

A 3' UTR Deletion Is a Leading Candidate Causal Variant at the *TMEM106B* Locus Reducing Risk for FTL D-TDP

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Abstract

Single nucleotide variants (SNVs) near *TMEM106B* have been associated with risk of frontotemporal lobar dementia with TDP pathology (FTLD-TDP) but the causal variant at this locus has not yet been isolated. The initial leading FTLD-TDP genome-wide association study (GWAS) hit at this locus, rs1990622, is intergenic and is in linkage disequilibrium (LD) with a *TMEM106B* coding SNV, rs3173615. We developed a long-read sequencing (LRS) dataset of 407 individuals in order to identify structural variants associated with neurodegenerative disorders. We identified a prevalent 322 base pair deletion on the *TMEM106B* 3' untranslated region (UTR) that was in perfect linkage with rs1990622 and near-perfect linkage with rs3173615 (genotype discordance in two of 274 individuals who had LRS and short-read next-generation sequencing). In Alzheimer's Disease Sequencing Project (ADSP) participants, this deletion was in greater LD with rs1990622 ($R^2=0.920916$, $D'=0.963472$) than with rs3173615 ($R^2=0.883776$, $D'=0.963575$). rs1990622 and rs3173615 are less closely linked ($R^2=0.7403$, $D'=0.9915$) in African populations. Among African ancestry individuals in the ADSP, the deletion is in even greater LD with rs1990622 ($R^2=0.936841$, $D'=0.976782$) than with rs3173615 ($R^2=0.764242$, $D'=0.974406$). Querying publicly available genetic datasets with associated mRNA expression and protein levels, we confirmed that rs1990622 is consistently a protein quantitative trait locus but not an expression quantitative trait locus, consistent with a causal variant present on the *TMEM106B* 3'UTR. In summary, the *TMEM106B* 3' UTR deletion is a large genetic variant on the *TMEM106B* transcript that is in higher LD with the leading GWAS hit rs1990622 than rs3173615 and may mediate the protective effect of this locus in neurodegenerative disease.

Introduction

Next-generation sequencing (NGS) has enabled genome-wide identification of single nucleotide variants (SNVs) associated with heritable diseases ranging from cancer to neurodegeneration. Variants identified as disease risk-modulating in genome-wide association studies (GWAS) of NGS data are frequently intergenic or intronic; such GWAS hits may be the causal variant themselves or may be in linkage with a nearby genetic feature that is the true disease risk-modifying variant.

Structural variants (SVs) – which include large insertions, deletions, duplications, and other genomic features greater than 50 base pair (bp) in length – are a source of genetic diversity whose impact on protein function is often readily interpretable due to their large size. SVs are presently challenging to identify with NGS. Whereas the 150 bp read length used by most NGS approaches is able to obtain high coverage of SNVs and some small insertions and deletions, SVs that exceed this read length are principally detected in NGS data by analyzing paired and split-read evidence as well as changes in sequencing depth^{1–3}. Emerging long-read sequencing (LRS) technology utilizes reads of greater than 10 kilobases (kb), enabling large SVs to be directly sequenced and properly aligned to the genome⁴. LRS greatly improves on SV discovery over short-read NGS approaches and is able to identify more than twice as many SVs as ensemble methods operating on short-read NGS data; up to 83% of insertions identified by LRS are not detected by NGS algorithms⁵.

We carried out LRS and SV calling for participants enrolled in the Stanford Alzheimer's Disease Research Center (ADRC) and/or the Stanford Aging and Memory Study (SAMS)⁶. After filtering for variants overlapping genes involved in Alzheimer's Disease (AD) and neurological function, we identified a highly prevalent SV on *TMEM106B*. A set of SNVs on and near *TMEM106B* have been associated with a strong protective effect in neurodegenerative diseases including

frontotemporal lobar dementia with TAR DNA-binding protein pathology (FTLD-TDP) and Alzheimer's Disease (AD) (though the effect size in AD is considerably smaller)⁷. We pursued the possibility that SVs on *TMEM106B* may mediate the protective effect of this locus.

The protective association of *TMEM106B* SNVs in FTLD-TDP was first described in a 2010 GWAS of FTLD-TDP cases and controls that identified three significant SNVs (rs1990622, rs6966915, rs1020004) in high linkage disequilibrium (LD) with one another, all on or near *TMEM106B*⁸. For the leading SNV, rs1990622, the major allele (rs1990622_A) was risk-increasing in FTLD-TDP (odds ratio (OR)=1.64) while the minor allele (rs1990622_G) was protective (OR=0.61). The minor allele of rs1990622 is quite common across populations in gnomAD⁹ (allele frequency (AF)=0.4989) and has been associated with increased plasma levels of progranulin (GRN) in controls, suggesting that *TMEM106B* variants may protect against FTLD-TDP by rescuing *GRN* haploinsufficiency^{10,11}. In the latest, largest AD GWAS a *TMEM106B* SNV (rs13237518, chr7:12229967, minor/major allele A/C) is found to be protective (OR=0.96)⁷, though less so than rs1990622 in FTLD-TDP. In LDpair¹², rs13237518 is in LD with rs1990622 ($R^2 = 0.8779$, $D' = 0.9409$).

The mechanism by which variants at the *TMEM106B* locus affect FTLD-TDP risk remains unclear. The genome-wide significant *TMEM106B* SNVs identified in Van Deerlin et al. are either intronic or intergenic. The only coding SNV in high LD with rs1990622 is the missense variant rs3173615, which results in a p.T185S amino acid change in exon 6 of *TMEM106B*. An *in vitro* study found that overexpression of *TMEM106B* S185 in HeLa cells resulted in a smaller increase in *TMEM106B* protein levels than overexpression of WT *TMEM106B*, indicating that rs3173615 may hasten protein degradation¹³. However, an *in vivo* study using a *GRN*^{-/-} mouse model homozygous for *TMEM106B* S186 (the conserved residue in mice) did not observe a change in *TMEM106B* protein levels relative to wild-type, nor amelioration of microgliosis or the

pathological lysosomal phenotype¹⁴. In addition, an FTLT-TDP GWAS evaluating both rs1990622 and rs3173615 found that rs1990622 was the most significant SNV at the *TMEM106B* locus following meta-analysis¹⁵. Thus, the evidence for rs3173615 as the causative variant on *TMEM106B* remains mixed.

In this study, we developed and queried a large LRS dataset and identified an SV on *TMEM106B* that may be the protective variant at the *TMEM106B* locus in FTLT and AD.

Methods

Study participants

Study protocols were approved by the Stanford University Institutional Review Board. The Stanford Alzheimer's Disease Research Center (ADRC) is a cohort of healthy older controls and patients with AD and related neurological disorders (n=274 with LRS and NGS, ages 36-93, 145 F, 129 M, healthy controls = 133, mild cognitive impairment cases = 47, AD cases = 22, other diagnosis = 72). All participants underwent a history and neurological exam, cognitive testing, and blood draw. Most participants also underwent brain imaging including MRI and amyloid PET scanning. Roughly 1/3 of participants also provided cerebrospinal fluid (CSF). Diagnoses were determined in a consensus conference meeting comprised of neurologists and neuropsychologists using standard clinical criteria for AD, MCI, and related disorders such as Parkinson's disease and Lewy body disease.

The Stanford Aging and Memory Study (SAMS) is a cohort of cognitively unimpaired older individuals (n=133 with LRS, ages 60-88, 73 F, 60 M). SAMS eligibility criteria include normal or corrected vision and hearing, native English speaking, no neurologic or psychiatric disease history, Clinical Dementia Rating score of zero, and normal performance on standardized

neuropsychological testing. Participants underwent CSF and plasma collection and brain imaging including MRI and amyloid PET. Unimpaired cognitive status was confirmed in a consensus conference meeting comprised of neurologists and neuropsychologists using standard clinical criteria.

Long-read sequencing, alignment and SV calling

High molecular weight DNA was extracted from primary blood mononuclear cells (PBMC's) that had been stored at -80C using a Puregene kit (Qiagen, Germany). DNA was sheared using a G-tube (Covaris LLC, Massachusetts). Sequencing libraries were prepared using Nanopore LSK-110 and sequenced on the PromethION48 (Oxford Nanopore Technologies, United Kingdom). An average of 50.4 gigabases were sequenced per sample, with a read length N50 of 18 kb. Sequencing data were base called using Guppy (High Accuracy, version 6.3), and aligned to HG38 using Minimap2¹⁶. Structural variants were called using Sniffles2¹⁷ in population mode. Variants with start position overlapping *TMEM106B* were extracted.

Short-read next-generation sequencing

TMEM106B SNV genotypes were determined from short-read NGS performed at either the Beijing Genomics Institute (BGI) in Shenzhen, China or as part of the Stanford Extreme Phenotypes in Alzheimer's Disease project with sequencing performed at the Uniformed Services University of the Health Sciences (USUHS) on an Illumina HiSeq platform. Among the 274 ADRC participants, 29 participants were sequenced via USUHS and 245 via BGI. The Genome Analysis Toolkit (GATK) workflow Germline short variant discovery was used to map genome sequencing data to the reference genome (GRCh38) and to produce high-confidence variant calls using joint-calling¹⁸.

3' UTR deletion dose curation

The genotypes of rs1990622, rs3173615, and the 3' UTR deletion were extracted for participants for whom whole genome LRS and NGS were available. Participants with discordant doses of the three variants in LRS – where the dose of any of the three variants differed from any other – were identified for manual curation. For these participants, LRS genome alignments were visualized in IGV¹⁹ and the dose of the *TMEM106B* 3' UTR deletion was determined by the following criteria: (1) the dose was set to 0 if no reads contained the deletion, (2) the dose was set to 1 if at least one but not all reads contained the deletion, and (3) the dose was set to 2 if all reads contained the deletion.

Alzheimer's Disease Sequencing Project (ADSP) LD analysis

ADSP R3 SNVs and Biograph²⁰ SV calls were downloaded from NIAGADS (<https://dss.niagads.org/datasets/ng00067/#data-releases>). SNVs were subset to rs3173615 (7:12229791:C:G) and rs1990622 (7:12244161:A:G) using Plink 1.9²¹. The *TMEM106B* 3' UTR deletion (chr7:12242077; SVLEN=-322; SVTYPE=DEL) was identified in Biograph SV calls. Samples present in both SV and SNV data were identified, VCF files for both datasets were subset to these samples, and the files were concatenated using bcftools²². LD was computed using Plink 1.9 with the --r2 dprime flag.

To identify African ancestry individuals in the ADSP, ancestries of all ADSP individuals were determined using SNPWeights v2²³ using reference populations from the 1000 Genomes Consortium²⁴. Individuals with greater than 75% African ancestry were classified as African ancestry.

eQTL and pQTL analysis

All expression quantitative trait locus (eQTL) effect sizes and p-values were queried for rs1990622 from summary statistics (Sieberts meta-analysis²⁵, CommonMind Consortium²⁶,

GTEX²⁷, Wingo²⁸, MetaBrain²⁹, eQTLGen³⁰). Protein quantitative trait locus (pQTL) effect sizes and p-values for ARIC³¹, DECODE³², Wingo, and Banner³³ were also queried for rs1990622 from summary statistics. See Data Availability Statement for direct links to summary statistics queried. For ROSMAP brain areas BA9, BA6, and BA37, processed TMT quantitated protein abundance data from Synapse projects syn25006657 and syn2580853 were used to calculate effect size and p-values using a multiple linear regression in R. The *lm* function was used to fit a linear regression model to combine AMP-AD WGS and SNP array data for rs1990622 against proteomics data, covarying out the first three genetic principal components, *APOE* status, and diagnosis. The same linear model was computed for MSBB BA36 using processed TMT proteomics data from Synapse project syn25006647. See Data Availability Statement for direct links to raw protein abundance data used from Synapse.

Results

We carried out whole genome LRS and SV calling for 407 participants enrolled in the Stanford ADRC and/or SAMS. Two unique SVs overlapping *TMEM106B* were identified, as summarized in Figure 1a. One SV, a 322 base pair (bp) deletion located in the 3' untranslated region (UTR) of *TMEM106B*, is highly prevalent with AF=0.4568 in our LRS dataset, comparable to the AF in gnomAD of rs1990622 (AF=0.4989) and rs3173615 (AF=0.4902). The *TMEM106B* 3' UTR deletion was detected in both LRS and NGS (Figure 1b). The second SV was much less prevalent (AF=0.1096) than rs1990622 and rs3173615. We linked LRS data to high coverage NGS data for 274 Stanford ADRC participants in order to evaluate the LD between the 3' UTR deletion, rs1990622 and rs3173615. The *TMEM106B* 3' UTR deletion was in perfect concordance with rs1990622 and was concordant with rs3173615 in all but two individuals.

The LD between the *TMEM106B* 3' UTR deletion, rs1990622, and rs3173615 was established in a large cohort by querying the ADSP database. 16882 samples were genotyped at both

SNVs rs1990622 and rs3173615. The *TMEM106B* 3' UTR deletion was identified in 12120 of 16841 samples (AF=0.4977) in the Biograph SV callset provided by ADSP. The SV was in greater LD with rs1990622 ($R^2=0.920916$, $D'=0.963472$) than with rs3173615 ($R^2=0.883776$, $D'=0.963575$). In LDpair, rs1990622 and rs3173615 are in high LD when assessed across all populations ($R^2=0.91$, $D'=0.9905$). However, we noted that these SNVs are not as closely linked in LDpair in African populations ($R^2=0.7403$, $D'=0.9915$). In African ancestry individuals in ADSP, the *TMEM106B* 3' UTR deletion was in even greater LD with rs1990622 ($R^2=0.936841$, $D'=0.976782$) than with rs3173615 ($R^2=0.764242$, $D'=0.974406$). Taken together, these data indicate that the *TMEM106B* 3' UTR deletion is more closely associated with rs1990622 than rs3173615 across populations and may underlie the slightly greater significance of rs1990622 over rs3173615 in reducing risk of FTLT-DTP.

We evaluated the effect of rs1990622 on *TMEM106B* expression and protein levels in eQTL and pQTL datasets (Table 1). rs1990622 does not result in a significant effect on *TMEM106B* expression levels in seven datasets and has a significant effect in three datasets. Across eQTL datasets, the direction of the effect of rs1990622 varies ($\beta < 0$ in two datasets, $\beta > 0$ in five datasets). rs1990622 results in a statistically significant effect on *TMEM106B* protein levels in ten datasets and does not have a significant effect in one dataset (Wingo meta-analysis, $p=0.1005$). The effect size is negative in eight pQTL datasets and positive in three. Our observation of significant pQTL effects in the absence of a consistent eQTL effect is most consistent with a model in which the *TMEM106B* causative variant exerts its protective effect after transcription of *TMEM106B*.

Discussion

The *TMEM106B* 3' UTR deletion is a previously unreported variant that is a potential candidate to mediate the protective effect of the *TMEM106B* locus in FTLT-DTP. At present, the

candidates for the causal variant at this locus are (1) the *TMEM106B* 3' UTR deletion; (2) rs3173615, the only coding SNV in this linkage block; (3) rs1990622, the leading GWAS hit; or (4) another variant in this linkage block. Recent *in vivo* work using a mouse model demonstrated that homozygous *TMEM106B* S186 mice had no difference in level of *TMEM106B* protein relative to wild-type and that homozygous *Grn*^{-/-} *TMEM106B*^{S186/S186} mice did not have ameliorated lysosomal proliferation or microgliosis relative to *Grn*^{-/-} mice, making it less likely that the rs3173615 variant is causal¹⁴. Moreover, rs1990622 was found to be more significant than rs3173615 following meta-analysis in a recent FTLT-TDP GWAS, which would be unexpected if the protective effect of the rs1990622 minor allele was due to its linkage with rs3173615.

We used publicly available expression and protein datasets to establish that the rs1990622 minor allele typically acts as a pQTL but not as an eQTL. This suggests that the protective effect at the *TMEM106B* locus is likely mediated by a genetic variant that acts after transcription to reduce *TMEM106B* protein levels. Because intronic and intergenic variants are not incorporated into the processed mRNA molecule it is less likely that such variants, including rs1990622, are mediating the protective effect of this allele. Furthermore, the pQTL finding suggests that the *TMEM106B* locus may exert its protective effect through an effect on protein availability rather than changes in function due to an amino acid change, reducing the likelihood that rs3173615 is the causal variant. That said, the Nicholson et al. study suggested that the *TMEM106B* S185 protein is less stable than wild-type in an *in vitro* setting and may result in reduced protein levels as a result¹³.

Lastly, we found in our LRS dataset, as well as in ADSP, that the *TMEM106B* 3' UTR deletion is in higher LD with rs1990622 than rs3173615, which is consistent with a model in which the

respective significance of these two SNVs is related to their linkage with the *TMEM106B* 3' UTR deletion.

There are several possibilities for how the *TMEM106B* 3' UTR deletion could mediate a protective effect against FTLD-TDP pathogenesis. The pQTL evidence indicates that the minor allele at the *TMEM106B* locus decreases TMEM106B protein levels. The deletion may result in selective enrichment of an alternate transcript polyadenylation site, changing the 3' UTR. Such a change in the 3' UTR could disrupt protein binding, which may in turn decrease translational efficiency, alter the subcellular localization of the RNA, or impair protein routing to the endoplasmic reticulum³⁴. Identifying a clear-cut mechanism linking the deletion to reduced TMEM106B protein levels (and increased progranulin protein levels) is still required to confirm that this is the causal variant at the locus.

In summary, we report a large, prevalent SV on *TMEM106B* that is in perfect LD with rs1990622 and near-perfect LD with rs3173615 in a large LRS dataset. LRS provides a valuable tool for detection of large genomic variants that can aid the interpretation of GWAS results and elucidate the genetic drivers of disease.

Conflicts of interest

The authors have no conflicting interests.

Data Availability

Sieberts eQTL meta-analysis: <https://www.synapse.org/#!/Synapse:syn17015233>

Wingo eQTL meta-analysis: <https://www.synapse.org/#!/Synapse:syn31826294>

GTEX eQTL summary statistics:

http://ftp.ebi.ac.uk/pub/databases/spot/eQTL/imported/GTEX_V8/

CommonMind Consortium eQTL summary statistics:

<https://www.synapse.org/#!/Synapse:syn4622659>

Banner pQTL summary statistics: <https://www.synapse.org/#!/Synapse:syn24847777>

ARIC pQTL summary statistics: <http://nilanjanchatterjeelab.org/pwas/>

MetaBrain eQTL summary statistics: <https://www.metabrain.nl>

eQTLgen eQTL summary statistics: <https://www.eqtlgen.org>

DECODE pQTL summary statistics (Supplementary Tables):

<https://www.nature.com/articles/s41588-021-00978-w#MOESM4>

ROSMAP proteomics BA6 and BA37: <https://www.synapse.org/#!/Synapse:syn25335376>

ROSMAP proteomics BA9: <https://www.synapse.org/#!/Synapse:syn25006657>

Mount Sinai Brain Bank proteomics: <https://www.synapse.org/#!/Synapse:syn25006647>

AMP-AD WGS & SNP array: <https://dss.niagads.org/sample-sets/snd10011/>

The NGS and LRS genomes will be made available in a research repository after publication.

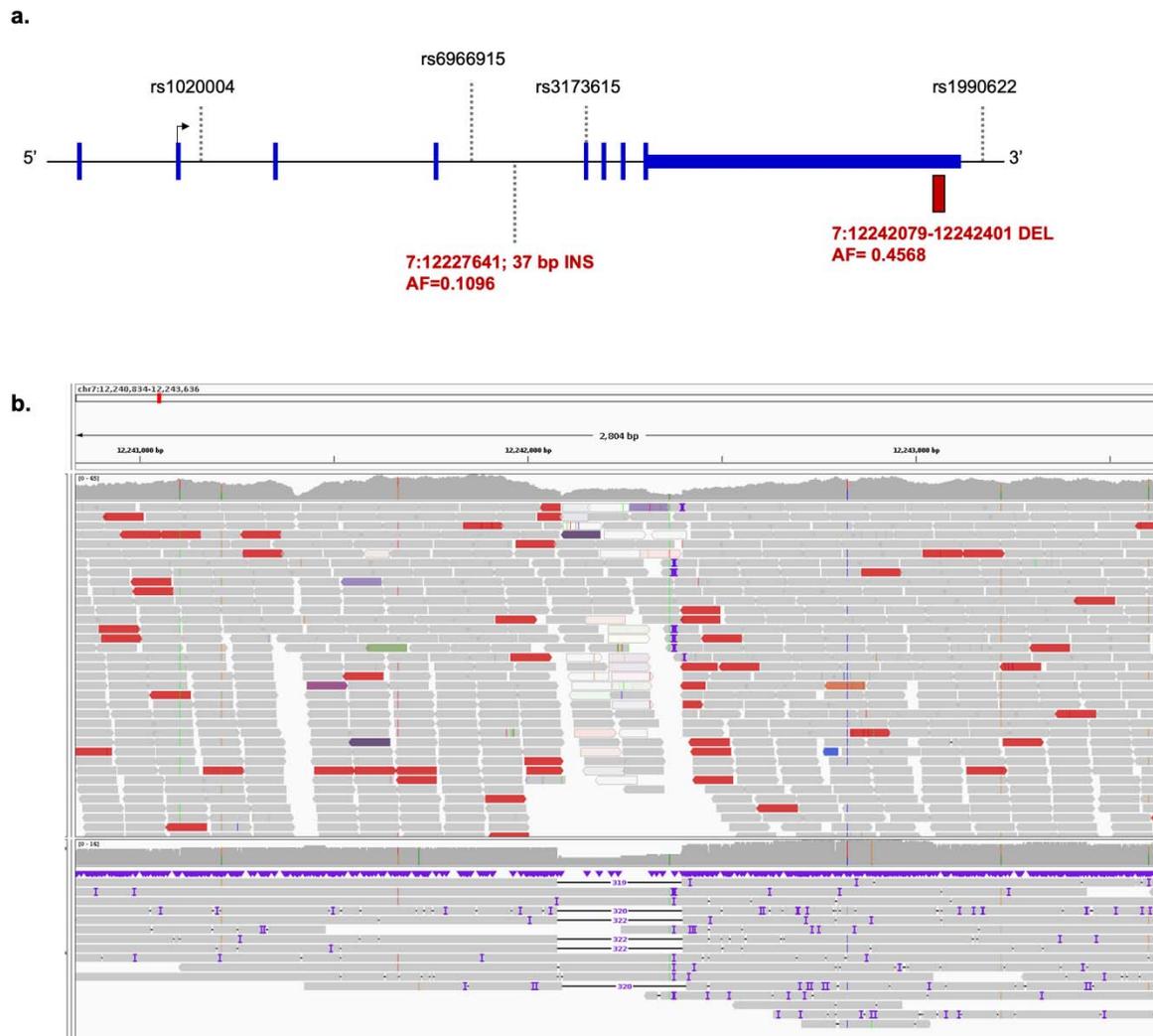


Figure 1. TMEM106B structural variants (a) Two structural variants were identified within 20 kilobases of *TMEM106B*. (b) The *TMEM106B* 3' UTR deletion was detected in both next-generation sequencing (top panel) and long-read sequencing (bottom panel). Both sequencing modalities are displayed here for a representative cohort participant carrying one copy of the *TMEM106B* 3' UTR deletion.

Dataset	tissue	sample size	eQTL		pQTL	
			beta	p-value	beta	p-value
GTEX	Blood	670	-	0.0000	-	-
GTEX	CTX	205	-	0.0000	-	-
Mayo	CER	275	0.3055	0.0001	-	-
Mayo	TCX	276	0.1529	0.0542	-	-
Wingo	DLPFC & PHG	722	0.0563	0.2416	-0.0773	0.1005
Metabrain	CTX	6601	0.0268	0.3115	-	-
eQTLGen	Blood	31427	-	0.0174	-	-
CommonMind	DLPFC	590	-0.0084	0.4044	-	-
Sieberts	CTX	1433	0.0260	0.4803	-	-
ROSMAP	DLPFC	269	-0.0214	0.7177	0.0093	0.0117
DECODE	Blood	35371	-	-	-0.1106	0.0000
Banner	DLPFC	129	-	-	-0.1786	0.0000
ROSMAP	DLPFC	116	-	-	0.0814	0.0000
ROSMAP	FC (BA6)	101	-	-	-0.1127	0.0063
ROSMAP	FC (BA9)	310	-	-	-0.2594	0.0000
MSBB	PHG	102	-	-	-0.3571	0.0000
ARIC AA	Plasma	1871	-	-	0.3147	0.0000
ARIC EA	Plasma	7213	-	-	-0.2547	0.0000
ROSMAP	TCX (BA37)	101	-	-	-0.1281	0.0012

Table 1. Effect of rs1990622 as a TMEM106B expression quantitative trait locus (eQTL) or protein quantitative trait locus (pQTL). Betas are those reported by respective studies and thus may be on different scales given different normalization procedures for transcriptomic and proteomic data.

Abbreviations: Cortex (CTX), cerebellum (CER), temporal cortex (TCX), DLPFC (dorsolateral prefrontal cortex), parahippocampal gyrus (PHG), frontal cortex (FC)

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The ADGC cohorts include: Adult Changes in Thought (ACT) (U01 AG006781, U19 AG066567), the Alzheimer's Disease Research Centers (ADRC) (P30 AG062429, P30 AG066468, P30 AG062421, P30 AG066509, P30 AG066514, P30 AG066530, P30 AG066507, P30 AG066444, P30 AG066518, P30 AG066512, P30 AG066462, P30 AG072979, P30 AG072972, P30 AG072976, P30 AG072975, P30 AG072978, P30 AG072977, P30 AG066519, P30 AG062677, P30 AG079280, P30 AG062422, P30 AG066511, P30 AG072946, P30 AG062715, P30 AG072973, P30 AG066506, P30 AG066508, P30 AG066515, P30 AG072947, P30 AG072931, P30 AG066546, P20 AG068024, P20 AG068053, P20 AG068077, P20 AG068082, P30 AG072958, P30 AG072959), the Chicago Health and Aging Project (CHAP) (R01 AG11101, RC4 AG039085, K23 AG030944), Indiana Memory and Aging Study (IMAS) (R01 AG019771),

Indianapolis Ibadan (R01 AG009956, P30 AG010133), the Memory and Aging Project (MAP) (R01 AG17917), Mayo Clinic (MAYO) (R01 AG032990, U01 AG046139, R01 NS080820, RF1 AG051504, P50 AG016574), Mayo Parkinson's Disease controls (NS039764, NS071674, 5RC2HG005605), University of Miami (R01 AG027944, R01 AG028786, R01 AG019085, IIRG09133827, A2011048), the Multi-Institutional Research in Alzheimer's Genetic Epidemiology Study (MIRAGE) (R01 AG09029, R01 AG025259), the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) (U24 AG021886), the National Institute on Aging Late Onset Alzheimer's Disease Family Study (NIA- LOAD) (U24 AG056270), the Religious Orders Study (ROS) (P30 AG10161, R01 AG15819), the Texas Alzheimer's Research and Care Consortium (TARCC) (funded by the Darrell K Royal Texas Alzheimer's Initiative), Vanderbilt University/Case Western Reserve University (VAN/CWRU) (R01 AG019757, R01 AG021547, R01 AG027944, R01 AG028786, P01 NS026630, and Alzheimer's Association), the Washington Heights-Inwood Columbia Aging Project (WHICAP) (RF1 AG054023), the University of Washington Families (VA Research Merit Grant, NIA: P50AG005136, R01AG041797, NINDS: R01NS069719), the Columbia University Hispanic Estudio Familiar de Influencia Genetica de Alzheimer (EFIGA) (RF1 AG015473), the University of Toronto (UT) (funded by Wellcome Trust, Medical Research Council, Canadian Institutes of Health Research), and Genetic Differences (GD) (R01 AG007584). The CHARGE cohorts are supported in part by National Heart, Lung, and Blood Institute (NHLBI) infrastructure grant HL105756 (Psaty), RC2HL102419 (Boerwinkle) and the neurology working group is supported by the National Institute on Aging (NIA) R01 grant AG033193.

The CHARGE cohorts participating in the ADSP include the following: Austrian Stroke Prevention Study (ASPS), ASPS-Family study, and the Prospective Dementia Registry-Austria (ASPS/PRODEM-Aus), the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Erasmus Rucphen Family Study (ERF), the Framingham Heart Study (FHS), and the Rotterdam Study (RS). ASPS is funded by the Austrian Science Fond (FWF) grant number P20545-P05 and P13180 and the Medical University of Graz. The ASPS-Fam is funded by the Austrian Science Fund (FWF) project I904), the EU Joint Programme – Neurodegenerative Disease Research (JPND) in frame of the BRIDGET project (Austria, Ministry of Science) and the Medical University of Graz and the Steiermärkische Krankenanstalten Gesellschaft. PRODEM-Austria is supported by the Austrian Research Promotion agency (FFG) (Project No. 827462) and by the Austrian National Bank (Anniversary Fund, project 15435. ARIC research is carried out as a collaborative study supported by NHLBI contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). Neurocognitive data in ARIC is collected by U01 2U01HL096812, 2U01HL096814, 2U01HL096899, 2U01HL096902, 2U01HL096917 from the NIH (NHLBI, NINDS, NIA and NIDCD), and with previous brain MRI examinations funded by R01-HL70825 from the NHLBI. CHS research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the NHLBI with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was

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The FUS cohorts include: the Alzheimer's Disease Research Centers (ADRC) (P30 AG062429, P30 AG066468, P30 AG062421, P30 AG066509, P30 AG066514, P30 AG066530, P30 AG066507, P30 AG066444, P30 AG066518, P30 AG066512, P30 AG066462, P30 AG072979, P30 AG072972, P30 AG072976, P30 AG072975, P30 AG072978, P30 AG072977, P30 AG066519, P30 AG062677, P30 AG079280, P30 AG062422, P30 AG066511, P30 AG072946, P30 AG062715, P30 AG072973, P30 AG066506, P30 AG066508, P30 AG066515, P30 AG072947, P30 AG072931, P30 AG066546, P20 AG068024, P20 AG068053, P20 AG068077, P20 AG068082, P30 AG072958, P30 AG072959), Alzheimer's Disease Neuroimaging Initiative (ADNI) (U19AG024904), Amish Protective Variant Study (RF1AG058066), Cache County Study (R01AG11380, R01AG031272, R01AG21136, RF1AG054052), Case Western Reserve University Brain Bank (CWRUBB) (P50AG008012), Case Western Reserve University Rapid Decline (CWRURD) (RF1AG058267, NU38CK000480), CubanAmerican Alzheimer's Disease Initiative (CuAADI) (3U01AG052410), Estudio Familiar de Influencia Genetica en Alzheimer (EFIGA) (5R37AG015473, RF1AG015473, R56AG051876), Genetic and Environmental Risk Factors for Alzheimer Disease Among African Americans Study (GenerAAtions) (2R01AG09029, R01AG025259, 2R01AG048927), Gwangju Alzheimer and Related Dementias Study (GARD) (U01AG062602), Hillblom Aging Network (2014-A-004-NET, R01AG032289, R01AG048234), Hussman Institute for Human Genomics Brain Bank (HIHGBB) (R01AG027944, Alzheimer's Association "Identification of Rare Variants in Alzheimer Disease"),

Ibadan Study of Aging (IBADAN) (5R01AG009956), Longevity Genes Project (LGP) and LonGenity (R01AG042188, R01AG044829, R01AG046949, R01AG057909, R01AG061155, P30AG038072), Mexican Health and Aging Study (MHAS) (R01AG018016), Multi-Institutional Research in Alzheimer's Genetic Epidemiology (MIRAGE) (2R01AG09029, R01AG025259, 2R01AG048927), Northern Manhattan Study (NOMAS) (R01NS29993), Peru Alzheimer's Disease Initiative (PeADI) (RF1AG054074), Puerto Rican 1066 (PR1066) (Wellcome Trust (GR066133/GR080002), European Research Council (340755)), Puerto Rican Alzheimer Disease Initiative (PRADI) (RF1AG054074), Reasons for Geographic and Racial Differences in Stroke (REGARDS) (U01NS041588), Research in African American Alzheimer Disease Initiative (REAAADI) (U01AG052410), the Religious Orders Study (ROS) (P30 AG10161, P30 AG72975, R01 AG15819, R01 AG42210), the RUSH Memory and Aging Project (MAP) (R01 AG017917, R01 AG42210Stanford Extreme Phenotypes in AD (R01AG060747), University of Miami Brain Endowment Bank (MBB), University of Miami/Case Western/North Carolina A&T African American (UM/CASE/NCAT) (U01AG052410, R01AG028786), and Wisconsin Registry for Alzheimer's Prevention (WRAP) (R01AG027161 and R01AG054047).

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