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Genome-wide analyses as part of the international FTLD-TDP whole genome sequencing consortium reveals novel disease risk factors and increases support for immune dysfunction in FTLD

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Abstract

Frontotemporal lobar degeneration with neuronal inclusions of the TAR DNA-binding protein 43 (FTLD-TDP) represents the most common pathological subtype of FTLD. We established the international FTLD-TDP whole genome sequencing consortium to thoroughly characterize the known genetic causes of FTLD-TDP and identify novel genetic risk factors. Through the study of 1,131 unrelated Caucasian patients, we estimated that *C9orf72* repeat expansions and *GRN* loss-of-function mutations account for 25.5% and 13.9% of FTLD-TDP patients, respectively. Mutations in *TBK1* (1.5%) and other known FTLD genes (1.4%) were rare, and the disease in 57.7% of FTLD-TDP patients was unexplained by the known FTLD genes. To unravel the contribution of common genetic factors to the FTLD-TDP etiology in these patients, we conducted a two-stage association study comprising the analysis of whole-genome sequencing data from 517 FTLD-TDP patients and 838 controls, followed by targeted genotyping of the most associated genomic loci in 119 additional FTLD-TDP patients and 1653 controls. We identified three genome-wide significant FTLD-TDP risk loci: one new locus at chromosome 7q36 within the *DPP6* gene led by rs118113626 (pvalue=4.82e-08, OR=2.12), and two known loci: *UNC13A*, led by rs1297319 (pvalue=1.27e-08, OR=1.50) and *HLA-DQA2* led by rs17219281 (pvalue=3.22e-08, OR=1.98). While *HLA* represents a locus previously implicated in clinical FTLD and related neurodegenerative disorders, the association signal in our study is independent from previously reported associations. Through inspection of our whole genome sequence data for genes with an excess of rare loss-of-function variants in FTLD-TDP patients (n = 3) as compared to controls (n=0), we further discovered a possible role for genes functioning within the *TBK1*-related immune pathway (e.g. *DHX58*, *TRIM21*, *IRF7*) in the genetic etiology of FTLD-TDP. Together, our study based on the largest cohort of unrelated FTLD-TDP patients assembled to date provides a comprehensive view of the genetic landscape of FTLD-TDP, nominates novel FTLD-TDP risk loci, and strongly implicates the immune pathway in FTLD-TDP pathogenesis.

Keywords

Whole-genome sequencing FTLD-TDP; TBK1; DPP6; UNC13A; HLA; Immunity

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Informed consent was obtained from all individual participants included in the study.

Introduction

Frontotemporal lobar degeneration (FTLD) is one of the leading causes of dementia in individuals younger than 65 years but can also affect individuals later in life. In the past two decades, a number of monogenic causes and genetic risk factors of FTLD have been described. Mutations in the genes encoding microtubule associate protein tau (*MAPT*) and progranulin (*GRN*), and repeat expansions in the chromosome 9 open reading frame 72 (*C9orf72*) gene were identified as the most common genetic causes of FTLD [37,4,16]; however, the majority of patients remain genetically unexplained. FTLD is an umbrella term for a highly heterogeneous group of clinical syndromes that result from selective dysfunction and typically atrophy of the frontal and temporal lobes. The predominant clinical presentations of FTLD are behavior and language dysfunction resulting in behavioral variant (bvFTD) [74], semantic variant of primary progressive aphasia (svPPA) or agrammatic variant of primary progressive aphasia (agPPA) [31]; however, related clinical syndromes such as frontotemporal dementia with motor neuron disease (FTD-MND), progressive supranuclear palsy (PSP) syndrome, and corticobasal syndrome (CBS) can occur. Patients with FTLD may further present with psychiatric symptoms and conditions such as compulsive behavior, mood disorders, and schizophrenia [101]. FTLD neuropathological studies have also revealed heterogeneity and pathological classification is now defined by the main component of the protein aggregates in a patient's brain at post mortem examination. FTLD with neuronal and cytoplasmic aggregates of the DNA and RNA binding protein TDP-43 (FTLD-TDP) is most common and based on the distribution of the neuronal cytoplasmic TDP-43-positive inclusions and dystrophic neurites in the cortical layers at least five distinct FTLD-TDP pathological subtypes (A-E) are described [54,44]. Interestingly, while the nature of the underlying FTLD pathology is challenging to predict in most clinically diagnosed FTLD patients, mutations in *GRN* invariably lead to FTLD-TDP type A while repeat expansions in *C9orf72* are mainly associated with FTLD-TDP type B [53]. In contrast, patients with mutations in *MAPT* accumulate pathological tau proteins (FTLD-tau) and do not have FTLD-TDP pathology [9]. These correlations between specific gene mutations and pathology subgroups provides validation for the pathological classification system and suggests that, at least in part, distinct molecular pathways could contribute to disease in the various FTLD pathological subtypes. Consequently, genetic studies focused on large clinical cohorts may have reduced power due to undesirable heterogeneity and analysis of specific FTLD pathological subtypes may be beneficial. Indeed, the inclusion of clinical patients in genome-wide association study (GWAS) has been successful by identifying one genome-wide significant association signal for FTLD at the HLA locus on chromosome 6 but required more than 2,000 FTLD patients and 4,000 controls [26]. On the contrary, the use of a much smaller cohort of pathologically confirmed FTLD-TDP patients identified *TMEM106B* as a genetic risk factor [85]. The latter study, however, was performed more than 8 years ago, only focused on common genetic variants, and included a significant number of patients with pathogenic *GRN* mutations and repeat expansions in *C9orf72*, underscoring the need for additional studies.

Here we present the results of our newly established International FTLD-TDP whole genome sequencing (WGS) consortium, in which we identified more than 1,150 unrelated

pathologically confirmed FTLN-TDP patients from 23 participating sites. Genome-wide association studies in 517 Caucasian FTLN-TDP patients without mutations in the known genes and 838 controls identified common risk variants at one novel (*DPP6*) and two known loci (*UNC13A* and *HLA-DQA2*) and suggests that rare damaging variants in the *TBKI*-related innate immune pathway are enriched in FTLN-TDP patients as compared to controls. These findings extend the genetic landscape of FTLN-TDP and further highlight immune dysfunction as a potential contributing factor in the development of FTLN-TDP.

Methods

Study subjects and basic genetic and pathological characterization

International FTLN-TDP whole genome sequencing (WGS) Consortium.—To identify novel genetic factors implicated in FTLN-TDP, we established the International FTLN-TDP WGS Consortium. Previously ascertained FTLN-TDP patients from 23 European, North American and Australian participating sites were included, one patient per family (Table 1). The pathological diagnosis of FTLN-TDP was considered sufficient for inclusion, irrespective of the clinical diagnosis of the patient. Patients diagnosed with FTLN-TDP and concomitant signs of motor neuron disease were also included. The availability of DNA for basic genetic characterization was considered an additional inclusion criterion such that the presence of a *C9orf72* expansion could be determined in all patients, mutations in *GRN* could be determined in all FTLN-TDP type A patients and *VCP* could be screened in all FTLN-TDP type D patients. For those patients in which mutation status was not yet available at the start of the study, *C9orf72* repeat expansions were analyzed using our previously reported two-step protocol and Sanger sequencing was used for *GRN* and *VCP* mutation screening [20,4,96]. Information on the presence of known disease mutations in other neurodegenerative disease genes within the cohort was requested from all participating sites but not all patients were systematically screened. A total of 1,134 Caucasian and 20 non-Caucasian FTLN-TDP patients were identified.

Discovery cohort.—The discovery stage included 554 genetically unexplained self-declared Caucasian FTLN-TDP patients with sufficient DNA quality and quantity available for WGS. Participating sites were required to provide the FTLN-TDP pathological subtyping for each patient according to the harmonized classification system or had to provide unstained fixed tissue slides such that phospho-TDP-43 immunostaining could be performed as part of this study. WGS was also available for 982 control individuals from the Mayo Clinic Biobank which is a convenience population collected at Mayo Clinic with detailed clinical records on each participant (Table 2) [67]. After quality control measures (see below), 517 FTLN-TDP patients and 838 controls were included in the genetic analyses.

Replication cohort.—For the replication stage, a total of 119 FTLN-TDP patients were available, including both newly ascertained FTLN-TDP patients and patient samples that failed quality control measures at the discovery stage, e.g. low coverage and low call rate (Table 2). FTLN-TDP patient samples from the discovery stage that failed quality control measures due to contamination, race, sex error or duplicates were not selected for the replication cohort. FTLN-TDP patients known to be related to individuals included in the

discovery cohort were excluded. Similar to the discovery cohort, all patients were negative for *C9orf72* repeat expansions and FTLN-TDP type A patients were negative for mutations in *GRN*. A total of 1,653 controls including a set of 249 pathologically confirmed normal controls as well as 1,404 clinical controls free of neurodegenerative disorder were used as the control cohort. Patients and controls were all reported Caucasian and originated from multiple sites (Table 2, suppl. table 2 Online Resource 1).

WGS discovery cohort

Whole blood or brain-derived DNA from 499 of the 554 unrelated FTLN-TDP patients from the discovery cohort and 982 individuals from the Mayo Clinic Biobank Study were whole genome sequenced at HudsonAlpha. Approximately 1000ng DNA from each sample was sheared on a Covaris LE220 focused-ultrasonicator (Woburn, MA, USA) with a target yield of 350bp fragment size. Following sonication, the fragmented DNA was taken into standard library preparation protocol using NEBNext® DNA Library Prep Master Mix Set for Illumina® (New England BioLabs Inc., Ipswich, MA, USA) with slight modifications. The post-ligated samples were individually barcoded with unique in-house primers and amplified through 6 cycles of PCR using KAPA HiFi HotStart Ready Mix (Kapa Biosystems, Inc., Woburn, MA, USA). Concentration of the libraries was assessed by Qubit® 2.0 Fluorometer, and the quality of the libraries was estimated by utilizing a DNA 5K chip on a Caliper GX, respectively. Accurate quantification was determined using the qPCR-based KAPA Biosystems Library Quantification kit (Kapa Biosystems, Inc., Woburn, MA, USA). Each sample was sequenced on one lane of Illumina's HiSeq X instrument using v2 flow cells and reagents to target 30X genomic coverage. Fastq files previously generated on an Illumina HiSeq X for 55 FTLN-TDP patients were obtained from 3 sites: UCSF (n=36) [30], DZNE (n=14) and NSW (n=5) leading to a total of 554 FTLN patients whole genome sequenced.

For all FTLN-TDP patients and controls fastq files were transferred to Mayo Clinic and processed through the Mayo Genome GPS v4.0 pipeline in batches of up to 75 samples. Briefly, reads were mapped to the human reference sequence (GRCh38 build) using the Burrows–Wheeler Aligner, and local realignment around indels was performed using the Genome Analysis Toolkit (GATK) [46,56]. Variant calling was performed using GATK HaplotypeCaller followed by variant recalibration (VQSR) according to the GATK best practices recommendations [22,86].

WGS data quality assessment

Sample level quality control.—Samples with less than 30X coverage in more than 50% of the genome (n=17 patients, n=41 controls), call rate below 85% (n=1 patient, n=0 controls), sex error (n=4 patients, n=2 controls) or contamination defined by a FREEMIX score above 0.03 (n=6 patients, n=4 controls) were removed. Non-Caucasian samples (n=6 patients, n=3 controls) were also removed. At this step, joint genotyping on all samples was performed, a final relatedness measurement was calculated using PREST [57], and duplicate samples (n=3 patients, n=0 control) as well as related ones (n=0 patient, n=25 controls) were removed. In total, 517 pathologically confirmed FTLN-TDP samples and 907 controls passed all quality control measures. After removing 69 controls with a possible clinical

diagnosis or family history of a neurodegenerative disorder as per clinical chart review, 517 FTLT-DTP patients and 838 controls were included in genetic association analyses.

Variant level quality control.—Genotype calls with GQ<10 and/or depth (DP) <10 were set to missing, and variants with ED > 4 were removed from all subsequent analyses. For all analyses, only variants that pass VQSR and with a call rate >95% were considered unless otherwise specified. The transition/transversion ratio for this final variant call set is 2.04. Functional annotation of variants was performed using ANNOVAR (version2016Feb01) [94]. Genotypes generated at the discovery phase for the top single nucleotide polymorphisms (SNPs, rs118113626, rs17219281 and rs12973192) were validated by independent Taqman assays (C_11514504_10 for rs12973192 – custom assays were designed for rs118113626 and rs17219281) on 466 FTLT-DTP patients and 837 controls included in the discovery phase with DNA available. All genotypes from the whole genome sequencing phase were confirmed by an orthogonal method. In addition, rs4726389 and rs118113626 were Sanger sequenced in a subset of 46 FTLT-DTP patients and 46 controls from the discovery phase and all genotypes were confirmed (primers available upon request). Rare loss of function variants (frameshift insertion/deletion/block substitution, stopgain, stoploss and splicing single nucleotide variants - SNVs) in *TBK1*, *TRIM21*, *DHX58*, *IRF7*, *IRF8*, *IRF3* and *NOD2* were confirmed by Sanger sequencing (primers available upon request).

Genotyping replication cohort

13 suggestive loci ($p < 1e-05$) were nominated for follow-up in the replication stage. For all suggestive loci, the lead variant and/or one proxy were included in a multiplex MassArray design (Agena Bioscience, San Diego, CA, USA) leading to a maximum of two variants per loci at the design stage. Two loci failed the design (rs148048968, rs3952538). Twenty nanograms of DNA as measured by spectrophotometer (Nanodrop; Wilmington, DE, USA) were used for genotyping on the MassArray iPLEX system (Agena Bioscience, San Diego, CA, USA) following the manufacturer's protocol. Variants with a call rate <95% or failing Hardy-Weinberg equilibrium in controls ($p < 0.05$) were subsequently removed from the analysis (one variant, rs9818987). Both genome-wide significant *DPP6* variants and one variant at each other locus (either the lead variant or a proxy) were retained for statistical analysis resulting in a total of 11 loci. Individuals with a genotyping rate <95% were removed from the analysis.

RNAseq analysis

RNA from frontal cortex tissue of 44 FTLT-DTP patients without known gene mutations and 24 pathologically confirmed normal controls was extracted using the RNeasy Plus mini kit (Qiagen, Venlo, Netherlands). RNA quality and quantity were determined with an Agilent 2100 Bioanalyzer using the RNA Nano Chip (Agilent Technologies). Only high-quality RNA samples were included (median RIN=9.3, IQR=8.8–9.8). Library preparation was performed using Illumina TruSeq mRNA v2 prep and sequenced at 10 samples/lane as paired-end 101 base pair reads on the HiSeq 4000 (Illumina, San Diego, CA). Raw RNAseq reads were aligned to the human reference genome (GRCh38) using the spliced transcripts alignment to a reference (STAR, v2.5.2b) [25]. Library quality was assessed using the

RSeQC (v3.0.0) package [95]. Gene-level expression was quantified using the featureCounts command in the Subread package (v1.5.1) [49]. An in-house R pipeline was used to obtain differentially expressed genes. Briefly, the R pipeline includes conditional quantile normalization (CQN), principal component (PC) analysis, source of variation (SOV) analysis, and differential expression analysis of genes. Differential expression analysis was performed using multivariable linear regression models adjusted for potential confounders. A Benjamini–Hochberg FDR correction was used for multiple testing. Analyses were performed with or without the incorporation of surrogate markers for five major cell types as covariates: neurons (*ENO2*), microglia (*CD68*), astrocytes (*GFAP*), oligodendrocytes (*OLIG2*), and endothelial cells (*CD34*), as described elsewhere [32,18,3]. All analyses also included the following covariates: RNA integrity number (RIN), sex, age, and plate.

DPP6 mRNA expression analyses

mRNA expression analysis of *DPP6* was conducted in one FTLD-TDP patient carrying a possible LOF variant and two neuropathologically normal controls in which *DPP6* LOF variants were excluded. RNA was extracted and quality was measured as described before. All RNA obtained had a RIN>8 and was subsequently reverse transcribed using the Superscript III system (Life Technologies, Carlsbad, CA, USA). Quantitative real-time PCR was performed in quadruplicate for each sample on an ABI7900 PCR system (Applied Biosystems, Foster City, CA, USA), using TaqMan gene expression assays (Life Technologies, Carlsbad, CA, USA) and following the manufacturer's recommendations. *DPP6* transcripts were measured using the probe Hs00736294_m1 for all *DPP6* transcripts from Invitrogen (Carlsbad, CA, USA). *MAP2* (probe Hs00258900_m1) and *GAPDH* (probe Hs02758991_g1) were used as reference genes. Results were analyzed using SDS software version 2.2 (Life Technologies, Carlsbad, CA, USA). *DPP6* transcript levels were assessed using the Ct method normalized by the geometric mean of *MAP2* and *GAPDH* transcripts to account for both total cell number and the contribution of neuronal cells specifically. In addition, after reverse transcription, a cDNA fragment containing the splice site mutation as well as two known common variants (rs2293353 and rs2230064) was amplified by PCR and Sanger sequenced from the patient carrying the potential LOF *DPP6* variant and one healthy control. Presence of heterozygous status for rs2293353 and rs2230064 was confirmed at the gDNA level by Sanger sequencing (primers are available upon request).

Statistical analyses

Age at onset, death and survival after onset analyses.—Distribution of ages at onset, ages at death and survival after onset were compared between patients included in the discovery phase with an FTLD-TDP subtype A, B and C by using a Kruskal-Wallis test followed by a post-hoc Dunn's test. Pvalues of the Dunn's test are provided after Bonferroni correction.

Generation of principal components.—Prior to running genetic association analyses, PC analysis was performed using a subset of variants meeting the following criteria: minor allele frequency (MAF) >5% and full sample Hardy Weinberg Equilibrium (HWE) $p > 1e-5$. For the PC analysis and common variant genome-wide association analyses, multi-allelic

variants were split into multiple variables (i.e. rows in the genotype dataset), where each variable/row represents the count of a specific alternate allele with samples carrying other alternate alleles being set to missing. Influential regions such as the HLA region were removed, and SNPs were pruned by LD with r^2 threshold of 0.1 prior to PC analysis. This analysis identified 4 PCs that were significantly associated with patient control status, which were subsequently used as covariates in all genetic association analyses.

SNP-level analysis of common variants.—For the common variant GWAS, SNPs with $MAF > 0.01$ in patients or controls and $HWE\ p > 1e-6$ in controls were analyzed. Multi-allelic markers were encoded as described above. In addition, since WGS of FTLD-TDP patients was performed at HudsonAlpha in 5 batches, a test was performed to identify SNPs with significant differences in genotype distributions between sequencing batches, and SNPs showing evidence of batch effects ($p < 0.05$) were removed.

For all remaining variants, association of genotypes with the patient/control status was assessed using logistic regression with allele dosage as the predictor assuming log-additive allele effects. Sex and the first four PCs were included as covariates in the models. Following the primary analysis comparing SNP genotypes between all FTLD-TDP patients and controls, exploratory analyses within pathological FTLD-TDP strata were performed to evaluate SNP association with FTLD-TDP type A, type B and type C. The SNP-level analyses were performed using PLINK v1.90b6.5 64-bit (13 Sep 2018) [72]. Meta-analyses of the discovery and replication results were performed under a fixed effects model. The I^2 heterogeneity statistics is provided to evaluate the degree of heterogeneity of the effects in the discovery and replication stages.

Gene-level analysis of rare variants: Association of rare variants with the patient/control status was assessed using an unweighted burden test implemented using the SKAT_1.2.1 R package [97]. For the rare variant analyses multi-allelic markers were split into multiple “variants” or variables (i.e. multiple rows in the genotype file), with a particular alternate allele being counted for each variable, and with genotypes corresponding to other alternate alleles being set to 0. Thus, each row in the data file represents a count of a particular alternate allele and is only missing when no alleles were called. Only VQSR pass variants with call rate $> 90\%$, $ED = 4$, and $MAF < 0.01$ in either patients or controls were included in these rare-variant gene-based analyses. Two sets of analyses were performed: The first included only frameshift (insertion/deletion/block substitution), stopgain, stoploss and splicing SNVs (jointly defined as loss-of-function (LOF) variants), while the second included all variants captured in the first analysis as well as non-synonymous SNVs and non-frameshift indels or block substitutions that were predicted to be probably damaging by Polyphen 2 and deleterious by SIFT [1,61]. Sex and the first four PCs were used as covariates in the model. As with the common SNP GWAS, analyses were performed with all FTLD-TDP patients, followed by exploratory analyses in pathological FTLD-TDP strata. Because adjustment for PCs in extremely rare variants might lead to spurious associations due to the rarity of mutation carriers, p-values are provided with and without the incorporation of PCs in the models. Finally, for the rare variant analysis in *DPP6* we included all missense variants and LOF variants passing quality control and with a

MAF<0.01 in either patients or controls and performed a SKAT test as implemented in the SKAT R package allowing bidirectionality of the effect of variants. The association was assessed with and without adjustment for the associated common SNPs in *DPP6* (rs4726389 and rs118113626). Exome-wide significance was defined as a pvalue<2.5e-06 (Bonferroni correction for 20,000 genes).

Gene prioritization

All genes with nominal significance identified through burden test with rare LOF mutations or coding variants predicted to be pathogenic by Polyphen2 and SIFT prediction software were subject to a gene ontology (biological processing) and KEGG pathway analysis using Webgestalt [102,93]. In addition, for all genes where at least 3 such variants were identified in FTLD-TDP patients and none in controls, a similarity analysis using the ToppGene database was performed [10]. As training set, we used the following genes: *C9orf72*, *GRN*, *TBK1*, *OPTN*, *VCP*, *TARDBP*, *CHCHD10*, *SQSTM1*, *UBQLN2*, *hnRNPA1*, *hnRNPA2B1*, *CSF1R*, *FUS*, *CHMP2B*, *LRRK2* and *TIA1*. The default training parameters were used as follows: Gene ontology molecular function, biological process and cellular component; human and mouse phenotype; pathway (BIOCYC, KEGG, Pathway Interaction Database, REACTOME, GenMAPP, MSigDB C2 BIOCARTA (v6.0), PantherDB, Panther Ontology, SMPDB); PubMed and Disease. For each gene false discovery rate qvalues are reported.

Results

Frequency of known gene mutations within the International FTLD-TDP WGS Consortium

Through collaborative efforts across North America, Europe and Australia, we established the International FTLD-TDP WGS Consortium and identified 1,154 unrelated patients with a pathologically confirmed diagnosis of FTLD-TDP and a source of DNA available for basic genetic characterization (Table 1). Analyses of common known genetic causes of FTLD-TDP revealed *C9orf72* repeat expansions in 25.5% and *GRN* mutations in 13.8% of Caucasian patients, together explaining the disease in nearly 40% of our Caucasian FTLD-TDP cohort. *TBK1* and other rare gene mutations accounted for an additional 0.9% and 1.2% of Caucasian patients, respectively, with the caveat that these genes were only analyzed in a subset of patients. In the non-Caucasian population, *GRN* mutations were the most frequent, explaining 20.0% of patients, compared to only 10.0% of FTLD-TDP patients with the *C9orf72* repeat expansion.

To identify genetic factors contributing to the disease in Caucasian FTLD-TDP patients without a known gene mutation, we next performed WGS on 554 patients with sufficient DNA quality and quantity available. A total of 982 individuals from the Mayo Clinic Biobank Study underwent WGS at the same time (Table 2). For each patient, pathological FTLD-TDP subtyping was available based on TDP-43 immunostaining. FTLD-TDP type B was most common (n=199), followed by FTLD-TDP type A (n=171) and C (n=161). FTLD-TDP type D (*VCP* gene negative) was observed in 4 patients and in 19 patients the subtyping was ambiguous. The distribution of ages at onset, ages at death and survival after onset were significantly different between the FTLD-TDP subtypes (Kruskal-Wallis test $p_{\text{value}}_{\text{age at onset}}=6.2\text{e-}10$; $p_{\text{value}}_{\text{age at death}}=2.0\text{e-}14$; $p_{\text{value}}_{\text{survival after onset}} < 2.2\text{e-}16$, Figure

1, Table 3). In particular, FTLD-TDP type A patients were the oldest at onset with a median age of 68.0 years ($IQR_{TDP A}=59.0-75.8$), which was significantly different from FTLD-TDP type B and type C patients ($AAO_{TDP B}=62.0$, $IQR_{TDP B}=55.0-69.0$, $pvalue=5.3e-07$; $AAO_{TDP C}=60.8$, $IQR_{TDP C}=55.8-65.4$, $pvalue=4.9e-09$). FTLD-TDP type B patients died significantly younger ($AAD_{TDP B}=67.0$, $IQR_{TDP B}=60.0-74.0$) as compared to FTLD-TDP type A patients ($AAD_{TDP A}=78.0$, $IQR_{TDP A}=67.0-85.0$, $pvalue=8.4e-15$) and FTLD-TDP type C patients ($AAO_{TDP C}=72.0$, $IQR_{TDP C}=66.7-76.0$, $pvalue=4.9e-05$) and FTLD-TDP type C patients had the longest survival after onset with a median survival of 11.0 years ($IQR_{TDP C}=8.1-13.2$) as compared to 7.2 years ($IQR_{TDP A}=4.0-11.0$, $pvalue=9.0e-08$) in FTLD-TDP type A and 3.8 years ($IQR_{TDP B}=2.0-6.0$, $pvalue=2.7e-35$) in FTLD-TDP type B.

After quality control, 517 FTLD-TDP patients and 838 controls free of neurodegenerative disorders were retained and included in genetic studies. An average sequencing depth of 40X was achieved for both patients and controls that passed quality controls. To further characterize the presence of known gene mutations within our FTLD-TDP cohort, we first queried the whole-genome dataset for variants observed in FTLD-TDP patients with a minor allele frequency $<0.1\%$ in the EXAC database and absent from our control dataset in the following genes: *GRN*, *TBK1*, *OPTN*, *VCP*, *TARDBP*, *CHCHD10*, *SQSTM1*, *UBQLN2*, *hnRNPA1*, *hnRNPA2B1*, *CSF1R*, *FUS*, *CHMP2B*, *LRRK2*, and *TIA1*. This led to the identification of LOF mutations in *TBK1* in 7 additional FTLD-TDP patients ($pvalue_{burden}=5.15e-03$). Together with the 10 *TBK1* carriers that were already known at the initiation of the International FTLD-TDP WGS Consortium, this brings the total to 17 *TBK1* mutation carriers versus none in controls. We also detected two variants in *OPTN*, one variant in *GRN* and one variant in *CHMP2B*, each of which was defined by the American College of Medical Genetics and Genomics (ACMG) as pathogenic (Table 4, Online Resource 2). Of note, the pathogenic *GRN* variant was a splicing variant c.708+6_+9delTGAG which had been missed due to its position near the 3' splice site of exon 7.[6] By adding these newly identified pathogenic variants to the overall cohort, *C9orf72* repeat expansions explained 25.5% of our Caucasian FTLD-TDP cohort, 13.9% of the patients carried a *GRN* mutation, 1.5% carried a *TBK1* mutation and 1.4% carried a mutation in another known disease gene (suppl. table 1 Online Resource 1). Conversely, 57.7% of Caucasian FTLD-TDP patients were not explained by mutations in the known genes.

Identification of common FTLD-TDP genetic risk factors

To identify novel common genetic risk factors for FTLD-TDP, we next performed single variant genome-wide association for the 7,083,292 common variants ($MAF>0.01$ in either FTLD-TDP patients or controls) that passed quality control in the 517 FTLD-TDP patients and 838 controls. Genomic inflation was moderate ($\lambda=1.05$). Logistic regression adjusting for sex and first four PCs identified one genome-wide significant locus at chromosome 7q36.2. The signal was driven by two variants: rs4726389 and rs118113626 located in the *DPP6* intron 1 region (Table 5, Figure 2; $p=4.63e-08$, $OR=2.453$; $p=4.88e-08$, $OR=2.481$). Both variants at the *DPP6* locus were in linkage disequilibrium ($r^2=0.77$ and $D'=0.88$) and conditional analysis on rs4726389 abolished the significant association of rs118113626. No additional common variants were detected with an $r^2>0.5$ at the 7q36.2 locus. Variants

rs4726389 and rs118113626 were not reported as expression quantitative trait loci (eQTL) in public databases and rs4726389 had a Regulomedb score of 6 suggesting minor effect on regulatory elements. Moreover, analysis of *DPP6* mRNA expression in a custom RNAseq dataset of frontal cortex tissue samples of FTLD-TDP patients (n=68) did not show an effect of rs4726389 on *DPP6* mRNA expression (pvalue=0.88 after FDR correction, fold change = 1.01; Figure 2). Compared to control tissue samples, we did observe a decrease in *DPP6* mRNA expression in FTLD-TDP patients; however, after correction for cell type composition, this association was no longer significant (pvalue=0.89 after FDR correction, Figure 2). By investigating the presence of rare variants in *DPP6* in our dataset, we further identified one LOF variant in our FTLD-TDP patients: the splice variant c.1345+1G>T (cDNA positions is provided according to NM_130797), whereas LOF variants were not observed in our control cohort. The LOF variant reduced cortex mRNA expression of *DPP6* by 41% and cDNA sequencing suggested degradation of the mutant allele by nonsense mediated decay (suppl. figure 1 Online Resource 1). We also identified 26 *DPP6* non-synonymous variants with a MAF less than 1% in either patients or controls. A SKAT test including the two LOF variants and the 26 missense variants resulted in a trend towards association of *DPP6* rare damaging variants with FTLD-TDP but failed to reach significance (pvalue=0.07). After adjustment for the top common SNPs this result did not change substantially suggesting the *DPP6* common variant and rare variant associations with FTLD-TDP are independent from one another.

In addition to the *DPP6* locus, 12 suggestive loci with a pvalue <1e-05 were identified (Table 5). Of the 13 loci, 11 were successfully followed-up in an independent replication cohort of newly ascertained FTLD-TDP patients (n=119) and controls (249 pathologically confirmed normal controls and 1,404 clinical controls). A meta-analysis combining the discovery and the replication stages resulted in three loci with genome-wide significance (Table 5). Rs118113626 at the *DPP6* locus remained genome-wide significant despite some heterogeneity between the two stages. The strongest signal however was found at chromosome 19p13.11 at the *UNC13A* locus, with top SNP rs12973192 (pvalue=1.27e-08, OR=1.50) followed by rs17219281 on chromosome 6p21.32 at the *HLA-DQA2* locus (pvalue= 3.22e-08, OR=1.98). According to the GTex database, rs17219281 is reported as an eQTL for *HLA-DQB2* and *HLA-DQA2* with the most significant association found with their expression in cortex for *HLA-DQB2* (pvalue=6.2e-07) and in amygdala for *HLA-DQA2* (pvalue=2.7e-03); the rare allele consistently increasing *HLA-DQB2* and *HLA-DQA2* gene expression.

In exploratory analysis, we next performed genome-wide association analyses within each FTLD-TDP strata (FTLD-TDP type A, B and C). Loci which showed suggestive association (pvalue <1e-05) in at least one of the strata are shown in suppl. table 3 (Online Resource 1). Interestingly, rs12973192 in *UNC13A* reached genome-wide significance within FTLD-TDP type B (pvalue=4.67e-08, OR=1.95), with virtually no association of rs12973192 with FTLD-TDP type A and C (pvalue_{TDP A}=1.90e-02, OR=1.35; pvalue_{TDP C}=4.94e-01, OR=1.10). In contrast, within FTLP-TDP type A patients, the *GRN* locus on chromosome 17 showed most significance. While the additive model resulted in a suggestive pvalue of 1.69e-07 at this locus in FTLD-TDP type A (rs708384), further investigation under different modes of inheritance, showed a much stronger and genome-wide significant signal using a

recessive model with most significance at rs5848 located in the 3' untranslated region of *GRN* ($p_{\text{value}}=4.99\text{e-}12$; $\text{OR}=5.16$). No genome-wide significant associations were found in FTLD-TDP type C.

Finally, KEGG pathway enrichment analysis including all suggestive loci in either the overall FTLD-TDP cohort or pathological strata (suppl. table 2 Online Resource 1) highlighted a significant overrepresentation of the inflammatory pathway ($p_{\text{value}_{\text{FDR}}}=2.79\text{e-}02$) represented by the presence of the *HLA* locus, *MAF* and *TLR4* genes.

Identification of rare FTLD-TDP genetic risk factors

To identify genes carrying rare FTLD-TDP risk variants we first focused only on genes that carried LOF variants. Gene-burden analyses including only these variants did not show exome-wide significant association (Online Resource 3) and gene ontology and pathway enrichment analyses on all nominally significant genes revealed no particular enrichment in biological processes (data not shown). We then focused on 61 genes in which we observed LOF variants in at least 3 patients and none in controls. *TBK1* showed the most LOF mutation carriers ($n=7$, as discussed above). Prioritization of the remaining 60 genes based on similarities with known FTLD-TDP genes using ToppGene identified *TRIM21* as the top gene based on its functional role within the *TBK1*-regulated innate immunity pathway (three LOF carriers, $p_{\text{value}_{\text{ToppGene}}}=1.97\text{e-}04$ after FDR correction, Online Resource 4). Two additional genes which are known to function in the same pathway showed significant similarity to known FTLD-TDP genes according to ToppGene: *IRF-7* (three LOF carriers, $p_{\text{value}_{\text{ToppGene}}}=8.68\text{e-}04$ after FDR correction) and *DHX58* (four LOF carriers, $p_{\text{value}_{\text{ToppGene}}}=1.43\text{e-}02$ after FDR correction) (Online Resource 5). Interestingly, manual inspection of the LOF variants observed in our WGS cohort also revealed one FTLD-TDP patient each with LOF variants in *IRF8*, *IRF3* and *NOD2* and no LOF variants in controls in these genes, further highlighting the importance of the *TBK1*-regulated innate immunity pathway in FTLD-TDP (suppl. figure 2 Online Resource 1). As a second analysis we broadened our filtering criteria allowing variants predicted to be pathogenic by two prediction algorithms (Polyphen-2 and SIFT) to be added to the LOF variants (Online Resource 3). This resulted in one exome-wide significant gene in the burden analysis (*OSBPL3*), however, this association did not remain significant when PCs were excluded from the model ($p_{\text{value}}=7.61\text{e-}07$, $p_{\text{value}_{\text{noPC}}}=2.24\text{e-}04$). Again, gene ontology and pathway analyses on the nominally significant genes failed to detect enrichment in biological processes (data not shown). When we selected genes which carried LOF variants and coding variants predicted to be pathogenic in at least three patients and no controls, similarity analysis with ToppGene ranked *NPC1* as the gene most closely resembling known FTLD-TDP genes (three variants, $p_{\text{value}_{\text{ToppGene}}}=6.33\text{e-}05$ after FDR correction, Online Resource 4).

Discussion

The significant heterogeneity in clinical and pathological presentations among FTLD patients and the strong correlations between known gene mutations and pathological FTLD

subgroups prompted our initiative to establish the international FTLN-TDP whole genome sequencing consortium. This allowed us to provide a comprehensive overview of the most common known genetic causes of FTLN-TDP and it formed the basis of an unbiased genome-wide association study which implicated both common variants at the *DPP6*, *UNC13A* and *HLA-DQA2* genomic loci and rare LOF variants in genes involved in the TBK1-immunity pathway in the genetic etiology of FTLN-TDP. The careful pathological classification of each patient included in the WGS study into an FTLN-TDP pathological subtype, also confirmed rs5848 located in the 3'UTR of *GRN* as a major risk factor for FTLN-TDP, specifically in FTLN-TDP type A.

Focusing on the known genes, combined analysis of 1,151 FTLN-TDP patients across 23 international sites clearly established the *C9orf72* repeat expansion as the most common known genetic cause of FTLN-TDP in Caucasian populations, explaining 25.5% of patients. We observed a wide range of *C9orf72* mutation frequencies across sites (from 10.3% to 45.5%), which likely reflects the specialized nature of certain clinics and the relative number of FTLN-MND patients that are followed. While we carefully excluded known relatives, the presence of founder effects may have also inflated the *C9orf72* disease frequency in certain populations, as suggested elsewhere [87]. Regardless, the high frequency of *C9orf72* repeat expansions in FTLN-TDP patients underscores the importance of understanding the multiple disease mechanisms associated with this mutation such that effective therapies can be developed for this significant patient subgroup.[5] Interestingly, in our non-Caucasian FTLN-TDP patients, only 10.0% carried a *C9orf72* repeat expansion, compared to 20.0% of patients with a *GRN* mutation. While we cannot draw definitive conclusions due to the small sample size, *GRN* mutations thus appear to be the major known genetic cause of FTLN-TDP among non-Caucasian populations.

We next focused on the 57.7% of Caucasian FTLN-TDP patients who remained unexplained after careful analysis for the presence of pathogenic mutations in known genes. WGS was performed on all patients with sufficient DNA quality and quantity available and 517 FTLN-TDP patients and 838 controls were eventually included in unbiased genetic association studies. At the discovery stage, a common variant genome-wide analysis identified the *DPP6* locus at chromosome 7q36 as a novel FTLN-TDP risk locus, with further validation at the meta-analysis stage which included 119 additional FTLN-TDP patients and 1,653 controls. *DPP6* is a type II transmembrane protein exclusively expressed in neurons [13]. It is a binding partner of the Kv4-containing A-type K⁺ channels which are important for determining cellular excitability. Based on available studies in mouse and human, one could hypothesize that FTLN-TDP associated risk variants reduce the amount of functional *DPP6*: *Dpp6* knockout mice show a reduction of hippocampal glutamatergic synapses and impaired hippocampus-dependent learning behavior and memory [50,81], *DPP6* LOF mutations in humans have been associated with neurodevelopmental disorders [48] and autoantibodies against *DPP6* were found to be the cause of a multifocal neurologic disorder of the central and autonomic nervous system [7,34,83]. The identification of two *DPP6* LOF mutations in our FTLN-TDP patient cohort is further in line with a LOF disease-risk mechanism. However, no effect of our top variants (rs4726389 and rs118113626) on brain *DPP6* mRNA expression levels could be detected, using either publically available data or a custom-derived frontal cortex RNAseq dataset. Given that these measures were obtained from

relatively small numbers of post-mortem tissue samples, using bulk RNAseq, these findings do not exclude an effect of these variants on DPP6 expression in (specific) neuronal populations. In addition, while our lead SNPs are located in intron 1 of *DPP6*, we cannot rule out an effect on more distantly located genes. It also remains possible that the observed risk variants tag a rarer functional variant, only present in a subset of patients, which would have hampered our ability to detect an effect. Such functional variant(s) may well be a complex rearrangement given that the chromosome 7q36 region is enriched with low copy repeats which increase the chance of recombination and chromosomal rearrangements, as described [24]. Long-range next-generation sequencing technologies would be needed to test this hypothesis in future studies. In fact, in an independent study, paired end and long reads Nanopore WGS in a previously unresolved autosomal dominant early-onset dementia family linked to 7q36 led to the identification of a large ~4Mb chromosomal inversion disrupting *DPP6*, further implicating loss of *DPP6* expression and/or function in early-onset dementia (Cacace et al., submitted, 2018). Finally, while the *DPP6* locus has been previously associated with ALS in some but not all studies [89,15,21,17,28,12,71,91,63], the top ALS-associated variant (rs10260404) is not in LD with our top SNPs (rs4726389 and rs10260404: $R^2=0.004$).

A meta-analysis of the discovery and replication stages revealed two additional genome-wide significant loci: *UNC13A* and *HLA*. The *UNC13A* locus signal was driven by rs12973192 which is in strong LD with rs12608932, a variant associated with ALS [90]. In fact, a meta-analysis of ALS and an earlier performed FTLD-TDP GWAS reached genome-wide association at the *UNC13A* locus [23], but failed to reach significance in the FTLD-TDP cohort alone [85,23]. More recently, *UNC13A* variant rs12608932 was shown to act as a phenotypic modifier in ALS patients by increasing the risk for frontotemporal cortical atrophy and impaired cognitive performance, reminiscent of an FTLD clinical presentation [69]. We also detected a genome-wide significant signal at the *HLA* locus on chromosome 6p21.32 led by rs17219281 which is located upstream of the *HLA-DQA2* and *HLA-DQB2* genes and has been reported as an eQTL with the rare allele (associated with FTLD-TDP risk) robustly increasing the expression of *HLA-DQA2* and *HLA-DQB2* transcripts in several brain regions. Even though most *HLA* genes are highly polymorphic, the *HLA-DQA2* and *HLA-DQB2* genes are poorly polymorphic, yet their biological function has not been well characterized [45]. Importantly, the *HLA* locus has previously been implicated in other neurodegenerative disorders including Alzheimer's and Parkinson's disease (PD) [42,60,33]. Moreover, a large GWAS identified rs9268877 at the *HLA-DRA* locus on 6p21.32 as a risk factor for clinical FTLD [26] with subsequent studies emphasizing a possible genetic overlap between FTLD and PD through rs9268877 [26,27]. Our FTLD-TDP associated lead SNP (rs17219281) is not in LD with rs9268877 ($R^2=0.03$) and in contrast to our lead SNP, the FTLD and PD-associated risk allele of rs9268877 was associated with reduced expression of *HLA-DQA2* transcripts [27]. Therefore, even though we identified association of FTLD-TDP with a known FTLD risk locus, the specific risk variant(s) and the associated disease mechanisms may vary across diseases and give rise to distinct neuropathologies. It will likely require the use of dedicated bioinformatic pipelines designed to analyze the high complexity of the HLA region to further clarify this issue.

The requirement of TDP-43 immunohistochemistry and pathological FTLD-TDP subtyping for all patients included in the WGS provided a level of quality control in terms of patient inclusion above and beyond that of previous studies. It also provided the first opportunity to test the hypothesis that different genetic factors influence disease risk in distinct FTLD-TDP pathological strata. Excitingly, using only 184 FTLD-TDP type B patients, we identified genome-wide significant association with variants in *UNC13A*, illustrating the power of our approach and confirming the overlap between ALS and FTLD-TDP type B in particular. In patients with FTLD-TDP type A, a more than 5-fold increased risk was detected for patients homozygous for the rare T-allele of rs5848 located in a microRNA binding site within the *GRN* 3'UTR [73] ($p_{\text{value}_{\text{REC}}}=4.99\text{e-}12$; OR=5.16), whereas no risk was observed in FTLD-TDP type B ($p_{\text{value}_{\text{REC}}}=2.04\text{e-}2$; OR=1.44) and type C ($p_{\text{value}_{\text{REC}}}=8.94\text{e-}1$; OR=1.05) patients. Pathological heterogeneity within previously studied patient cohorts (e.g. variable proportions of FTLD-TDP type A patients) likely contributed to the discrepant reports on the role of rs5848 in FTLD published in the last decade [11,73,75,80]. Importantly, however, we previously showed that the rs5848 risk allele is associated with reduced *GRN* expression in cerebellar tissue samples to a level intermediate between *GRN* mutation carriers and controls [73] and similar effects of rs5848 on expression were reported in plasma and cerebrospinal fluid [36,62]. These findings suggest that reduced *GRN* levels (resulting from rs5848) may contribute to FTLD-TDP type A disease risk in at least a subset of the patients without *GRN* LOF mutations. This would expand the overall contribution of *GRN* dysfunction to FTLD-TDP with likely significant implications once *GRN*-related therapies become available. For FTLD-TDP type C, best known for its lack of a positive family history in most patients, we did not identify any genome-wide significant risk factors; however, this may have been a result of a lack of power (only 143 FTLD-TDP type C in association study).

Finally, our study also sheds new light on *TBK1* and the role of the innate immune signaling in FTLD. Neuroinflammation and immunity have previously been reported in the context of FTLD, and the idea that immune dysfunction may contribute to FTLD risk is not new. Microglial activation is a pathological hallmark of patients with FTLD [43,66,82] and a key feature of genetic FTLD mouse models [40,52,79,99] and both *GRN* and *C9orf72* have been extensively linked to neuroinflammation and microglial activation [41,55,65,99]. Genetic overlap between immune-mediated diseases and clinical FTLD was recently reported [8] and independent studies found an increased prevalence of autoimmune conditions in patients with *GRN* and *C9orf72* mutations, and in clinical FTLD patients predicted to have an underlying FTLD-TDP pathology (svPPA and FTLD-MND) [58,59]. In our study, we confirmed the importance of *TBK1* by establishing it as the third most common genetic cause of FTLD-TDP, with LOF variants in 1.5% of Caucasian FTLD-TDP patients. Moreover, even though we did not have statistical power to detect exome-wide significant association in our rare variant burden analysis, prioritization of genes with an excess of LOF variants in FTLD-TDP patients (n = 3) versus controls (n=0) detected several other genes involved in the regulation of inflammation and immunity through *TBK1* (*TRIM21*, *DHX58*, *IRF7*, *IRF3*, *IRF8* and *NOD2*). Segregation data as well as independent replication is necessary to infer a causal or risk effect of such LOF variants. *TBK1* encodes a protein kinase involved in regulation of the immune response, autophagy and inflammation [35]. In

the innate immune signaling pathway, TBK1 can be activated through multiple pathways including double stranded (ds)RNA (TLR3-TRIF), lipopolysaccharides (TLR4-TRIF), viral RNA (RIG-I-MAVs) and dsDNA (cGAS-STING) resulting in the phosphorylation and activation of IRF3/IRF7 (suppl. figure 2 Online Resource 1) [51,47,64]. *TBK1* mutations observed in ALS and FTLN-TDP patients were previously shown to reduce the activation of IRF3 [29,38,84,19], and our observation of LOF variants in *IRF3*, *IRF7* and *IRF8* in FTLN-TDP patients thus points to alternative genetic insults that may have similar consequences. TRIM21, with LOF variants in 3 FTLN-TDP patients, also positively regulates innate immunity by facilitating the recruitment of TBK1 to MAVS through the regulation of MAVS polyubiquitination [98]. DHX58 (mutated in 4 FTLN-TDP patients) was originally thought to be a negative regulator of the RIG-I-like receptor family [77,39,100,76]; however, more recent work has shown the importance of DHX58 in the enhancement of MDA5-mediated antiviral signaling *in vivo* [78,92]. In fact, studies in *Dhx58* knock-out mice found that Dhx58 was essential for type I IFN production in response to picornaviridae infection [78]. In combination, our findings suggest a critical role for impaired interferon production in FTLN-TDP; however, TBK1 is also well known for its role in the autophagy pathway through interactions with OPTN and SQSTM1, two other proteins implicated in FTLN-TDP etiology [2]. Future studies should therefore decipher the respective roles of IFN signaling and autophagy in TBK1-related FTLN-TDP and the possible crosstalk between these two pathways [78].

Our study also has some limitations. Since we only recruited 20 non-Caucasian FTLN-TDP patients, the *GRN* and *C9orf72* mutation frequencies in this cohort may be unreliable and novel gene discovery was not possible. Second, while we included the analyses of both common and rare variants, this study only focused on highly selected rare variants and we did not analyze copy number and structural variants. Further studies are therefore necessary to provide a full overview of the genetic factors contributing to FTLN-TDP. Third, the relatively small sample size of our discovery and replication cohorts may have resulted in a lack of statistical power to detect weak genetic associations with disease risk. The use of clinical FTLN cohorts, enriched for patients with certain clinical FTLN subtypes, may be one possible avenue for future replication studies; however, while some genetic risk factors will benefit from such approach, some true genetic risk factors may fail to replicate due to an increased heterogeneity of underlying pathologies.

In conclusion, our analysis of the largest cohort of pathologically characterized patients with FTLN-TDP in which mutations in the known causal genes *GRN* and *C9orf72* were excluded, identified three genomic loci harboring common FTLN-TDP risk variants: *DPP6*, *UNC13A* and *HLA-DQA2*, and an excess of rare LOF variants in the TBK1-related innate immunity pathway in FTLN-TDP patients as compared to controls. Future work will focus on the identification of functional variants and their associated disease mechanism at each of the associated loci; however, we nominate *DPP6* with its modulating effect on K-channel activation as a possible novel FTLN-TDP risk gene and we strongly implicate the immune pathway in FTLN-TDP pathogenesis.

Supplementary Material

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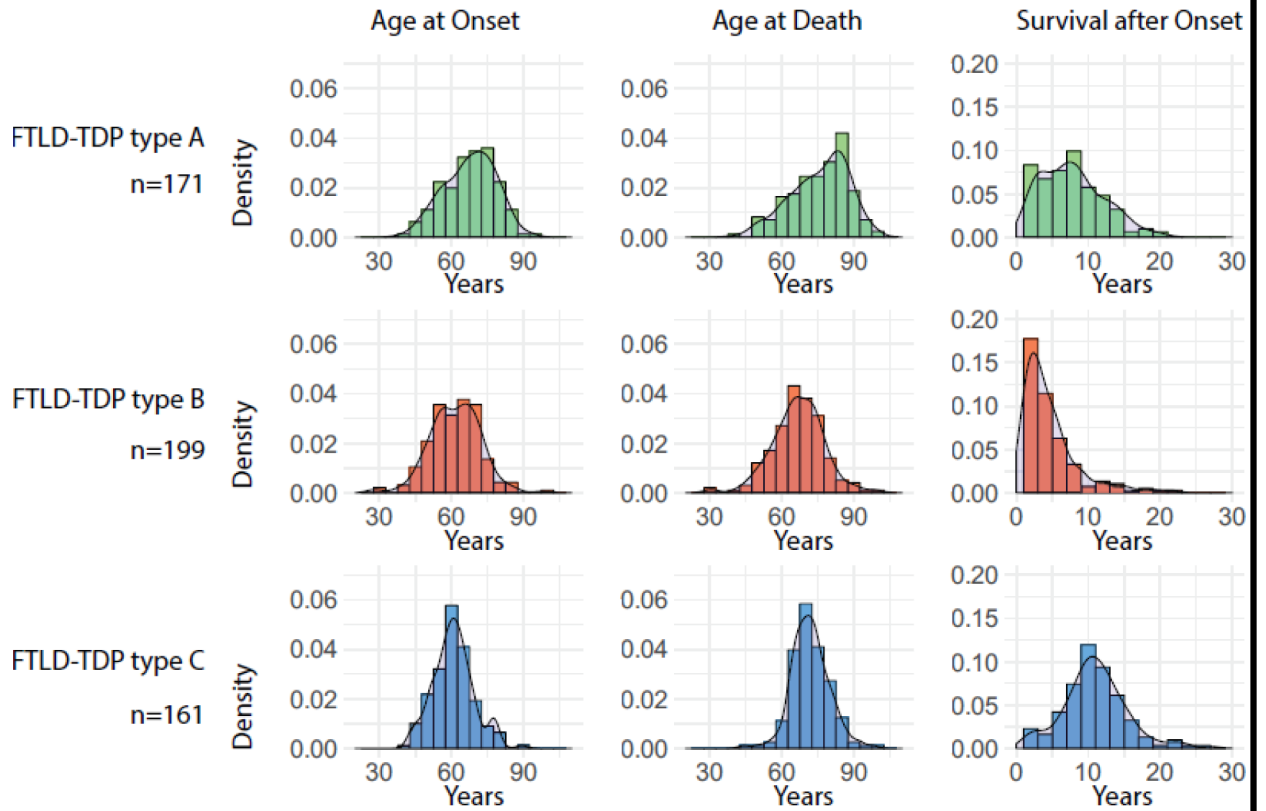


Figure 1. Age distributions in each FTLD-TDP pathological subtype

Age at onset, age at death and survival after onset are represented as histogram per FTLD-TDP pathological subtype. A density curve is superimposed to the histograms.

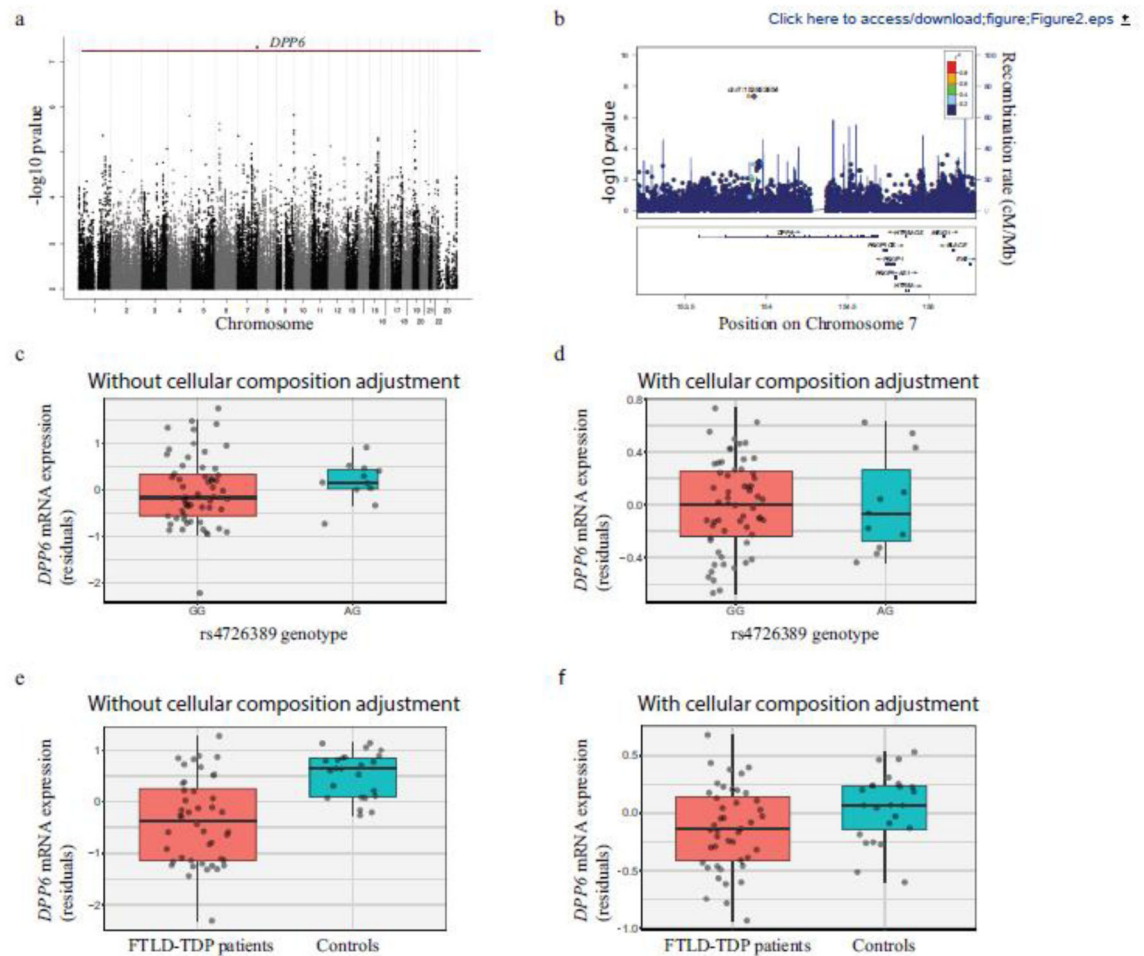


Figure 2. Common variant whole genome sequencing association study and *DPP6* locus
(a) Manhattan plot of the FTLD-TDP patients versus control association study. The red dotted line represents the genome-wide significance level ($p=5e-08$). **(b)** Regional association (locus zoom) plot of the *DPP6* locus. Each dot represents a genotyped variant. The purple dot is the most significant variant (rs4726389) among variants in the region. Dots are colored from red to blue according to their r^2 value, showing their degree of linkage disequilibrium with rs4726389 (grey indicates an r^2 of zero). The light blue line shows the estimated recombination rate. **(c)** *DPP6* mRNA expression levels in function of the rs4726389 genotypes without correction for cellular composition in custom RNAseq frontal cortex dataset. **(d)** *DPP6* mRNA expression levels in function of the rs4726389 genotypes with correction for cellular composition in custom RNAseq frontal cortex dataset. **(e)** Differential *DPP6* mRNA expression levels in FTLD-TDP patients and controls without correction for cellular composition. **(f)** Differential *DPP6* mRNA expression levels in FTLD-TDP patients and controls with correction for cellular composition.

Table 1. Overview of genetic status in FTL D-TDP patients within the international FTL D-TDP whole genome sequencing consortium.

The genetic status of FTL D-TDP patients per site per ethnicity is presented for each of the major FTL D-TDP genes (*GRN*, *C9orf72*, *TBKI*) and for other genes which includes *VCP* (n=5), *CHMP2B* (n=1), *CHCHD10* (n=2), *FUS* (n=1), *CSF1R* (n=1) and *TIAI* (n=1).

Site	Number of FTL D-TDP	Caucasian				Non-Caucasian			
		GRN	<i>C9orf72</i>	<i>TBKI</i>	Other genes	GRN	<i>C9orf72</i>	<i>TBKI</i>	Other genes
Banner Sun Health Research Institute	29	6	3	0	0	0	0	0	0
Columbia University	25	2	3	0	0	0	0	0	0
Emory University	48	6	13	0	0	1	1	0	0
Erasmus University	93	5	22	0	2	0	0	0	0
German Center for Neurodegenerative Diseases	24	2	7	1	0	0	0	0	0
Indiana University	32	13	4	1	0	0	0	0	0
Karolinska University	44	4	20	1	1	0	0	0	0
King's College London	50	3	6	0	0	0	0	0	0
Ludwig-Maximilians-University Munich	40	4	16	1	3	0	0	0	0
Mayo Clinic Jacksonville/Rochester	207	27	51	1	0	0	0	0	0
Northwestern University	69	16	8	1	0	0	0	0	0
University College London	54	13	13	0	0	0	0	0	0
University of British Columbia	72	9	26	0	0	5	1	0	0
University of California San Diego	15	2	3	0	0	0	0	0	0
University of New South Wales	47	9	20	0	2	0	0	0	0
University of Pennsylvania	95	17	29	3	2	3	0	0	0
University of San Francisco	101	9	22	1	0	1	2	1	0
University of Toronto	18	1	5	0	1	0	0	0	0
University of Pittsburgh Medical Center	20	2	3	0	0	0	0	0	0
University Texas Southwestern Medical Center	41	5	9	0	0	0	0	0	0
Washington University School of Medicine	24	2	4	0	0	1	0	0	0
University of Western Ontario	6	0	2	0	0	1	0	0	0
TOTAL	1154	157 (13.8%)	289 (25.5%)	10 (0.9%)	11 (1.0%)	14 (70.0%)	4 (20.0%)	2 (10.0%)	0

Table 2.
Demographics of FTL D-TDP patients and controls included in the association studies.

Median age at onset, age at death and age at last healthy visit of patients and controls included in the discovery and replication stages are presented. N: number of individuals, IQR: interquartile range, NA: not applicable,

Group	Discovery			Replication				
	Age at onset* (IQR)	Age at death (IQR)	% female (N)	Total	Age at onset* (IQR)	Age at death (IQR)	% female (N)	Total
FTLD-TDP	63 (56.0 – 70.0)	71 (64.0 – 78.0)	68.7% (226)	554	59 (52.0 – 66.0)	71 (64.0 – 75.6)	42.0% (50)	119
Controls	66 (57.1 – 76.3)	NA	50.0% (491)	982	68.1 (58.7 – 80.5)	NA	48.4% (800)	1653

* : indicates the age at last visit for the controls.

Table 3.
Demographics of FTL D-TDP patients included in the whole-genome sequencing.

Median age at onset, age at death and age at last healthy visit of patients are presented (in years). IQR: interquartile range.

Group	Age at Onset (IQR)	Age at Death (IQR)	Survival after onset (IQR)
FTLD-TDP type A (n=171)	68.0 (59.0 – 75.8)	78.0 (67.0 – 85.0)	7.2 (4.0 – 11.0)
FTLD-TDP type B (n=199)	62.0 (55.0 – 69.0)	67.0 (60.0 – 74.0)	3.8 (2.0 – 6.0)
FTLD-TDP type C (n=161)	60.8 (55.8 – 65.4)	72.0 (66.7 – 76.0)	11.0 (8.1 – 13.2)

Table 4.

Pathogenic variants identified by whole-genome sequencing.

Pathogenic variants according to the ACMG criteria are presented along with their predicted pathogenicity (CADD score) and frequency in ExAC non-Finnish European (ExAC NFE). Phenotypic data is presented for each FTL-D-TDP carrier and includes sex, age at death, age at onset and the TDP-43 pathological subtype.

Gene name	Variant category	Variant cDNA	Variant Protein	CADD score	ExAC NFE	Sex	Age at Death	Age at Onset	TDP-43 Type	Publications
<i>CHMP2B</i>	nonsynonymous SNV	NM_014043:c.A618T	p.Q206H	23.9	1.51E-05	F	71	62	C	[14] [68]
<i>GRN</i>	splice	NM_002087:c.708+6_+9delTGAG		.	.	M	72	62	A	
<i>OPTN</i>	frameshift insertion	NM_001008212:c.381_382msAG	p.D127fs	24.3	2.00E-04	M	70	62.2	A	
<i>OPTN</i>	stopgain	NM_001008212:c.C703T	p.Q235X	39	.	M	70	64.3	A	[70]
<i>TBK1</i>	stopgain	NM_013254:c.C349T	p.RHTX	39	.	M	72	68	A	[70] [88]
<i>TBK1</i>	stopgain	NM_013254:c.C1330T	p.R444X	45	1.51E-05	M	60	52	B	[84]
<i>TBK1</i>	frameshift deletion	NM_013254:c.I328_I331del	p.I443fs	35	.	M	75	66	A	
<i>TBK1</i>	stopgain	NM_013254:c.C379T	p.R127X	38	.	F	75	68	A	[88]
<i>TBK1</i>	stopgain	NM_013254:c.I272delT	p.Y424X	25.8	.	F	54	50	B	
<i>TBK1</i>	frameshift deletion	NM_013254:c.I886_I889del	p.Q629fs	35	.	F	78	68	A	
<i>TBK1</i>	splice	NM_013254:c.992+G>A		24.5	.	M	71	68	B	[88]

Table 5. Associations within the discovery, replication and meta-analysis stages for loci identified in the FTLD-TDP patients versus control analysis.

Each locus is presented with the variants followed-up in the replication along with their position, the closest gene (Locus Name), the minor allele frequency in controls and patients (MAF Controls/Patients), its odds ratio (OR) and pvalue.

SNP ID	Position	Major/minor allele	Locus name	Discovery stage			Replication Stage			Meta-analysis		
				MAF in controls/patients	OR (95% CI)	p value	MAF in controls/patients	OR (95% CI)	p value	OR	p value	I ²
rs61831315	chr1:186126654	A/G	<i>HMCN1</i>	0.05/0.02	0.31 (0.18–0.51)	4.28E-06	0.05/0.06	1.07 (0.60–1.92)	8.13E-01	0.52	9.06E-04	90.17
rs61707463	chr4:169787358	A/G	<i>C4orf27</i>	0.02/0.05	3.58 (2.13–6.03)	1.60E-06	0/0	NA	NA	NA	NA	NA
rs11132244	chr4:184422579	A/G	<i>IRF2</i>	0.3/0.23	0.64 (0.53–0.78)	9.17E-06	0.27/0.3	1.14 (0.84–1.55)	3.93E-01	0.76	1.06E-03	89.67
rs17219281	chr6:32707868	G/A	<i>HLA-DQA2</i>	0.06/0.11	2.06 (1.52–2.79)	3.19E-06	0.07/0.13	1.84 (1.24–2.74)	2.58E-03	1.98	3.22E-08	0
rs6463679	chr7:7305371	G/A	<i>LOC101927354</i>	0.34/0.42	1.49 (1.26–1.77)	4.39E-06	0.36/0.41	1.23 (0.93–1.63)	1.43E-01	1.42	2.81E-06	23.39
rs10267171	chr7:110723284	T/C	<i>IMMP2L</i>	0.19/0.25	1.61 (1.31–1.98)	6.53E-06	0.2/0.16	0.76 (0.53–1.1)	1.41E-01	1.34	1.34E-03	91.82
rs118113626	chr7:154194746	C/T	<i>DPP6</i>	0.05/0.10	2.48 (1.79–3.44)	4.88E-08	0.07/0.09	1.51 (0.94–2.43)	8.91E-02	2.12	4.82E-08	64.89
rs4726389	chr7:154225769	G/A	<i>DPP6</i>	0.05/0.10	2.45 (1.78–3.39)	4.63E-08	0.07/0.08	1.27 (0.77–2.08)	3.44E-01	2.02	3.50E-07	79.20
rs13283101	chr9:135082911	G/A	<i>OLFMI</i>	0.12/0.20	1.74 (1.38–2.20)	3.30E-06	0.15/0.16	1.01 (0.70–1.45)	9.73E-01	1.49	8.06E-05	83.56
rs12425381	chr12:15176736	C/G	<i>RERG</i>	0.24/0.17	0.65 (0.53–0.81)	6.78E-05	0.18/0.13	0.69 (0.39–1.24)	2.14E-01	0.66	3.08E-05	0
rs4240777	chr15:85946519	C/G	<i>MIR548AP</i>	0.31/0.40	1.51 (1.27–1.81)	4.97E-06	0.37/0.41	1.16 (0.88–1.53)	3.02E-01	1.40	1.04E-05	59.59
rs12973192	chr19:17642430	C/G	<i>UNC13A</i>	0.37/0.45	1.48 (1.25–1.75)	3.44E-06	0.34/0.44	1.57 (1.20–2.04)	9.22E-04	1.5	1.27E-08	0