TMEM106B regulates progranulin levels and the penetrance of FTLD in *GRN* mutation carriers

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ABSTRACT

Objectives: To determine whether *TMEM106B* single nucleotide polymorphisms (SNPs) are associated with frontotemporal lobar degeneration (FTLD) in patients with and without mutations in progranulin (*GRN*) and to determine whether *TMEM106B* modulates *GRN* expression.

Methods: We performed a case-control study of 3 SNPs in *TMEM106B* in 482 patients with clinical and 80 patients with pathologic FTLD–TAR DNA-binding protein 43 without *GRN* mutations, 78 patients with FTLD with *GRN* mutations, and 822 controls. Association analysis of *TMEM106B* with GRN plasma levels was performed in 1,013 controls and *TMEM106B* and *GRN* mRNA expression levels were correlated in peripheral blood samples from 33 patients with FTLD and 150 controls.

Results: In our complete FTLD patient cohort, nominal significance was identified for 2 *TMEM106B* SNPs (top SNP rs1990622, $p_{allelic} = 0.036$). However, the most significant association with risk of FTLD was observed in the subgroup of *GRN* mutation carriers compared to controls (corrected $p_{allelic} = 0.0009$), where there was a highly significant decrease in the frequency of homozygote carriers of the minor alleles of all *TMEM106B* SNPs (top SNP rs1990622, CC genotype frequency 2.6% vs 19.1%, corrected $p_{recessive} = 0.009$). We further identified a significant association of *TMEM106B* SNPs with plasma GRN levels in controls (top SNP rs1990622, corrected $p = 0.002$) and in peripheral blood samples a highly significant correlation was observed between *TMEM106B* and *GRN* mRNA expression in patients with FTLD ($r = -0.63$, $p = 7.7 \times 10^{-5}$) and controls ($r = -0.49$, $p = 2.2 \times 10^{-10}$).

Conclusions: In our study, *TMEM106B* SNPs significantly reduced the disease penetrance in patients with *GRN* mutations, potentially by modulating GRN levels. These findings hold promise for the development of future protective therapies for FTLD. **Neurology® 2011;76:467–474**

GLOSSARY

FTLD = frontotemporal lobar degeneration; **GWAS** = genome-wide association study; **SNP** = single nucleotide polymorphism; **TDP** = TAR DNA-binding protein.

Frontotemporal lobar degeneration (FTLD) is a progressive neurodegenerative disorder accounting for 5%–10% of all patients with dementia and 10%–20% of patients with dementia with an onset before age 65 years.[1,2] In recent years, major advances have been made in our understanding of both the neuropathologic and genetic bases of FTLD.[3] Mutations in the genes encoding the microtubule-associated protein tau (*MAPT*)[4–6] and progranulin (*GRN*)[7,8] together explain 10%–25% of familial FTLD and 5%–10% of all FTLD cases.[9] While patients with *MAPT*

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mutations invariably show FTLD with tau pathology, those with loss-of-function mutations in *GRN* are found to have FTLD with intracellular deposits of hyperphosphorylated, ubiquitinated, and C-terminally truncated fragments of TAR DNA-binding protein 43 (TDP-43) (FTLD-TDP).

The recent ability to subclassify FTLD based on the underlying molecular pathology initiated a large collaborative genome-wide association study (GWAS) to identify genetic risk factors for FTLD-TDP.[10] Using a combination of autopsy-confirmed patients with FTLD-TDP and *GRN* mutation carriers, this study identified genome-wide association of FTLD-TDP with 3 single nucleotide polymorphisms (SNPs) in the uncharacterized transmembrane protein TMEM106B. It was suggested that *TMEM106B* variants confer risk for FTLD-TDP by increasing TMEM106B expression; however, the exact pathologic mechanisms leading to FTLD-TDP remain unknown.

In this study, we aimed to replicate the association of *TMEM106B* SNPs using our large series of patients with FTLD with and without *GRN* mutations. We also performed in vivo studies in plasma and peripheral blood to test the hypothesis that *TMEM106B* SNPs regulate GRN expression levels and influence FTLD risk by modulating GRN expression.

METHODS

Study populations. A total of 640 patients with FTLD were included in the initial genetic association studies, including 482 patients with clinical FTLD of unknown pathologic subtype without *GRN* mutations (255 male, 227 female), 80 patients with FTLD with pathologically confirmed FTLD-TDP without *GRN* mutations (43 male, 37 female), and 78 probands (33 male, 45 female) of genealogically unrelated families carrying a total of 31 different *GRN* mutations. None of these patients were included in the previous FTLD-TDP GWAS.[10] The mean age at diagnosis was 64.9 ± 9.4 years (range 26–90) for the clinical series and 68.4 ± 12.9 years (range 35–90) for the pathologically confirmed patients, while the mean age at onset in mutation carriers was 60.6 ± 8.5 years (range 43–82). For the clinical series, age at diagnosis was determined as the age at which the patient was first diagnosed with FTLD by a clinician, while the age at autopsy was defined as the age at diagnosis for pathologically confirmed patients. A total of 34 additional symptomatic *GRN* mutation carriers related to probands from *GRN* families already included in the analysis were also available for study. Patients were selected according to the following criteria: a clinical diagnosis of behavioral variant FTD, semantic dementia or progressive nonfluent aphasia or a pathologic diagnosis of FTLD-TDP, and a Caucasian ancestry and a DNA sample available for genetic studies. Patients with FTLD with mutations in *MAPT* were excluded from all analysis, while patients with FTLD with *GRN* mutations were included in the initial analysis followed by a stratified analysis in subseries of patients with clinical FTLD without *GRN* mutations, patients with FTLD-TDP without *GRN* mutations, and patients with *GRN* mutations. Patients with FTLD were ascertained from a total of 12 centers between 1995 and 2010: Mayo Clinic Jacksonville (n = 190), Mayo Clinic Rochester (n = 131), Mayo Clinic Scottsdale (n = 10), University of California, San Francisco (n = 132), University of California, Los Angeles (n = 3), Northwestern University Feinberg School of Medicine (n = 18), Drexel University College of Medicine (n = 30), University of British Columbia, Canada (n = 18), Harvard Brain Bank (n = 3), University of Western Ontario, Canada (n = 31), and IRCCS "Centro S. Giovanni di Dio-Fatebenefratelli," Brescia, Italy (n = 44). An additional 30 patient samples were obtained from the Mayo Clinic Jacksonville Brain Bank.

Patients with FTLD were age- and sex-matched to a group of 822 neurologically normal controls (434 male, 388 female) for genetic association studies. The mean age at draw for controls was 67.0 ± 9.9 years (range 20–95). All controls were of Caucasian ancestry and ascertained at Mayo Clinic Jacksonville, FL (n = 645), Mayo Clinic Scottsdale, AZ (n = 54), or University of California, San Francisco (n = 123).

For GRN ELISA assays, plasma samples of 2 sets of healthy controls ascertained at Mayo Clinic Jacksonville and Mayo Clinic Rochester were included. The initial series consisted of 518 individuals (200 male, 318 female) with a mean age at draw of 73.1 ± 4.3 years (range 60–80). The follow-up series included 495 individuals (210 male, 284 female) with a mean age at draw of 77.9 ± 7.5 years (range 60–90).

For mRNA expression studies, a total of 150 healthy controls (63 male, 87 female, mean age at draw 65 ± 11 years, range 25–88) and 33 patients with FTLD (16 male, 17 female, mean age at draw 61 ± 10 years, range 29–76) were collected at the University of California, San Francisco.

All patients agreed to be in the study and biological samples were obtained after informed consent with ethical committee approval from the respective institutions.

Genetic analyses. *TMEM106B* genotypes for rs1020004 and rs1990622 for the initial ELISA series were previously generated using Illumina HumanHap300 BeadChips.[11] In all other series *TMEM106B* SNPs were genotyped using Taqman SNP genotyping assays on the 7900HT Fast Real Time PCR system. Genotype calls were made using the SDS v2.2 software (Applied Biosystems, Foster City, CA). SNPs rs1020004, rs1990622, and rs3173615 were genotyped using inventoried Taqman assays (C___7604953_10, C__11171598_10, and C__27465458_10). For rs6966915, comparison of genotypes generated using Illumina BeadChips and the inventoried Taqman assay suggested the presence of a polymorphic variant adjacent to rs6966915 which interfered with the genotyping in the Taqman assay. Sequencing analysis in samples with discordant genotypes revealed a rare G>A polymorphism 11 bp downstream of rs6966915, likely overlapping with one of the Taqman primers. We therefore designed a custom Taqman assay for rs6966915 (PCR primers F:GTGTGTTTCTTAGGACATTGTTTTT and R:CCTCTCTAAGGTTTTTGTTTGTTTTGTTTTC) with reporter primers AGGCTACACGGTCCTT(VIC) and AGGCTACACAGTCCTT(FAM) and confirmed accurate genotyping using this new assay (figure e-1 on the *Neurology*® Web site at www.neurology.org). The custom rs6966915 Taqman assay was used to genotype all series. For sequencing analysis of *TMEM106B*, all coding and noncoding exons were PCR amplified using flanking

Table 1 Association analysis of TMEM106B in complete FTLD case-control series

SNP IDs	Controls (n = 822) No.	Controls (n = 822) %	FTLD (n = 640) No.	FTLD (n = 640) %	Allelic association Uncorrected *p* value	Allelic association Corrected *p* value	Genotypic association Model	OR (95% CI)	Uncorrected *p* value	Corrected *p* value
rs1020004										
AA	407	49.5	304	47.6	0.793	N/A	ADD	1.04 (0.89–1.21)	0.661	N/A
AG	328	39.9	274	42.9			DOM	1.09 (0.89–1.35)	0.404	N/A
GG	87	10.6	61	9.5			REC	0.93 (0.66–1.31)	0.673	N/A
rs6966915										
CC	294	35.8	224	35.1	0.279	N/A	ADD	0.94 (0.81–1.09)	0.381	N/A
CT	374	45.5	324	50.8			DOM	1.04 (0.83–1.29)	0.735	N/A
TT	154	18.7	90	14.1			REC	0.74 (0.56–0.98)	0.038	0.114
rs1990622										
TT	293	35.6	223	34.9	0.281	N/A	ADD	0.93 (0.80–1.08)	0.370	N/A
CT	372	45.3	324	50.7			DOM	1.04 (0.83–1.29)	0.736	N/A
CC	157	19.1	92	14.4			REC	0.74 (0.56–0.98)	0.036	0.108

Abbreviations: CI = confidence interval; FTLD = frontotemporal lobar degeneration; OR = odds ratio; SNP = single nucleotide polymorphism.

intronic primers tailed with M13 sequences. PCR products were purified using AMPure (Agencourt Biosciences, Beverly, MA) then sequenced in both directions using M13 primers and the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were purified using CleanSEQ (Agencourt Biosciences) and analyzed on an ABI3730 Genetic Analyser (Applied Biosystems). *TMEM106B* sequencing was performed in 2 *GRN* mutation carriers (one homozygous for the rare alleles of all 3 *TMEM106B* SNPs and one homozygous for the rare alleles of rs1990622 and rs6966915, but heterozygous for rs1020004) as well as 24 controls selected to represent all possible combinations of genotypes for the 3 *TMEM106B* SNPs.

Expression studies. To determine GRN expression levels in human plasma samples of healthy controls, we used the Human Progranulin Quantikine ELISA Kit (R&D Systems) using undiluted plasma samples. Samples were analyzed as part of an initial series (n = 518) or follow-up series (n = 495). To increase accuracy, all samples were analyzed in duplicate, independent experiments and readings from duplicate samples with a CV >21.06% were excluded from further analysis. Six interplate control samples were used to adjust for plate-to-plate variation. Recombinant human GRN provided with the ELISA kit was used as a standard.

For mRNA expression analysis, peripheral blood samples were drawn in 2 PAXgene tubes, stored at room temperature for at least 2 hours, and then at 4°C. Total RNA was extracted using the PAXgene blood RNA kit (PreAnalytix GmbH, QIAGEN, Germany). RNA quantity was assessed with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE), and quality with an Agilent Bioanalyzer Nanochips. Total RNA (200 ng) was amplified, labeled, and hybridized on Illumina HumanRef-8 v3 Expression BeadChips (Illumina Inc., San Diego, CA), querying the expression of approximately 24,000 RefSeq-curated transcripts, including *TMEM106B* (probe ILMN_2067607) and *GRN*, interrogated by 2 probes: ILMN_1811702 (mapping to the 3′UTR) and ILMN_1724250 (mapping to *GRN* exon 12).

Statistical analyses. All tests of association were performed using PLINK unless otherwise noted.[12] Logistic regression analyses were employed to test for association among the 3 *TMEM106B* SNPs of interest (rs1020004, rs1990622, and rs6966915) and risk of disease in the patient-control series using allelic and genotypic (additive, dominant, and recessive) models, with sex and age at diagnosis (for patients) and age at blood draw (for controls) as covariates. Linear regression analysis of age at onset, with sex as covariate, was used to determine if the SNPs of interest had an effect on age at onset in *GRN* mutation carriers. Given that age at onset was only available for a subset of non-*GRN* mutation carriers, in this subgroup linear regression analysis of age at diagnosis, with sex as covariate, was used to determine if the SNPs of interest had an effect on age at onset. Linear regression analyses were also employed to test for association between GRN plasma levels and the 3 *TMEM106B* SNPs (rs1020004, rs1990622, and rs6966915) using an additive model with age at blood draw, series, and sex as covariates. For all analyses resulting in an uncorrected p value < 0.05, a correction for multiple testing was performed using a conservative Bonferroni correction to adjust p values for the 3 SNPs tested.

mRNA expression data analysis was performed using *R* (www.r-project.org) and Bioconductor (www.bioconductor.org) packages. Absolute expression values were log2 transformed and normalized using quantile normalization. The Pearson correlation coefficient (r) was computed using the *R* function "cor.test," with default parameters.

RESULTS In our complete cohort including patients with FTLD with and without *GRN* mutations, association analysis using 3 SNPs in *TMEM106B* (rs1020004, rs1990622, and rs6966915) showed nominal significance for rs6966915 and rs1990622 (table 1). Since the *TMEM106B* association identified in the original FTLD-TDP GWAS was more significant in *GRN* mutation carriers, we further performed association analysis in our subpopulation of 78 genealogically unrelated *GRN* mutation carriers. In this cohort, we identified a highly significant decrease in the frequency of the minor alleles of all

Table 2 Association analysis of *TMEM106B* SNPs in subgroup of *GRN* mutation carriers

	Controls (n = 822)		FTLD-GRN (n = 78)		Allelic association		Genotypic association			
SNP IDs	No.	%	No.	%	Uncorrected *p* value	Corrected *p* value	Model	OR (95% CI)	Uncorrected *p* value	Corrected *p* value
rs1020004										
AA	407	49.5	50	64.1	0.002	0.006	ADD	0.56 (0.37–0.86)	0.007	0.021
AG	328	39.9	27	34.6			DOM	0.59 (0.36–0.98)	0.040	0.120
GG	87	10.6	1	1.3			REC	0.11 (0.02–0.83)	0.032	0.096
rs6966915										
CC	294	35.8	38	48.7	0.0004	0.001	ADD	0.57 (0.39–0.83)	0.003	0.009
CT	374	45.5	38	48.7			DOM	0.66 (0.41–1.07)	0.094	N/A
TT	154	18.7	2	2.6			REC	0.12 (0.03–0.49)	0.003	0.009
rs1990622										
TT	293	35.6	38	48.7	0.0003	0.0009	ADD	0.57 (0.39–0.82)	0.003	0.009
CT	372	45.3	38	48.7			DOM	0.65 (0.40–1.07)	0.088	N/A
CC	157	19.1	2	2.6			REC	0.12 (0.03–0.48)	0.003	0.009

Abbreviations: CI = confidence interval; FTLD = frontotemporal lobar degeneration; OR = odds ratio; SNP = single nucleotide polymorphism.

TMEM106B SNPs in mutation carriers compared to controls (top SNP rs1990622 C-allele frequency of 26.9% vs 41.7%, corrected $p = 0.0009$); more specifically, there were fewer homozygous carriers of these minor alleles (top SNP rs1990622, CC-genotype frequency of 2.6% vs 19.1%, corrected $p = 0.009$ in a recessive model) (table 2). Of the 78 probands, only 2 were homozygous for the minor alleles of rs1990622 and rs6966915 (one of which was also homozygous for the minor allele of rs1020004) and none of 34 other symptomatic *GRN* mutation carriers (related to the probands) were homozygous carriers of the minor alleles of *TMEM106B* SNPs. Both of the probands who were homozygous for the rare alleles of rs1990622 and rs6966915 presented with disease at a late onset age. One patient (MY04172) was first evaluated at the age of 74 for progressive forgetfulness and confusion of about 5 years duration and died at age 85, while the other patient (FAM352 II:1) was diagnosed with dementia at age 77 and is currently alive at 79 years. The latter patient was also homozygous for rs1020004. Linear regression analyses using age at diagnosis in the 78 *GRN* mutations carriers supported these findings and showed that the minor alleles of the *TMEM106B* SNPs were nominally associated with a later onset of FTLD (rs1990622 and rs6966915: $\beta = 12.53$, SE = 5.74, $p = 0.032$, corrected $p = 0.096$; and rs1020004: $\beta = 19.63$, SE = 8.08, $p = 0.018$, corrected $p = 0.054$) in a recessive model. Finally, we genotyped *TMEM106B* SNPs in the probands of 22 genealogically unrelated families carrying a *MAPT* mutation. In this group, we identified 4/22 (18.1%) patients homozygous for the minor alleles of rs1990622 and rs6966915, a similar frequency to controls. This suggests that the protective effect of *TMEM106B* SNPs is specific to *GRN* mutation carriers. Also, no association with *TMEM106B* SNPs was detected in the subpopulation of patients with clinical FTLD (table e-1) or patients with FTLD-TDP (table e-2) alone. Linear regression analyses also failed to identify an effect on age at diagnosis for the 3 SNPs in the complete FTLD patient series without *GRN* mutations, although suggestive significance was observed for all 3 SNPs using a recessive model (rs1990622: $p = 0.116$; rs6966915: $p = 0.117$; and rs1020004: $p = 0.060$).

We next determined whether *TMEM106B* SNPs were associated with GRN expression levels in plasma samples from healthy controls. Using an initial cohort of 518 plasma samples a significant association with increased GRN protein levels was identified for all 3 *TMEM106B* SNPs tested. The most significant association was obtained with rs1990622 using an additive model (table 3). Analyses of a replication cohort of 495 independent plasma samples from controls also showed in an additive model suggestive association of the minor alleles of rs1990622 and rs6966915 with increased GRN protein levels (table 3). In the combined plasma cohort, rs1990622 showed the most significant association with GRN expression levels, which remained significant after Bonferroni correction for the 3 *TMEM106B* SNPs tested (table 3).

Since the original publication[10] reported that *TMEM106B* SNPs correlated with *TMEM106B* mRNA expression and our current data suggest a correlation between *TMEM106B* SNPs and GRN protein levels, we further queried peripheral blood

Table 3 Association analysis of *TMEM106B* SNPs with plasma GRN levels using multivariate regression analysis

rs number (minor allele)	Chr7 position (NCBI build 36)	Study population	GRN plasma levels			
			β	SE	Uncorrected *p* value	Corrected *p* value
rs1020004 (G)	12,222,303	Initial series	0.034	0.014	0.013	0.039
		Replication series	0.012	0.015	0.433	N/A
		Combined series	0.022	0.010	0.026	0.078
rs6966915 (T)	12,232,513	Initial series	0.040	0.013	0.003	0.009
		Replication series	0.021	0.013	0.117	N/A
		Combined series	0.030	0.009	0.001	0.003
rs1990622 (C)	12,250,312	Initial series	0.043	0.013	0.001	0.003
		Replication series	0.022	0.013	0.104	N/A
		Combined series	0.032	0.009	0.0007	0.002

Abbreviations: SNP = single nucleotide polymorphism.

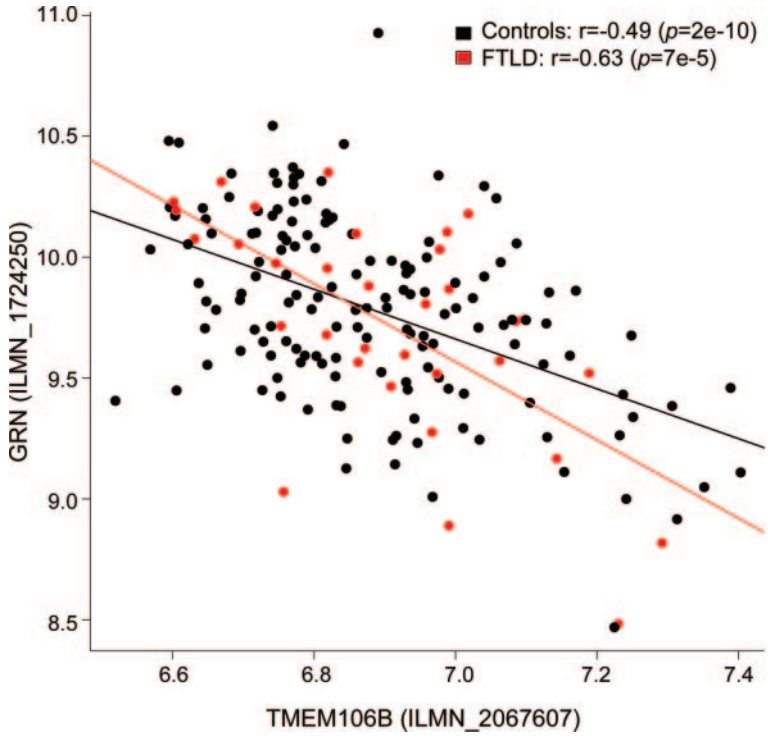
mRNA expression data available to us from a large collection of healthy controls and patients with FTLD. Using one probe for *TMEM106B* and 2 independent *GRN* probes, we identified a correlation between *TMEM106B* and *GRN* mRNA levels with higher levels of *GRN* mRNA in individuals with reduced levels of *TMEM106B* mRNA in both patients with FTLD (n = 150, $r = -0.63$, $p = 7.7 \times 10^{-5}$) and controls (n = 33, $r = -0.49$, $p = 2.2 \times 10^{-10}$, probe ILMN_1724250) (figure 1).

Finally, to identify possible functional variants in the *TMEM106B* coding region which could affect GRN expression levels, we performed sequencing analysis of all coding and noncoding exons of *TMEM106B* in the 2 *GRN* mutation carriers homozygous for the minor alleles of rs1990622 and rs6966915 (MY04172 and FAM352 II:1) and in 24 controls. We identified 2 coding variants: one rare variant which was observed in a single control (c.401G>A; p.S134N) and one common variant (rs3173615, pT185S). Further genotyping revealed that rs3173615 was in complete linkage disequilibrium with rs1990622 and rs6966915 in our cohort of *GRN* mutation carriers.

DISCUSSION The first GWAS study designed to identify susceptibility loci for FTLD was recently performed on a cohort of patients with FTLD with TDP-43 pathology and identified genetic variation in the uncharacterized transmembrane protein *TMEM106B* as a genetic risk factor for FTLD-TDP.[10] In addition to patients with pathologically confirmed FTLD-TDP, that study included patients carrying *GRN* mutations, who are known to consistently have TDP-43 pathology at autopsy. To confirm the association of *TMEM106B* SNPs with FTLD, we performed association analyses in a large cohort including 482 clinical and 80 newly collected patients with FTLD-TDP without *GRN* mutations and 78 symptomatic *GRN* mutation carriers.

Only nominal significant association with *TMEM106B* was detected in our complete patient cohort. However, in the subgroup of 78 probands of genealogically unrelated families carrying 31 different *GRN* mutations, we identified a highly significant association of all 3 *TMEM106B* SNPs with FTLD, suggesting that genetic variation in *TMEM106B* may specifically modify the development of FTLD in the presence of a *GRN* mutation. Importantly, genome-wide significance at chromosome 7p21 in the original FTLD-TDP GWAS was only identified when *GRN* mutation carriers were included in the analysis. In our series, the 3 SNPs tested showed significantly reduced frequencies of *GRN* mutation carriers homozygous for the minor alleles of *TMEM106B* SNPs. In contrast to a genotype frequency of approximately 19% in controls, only 2 patients with *GRN* mutations (2.6% of the population) were homozygous for the minor alleles of rs1990622 and rs6966915. These 2 patients each developed FTLD at a late age (69 and 77 years) compared to the average onset of 60 years in our population of *GRN* mutation carriers and previously published estimates.[13] These data suggest that *GRN* mutation carriers with 2 copies of the minor alleles of rs1990622 and rs6966915 have a significantly reduced disease penetrance or have the onset significantly delayed. In support of our findings, none of 32 additional *GRN* mutation carriers previously included in the FTLD-TDP GWAS and available for genotyping to us or 34 symptomatic *GRN* mutation carriers related to probands already included in the study (including one first-degree relative of

Figure 1 Peripheral blood *TMEM106B* and *GRN* mRNA expression levels in patients with FTLD and controls



Scatterplot depicting peripheral blood *GRN* (as detected by Illumina probe ILMN_1724250) and *TMEM106B* mRNA expression levels in 33 patients with FTLD and 150 healthy controls. Data are expressed in log2-transformed normalized expression values. Regression lines are shown for patients (red) and controls (black). Similar results are obtained with probe ILMN_1811702.

MY04172) were homozygous carriers of the minor alleles of *TMEM106B* SNPs. Finally, we performed sequencing analysis of the complete *TMEM106B* coding region and identified a coding variant located in *TMEM106B* exon 6, predicted to result in p.T185S (rs3173615). Both patients homozygous for the minor alleles of rs1990622 and rs6966915 carried 2 copies of serine at position 185 and additional genotyping confirmed complete linkage disequilibrium of rs3173615 with rs1990622 and rs6966915 in our population of *GRN* mutation carriers.

In contrast to the subpopulation of *GRN* mutation carriers, no significant association with *TMEM106B* was observed in patients with clinical FTLD or patients with FTLD-TDP without *GRN* mutations. A lack of association of *TMEM106B* SNPs was also previously reported in a series of 192 clinical patients as part of the original FTLD GWAS.[10] The lack of association in clinical FTLD series could be due to the significant heterogeneity predicted for this patient group, with at least 3 different FTLD molecular pathologies and possibly other neurodegenerative disorders underlying the clinical FTLD phenotype in these patients.[14–17] With only 80 patients with confirmed FTLD-TDP in the current study, we also had limited power to detect an OR comparable to the original study, which may explain why our results did not reach significance in the FTLD-TDP subpopulation.

GRN is a secreted growth factor with diverse roles in development, wound repair, inflammation, and tumor formation.[18,19] Moreover, recent studies suggested that GRN is a neurotrophic factor involved in maintaining neuronal function during aging.[20,21] Since a 50% loss of GRN expression is sufficient to cause FTLD-TDP in *GRN* mutation carriers[7,8] and partially reduced expression levels of GRN have been shown to increase the risk for FTLD-TDP,[22,23] we hypothesized that *TMEM106B* SNPs could influence FTLD-TDP risk by modulating GRN expression.

We previously reported that *GRN* mRNA and protein is detectable both in the central and peripheral compartments and can be used to identify symptomatic and asymptomatic *GRN* mutation carriers.[24–26] Using a GRN ELISA, we now show that the minor alleles of the *TMEM106B* SNPs (associated with a protective effect in *GRN* mutation carriers) are associated with a modest but significant increase in GRN expression levels in plasma of healthy controls in 2 independent series. Furthermore, in peripheral blood samples of patients with FTLD and controls, increased levels of *TMEM106B* mRNA were associated with reduced levels of *GRN* mRNA. Together with the previously published observation that *TMEM106B* variants confer FTLD-TDP risk by increasing *TMEM106B* expression, these data strongly support a hypothesis whereby the common risk alleles of *TMEM106B* SNPs increase expression of *TMEM106B*, leading to a decrease in GRN mRNA and protein levels, explaining the increased FTLD-TDP risk. Conversely, *GRN* mutation carriers homozygous for the minor alleles of *TMEM106B* SNPs may have GRN expression levels which are sufficiently elevated to reach a critical threshold, reducing the disease penetrance and protecting them from developing FTLD. Whether p.T185S identified in this study or another functional variant in *TMEM106B* outside the coding region is responsible for altering TMEM106B expression remains unknown and should be the focus of future functional studies.

Importantly, both in the current study and in the FTLD-TDP GWAS study, the association of *TMEM106B* SNPs with FTLD was statistically more significant in patients with FTLD with *GRN* mutations compared to those without mutations. This strongly argues that TMEM106B functionally interacts, either directly or indirectly, with GRN to affect FTLD pathogenesis. The study of TMEM106B and

its relation to GRN holds promise for the development of protective therapies for FTLD in the future.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ. Clinical and pathological diagnosis of frontotemporal dementia: report of the Work Group on Frontotemporal Dementia and Pick's Disease. Arch Neurol 2001;58:1803–1809.
2. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546–1554.
3. Mackenzie IR, Rademakers R. The molecular genetics and neuropathology of frontotemporal lobar degeneration: recent developments. Neurogenetics 2007;8:237–248.
4. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998;393:702–705.
5. Poorkaj P, Bird TD, Wijsman E, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 1998;43:815–825.
6. Spillantini MG, Goedert M, Crowther RA, Murrell JR, Farlow, MR, Ghetti B. Familial multiple system tauopathy with presenile dementia: a disease with abundant neuronal and glial tau filaments. Proc Natl Acad Sci USA 1997;94:4113–4118.
7. Baker M, Mackenzie IR, Pickering-Brown SM, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006;442:916–919.
8. Cruts M, Gijselinck I, van der Zee J, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006;442:920–924.
9. Rademakers R, Hutton M. The genetics of frontotemporal lobar degeneration. Curr Neurol Neurosci Rep 2007;7:434–442.
10. Van Deerlin VM, Sleiman PM, Martinez-Lage M, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat Genet 2010;42:234–239.
11. Carrasquillo MM, Zou F, Pankratz VS, et al. Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. Nat Genet 2009;41:192–198.
12. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.
13. Cruts M, Van Broeckhoven C. Loss of progranulin function in frontotemporal lobar degeneration. Trends Genet 2008;24:186–194.
14. Deramecourt V, Lebert F, Debachy B, et al. Prediction of pathology in primary progressive language and speech disorders. Neurology 2010;74:42–49.
15. Forman MS, Farmer J, Johnson JK, et al. Frontotemporal dementia: clinicopathological correlations. Ann Neurol 2006;59:952–962.
16. Hodges JR, Davies RR, Xuereb JH, et al. Clinicopathological correlates in frontotemporal dementia. Ann Neurol 2004;56:399–406.
17. Knibb JA, Xuereb JH, Patterson K, Hodges JR. Clinical and pathological characterization of progressive aphasia. Ann Neurol 2006;59:156–165.
18. He Z, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. J Mol Med 2003;81:600–612.
19. Zhu J, Nathan C, Jin W, et al. Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. Cell 2002;111:867–878.
20. Ahmed Z, Sheng H, Xu YF, et al. Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggests a role for progranulin in successful aging. Am J Pathol Epub 2010.
21. Van Damme P, Van Hoecke A, Lambrechts D, et al. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. J Cell Biol 2008;181:37–41.
22. Rademakers R, Eriksen JL, Baker M, et al. Common variation in the miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. Hum Mol Genet 2008;17:3631–3642.
23. Shankaran SS, Capell A, Hruscha AT, et al. Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. J Biol Chem 2008;283:1744–1753.
24. Coppola G, Karydas A, Rademakers R, et al. Gene expression study on peripheral blood identifies progranulin mutations. Ann Neurol 2008;64:92–96.
25. Finch N, Baker M, Crook R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain 2009;132:583–591.
26. Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. Neurology 2008;71:1235–1239.