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TMEM106B protects *C9ORF72* expansion carriers against frontotemporal dementia

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Conflicts of Interest

Mrs. DeJesus-Hernandez and Dr. Rademakers hold a patent on methods to screen for the hexanucleotide repeat expansion in the *C9ORF72* gene; the other authors declare that they have no conflict of interest.

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Abstract

Variants in transmembrane protein 106 B (*TMEM106B*) modify the disease penetrance of frontotemporal dementia (FTD) in carriers of progranulin (*GRN*) mutations. We investigated whether TMEM106B is also a genetic modifier of disease in carriers of chromosome 9 open reading frame 72 (*C9ORF72*) expansions. We assessed the genotype of 325 *C9ORF72* expansion carriers (cohort 1), 586 FTD patients lacking *C9ORF72* expansions (with or without motor neuron disease [MND]; cohort 2), and a total of 1,302 controls for *TMEM106B* variants (rs3173615 and rs1990622) using MassArray iPLEX and Taqman genotyping assays. For our primary analysis, we focused on functional variant rs3173615, and employed a recessive genotypic model. In cohort 1, patients with *C9ORF72* expansions showed a significantly reduced frequency of carriers homozygous for the minor allele as compared to controls (11.9% versus 19.1%, odds ratio (OR): 0.57, p=0.014; same direction as carriers of *GRN* mutations). The strongest evidence was provided by FTD patients (OR: 0.33, p=0.009) followed by FTD/MND patients (OR: 0.38, p=0.017), whereas no significant difference was observed in MND patients (OR: 0.85, p=0.55). In cohort 2, the frequency of carriers homozygous for the minor allele was not significantly reduced in patients as compared to controls (OR: 0.77, p=0.079); however, a significant reduction was observed when focusing on those patients with frontotemporal lobar degeneration and TAR DNA-binding protein 43 inclusions (FTLD-TDP; OR: 0.26, p<0.001).

Our study identifies *TMEM106B* as the first genetic factor modifying disease presentation in *C9ORF72* expansion carriers. Homozygosity for the minor allele protects carriers from developing FTD, but not from developing MND; similar effects are seen in FTLD-TDP patients with yet unknown genetic causes. These new findings show that the protective effects of *TMEM106B* are not confined to carriers of *GRN* mutations, and might be relevant for prognostic testing, and as a promising therapeutic target for the entire spectrum of FTLD-TDP.

Keywords

C9ORF72; TMEM106B; frontotemporal dementia; motor neuron disease; amyotrophic lateral sclerosis; disease modifier

Introduction

Repeat expansions in chromosome 9 open reading frame 72 (*C9ORF72*) are the most common known genetic cause of frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), and a combination of both diseases (FTD with motor neuron disease [FTD/MND]) [7,21]. Previously, we showed that the repeat length of these expansions varies between tissues, and that repeat lengths in the cerebellum are associated with survival after disease onset [26]. Moreover, we demonstrated that additional mutations in FTD-associated genes (progranulin [*GRN*] and microtubule-associated protein tau [*MAPT*]) might also contribute to the phenotypic variability that is detected in patients with *C9ORF72* repeat expansions [25].

To date, other genetic factors that could act as disease modifiers have not been reported in *C9ORF72* expansion carriers. For this reason, we investigated a promising candidate: transmembrane protein 106 B (TMEM106B). Single nucleotide polymorphisms (SNPs) in *TMEM106B* are associated with the risk of frontotemporal lobar degeneration with TAR DNA-binding protein 43 inclusions (FTLD-TDP) [28], which is the predominant pathology in many FTD patients [18,15]. Interestingly, this association is most prominent in patients

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with *GRN* mutations; in these patients, two copies of the minor allele of *TMEM106B* SNPs (top SNP rs1990622) appear to reduce disease penetrance, protecting individuals from developing FTD [28,8]. Variant rs3173615, which is in linkage disequilibrium (LD) with rs1990622, dictates a change from threonine to serine at position 185 (p.T185S) [19,5,29]. This variant has been shown to regulate TMEM106B protein levels, and was proposed as the functional variant underlying the genetic association with FTLD-TDP [19].

In our present study, we assessed the frequency of *TMEM106B* SNPs rs3173615 and rs1990622 in a large cohort of *C9ORF72* expansion carriers (n=325), in an additional cohort of patients with FTD or FTD/MND without *C9ORF72* expansions (n=586), and in control subjects (n=1,302). Our results reveal that variants in *TMEM106B* protect against developing FTD in carriers of *C9ORF72* repeat expansions, and also in patients with currently unknown causes of FTLD-TDP, emphasizing crucial similarities between *C9ORF72*-related FTLD-TDP, *GRN*-related FTLD-TDP, and other causes of FTLD-TDP.

Materials and Methods

Subjects

For cohort 1 we included all 325 carriers of *C9ORF72* repeat expansions available to us. These subjects were obtained through North American/Canadian institutions: the Mayo Clinic (n=121), Coriell Research Institute (n=71), University of British Columbia, Canada (n=58), University of California, San Francisco (n=38), Robarts Research Institute (n=11), Northwestern University Feinberg School of Medicine (n=9), Drexel University College of Medicine (n=7), University of Western Ontario, Canada (n=7), and Banner Sun Health Research Institute (n=3). Subjects were clinicopathologically diagnosed with FTD (n=86), FTD/MND (n=78) or MND (n=127), with another diagnosis (n=7; e.g. Alzheimer's disease, alcohol abuse or behavioral impairment), or they were asymptomatic at time of last evaluation (n=27; age at evaluation: 43.6±12.7). Our primary analysis focused on the 260 unrelated probands with FTD (n=69), FTD/MND (n=71) or MND (n=120), and a group of neurologically normal controls of similar age and gender provided by the Mayo Clinic (n=376). In secondary analyses, we also examined the sensitivity of our results by including the 65 remaining expansion carriers who were family members or had received another diagnosis, and by including additional controls from our major sites (n=410; Coriell Research Institute [n=264], University of British Columbia, Canada [n=17], University of California, San Francisco [n=116], and Robarts Research Institute [n=13]). All *C9ORF72* expansion carriers and control subjects were screened for *TMEM106B* SNPs rs3173615 and rs1990622.

Cohort 2 was previously screened for *TMEM106B* SNP rs1990622 [8,28]; however, these subjects were investigated before the identification of repeat expansions in *C9ORF72*. Patients in this cohort 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. Based on our current findings, we reanalyzed our genotype data in this cohort after exclusion of patients with *C9ORF72* repeat expansions and *GRN* mutations, and we also genotyped all patients and controls for rs3173615. Furthermore, we extended this cohort to include all additional pathologically confirmed FTLD-TDP and FTLD/MND patients without *C9ORF72* repeat expansions or *GRN* mutations (n=31), who were recruited at our Mayo Clinic Brain Bank since our original study in 2010. In total, cohort 2 comprised 586 patients who were ascertained at the Mayo Clinic (n=380), University of California, San Francisco (n=117), University of Western Ontario, Canada (n=31), Drexel University College of Medicine (n=21), Northwestern University Feinberg School of Medicine (n=21), University of British Columbia, Canada (n=10), and University of Texas Southwestern Medical Center (n=6). All control subjects in

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cohort 2 (n=765) were obtained from the Mayo Clinic (not included in cohort 1: n=516, included in cohort 1: n=133) and University of California, San Francisco (n=116) [8]. In addition, we performed a joint analysis including the controls from cohort 1 (total number of controls: n=1,302).

Characteristics of cases and controls included in both cohorts are shown in Table 1. All subjects agreed to be in the study, and biological samples were obtained after informed consent with ethical committee approval from the respective institutions.

Genotyping

TMEM106B SNPs rs3173615 and rs1990622 were genotyped in cohort 1 on a Sequenom MassArray iPLEX platform (San Diego, CA) and analyzed with Typer 4.0 software. In cohort 2, rs3173615 and rs1990622 were genotyped using Taqman SNP genotyping assays (C_27465458_10, C__11171598_10; Invitrogen, Carlsbad, CA) on the 7900HT Fast Real Time PCR system. Genotype calls were made using SDS v2.2 software (Applied Biosystems, Foster City, CA). In control subjects, there was no evidence of a departure from Hardy-Weinberg equilibrium for rs3173615 or rs1990622 in both cohorts (all $p>0.010$). The two SNPs were in almost complete LD ($r^2 \geq 0.97$), and given this strong LD, for simplicity we focused our analysis on functional variant rs3173615.

Immunohistochemistry

Immunohistochemistry was performed in a blinded fashion on a preliminary group of nine patients who were diagnosed with FTLD-TDP and did not show signs of MND. We included all three patients from the Mayo Clinic Brain Bank for whom fixed tissue was available and who were homozygous for the minor protective *TMEM106B* allele (GG genotype for rs3173615; *C9ORF72* repeat expansion: n=1, without *C9ORF72* repeat expansion: n=2). In addition, we also randomly selected six FTLD-TDP patients without MND who were homozygous for the major risk *TMEM106B* allele (CC genotype for rs3173615; *C9ORF72* repeat expansion: n=3, without *C9ORF72* repeat expansion: n=3), to allow comparisons of TDP-43 burden between patients homozygous for the minor or major allele.

We stained 5-μm-thick sections from the frontal cortex for TDP-43 (pS409/410, 1:5,000, mouse monoclonal, Cosmobio Co., Tokyo, Japan), and repeat-associated non-ATG translation peptides (C9RANT, Rb5823, 1:5,000, Mayo Clinic) [1], the latter has recently been shown to be distinctive for *C9ORF72* expansions [1,17]. Sections were cut from formalin-fixed paraffin-embedded blocks, deparaffinized in xylene, rehydrated in a graded series of ethanol, and washed in distilled water. To process stains, we used DAKO Autostainer Plus (DAKO, Carpinteria, CA) and DAKO EnVision+ System–horseradish peroxidase (diaminobenzidine). Immunostains were subsequently counterstained with Lerner's hematoxylin, dehydrated, and coverslipped. Stained slides were viewed using an Olympus BX40 microscope (Olympus Corporation of the Americas, Center Valley, PA). TDP-43 pathologic subtype was assigned in accordance with the harmonized criteria (Type A-C) [14]. Neuronal lesion counts were performed in a blinded fashion on layer 2 of the midfrontal gyrus. Counts were performed in six 10x microscopic fields randomly selected over the gyrus, and averaged for a composite pathologic burden score.

Statistical Analysis

Separately for both cohorts, comparisons of *TMEM106B* SNPs with controls were made using logistic regression models; odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Given our previous studies related to *TMEM106B* in carriers of *GRN* mutations [19,8], we hypothesized that *TMEM106B* SNPs would affect disease penetrance in

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C9ORF72 expansion carriers under a recessive genotypic model (presence versus absence of two copies of the minor allele). For this reason, we utilized this model in our primary analysis, though in secondary analyses we also examined other genotypic models (additive, dominant, and co-dominant).

In cohort 1, models were adjusted for gender, and the primary analysis involved unrelated probands with FTD, FTD/MND, MND, and controls. Comparisons with controls were made for all patients, and also separately for FTD, FTD/MND, and MND subgroups. Sensitivity of results to the inclusion of related family members and individuals with other diagnoses, and to the inclusion of additional controls were considered in secondary analyses. For cohort 2, models were adjusted for gender and age (age at blood draw in controls, age at diagnosis in clinically diagnosed patients, age at death in pathologically diagnosed patients). We compared controls to all patients, and additionally, we made comparisons separately for the FTD subgroup and according to the method of diagnosis (clinical or pathological).

To evaluate associations of *TMEM106B* SNPs with age at onset, we also used linear regression models adjusted for gender and disease subgroup for cohort 1 (using only FTD, FTD/MND, and MND probands); our primary analysis involved a recessive genotypic model, while other genotypic models (additive, dominant, and co-dominant) were examined in secondary analyses.

In order to adjust for multiple testing in our main disease-association analysis using recessive genotypic models, we utilized a Holm step-down adjustment [11] separately for both cohorts. After this adjustment, $p \leq 0.025$ (cohort 1) and $p \leq 0.0125$ (cohort 2) were considered statistically significant. All statistical analyses were performed with R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

Cohort 1

We first compared the frequency of carriers homozygous for the minor rs3173615 *TMEM106B* allele (corresponding to homozygous p.185S carriers [GG genotype]) between our FTD, FTD/MND or MND probands with *C9ORF72* expansions and our control subjects (Table 1). Homozygosity for the minor allele was detected in 11.9% of the 260 cases as compared to 19.1% of the 376 controls (OR: 0.57, p=0.014; Table 2). When including all 65 family members and individuals with other diagnoses who harbored *C9ORF72* expansions, the frequency of carriers homozygous for the minor allele remained lower in individuals with *C9ORF72* expansions than in controls (11.4% versus 19.1%).

Subsequently, we determined whether the lower frequency of homozygous carriers was present in all disease subgroups (Table 2). Interestingly, the lowest frequency was encountered in FTD patients (7.2%, OR: 0.33, p=0.009) followed by FTD/MND patients (8.5%, OR: 0.38, p=0.017), whereas no significant difference was detected in MND patients (16.7%, OR: 0.85, p=0.55). These findings were consistent when including family members (FTD: 7.0%, FTD/MND: 9.0%, and MND: 16.7%).

A similar trend was seen for all aforementioned differences with controls, when including 410 extra controls from other sites to form a combined control group of 786 individuals (Online Resource Table 1), and when additionally adjusting the logistic regression models for age in the subset of individuals with that information available (data not shown). We also considered other genotypic models (additive, dominant, and co-dominant), but the differences stayed most significant under the recessive genotypic model (Online Resource Table 2).

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Furthermore, we investigated whether homozygosity for the minor allele might influence age at onset. In the 240 FTD, FTD/MND or MND probands with age at onset information available, the mean age at onset was 1.26 years lower in patients homozygous for the minor allele in comparison to other patients, when utilizing a recessive genotypic model (95% CI: 4.78 years lower to 2.25 years higher; Table 3); it is important to realize, however, that this did not approach significance (p=0.48). The lack of association between rs3173615 and age at onset was also apparent when examining other genotypic models (additive, dominant, and co-dominant; Table 3). All association analyses regarding disease and age at onset were comparable for rs1990622 (data not shown).

Cohort 2

Before the identification of repeat expansions in *C9ORF72*, we assessed a large cohort of FTD patients, FTD/MND patients, and control subjects for *TMEM106B* SNPs [28,8]. For our present study, we excluded all carriers of *C9ORF72* expansions and *GRN* mutations from this cohort, and reanalyzed our data. In addition, we extended our cohort with 31 newly ascertained FTLD-TDP patients. In this cohort of 586 patients and 765 controls, we showed that the frequency of carriers homozygous for the minor rs3173615 *TMEM106B* allele (GG genotype) did not differ significantly between groups, when using a recessive genotypic model (15.0% versus 18.7%, OR: 0.77, p=0.079; Table 2). Because our findings in cohort 1 were most pronounced in FTD patients, we subsequently focused our analysis on patients with FTD (without signs of MND); however, in this group we also did not detect a significant difference between patients and controls (14.9% versus 18.7%, OR: 0.76, p=0.071). Whilst this group included both clinically diagnosed patients (n=430) and pathologically diagnosed patients (n=101), we next investigated these subgroups separately. Although no significant differences were observed when comparing clinically diagnosed patients and controls (17.0% versus 18.7%, OR: 0.91, p=0.56; reported diagnosis of behavioral variant FTD: 14.3%; remaining FTD patients: 18.1%), the frequency of carriers homozygous for the minor allele was significantly lower in patients who received a diagnosis of FTLD-TDP than in controls (5.9% versus 18.7%, OR: 0.26, p<0.001), even though patients with *C9ORF72* expansions and *GRN* mutations had already been excluded.

Comparable results were obtained after inclusion of all additional controls from cohort 1 to form a combined control group of 1,302 subjects (Online Resource Table 1). Differences between patients and controls were also examined under other genotypic models (additive, dominant, and co-dominant); however, differences were not as strong as those observed under a recessive genotypic model (Online Resource Table 2). All results were similar when evaluating associations with *TMEM106B* rs1990622 (data not shown).

Immunohistochemistry

All nine patients for whom immunohistochemistry was performed displayed TDP-43 pathology (Table 4, Fig. 1). Only one patient had pathologic lesions consistent with TDP-43 Type C (predominant long dystrophic neuritis; patient i), and therefore, this patient was excluded from further analyses. The remaining eight patients showed pathologic lesions consistent with TDP-43 Type A (neuronal cytoplasmic and intranuclear inclusions and small dystrophic neuritis; patients a–h). When performing a very preliminary comparison in TDP-43 Type A burden within each of the cohorts, the lowest pathologic burden was consistently present in homozygous carriers of the minor, protective, *TMEM106B* allele. Of note, all patients with *C9ORF72* repeat expansions demonstrated C9RANT pathology, characterized by neuronal cytoplasmic inclusions, as opposed to patients without these expansions.

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Discussion

Our results identify rs3173615 (corresponding to *TMEM106B* p.T185S) as an important genetic modifying factor in *C9ORF72* expansion carriers. We focused our analysis on a recessive genotypic model, and this model revealed that individuals who carry a *C9ORF72* repeat expansion and harbor two copies of the minor *TMEM106B* allele (GG genotype) are less likely to develop disease symptoms (OR: 0.57), in particular FTD (OR: 0.33). Although these findings suggest that the protective effects of *TMEM106B* variants are not limited to previously reported individuals with *GRN* mutations, these protective effects seem more pronounced in carriers of *GRN* mutations, who demonstrated a frequency of homozygous carriers as low as 2% [19,30,8], corresponding to an OR of approximately 0.12 [8].

Repeat expansions in *C9ORF72* and mutations in *GRN* are common causes of FTD: they account for up to 50% of familial FTD patients [27,16,2,6,9]. Importantly, in our second patient cohort in which all carriers of *C9ORF72* expansions and *GRN* mutations were excluded, the frequency of two copies of the minor *TMEM106B* allele in FTLD-TDP patients remained significantly lower as compared to the more heterogeneous group of clinically diagnosed FTD patients or controls. These findings suggest that the protective effects of *TMEM106B* variants are not confined to *C9ORF72*-related FTLD-TDP or *GRN*-related FTLD-TDP, but that they can also be encountered in the remaining FTLD-TDP patients with a presently unknown cause.

Interestingly, other well-known types of FTD, such as FTLD-tau caused by *MAPT* mutations, do not appear to show a low frequency of carriers homozygous for minor *TMEM106B* alleles (18%) [8]. Moreover, we found no significant differences in *TMEM106B* variants between MND patients with *C9ORF72* repeat expansions and controls, which is in line with a report that assessed these variants in a cohort of ALS patients and controls [30]. Importantly, while our analyses focused on the protective effect of the minor allele of *TMEM106B* SNPs, our findings are compatible with previous studies which identified the major allele of *TMEM106B* SNPs as potential risk-alleles for the development of FTD [28,29,8,23,30,5]. In fact, given our new findings, it is also important to emphasize that differences in the composition of cohorts (e.g. percentage of patients with *C9ORF72* repeat expansions, *GRN* mutations, other causes of FTLD-TDP, or the presence of MND), may have greatly impacted the results of these previously published *TMEM106B* association studies, and likely explain the reported conflicting results related to *TMEM106B* risk in FTD [28,29,8,23,30,5].

TMEM106B is a 274 amino acid glycosylated type-II transmembrane protein that localizes in late endosomes and lysosomes, and co-localizes with the progranulin protein (PGRN) [3,4,19,12]. Elevated levels of TMEM106B have been shown to result in abnormal lysosomal morphology, and are found to delay the degradation of endocytic cargoes [3]. Although the effects of TMEM106B overexpression on PGRN levels seem small, PGRN levels in plasma appear to correlate with *TMEM106B* variants [3,19,4,8,5]. *In vitro* experiments that assessed the risk isoform (T185) and protective isoform (S185) of TMEM106B, showed that the protective isoform is consistently expressed at lower levels (as low as half) [19]. It has also been revealed that this probably results from a more rapid protein degradation (nearly 4-fold), potentially caused by an abnormal glycosylation of S185 [19]. Taken together, these findings suggest that lysosomal pathways may play a critical role in the pathogenesis of FTD; for instance, they may affect the degradation and subsequent aggregation of TDP-43, and thereby, they could influence the likelihood of developing FTD.

Because we did detect homozygous carriers of the minor *TMEM106B* allele in a small percentage of FTLD-TDP patients, we postulate that another as yet unknown factor (or

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combination of factors) has such a strong effect in these patients that the protective effects of the minor allele are lessened. Importantly, our highly preliminary immunohistochemical findings may support the hypothesis that *TMEM106B* variants affect TDP-43: patients with the protective allele who did develop FTLD-TDP seemed to show less TDP-43 burden than patients with the risk allele. Since homozygous carriers of protective alleles are rare in FTD patients, however, we were only able to investigate a very limited number of samples, and consequently, additional cases should be assessed to confirm these suggestive findings.

Based on our present results, and the aforementioned studies, we speculate that a cascade of lysosomal dysfunction might contribute to *C9ORF72*-related FTD, and possibly, to other types of FTD. In fact, it has already been suggested that FTD-associated genes valosin containing protein (*VCP*) and charged multivesicular body protein 2b *(CHMP2B)*, which account for a small subgroup of FTD patients, are involved in accumulation of autophagosomes, affect endolysosomal sorting, regulate the maturation and size of early endosomes, impair endosome-lysosome fusion, and delay the degradation process of cargo proteins [20,24,10,13,22], and thus, lysosomal dysfunction could represent a shared mechanism that underlies many types of FTD.

Our study has several limitations. Despite the relatively large sample size, for example, the possibility of a type II error (i.e. false-negative association) should be considered. Moreover, due to the low frequency of two copies of the minor *TMEM106B* allele, especially in FTD patients, detailed analysis of clinical and pathological characteristics was obviously hampered. This could have also affected differences in frequencies detected when utilizing a recessive genotypic model, and may have contributed to the lack of association with age at onset. Furthermore, although we excluded individuals with known familial relationships from our primary analysis, and *C9ORF72* and *TMEM106B* are located on different chromosomes, we cannot exclude the possibility that some subjects may be distantly related and share loci. Nevertheless, our study was able to thoroughly assess *TMEM106B* variants in a unique cohort of both clinically and pathologically diagnosed *C9ORF72* expansion carriers, in an additional large cohort of FTD patients and controls, and in a disease spectrum ranging from FTD to MND.

To summarize, our findings reveal a new link between *C9ORF72*-related FTLD-TDP, *GRN*-related FTLD-TDP, and other causes of FTLD-TDP: a decreased frequency of carriers homozygous for the minor G-allele of *TMEM106B* rs3173615, suggesting a protective effect of this particular genotype. In *C9ORF72* mutation carriers, *TMEM106B* protects specifically against the development of FTD, but not MND; providing the first genetic modifier factor involved in disease presentation in this important genetic subgroup. We hypothesize that *TMEM106B* variants might protect *C9ORF72* expansion carriers from developing FTD, potentially by affecting lysosomal pathways. These findings provide novel opportunities for the development of prognostic tests, and for new treatment strategies aiming at *C9ORF72* expansion carriers, and the larger subgroup of patients within the disease spectrum of FTD with TDP-43 pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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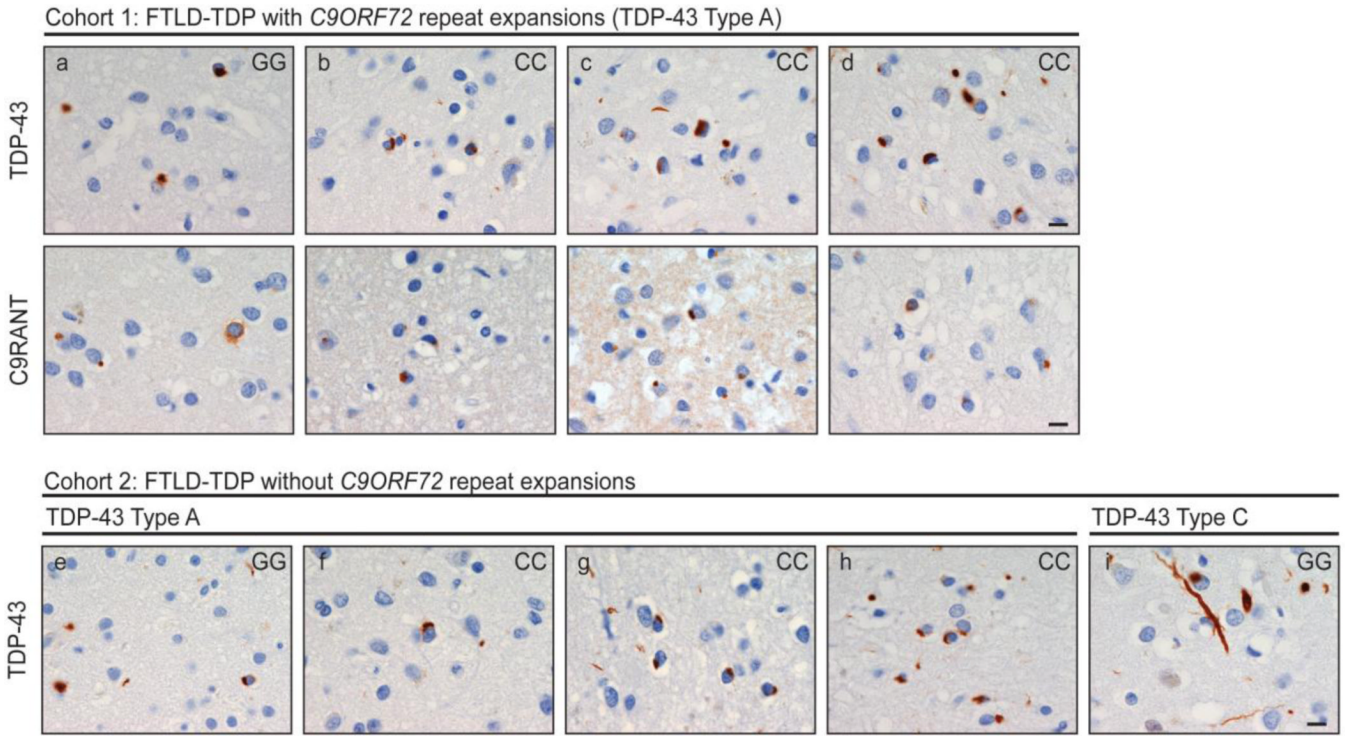

Fig. 1. Immunohistochemistry of patients homozygous for minor or major *TMEM106B* allele

TAR DNA-binding protein 43 (TDP-43; cohort 1 and cohort 2) and dipeptide-repeat protein (C9RANT; cohort 1) pathology in the midfrontal gyrus (n=9; 3 homozygous carriers of the minor allele [GG], and 6 homozygous carriers of the major allele [CC] in rs3173615). The case identifier (Table 4) is shown in the upper left corner, and the genotype is shown in the upper right corner. [measure bar = 10 μm]

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Table 1

Characteristics of study cohorts

Group	N	Female gender	Age	Age at onset	FTD only	Pathological diagnosis
Cohort 1 – controls and *C9ORF72* repeat expansion carriers (FTD, FTD/MND, and MND, or other diagnosis)						
Controls	376	173 (46.0%)	61.2 ± 10.2 (35–90)	N/A	N/A	N/A
All repeat expansion carriers	325	148 (45.5%)	59.2 ± 9.9 (35–90)	56.8 ± 9.2 (34–83)	86 (26.5%)	118 (36.3%)
FTD, FTD/MND, and MND probands	260	114 (43.8%)	59.5 ± 10.0 (35–90)	56.9 ± 9.0 (34–83)	69 (26.5%)	107 (41.2%)
FTD probands	69	28 (40.6%)	63.1 ± 12.3 (35–90)	58.1 ± 9.6 (34–79)	69 (100.0%)	40 (58.0%)
FTD/MND probands	71	25 (35.2%)	60.6 ± 8.5 (39–80)	56.2 ± 9.0 (34–74)	0 (0.0%)	51 (71.8%)
MND probands	120	61 (50.8%)	56.9 ± 8.6 (37–83)	56.5 ± 8.7 (37–83)	0 (0.0%)	16 (13.3%)
Cohort 2 – controls and FTD or FTD/MND patients without *C9ORF72* repeat expansions or *GRN* mutations						
Controls	765	363 (47.4%)	67.1 ± 10.0 (20–95)	N/A	N/A	N/A
All FTD or FTD/MND patients	586	273 (46.6%)	66.8 ± 10.4 (26–99)	62.4 ± 9.8 (26–95)	531 (90.6%)	141 (24.1%)
All FTD patients	531	251 (47.3%)	67.1 ± 10.1 (26–99)	62.7 ± 9.5 (26–86)	531 (100.0%)	101 (19.0%)
Pathologically diagnosed	101	48 (52.5%)	74.0 ± 11.2 (49–99)	64.5 ± 11.1 (39–86)	101 (100.0%)	101 (100.0%)
Clinically diagnosed	430	203 (47.2%)	65.5 ± 9.1 (26–90)	62.4 ± 9.1 (26–85)	430 (100.0%)	0 (0.0%)

Continuous variables are summarized with the sample mean ± standard deviation (range). For cohort 1, the age provided is age at blood draw in controls, age at onset in clinically diagnosed patients, and age at death in pathologically diagnosed patients. For cohort 2, the age provided is age at blood draw in controls, age at diagnosis in clinically diagnosed patients, and age at death in pathologically diagnosed patients. In cohort 1, information was unavailable regarding age (n=42) and age at onset (n=57). In cohort 2, information was unavailable regarding age at onset (n=95).

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Table 2

Associations of *TMEM106B* rs3173615 with disease

		***TMEM106B* rs3173615 genotype information**				**Comparison with controls under a recessive model**	
Group	**N**	**MAF**	**CC**	**CG**	**GG**	**OR (95% CI)**	**P-value**
Cohort 1 – controls and *C9ORF72* repeat expansion carrier probands (FTD, FTD/MND, MND)							
Controls	376	43.2%	123 (32.7%)	181 (48.1%)	72 (19.1%)	1.00 (reference)	N/A
FTD, FTD/MND, and MND patients	260	38.8%	89 (34.2%)	140 (53.8%)	31 (11.9%)	0.57 (0.36 – 0.90)	0.014
FTD patients	69	35.5%	25 (36.2%)	39 (56.5%)	5 (7.2%)	0.33 (0.13 – 0.85)	0.009
FTD/MND patients	71	42.3%	17 (23.9%)	48 (67.6%)	6 (8.5%)	0.38 (0.16 – 0.92)	0.017
MND patients	120	38.8%	47 (39.2%)	53 (44.2%)	20 (16.7%)	0.85 (0.49 – 1.46)	0.55
Cohort 2 – controls and FTD or FTD/MND patients without *C9ORF72* repeat expansions or *GRN* mutations							
Controls	765	41.0%	280 (36.6%)	342 (44.7%)	143 (18.7%)	1.00 (reference)	N/A
FTD and FTD/MND patients	586	39.3%	213 (36.3%)	285 (48.6%)	88 (15.0%)	0.77 (0.58 – 1.03)	0.079
FTD patients	531	39.8%	187 (35.2%)	265 (49.9%)	79 (14.9%)	0.76 (0.56 – 1.03)	0.071
Pathologically diagnosed	101	30.6%	45 (44.6%)	50 (49.5%)	6 (5.9%)	0.26 (0.11 – 0.61)	<0.001
Clinically diagnosed	430	42.0%	142 (33.0%)	215 (50.0%)	73 (17.0%)	0.91 (0.67 – 1.25)	0.56

FTD=frontotemporal dementia; MND=motor neuron disease; OR=odds ratio; CI=confidence interval; MAF=minor allele frequency. ORs, 95% CIs, and p-values result from logistic regression models where rs3173615 was considered under a recessive model. For cohort 1, models were adjusted for gender. For cohort 2, models were adjusted for age (age at blood draw in controls, age at diagnosis in clinically diagnosed patients, and age at death in pathologically diagnosed patients) and gender. After applying a Holm step-down adjustment for multiple testing, $p \leq 0.025$ are considered as statistically significant in cohort 1, and $p \leq 0.0125$ are considered as statistically significant in cohort 2.

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Table 3

Associations of *TMEM106B* rs3173615 with age at onset for probands in cohort 1

	Association between rs3173615 and age at onset	
Model	**Regression coefficient (95% CI)**	**P-value**
Recessive (GG vs. CC or CG)	−1.26 (−4.78, 2.25)	0.48
Additive (effect of each additional G allele)	−0.24 (−2.04, 1.55)	0.79
Dominant (CG or GG vs. CC)	0.16 (−2.30, 2.63)	0.90
Co-dominant		
CG vs. CC	0.45 (−2.12, 3.02)	0.73
GG vs. CC	−0.99 (−4.84, 2.85)	0.61

Regression coefficients, 95% CIs, and p-values result from linear regression models adjusted for age and disease group. Regression coefficients are interpreted as the change in mean age at onset corresponding to presence of the GG genotype for rs3173615 (recessive model), to each additional G allele for rs3173615 (additive model), and to presence of the CG or GG genotype for rs3173615 (dominant model). For the co-dominant model, regression coefficients are interpreted as the change in mean age at onset in comparison to the CC genotype for rs3173615.

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Table 4

TDP-43 burden of patients with TDP-43 Type A pathology

Group		*C9ORF72* expansion	Genotype	Age at Death	Gender	TDP-43 Count
Cohort 1	**a**	Yes	GG	90	F	7.7
	b	Yes	CC	83	F	10.5
	c	Yes	CC	74	M	11.0
	d	Yes	CC	71	M	20.5
Cohort 2	**e**	No	GG	90	F	4.5
	f	No	CC	84	M	6.0
	g	No	CC	86	M	16.7
	h	No	CC	77	M	24.2

Subjects are homozygous carriers of the minor *TMEM106B* allele (GG), or of the major *TMEM106B* allele (CC) in rs3173615. TDP-43 counts were performed in six 10× microscopic fields randomly selected over the gyrus, and averaged for a composite pathologic burden score. Identifiers correspond to Fig. 1.