Potential genetic modifiers of disease risk and age at onset in patients with frontotemporal lobar degeneration and *GRN* mutations: a genome-wide association study

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Summary

Background Loss-of-function mutations in *GRN* cause frontotemporal lobar degeneration (FTLD). Patients with *GRN* mutations present with a uniform subtype of TAR DNA-binding protein 43 (TDP-43) pathology at autopsy (FTLD-TDP type A); however, age at onset and clinical presentation are variable, even within families. We aimed to identify potential genetic modifiers of disease onset and disease risk in *GRN* mutation carriers.

Methods The study was done in three stages: a discovery stage, a replication stage, and a meta-analysis of the discovery and replication data. In the discovery stage, genome-wide logistic and linear regression analyses were done to test the association of genetic variants with disease risk (case or control status) and age at onset in patients with a *GRN* mutation and controls free of neurodegenerative disorders. Suggestive loci ($p<1\times10^{-5}$) were genotyped in a replication cohort of patients and controls, followed by a meta-analysis. The effect of genome-wide significant variants at the *GFRA2* locus on expression of *GFRA2* was assessed using mRNA expression studies in cerebellar tissue samples from the Mayo Clinic brain bank. The effect of the *GFRA2* locus on progranulin concentrations was studied using previously generated ELISA-based expression data. Co-immunoprecipitation experiments in HEK293T cells were done to test for a direct interaction between GFRA2 and progranulin.

Findings Individuals were enrolled in the current study between Sept 16, 2014, and Oct 5, 2017. After quality control measures, statistical analyses in the discovery stage included 382 unrelated symptomatic *GRN* mutation carriers and 1146 controls free of neurodegenerative disorders collected from 34 research centres located in the USA, Canada, Australia, and Europe. In the replication stage, 210 patients (67 symptomatic *GRN* mutation carriers and 143 patients with FTLD without *GRN* mutations pathologically confirmed as FTLD-TDP type A) and 1798 controls free of neurodegenerative diseases were recruited from 26 sites, 20 of which overlapped with the discovery stage. No genome-wide significant association with age at onset was identified in the discovery or replication stages, or in the meta-analysis. However, in the case-control analysis, we replicated the previously reported *TMEM106B* association (rs1990622 meta-analysis odds ratio [OR] 0·54, 95% CI 0·46–0·63; $p=3\cdot54\times10^{-16}$), and identified a novel genome-wide significant locus at *GFRA2* on chromosome 8p21.3 associated with disease risk (rs36196656 meta-analysis OR 1·49, 95% CI 1·30–1·71; $p=1\cdot58\times10^{-8}$). Expression analyses showed that the risk-associated allele at rs36196656 decreased *GFRA2* mRNA concentrations in cerebellar tissue (p=0·04). No effect of rs36196656 on plasma and CSF progranulin concentrations was detected by ELISA; however, co-immunoprecipitation experiments in HEK293T cells did suggest a direct binding of progranulin and GFRA2.

Interpretation TMEM106B-related and GFRA2-related pathways might be future targets for treatments for FTLD, but the biological interaction between progranulin and these potential disease modifiers requires further study.

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***TMEM106B* and *GFRA2* might also provide opportunities to select and stratify patients for future clinical trials and, when more is known about their potential effects, to inform genetic counselling, especially for asymptomatic individuals.**

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Introduction

Frontotemporal lobar degeneration (FTLD) represents a collection of neurodegenerative diseases accounting for 5–10% of all patients with dementia and 10–20% of patients with onset of dementia before 65 years of age.[1] Three clinical variants of FTLD have been described: a behavioural variant and two language variants, the non-fluent and the semantic variants of primary progressive aphasia. The most common pathological subtype of FTLD is characterised by aggregates of the TAR DNA-binding protein 43 (TDP-43; FTLD-TDP).[2,3] Four different FTLD-TDP pathological subtypes have been defined based on the morphology and anatomical distribution of TDP-43 pathology (types A–D).[2]

Mutations in *GRN* are the second most common genetic cause of FTLD-TDP, accounting for 5–20% of FTLD cases with positive family history.[4–6] All known heterozygous pathogenic *GRN* mutations cause disease through a uniform disease mechanism: the loss of 50% of functional progranulin, leading to haploinsufficiency.[4] Additionally, all patients with *GRN* mutations present with FTLD-TDP type A at autopsy.[2] Despite this uniform disease mechanism and pathological presentation, according to clinical findings, the age at symptom onset and clinical phenotype associated with *GRN* mutations are variable, even within the same family, and the penetrance of *GRN* mutations is not complete, even at old age.[7,8] A genome-wide association study (GWAS) from 2010[9] reported variants in *TMEM106B* as risk factors for FTLD-TDP, and subsequent studies[10,11] established *TMEM106B* as a modifier of disease risk in individuals with *GRN* mutations.[9–11] Identification of additional genetic modifiers of *GRN*-associated FTLD could lead to improved genetic counselling, and could help to identify potential new targets for disease-modifying treatments. We therefore aimed to identify additional

Research in context

Evidence before this study

Mutations in *GRN* are an important cause of frontotemporal lobar degeneration (FTLD) with TAR DNA-binding protein 43 (TDP-43) pathology (FTLD-TDP). Pathogenic mutations are heterozygous and cause disease through a uniform mechanism leading to a 50% loss of functional progranulin. We searched PubMed on Jan 30, 2018, for the terms "GRN" OR "PGRN" AND "onset age variability", without language restrictions and including all publications from database inception. We identified seven studies, which reported large variability in age at onset among *GRN* mutation carriers, suggesting that genetic modifiers might be in part responsible for the phenotypic presentation. We also searched PubMed on Jan 30, 2018, for the terms "GRN" OR "PGRN" AND "genome-wide association study", without language restrictions and including all publications from database inception. We identified one previous study that focused on FTLD-TDP, which included 80 *GRN* mutation carriers in a genome-wide association analysis. That study identified *TMEM106B* as a risk factor in patients with FTLD-TDP, with a particularly strong effect in *GRN* mutation carriers, suggesting an effect of *TMEM106B* variants on disease penetrance in individuals with *GRN* mutations. We found no other genome-wide association studies in *GRN* mutation carriers done before the current study.

Added value of this study

Through international collaborations, our cohort of patients with *GRN* mutations was five times larger than that used in the previous genome-wide association study. Using a two-stage association study, we confirmed the *TMEM106B* locus as the most important modifier of disease risk in *GRN* mutation carriers and we were able to estimate that *GRN* carriers of the *TMEM106B* protective haplotype (tagged by the G allele of rs3173615) have 50% lower odds of developing disease symptoms than carriers of the non-protective haplotype. We also newly identified the *GFRA2* locus on chromosome 8p21.3 as a potential genome-wide significant modifier of disease risk in patients with *GRN* mutations. The lead variant at the *GFRA2* locus (rs36196656) is located within *GFRA2* intron 3 and affected the expression profile of *GFRA2*. Functional studies also showed that progranulin binds to GFRA2 in vitro.

Implications of all the available evidence

The identification of genetic variants in *TMEM106B* and *GFRA2* as modifiers of disease risk in patients with *GRN* mutations provides new avenues towards biomarker discovery and the development of therapeutic approaches for patients with FTLD. These genetic variants might further inform genetic counselling in families and could aid in future clinical trial designs.

genetic modifiers in *GRN* mutation carriers through genome-wide association analyses in unrelated symptomatic patients with *GRN* mutations.

Methods

Study design and participants

The study was done in three stages: a discovery stage, a replication stage, and finally a meta-analysis of the discovery and replication data. Participants were recruited at 40 international clinical or pathological research centres in Italy, the USA, France, Spain, the UK, Canada, the Netherlands, Sweden, Australia, Denmark, Poland, and Germany between Sept 16, 2014, and Oct 5, 2017 (appendix). No restriction in terms of age, sex, or race was applied to the initial selection; however, the statistical analyses only included white individuals to limit genetic heterogeneity (appendix). Identification of *GRN* mutations and assessment of TDP-43 pathological subtype was done at each individual site. In the discovery stage, we obtained DNA from 33 centres from symptomatic *GRN* carriers from the USA, Canada, Europe, and Australia, and healthy controls from Italy and Spain. We also obtained genetic data from 1986 controls free from neurodegenerative diseases from the Genome-Wide Association Study of Parkinson Disease: Genes and Environment from the Center for Inherited Disease Research (CIDR) consortium (NCBI dbGaP phs000196.v3.p1;[12] hereafter referred to as the CIDR dataset and considered one site; appendix). Additional and non-overlapping patients and controls free from neurodegenerative diseases were recruited for the replication stage from 26 centres, 20 of which overlapped with the discovery stage and six of which were newly identified (appendix).

Age at onset was defined as the age at which the first disease symptoms appeared, including initial cognitive dysfunction in judgment, language, or memory, or changes in behaviour or personality. Written informed consent for genetic studies was given by patients and controls who were alive, or by next of kin at the time of death for autopsy material, with approval from each institution's institutional review board.

Procedures and statistical analysis

Genotyping and quality control procedures for the discovery stage are described in detail in the appendix. Genome-wide association analyses, using logistic and linear regressions, were done to test the association of genetic variants with patient or control status (disease risk) and age at onset, respectively, under an additive model for allele effects and adjusting for age, sex, and the first two principal components of genetic variation when appropriate (appendix). Minor alleles were treated as effect alleles. As exploratory analyses, association of variants with absence or presence of specific first clinical symptoms (memory, behaviour, or language impairment) or presence of parkinsonism at any time during the course of the disease was tested among patients by logistic regression adjusting for age, sex, and the first two principal components (appendix). Association of previously reported putative genetic modifier variants in known neurodegenerative disease genes with disease presentation and age at onset were also established.

Lead variants or a proxy associated at a p value of less than 1×10^{-5} with disease risk or age at onset in the discovery stage were selected for the replication stage. Genotyping and quality control measures for this stage are described in the appendix. Association analyses were done using logistic or linear regressions to replicate association of genetic variants associated suggestively with disease risk or age at onset, adjusting for age and sex when appropriate under an additive model. 36 variants at 34 loci were analysed in the replication stage, and thus a Bonferroni-corrected significance threshold of p less than $1{\cdot}5\times10^{-3}$ was used in this stage. A meta-analysis of the discovery and replication results was done under a fixed-effects model. We also calculated I^2 heterogeneity statistics to assess the degree of heterogeneity of the effects in the discovery and replication stages; for single nucleotide polymorphisms with an I^2 value suggestive of moderate or high heterogeneity ($I^2>0{\cdot}3$) we also did a random effects meta-analysis to verify that conclusions regarding associations would not change under this model. Using the discovery data, a test of interaction was done for the

	Discovery stage		Replication stage		
	GRN mutation carriers (n=382)	Controls (n=1146)	*GRN* mutation carriers (n=67)	Controls (n=1798)	*GRN*-negative FTLD-TDP type A (n=143)
Age (years)					
At onset	60·0 (55·0–66·0)	NA	59·0 (55·0–65·0)	NA	70·0 (62·0–76·8)
At death	66·0 (61·0–73·0)	NA	65·0 (60·8–71·0)	77·0 (64·0–81·0)	79·0 (68·0–85·0)
At last healthy visit	NA	62·0 (56·0–67·0)	NA	62·0 (53·0–71·0)	NA
Sex					
Women	211 (55%)	630 (55%)	35 (52%)	853 (47%)	61 (43%)
Men	171 (45%)	516 (45%)	32 (48%)	945 (53%)	82 (57%)

Data are median (IQR) or number (%). NA=not applicable. FTLD-TDP=frontotemporal lobar degeneration with TAR DNA-binding protein 43.

Table 1: **Demographics**

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genome-wide significant loci found to modify disease risk in *GRN* mutation carriers. Specifically, using the top variants from *TMEM106B* and *GFRA2*, a logistic regression model was fitted with both variant genotypes and their multiplicative effect as predictors of risk, and a likelihood ratio test of the multiplicative term was done to assess the effect of the variant interaction on disease risk.

To establish the effect of the lead variant at the *GFRA2* locus on brain *GFRA2* mRNA expression, quantitative real-time PCR was done in cerebellar tissue samples from AA and CC carriers from the Mayo Clinic brain bank (appendix). The effect of the lead variant on progranulin concentrations in plasma and CSF was assessed by Taqman genotyping of individuals for whom concentrations of progranulin were previously measured by ELISA,[13] by linear regression adjusting for age and sex. Whole-genome sequence data from control individuals from the Mayo Clinic biobank were used to estimate linkage disequilibrium measures (D' and r^2) between all variants at the *GFRA2* locus and the lead variant.

To study the direct interaction between progranulin and GFRA2, HEK293T cells were co-transfected with GFRA2 and progranulin. Cell lysates were collected and subjected to immunoprecipitation (appendix).

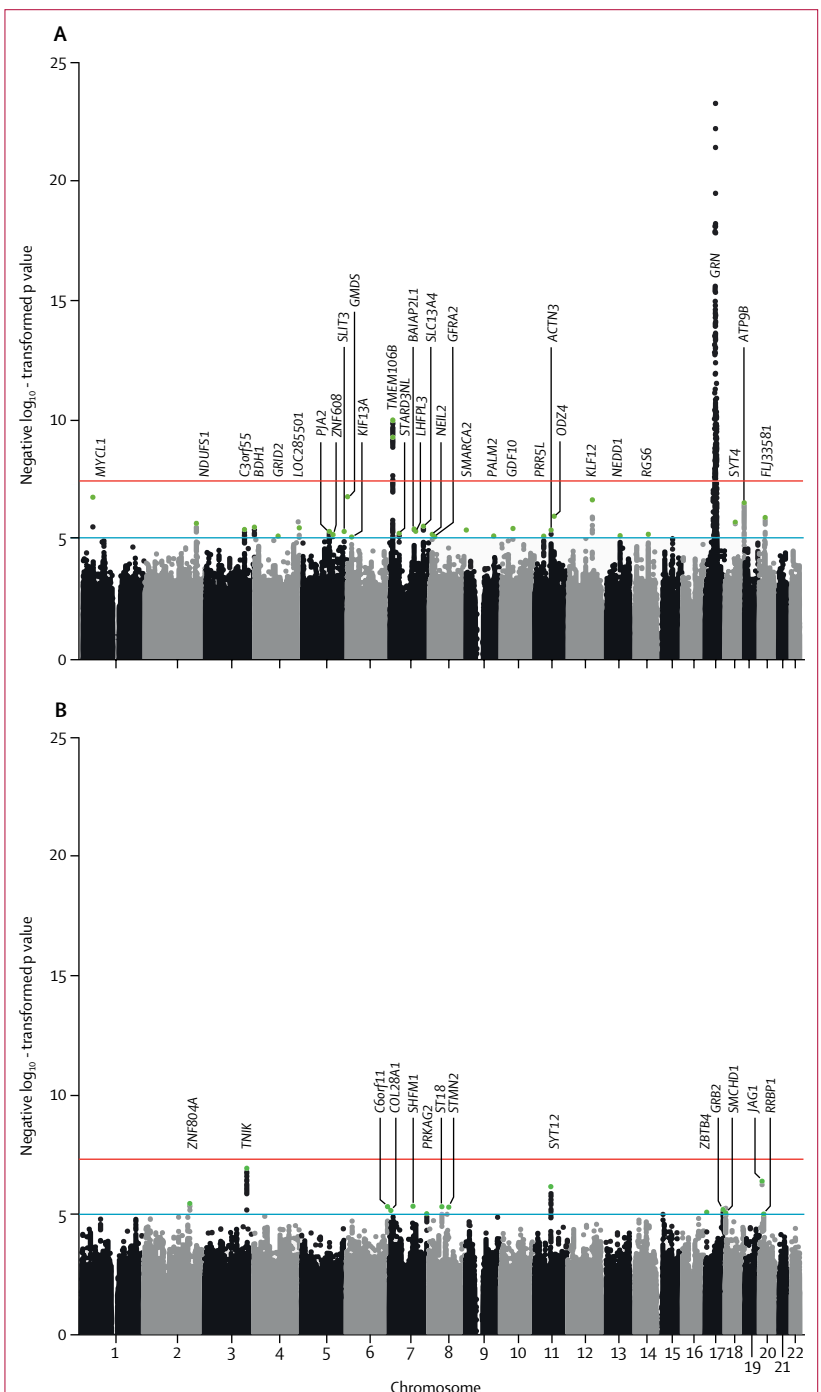

Figure 1: **Manhattan plots of the case-control (A) and age at onset (B) analyses in the discovery phase**
The red lines represent the genome-wide significance threshold ($p<5\times10^{-8}$). The blue line denotes $p=1\times10^{-5}$. Green dots represent the variants that were included in the replication stage. At some loci a proxy of the top variant was selected for genotyping in the replication stage.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
In the discovery stage, we obtained DNA samples from 493 patients carrying 120 different loss-of-function mutations in *GRN* and 505 controls from Italy and Spain. We also obtained genetic data from 1986 controls from the CIDR dataset (appendix). Three mutations were identified in more than 20 patients: Thr272Serfs*10 (n=97), Arg493* (n=35), and the chromosomal mutation 709-1G>A (n=31). After quality control, the discovery stage included 382 unrelated symptomatic *GRN* mutation carriers and 1146 unrelated controls. Patients had a median age at onset of 60·0 years (IQR 55·0–66·0) and 211 (55%) were women (table 1). Large variability in the age at onset was detected even among patients with the same mutation. Among patients with the most frequent mutation, Thr272Serfs*10, ages at onset ranged from 39 years to 82 years, with a median age at onset of 62·0 years (IQR 56·0–66·0). Genome-wide logistic regression analysis in the discovery stage identified an expected significant association with variants at the *GRN* locus on chromosome 17q21 (figure 1). Haplotype analyses using 16 variants around *GRN* showed that this association was driven by distantly related individuals sharing founder haplotypes corresponding to the most common mutations in our cohort. Within the quality control dataset, we estimated the presence of a shared haplotype in 22 (100%) of 22 patients carrying the 709-1G>A mutation and in 63 (81%) of 78 patients carrying the Thr272Serfs*10 mutation, whereas 18 (60%)

of 30 patients with Arg493* were estimated to carry one of two founder haplotypes. We also detected the known *TMEM106B* locus, including 93 variants with genome-wide significant association and in strong linkage disequilibrium (D'>0·8, r^2>0·6) with the lead variant rs7791726 (odds ratio [OR] 0·53, 95% CI 0·44–0·64; p=1·53×10^{-10}; figure 1; appendix). In particular, rs7791726 was in strong linkage disequilibrium with the previously reported *TMEM106B* variants rs1990622, rs3173615, and rs1990620 (D'=1, r^2>0·8). In the discovery cohort, 163 (14%) of 1146 controls were homozygous carriers of the rare allele rs3173615, whereas only four (1%) of 382 were homozygous in the *GRN* mutation carriers cohort. No additional genome-wide significant association signals were detected; however, 29 additional loci showed suggestive association (p<1×10^{-5}; figure 1; appendix). After adjustment with the lead variant on chromosome 17q21 (rs141568868), these suggestive associations did not change substantially.

A separate genome-wide linear regression analysis of onset age within the patient cohort did not identify any genome-wide significant association signals; however, 14 loci showed suggestive associations (p<1×10^{-5}; figure 1, table 2; appendix). Since only the wild-type copy of *GRN* is expressed in patients with *GRN* mutations, we analysed the effect of rs5848 located in the 3′ untranslated region of *GRN*, comparing patients homozygous for the common (C) and rare (T) alleles at this marker; no significant association with onset age was noted (p=0·36). No association with age at onset was detected with rs3173615 at the *TMEM106B* locus (beta –0·12, 95% CI –1·59 to 1·35; p=0·87).

The replication stage of the association study, which included 210 patients (67 symptomatic *GRN* mutation carriers and 143 patients without known mutations in *GRN* and C9ORF72 with pathologically confirmed FTLD-TDP type A) and 1798 controls (table 1), identified significant association at the Bonferroni-corrected level of p less than 1·5×10^{-3} for two loci nominated by the case-control discovery GWAS (*TMEM106B* and *GFRA2*; table 3). None of the loci nominated through the discovery GWAS of age at disease onset were significant after Bonferroni correction (table 2). The strongest signal in the case-control analysis was at the *TMEM106B* locus with marker rs3173615 (OR 0·53, 95% CI 0·42–0·67; p=8·97×10^{-8}; table 3). The lead variant at the second locus was rs36196656 located within intron 3 of *GFRA2* (MAF$_{patients}$=0·44, MAF$_{controls}$=0·35; OR 1·46, 95% CI 1·18–1·80; p=0·00044). In the meta-analysis of discovery and replication stages, both the *TMEM106B* and *GFRA2* loci achieved genome-wide significance (*TMEM106B*, rs3173615, OR 0·54, 95% CI 0·47–0·63; p=3·78×10^{-16}; *GFRA2*, rs36196656 OR 1·49, 95% CI 1·30–1·71; p=1·58×10^{-8}; table 3). For both loci, the I^2 heterogeneity statistic showed no heterogeneity of effects between the two stages. No other loci had a p value less than 5×10^{-8} in the meta-analysis. For loci showing high heterogeneity

	Position*	Major/minor allele	Locus name	Discovery stage			Replication stage			Meta-analysis		
				MAF in patients	Beta (95% CI)	p value	MAF in patients	Beta (95% CI)	p value	Beta (95% CI)	p value	I^2
rs116316277	2:185834886	C/T	*ZNF804A*	0·03	8·09 (4·72 to 11·46)	3·58×10^{-6}	0·08	–1·03 (–6·79 to 4·72)	0·73	5·76 (2·85 to 8·67)	0·00010	86·1
rs6809184	3:170888198	C/T	*TNIK*	0·05	–6·78 (–9·24 to –4·32)	1·22×10^{-7}	0·09	–0·54 (–5·29 to 4·21)	0·82	–5·46 (–7·64 to –3·27)	1·01×10^{-6}	80·9
rs12189587	6:165332257	C/T	*C6orf11*	0·11	–4·05 (–5·76 to –2·34)	4·83×10^{-6}	0·13	1·40 (–2·32 to 5·11)	0·46	–3·10 (–4·65 to –1·54)	9·44×10^{-5}	85·3
rs6962939	7:7524226	T/A	*COL28A1*	0·04	–6·02 (–8·61 to –3·43)	7·00×10^{-6}	0·06	–6·15 (–12·56 to 0·25)	0·061	–6·04 (–8·43 to –3·64)	8·18×10^{-7}	0
rs2922921	7:96398079	G/A	*SHFM1*	0·02	9·65 (5·58 to 13·72)	4·65×10^{-6}	0·06	–0·54 (–7·77 to 6·70)	0·88	7·20 (3·65 to 10·75)	6·93×10^{-5}	82·7
rs77466830	7:151529171	C/A	*PRKAG2*	0·32	2·91 (1·64 to 4·18)	9·49×10^{-6}	0·43	–0·13 (–2·36 to 2·11)	0·91	2·17 (1·06 to 3·27)	0·00012	81·3
rs9792144	8:53081551	C/G	*ST18*	0·12	3·99 (2·30 to 5·68)	4·88×10^{-6}	0·18	2·99 (–0·26 to 6·24)	0·073	3·78 (2·28 to 5·28)	7·55×10^{-7}	0
rs3922636	8:80383502	G/A	*STMN2*	0·19	3·28 (1·89 to 4·67)	5·08×10^{-6}	0·31	0·49 (–2·23 to 3·21)	0·72	2·70 (1·47 to 3·94)	1·83×10^{-5}	68·4
rs12943707	17:73317510	C/G	*GRB2*	0·29	–2·80 (–4·00 to –1·60)	6·40×10^{-6}	0·40	–0·41 (–2·80 to 1·98)	0·74	–2·32 (–3·39 to –1·25)	2·22×10^{-5}	67·5
rs1561819	18:2712629	G/A	*SMCHD1*	0·49	–2·41 (–3·46 to –1·36)	8·96×10^{-6}	0·51	–0·81 (–2·97 to 1·35)	0·46	–2·11 (–3·05 to –1·16)	1·23×10^{-5}	41·5
rs6108746	20:10902771	T/C	*JAG1*	0·19	3·54 (2·19 to 4·89)	4·23×10^{-7}	0·25	1·69 (–1·11 to 4·48)	0·24	3·19 (1·98 to 4·41)	2·59×10^{-7}	27·4
rs6111609	20:17664546	C/A	*RRBP1*	0·22	2·86 (1·61 to 4·11)	9·83×10^{-6}	0·22	2·88 (–0·03 to 5·80)	0·054	2·86 (1·71 to 4·01)	1·05×10^{-6}	0

The locus name is determined by the closest gene to the significant variant. MAF=minor allele frequency. *Based on the Human Genome version 38.

Table 2: **Loci identified in the age at onset analyses and followed up in the replication stage**

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	Position*	Major/minor allele	Locus name	Discovery stage			Replication stage			Meta-analysis		
				MAF in patients/controls	OR (95%CI)	p value	MAF in patients/controls	OR (95%CI)	p value	OR (95%CI)	p value	I^2
rs13393316	2:206999339	A/G	*NDUFS1*	0·10/0·16	0·50 (0·38–0·67)	$2·65×10^{-6}$	0·12/0·14	0·81 (0·59–1·13)	0·21	0·62 (0·50–0·77)	$1·34×10^{-5}$	78·5
rs4680382	3:157324261	G/A	*C3orf55*	0·59/0·32	1·50 (1·26–1·78)	$4·75×10^{-6}$	0·35/0·35	1·00 (0·80–1·24)	0·99	1·28 (1·12–1·47)	0·00035	87·7
rs13072484	3:197136822	G/A	*BDH1*	0·29/0·21	1·54 (1·28–1·85)	$3·79×10^{-6}$	0·23/0·22	1·03 (0·81–1·32)	0·80	1·34 (1·15–1·55)	0·00012	84·5
rs7905029	5:108855306	C/G	*PJA2*	0·03/0·08	0·35 (0·23–0·55)	$5·72×10^{-6}$	0·07/0·08	0·99 (0·66–1·49)	0·96	0·62 (0·46–0·84)	0·0020	91·0
rs146261599	5:123600139	T/G	*ZNF608*	0·05/0·02	2·91 (1·82–4·64)	$7·64×10^{-6}$	0·02/0·03	1·09 (0·54–2·17)	0·81	2·13 (1·45–3·15)	0·00012	81·3
rs181675566	5:168651912	T/C	*SLIT3*	0·04/0·01	3·86 (2·15–6·90)	$5·72×10^{-6}$	0·01/0·02	0·60 (0·24–1·53)	0·29	2·29 (1·40–3·76)	0·0010	90·8
rs6904835†	6:17810195	T/C	*KIF13A*	0·32/0·24	1·50 (1·25–1·80)	$9·67×10^{-6}$	0·29/0·27	1·08 (0·86–1·36)	0·51	1·32 (1·15–1·53)	$9·94×10^{-5}$	79·5
rs3173615†‡	7:12269417	C/G	*TMEM106B*	0·27/0·39	0·55 (0·45–0·66)	$7·81×10^{-10}$	0·27/0·42	0·53 (0·42–0·67)	$8·97×10^{-8}$	0·54 (0·47–0·63)	$3·78×10^{-16}$	0
rs7791726†‡	7:12283329	G/C	*TMEM106B*	0·26/0·39	0·53 (0·44–0·64)	$1·53×10^{-10}$	0·28/0·42	0·55 (0·44–0·70)	$4·71×10^{-7}$	0·54 (0·46–0·63)	$3·80×10^{-16}$	0
rs1990622†‡	7:12283787	A/G	*TMEM106B*	0·26/0·39	0·53 (0·44–0·65)	$1·61×10^{-10}$	0·28/0·42	0·55 (0·44–0·70)	$4·09×10^{-7}$	0·54 (0·46–0·63)	$3·54×10^{-16}$	0
rs62443267	7:38153313	C/T	*STARD3NL*	0·19/0·19	0·62 (0·50–0·76)	$6·83×10^{-6}$	0·25/0·25	0·93 (0·73–1·20)	0·59	0·74 (0·63–0·86)	0·00016	84·1
rs141226303	7:104251213	A/G	*LHFPL3*	0·04/0·01	3·73 (2·11–6·59)	$5·61×10^{-6}$	0·02/0·01	1·06 (0·47–2·38)	0·89	2·46 (1·55–3·93)	0·00015	83·9
rs3110811†	7:135402648	A/G	*SLC13A4*	0·29/0·20	1·55 (1·29–1·87)	$3·50×10^{-6}$	0·21/0·23	0·82 (0·64–1·06)	0·14	1·25 (1·07–1·45)	0·0039	93·5
rs10101195†	8:11623212	C/A	*NEIL2*	0·18/0·26	0·62 (0·51–0·77)	$7·50×10^{-6}$	0·20/0·23	0·79 (0·61–1·02)	0·071	0·68 (0·58–0·80)	$3·71×10^{-6}$	47·9
rs36196656†‡	8:21621247	C/A	*GFRA2*	0·46/0·37	1·51 (1·26–1·82)	$9·44×10^{-6}$	0·44/0·35	1·46 (1·18–1·80)	0·00044	1·49 (1·30–1·71)	$1·58×10^{-8}$	0
rs10816848	9:112421435	T/A	*PALM2*	0·42/0·49	0·68 (0·57–0·80)	$8·74×10^{-6}$	0·46/0·49	0·87 (0·70–1·07)	0·19	0·69 (0·65–0·85)	$6·37×10^{-6}$	69·4
rs78781776	11:36466533	A/G	*PRR5L*	0·10/0·05	2·00 (1·47–2·72)	$8·88×10^{-6}$	0·09/0·07	1·27 (0·86–1·86)	0·23	1·68 (1·32–2·13)	$2·37×10^{-5}$	69·8
rs10791882†	11:66319313	G/A	*ACTN3*	0·46/0·37	1·49 (1·26–1·77)	$5·01×10^{-6}$	0·40/0·39	1·06 (0·85–1·31)	0·62	1·30 (1·14–1·49)	0·00012	83·5
rs10860097	12:97199656	A/T	*NEDD1*	0·05/0·02	3·43 (2·14–5·50)	$2·88×10^{-7}$	0·03/0·02	1·29 (0·70–2·37)	0·41	2·38 (1·64–3·45)	$5·15×10^{-6}$	83·9
rs61965655	13:74712915	T/A	*KLF12*	0·07/0·04	2·33 (1·61–3·39)	$8·52×10^{-6}$	0·04/0·05	0·91 (0·55–1·52)	0·73	1·71 (1·24–2·27)	0·00042	88·2
rs847358	14:72780521	G/A	*RGS6*	0·53/0·44	1·46 (1·24–1·73)	$7·43×10^{-6}$	0·45/0·46	0·97 (0·79–1·20)	0·78	1·25 (1·10–1·42)	0·00084	88·9
rs12605286	18:41150167	G/A	*SYT4*	0·23/0·31	0·60 (0·48–0·74)	$2·37×10^{-6}$	0·29/0·26	1·20 (0·95–1·51)	0·13	0·82 (0·70–0·96)	0·014	94·6
rs7240419†	18:76928989	G/A	*ATP9B*	0·31/0·22	1·62 (1·34–1·94)	$3·80×10^{-7}$	0·25/0·23	1·10 (0·86–1·41)	0·44	1·41 (1·21–1·63)	$5·96×10^{-6}$	83·0
rs6076187	20:24082578	G/A	*FLJ33581*	0·07/0·03	2·47 (1·71–3·57)	$1·53×10^{-6}$	0·04/0·04	0·87 (0·50–1·53)	0·64	1·80 (1·32–2·45)	0·00017	89·2

The locus name is determined by the closest gene to the significant variant. MAF=minor allele frequency. OR=odds ratio. *Based on the Human Genome version 38. †Variants annotated as expression quantitative loci in the GTex database. ‡Variants that are study-wide significant at the replication stage after Bonferroni correction.

Table 3: **Loci identified in the case-control analysis and followed up in the replication stage**

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under a fixed-effects model, a meta-analysis using a random-effect model was done and no substantial differences in association were detected compared with the fixed-effects model. Conditional analysis adjusted for the *TMEM106B* variant rs3173615 in the discovery stage had no effect on the association at the *GFRA2* variant rs36196656 (OR 1·54, 95% CI 1·28–1·85; p=5·80×10^{-6}). Moreover, tests of interactions between these variants provided no evidence for interaction effects on disease risk ($p_{interaction}$>0·1; data not shown), suggesting that the effect of the *GFRA2* variant on disease risk is not modified by the *TMEM106B* genotype that a person carries, and vice versa.

At the putative novel *GFRA2* locus, both patients with *GRN* mutations and those with FTLD-TDP type A without known mutations contributed to the observed association in the replication stage (appendix). A significant association was detected when only patients with *GRN* mutations were included (OR 1·69, 95% CI 1·19–2·40; p=0·0031; appendix); however, patients with FTLD-TDP type A showed a comparable allele frequency and OR at rs36196656 (OR 1·40, 95% CI 1·08–1·82; p=0·011; appendix).

To identify possible functional variants at the newly identified putative *GFRA2* locus, we analysed publicly available data and whole-genome sequence data from 959 control individuals from the Mayo Clinic biobank, which showed two single nucleotide polymorphisms (rs144692383 and rs150047054) and a deletion of three base pairs (rs36144451) to be in strong linkage disequilibrium (r^2>0·8) with the lead variant rs36196656 (figure 2; appendix). All four variants are located in close proximity within *GFRA2* intronic regions: intron 3 of *GFRA2* transcript variant A (NM_001495), intron 2 of *GFRA2* transcript variant B (NM_001165038), and intron 1 of *GFRA2* transcript variant C (NM_001165039), depending on alternative splicing at the *GFRA2* locus (figure 2). Several of these variants are predicted to affect transcription factor binding sites and histone marks and they all are expression quantitative loci for *GFRA2* in testis (p=1·80×10^{-14} according to the GTex database). Indeed, *GFRA2* RNA expression analyses in cerebellar tissue samples from individuals with rs36196656 CC

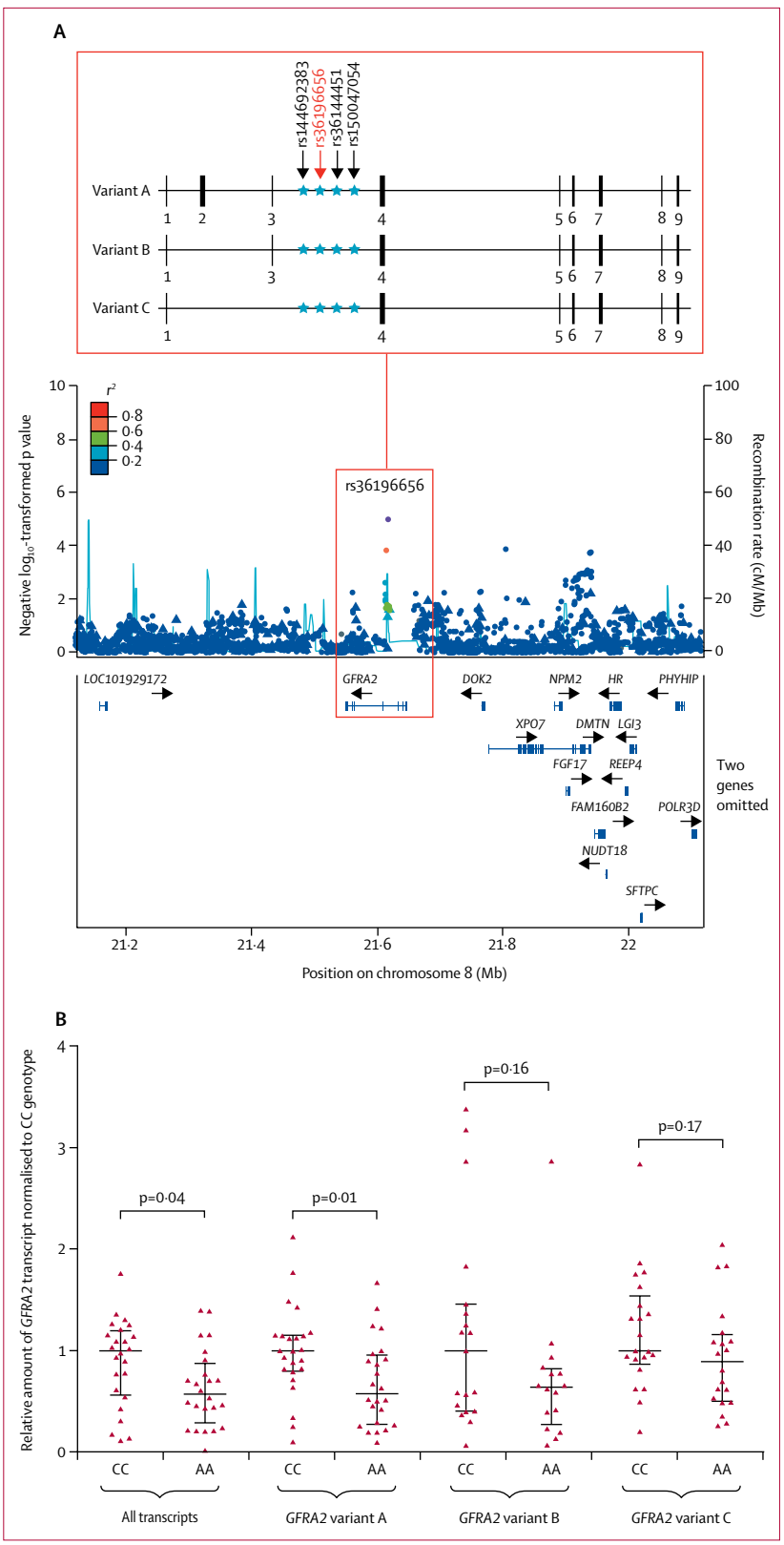

***Figure 2:* GFRA2 genetic locus and expression studies**
(A) The top panel presents the *GFRA2* gene and its three GFRA2 transcripts. Exons are represented as small black boxes and non-coding regions as a straight line. The location of the three variants in strong linkage disequilibrium (black arrows) with rs36196656 (red arrow) are represented as blue stars across the different *GFRA2* transcripts. The *GFRA2* locus zoom plot is presented on the bottom panel. Each dot represents a genotyped (triangle) or imputed (circle) variant. The purple dot is the most significant variant (rs36196656) among variants in the region. Dots are coloured from red to blue according to their r^2 value, showing their degree of linkage disequilibrium with rs36196656 (grey indicates an r^2 of zero). The light blue line shows the estimated recombination rate. (B) Cerebellar mRNA expression of *GFRA2* transcripts stratified by rs36196656 genotype. All values are normalised to two reference genes and within each assay, expression is shown normalised to homozygous rs36196656-CC carriers. The median (horizontal bar) and IQR (whiskers) is presented for each group.

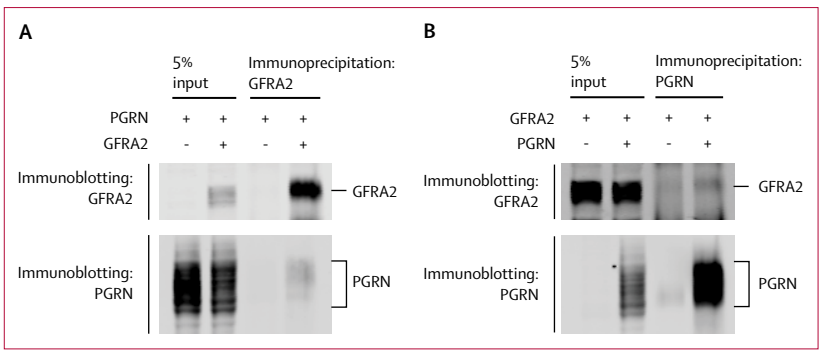

Figure 3: **Interaction of progranulin and GFRA2**
GFRA2 and progranulin immunoblots are displayed after immunoprecipitation with anti-GFRA2 antibody (A) and anti-progranulin antibody (B) from cell lysates of HEK293T cells co-transfected with untagged GFRA2 and untagged progranulin or vector control. 5% input=5% of the total amount of cell lysates used for immunoprecipitation.

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(n=24) and AA (n=24) genotypes available from the Mayo Clinic brain bank showed substantial variability in expression among individuals, but confirmed a 40% reduction in all *GFRA2* transcripts in brains of homozygous carriers of the risk allele (AA) compared with CC carriers, which was significant when analysing all *GFRA2* variants (p=0·04) or variant A individually (p=0·01; figure 2). *GFRA2* transcript variant A was consistently the predominant transcript expressed in cerebellar tissue (appendix), and no significant difference in the ratio of *GFRA2* transcripts (A, B, and C) was noted between AA and CC carriers (data not shown). Since the potential functional variant or variants underlying the observed association could also be less frequent than the lead variant, we further identified all variants with *D'* greater than 0·8, which resulted in an additional 130 single nucleotide variants, none of which were coding (data not shown).

To assess a potential direct effect of *GFRA2* markers on progranulin expression in plasma and CSF, we did a linear regression adjusting for age and sex, which showed that rs36196656 is not associated with progranulin concentrations in plasma or CSF in 345 individuals (p=0·61 and p=0·67 respectively; appendix). We next hypothesised that GFRA2 might directly interact with progranulin and serve as a receptor for progranulin. Using transient overexpression of untagged progranulin and GFRA2 in HEK293T cells, immunoprecipitation of GFRA2 pulled down progranulin in cell lysates. Reciprocally, immunoprecipitation of progranulin pulled down GFRA2 (figure 3).

Discussion

Using an unbiased two-stage GWAS in what is, to our knowledge, the largest available collection of unrelated patients with FTLD with pathogenic *GRN* mutations, we identified two association signals: one at the known *TMEM106B* locus and one at a novel putative locus encompassing *GFRA2*. *GRN* mutations are a rare cause of FTLD and, despite the international nature of our collaboration, we were limited by the number of *GRN* carriers we were able to identify. In the discovery stage, we therefore relied on the uniform loss-of-function disease mechanism associated with pathogenic *GRN* mutations and combined genetic analysis of patients with 120 distinct mutations. In the replication stage, newly identified *GRN* mutation carriers were combined with patients with FTLD-TDP type A with unknown genetic cause, who are pathologically indistinguishable from *GRN* carriers and possibly share disease mechanisms. Using this approach, genome-wide significant associations were detected when symptomatic patients were compared with healthy controls, suggesting that *TMEM106B* and *GFRA2* are able to modify disease risk. Moreover, the allele at the lead *GFRA2* variant (rs36196656) associated with reduced disease risk correlated with increased brain mRNA expression of *GFRA2* transcripts.

Our study confirms *TMEM106B* as the strongest modifier of disease risk in *GRN* mutation carriers and patients with *GRN*-negative FTLD-TDP type A. Published studies had already established that variants associated with the *TMEM106B* risk haplotype correlate with increased expression of *TMEM106B*[9] and increases in the amount of TMEM106B are detrimental to lysosomal health and function.[14–16] Among the variants in strong linkage disequilibrium, several functional candidates have been reported, including rs3173615 encoding TMEM106B Thr185Ser and the non-coding variant rs1990620, which is suggested to affect higher-order chromatin architecture at the *TMEM106B* locus.[16,17] We estimated that *GRN* carriers of the *TMEM106B* protective haplotype (tagged by the G allele of rs3173615) have about 50% lower odds of developing disease symptoms compared with carriers of the non-protective haplotype. Indeed, despite a population frequency of 14% in our control discovery cohort, only 1% of unrelated symptomatic patients were homozygous rs3173615 GG carriers, suggesting that many *GRN* mutation carriers who are also homozygous for the protective *TMEM106B* haplotype do not have symptoms. This is an important finding for a disease gene once thought to be nearly fully penetrant and prompts the important question as to whether *TMEM106B* genotyping should be done routinely when *GRN* genetic testing is requested, or whether it should at least be discussed as a crucial component of predictive *GRN* genetic testing and counselling protocols, especially in asymptomatic individuals.

The *GFRA2* locus was identified as a second independent potential modifier of disease risk, with a significant association in the meta-analysis of our combined discovery and replication stages. Both *GRN* carriers and patients with FTLD-TDP type A without mutations contributed to the observed association. Expression data points to a potential disease mechanism in which risk-associated variants at the *GFRA2* locus decrease brain mRNA expression of *GFRA2*. Whether

these variants similarly affect GFRA2 protein expression remains to be tested. GFRA2 is the preferential coreceptor for neurturin, one of four members of the GDNF family ligands with an important role in neuronal differentiation, proliferation, and survival.[18] Neurturin further requires the transmembrane signalling receptor tyrosine kinase RET to assemble as a multi-component receptor system. Upon binding of neurturin to GFRA2, RET activates downstream signalling pathways including mitogen activated protein kinase, extracellular signal-regulated kinase 1/2, and AKT. In vitro, we obtained evidence of a direct binding of progranulin to GFRA2, which could suggest that GFRA2 might be a signalling receptor for progranulin; however, future experiments both in vitro and in vivo will be needed to establish the functional consequences of this interaction. If GFRA2 is confirmed to serve as a receptor for progranulin, one possible future therapeutic avenue could be to enhance their binding, for example by using small molecules or compounds that target the progranulin-GFRA2 interaction. Another possibility, which is not mutually exclusive, is that progranulin and GFRA2 are part of independent neurotrophic signalling pathways. In this scenario, reduced neurotrophic signalling in *GFRA2* risk allele carriers might facilitate the development of symptoms in *GRN* mutation carriers, who are already vulnerable to neuronal loss as a result of reduced neurotrophic progranulin signalling. A loss of neurotrophic GFRA2 signalling might also affect patients with FTLD-TDP type A without *GRN* mutations, especially since *GFRA2* expression seems to be enriched in the frontal and motor cortices, which are highly vulnerable regions in FTLD (appendix). The finding of impaired behaviour and memory deficits in *GFRA2* knockout mice further supports this theory.[19] GDNF, which preferentially binds to GFRA1, and neurturin have been studied extensively for their neuroprotective potential in Parkinson's disease models, and clinical trials in patients with Parkinson's disease have been done by delivery of GDNF and neurturin as purified proteins or by viral-vector-mediated gene delivery to the brain.[20–22] Although none of these proteins have shown efficacy in clinical trials, the delivery of GDNF family ligands to the brain was safe and provides hope that modified gene therapy approaches to boost GFRA2/neurturin signalling could be developed and tested in patients with sporadic FTLD or FTLD associated with mutations in *GRN*.

Our study did not identify genome-wide significant associations with age at disease onset. Variability in the clinical presentation of FTLD and the subjective nature of defining disease onset might have contributed to this absence of association, especially since 40 clinical centres contributed data. The focus on unrelated symptomatic patients as opposed to extended families, in which a smaller number of genetic factors are expected to contribute to disease onset, might have further restricted our ability to identify significant associations. A previous study[11] in four large families reported a 13-year decrease in onset age for carriers of the *TMEM106B* risk allele; however, no association with age at onset was noted for *TMEM106B* in our study.

Our study has several limitations. First, only symptomatic unrelated *GRN* mutation carriers were included in the analysis. Individual *GRN* families were generally small with few symptomatic and informative asymptomatic carriers available, which restricts the ability to perform family-based studies. Second, since patient samples were collected in various countries, population stratification could bias the results. To address this issue, we combined publicly available control genotype data with newly generated genotypes from control individuals ascertained in Italy and Spain, allowing each patient to be matched to three controls from the same country, followed by standard methods to correct for any remaining bias. Detailed analysis at the newly identified putative *GFRA2* locus across geographical populations showed consistent ORs associated with the lead variant (rs36196656; appendix). Third, patients with FTLD-TDP type A without *GRN* mutations were included in the replication stage. Although this broadens the potential effect of *TMEM106B* and *GFRA2* associations to patients with sporadic FTLD, our approach probably discounted several genetic modifiers specific to *GRN* mutation carriers. Finally, our functional studies were limited to GFRA2 and thus other genes in addition to *GFRA2* might contribute to the observed association on chromosome 8.

In conclusion, two loci, *TMEM106B* and *GFRA2*, harboured genetic variants able to modify disease risk. These modifiers are likely to inform genetic counselling in families and could aid in future clinical trial designs. More importantly, identification of these modifiers in human beings supports TMEM106B-related and GFRA2-related pathways as potential targets for treatment. Accordingly, increasing GFRA2 expression or signalling, or improving lysosomal function, or both, in FTLD-relevant brain areas might be important areas for future research that could complement the current translational research efforts focused on increasing progranulin concentrations.[23–25]

Contributors

RR designed and oversaw the study. RR, CP, XZ, and JMB did the primary data interpretation. RR, CP, and XZ wrote the paper, and JMB contributed substantial edits. RBP generated and CP analysed the genotypes for the replication stage. JMB supervised and CP, GDJ, and DJS participated in quality control and statistical analysis of the discovery stage, replication stage, and meta-analysis. MB did the GFRA2 mRNA expression analyses and CP did statistical analyses of the data. CP, MvB, and YR did bioinformatic analyses of the GFRA2 locus. XZ led and AN, TMP, NAF, MD-H, and RBP assisted in the cell biological analysis of GFRA2 and progranulin. EC was responsible for sample organisation and data curation. All other authors recruited or clinically or neuropathologically characterised patients and controls for the discovery and replication stages of the study. RR acquired funding for the discovery and replication stages, and functional characterisation of candidate genes. All authors contributed to and reviewed the final version of the manuscript.

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See **Online** for appendix

For the **GTex database** see https://www.gtexportal.org/

Declaration of interests
MNS reports grants from Avid Radiopharmaceuticals, Genentech, Merck, Pfizer, Roche, and Suven Life Sciences; reports grants and personal fees from Axovant Sciences, Biogen, Eli Lilly, and vTv Therapeutics; reports personal fees from Grifols and Sanofi; and holds stock in Brain Health, Muses Labs, and Versanum. ALB reports grants from the National Institutes of Health (NIH), Bluefield Project to Cure Frontotemporal Dementia, CBD Solutions, the Tau Consortium, Biogen, Bristol-Myers Squibb, C2N Diagnostics, FORUM Pharmaceuticals, Genentech, Roche, TauRx Therapeutics, and Association for Frontotemporal Degeneration; personal fees from AbbVie, Delos Pharmaceuticals, Denali Therapeutics, Alector, Janssen Pharmaceutica, Celgene, Merck, Novartis, Toyama Chemical, and UCB; and grants and non-financial support from Eli Lilly. GMH reports grants from the Australian National Health and Medical Research Council (grant numbers 1037747, 1079679). ZKW reports grants from the NIH/National Institute of Neurological Disorders and Stroke (NINDS; grant number P50 NS072187). RCP reports personal fees from Roche, Merck, Genentech, and Biogen. All other authors declare no competing interests.

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