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Granulin mutation drives brain damage and reorganization from preclinical to symptomatic FTLD

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Abstract

Granulin (*GRN*) mutations have been identified as a major cause of frontotemporal lobar degeneration (FTLD) by haploinsufficiency mechanism, although their effects on brain tissue dysfunction and damage still remain to be clarified. In this study, we investigated the pattern of neuroimaging abnormalities in FTLD patients, carriers and noncarriers of *GRN Thr272fs* mutation, and in presymptomatic carriers. We assessed regional gray matter (GM) atrophy, and resting (RS)-functional magnetic resonance imaging (fMRI). The functional connectivity maps of the salience (SN) and the default mode (DMN) networks were considered. Frontotemporal gray matter atrophy was found in all FTLD patients (more remarkably in those *GRN Thr272fs* carriers), but not in presymptomatic carriers. Functional connectivity within the SN was reduced in all FTLD patients (again more remarkably in those mutation carriers), while it was enhanced in the DMN. Conversely, presymptomatic carriers showed increased connectivity in the SN, with no changes in the DMN. Our findings suggest that compensatory mechanisms of brain plasticity are present in *GRN*-related FTLD, but with different patterns at a preclinical and symptomatic disease stage.

Keywords: Progranulin; Granulin; Frontotemporal lobar degeneration; Preclinical; Voxel based morphometry; Resting state fMRI

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disorder characterized by behavioral abnormalities, language impairment, and deficits of executive functions as most typical clinical features (McKhann et al., 2001; Neary et al., 1998). FTLD has a strong genetic background with about 50% of patients showing a positive family history for dementia (Rademakers and Rovelet-Lecrux, 2009). FTLD pathophysiology has long been referred to the presence of mutations in microtu-

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bule-associated protein tau (*MAPT*) (Hutton et al., 1998; Spillantini and Goedert, 1998), which were first identified in families with FTLD-Parkinsonism and tau-positive inclusions, as assessed by postmortem investigation. For a decade, gene mutations for *MAPT* have been regarded as the key player for monogenic FTLD, with more than 40 mutations that have been identified so far (http://www.molgen.ua.ac.be/FTDmutations). More recently, an exciting breakthrough in the search of novel causal FTLD genes was provided by identification of loss-of-function mutations for Granulin (*GRN*) (Baker et al., 2006; Cruts et al., 2006). To be considered pathogenetic, these mutations are expected to induce a loss of 50% functional progranulin (PGRN), with a mechanism of haploinsufficiency (Rademakers and Rovelet-Lecrux, 2009). In less than 4 years, more than 60 different pathogenetic mutations for *GRN* have been reported in literature (http://www.molgen.ua.ac.be/FTDmutations). In the presence of *GRN* gene mutation, FTLD segregates in a Mendelian fashion, which is compatible with an autosomal dominant inheritance (Cruts and Van, 2008). The physiological role, as well as the effect of reduction of PGRN in the brain tissue are still largely unknown, although it has been recently suggested that PGRN might act as a neurotrophic factor (Van Damme et al., 2008). Soon after the discovery of *GRN* mutations, the nuclear protein TAR DNA-binding protein 43 (TDP-43) was identified as the major protein that plays a pathogenetic role in all FTLD cases associated with *GRN* mutations (Neumann et al., 2006). The underlying mechanism from which PGRN haploinsufficiency determines TDP-43 inclusions and, subsequently, brain damage and the clinical onset of disease is unknown. The behavioral (bvFTD) and the progressive nonfluent aphasia (PNFA) variants of FTLD are the most typical presentations in *GRN* mutation carriers, with a clinical onset in the 5th and 6th decades of life (LeBer et al., 2008; Masellis et al., 2006; Mesulam et al., 2007; Rademakers et al., 2007; Snowden et al., 2006; Van Deerlin et al., 2007).

Against this large background of improvements in characterizing FTLD genetics, the relationship between molecular aspects of pathogenesis, and structural and functional modifications of the brain tissue still remains to be clarified. Imaging genetics is a rapidly emerging field that is opening up a new landscape of discovery in neuroscience (Thompson et al., 2010). In this context, magnetic resonance imaging (MRI) has become an increasingly popular tool for human brain investigation in vivo. MRI has the unique ability to provide quantitative information on both brain tissue structure and functioning. Voxel-based morphometry (VBM) is currently regarded as a robust magnetic resonance technique suitable for assessing structural gray matter (GM) modifications in an unbiased fashion (Bozzali et al., 2008; Gorno-Tempini et al., 2004). On the other hand, resting state functional MRI (fMRI) has shown the ability to provide measures of functional brain connectivity (Biswal et al., 1995; De Luca et al., 2005; Fox and Raichle, 2007). Functional connectivity is a concept based on the evidence that different brain regions present with synchronous patterns of activity at rest. Those regions are likely to be part of common networks subserving complex brain functions. In the presence of pathology, the loss of brain connectivity may account for some cognitive disabilities, and even for some gray matter loss secondary to neuronal disconnection (Gili et al., 2011). From resting state fMRI data (i.e., fMRI time series collected while subjects lie vigilant but at rest in the scanner), several networks can be extracted at the same time in a data-driven fashion, using independent component analysis (Greicius et al., 2003). The default mode network (DMN) is by far the most extensively studied network. This is believed to be relevant for specific higher level functions, such as the working memory, mind wandering, and goal-directed behaviors (Fox and Raichle, 2007). The so-called salience network (SN) is another interesting resting state fMRI component, which is believed to be particularly informative when investigating patients with FTLD (Zhou et al., 2010). It is characterized by a more anterior anatomical distribution, and it has been related to behavioral and emotional functions. In a recent work, Zhou and coworkers (Zhou et al., 2010) have assessed changes in both the DMN and the SN in patients with FTLD and Alzheimer's disease (AD), demonstrating a reversed pattern of abnormalities in the 2 diseases. AD, as also demonstrated by others (Gili et al., 2011; Greicius et al., 2004), is characterized by a remarkable disruption of the DMN. In contrast, SN has been reported to be selectively damaged in patients with FTLD. The hypothesis of a selective involvement of a specific network in either form of dementia is supported by the observation that the pattern of atrophy typically observed in AD overlaps with the DMN in healthy subjects (Seeley et al., 2009), while the pattern of atrophy observed in FTLD overlaps with the SN (Zhou et al., 2010). Furthermore, an increase of connectivity within the DMN (Zhou et al., 2010) has been reported in FTLD, a result which could be suggestive of a compensatory mechanism. However, this interpretation would need to be corroborated by data obtained in patients at early (or preclinical) stages.

Monogenic FTLD represents a unique opportunity to investigate the pathophysiology of FTLD in its preclinical stages, thanks to the possibility to identify carriers of pathogenetic mutations as at-risk individuals. Our group has previously identified a genetically coalescent cohort of families with *GRN Thr272fs* in Italian patients with FTLD (Borroni et al., 2008a, 2008b, 2011a), and has demonstrated that these families harbor a common ancestor dating back to the Neolithic era (Borroni et al., 2011a).

Taking advantage from a unique large pedigree with different generations available, principal aims of the current study were: (1) to confirm on a larger population of subjects, structural and functional changes that have been previously

reported in patients with FTLD byZhou et al. (2010); and (2) to investigate the effect of *GRN* mutation on both brain tissue structure and function, moving from the preclinical to the manifest stage of FTLD.

2. Methods

2.1. Subjects

Sixty-one individuals, recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, at University of Brescia (Brescia, Italy), were enrolled for the current study. For the aim of the work, subjects' recruitment followed the strategy summarized in Fig. 1A. From a large pool of almost 250 patients with FTLD (all genetically characterized for the presence/absence of *GRN* and *MAPT* mutations), those identified as carriers of *GRN Thr272fs* mutation were invited to take part in the current study, and 7 of them accepted. Their asymptomatic siblings were also invited, this implying for them to undergo MRI scanning as well as genetic assessment of mutation for *GRN Thr272fs*. As previously demonstrated, all individual carriers of *GRN Thr272fs* mutation (asymptomatic or symptomatic) within a large geographic area of northern Italy, had a common ancestor who is considered as belonging to the same pedigree (Borroni et al., 2011a). Among 22 available asymptomatic siblings, 9 of them were found to be *GRN Thr272fs* mutation carriers. The remaining 13 (noncarriers of mutation) served as control group for the asymptomatic carriers. Asymptomatic mutation carriers and noncarriers were part of a genetically homogeneous population, as they all came from the same 6 families. This latter aspect makes our experimental design well controlled, as the presence/absence of *GRN Thr272fs* mutation is the only critical variable between the 2 groups. Twenty-one FTLD patients (taken from the same pool of 250 individuals), noncarriers of *GRN Thr272fs* mutation, were also enrolled in the study. They had to match for age, gender, and phenotype with the group of FTLD *GRN Thr272fs* mutation carriers. Finally, 11 healthy elderly individuals, unrelated to the patients, were also recruited and served as controls for both FTLD patient carriers and noncarriers of *GRN Thr272fs* mutation. Five subjects (all patients with FTLD, noncarriers of *GRN Thr272fs* mutation) were excluded from MRI analysis, due to the poor quality of their imaging data (movement artifacts). The remaining 56 subjects were therefore divided in the following experimental groups (Fig. 1B): (1) FTLD patient carriers of *GRN Thr272fs* mutation ($n = 7$) (S+m+); (2) FTLD patient noncarriers of *GRN Thr272fs* mutation ($n = 16$) (S+m-); (3) asymptomatic at-risk individual carriers of *GRN Thr272fs* mutation ($n = 9$) (S-m+); (4) asymptomatic individual nonmutation carriers ($n = 13$) (S-m-); (5) healthy elderly subjects ($n = 11$). Main demographic, genetic, and clinical characteristics of the entire population of subjects who were considered for MRI analyses are summarized in Table 1.

All FTLD patients met current clinical diagnostic criteria for behavioral variant frontotemporal dementia (15 cases) (McKhann et al., 2001; Neary et al., 1998) and PNFA (8 cases) (Gorno-Tempini et al., 2011), with a similar distribution between carriers and noncarriers of *GRN Thr272fs* mutation (see Table 1). To increase as much as possible the confidence of a correct diagnosis of FTLD in patient noncarriers of *GRN Thr272fs* mutation, they had to be clinically and neuropsychologically followed-up for at least 2 years, at the time of recruitment. In all FTLD patients and asymptomatic siblings (carriers and noncarriers of *PGRN Thr272fs* mutation), serum dosage of PGRN was carried out before MRI scanning. Due to the haploinsufficiency mechanism that has been shown to underlie *GRN* gene mutations, a remarkable reduction of PGRN levels is expected by definition in mutation carriers (Sleegers et al., 2009).

Finally, all patients had an extensive neurological and neuropsychological evaluation, a routine laboratory examination, and conventional brain MRI before entering this study, to rule out any potential alternative diagnosis. With respect to exclusion of signs suggestive of a concomitant cerebrovascular disease, exclusion criteria proposed in previous studies were applied in all subjects (Serra et al., 2010).

In all recruited subjects, family history for dementia was carefully investigated. As previously proposed by Goldman et al. (2005), subjects were given a "Goldman score" ranging from 1 to 4, where 1 identifies an autosomal dominant family history of dementia, 2 identifies a familial aggregation of 3 or more family members with dementia, 3 identifies 1 other first degree relative with dementia, and 4 identifies no or unknown family history for dementia.

Written informed consent (from the subject or from the responsible guardian if the subject was incapable) was obtained, for each procedure, before study initiation, including blood collection from venous puncture, genetic analysis, and MRI scanning. The work conformed to the Helsinki Declaration and was approved by the local Ethic Committee.

2.2. Neuropsychological assessment

2.2.1. FTLD patients

A neuropsychological battery was administered to each patient by 2 trained neuropsychologists 48 hours before the acquisition of the MRI. It included a general cognitive evaluation, using the Mini Mental State Examination (MMSE) (Folstein et al., 1975), and tests specific for each cognitive domain (as reported in Table 2): Raven's Coloured Progressive Matrices (Bingham et al., 1966), as a measure of reasoning; Controlled Oral Word Association Test and Category Fluency test (Isaacs and Kennie, 1973), as a measures of verbal fluency; Rey's Complex Figure Copy and Recall (Loring et al., 1990), as a measures of visuospatial abilities and episodic long-term memory; the Story Recall Test (Babcock and Levy, 1940), as

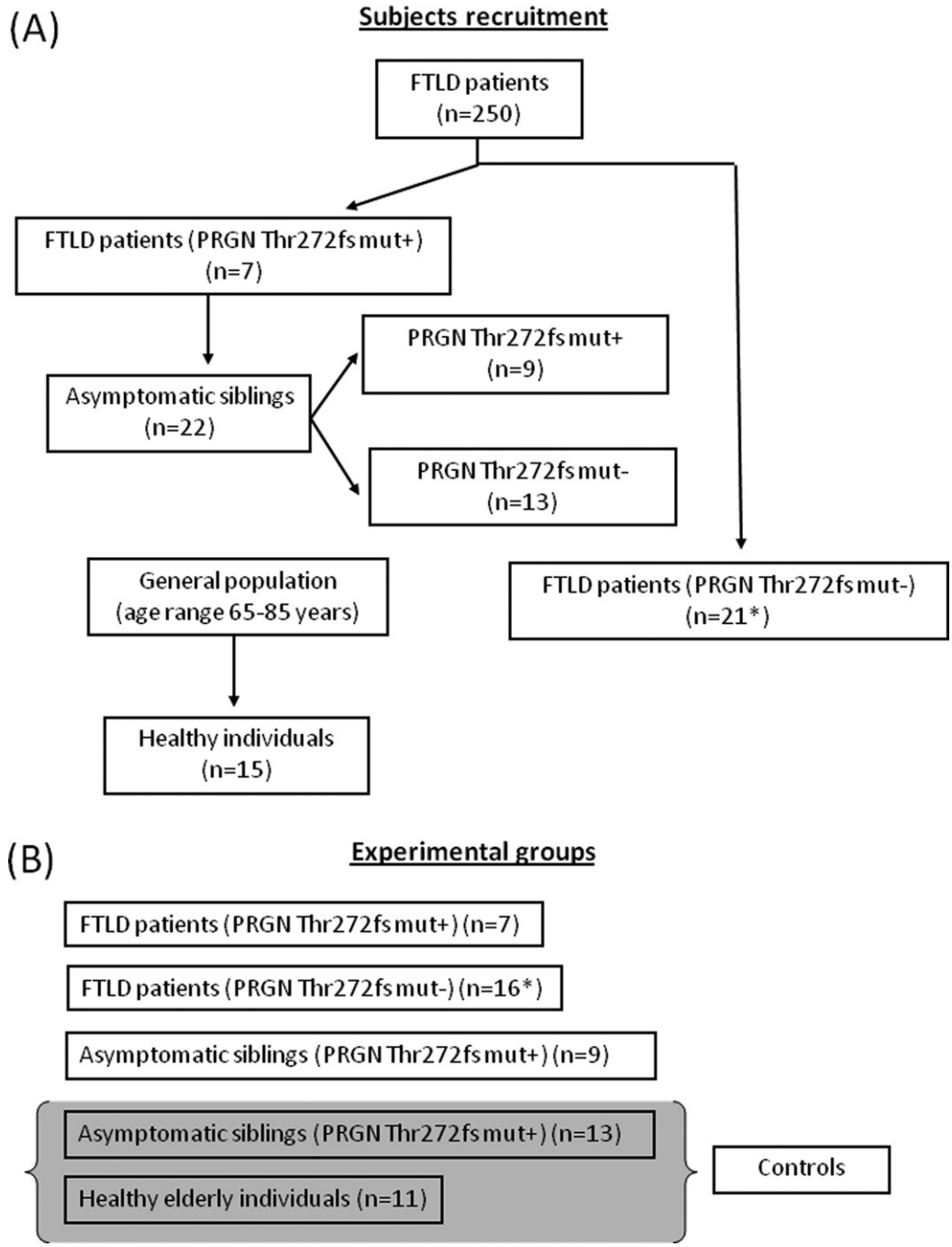

Fig. 1. Recruitment strategy used for the current study (A). From a pool of almost 250 patients with frontotemporal lobar degeneration (FTLD) (all genetically characterized for the presence of *GRN* and *MAPT* mutations), we recruited 7 patient carriers of *GRN Thr272fs* mutation. From their families, we recruited 22 asymptomatic siblings, 9 of them carriers of *GRN Thr272fs* mutation (m+), and 13 noncarriers (m-). These latter individuals were used as matched healthy controls for the asymptomatic mutation carriers. From the same pool of 250 patients, we also recruited 21 individuals with FTLD noncarrier of *GRN Thr272fs* mutation. Five of them were excluded from MRI analyses, due to low quality imaging data (*). Finally, a group of 11 healthy elderly subjects were recruited from the general population to act as matched controls for FTLD patients. Subjects were grouped as shown in (B).

a measure of verbal episodic long-term memory; the Digit Span test (Blackburn and Benton, 1957), as a measure of short-term memory; the Trail Making Test A and B (Reitan, 1958), as measures of executive functions; and the Token Test (De Renzi and Vignolo, 1962), as a measure of language comprehension. For each administered test appropriate adjustments for age and education were applied according to the Italian normative data. Instrumental Activities of Daily Living (IADL) (Lawton and Brody, 1969), and Basic Activities of Daily Living (BADL) (Sheikh et al., 1979) were also assessed.

Table 1
Main demographic, clinical characteristics, and serum PGRN dosage of studied subjects

Variable	FTLD m+ (n = 7)	FTLD m- (n = 16)	Elderly controls (n = 11)	Asymptomatic m+ (n = 9)	Asymptomatic m- (n = 13)
Age at evaluation, y	59.9 ± 4.9	66.8±7.7	61.0 ± 9.9	40.1±11.7	40.5±7.8
Gender, female % (n)	57.1 (4)	31.2 (5)	72.7 (8)	15.4 (2)	84.6 (11)
Age at onset, y	58.1 ± 5.4	64.0±7.4	—	—	—
Phenotype	4 FV; 3 PNFA	9 FV; 7 PNFA	—	—	—
Disease duration, y	1.71 ± 0.8	2.8±2.7	—	—	—
Education, y	8.0 ± 2.3	7.1±2.5	9.5±5.8	11.6±3.4	10.7±3.1
FH, % (n)	71.4 (5)	0 (0)	0 (0)	—	—
Serum PGRN, pg/mL[a]	42.4 ± 13.3	171.3±51.3	NA	43.8±7.2	164.0±30.1

No significant differences were found between patients with FTLD m+ and FTLD m-, with the exception of FH ($p < 0.001$) and serum PGRN levels ($p < 0.001$). Gender distribution was different between elderly controls and FTLD m- patients ($p = 0.041$). Asymptomatic m+ and asymptomatic m- individuals differed for gender distribution ($p = 0.003$) and serum PGRN levels ($p < 0.001$). Group comparisons were performed by Mann-Whitney test or χ^2 test (statistical threshold: $p \leq 0.05$). See text for further details.

Key: FH, family history as per Goldman's score = 1 (autosomal dominant disease); FTLD, frontotemporal lobar degeneration; FV, frontal variant; m+/-, presence/absence of *GRN Thr272fs* mutation; PGRN, progranulin; PNFA, progressive nonfluent aphasia.

[a] Serum PGRN levels was available in 19 FTLD (5 FTLD m+ and 14 FTLD m-) patients and 18 asymptomatic siblings of FTLD m+ patients (8 asymptomatic m+ and 10 asymptomatic m-).

Behavioral and psychiatric disturbances were evaluated by Neuropsychiatry Inventory (NPI) (Cummings et al., 1994), and Frontal Behavioral Inventory (FBI, Part A and B) (Alberici et al., 2007).

Table 2
Assessment of neuropsychological and behavioral profiles, and ability to perform daily living activities in FTLD patients

Test	FTLD m+ (n=7)	FTLD m- (n=16)	p value [a]
A) Neuropsychological assessment			
MMSE	16.3 ± 12.1	23.0 ± 6.4	0.278
Short story	3.2 ± 3.8	7.8 ± 4.4	0.062
Rey figure, copy	9.1 ± 11.1	17.1 ± 14.0	0.222
Rey figure, recall	1.9 ± 2.9	7.5 ± 8.0	0.106
Raven Coloured matrices	10 ± 8.1	18.9 ± 10.7	0.047
Digit span	3.7 ± 2.1	4.4 ± 1.5	0.449
Token test	17.7 ± 13.6	25.9 ± 9.0	0.149
Trail making test A	279.7 ± 218.1	166.7 ± 167.6	0.249
Trail making test B	500.0 ± 124.0	379.3 ± 170.1	0.197
Phonological fluency	10.0 ± 12.1	16.3 ± 10.2	0.175
Semantic fluency	14.0 ± 10.0	24.1 ± 13.3	0.076
Clock drawing	4.67 ± 2.7	6.4 ± 2.7	0.203
B) Behavioral assessment			
FBI A	16.9 ± 8.3	7.4 ± 5.0	0.012
FBI B	5.1 ± 4.1	2.2 ± 1.8	0.110
FBI AB	22.0 ± 11.7	9.6 ± 6.0	0.020
NPI	15.6 ± 13.3	10.94 ± 6.5	0.720
C) Assessment of performance in daily living activities			
IADL (lost)	2.86 ± 2.2	0.88 ± 1.7	0.022
BADL (lost)	1.3 ± 1.8	0.5 ± 1.3	0.175

Reported are mean ± standard deviation scores obtained by patients with FTLD m+ and those with FTLD m-. Bonferroni correction is computed as follows: $p <$ alpha/n in the case of $n = 18$ multiple comparisons and alpha = 0.05, Bonferroni correction would lead to $p = 0.003$. None of the differences between the 2 subgroups (FTLD m+ and FTLD m-) reached the statistical threshold ($p <= 0.003$). See text for further details.

Key: BADL, basic activities of daily living; FBI, frontal behavior inventory; FTLD, frontotemporal lobar degeneration; IADL, instrumental activity for daily living; m+/-, presence/absence of *GRN Thr272fs* mutation; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

[a] Mann-Whitney test.

2.2.2. Asymptomatic siblings (PGRN mutation carriers and noncarriers)

These subjects were screened using a shorter neuropsychological assessment, including the following tests: MMSE, Controlled Oral Word Association Test and Category Fluency, Wisconsin Card Sorting test (WCST), and Iowa Gambling test.

2.2.3. Healthy elderly subjects

A brief formal neuropsychological assessment was used, in each healthy elderly control, to exclude the presence of signs suggestive for the presence of subclinical cognitive impairment. They were all administered the MMSE (for which they had to report a score of 28.0 or higher) and the IADL (for which no sign of impairment was accepted).

2.3. Granulin sequencing and serum progranulin levels

Genomic DNA was extracted from peripheral blood using a standard procedure. All the 12 exons plus exon 0 of *GRN*, and at least 30 base pairs (bp) of their flanking introns were evaluated by polymerase chain reaction (PCR) and subsequent sequencing. *GRN Thr272fs* (*g.1977_1980 delCACT*) was tested as previously described (Borroni et al., 2008a, 2008b).

Serum levels of PGRN were measured, in duplicate, using commercial enzyme-linked immunosorbent assay (ELISA) kit (Human Progranulin ELISA kit; Adipogen Inc., Seoul, Korea). All tests for each sample were performed with ELISA kits of the same lot to reduce assay variability. Biological measurements were blinded to clinical diagnoses.

2.4. Statistics

SPSS package (v. 17.0, Chicago, IL, USA) was used to run statistics for group differences in demographic and clinical characteristics, and in neuropsychological, behav-

ioral, and laboratory measures. Group comparisons were assessed by Mann-Whitney test or χ^2 test, setting the statistical threshold to p values ≤ 0.05. When testing for differences in neuropsychological and behavioral characteristics (for which multiple measures are considered), Bonferroni's correction for multiple comparisons was applied.

2.5. MRI acquisition

All imaging was obtained using a 1.5 T magnetic resonance scanner (Siemens Symphony, Erlangen, Germany), equipped with a circularly polarized transmit-receive coil. In a single session, the following scans were collected from each studied subject:

(1) Dual-echo turbo spin echo (TSE) (repetition time [TR] = 2500 ms, echo time [TE] = 50 ms), to exclude the presence of macroscopic brain abnormalities, according to exclusion criteria; (2) 3D magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted scan (TR = 2010 ms, TE = 3.93 ms, matrix = 1 × 1 × 1, in-plane field of view [FOV] = 250 × 250 mm^2, slice thickness = 1 mm, flip angle = 15°); and (3) T2*-weighted echo planar (EPI) sensitized to blood oxygen level dependent (BOLD) contrast (TR = 2500 ms, TE = 50 ms, 29 axial slices parallel to anterior commisure–posterior commisure line (AC-PC) line, matrix = 64 × 64, field of view = 224 mm, slice thickness = 3.5 mm) for resting state fMRI. Blood oxygen level dependent EPI images were collected during rest for an 8-minute period, resulting in a total of 195 volumes. During this acquisition, subjects were instructed to keep their eyes closed, not to think of anything in particular, and not to fall asleep.

2.6. Image analysis

Dual echo turbo spin echo images were carefully reviewed to exclude the presence of signs suggestive of cerebrovascular disease, according to the inclusion criteria (Serra et al., 2010).

T1-weighted images from all recruited subjects were visually inspected for a qualitative assessment of macroscopic atrophy, and to check for the quality of data before carrying out a quantitative volumetric analysis.

2.7. Voxel-based morphometry (VBM)

Magnetization-prepared rapid gradient echo data were processed using the VBM protocol in Statistical Parametric Mapping 8 (SPM8; Wellcome Department of Imaging Neuroscience; www.fil.ion.ucl.ac.uk/spm/). For each subject, an iterative combination of segmentations and normalizations (implemented within the "Segment" SPM8 module) produced a GM probability map (Ashburner and Friston, 2005) in Montreal Neurological Institute (MNI) coordinates. To compensate for compression or expansion during warping of images to match the template, GM maps were "modulated" by multiplying the intensity of each voxel by the local value derived from the deformation field (Jacobian determinants) (Ashburner and Friston, 2001). All data were then smoothed using a 12-mm full width half maximum (FWHM) Gaussian kernel. Modulated and smoothed GM were analyzed in SPM8, using a full factorial design. Subjects were modeled in 5 separate groups: patients with FTLD *GRN Thr272fs* mutation carriers ($n = 7$); patients with FTLD nonmutation carriers ($n = 17$); healthy elderly subjects ($n = 11$); asymptomatic subjects nonmutation carriers (young healthy subjects; $n = 13$); and asymptomatic subjects FTLD *GRN Thr272fs* mutation carriers ($n = 9$). Additionally, age, gender, and years of formal education were added as covariates of no interest. The GM maps analysis was also adjusted for the total intracranial volume (ICV = GM volume + white matter volume + cerebrospinal fluid volume). Contrasts were designed to assess (1) the effect of symptoms and mutation in regional GM volumes by comparing FTLD patients with healthy elderly subjects, (2) the effect of mutation in preclinical stages by comparing asymptomatic subjects *GRN Thr272fs* mutation carriers against those nonmutation carriers (young healthy subjects); and (3) the interaction between the presence of symptoms and the presence of *GRN* Thr272fs mutation.

For every T-contrast, we applied family-wise error (FWE) correction for multiple comparisons, and we accepted as significant p values of less than 0.005 at cluster level.

2.8. fMRI data analysis

Resting state fMRI data were preprocessed using SPM8 for image preprocessing and statistical comparison, and the Group independent component analysis (ICA) for fMRI toolbox (GIFT, icatb.sourceforge.net/) for ICA.

For each subject the first 4 volumes of the fMRI series were discarded to allow for T1 equilibration effects. The preprocessing steps included correction for head motion, compensation for slice-dependent time shifts, normalization to the EPI template in Montreal Neurological Institute coordinates provided with SPM8, and smoothing with a 3D Gaussian Kernel with 8 mm^3 FWHM. Then, all images were filtered by a phase-insensitive bandpass filter (pass band 0.01–0.08 Hz) to reduce the effect of low frequency drift and high frequency physiological noise. ICA analysis was employed to identify 20 independent components. Briefly, group ICA for fMRI toolbox first concatenates the individual data across time, and then produces a computation of subject specific components and time courses. For all subjects grouped together, the toolbox performed the analysis in 3 steps: (1) data reduction, (2) application of the FastICA algorithm, and (3) back-reconstruction for each individual subject (De Luca et al., 2006). Results were converted to Z-scores. The 20 components were reviewed, and compared, by computing the spatial correlation coefficient, to customized templates of the SN and of the DMN. These templates were obtained from an independent sample of 28 healthy subjects (13 females, mean age 38.6 years, SD

7.5 years). This procedure was performed using the tool for spatial sorting of the components available with GIFT. Every subject's *Z*-score maps corresponding to these 2 resting state networks were used for cross-subject analyses. A random-effect analysis was carried out using a full factorial design in SPM8. As for the VBM analysis, a full factorial design was used for both SN and DMN. Subjects were divided into 5 separate groups: patients with FTLD *GRN Thr272fs* mutation carriers ($n = 7$); patients with FTLD nonmutation carriers ($n = 16$); healthy elderly subjects ($n = 11$); asymptomatic subjects nonmutation carriers (young healthy subjects; $n = 13$); and asymptomatic subjects FTLD *GRN Thr272fs* mutation carriers ($n = 9$). Age, gender, years of education, and total GM volumes (as derived by VBM analysis) were entered as covariates of no interest.

For each considered network (SN and DMN), contrasts were designed to assess (1) the effect of symptoms and mutation in functional connectivity by comparing FTLD patients with healthy elderly subjects, (2) the effect of mutation in preclinical stages of disease by comparing asymptomatic subjects *GRN Thr272fs* mutation carriers with those nonmutation carriers (young healthy controls); and (3) the interaction between the presence of symptoms and the presence of *GRN Thr272fs* mutation on the functional brain connectivity.

In patients only, we performed a multiple regression analysis to assess the presence of correlations between the MMSE (a measure of global cognition) and functional connectivity within the SN and the DMN. The analysis was adjusted for age, gender, years of education, and total GM volume. p values were considered significant if less than 0.05, after FWE correction at cluster level.

3. Results

3.1. Demographic, clinical, and laboratory characteristics of studied subjects

There was no significant difference in age ($p = 0.08$), gender ($\chi^2 = 1.05$; $df = 1$; $p = 0.31$), age of clinical onset ($p = 0.10$), disease duration ($p = 0.54$), and clinical phenotypes distribution ($\chi^2 = 0.0016$, $df = 1$; $p = 0.97$) between FTLD patients carriers and noncarriers of *GRN Thr272fs* mutation (see Table 1). As expected, FTLD carriers of *GRN Thr272fs* mutation had a higher rate of positive family history for dementia (Goldman's score = 1; 66.4%) than FTLD noncarriers (0%; $p < 0.001$) (see Table 1). Consistent with genetic assessments, the former group had significantly lower serum levels of PGRN than the latter group ($p < 0.001$) (see Table 1).

There was no significant age difference between asymptomatic subjects with and without *GRN Thr272fs* mutation. Conversely, gender distributions were different between the 2 groups. As expected, asymptomatic *GRN Thr272fs* mutation carriers had lower serum levels of PGRN than asymptomatic noncarriers ($p < 0.001$) (see Table 1).

3.2. Neuropsychological and behavioral measures, and assessment of performances in daily living activities

As reported in Table 2, the level of global cognitive impairment as well as that of functional impairment were comparable between the 2 groups of patients with FTLD. Specific neuropsychological tests and behavioral measures did not reveal any significant difference between the 2 groups.

According to inclusion criteria, all elderly healthy controls had to report MMSE scores higher than 28.0, and normal performance at IADL.

All asymptomatic subject carriers of *GRN Thr272fs* mutation reported the maximum score at the MMSE, and performed within the normal range at any other administered test. The between group comparison did not reveal any difference at any test.

3.3. MRI data

3.3.1. Visual inspection of T1-weighted images

T1-weighted images from the FTLD patient carriers of *GRN Thr272fs* mutation are shown in Fig. 2. Visual inspection revealed an asymmetric distribution of atrophy (more remarkable on the left hemisphere) in 4 out of 7 patients (subjects 1, 2, 5, and 7). Five out of 7 patients showed a more severe atrophy in the frontal lobes (subjects 1, 2, 4, 5, and 7), while the other 2 had a more posterior distribution of atrophy (subjects 3 and 6). Patients with sporadic FTLD revealed patterns of frontotemporal atrophy consistent with the diagnosis. T1-weighted images from asymptomatic *GRN Thr272fs* mutation carriers did not reveal any macroscopic abnormality, and, according to experienced radiologists were nondistinguishable from those of healthy subjects of comparable age. Consistent with aging related processes, elderly healthy subjects had (on average) larger ventricles than younger controls, in the absence of pathological changes.

As mentioned above, scans from 5 patients with sporadic FTLD revealed the presence of movement artifacts, and were excluded from VBM analysis.

3.3.2.VBM

Patients with FTLD (S+m+ and S+m-) compared altogether with healthy age matched elderly controls (ES-m-) had reduced GM volume in the left frontotemporal cortex (Fig. 3A). An additional reduction of GM volume was found in the left frontal cortex of FTLD patients *GRN* mutation carriers when compared with those without *GRN* pathogenetic mutations (S+m+ < S+m-) (see Fig. 3A).

Asymptomatic carriers of *GRN Thr272fs* mutation (S-m+) compared with asymptomatic noncarriers (S-m-) did not reveal any significant difference in regional GM volumes. The symptom by *GRN* Thr272fs mutation interaction revealed a negative effect of mutation on the GM volumes in the left insular cortex that, however, did not survive FWE correction (p uncorrected < 0.001).

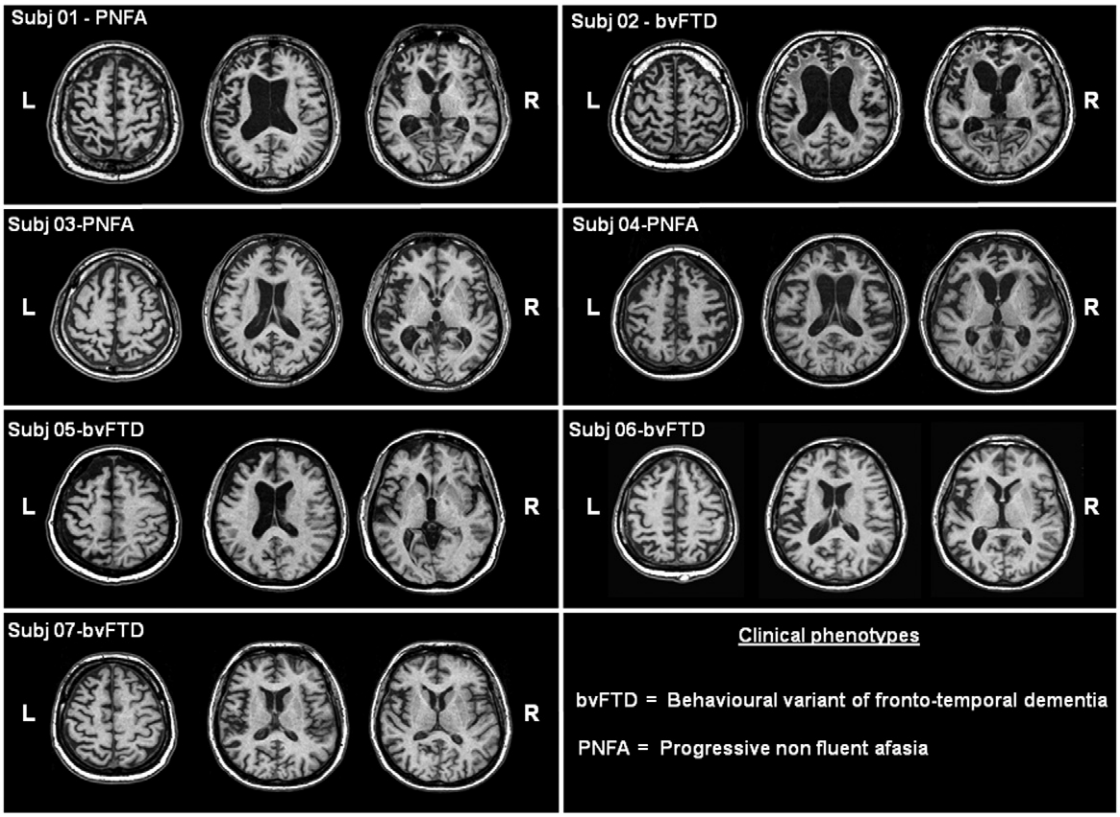

Fig. 2. Axial T1-weighted images from the frontotemporal lobar degeneration (FTLD) patients carriers of *GRN Thr272fs* mutation. Visual inspection of images reveals an asymmetric distribution of atrophy (left more than right hemisphere) in 4 out of 7 patients (subject 1, 2, 5, and 7). Frontal involvement is prominent in subjects 1, 2, 4, 5, and 7. Conversely, subjects 3 and 6 show a pattern of brain atrophy with a more posterior distribution. Abbreviations: L, left; R, right.

3.3.3. Resting state fMRI

Among the 20 components modeled in the ICA analysis, several well-known resting state networks were identified, including the SN, the DMN, the sensory-motor network, and the visual network.

The main effect of group (including all studied subjects) for the salience and the default mode network are shown in Fig. 4.

The analysis of the SN revealed 4 clusters of reduced functional connectivity in patients with FTLD compared altogether (S+m+ and S+m-) against elderly healthy controls. These clusters of reduced functional connectivity were localized in the frontal lobe of FTLD patients bilaterally (Fig. 5A). In contrast, the asymptomatic mutation carriers (S-m+) compared with age-matched young healthy controls (Y S-m-) showed a medial frontal area of increased connectivity (Fig. 5B).

The direct comparison between FTLD patients *GRN Thr272fs* mutation carriers and those nonmutation carriers revealed a reduction of connectivity in the left prefrontal cortex of the former group (i.e., S+m+ $<$ S+m-; p uncorrected $<$ 0.001) that, however, does not survive FWE correction.

The symptom by *GRN* Thr272fs mutation interaction revealed a negative effect of mutation on the functional connectivity in the left prefrontal cortex that, again, did not survive FWE correction (p uncorrected $<$ 0.001).

The analysis of the DMN revealed a significant increase of functional connectivity in the left angular gyrus of FTLD patients compared altogether (S+m+ and S+m-) with elderly healthy controls (Fig. 6A). A significant symptom by *GRN Thr272fs* mutation interaction (p-FWE cluster level corrected $<$ 0.05) was observed in the posterior cingulate cortex and in the left temporal lobe (Fig. 6B).

No significant correlation was found between functional connectivity within the SN and the DMN and patients' MMSE score.

4. Discussion

In this MRI study, we recruited a large population of patients, part of them suffering from the sporadic form of FTLD, some of them carriers of *GRN Thr272fs* mutation. Further, for the first time, we investigated a group of *GRN Thr272fs* mutation carriers in a presymptomatic stage of disease. This latter aspect is the main strength of the current

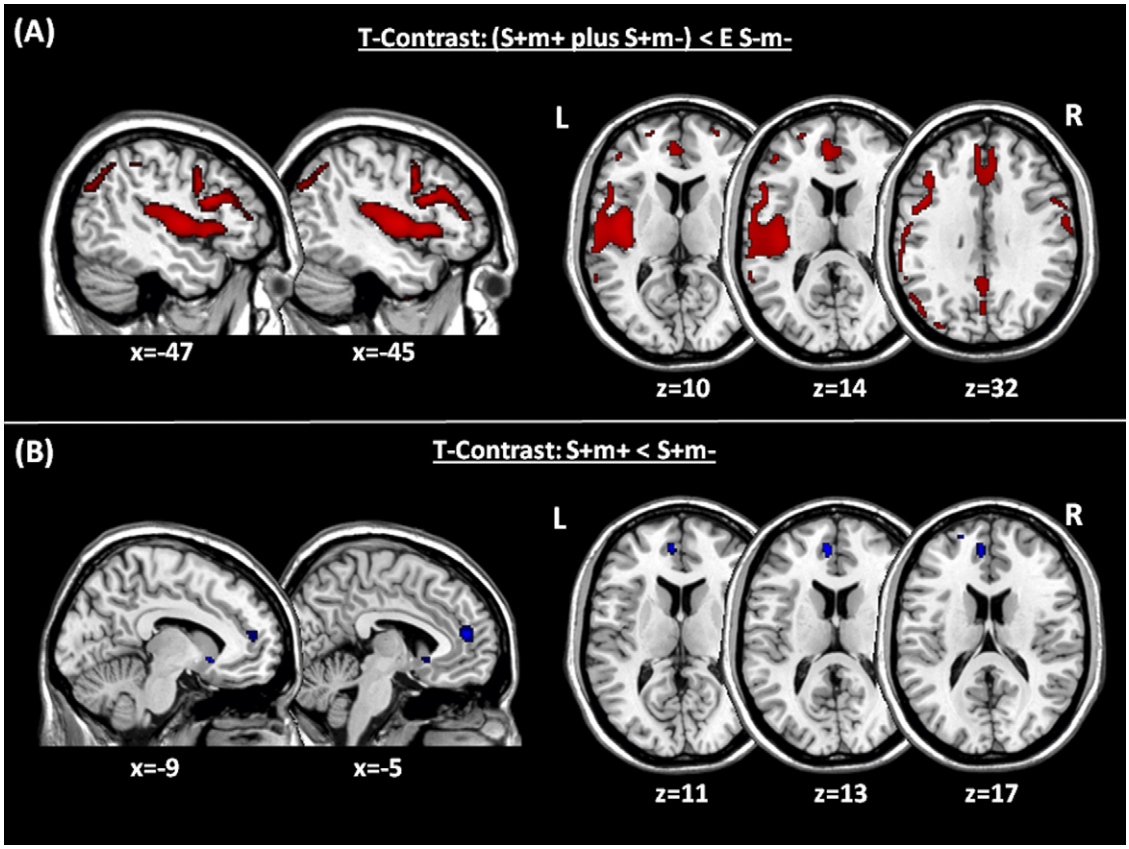

Fig. 3. VBM analysis. (A) Main effect of frontotemporal lobar degeneration (FTLD) showing an extensive pattern of gray matter (GM) atrophy which predominantly involves the left prefrontal cortex; (B) additional GM loss in the left prefrontal cortex of FTLD patients as an effect of being *GRN Thr272fs* mutation carriers. Statistical threshold: p values FWE cluster level corrected < 0.005. See text for further details. Abbreviations: ES-m-, elderly healthy subjects; FWE, family-wise error; S-m+, asymptomatic carriers of *GRN Thr272fs* mutation; S+m+, patients with FTLD carriers of *GRN Thr272fs* mutation; S+m-, FTLD patients nonmutation carriers; Y S-m-, young healthy subjects.

study, as we had the opportunity to enroll, from different nuclear kindreds, a relatively large group of family members bearing the same pathogenic *GRN Thr272fs* mutation, all descending from a common ancestor. This represents an extraordinary naturalistic experimental model to reconstruct, by advanced MRI techniques, the impact of *GRN Thr272fs* mutation on the brain tissue structural and functional characteristics.

When investigating the main effect of symptoms (regardless of presence/absence of *GRN Thr272fs* mutation), VBM analysis revealed a well defined pattern of regional GM atrophy, which mainly involved the left frontotemporal areas (see Fig. 3A). This anatomical distribution of structural brain damage is consistent with that reported by others (Beck et al., 2008; Rohrer et al., 2010), and fits well with the cognitive and behavioral aspects typically observed in FTLD. Moreover, as shown here for the first time, this damage is remarkably prominent in the presence of *GRN Thr272fs* mutation. Although, due to our selection criteria, cognitive and behavioral disabilities were not significantly different between FTLD groups (with and without *GRN Thr272fs* mutation), our findings suggest that the presence of *GRN Thr272fs* mutation may act as an independent contributor in determining brain tissue damage. This is in line with recent evidence of a worse prognosis in *GRN* mutation carriers as compared with sporadic FTLD (Borroni et al., 2011b).

Consistent with the findings recently reported by Zhou et al. (2010), our resting state fMRI analysis revealed a selective disruption of the SN in the presence of FTLD pathology (Fig. 5A). This network is believed to be implicated in behavioral functioning and emotion processing, thus accounting for some critical features of FTLD. Similarly to the distribution of GM atrophy, the involvement of the SN was also more marked in FTLD mutation carriers than in mutation noncarriers (Fig. 5A). Asymptomatic GRN mutation carriers showed an increase in functional connectivity within the SN (Fig. 5B). Despite the absence of direct associations between this change in functional connectivity and cognitive data, we can speculate that this enhancement may compensate for the effects of reduced PGRN at early stages, thus accounting for the absence of symptoms at preclinical stages.

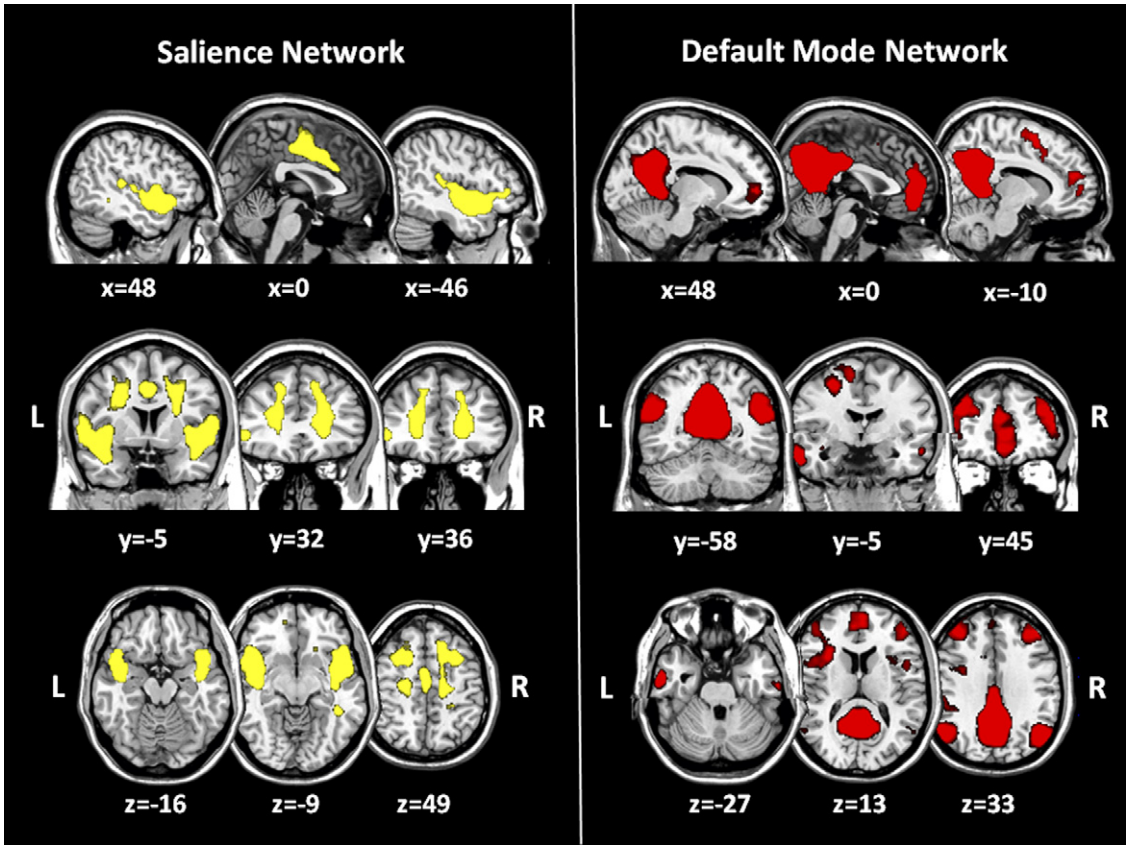

Fig. 4. Main effect of group (including all studied subjects) for the salience (left side) and the default mode network (right side). Statistical threshold: p family-wise error corrected < 0.005.

The DMN, consistent with previous findings (Zhou et al., 2010), was found to be enhanced in the presence of FTLD (Fig. 6A). Again, we were unable to demonstrate any direct association between patients' cognitive data and changes in functional connectivity within the DMN. Nevertheless, this enhanced connectivity within the DMN of FTLD patients might represent a compensation mechanism of brain plasticity in the presence of a disease that selectively targets neurons in the frontotemporal areas. Further, the enhancement of the DMN (in contrast with the disruption typically observed in patients with AD) is in line with the typical cognitive profile observed in FTLD patients, which is characterized by a relative preservation of memory functions. As observed in the VBM analysis, the presence of *GRN Thr272fs* mutation seems to make the pattern of functional brain abnormalities worse. Indeed, an area of negative group × mutation interaction was observed within the DMN (Fig. 6B). This suggests an early enhancement of connectivity at preclinical stages (*GRN Thr272fs* mutation carriers), but a reduction in FTLD patients *GRN Thr272fs* mutation carriers as compared with mutation noncarriers. This finding, taken together with the evidence of increase connectivity in the SN of asymptomatic carriers, allows us to speculate that 2 different compensatory mechanisms may coexist in FTLD brains. As reported in Fig. 7, in a preclinical stage, we observe an enhancement in the SN, which is the main target of pathology. This mechanism is likely to be temporary, and, when it fails, the symptoms become evident. At this stage, a second mechanism of compensation may be observed within the DMN.

The relationship between our current neuroimaging findings and the molecular mechanisms underlying neuronal firing patterns closer to PGRN action, are complex and still not elucidated. The *GRN* gene encodes a secreted multifunctional growth factor involved in tissue remodeling, wound repair, and inflammation (Baker et al., 2006; Cruts et al., 2006). In the brain tissue, where PGRN is expressed in both neurons and microglia, its functions have not yet been studied extensively. However, it has been recently suggested that PGRN may play a role in neuronal development and in neuronal survival (Baker et al., 2006; Cruts et al., 2006; Kleinberger et al., 2010).

As already mentioned above, more than 60 pathogenetic *GRN* mutations have been described so far, and they are all expected to cause *PGRN* haploinsufficiency (Alzheimer Disease and Frontotemporal Dementia Database; http://www.molgen.ua.ac.be/FTDmutations/). The mechanisms by which PGRN loss-of function leads to the neuropathological hallmark of *GRN*-related FTLD, namely TDP-43

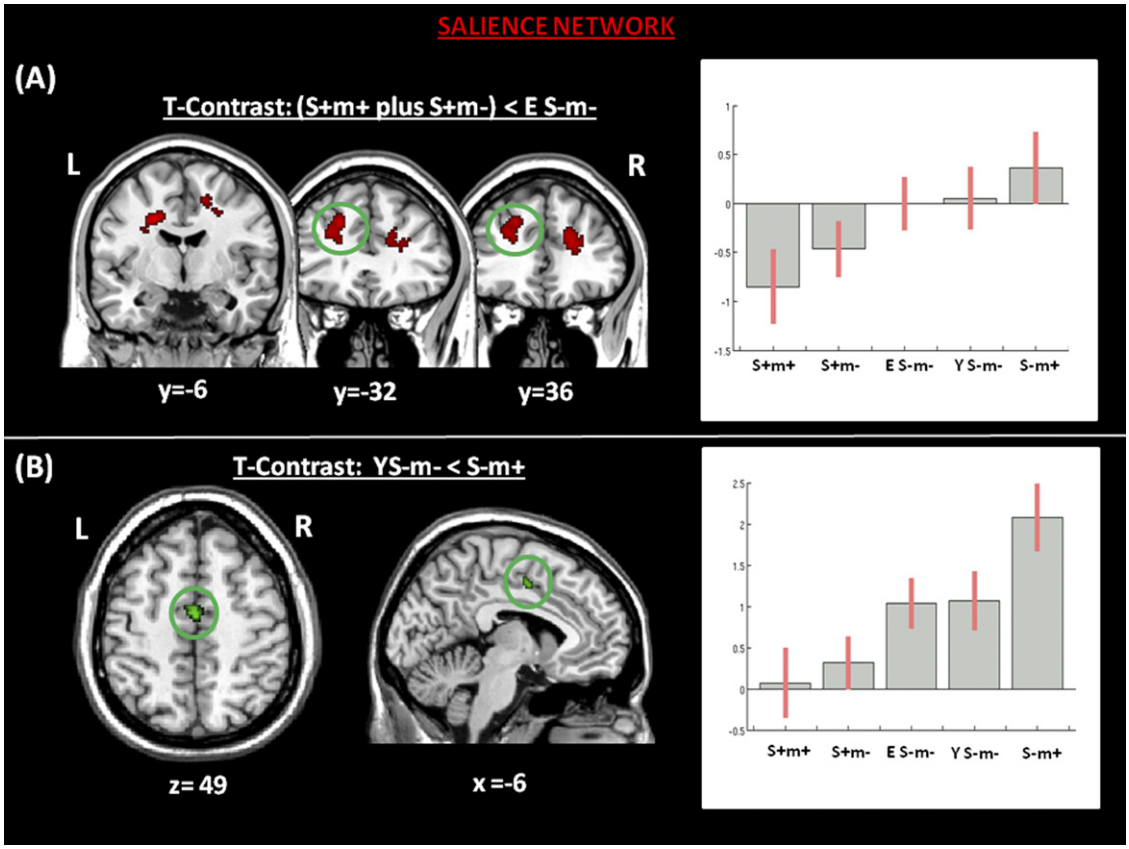

Fig. 5. Salience network functional connectivity. (A) Pattern of reduced connectivity in all patients with frontotemporal lobar degeneration (FTLD) as compared with age- and gender-matched healthy controls (main effect of symptoms), together with a plot of contrast estimates at the maximum *T* values. (B) A region of the salience network where asymptomatic carriers showed increased connectivity compared with matched healthy controls. For each panel, the green circle highlights the cluster to which the plot refers. Statistical threshold: *p* values cluster level family-wise error corrected < 0.05. See text for further details. Abbreviations: ES-m-, elderly healthy subjects; S-m+, asymptomatic carriers of *GRN Thr272fs* mutation; S+m+, patients with FTLD carriers of *GRN Thr272fs* mutation; S+m-, FTLD patients nonmutation carriers; YS-m-, young healthy subjects.

positive neuronal cytoplasmatic and intranuclear inclusions (Neumann et al., 2006), is still largely unknown.

The present study, for the first time, provides a model to characterize in vivo the brain changes occurring in cases of *PGRN* inherited disorder, from the preclinical to the symptomatic stages.

The presymptomatic subjects, carriers of *GRN Thr272fs* mutation, showed normal performances at an extensive cognitive and behavioral assessment, as further supported by the follow-up evaluation of the original group sample (Borroni et al., 2008a). Conversely, in preclinical carriers of *MAPT* mutation, deficits in language and executive function were detectable even 20 years before clinical onset (Geschwind et al., 2001). A possible explanation for these observations is that, in *PGRN* presymptomatic subjects, normal cognition is maintained by the compensatory mechanism of increased connectivity within the SN. At present, we are unable to hypothesize which possible patterns of functional connectivity might be present in the brain of asymptomatic *MAPT* mutation carriers. Future studies, including subjects with different kinds of mutation, are needed to clarify whether similar or distinct network rearrangements are modulated by different genetic defects. For example, it remains to be clarified whether the mechanisms of brain plasticity that we observed in the current study, occur exclusively in cases of "loss of function," such as in PGRN haploinsufficiency, or also in others. Another point that needs addressing, is how the human brain may cope with different molecular mechanisms, such as the abnormal tau deposition and protein gain of function observed in *MAPT* disease.

As already stated, this is the first study that used resting state fMRI to investigate asymptomatic carriers of *GRN* mutations. There is only a previous report that used VBM and diffusion tensor MRI to respectively assess changes in GM and white matter of a small group of asymptomatic carriers. Consistent with our current findings, no changes were found in regional GM volumetrics. Conversely, microscopic abnormalities were detectable in the left uncinate fasciculus and in the inferior fronto-occipital fasciculus (Borroni et al., 2008a).

This study entails some limitations. First, it was only focused on a homogeneous kindred, carrying the *GRN Thr272fs* mutation. This means that our results cannot be

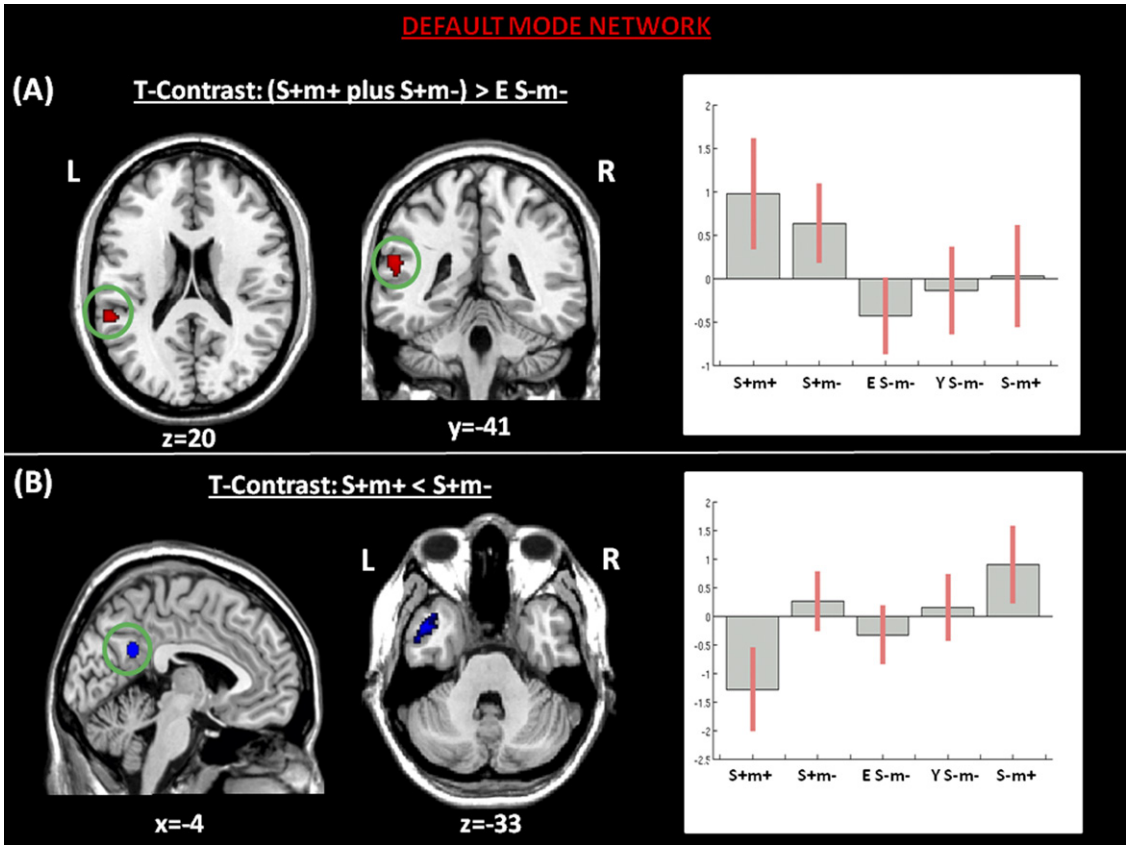

Fig. 6. Default mode network (DMN) functional connectivity. (A) Pattern of increased connectivity in all patients with frontotemporal lobar degeneration (FTLD) as compared with age- and gender-matched healthy controls (main effect of symptoms), together with a plot of contrast estimates at the maximum *T* values. (B) Areas of the DMN, in which FTLD patients *GRN Thr272fs* mutation carriers compared with FTLD patients nonmutation carriers showed reduced connectivity, and relative plot of contrast estimates in the 5 groups. For each panel, the green circle highlights the cluster to which the plot refers. Statistical threshold: *p* values cluster level family-wise error corrected < 0.05. See text for further details. Abbreviations: ES-m-, Elderly healthy subjects; S+m+, patients with FTLD carriers of *GRN Thr272fs* mutation; S+m-, FTLD patients nonmutation carriers; S-m+, asymptomatic carriers of *GRN Thr272fs* mutation. YS-m-, young healthy subjects.

generalized to all different *GRN* mutations reported in the literature. Nevertheless, as *GRN* mutations all share a common loss-of-function effect, it is likely that similar MRI changes might be found also in other pathogenetic variations. Second, in asymptomatic carriers a more detailed cognitive assessment would be needed, in order to evaluate in-depth subtle cognitive deficits, such as executive dysfunctions. Third, if the diagnostic process was relatively easy in FTLD patients bearing *GRN* mutation, autopsy confirmation in cases suffering from a nonmonogenic disease would be warranted. Nevertheless, this latter group did not represent the main focus of the current work. Moreover, the pattern of MRI changes we observed in our group of patients without *GRN*-mutation is comparable with that reported in previous studies (Beck et al., 2008; Rohrer et al., 2010), thus suggesting the accuracy of the diagnostic process. Fourth, we were unable to detect associations between measures of global cognition and the strength of connectivity in the SN and in the DMN of FTLD patients. This lack of correlation might be due to the small sample size, and needs to be further investigated in future studies on larger populations. Finally, a comprehensive work evaluating both white matter abnormalities and functional connectivity changes would be desirable.

In conclusion, this study has tried to unravel the pattern of neuroimaging abnormalities in FTLD patients, carriers, and noncarriers of *GRN Thr272fs* mutation, and in presymptomatic carriers. Understanding the *GRN*-associated preclinical changes and how the molecular process influences functional brain connectivity, is a crucial step to identify a potential target for any evidenced-based treatment for at-risk individuals, and to monitor the effects of intervention. To meet this goal, it is mandatory to establish whether *GRN* mutations lead to brain plasticity compensatory mechanisms and how these abnormalities converge into neurodegeneration in later life.

Disclosure statement

The authors disclose no conflicts of interest.

Written informed consent was obtained from the subject

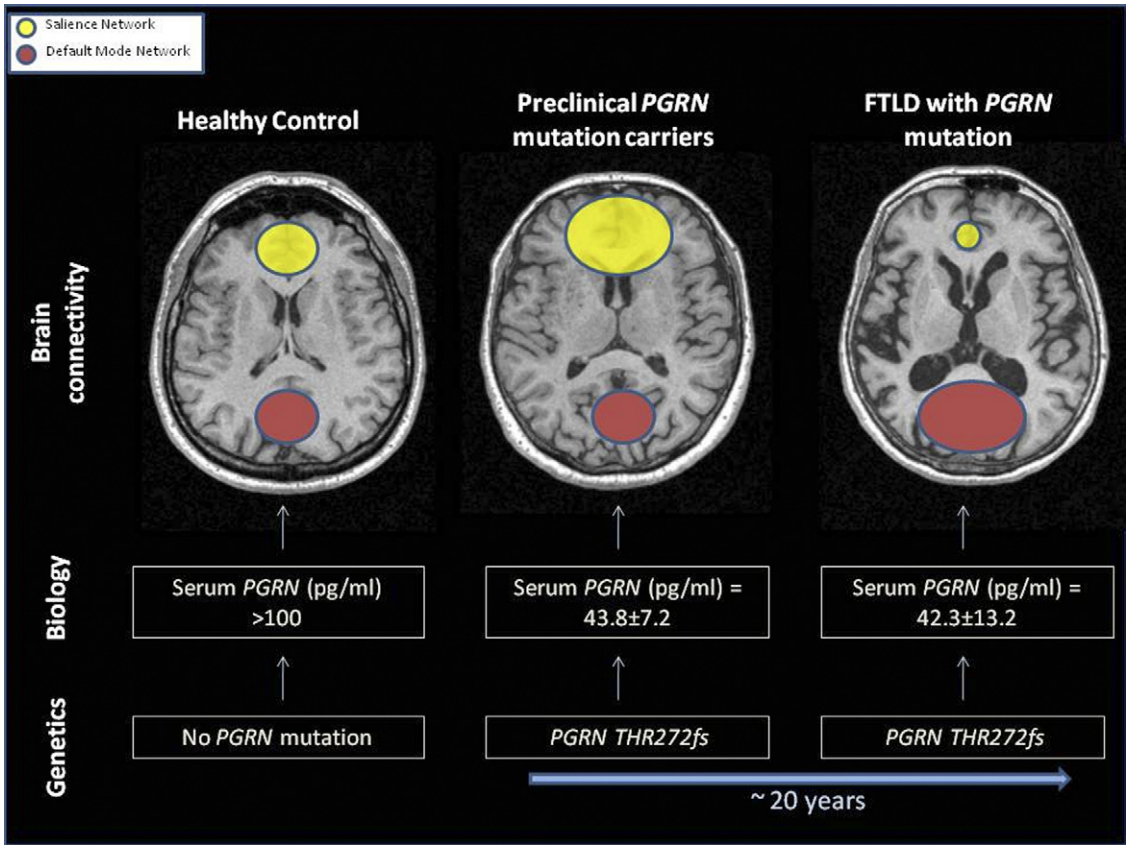

Fig. 7. Schematic representation of the brain functional connectivity changes in *GRN Thr272fs* carriers from preclinical to symptomatic stages of frontotemporal lobar degeneration (FTLD). At preclininical stages, when no gray matter (GM) loss is detectable, the salience network is enhanced while the default mode network is unchanged. After conversion to clinical FTLD, the salience network becomes disrupted and the default mode network is enhanced. See text for further details.

or from the responsible guardian if the subject was incapable. The work conformed to the Helsinki Declaration and was approved by the local Ethic Committee.

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References

Alberici, A., Geroldi, C., Cotelli, M., Adorni, A., Calabria, M., Rossi, G., Borroni, B., Padovani, A., Zanetti, O., Kertesz, A., 2007. The Frontal Behavioural Inventory (Italian version) differentiates frontotemporal lobar degeneration variants from Alzheimer's disease. Neurol. Sci. 28, 80–86.

Ashburner, J., Friston, K.J., 2001. Why voxel-based morphometry should be used. Neuroimage 14, 1238–1243.

Ashburner, J., Friston, K.J., 2005. Unified segmentation. Neuroimage 26, 839–851.

Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A.D., Rollinson, S., Cannon, A., Dwosh, E., Neary, D., Melquist, S., Richardson, A., Dickson, D., Berger, Z., Eriksen, J., Robinson, T., Zehr, C., Dickey, C.A., Crook, R., McGowan, E., Mann, D., Boeve, B., Feldman, H., Hutton, M., 2006. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 442, 916–919.

Blackburn, H.L., Benton, A.L., 1957. Revised administration and scoring of the digit PNFAn test. J. Consult. Psychol. 21, 139–143.

Beck, J., Rohrer, J.D., Campbell, T., Isaacs, A., Morrison, K.E., Goodall, E.F., Warrington, E.K., Stevens, J., Revesz, T., Holton, J., Al-Sarraj, S., King, A., Scahill, R., Warren, J.D., Fox, N.C., Rossor, M.N., Collinge, J., Mead, S., 2008. A distinct clinical, neuropsychological and radiological phenotype is associated with progranulin gene mutations in a large UK series. Brain 131, 706–720.

Bingham, W.C., Burke, H.R., Murray, S., 1966. Raven's Progressive Matrices: construct validity. J. Psychol. 62, 205–209.

Biswal, B., Yetkin, F.Z., Haughton, V.M., Hyde, J.S., 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn. Reson. Med. 34, 537–541.

Borroni, B., Alberici, A., Premi, E., Archetti, S., Garibotto, V., Agosti, C., Gasparotti, R., Di Luca, M., Perani, D., Padovani, A., 2008a. Brain magnetic resonance imaging structural changes in a pedigree of asymptomatic progranulin mutation carriers. Rejuvenation Res. 11, 585–595.

Borroni, B., Archetti, S., Alberici, A., Agosti, C., Gennarelli, M., Bigni, B., Bonvicini, C., Ferrari, M., Bellelli, G., Galimberti, D., Scarpini, E., Di Lorenzo, D., Caimi, L., Caltagirone, C., Di Luca, M., Padovani, A., 2008b. Progranulin genetic variations in frontotemporal lobar degeneration: evidence for low mutation frequency in an Italian clinical series. Neurogenetics 9, 197–205.

Borroni, B., Bonvicini, C., Galimberti, D., Tremolizzo, L., Papetti, A., Archetti, S., Turla, M., Alberici, A., Agosti, C., Premi, E., Appollonio, I., Rainero, I., Ferrarese, C., Gennarelli, M., Scarpini, E., Padovani, A., 2011a. Founder effect and estimation of the age of the Progranulin Thr272fs mutation in 14 Italian pedigrees with frontotemporal lobar degeneration. Neurobiol. Aging, 32:555.e1–8.

Borroni, B., Grassi, M., Archetti, S., Papetti, A., Del Bo, R., Bonvicini, C., Comi, G.P., Gennarelli, M., Bellelli, G., Di Luca, M., Padovani, A., 2011b. Genetic background predicts poor prognosis in frontotemporal lobar degeneration. Neurodegener. Dis. 8, 289–295.

Bozzali, M., Cercignani, M., Caltagirone, C., 2008. Brain volumetrics to investigate aging and the principal forms of degenerative cognitive decline: a brief review. Magn. Reson. Imaging 26, 1065–1070.

Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J.J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P.P., Kumar-Singh, S., Van Broeckhoven, C., 2006. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 442, 920–924.

Cruts, M., Van Van Broeckhoven, C., 2008. Loss of progranulin function in frontotemporal lobar degeneration. Trends Genet. 24, 186–194.

Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A., Gornbein, J., 1994. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 44, 2308–2314.

De Luca, M., Beckmann, C.F., De Stefano, N., Matthews, P.M., Smith, S.M., 2006. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. Neuroimage 29, 1359–1367.

De Luca, M., Smith, S., De Stefano, N., Federico, A., Matthews, P.M., 2005. Blood oxygenation level dependent contrast resting state networks are relevant to functional activity in the neocortical sensorimotor system. Exp. Brain Res. 167, 587–594.

De Renzi, E., Vignolo, L.A., 1962. The Token Test: a sensitive test to detect receptive disturbances in aphasics. Brain 85, 665–678.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-Mental State". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12, 189–198.

Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. 8, 700–711.

Geschwind, D.H., Robidoux, J., Alarcón, M., Miller, B.L., Wilhelmsen, K.C., Cummings, J.L., Nasreddine, Z.S., 2001. Dementia and neurodevelopmental predisposition: cognitive dysfunction in presymptomatic subjects precedes dementia by decades in frontotemporal dementia. Ann. Neurol. 50, 741–746.

Gili, T., Cercignani, M., Serra, L., Perri, R., Giove, F., Maraviglia, B., Caltagirone, C., Bozzali, M., 2011. Regional brain atrophy and functional disconnection across Alzheimer's disease evolution. J. Neurol. Neurosurg., Psychiatry 82, 58–66.

Goldman, J.S., Farmer, J.M., Wood, E.M., Johnson, J.K., Boxer, A., Neuhaus, J., Lomen-Hoerth, C., Wilhelmsen, K.C., Lee, V.M., Grossman, M., Miller, B.L., 2005. Comparison of family histories in FTLD subtypes and related tauopathies. Neurology 65, 1817–1819.

Gorno-Tempini, M.L., Dronkers, N.F., Rankin, K.P., Ogar, J.M., Phengrasamy, L., Rosen, H.J., Johnson, J.K., Weiner, M.W., Miller, B.L., 2004. Cognition and anatomy in three variants of primary progressive aphasia. Ann. Neurol. 55, 335–346.

Gorno-Tempini, M.L., Hillis, A.E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S.F., Ogar, J.M., Rohrer, J.D., Black, S., Boeve, B.F., Manes, F., Dronkers, N.F., Vandenberghe, R., Rascovsky, K., Patterson, K., Miller, B.L., Knopman, D.S., Hodges, J.R., Mesulam, M.M., Grossman, M., 2011. Classification of primary progressive aphasia and its variants. Neurology 76, 1006–1014.

Greicius, M.D., Krasnow, B., Reiss, A.L., Menon, V., 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc. Natl. Acad. Sci. U. S. A. 100, 253–258.

Greicius, M.D., Srivastava, G., Reiss, A.L., Menon, V., 2004. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: Evidence from functional MRI. Proc. Natl. Acad. Sci. U. S. A. 101, 4637–4642.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R.C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Joosse, M., Kwon, J.M., Nowotny, P., Che, L.K., Norton, J., Morris, J.C., Reed, L.A., Trojanowski, J., Basun, H., Lannfelt, L., Neystat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P.R., Hayward, N., Kwok, J.B., Schofield, P.R., Andreadis, A., Snowden, J., Craufurd, D., Neary, D., Owen, F., Oostra, B.A., Hardy, J., Goate, A., van Swieten, J., Mann, D., Lynch, T., Heutink, P., 1998. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 393, 702–705.

Isaacs, B., Kennie, A.T., 1973. The Set test as an aid to the detection of dementia in old people. Br. J. Psychiatry 123, 467–470.

Kleinberger, G., Wils, H., Ponsaerts, P., Joris, G., Timmermans, J.P., Van Broeckhoven, C., Kumar-Singh, S., 2010. Increased caspase activation and decreased TDP-43 solubility in progranulin knockout cortical cultures. J. Neurochem. 115, 735–747.

Lawton, M.P., Brody, E.M., 1969. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 9, 179–186.

LeBer, I., Camuzat, A., Hannequin, D., Pasquier, F., Guedj, E., Rovelet-Lecrux, A., Hahn-Barma, V., van der Zee, J., Clot, F., Bakchine, S., Puel, M., Ghanim, M., Lacomblez, L., Mikol, J., Deramecourt, V., Lejeune, P., de la Sayette, V., Belliard, S., Vercelletto, M., Meyrignac, C., Van Broeckhoven, C., Lambert, J.C., Verpillat, P., Campion, D., Habert, M.O., Dubois, B., Brice, A. French Research Network on FTD/FTD-MND, 2008. Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain 131, 732–746.

Loring, D.W., Martin, R.C., Meador, K.J., Lee, G.P., 1990. Psychometric construction of the Rey-Osterrieth Complex Figure: methodological considerations and interrater reliability. Arch. Clin. Neuropsychol. 5, 1–14.

Masellis, M., Momeni, P., Meschino, W., Heffner, R., Jr., Elder, J., Sato, C., Liang, Y., St George-Hyslop, P., Hardy, J., Bilbao, J., Black, S., Rogaeva, E., 2006. Novel splicing mutation in the progranulin gene causing familial corticobasal syndrome. Brain 129, 3115–3123.

McKhann, G.M., Albert, M.S., Grossman, M., Miller, B., Dickson, D., Trojanowski, J.Q., Work Group on Frontotemporal Dementia and Pick's Disease, 2001. Clinical and pathological diagnosis of frontotemporal dementia: report of the Work Group on Frontotemporal Dementia and Pick's Disease. Arch. Neurol. 58, 1803–1809.

Mesulam, M., Johnson, N., Krefft, T.A., Gass, J.M., Cannon, A.D., Adamson, J.L., Bigio, E.H., Weintraub, S., Dickson, D.W., Hutton, M.L., Graff-Radford, N.R., 2007. Progranulin mutations in primary progressive aphasia: the PPA1 and PPA3 families. Arch. Neurol. 64, 43–47.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P.H., Albert, M., Boone, K., Miller, B.L., Cummings, J., Benson, D.F., 1998. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51, 1546–1554.

Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., McCluskey, L.F., Miller, B.L., Masliah, E., Mackenzie, I.R., Feldman, H., Feiden, W., Kretzschmar, H.A., Trojanowski, J.Q., Lee, V.M., 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.

Rademakers, R., Baker, M., Gass, J., Adamson, J., Huey, E.D., Momeni, P., Spina, S., Coppola, G., Karydas, A.M., Stewart, H., Johnson, N., Hsiung, G.Y., Kelley, B., Kuntz, K., Steinbart, E., Wood, E.M., Yu, C.E., Josephs, K., Sorenson, E., Womack, K.B., Weintraub, S., Pickering-Brown, S.M., Schofield, P.R., Brooks, W.S., Van Deerlin, V.M., Snowden, J., Clark, C.M., Kertesz, A., Boylan, K., Ghetti, B., Neary, D., Schellenberg, G.D., Beach, T.G., Mesulam, M., Mann, D., Grafman, J., Mackenzie, I.R., Feldman, H., Bird, T., Petersen, R., Knopman, D., Boeve, B., Geschwind, D.H., Miller, B., Wszolek, Z., Lippa, C., Bigio, E.H., Dickson, D., Graff-Radford, N., Hutton, M., 2007. Phenotypic variability associated with progranulin haploinsufficiency in patients with the common 1477C–>T (Arg493X) mutation: an international initiative. Lancet Neurol. 6, 857–868.

Rademakers, R., Rovelet-Lecrux, A., 2009. Recent insights into the molecular genetics of dementia. Trends Neurosci. 32, 451–461.

Reitan, R.M., 1958. Validity of the Trail Making Test as an indicator of organic brain damage. Percept. Mot. Skills 8, 271–276.

Rohrer, J.D., Ridgway, G.R., Modat, M., Ourselin, S., Mead, S., Fox, N.C., Rossor, M.N., Warren, J.D., 2010. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. Neuroimage 53, 1070–1076.

Seeley, W.W., Crawford, R.K., Zhou, J., Miller, B.L., Greicius, M.D., 2009. Neurodegenerative diseases target large-scale human brain networks. Neuron 62, 42–52.

Serra, L., Perri, R., Cercignani, M., Spanò, B., Fadda, L., Marra, C., Carlesimo, G.A., Caltagirone, C., Bozzali, M., 2010. Are the behavioral symptoms of Alzheimer's disease directly associated with neurodegeneration? J. Alzheimers Dis. 21, 627–639.

Sheikh, K., Smith, D.S., Meade, T.W., Goldenberg, E., Brennan, P.J., Kinsella, G., 1979. Repeatability and validity of a modified activities of daily living (ADL) index in studies of chronic disability. Int. Rehabil. Med. 1, 51–58.

Sleegers, K., Brouwers, N., Van Damme, P., Engelborghs, S., Gijselinck, I., van der Zee, J., Peeters, K., Mattheijssens, M., Cruts, M., Vandenberghe, R., De Deyn, P.P., Robberecht, W., Van Broeckhoven, C., 2009. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. Ann. Neurol. 65, 603–609.

Snowden, J.S., Pickering-Brown, S.M., Mackenzie, I.R., Richardson, A.M., Varma, A., Neary, D., Mann, D.M., 2006. Progranulin gene mutations associated with frontotemporal dementia and progressive non-fluent aphasia. Brain 129, 3091–3102.

Spillantini, M.G., Goedert, M., 1998. Tau protein pathology in neurodegenerative diseases. Trends Neurosci. 21, 428–433.

Thompson, P.M., Martin, N.G., Wright, M.J., 2010. Imaging genomics. Curr. Opin. Neurol. 23, 368–373.

Van Damme, P., Van Hoecke, A., Lambrechts, D., Vanacker, P., Bogaert, E., van Swieten, J., Carmeliet, P., Van Den Bosch, L., Robberecht, W., 2008. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. J. Cell Biol. 181, 37–41.

Van Deerlin, V.M., Wood, E.M., Moore, P., Yuan, W., Forman, M.S., Clark, C.M., Neumann, M., Kwong, L.K., Trojanowski, J.Q., Lee, V.M., Grossman, M., 2007. Clinical, genetic, and pathologic characteristics of patients with frontotemporal dementia and progranulin mutations. Arch. Neurol. 64, 1148–1153.

Zhou, J., Greicius, M.D., Gennatas, E.D., Growdon, M.E., Jang, J.Y., Rabinovici, G.D., Kramer, J.H., Weiner, M., Miller, B.L., Seeley, W.W., 2010. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. Brain 133, 1352–1367.