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C9orf72 and UNC13A are shared risk loci for ALS and FTD: a genome-wide meta-analysis

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Abstract

Objective—Substantial clinical, pathological and genetic overlap exists between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 inclusions have been found in both ALS and FTD cases (FTD-TDP). Recently, a repeat expansion in *C9orf72* was identified as the causal variant in a proportion of ALS and FTD cases. We sought to identify additional evidence for a common genetic basis for the spectrum of ALS-FTD.

Methods—We used published GWAS data of 4,377 ALS patients and 13,017 controls and 435 pathology-proven FTD-TDP cases and 1,414 controls for genotype imputation. Data were analyzed in a joint meta-analysis, by replicating topmost associated hits of one disease in the other, and by using a conservative rank products analysis, allocating equal weight to ALS and FTD-TDP sample sizes.

Results—Meta-analysis identified 19 genome-wide significant single nucleotide polymorphisms (SNPs) at *C9orf72* on chromosome 9p21.2 (lowest $p=2.6\times 10^{-12}$) and one SNP in *UNC13A* on chromosome 19p13.11 ($p=1.0\times 10^{-11}$) as shared susceptibility loci for ALS and FTD-TDP. Conditioning on the 9p21.2 genotype increased statistical significance at *UNC13A*. A third signal, on chromosome 8q24.13 at the *SPG8* locus coding for strumpellin, ($p=3.91\times 10^{-7}$) was replicated in an independent cohort of 4,056 ALS patients and 3,958 controls ($p=0.026$; combined analysis $p=1.01\times 10^{-7}$).

Interpretation—We identified common genetic variants at *C9orf72*, but in addition in *UNC13A* that are shared between ALS and FTD. *UNC13A* provides a novel link between ALS and FTD-TDP, and identifies changes in neurotransmitter release and synaptic function as a converging mechanism in the pathogenesis of ALS and FTD-TDP.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive muscle weakness due to the loss of motor neurons in both brain and spinal cord. No cure exists and disease etiology has not yet been fully elucidated. Important overlap exists with frontotemporal dementia (FTD), which is characterized by changes in cognition, behavior and language. Clinically, approximately 5–15% of patients with ALS have FTD, while about 3–14% of FTD patients also fulfill the criteria for ALS.^{1,2} Neuropathologically, the majority of FTD cases can be divided in two subtypes, characterized by cellular inclusions of either tau (FTD-tau) or TDP-43 (FTD-TDP). TDP-43 inclusions have been found in neurons of both ALS and FTD-TDP patients.³ Lastly, substantial genetic overlap between ALS and FTD has been reported. Linkage studies identified a locus of several megabases on chromosome 9p21 in families of patients with both ALS and FTD.^{4–6} Previous genome-wide association studies (GWAS) of non-familial ALS helped fine-map this region, and recently, a hexanucleotide repeat expansion in *C9orf72* was discovered in this region.^{7–11} The *C9orf72* repeat expansion is present in approximately 6% of sporadic ALS and sporadic FTD patients, and in up to 37% and 25% of familial ALS and FTD cases, respectively.¹² Additionally, mutations in *VCP* have been implicated in both ALS patients and in FTD.¹³ Furthermore, mutations in the gene for TDP-43 (*TARDBP*) have been found in ALS and FTD with motor neuron degeneration (FTD-MND), but are rarely present in FTD-TDP cases without motor neuron symptoms.^{14,15}

The majority of gene mutations have been found in familial cases of ALS and FTD, but these mutations are less frequent in cases without a positive family history.^{11,12,16} Meta-analysis of GWAS data is a powerful tool to discover new susceptibility loci for non-familial disease.¹⁷ The association signals from a GWAS may represent common variants acting as ‘genetic risk factors’, but may also form a proxy for rare, moderately penetrant genetic variants, such as the repeat expansion in *C9orf72*.^{7,9} The discovery of the *C9orf72* repeat expansion has, additionally, shown that intronic, non-coding variation may be causal to disease. Previously, the most recent and largest GWAS of sporadic ALS identified the locus on chromosome 9p21.2 (comprising *C9orf72*) and *UNC13A* as susceptibility loci.^{10,11} Recently, the first GWAS of FTD-TDP patients has been published, identifying three common variants in *TMEM106B* associated with susceptibility to sporadic FTD.¹⁶ The association with *TMEM106B* variants has now been replicated in independent cohorts including FTD-TDP patients.^{18,19}

Both ALS and FTD may form parts of a spectrum of neurodegenerative disease. This spectrum ranges from pure motor ALS, to ALS with mild cognitive impairment, to FTD-MND, and ultimately, to pure FTD without motor neuron symptoms.²⁰ In the present study, we sought to identify a common genetic basis for this spectrum of neurodegenerative disease. Therefore, we conducted a meta-analysis of all available GWAS data in ALS and TDP-43 positive FTD aimed at the discovery of additional common variants that would affect susceptibility to both neurodegenerative diseases.

Methods

Subjects

ALS cohorts were derived from all available previously published GWAS of ALS patients.^{10,11} We included 16 cohorts of Caucasian sporadic ALS patients ($n = 4,638$) and/or unaffected controls ($n = 14,038$) from six European countries and the USA for whom genome-wide genotype data were available. Previous replication cohorts with selected SNP sets (for example obtained by TaqMan genotyping) could not be included. For all cohorts, the diagnosis of probable or definite ALS was made according to the revised El Escorial criteria.²¹

We obtained raw genotype data for 658 individuals that were originally genotyped for the FTD-TDP GWAS, and were recruited from 11 countries in Europe, USA, Canada and Australia.¹⁶ In the original publication, 598 cases with FTD-TDP pathology matched the inclusion criteria, of which 515 were used for analysis. For the present study, we only included cases with FTD-TDP confirmed by TDP-43 immunohistochemistry, a single proband per pedigree, and only individuals of European descent. We excluded cases with mutations in *GRN* or *VCP*, resulting in 453 FTD-TDP cases that remained for further quality control. Clinical data on the presence of motor neuron symptoms were recorded. The Wellcome Trust Case Control Consortium (WTCCC) 1958 Birth Cohort was used for population controls.¹⁶

For replication purposes, we collected genomic DNA from a total of 2,218 sporadic ALS patients and 2,261 unaffected controls from The Netherlands, Germany, Sweden and Switzerland. In addition, *in silico* genotypes were obtained from Illumina beadchip data for 1,838 sporadic ALS patients and 1,697 controls from Italy.

Dutch patients were recruited by neuromuscular centers at the University Medical Center Utrecht, the Radboud University Nijmegen Medical Center, and the Academic Medical Center Amsterdam, as part of an ongoing population-based study of ALS in The Netherlands. Unrelated control samples without any neuromuscular disease were matched for age and gender. Swedish samples were included from the Umeå University ALS Clinic that had not yet participated in previous GWAS included in the present meta-analysis. For the Swiss stratum, patients were recruited at Kantonsspital St. Gallen. German ALS patients were recruited through Ulm University Hospital and Charité University Hospital, Berlin. Control samples were unrelated individuals, free of any neuromuscular disease. Italian ALS patients were included through different Italian ALS centers as part of the Italian SLAGEN Consortium. Controls consisted of Italian healthy individuals who did not have a personal or family history for neurodegenerative diseases.

For all strata, patients with ALS fulfilled the revised El Escorial Criteria for possible, probable, or definite ALS. Cases with a family history of ALS or non-Caucasian descent were excluded. As the discovery ALS and FTD GWAS samples include individuals with *C9orf72* repeat expansions (no complete data available), we did not exclude *C9orf72* repeat expansion carriers from the replication sets.

All participants gave written informed consent and approval was obtained from the local institutional review boards. More detailed information on ALS or FTD subject selection methods has been published previously and can be found in Supplementary Table 1.^{10,11,16}

Genotypes and quality control

For each cohort, raw Illumina beadchip genotype data were obtained. The following quality control measures were applied to each cohort separately. We removed A/T and C/G SNPs in order to prevent allele swaps, SNPs with a minor allele frequency (MAF) < 5%, a genotyping call rate < 95%, or with deviation from Hardy-Weinberg equilibrium (HWE) in controls ($p < 0.001$). Samples with missing phenotype data, a genotyping call rate < 95%, high (inbreeding coefficient $F > 0.05$) or low ($F < -0.025$) heterozygosity rates, or where the clinically reported gender did not match the genotypic gender (based on chromosome X markers), were removed. SNP identifiers and positions were mapped to dbSNP 126 and NCBI genome build 36. For the WTCCC 2 cohort, markers and samples listed for exclusion (as provided by the WTCCC), and samples previously included in the WTCCC 1 cohort, were removed. Cohorts consisting of control samples only were merged with cohorts that included affected patients from the same country, ultimately forming twelve balanced strata for ALS and one for FTD. Per stratum, we excluded SNPs with differing missing rates between cases and controls ($p < 1 \times 10^{-3}$), or SNPs that, as determined by flanking SNPs, are missing higher for one allele ($p < 1 \times 10^{-10}$). For population stratification analysis purposes, strata were merged into a separate dataset containing only SNPs common to all strata. Population substructure was determined by using principal components analysis in EIGENSTRAT, also incorporating HapMap 3 release 2 samples.²² After the removal of population outliers (based on deviation from the CEU+TSI population cluster in a plot of the first two principal components, duplicate (PI_HAT value > 0.9), and related samples (PI_HAT > 0.2), new principal components were calculated. For the ALS and FTD meta-analysis, we removed duplicate and related individuals across disease datasets. See Supplementary Table 2 for Hardy-Weinberg equilibrium (HWE) p values and call rates for significantly associated SNPs per stratum.

For the replication of association with the chromosome 8q24.13 locus, we used KASPar (KBioscience) and TaqMan (Applied Biosystems) assays to determine rs13268726 and rs12546767 genotypes in the replication set, according to the manufacturer's protocols. We used an ABI Prism 7900HT analyzer (Applied Biosystems) and SDS v2.3 software (Applied Biosystems) to determine genotype clusters, and outliers were excluded from further analyses. For the Italian SLAGEN cohort, *in silico* genotypes were obtained from Illumina Human-660W Quad Beadchips for rs12546767, and rs13268726 genotypes were imputed using IMPUTE v2 and HapMap3 release 2 and 1000 Genomes pilot reference panels.

Statistical analysis

Because of the use of many different genotyping platforms, and a relatively small number of markers with genotypes in all strata, we used genome-wide SNP imputation to extend genome-wide coverage and to increase comparability.²³ Imputation was carried out using IMPUTE2 v2.1.2 in genomic *chunks* of 5 Mb, leaving all options at the program's defaults. We preferred the HapMap3r2 CEU+TSI reference (~1.4M markers) as a reference panel,

because of the relatively large number of reference haplotypes ($n = 410$). We, additionally, imputed using the HapMap2 reference (120 haplotypes, ~2.5M markers), to determine if we would not miss important associations compared to the HapMap3r2 reference. Imputation using the HapMap2 reference did not yield additional significant results (data not shown).

Imputed genotypes were stored as continuous allele dosage data, which are continuous numerical values indicating the estimated number of minor alleles (ranging from 0 to 2), thus incorporating a measure of imputation uncertainty.

Associations between genotypes and disease susceptibility, were tested in logistic regression models for each of the strata in PLINK. Gender and principal components (PCs) that were strongly ($p < 1 \times 10^{-5}$) associated with disease status were included as covariates in the logistic regression analyses (seven PCs for ALS, two PCs for FTD). Results from each of the strata were joined in a fixed effect inverse variance meta-analysis in PLINK, both per disease (ALS or FTD) separately, and combined.^{24,25} See Supplementary Table 2 for allele and genotype frequencies per stratum for the topmost associated SNPs. We calculated genotypic odds ratios (OR_{geno}) for top-associated markers in heterozygotes (1 minor allele vs. 2 major allele carriers) and homozygotes (2 minor allele vs. 2 major allele carriers) using logistic regression, and incorporating the same covariates as were used in the main disease susceptibility analyses.

We used the GCTA software package to calculate trait heritability estimates for ALS and FTD-TDP cohorts, as well as to estimate the cross-traits heritability, using a bivariate restricted maximum likelihood analysis, as has been described previously.^{26,27} Imputed dosage data were used as input, excluding SNPs with a poor imputation quality score ($R^2 < 0.3$) and minor allele frequency < 0.01 . The first seven principal components calculated from a combined dataset of ALS and FTD were included as covariates. Additionally, we performed a conditional and joint multiple-SNP analysis of the ALS and FTD meta-analysis results in order to determine possible independent association signals within a genomic locus reaching genome-wide significance.²⁸ The conditional and joint analyses were carried out after converting imputed dosage data to hard-called genotypes (as required by the GCTA software).

As an additional approach, in order to determine SNPs with shared susceptibility to both ALS and FTD, we selected SNPs with $p < 1 \times 10^{-4}$ from the above ALS meta-analysis and used the FTD-TDP data to replicate the associations with these SNPs. We used linkage disequilibrium (LD)-based *clumping* of SNPs in PLINK in order to cluster multiple genotyped and imputed SNPs within a region of strong LD, thus determining independent loci.²⁵ Per clump, we looked up p values of SNPs from the above logistic regression results in the FTD analysis. We applied a Bonferroni multiple-testing correction for the number of independent loci (clumps) tested. Subsequently, SNPs with p values below the threshold of 1×10^{-3} in the FTD analysis were selected for replication in the ALS data. For the selection of SNPs from the FTD analysis, we used a less stringent p value threshold ($p < 1 \times 10^{-3}$) in order to avoid the omission of false-negative associations due to limited statistical power of the FTD-TDP data.

Furthermore, as sample sizes differ substantially between the joined ALS strata and the FTD-TDP stratum, we would be almost exclusively measuring the GWAS signal from the ALS patients. We, therefore, used a conservative rank products (RP) analysis to compare results from both ALS and FTD.²⁹ The rank products method originates from the analysis of multiple expression micro-array experiments, and provides a non-parametric test that is independent of sample size. For the RP analysis, we ranked SNPs by increasing p value for each disease (ALS or FTD). Only SNPs with the same direction of effect were included. For each SNP we calculated the RP. Ultimately, SNPs were sorted by increasing rank product, and a permutation test was used to determine statistical significance for each RP. In order to obtain empirical p values, we permuted the ranks of each SNP 100,000,000 times and counted the number of times the permuted RP was equal to or higher than the observed RP. SNPs with an empirical p value $< 5 \times 10^{-8}$ were considered to be significantly top-ranked for both diseases.

Replication of association with locus 8q24.13

In the replication set of sporadic ALS patients and unaffected controls, for each stratum, association between SNP and disease status was tested in a logistic regression model corrected for gender. Subsequently, results were analyzed in a weighted inverse variance meta-analysis in PLINK.

Results

After quality control, there were twelve ALS strata (4,377 cases, 13,017 controls), and one FTD stratum (435 cases, 1,414 controls). For some cohorts, the total number of cases and controls differed from the original publications, due to different quality control methods. Genomic inflation factors (λ_{GC}) ranged from 1.01 – 1.07, indicating adequate quality control and correction for population stratification (Supplementary Table 1). Associations did not reach genome-wide significance ($p < 5 \times 10^{-8}$) in any of the 13 strata separately.

Analysis of ALS and FTD separately

First, we inspected a meta-analysis of the ALS strata. Consistent with previous findings, we found genome-wide significant hits near *C9orf72* on chromosome 9p21.2 (top SNP rs3849943) and in gene *UNC13A* (top SNP rs12608932) on chromosome 19p13.11 (Fig 1 and 2; Table 1).^{10,11}

Subsequently, we analyzed the separate FTD-TDP stratum. We found no genome-wide significant associations, which was consistent with the results for patients without progranulin (*GRN*) mutations in the original publication (Fig 1).¹⁶ The exact association results differed minimally from the original publication, most probably due to the use of a partly different control cohort, and different methods for quality control and statistical analysis.

Combined ALS and FTD analysis

To examine common genetic variants that are shared in ALS and FTD-TDP we applied three complementary methods to avoid only picking up ALS effects, considering the

imbalance in cohort size. First, all strata were joined into a single meta-analysis. Not only was the signal at 9p21.2 (*C9orf72*) greatly enhanced, but also at *UNC13A* (Fig 1). For rs3849943 at 9p21.2, the genotypic odds ratio (OR_{geno}) in heterozygotes is 1.25 ($p = 3.19 \times 10^{-7}$), in homozygotes the OR_{geno} is 1.19 ($p = 6.76 \times 10^{-5}$); while for rs12608932 in *UNC13A*, the heterozygote OR_{geno} and homozygote OR_{geno} are 1.12 ($p = 0.014$) and 1.29 ($p = 3.33 \times 10^{-15}$), respectively. Results for markers previously associated with sporadic ALS or FTD-TDP can be found in Supplementary Table 3. We did not identify any new genome-wide significant associations in the joint meta-analysis, although one new locus nearing the genome-wide significance threshold emerged at chromosome 8q24.13 (Fig 1).

Using a restricted maximum likelihood analysis, we estimated trait heritability for both ALS and FTD cohorts separately, as well as the cross-traits heritability between ALS and FTD-TDP. We estimated a SNP heritability for ALS of 0.21 (standard error 0.02), while for FTD-TDP no reliable heritability estimate could be calculated due to lack of statistical power. The genetic correlation between ALS and FTD-TDP was modest, but significant (0.20, standard error 0.098, $p = 0.012$). The SNP-based coheritability was estimated at 0.02 (standard error 0.007).

To further demonstrate shared susceptibility to both ALS and FTD-TDP for the *C9orf72* and *UNC13A* loci, we selected top-associated SNPs from one disease and tried to replicate their association in the other disease. We selected 191 SNPs with $p < 1 \times 10^{-4}$ in 75 independent loci from the ALS meta-analysis, and looked up association results for these SNPs in the FTD analysis, applying a Bonferroni multiple-testing correction for the number of independent loci tested. We thus identified six SNPs in two loci (*UNC13A* and *C9orf72*) that were significantly replicated in the FTD-TDP data (Table 2). Conversely, we selected 1,450 SNPs with $p < 1 \times 10^{-3}$ in 658 independent loci from the FTD analysis and replicated these SNPs in the ALS data. We, again, identified two loci (seven SNPs near *C9orf72* and one in *UNC13A*) that were significantly replicated in the ALS data (Table 2).

Subsequently, we used a third approach to investigate whether the associations near *C9orf72* and *UNC13A* were not exclusively driven by one of the disease cohorts. Because of the large sample size of the ALS strata, the combined meta-analysis signals might be largely driven by ALS patients. To take this into account, we used a conservative, sample size-independent, rank products (RP) analysis. This analysis identifies SNP associations whose p values that are ranked highest in both diseases, thus allocating equal weight to both ALS and FTD results. Supplementary Table 4 shows the top ten SNPs from this analysis, arranged according to increasing RP. The $-\log_{10} p$ values of SNPs with the lowest RPs are ranked highest in both diseases. Empirical p values for the rank products, as determined by 100,000,000 permutations, were highly significant for the top six SNPs ($p < 5 \times 10^{-8}$). SNP rs12608932 in *UNC13A* had the strongest signal in both diseases. The following nine most significant SNPs were all in the *C9orf72* locus. Fig 3 shows a more visual representation of the results from both studies; associations present in both ALS and FTD strata clearly stand out from the disease-specific and non-significant associations.

Our study most likely includes a substantial number of *C9orf72* repeat expansion carriers, however, we are not able to retrieve repeat expansion status for all patients included. As a

proxy for *C9orf72* repeat expansion status, we adjusted association tests for rs3849943 genotype (the top SNP at the 9p21.2 locus). The association at *UNC13A* was not dependent on rs3849943 SNP genotype in ALS (OR 1.18, $p = 8.64 \times 10^{-9}$), FTD (OR 1.45, $p = 9.09 \times 10^{-6}$), and the ALS-FTD meta-analysis (OR 1.21, $p = 5.68 \times 10^{-12}$). Instead, the statistical significance of the association with *UNC13A* increased. Similarly, for the 8q24.13 locus (top SNP rs13268726), association results that were corrected for rs3849943 genotype in ALS (OR 0.80, $p = 6.04 \times 10^{-6}$), FTD (OR 0.71, $p = 0.029$), and the ALS-FTD meta-analysis (OR 0.79, $p = 6.47 \times 10^{-7}$) were very similar to the unadjusted results (Table 1). Furthermore, we performed a systematic, genome-wide conditional analysis to identify possible additional, independent association signals in our meta-analysis of ALS and FTD-TDP. The conditional analysis did not yield any additional independent association signals at the chromosome 9p21.2 locus, gene *UNC13A* and chromosome 8q24.13. For each locus, association signals were driven by the SNP with the lowest p value. Adjusted, joint p values, did not differ notably from the original p values, indeed indicating these three SNPs represent true independent signals (data not shown).

Analyses including only FTD-TDP cases without motor neuron symptoms

As noted previously, a substantial proportion of FTD-TDP patients also have motor neuron symptoms. In order to determine if association signals from the FTD-TDP cohort were not solely driven by patients with motor neuron symptoms, we repeated the ALS and FTD meta-analysis after removing all FTD-TDP patients with signs of motor neuron disease ($n = 99$) from the FTD stratum. Of course, statistical power was attenuated for the set of FTD-only patients. However, signals at *C9orf72* and *UNC13A* were still enhanced by adding the FTD-only cases into the meta-analysis (Fig 1). Also, the rank products analysis showed consistent ranking of the top 6 markers (Supplementary Table 4).

New signal on chromosome 8q24.13

In the combined ALS and FTD meta-analysis, there was one new signal on chromosome 8q24.13 with top SNPs rs13268726 (imputed, OR 0.79, $p = 3.91 \times 10^{-7}$), and rs12546767 (genotyped, OR 0.80, $p = 4.68 \times 10^{-7}$) and mapping to a region with strong LD comprising genes *SQLE*, *KIAA0196*, and *NSMCE2* (Fig 4). Both top SNPs are in strong LD ($r^2 = 0.95$, $D' = 0.975$). Additionally, in the analysis of FTD-TDP cases without motor neuron symptoms, we found consistent associations for rs13268726 (OR 0.69, $p = 0.029$) and rs12546767 (OR 0.67, $p = 0.022$) compared to the full FTD stratum (Table 3).

We followed up rs13268726 (in *SQLE*) and rs12546767 (in *KIAA0196*) in a replication set of 4,056 sporadic ALS patients and 3,958 controls. We replicated the associations between the two SNPs in this locus and ALS susceptibility, with the lowest p value for rs12546767 (OR 0.88, $p = 0.026$) in gene *KIAA0196* (Fig 2). Joint analysis of both genome-wide and replication data reached a p value of 1.01×10^{-7} for this locus, but did not reach genome-wide significance ($p < 5 \times 10^{-8}$). Detailed results are shown in Table 3.

Discussion

In the present study, we conducted a large genome-wide meta-analysis in a combined cohort of nearly 5,000 patients and over 14,000 controls. We aimed at finding new genetic variants that would affect susceptibility to both sporadic ALS and TDP-43 positive FTD. With three complementary methods, to avoid only picking up effects of the larger ALS cohort, we identified not only the known 9p.21 locus including *C9orf72*, but also the *UNC13A* locus as shared between both neurodegenerative diseases. By combining results from ALS and FTD datasets in a joint meta-analysis, we found a strong increase of association signals at both loci. Furthermore, by replication of top-associated SNPs from one disease in the other and vice versa, we found both the *C9orf72* and *UNC13A* loci to be significantly associated in the ALS as well as in the FTD analysis. We also showed that signals at both loci are not solely driven by the relatively large number of ALS patients. Furthermore, by repeating the meta-analysis selecting only FTD patients without motor neuron disease, we demonstrated that the signals from the meta-analysis are not unique to individuals with motor neuron symptoms in both groups. Lastly, we found a modest, but significant genetic correlation between ALS and FTD-TDP.

The observation that the *UNC13A* association signal is shared between ALS and FTD-TDP cohorts, highlights *UNC13A* not only as susceptibility gene in ALS, but also as a susceptibility factor for FTD-TDP, further corroborating the role of *UNC13A* in neuronal degeneration. Previously we have shown that rs12608932 acts as a modifier of survival in ALS, which was recently replicated in an Italian cohort of ALS patients.^{30,31} *UNC13A*, therefore, poses an interesting therapeutic target. The protein encoded by *UNC13A* is a member of a family of presynaptic proteins present throughout the nervous system and involved in the priming of presynaptic vesicles containing neurotransmitters before their release.³² Aberrant function of *UNC13A* disrupts the exocytosis of excitatory and inhibitory neurotransmitters. This not only affects the biochemical communication between neurons, but also triggers structural changes in existing neuronal networks.³² Furthermore, changes in the release of neurotransmitters as a consequence of defects in *UNC13A* are in line with the glutamate excitotoxicity hypothesis, previously implicated in motor neuron degeneration. Thus, our findings implicate changes in neurotransmitter release and synaptic function as a converging mechanism in the pathogenesis of ALS and FTD.³³

The highly significant signal generated by 19 SNPs at the chromosome 9p21.2 locus is most likely linked to *C9orf72* repeat expansion status. As we have *C9orf72* repeat expansion status available for only a few of the strata included in the present meta-analysis, we were not able to perform an analysis with *C9orf72* wild-type carriers only to search for a residual effect of this locus in ALS-FTD. As a proxy for *C9orf72* repeat expansion status, we conditioned on rs3849943 genotype and showed that the association signal at *UNC13A* increased. Moreover, it is interesting to note that *C9orf72* is structurally related to DENN RabGEFs and that Rabs cooperate with *UNC13A* to regulate synaptic functions.³⁴⁻³⁶ Therefore, these observations hint at the exciting possibility that defects in *UNC13A* and *C9orf72* in ALS and FTD converge upon the same synaptic mechanisms.

The present study identified one locus with increased disease association signal in the combined ALS and FTD meta-analysis on chromosome 8q24.13, nearing the genome-wide significance threshold. We successfully replicated disease association for the two top SNPs in this locus (rs13268726 and rs12546767) in an independent cohort of sporadic ALS patients. The 8q24.13 locus maps to a region of high LD encompassing *SQLE*, *KIAA0196* and *NSMCE2* (Fig 4). Of these genes *KIAA0196* appears to be of particular interest. First, mutations in *KIAA0196* cause hereditary spastic paraplegia, which shares clinical upper motor neuron dysfunction with ALS.³⁷ Second, *KIAA0196* (alias *SPG8*) encodes for strumpellin, a valosin-containing protein (VCP) binding partner in the human central nervous system.³⁸ Mutations in *VCP* have previously been identified in both ALS and FTD patients.¹³ Third, protein aggregates containing strumpellin have been found in patients with inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD).³⁸ Nevertheless, since the combined analysis of the discovery and replication samples did not show a genome-wide significant association ($p=1.01\times 10^{-7}$), further work is needed to establish the role of *KIAA0196* and other genes in the associated locus in ALS-FTD pathogenesis.

Our study is the largest GWAS including sporadic ALS and TDP-43 positive FTD patients in search of new loci for neurodegeneration. With over 4,300 ALS patients and over 14,000 controls the study was well powered to detect associations of common variants with modest effect size. For example, we estimated 97% power for the detection of an association similar to rs3849943 on chromosome 9p.21 at $\alpha = 5\times 10^{-8}$. In terms of sample sizes required for GWAS, the FTD-TDP cohort was relatively small, but unique due to the homogenous TDP-43 pathology. The relatively small increase in statistical power obtained by adding 435 TDP-43 positive FTD cases might provide an important explanation for why we did not identify new shared susceptibility loci with genome-wide significance.

Also, in order to replicate our findings in TDP-43 positive FTD cohorts, one would require a minimum of 1,000–8 1,950 TDP-43 pathology-proven FTD cases and controls to achieve 80% power for detecting an effect with OR 0.8 and 1.2 (with minor allele frequency 0.1 – 0.35 at $\alpha=0.05$), which is clearly challenging and requires further international collaborations. In addition, future studies implementing a combined analysis with large cohorts of patients with neurodegenerative disease including FTD, late-onset Alzheimer's disease or Parkinson's disease might add more statistical power and improve chances of finding new shared susceptibility loci for neurodegeneration.

For the FTD-TDP cohort, clinical information was available allowing us to identify patients with and without motor neuron signs, although we cannot definitively rule out the possibility that a small proportion of FTD cases classified as 'without motor neuron signs' still developed motor neuron disease after the last clinical follow-up. For the ALS strata, however, data on frontotemporal dementia or cognitive impairment in ALS patients were not available. Therefore, the extent to which signals from the meta-analysis are driven by signs of frontotemporal dementia or cognitive impairment is not known exactly, although previous studies have estimated a proportion of 5–10% of ALS patients also having FTD.^{1,2} Furthermore, previously, an association was reported of variants in *TMEM106B* with susceptibility to FTD and with cognitive impairment in ALS patients.^{18,19} In the present

meta-analysis we did find this locus in FTD, but we did not find a significant association of variants in *TMEM106B* with ALS. It is possible that an association with *TMEM106B* exists within a subset of ALS patients with cognitive impairment. Careful deep phenotyping of samples in future GWAS studies will help to shed light on the genetic determinants of motor neuron dysfunction versus cognitive impairment.

In conclusion, our meta-analysis identifies *UNC13A* as a novel link between ALS and TDP-43 positive FTD, which identifies synaptic defects as a shared disease mechanism and further corroborates the role of *UNC13A* and synaptic mechanisms in neuronal degeneration. Our results provide a novel starting point for further dissection of shared pathogenic pathways underlying ALS and FTD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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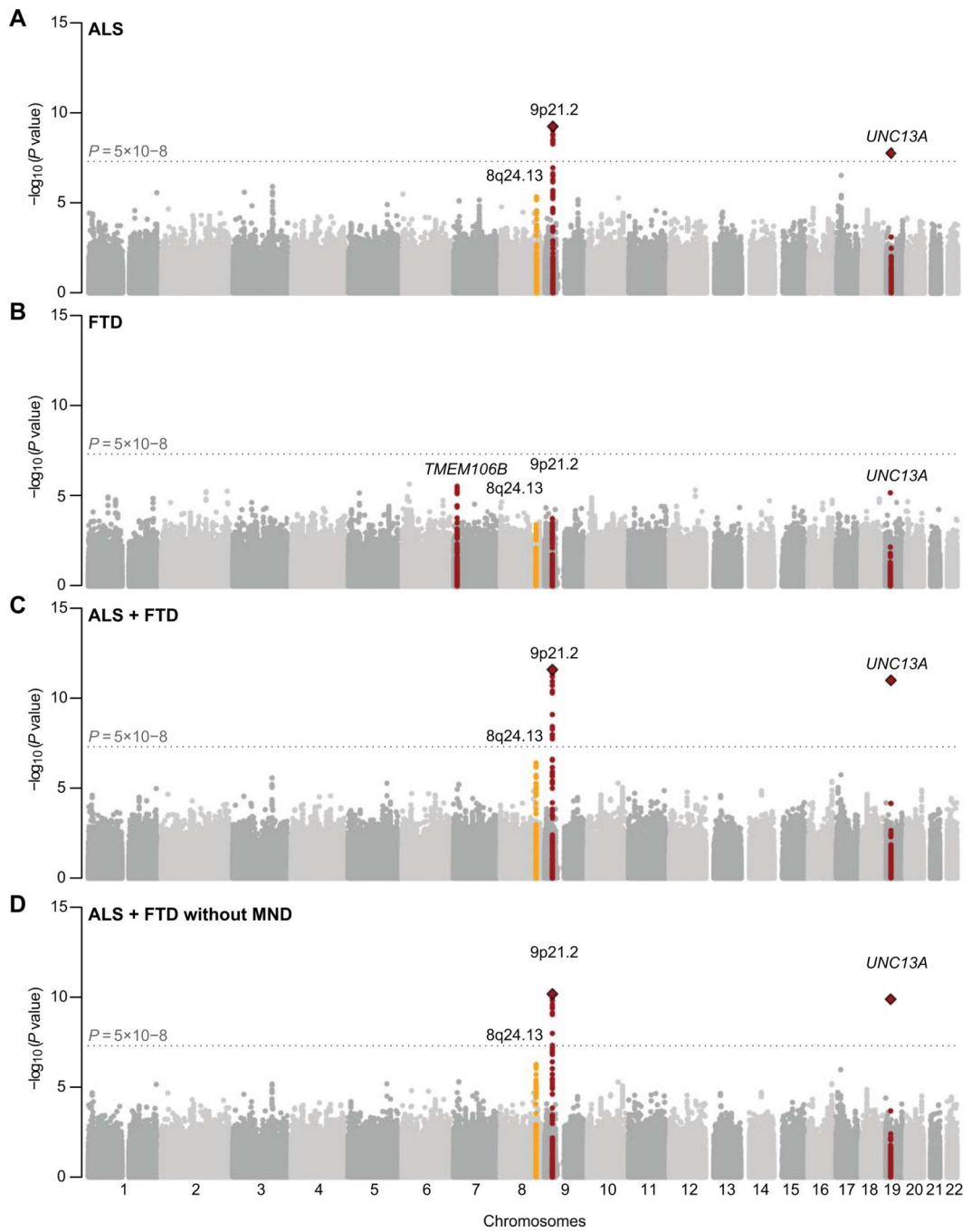


Figure 1.

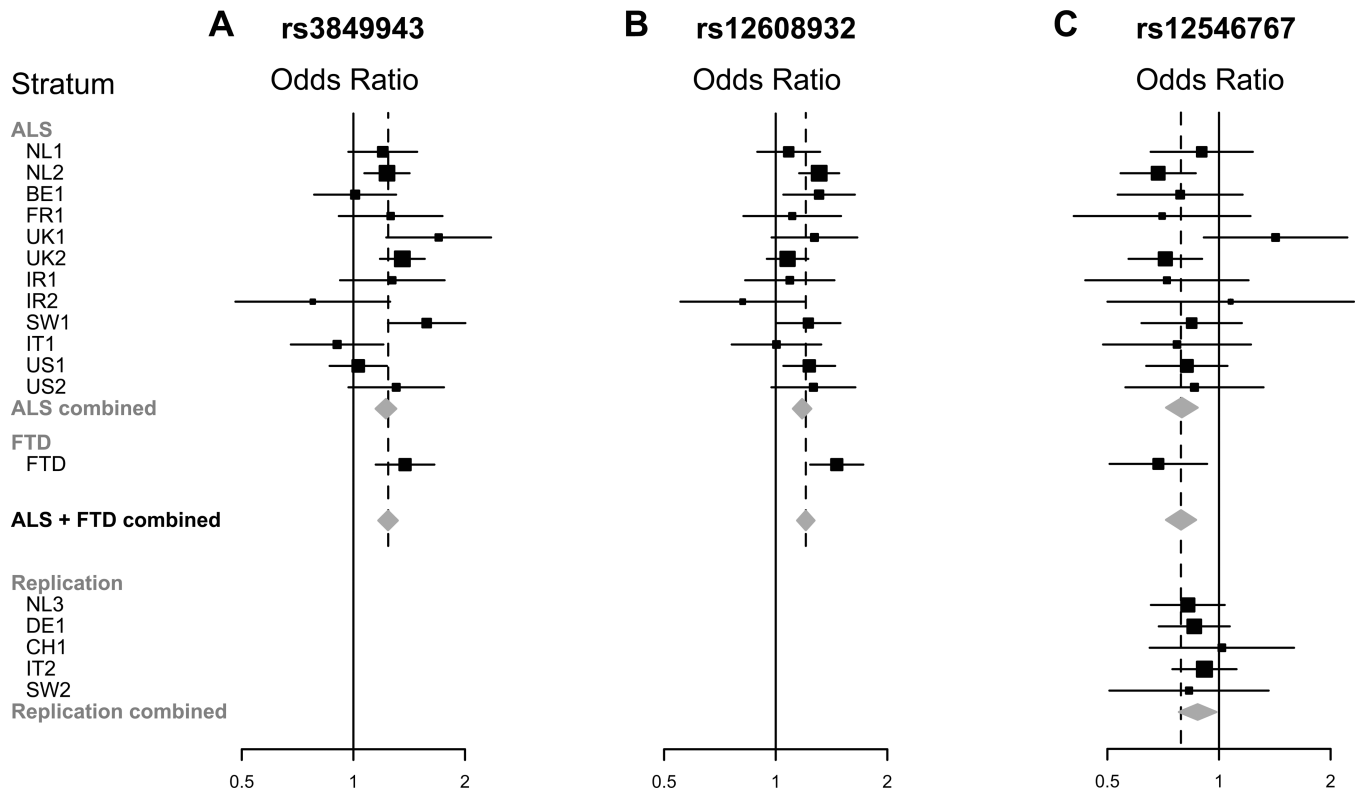


Figure 2.

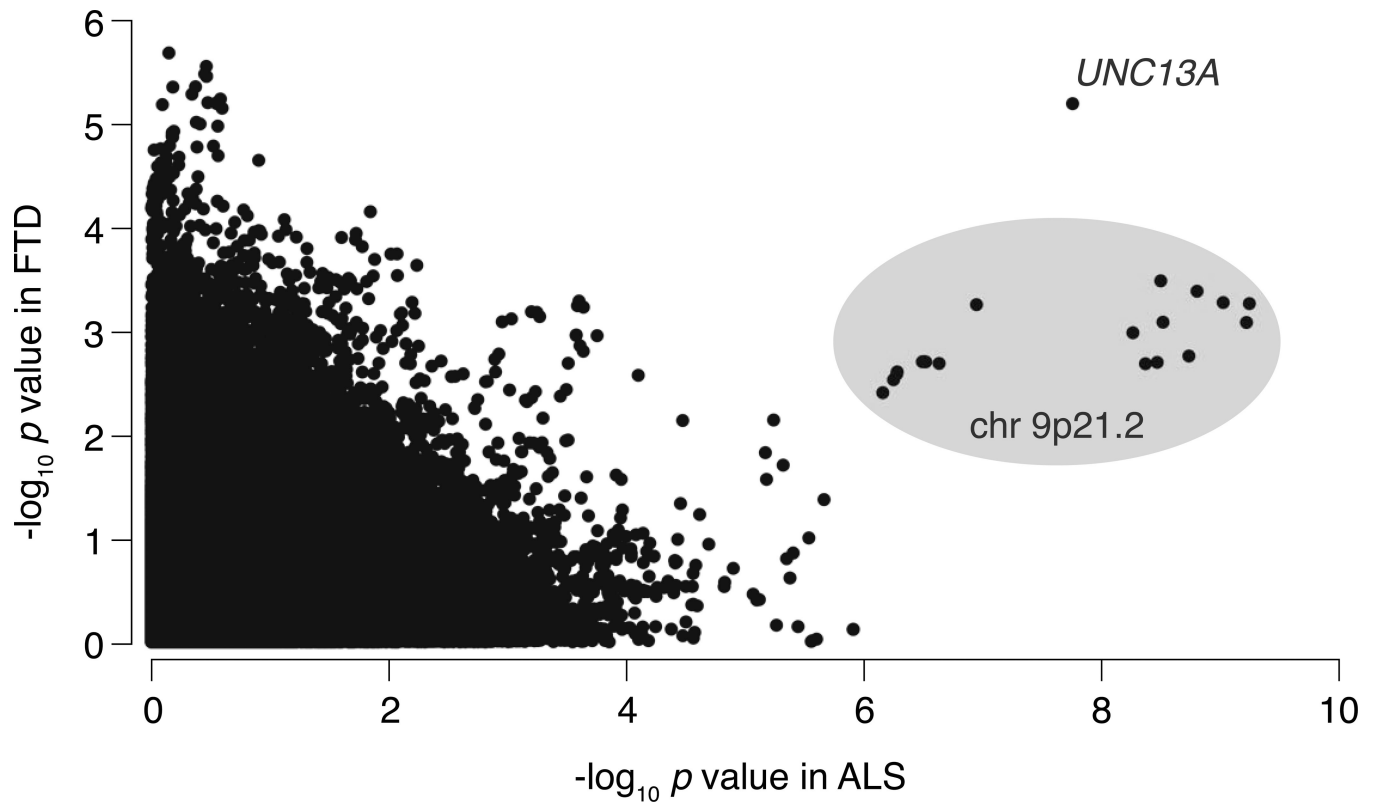


Figure 3.

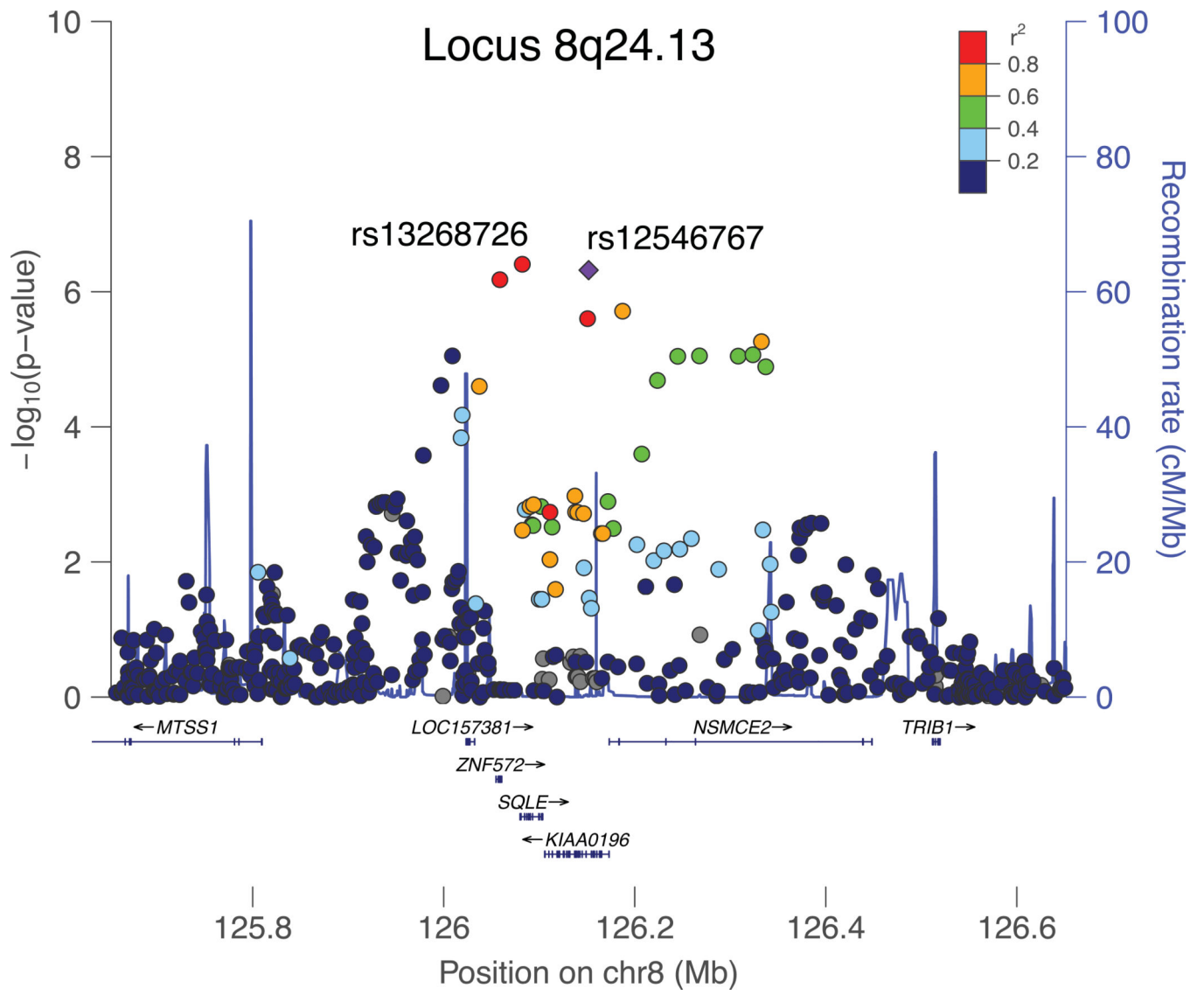


Figure 4.

TABLE 1

Top association results for independent loci containing SNPs, per disease and in the joint meta-analysis

Chr	Locus	n SNPs	Top SNP	Minor allele	MAF	ALS analysis		FTD analysis		Meta-analysis	
						OR	p	OR	p	OR	p
9	9p21.2	29	rs3849943	C	0.24	1.22	5.48×10 ⁻¹⁰	1.38	5.53×10 ⁻⁴	1.24	2.60×10 ⁻¹²
19	UNC13A	1	rs12608932	C	0.35	1.18	1.70×10 ⁻⁸	1.46	6.57×10 ⁻⁶	1.21	1.02×10 ⁻¹¹
8	8q24.13	11	rs13268726	G	0.10	0.80	4.69×10 ⁻⁶	0.70	0.020	0.79	3.91×10 ⁻⁷
17	CENPV	1	rs7477	A	0.49	1.16	2.91×10 ⁻⁷	0.98	0.785	1.14	1.82×10 ⁻⁶
3	TXNDC6	8	rs7638688	A	0.29	0.85	1.20×10 ⁻⁶	0.97	0.757	0.87	2.66×10 ⁻⁶
16	KIAA0513	3	rs16975170	T	0.12	1.17	2.27×10 ⁻⁴	1.53	5.99×10 ⁻⁴	1.20	4.20×10 ⁻⁶
5	FAM13B	1	rs9327807	C	0.18	0.85	1.24×10 ⁻⁵	0.87	0.196	0.85	5.25×10 ⁻⁶
7	7p21.1	2	rs10233425	C	0.01	1.88	7.46×10 ⁻⁶	1.44	0.391	1.83	6.15×10 ⁻⁶
10	BUB3	1	rs11248416	G	0.11	1.23	3.64×10 ⁻⁵	1.27	0.103	1.24	9.19×10 ⁻⁶
17	17p13.2	1	rs12950017	C	0.24	1.16	1.47×10 ⁻⁵	1.11	0.293	1.15	9.19×10 ⁻⁶

Per locus, the number of SNPs with $p < 1 \times 10^{-5}$ is indicated, and association results for the SNP with the most significant p value (top SNP) are presented. The ALS analysis was based on 4,377 sporadic ALS patients and 13,017 controls. The FTD analysis was based on 435 sporadic FTD-TDP cases and 1,414 population controls. The meta-analysis comprises a total of 4,811 patients with either ALS or FTD, and 14,428 controls. Chr = chromosome; MAF = weighted minor allele frequency across all datasets; OR = odds ratio.

TABLE 2

Replication of top SNPs from ALS analysis in FTD data and vice versa

Top SNPs from ALS replicated in FTD						
Chr	Locus	SNP	Minor allele	ALS analysis	FTD analysis	
				OR	p	p Bonferroni
19	<i>UNC13A</i>	rs12608932	C	1.18	1.70×10 ⁻⁸	4.93×10 ⁻⁴
9	9p21.2	rs2453554	T	1.21	3.06×10 ⁻⁹	0.025
9	9p21.2	rs700791	A	1.22	1.51×10 ⁻⁹	0.032
9	9p21.2	rs3849942	T	1.22	9.12×10 ⁻¹⁰	0.041
9	9p21.2	rs3849943	C	1.22	5.48×10 ⁻¹⁰	0.041
9	9p21.2	rs774359	C	1.18	1.09×10 ⁻⁷	0.042
Top SNPs from FTD replicated in ALS						
				FTD analysis	ALS analysis	
				OR	p	p Bonferroni
9	9p21.2	rs3849943	C	1.38	5.53×10 ⁻⁴	3.16×10 ⁻⁷
9	9p21.2	rs10967965	T	1.39	8.41×10 ⁻⁴	3.34×10 ⁻⁷
9	9p21.2	rs3849942	T	1.38	5.41×10 ⁻⁴	5.25×10 ⁻⁷
9	9p21.2	rs700791	A	1.39	4.21×10 ⁻⁴	8.71×10 ⁻⁷
9	9p21.2	rs17779457	G	1.36	8.35×10 ⁻⁴	1.69×10 ⁻⁶
9	9p21.2	rs2453554	T	1.39	3.35×10 ⁻⁴	1.76×10 ⁻⁶
19	<i>UNC13A</i>	rs12608932	C	1.46	6.57×10 ⁻⁶	9.77×10 ⁻⁶
9	9p21.2	rs774359	C	1.37	5.67×10 ⁻⁴	6.30×10 ⁻⁵
Top SNPs from ALS replicated in FTD without MND signs						
Chr	Locus	SNP	Minor allele	ALS analysis	FTD without MND signs analysis	
				OR	p	p Bonferroni
19	<i>UNC13A</i>	rs12608932	C	1.18	1.70×10 ⁻⁸	0.034

Top SNPs from FTD without MND signs replicated in ALS

		FTD without MND signs analysis		ALS analysis			
19	<i>UNC13A</i> rs12608932	C	1.39	4.52×10^{-4}	1.18	1.70×10^{-8}	8.63×10^{-6}

The upper part of the table shows SNPs with $p < 1 \times 10^{-4}$ in the ALS analysis and with $p < 0.05$ after Bonferroni correction for the number of independent loci ($n = 191$) tested in the FTD analysis. Conversely, the second part of the table shows SNPs with $p < 1 \times 10^{-3}$ in the FTD analysis and with $p < 0.05$ after Bonferroni correction for the number of independent loci ($n = 658$) tested in the ALS analysis. Subsequently, SNPs are shown with $p < 1 \times 10^{-4}$ in the ALS analysis, and with $p < 0.05$ after Bonferroni correction for the number of independent loci ($n = 191$) tested in the FTD-TDP without MND signs analysis. The lower part of the table shows SNPs with $p < 1 \times 10^{-3}$ in the FTD-TDP without MND signs analysis and with $p < 0.05$ after Bonferroni correction for the number of independent loci ($n = 1,374$) tested in the ALS analysis. Results are sorted by increasing Bonferroni-corrected p value. Chr = chromosome; OR = odds ratio; MND = motor neuron disease.

TABLE 3

Association results for top SNPs at the chromosome 8q24.13 locus

SNP	Gene	Minor allele	MAF	ALS analysis		FTD analysis		ALS + FTD meta-analysis		Replication ALS		Meta-analysis + replication combined	
				OR	p	OR	p	OR	p	OR	p	OR	p
rs13268726	<i>SQLE</i>	G	0.10	0.80	4.69×10^{-6}	0.79	0.020	0.79	3.91×10^{-7}	0.89	0.044	0.82	1.85×10^{-7}
rs12546767	<i>KIAA0196</i>	C	0.10	0.80	6.63×10^{-6}	0.69	0.015	0.79	4.78×10^{-7}	0.88	0.026	0.82	1.01×10^{-7}

Association results are shown for a weighted inverse-variance meta-analysis of 12 sporadic ALS cohorts (4377 ALS cases, 13017 controls), for logistic regression analysis in the FTD-TDP cohort (435 FTD-TDP cases, 1414 controls), for the combined ALS-FTD meta-analysis (4811 cases, 14428 controls), and for an independent replication cohort of sporadic ALS (4056 ALS, 3958 controls). In the righthand column, results for a combined analysis of both the ALS-FTD meta-analysis data and replication cohort are shown (8867 cases, 18386 controls). MAF = weighted minor allele frequency across all datasets; OR = odds ratio.