Effect of *TMEM106B* Polymorphism on Functional Network Connectivity in Asymptomatic *GRN* Mutation Carriers

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**IMPORTANCE** *Granulin (GRN)* mutations represent one of the most frequent genetic causes of inherited frontotemporal dementia. The study of asymptomatic carriers of *GRN Thr272fs* mutation (aGRN+) provides a unique opportunity to study the natural history of the disease and the role of modulating factors on disease onset. It has been demonstrated that the *TMEM106B* polymorphism is associated with *GRN*-related frontotemporal dementia and affects age at onset in *GRN* mutation carriers.

**OBJECTIVE** To ascertain whether *TMEM106B* genetic status modulates *GRN* disease by evaluating resting-state functional connectivity in aGRN+ individuals according to *TMEM106* genetic variation.

**DESIGN, SETTING, AND PARTICIPANTS** Academic tertiary referral center for neurodegenerative disorders in 17 asymptomatic carriers of aGRN+ and 14 healthy controls.

**MAIN OUTCOMES AND MEASURES** Changes in resting-state functional connectivity, focusing on the default mode network, ventral and dorsal salience networks, executive network, frontoparietal networks, and attentive network and the effect of *TMEM106B* genotypes in aGRN+ participants and healthy controls (statistical nonparametric mapping).

**RESULTS** aGRN+ participants showed decreased brain connectivity within the left frontoparietal network and increased connectivity in the executive network compared with healthy controls. The *TMEM106B* at-risk polymorphism (T/T) was associated with decreased connectivity within the ventral salience network (ie, middle frontal gyrus) and the left frontoparietal network (ie, left precuneus).

**CONCLUSIONS AND RELEVANCE** This study suggests that the *TMEM106B* polymorphism modulates brain connectivity in aGRN+ individuals, with additional damage of the ventral salience network and left frontoparietal network observed. Genotyping *TMEM106B* is of importance in aGRN+ individuals for prognostic purposes and to assess early brain damage.

Frontotemporal dementia (FTD) is a neurodegenerative disorder in which behavioral abnormalities, language impairment, and executive function deficits represent the most typical clinical features.1,2 One of the most common causes of inherited FTD is the loss-of-function mutations within the *granulin (GRN)* gene (OMIM 138945).3,4 More than 60 different pathogenetic mutations have been identified so far (http://www.molgen.ua.ac.be/FTD mutations). GRN mutations lead to FTD with TDP-43 inclusions5 and have a wide clinical phenotype. Age at disease onset is highly heterogeneous,6 and presenting symptoms frequently resemble either behavioral variant FTD or the agrammatic variant of primary progressive aphasia. Neuroimaging studies have demonstrated that patients carrying *GRN* mutations have an asymmetric gray matter loss in the frontal, posterior temporal, and parietal cortices.7,8 Notwithstanding, little is known about the preclinical stages of the disease, such as the early and specific brain regional damage of *GRN* disease and the factors antedating and modulating disease onset. Studies of those who will develop the disease at some point are essential to understanding the pattern and timing of brain damage and symptom onset.

Recent works by our group, carried out in a genetically coalescent cohort of families with the *GRN Thr272fs* mutation,9,10 have demonstrated early abnormalities of functional network connectivity even in presymptomatic stages of disease,11 without gray matter loss11 but with significant alterations in white matter bundles. In light of this, resting-state functional magnetic resonance imaging (fMRI) represents the most promising...
neuroimaging tool for disentangling the early functional abnormalities sustaining the progressive clinical impairment, and the study of networks involved in frontal functions (ie, the salience network [SN], executive network [EN], dorsal attention network, and frontoparietal networks [FPNs]), along with the most studied network involved in the more posterior and parietal functions (ie, the default mode network), might be of interest.\textsuperscript{11-13}

Furthermore, the \textit{TMEM106B} polymorphism has been recently identified as a key genetic modifying factor in FTD-TDP-43 cases, particularly in patients carrying \textit{GRN} mutations.\textsuperscript{14} Namely, the \textit{TMEM106B} rs1990622 C/T polymorphism was associated with a mean reduction of 13 years of age at disease onset for T/T carriers compared with noncarriers (\(\pm\): TC or CC).\textsuperscript{15} This finding has been further confirmed in an extended cohort.\textsuperscript{16} Furthermore, the link between \textit{GRN} mutation and \textit{TMEM106B} is supported at the biologic level since progranulin levels have been related to \textit{TMEM106B} expression.\textsuperscript{17}

With these caveats in mind, in the present study, we investigated the modulating effect of the \textit{TMEM106B} polymorphism on brain functional connectivity in a cohort of asymptomatic individuals carrying the \textit{GRN} Thr272fs mutation (\textit{aGRN+}) compared with age-similar healthy controls (HCs). This allowed us to assess early brain modification in at-risk individuals and evaluate the effect of \textit{TMEM106B} screening in predicting additional brain damage.

## Methods

### Participants

This work is part of an ongoing study that evaluates brain damage in preclinical inherited dementias. Seventeen \textit{aGRN+} participants, siblings of patients recruited from the Center for Ageing Brain and Neurodegenerative Disorders at the University of Brescia (Brescia, Italy), were enrolled in the present study. Moreover, 14 age-similar controls (noncarriers) were considered among first-degree relatives without a mutation. All participants had a neurologic and neuropsychologic evaluation, as previously published,\textsuperscript{18} and cognitive performances were unremarkable. Furthermore, they had no comorbidities that might affect brain structure. All included participants, both \textit{GRN} mutation carriers and noncarriers, were demonstrated to have a common genetic ancestor, as previously published.\textsuperscript{10}

Each participant underwent MRI scanning and blood sampling for genetic screening of the \textit{GRN} Thr272fs mutation, \textit{TMEM106B} rs1990622 T/C polymorphism, and \textit{apolipoprotein E (ApoE)} genotype.

Written informed consent was obtained from each participant for every procedure before study initiation, including blood collection from venous puncture, genetic analysis, and MRI scanning. The work conformed to the Declaration of Helsinki and was approved by a local ethics committee at Spedali Civili Brescia in Italy.

### Granulin Sequencing

Genomic DNA was extracted from peripheral blood using a standard procedure. All 12 exons, including exon 0 of \textit{GRN}, and at least 30 base pairs of their flanking introns were evaluated by polymerase chain reaction (PCR) and subsequent sequencing. \textit{GRN} Thr272fs (g.1977_1980delCACT) was tested as previously described.\textsuperscript{9,19}

### TMEM106B Genetic Analysis

Genomic DNA was extracted from whole peripheral blood according to standard procedures. Forward 5′-TCTTACCTGTATCCCGTTCC-3′ and reverse 5′-AAAAAAGAGCTTTAGCGCATA-3′ primers were used. The cycling program included an initial denaturation at 95°C for 2 minutes followed by 45 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and a final 5-minute extension at 72°C. The PCR products were separated on a 2% agarose gel, and the fragments were evaluated by denaturing high-performance liquid chromatography analysis for rs1990622 genetic variation (C/T substitution).

### ApoE Genotype Analysis

Genetic variation at the \textit{ApoE} locus was determined by restriction isotyping using PCR amplification and subsequent digestion with \textit{HhaI} (New England BioLabs).

### MRI Acquisition

All imaging was obtained using a 1.5-T magnetic resonance scanner (MAGNETOM Symphony; Siemens) equipped with a circularly polarized transmit-receive coil, as previously published.\textsuperscript{11} Resting-state fMRI data were preprocessed using Statistical Parametric Mapping (SPM8) (www.fil.ion.ucl.ac.uk/spm/) for image preprocessing and statistical comparison and the group independent component analysis (ICA) for the fMRI toolbox (GIFT 1.3j; http://mialab.mrn.org/software/) for network identification. For each participant, 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 echo planar imaging template in Montreal Neurological Institute coordinates provided with SPM8, and smoothing with a 3-dimensional gaussian kernel with 8-mm\(^3\) full-width at half maximum. 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 physiologic noise.

Briefly, the group ICA for the fMRI toolbox first concatenates the individual data across time and then produces a computation of subject-specific components and time courses. For all participants grouped together, the toolbox performed analyses in 3 steps: (1) data reduction, (2) application of the FastICA algorithm, and (3) back-reconstruction for each individual.\textsuperscript{20} The ICA analysis was used to identify 40 independent components, using the minimum description length criterion for the dimension determination.\textsuperscript{21} Statistical reliability of independent component decomposition was evaluated using the ICASSO Toolbox, implemented in \textit{GIFT}\textsuperscript{22} running the FastICA algorithm 10 times with different initial conditions and bootstrapped data sets. Results were converted to z scores. The 40 components were reviewed and compared by computing the spatial correlation coefficient with customized templates of the SN, default mode network, EN, FPNs, and attention network.\textsuperscript{13} This procedure was performed using...
the tool for spatial sorting of the components available with GIFT. Every participant’s z score maps, corresponding to resting-state networks, were used for cross-subject analyses. For the purpose of the present study, participants were divided into 2 separate groups: asymptomatic aGRN+ individuals (n = 17) and age-similar HCs (n = 14). Age, sex, and total gray matter volume were used as covariates of no interest.

Statistical Analysis
Group comparisons of demographic, clinical, and genetic variables were assessed by the Mann-Whitney, χ², or Fisher exact test, as indicated, setting the statistical threshold to P < .05 (SPSS, version 17.0; SPSS, Inc).

With regard to imaging analysis, we applied Statistical nonParametric Mapping (SnPM) (SPSS, version 17.0),23,24 and a threshold of P < .001 was set. Results are presented as SnPM pseudo-t maps superimposed on a 3-dimensional MRI brain template, with a voxel threshold of 30 voxels.25

For each network considered, contrasts were designed to assess (1) the difference in brain connectivity between aGRN+ and HC groups (nonparametric 2-sample t test), (2) the effect of TMEM106B rs1990622 T/T vs *C (T/C or C/C) on brain connectivity in the aGRN+ group (nonparametric 2-sample t test) to assess the outcome of genetic variation in preclinical disease, and (3) the effect of TMEM106B rs1990622 T/T vs *C (T/C or C/C) on brain connectivity in HCs (nonparametric 2-sample t test) to ascertain the result of genetic variation on brain connectivity in the healthy condition. To ensure the highest reliability, we reported only the results included in the network templates.

Results
Demographic and clinical characteristics of aGRN+ participants and HCs are shown in Table 1. The mean (SD) age of aGRN+ participants was 40.2 (9.6) years, and 47.1% were women. No significant differences in TMEM106B and ApoE genotypes, as well as allele frequencies between aGRN+ and HCs, were found.

We evaluated the effect of the TMEM106B polymorphism on brain functional connectivity and excluded the analysis of the ApoE genotype because of the low variability in our sample (only 1 participant carried at least 1 ε4 allele).

The direct comparison between aGRN+ participants and HCs revealed a significant reduced brain connectivity within the left FPN mainly involving the superior parietal lobule (nonparametric 2-sample t test; x, y, and z: -24, -70, and 56; pseudo-t, 4.66; P < .001; cluster size, 71 voxels) (Figure 2A). Furthermore, an increased connectivity within the EN (ie, right precentral gyrus) was evident (nonparametric 2-sample t test; x, y, and z: 56, 8, and 10; pseudo-t, 4.73; P < .001; cluster size, 55 voxels) (Figure 2B).

As shown in Table 2 and Figure 2, when aGRN+ participants were considered, a significant effect of the at-risk TMEM106B T/T polymorphism was observed in the precuneus within the left FP network compared with *C carriers (T/C or C/C) (nonparametric 2-sample t test; Figure 2A) and the right middle frontal gyrus in the ventral SN (Figure 2C). As shown in Figure 2B and 2D, TMEM106B T/T carriers showed decreased brain connectivity compared with *C carriers (nonparametric 2-sample t test).

No significant effect of the TMEM106B polymorphism on functional connectivity in the other considered networks was found. When the same analysis was carried out in HCs, no effect of the TMEM106B polymorphism was observed.

Discussion
Considerable progress has been made in recent years toward unraveling the genetic causes of FTD,26 but there is clearly still much to be learned. With regard to GRN-related FTD, several studies have carefully characterized presenting symptoms and related neuroimaging patterns,7,27,28 but few reports have investigated the early preclinical stages of the disease. Studying presymptomatic individuals carrying GRN pathogenetic mutations is indeed key to improving our understanding of the pattern and timing of the first brain changes and the likely modifiable factors affecting cerebral damage.29

In the cases of GRN mutations, penetrance increases with age, with 90% of carriers affected by age 70 years30; nevertheless, age of onset is highly variable within and between families.

Table 1. Demographic and Clinical Characteristics of aGRN+ Participants and Age-Similar HCs and TMEM106B Genotype and Allele Frequencies

<table>
<thead>
<tr>
<th>Variable</th>
<th>aGRN+ (n = 17)</th>
<th>HC (n = 14)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>42 (24-63)</td>
<td>42 (28-55)</td>
<td>.99</td>
</tr>
<tr>
<td>Female sex</td>
<td>8 (47.1)</td>
<td>11 (78.6)</td>
<td>.07</td>
</tr>
<tr>
<td>Education, median (range)</td>
<td>12 (5-17)</td>
<td>8 (8-17)</td>
<td>.51</td>
</tr>
<tr>
<td>ApoE genotype, ε4</td>
<td>1 (5.9)</td>
<td>0</td>
<td>.55</td>
</tr>
<tr>
<td>TMEM106B genotype frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3 (17.6)</td>
<td>2 (14.2)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>10 (58.8)</td>
<td>6 (42.9)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>4 (23.6)</td>
<td>6 (42.9)</td>
<td>.52</td>
</tr>
<tr>
<td>TMEM106B allele frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>16 (47.1)</td>
<td>10 (35.8)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>18 (52.9)</td>
<td>18 (64.2)</td>
<td>.41</td>
</tr>
</tbody>
</table>

Abbreviations: aGRN+, granulin Thr227fs mutation; ApoE, apolipoprotein E; HC, healthy control.

* Values are presented as number (percentage) unless otherwise indicated.

† Mann-Whitney test.

‡ χ² Test.

§ ε4 = ε4/ε4 or ε4/ε–.

* Fisher exact test.
and ranges from the mid-40s to mid-70s. Furthermore, mutation carriers still asymptomatic in their 80s have been reported. These observations strongly suggest that other genes and/or environmental factors affect the age at onset in GRN-related FTD. Evaluating preclinical brain damage in GRN mutation carriers might give new insights into disease pathogenetic mechanisms.

Since previous works found only white matter damage in preclinical disease stages,19,30 we performed a resting-state fMRI study to evaluate functional brain connectivity in those networks typically affected in FTD. Considering both aGRN+ participants and age-similar HCs, we confirmed the hypothesis of early functional changes in preclinical disease stages, as previously reported.11 In the present work, we considered a larger number of networks, and in aGRN+ individuals, we demonstrated a significant reduced connectivity of the left FPN and an increased connectivity within the EN.

According to this and other literature findings,13 the impairment of the FPN along with the SN could be specific for GRN-related FTD, with variable findings on the basis of the sample considered. Frontoparietal networks have been related to top-down modulation of attention and working memory,22 as well as in the selection of relevant environmental information that could be important for integrating environmental sensory stimuli and behavioral goals and expectations.32 Furthermore, we found a significant increase in connectivity within the EN in aGRN+ participants. The EN, involved in FTD progression,33 has been hypothesized to provide bias signals to other brain regions to improve cognitive control.34 Increased resting-state fMRI connectivity is still a matter of debate in neuroimaging studies.35-37 Although a compensatory mechanism has been proposed, the direct relationship between coexisting increased and reduced activations is still under examination.36

The recently suggested theoretical concept of molecular neupathies supports the idea that if a specific network is affected by a specific proteinopathy (ie, SN), other brain networks could be dynamically involved during the course of the disease (ie, EN).38 This seems to be particularly true in monogenic neurodegenerative disorders, as already demonstrated.39-42 In preclinical Alzheimer disease due to the presenilin 1 mutation39 and in other presymptomatic monogenic disorders,40-42 decreased connectivity of disease-specific brain networks is counteracted by increased connectivity of others in a compensative fashion. It might be argued that the simultaneous effects of the progressive disruption of key networks on one hand and the progressive failure of compensatory mechanisms on the other may contribute to the onset of clinical symptoms.

In the present study, we subsequently explored whether additional genetic factors might lead to further damage in brain...
connectivity in GRN-related disease. The TMEM106B gene was recently linked by genome-wide association to FTD-TDP.\textsuperscript{14,43} and the rs1990622 polymorphism was further associated with GRN disease, with the TT genotype antedating the age at disease onset.\textsuperscript{15} In vivo studies have demonstrated a convergence between progranulin and TMEM106B at molecular and cellular levels.\textsuperscript{17,43} The overexpression of TMEM106B correlated with elevated progranulin levels by attenuating degradation of progranulin itself.\textsuperscript{17,33,43} Thus, TMEM106B genetic variation was suggested to have important implications in GRN disease.\textsuperscript{44,45} In the present study, we confirmed and extended previous literature, demonstrating that the TMEM106B rs1990622 TT genotype determined additional brain damage in \textit{aGRN+} individuals, with reduced connectivity within the ventral SN and the left FPNs. From this perspective, our study suggests that the pathologic action of the at-risk TMEM106B genotype (rs1990622 TT), most likely biologically related to progranulin levels by attenuating its lysosomal degradation,\textsuperscript{17} was represented by a perturbation of resting-state functional connectivity, especially involving those networks primarily impaired when the disease is overt (ie, SN and FPNs). Even though further studies are warranted, it could be suggested that TMEM106B genetic variations might antedate the clinical onset by impairing the complex system of functional connectivity homeostasis of GRN-related disease. These biologic effects are mirrored by functional network abnormalities, and reduced connectivity in the target region was observed.\textsuperscript{12,35,36,46}

We acknowledge that our study has some limitations. In particular, the small sample size is a major weakness for statistical analysis, and confirmatory replication studies are warranted in the future.

In conclusion, taken together, these findings support previous literature data\textsuperscript{14,16,17} of a modulating effect of TMEM106B genetic variations in GRN-related FTD. TMEM106B genotyping may help to predict prognosis and disease onset in GRN mutation carriers.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Decreased Brain Connectivity in an \textit{aGRN+} Participant According to the TMEM106B rs1990622 Polymorphism}
\end{figure}
Effect of TMEM106B Polymorphism in GRN Carriers

Additional Contributions: We thank involved volunteers for participating in this study. Marco Bozzali, MD (Neuroimaging Laboratory, Santa Lucia Foundation, Rome, Italy), provided advice in neuroimaging procedures. No financial compensation was given.

REFERENCES