven, Leuven, Belgium
yLaboratory of Neurosciences, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
zFondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
{Nucl Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford, Oxford, UK
\[University of Oxford, Hauzen, Oxford, UK\]
\[Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK\]
\[Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France\]
\[Centre de référence des démences rares ou préocces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France\]
\[Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France\]
\[Reference Network for Rare Neurological Diseases (ERN-RND)\]

* Corresponding author at: Clinica Neurologica, Università degli Studi di Brescia, P.le Spedali Civili 1, 25123, Brescia, Italy. Phone: 0039 0303995632.
E-mail address: bborroni@unwind.it (B. Borroni).
\[see appendix for consortium authors\]
ARTICLE INFO

Article history:
Received 12 April 2021
Revised 28 August 2021
Accepted 1 September 2021
Available online 8 September 2021

Keywords:
Frontotemporal Dementia
GRN mutation
resting-state functional MRI
dynamic functional network connectivity
spatial chronnectome

ABSTRACT

The presymptomatic brain changes of granulin (GRN) disease, preceding by years frontotemporal dementia, has not been fully characterized. New approaches focus on the spatial chronnectome can capture both spatial network configurations and their dynamic changes over time. To investigate the spatial dynamics in 141 presymptomatic GRN mutation carriers and 282 noncarriers from the Genetic Frontotemporal dementia research Initiative cohort. We considered time-varying patterns of the default mode network, the language network, and the salience network, each summarized into 4 distinct recurring spatial configurations. Dwell time (DT) (the time each individual spends in each spatial state of each network), fractional occupancy (FO) (the total percentage of time spent by each individual in a state of a specific network) and total transition number (the total number of transitions performed by each individual in a specific state) were considered. Correlations between DT, FO, and transition number and estimated years from expected symptom onset (EYO) and clinical performances were assessed. Presymptomatic GRN mutation carriers spent significantly more time in those spatial states characterised by greater activation of the insula and the parietal cortices, as compared to noncarriers (p < 0.05, FDR-corrected). A significant correlation between DT and FO of these spatial states and EYO was found, the longer the time spent in the spatial states, the closer the EYO. DT and FO significantly correlated with performances at tests tapping processing speed, with worse scores associated with increased spatial states’ DT. Our results demonstrated that presymptomatic GRN disease presents a complex dynamic reorganization of brain connectivity. Change in both the spatial and temporal aspects of brain network connectivity could provide a unique glimpse into brain function and potentially allowing a more sophisticated evaluation of the earliest disease changes and the understanding of possible mechanisms in GRN disease.

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1. Introduction

Frontotemporal dementia (FTD) is a clinical and neuropathological heterogeneous disorder, characterized by language impairment, deficits in executive functions, and behavioural and/or personality disturbances (Gorno-Tempini et al., 2011; Rascovsky et al., 2011).

Pathogenetic mutations within the granulin (GRN) gene, one of the most common causes of familial FTD with TAR-DNA binding protein 43 (TDP-43) inclusions (Baker et al., 2006; Cruts et al., 2006), mostly present with behavioral variant FTD or nonfluent primary progressive aphasia (Moore et al., 2020). Clinical symptoms are preceded by a long accrual of subtle changes, which provide a unique time-window to study the earliest disease stages. (Chitramuthu et al., 2017; Panman et al., 2021). In particular, the study of preclinical brain changes might give novel insights into the pathophysiological processes, and into potential compensatory mechanisms occurring before the onset of symptoms, thus representing potential targets of intervention.

Only a few resting-state functional magnetic resonance imaging (MRI) connectivity studies are available so far, mainly suggesting the impairment of brain connectivity within the salience network (SN) and the default mode network (DMN) (Borroni et al., 2012; Dopper et al., 2013; Feis et al., 2019; Lee et al., 2019; Premi et al., 2014a; Premi et al., 2016). However, these studies hold a common assumption that each brain network is comprised of a fixed set of brain regions with a static spatial pattern over time (i.e., static functional network connectivity, sFNC) (Iraji et al., 2019a). This is indeed an oversimplification, as the resting brain is highly dynamic, with reoccurring variation in spatial patterns of brain functional organization during time (Calhoun et al., 2014; Iraji et al., 2020).

As such, while previous studies have focused on capturing dynamic brain network connectivity by assessing the variations in the temporal coupling between spatially static brain networks (Abrul et al., 2017; Allen et al., 2014; Calhoun et al., 2014; Armin Iraji et al., 2020; Miller et al., 2016), recent studies have explored the possibility to capture time-varying spatial patterns of brain networks (Iraji et al., 2019a, 2019b). In other words, functional brain networks are only transiently isolated, and each brain network may assume different configurations in space (i.e., “dissemination in space”) during the scan period (i.e., “dissemination in time”) (A. Iraji et al., 2020). This results in a number of identifiable spatial states, that may recruit distinct neuroanatomical brain regions, for each considered functional brain network (i.e., DMN or the SN). It is worth noting that spatial chronnectome focuses on variations in the spatial pattern of a given network (e.g., DMN) and leverages the spatial information to capture brain dynamics. This differs from performing dynamic functional network connectivity analysis on the subnetworks of a given network (e.g., evaluating dynamic functional network connectivity between subnetworks of DMN obtained from high model order ICA), which studies time-varying properties of the temporal couplings between subnetworks activity patterns (i.e., no spatial information is included in studying brain dynamics) (A Iraji et al., 2020; Iraji et al., 2019a).

In this view, a spatial chronnectome analysis may enable a more sophisticated evaluation of the spontaneously fluctuating nature of neuronal signals by assessing both spatial network configurations and their dynamic changes over time. Moreover, a spatial chronnectome analysis might be able to detect patterns of brain reorganization in the earliest phases of FTD pathology.

Therefore, the goal of the present study is to investigate whether the spatial chronnectome approach may reveal the early dynamic changes in presymptomatic FTD. To this, we considered presymptomatic subjects with GRN mutations from the Genetic Frontotemporal dementia Initiative (GENFI) (www.genfi.org) (Rohrer et al., 2015).
2. Methods

2.1. Participants

Data for this study were drawn from the GENFI 2 multicentre cohort study (data freeze 5), which consists of 26 research centres across Europe and Canada (www.genfi.org.uk). Inclusion and exclusion criteria have been previously described (Rohrer et al., 2015). Local ethics committees approved the study at each site, and all participants provided written informed consent according to the Declaration of Helsinki.

For the aim of the present work, we considered subjects carrying a pathogenic mutation within the GRN gene and, as control group, their first-degree relatives not carrying pathogenic GRN mutations, for whom an MRI scan acquired on a 3T scanner was available.

Estimated years from expected symptom onset in presymptomatic GRN mutation carriers were calculated as the age of the participant at the time of the study assessment minus the mean familial age at symptom onset, as previously reported (Rohrer et al., 2015). Each subject underwent a standardised neuropsychological assessment, as previously reported (Rohrer et al., 2015).

2.2. MRI acquisition

MRI protocol was common to all the GENFI sites and adapted for different scanners; no pre-study phantom harmonization was performed at local level. In summary, T2-weighted echo planar imaging (EPI) sequences sensitized to blood oxygenation level dependent (BOLD) contrast for rs-fMRI were considered in the present study (Premi et al., 2019). As the volume numbers (ranging from 140 to 200) varied across the GENFI centres, we considered only the first 140 volumes of the EPI images for each subject. In particular, we had 369 subjects with 200 timepoints that were cropped to 140, discharging the last 60 timepoints (mean acquisition time: 326.22 ± 20.54 seconds). From this point of view, Furthermore, differences in repetition times (TRs, ranging from 2200 microseconds to 2500 microseconds) (see Supplementary Table 1) has been considered in spatial chronnectome preprocessing and analysis. During scanning, subjects were asked to keep their eyes closed, not to think of anything in particular, and not to fall asleep.

2.3. Neuroimaging pre-processing and analyses

Functional data were preprocessed using the toolbox for Data Processing & Analysis for Brain Imaging (DPABI, http://rfmri.org/dpabi) (Yan et al., 2016) based on the Statistical Parametric Mapping (SPM12, https://www.fil.ion.ucl.ac.uk/spm/) software.

For each subject, the first 2 volumes of the fMRI series were discharged to account for magnetization equilibration. The remaining 138 volumes underwent slice-timing correction and were realigned to the first volume. Any subject who had a maximum displacement in any direction larger than 2.5 mm, or a maximum rotation (x,y,z) larger than 2.5°, was excluded. We considered frame-wise displacement (FD) (Power et al., 2012) as a nuisance variable accounting for head motion during MRI scanning. Data were subsequently spatially normalized to the EPI unified segmentation template (considering that EPI normalization is able to reduce variability across subjects) (Calhoun et al., 2017) in Montreal Neurological Institute coordinates derived from SPM12 software and resampled to 3 × 3 × 3 cubic voxels. Spatial smoothing with an isotropic Gaussian kernel with the full width at half-maximum of 6 mm was applied, followed pre-processing pipeline previously adopted for spatial chronnectome analysis (Iraji et al., 2019a).

2.4. Functional networks decomposition

The functional imaging data were processed using the GIFT (GIFT toolbox, http://trendscenter.org/software/gift), and a spatially constrained multivariate objective optimization ICA with reference (MOO-ICAR) (Du et al., 2015; Du and Fan, 2013) was used to obtain spatial maps for selected large-scale networks, namely the default mode network (DMN), the language network (LN) and the salience network (SN), from a recently published set of brain networks (Iraji et al., 2019a). Spatial maps are used as reference templates to calculate functional networks for each subject by maximizing independence in the context of the spatial constraint. These template maps include the brain networks with a not-artefactual neuronal origin and assign the remaining data to be noise. We have taken advantage of the recently published set of 12 spatial maps for our network selection, considering DMN, LN and SN as network of interest (Iraji et al., 2019a). The TR of each subject was entered in GIFT pre-processing, and we accounted for TR values differences among centres (143 subjects with TR = 2200 microseconds, 280 subjects with TR = 2500 microseconds) interpolating the data to the minimum TR (2200 ms).

These processed data were also used for sFNC statistical analysis, considering the DMN, LN, and SN large-scale networks derived from MOO-ICAR pre-processing analysis (see Supplementary Fig., panel A), as well as for spatial chronnectome analysis.

2.5. Dynamic coupling maps calculation (dCM), k-means clustering, dwell time (DT), fractional occupancy (FO) and total transitions number (TN) calculation

The spatial chronnectome analysis was achieved using the dynamic FNC toolbox implemented in GIFT (GIFT toolbox, http://trendscenter.org/software/gift). Sliding window length or number of clusters were chosen according to previous literature data on dynamic connectivity (Armin Iraji et al., 2020; Iraji et al., 2019a). The single time courses were detrended (to remove baseline drifts from the scanners and/or physiological pulsations), orthogonalized with respect to 12-motion parameters, despiked (replacement of outlier time points with 3rd order spline fitting to clean neighbouring points) and filtered using a 5th order Butterworth filter (0.01–0.15 Hz). For each considered brain network, the temporal coupling between a specific brain network and every voxel of the brain was calculated using the sliding-window correlation approach resulting in one dCM per window. This procedure takes all the potential associations into account and fully captures the relationship between each voxel and the brain network (for example, if a given voxel is highly correlated with two networks, correlation analysis allows the detection of both of these associations). We used the tapered window obtained by convolving a rectangle (width = 30 TRs) with a Gaussian (σ = 3 TRs) and the sliding step size of one TR resulting in 108 windows per subject (Armin Iraji et al., 2020; Iraji et al., 2019a).

k-means clustering was applied to summarize the dCMs of each brain network into a set of spatial states, which allows us to investigate the dynamic properties of the brain network via temporal variations of these distinct spatial states. The number of spatial states was set to 4, in line with Iraji et al. (Iraji et al., 2019a). For each brain network, k-means clustering was applied on the 45,684 (423 subjects × 108 windows) dCMs of the brain network. K-means clustering was repeated 100 times with different initializations using the k-means++ technique to increase the chances of escaping local minima (Arthur and Vassilvitskii, 2007). The correlation distance metric was used to measure the similarity between data points (i.e., the dCMs), as it is more effective in the detection of spatial patterns irrespective of voxel intensities. Using tempo-
ral profiles of the spatial states, the mean dwell time (DT), that is the average of the amount of time that subjects stay in a given state once entering that state), the fractional occupancy (FO), that is the total percentage of time that subjects spent in a given state, and the total transition number (TN), that is the total number of transitions among states performed by subjects for each network, were calculated for each network, as state-level dynamic indexes to summarize dynamic properties of each network.

2.6. Static functional network connectivity (sFNC) analysis

MOO-ICAR preprocessing was used a to estimate individual networks (Iraji et al., 2019a). Back-reconstruction step considered the estimation of subject-specific networks and their related time courses based on the selected 3 networks (DMN, LN, and SN) (Iraji et al., 2019a; Salman et al., 2019). Statistical analysis was then performed using SPM12, as follows: (1) between-group comparison (presymptomatic GRN carriers vs healthy controls), considering age, gender, site, FD Powers and insular volume (as % of TIV) as nuisance variables (p < 0.001 uncorrected for multiple comparisons and p < 0.05 family-wise error whole-brain); (2) multiple regression to assess the relationship between imaging variables and age at expected symptom onset (EYO) or neuropsychological tests, covarying for gender, age, FD Powers, site and insular volume (as % of TIV), as appropriate (p < 0.001 uncorrected for multiple comparisons and p < 0.05 FWE whole-brain).

Furthermore, on the whole group of subjects (presymptomatic GRN carriers and healthy controls) brain connectome was calculated to assess between-network connectivity. A connectogram was reported to show the correlations among considered networks (DMN, LN, and SN) using bezier curves and thumbnails of spatial maps.

2.7. Statistical analyses

Comparisons of demographic and clinical characteristics were assessed by Student’s t-test for continuous variables and χ² test for categorical variables.

A univariate general linear model (GLM) was adopted to study the main effect of group (GRN vs. HC) considering age, gender, FD, site and insular volume (as % of total intracranial volume [TIV]) as nuisance variables, and corrected for multiple comparisons (Benjamin and Hochberg, 1995).

Partial correlation analyses were used to assess the relationship between imaging variables and age at expected symptom onset (EYO) or neuropsychological tests, covarying for gender, age, FD Powers, site and insular volume (as % of TIV), as appropriate. A multiple stepwise regression analysis was run to predict EYO from gender, site, FD Powers, insular volume (as % of TIV) and dynamic indexes (separate analyses were performed for DT and FO) significantly correlated with EYO in partial correlation analysis.

All the statistical analysis was performed using IBM SPSS Statistics 22.0 (Chicago, USA) and statistical significance level set at p < 0.05.

3. Results

3.1. Participants

Four-hundred twenty-three participants were included in the present study, namely 141 with a pathogenic mutation in the GRN gene (age = 45.9 ± 11.9 years, female = 63.8%) and 282 noncarrier first-degree relatives, who therefore acted as controls within the study (age = 46.5 ± 13.2 years, female = 57.4%) (see Table 1 for demographic and clinical characteristics).

<table>
<thead>
<tr>
<th>Variable</th>
<th>HC N = 282</th>
<th>GRN N = 141</th>
<th>p-value⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.5 ± 13.2</td>
<td>45.9 ± 11.9</td>
<td>0.650</td>
</tr>
<tr>
<td>Female, % (number)</td>
<td>57.4 (162)</td>
<td>63.8 (90)</td>
<td>0.207⁵</td>
</tr>
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<td>Education (y)</td>
<td>14.3 ± 3.3</td>
<td>14.6 ± 3.5</td>
<td>0.395</td>
</tr>
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<td>Years from expected onset (y)</td>
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<tr>
<td>Framewise displacement (FD)</td>
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<td>Insular volume (% of TIV)</td>
<td>0.76 ± 0.07</td>
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<td>0.540</td>
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Cognitive assessment

| TMT A (sec) | 27.7 ± 12.5 | 28.5 ± 10.1 | 0.318⁶ |
| TMT B (sec) | 66.7 ± 33.7 | 65.9 ± 31.2 | 0.882⁶ |

Results are expressed as mean ± standard deviation, otherwise specified. Key: GRN, Granulin; HC, Healthy Controls; TMT, Trail Making Test; TN, total intracranial volume

⁴ Student t-test unless otherwise specified; ⁵ Chi-Square test; ⁶ univariate General Linear Model corrected for age and gender.

3.2. Assessing the spatial chronnectome

We first assessed time-varying information of each brain network into spatial patterns, termed spatial states, in the whole group of participants. For each brain network, we identified four distinct spatial states (from 1 to 4). Each spatial state consists of a hub, expected to be part of the referral network all the time, and other brain regions that selectively differentiate the brain network in the different spatial states. Additionally, anticorrelative connections may be identified in different segments of time in each spatial state, further underlying the existence of extra regions associated with specific networks at different moments in time.

For the purpose of the present study, we focused on the spatial dynamics within the DMN, the LN, and the SN, mainly involved in FTD pathology.

3.2.1. Default mode network (DMN)

As previously demonstrated (Iraji et al., 2019a), each of the four spatial states of the DMN are defined by posterior cingulate cortex hub, with spatial state 1 representing the “classical” already described DMN with the frontal cortex hub, that was substantially reduced in spatial state 2 (see Fig. 1 hot colour); moreover, spatial state 2 presents anti-correlative connections with the insula (see Fig. 1 cold colours). The spatial state 3 of DMN is characterised by reduced activation of posterior cingulate cortex hub and the lack of frontal region activation, as compared to “classical” DMN, while sensorimotor and occipital areas anticorrelated to DMN in spatial state 4 (see Fig. 1 cold colours).

3.2.2. Language network (LN)

The LN is overall characterised by parietal and posterior cingulate hub, bilaterally, clearly depicted in spatial state 1. Spatial state 2 is defined by greater tempo-parietal network activation and selective temporal pole activation along with anticorrelative connections with the bilateral frontal cortex; a more pronounced activation of the parietal and posterior cingulate hub represents spatial state 3, while an even more pronounced bilateral activation of temporo-parietal hub is the signature of spatial state 4 (see Fig. 2).

3.2.3. Salience network (SN)

The four spatial states of the SN are characterized by the frontal hub, with more pronounced signal in the insula region, bilaterally, in spatial state 1, and with anticorrelative connections with DMN regions in spatial state 1, 3 and 4 and with anticorrelative connections within medial frontal regions in spatial state 3 and 4. Notably,

Table 1 Demographic characteristics of included subjects

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anticorrelation with DMN was more pronounced in spatial state 4. Spatial state 2 is characterised by “classical” already described SN, with no extra regions of co-activation (see Fig. 3).

3.3. Dwell time (DT), fractional occupancy (FO) and total transitions number (TN) in presymptomatic GRN mutation carriers as compared to HC

We therefore analysed DT, FO, and TN, for each of the considered networks, in presymptomatic GRN as compared to HC. The mean DTs in GRN and HC groups are reported in Table 2, whereas FO and TN mean values are reported in Supplementary Table 1.

3.3.1. Default mode network (DMN)

When we considered DMN, presymptomatic GRN mutation carriers spent more time (DT) in spatial state 2, namely the spatial state with anti-correlative connections with the insula bilaterally ($p < 0.05$, FDR-corrected, see Fig. 1). Furthermore, considering FO, presymptomatic GRN carriers stayed for a lesser extent in spatial state 4 (FO) ($p < 0.05$, FDR-corrected, see Supplementary Table 2). We did not find any significant differences in TN.

3.3.2. Language network (LN)

When we considered LN, presymptomatic GRN mutation carriers spent more time (DT and FO) in spatial state 4, the spatial state with the more pronounced activation of the parietal hub and lacking the temporal pole activation ($p < 0.05$, FDR-corrected, see Fig. 2 and Supplementary Fig. 1). Moreover, limited to FO, presymptomatic GRN carriers spent less time in spatial state 2 ($p < 0.05$, FDR-corrected, see Supplementary Table 2). We did not find any significant differences in TN.

![Fig. 1. Spatial states of the default mode network (DMN) and mean dwell time differences between presymptomatic GRN mutation carriers and healthy controls. The spatial states of the default mode network: Hot and cold colours represent positive and negative associations to the default mode, respectively. Significant dwell-time differences between groups were reported ($p < 0.05$ FDR-corrected). Maps of each spatial states are displayed on a standardized axial T1 MRI template, z-axis coordinates are reported under each slice. DMN = default mode network; GRN = presymptomatic granulin mutation carriers; HC = healthy controls.](image-url)
3.3.3. Salience network (SN)

When we considered the SN, GRN mutation carriers spent more time (DT and FO) in spatial state 1, the one with the most pronounced activation of the insula region, and less time (DT and FO) in spatial state 4, one of the states associated with anti-correlative connections within regions belonging to DMN (frontal, parietal regions bilaterally, posterior cingulate cortex), as compared to HC (p < 0.05, FDR-corrected, see Fig. 3 and Supplementary Fig. 1).

3.3.4. Correlation between estimated years from expected symptom onset (EYO) and dwell time (DT), fractional occupancy (FO) and total transitions number (TN) in GRN mutations carriers

Correlation between DT, FO and TN of spatial states and EYO was analysed. For DT, we found significant correlations between (1) DT of DMN spatial state 2 and EYO, the longer the time spent in DMN spatial state 2, the closer the disease onset (r = 0.236, p < 0.05 FDR-corrected), (2) DT of LN spatial state 3 and EYO, the longer the time spent in LN spatial state 3 the further the disease onset (r = -0.190 p < 0.05 FDR-corrected) and, (3) DT of LN spatial state 4 and EYO, the longer the time spent in LN spatial state 4, the closer the disease onset (r = 0.286 p < 0.05 FDR-corrected). No significant association between DT and EYO for SN spatial states was observed. Regression analyses identified LN spatial state 4 (B = 0.804, β = 0.214, p = 0.016), as the best predictors of EYO in presymptomatic GRN mutation carriers.

When we considered FO, a comparable pattern of correlations were found between (1) FO of DMN spatial state 2 and EYO (r = 0.272, p < 0.05 FDR-corrected), (2) FO of LN spatial state 3 and EYO (r = -0.205 p < 0.05 FDR-corrected) and (3) FO of LN spatial state 4 and EYO (r = 0.332 p < 0.05 FDR-corrected). No significant association between FO and EYO for SN spatial states was observed.

Regression analyses identified LN spatial state 4 (B = 8.925, β = 0.236, p = 0.006), as the best predictors of EYO in presymptomatic GRN mutation carriers.

To further elucidate the role of LN state 4 in predicting EYO, regression analysis demonstrated that an increase in DT of one second was associated with a closer clinical onset equal to one month (B = 0.084, 30.66 days). Moreover, setting a cut-off value of DT equal to 60 seconds (for details see Supplementary Fig. 2), GRN carriers with higher values (18 subjects, mean value = 97.3 ± 15.7 seconds) vs GRN carriers with lower values (123 subjects, mean value = 14.4 ± 13.6 seconds) showed a significantly different EYO (-6.9 ± 9.2 years vs. -15.1 ± 12.2 years, p = 0.02, applying univariate GLM corrected for gender, site, FD Powers and insular volume as nuisance variables).

3.4. Correlation between neuropsychological assessment and dwell time (DT), fractional occupancy (FO) and total transitions number (TN) in GRN mutations carriers

To further explore the correlation between DT, FO, TN and cognitive performances, we considered tests demonstrated to be altered already before EYO 23, tapping executive functions and processing speed (Trail Making Test, part A and B) and naming (Boston Naming Test) (see Table 1 for mean scores).

A significant correlation was found between DT and FO of SN spatial state 1 and TMT part A (r = 0.235 and r = 0.286, respec-
Fig. 3. Spatial states of the salience network (SN) and mean dwell time differences between presymptomatic GRN mutation carriers and healthy controls. The spatial states of the salience network. Hot and cold colours represent positive and negative associations to the salience network, respectively. Significant dwell-time differences between groups were reported (p < 0.05 FDR-corrected). Maps of each spatial state are displayed on a standardized axial T1 MRI template, z-axis coordinates are reported under each slice. GRN, presymptomatic granulin mutation carriers; HC, healthy controls; SN, salience network.

oppositely, both p = 0.05 FDR-corrected), the longer the time spent in SN spatial state 1 the worse the score at TMT. For DMN spatial state 2, a significant correlation between FO and TMT part A was reported (r = 0.256, p = 0.05 FDR-corrected).

No significant correlations with the remaining neuropsychological tests were found. Moreover, no significant findings for TN were reported.

3.5. Static functional network connectivity - sFNC

When sFNC was assessed, the brain connectome (connectogram) showed a significant negative correlation between DMN and SN in the whole group of subjects, and with a lesser extent, a negative correlation between SN and LN and a positive correlation between LN and DMN (Supplementary Fig. 3, Panel B).

Within-network connectivity (between presymptomatic GRN carriers and healthy controls) of the three considered networks (DMN, LN, and SN) (Supplementary Fig. 3, Panel A) showed no significant differences at the pre-established thresholds (p < 0.001 uncorrected). Nonetheless, multiple regression analyses (to assess the potential relationship between sFNC and EYO as well as with cognitive performances) did not show any statistically significant cluster at the pre-established threshold (p < 0.001 uncorrected).

4. Discussion

The presymptomatic phase of neurodegenerative diseases lasts many years, with progressive modifications and potential compensative mechanisms potentially counteracting the ongoing pathological process. In the present study, we have examined the earliest brain changes in subjects with highly penetrant GRN mutations, and we have demonstrated a complex and dynamic network reorganization occurring before the onset of clinical symptoms.

We conducted a three steps study design: (1) firstly, we selected three networks of interest, namely the SN and the LN, as GRN-related disease usually presents with behavioural variant FTD or non-fluent primary progressive aphasia (Gorno-Tempini et al., 2011; Rascovsky et al., 2011), and the DMN, as previously demonstrated to be involved in FTD (Borroni et al., 2012; Lee et al., 2019; Zhou et al., 2010); (2) we applied an advanced spatial chronnectome approach (Iraji et al., 2019a), that allowed us to evaluate not only static FNC (i.e., SN, LN, DMN) but also to assess the distinct conformational spatial states of each individual network and their dynamic patterns over time (i.e., spatial state 1–4); and (3) we computed mean dwell times for each considered network, namely the time each subject spends in each spatial state, in GRN mutation carriers versus noncarriers.

One of the main findings of the present study is that, in presymptomatic disease stage, GRN mutation carriers significantly spend more time in those spatial states characterised by activation of cortical regions that will be involved in the symptomatic stages of disease (Rohrer et al., 2015). As compared to controls, GRN mutation carriers spent more time in spatial state 4 of the LN, characterised by greater activation of parietal cortex, as compared to the other spatial states of the LN, and in spatial state 1 of the SN, characterised by greater activation of the insula, as compared to the other spatial states of the SN. Indeed, it might be argued that the selective or the main
involvement of either insula or parietal regions might drive the development of clinical phenotype, namely behavioural variant FTD or nonfluent primary progressive aphasia.

Conversely, in line with previous literature data (Borroni et al., 2012; Dopper et al., 2013; Feis et al., 2019; Lee et al., 2019; Enrico Premi et al., 2014b; Premi et al., 2016), the analysis of sFNC between GRN mutation carriers and healthy controls did not yield any significant result. The lack of significant results of sFNC is somehow not surprising as it considers the mean overall rs-MRI signal of each network, in a disease stage with substantial absence of structural brain changes (Borrego-Écija et al., 2021).

The second main result of the present work is that the dynamic interplay between DMN and SN shows substantial changes in the presymptomatic stages of GRN disease. We reported that GRN mutation carriers spent more time in DMN spatial state 2, where DMN is anticorrelated with bilateral insular regions belonging to SN, and spent less time in SN spatial state 4, where SN is anticorrelated with all the nodes belonging to DMN, with an imbalance of the physiological relationship between DMN and SN.

Literature data on static between-networks’ connectivity (connectogram) (Allen et al., 2011; Fox et al., 2005; Uddin et al., 2009) (as well as the present work, Supplementary Fig. 1B) have already demonstrated the presence of significant negative correlations (anticorrelations) between SN and DMN, with an antagonistic interaction during social and self-related cognitive activities (Seeley et al., 2007).

Indeed, the present analysis, allowing the identification of regions not primarily belonging to the referral hub of the network, suggests a paradoxical behaviour of presymptomatic GRN disease with a widespread whole-brain connectivity breakdown of at-distance brain networks and derangement of the competitive relationships between SN and DMN (Tognoli and Kelso, 2014). Once again, GRN mutation carriers spent more time in those networks with greater engagement of regions involved in symptomatic disease, i.e., regions belonging to the SN and the insula.

The ability to measure the intrinsic functional architecture of the brain has grown exponentially over the last two decades (White and Calhoun, 2019). The assumption at the basis of spatial chronnectome is that connections within the brain can differentially fire between different regions at different times, and these differences can be quantified (Saha et al., 2021). Indeed, this is the first study assessing spatial chronnectome in presymptomatic monogenic FTD and in preclinical dementia in general, thus whether network dynamic connectivity reorganization acted to increase brain efficiency or is an early feature of Granulin haploinsufficiency is yet to be clarified (Lee et al., 2019). However, changes within the insula and parietal cortex have been described as the earliest signature of GRN disease (Cash et al., 2018; Lee et al., 2019; Panman et al., 2021; Rohrer et al., 2015), suggesting that the selective activation of functional connectivity of these brain regions might contribute to the maintenance of cognitive functions. The significant relationship between estimated years from expected symptom onset and spatial states’ dwell times further strengthens this hypothesis, the closer the onset of symptoms the longer the time spent in the spatial states with increased insula and parietal cortex connectivity (i.e., DMN spatial state 2 and LN spatial state 4); on the other side, the further estimated years from expected symptom onset were associated with longer time spent in spatial states resembling the “classical” hubs, with no anti-correlative extra-regions activation within the insula (i.e., DMN spatial state 3) and less activation of parietal cortex (i.e., LN spatial state 1).

Furthermore, TMT-A (Bowie and Harvey, 2006), directly correlated with the time spent in SN spatial state 1. As an index of preprocessing speed skill, TMT-A is one of the early cognitive markers in preclinical FTD (Rohrer et al., 2015). The absence of a significant correlation between TMT-A scores and meta-state measures in healthy controls further supports the idea that this finding is not age-driven but mutation-driven (data not shown).

The current study has a number of limitations. First, we considered only GRN mutations and the assessment and comparison with other monogenic FTD disorders should be considered in future work as well as the effect of different mutations within the same gene and the heterogeneity of clinical phenotypes. In addition, our analysis included the estimated age at onset, but we recognize that possible biases and possible discrepancies across mutations and families may occur (Moore et al., 2020). Moreover, we recognize possible variance in acquisition protocols in this multi-site neuroimaging study, even though a careful harmonization of sequences and data was carried out to reduce differences across scanning platforms. In particular, unstable wakefulness with the tendency to drift into sleep can affect fMRI acquisition (Tagliazucchi and Laufs, 2014; Wang et al., 2017). Recently, arousal fluctuations were linked to global waves of activity propagating throughout the brain, potentially contributing to fMRI signal fluctuations (Raut et al., 2021). Even if an objective measurement of arousal fluctuation was not available for our sample, during fMRI site harmonization the majority of subjects (<85%) were cropped to 140 timepoint (as described in details in the methods section), discharging the last 60 timepoints (that can be considered as the most critical part of fMRI acquisition with regard to arousal problems) (Allen et al., 2018; Damaraju et al., 2020; Tagliazucchi and Laufs, 2014; Wang et al., 2017). Finally, still unresolved issues regarding the application of dynamic functional connectivity analyses to resting fMRI data may limit the insights that can be gained from this promising new research area (Jurie et al., 2020).

In conclusion, presymptomatic GRN disease presents a complex perturbation of spatial chronnectome, detectable at whole-brain and at a network-level, despite the absence of sFNC abnormalities. Presymptomatic GRN disease spent more time in those networks primarily affected by earliest neuropathological changes (Gass et al., 2006) and presented an imbalance of the physiological competitive relationship between the DMN and the SN. Spatial chronnectome, evaluating both the spatial and temporal changes of brain network connectivity, provides a more sophisticated evaluation of the earliest disease changes leading to disease onset, and may help in understanding the possible causative mechanisms in GRN disease.

Disclosure statement

Dr Graff reported receiving grants from the Swedish Research Council JointProgramme–Neurodegenerative Disease Research GENFI-prox domain registration no. 2019-02248, the SwedishResearch Council Joint Programme–Neurodegenerative Disease Research Prefrontal domain registration no.2015-02926, the Swedish Research Council Dnr 208-02754, the Schörling Foundation Swedish FTD Initiative, theSwedish Alzheimer Foundation, the Swedish Brain Foundation, the Region Stockholm ALF-project, the KarolinskaInstitutet Doctoral and StratNeuro, and from the Swedish Dementia Foundation during the conduct of the study. Dr Maselli reported receiving grants from the Canadian Institutes of Health, the Cambridge Trust, the OntarioBrain Institute, the Weston Brain Institute, the Roche Clinical Trial, the Washington University Clinical Trial, and theAlector Clinical Trial; and personal fees from Arkuda Therapeutics Advisory Board, the Ionis Advisory Board, HenryStewart Talks Royalties, Alector Advisory Board, and Wake Life Sciences Advisory Board outside the submittedwork. Dr Rowe reported receiving grants from the National Institute for Health Research, Wellcome Trust, Janssen, AZ Medimmune, Lilly, and Medi-
cal Research Council; and personal fees from Biogen, Asceneuron, UCB, Althira,Astex, and SVHealth outside the submitted work. Dr Rowe also reported serving as Trustee for the Progressive Supranuclear Palsy Association, Darwin College, and Guarantor of Brain; and reported serving as an editor of Brain. Dr Le Ber reported receiving funding from the program “Investissements d’avenir” and from Agence Nationale de la Recherche/Direction Générale de l’Offre de Soins; serving as a member of the advisory board for PrevailTherapeutics; and receiving research grants from Agence Nationale de la Recherche, Direction Générale de l’Offre de Soins, Programme Hospitalier de Recherche Clinique, Vaincre Alzheimer Association, ARSla Association, Fondation Plan Alzheimer, and PRTS PrevDe-

mALS; personal fees from Prevail Therapeutics; and grants from Programme Hospitalier de Recherche Clinique FTLD exome, Programme Hospitalier de Recherche Clinique PredictPGRN, and ANR-10-IAIHU-06 outside the submitted work. Dr Sanchez Valle reported receiving grants from Fundación Marató de TV3 and personal fees from Wave Pharmaceuticals for participation in advisory board meetings and lonis for participation in advisory board meetings outside the submitted work. Dr Moreno reported receiving grants from Tau Consortium outside the submitted work. Dr Synofzik reported receiving personal fees from Actelion Pharmaceuticals and Orphanzyme outside the submitted work. Dr Santana reported receiving grants from GENFI and personal fees and travel funds from commercial sponsors outside the submitted work. Dr Levin reported receiving grants from Munich Cluster of Systems Neurology (SyNergy) and personal fees from ModagGmbH, Bayer Vital, Roche, Axon Neuroscience, Thieme medical publishers, and W. Kohlhammer GmbH medical publishers; and nonfinancial support from Abbvie outside the submitted work. Dr Otto reported receiving grants from BMBF during the conduct of the study. Dr Ghi-
doni reported receiving grants from the Italian Ministry of Health during the conduct of the study. Dr Rohrer reported performing medical advisory board work for AlecTor, Wave Life Sciences, and Prevail Therapeutics outside the submitted work. No other disclo-
sures were reported.

Acknowledgements

We thank our participant volunteers and their families for their participation; the radiographers/technologists and research nurses from all centers involved in this study for their invaluable support in data acquisition.

This work is supported by JPNF grant “GENFI-prox” (to MS, JvS, MO, CG, JR, and BB), the Centre d’Investigation Clinique (ICM, France), the Centre pour l’Acquisition et le Traitement des Images platform (CATI, France), the UK Medical Research Council, and the Canadian Institutes of Health Research as part of a Centres of Excellence in Neurodegeneration grant, a Canadian Institutes of Health Research operating grant, Fundació Marató de TV3, Spain.

Supplementary materials


Appendix – GENFI consortium

- Sónia Afonso Instituto Ciencias Nucleares Aplicadas a Saúde, Universidade de Coimbra, Coimbra, Portugal
- María Rosario Almeida Faculty of Medicine, University of Coimbra, Coimbra, Portugal
- Sarah Anderl-Straub Department of Neurology, University of Ulm, Ulm, Germany
- Christin Andersson Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
- Anna Antonell Alzheimer’s disease and Other Cognitive Disor-
ders Unit, Neurology Service, Hospital Clinic Barcelona, Spain
- Andrea Arighi Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
- Mircea Balasa Alzheimer’s disease and Other Cognitive Disor-
ders Unit, Neurology Service, Hospital Clinic, Barcelona, Spain
- Myriam Barandiaran Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain; Neuroscience Area, Biodonostia Health Re-

search Institute, San Sebastian, Gipuzkoa, Spain
- Nuria Bargalló Imaging Diagnostic Center, Hospital Clinic, Barcelona, Spain
- Robert Bartha Department of Medical Biophysics, The University of Western Ontario, London, Ontario, Canada; Centre for Functional and Metabolic Mapping, Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada
- Benjamin Bender Department of Diagnostic and Interventional Neuroradiology, University of Tübingen, Tübingen, Germany
- Maxime Bertoux Inserm 1172, Lille, France
- Anne Bertrand Sorbonne Université, Paris Brain Institute – Insti-
itut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
- Valentina Bessi Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
- Sandra Black Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
- Sergio Borrego-Ecija Alzheimer’s disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Barcelona, Spain
- Arabella Bouzigues Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
- Jose Bras Center for Neurodegenerative Science, Van Andel In-
ishitute, Grand Rapids, Michigan, MI 49503, USA
- Alexis Brice Sorbonne Université, Paris Brain Institute – Insti-
tut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
- Rose Bruffaerts Laboratory for Cognitive Neurology, Depart-
ment of Neurosciences, KU Leuven, Leuven, Belgium
- Agnès Camusat Sorbonne Université, Paris Brain Institute – Insti-
itut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
- Marta Cañada CITa Alzheimer, San Sebastian, Gipuzkoa, Spain
- Valentina Cantoni Centre for Neurodegenerative Disorders, University of Brescia, Brescia, Italy
- Paola Caroppo Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
- Miguel Castelo-Branco Faculty of Medicine, University of Coimbra, Coimbra, Portugal
- Olivier Colliot Sorbonne Université, Paris Brain Institute – Insti-
itut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
- Thomas Cope Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK
- Vincent Deramecourt Univ Lille, France
- Giuseppe Di Fede Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
- Alina Diez Neuroscience Area, Biodonostia Health Research In-
situte, San Sebastian, Gipuzkoa, Spain
- Diana Duro Faculty of Medicine, University of Coimbra, Coim-
bra, Portugal
• Chiara Fenoglio Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
• Camilla Ferrari Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
• Catarina B. Ferreira Laboratory of Neurosciences, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
• Nick Fox Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
• Morris Freedman Baycrest Health Sciences, Rotman Research Institute, University of Toronto, Toronto, Canada
• Giorgio Fumagalli Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Neurodegenerative Diseases Unit, Milan, Italy; University of Milan, Centro Dino Ferrari, Milan, Italy
• Aurélie Funkiewiez Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France
• Alazne Gabilondo Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain
• Serge Gauthier Alzheimer Disease Research Unit, McGill Centre for Studies in Aging, Department of Neurology & Neurosurgery, McGill University, Montreal, Québec, Canada
• Giorgio Giaccone Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
• Ana Gorostidi Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain
• Caroline Greaves Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
• Rita Guerreiro Center for Neurodegenerative Science, Van Andel Institute, Grand Rapids, Michigan, MI 49503, USA
• Carolin Heller Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
• Tobias Hoegen Neurologische Klinik, Ludwig-Maximilians-Universität München, Munich, Germany
• Begoña Indakoetxea Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain; Neuroscience Area, Biodonostia Health Research Institute, San Sebastian, Gipuzkoa, Spain
• Vesna Jelic Division of Clinical Geriatrics, Karolinska Institutet, Stockholm, Sweden
• Hans-Otto Karnath Division of Neuropsychology, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany
• Ron Keren The University Health Network, Toronto Rehabilitation Institute, Toronto, Canada
• Gregory Kuchinski Univ Lille, France
• Tobias Langheinrich Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK
• Thibaud Lebouvier Univ Lille, France
• Maria João Leitão Centre of Neurosciences and Cell Biology, Universidade de Coimbra, Coimbra, Portugal
• Albert Lladó Alzheimer’s disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Barcelona, Spain
• Gemma Lombardi Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
• Jolina Lombardi Department of Neurology, University of Ulm, Ulm
• Sandra Loosli Neurologische Klinik, Ludwig-Maximilians-Universität München, Munich, Germany
• Carolina Maruta Laboratory of Language Research, Centro de Estudos Egas Moniz, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
• Simon Mead MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK
• Lieve Meeter Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands
• Gabriel Miltenberger Faculty of Medicine, University of Lisbon, Lisbon, Portugal
• Rick van Minkelen Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands
• Sara Mitchell Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada
• Katrina Moore Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
• Benedetta Nacmias Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Florence, Italy
• Annabel Nelson Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London, UK
• Jennifer Nicholas Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK
• Linn Oijerstedt Center for Alzheimer Research, Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden
• Jaume Olives Alzheimer’s disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, Barcelona, Spain
• Sebastien Ourselin School of Biomedical Engineering & Imaging Sciences, King’s College London, London, UK
• Jessica Panman Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands
• Janne M. Papma Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands
• Yolande Pijnenburg Amsterdam University Medical Centre, Amsterdam Umc, Amsterdam, Netherlands
• Cristina Polito Department of Biomedical, Experimental and Clinical Sciences “Mario Serio”, Nuclear Medicine Unit, University of Florence, Florence, Italy
• Sara Prioni Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
• Catharina Pritz Neurologische Klinik, Ludwig-Maximilians-Universität München, Munich, Germany
• Rosa Rademakers Department of Neurosciences, Mayo Clinic, Jacksonville, Florida, USA
• Veronica Redaelli Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
• Daisy Rinaldi Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, AP-HP – Hôpital Pitié-Salpêtrière, Paris, France
• Tim Rittman Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
• Ekaterina Rogaeva Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada
• Adeline Rollin CHU, CNR-MAJ, Labex Distalz, LICEND Lille, France
• Pedro Rosa-Neto Translational Neuroimaging Laboratory, McGill Centre for Studies in Aging, McGill University, Montreal, Québec, Canada
• Giacomina Rossi Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy


