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Genome-wide Analyses Identify KIF5A as a Novel ALS Gene

Highlights

- Loss-of-function mutations in *KIF5A* are a cause of amyotrophic lateral sclerosis
- ALS-associated *KIF5A* mutations are distinct from HSP and CMT mutations in *KIF5A*
- Identification of *KIF5A* highlights the role of cytoskeleton in ALS pathogenesis

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In Brief

Using a large-scale genome-wide association study and exome sequencing, we identified *KIF5A* as a novel gene associated with ALS. Our data broaden the phenotype resulting from mutations in *KIF5A* and highlight the importance of cytoskeletal defects in the pathogenesis of ALS.

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Genome-wide Analyses Identify KIF5A as a Novel ALS Gene

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SUMMARY

To identify novel genes associated with ALS, we undertook two lines of investigation. We carried out a genome-wide association study comparing 20,806 ALS cases and 59,804 controls. Independently, we performed a rare variant burden analysis comparing 1,138 index familial ALS cases and 19,494 controls. Through both approaches, we identified *kinesin family member 5A* (*KIF5A*) as a novel gene associated with ALS. Interestingly, mutations predominantly in the N-terminal motor domain of KIF5A are causative for two neurodegenerative diseases: hereditary spastic paraplegia (SPG10) and Charcot-Marie-Tooth type 2 (CMT2). In contrast, ALS-associated mutations are primarily located at the C-terminal cargo-binding tail domain and patients harboring loss-of-function mutations displayed an extended survival relative to typical ALS cases. Taken together, these results broaden the phenotype spectrum resulting from mutations in *KIF5A* and strengthen the role of cytoskeletal defects in the pathogenesis of ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS; OMIM: 05400) is a neurodegenerative disorder clinically characterized by rapidly progressive muscle weakness and death due to respiratory failure, typically within 2 to 4 years of symptom onset (van Es et al., 2017). Although ALS is perceived as being rare, approximately 6,000 Americans die annually from the condition

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(Hirtz et al., 2007). Furthermore, the number of ALS cases across the globe will increase to nearly 400,000 in 2040, predominantly due to aging of the population (Arthur et al., 2016). This increase is anticipated to place an enormous socioeconomic burden on global healthcare systems, in particular because the annual healthcare cost per patient with ALS is among the highest for any neurological disease (Gladman and Zinman, 2015).

Approximately 10% of ALS cases display a family history (FALS), whereas the remaining 90% of ALS cases are sporadic (SALS) in nature. Driven in large part by advances in genotyping and sequencing technology, the genetic etiology of two-thirds of FALS cases and about 10% of SALS cases is now known (Chia et al., 2018; Renton et al., 2014). Mutations in *SOD1* were the first identified cause of ALS (Rosen et al., 1993), contributing to ~20% of FALS and ~2% of SALS. More recently, pathogenic

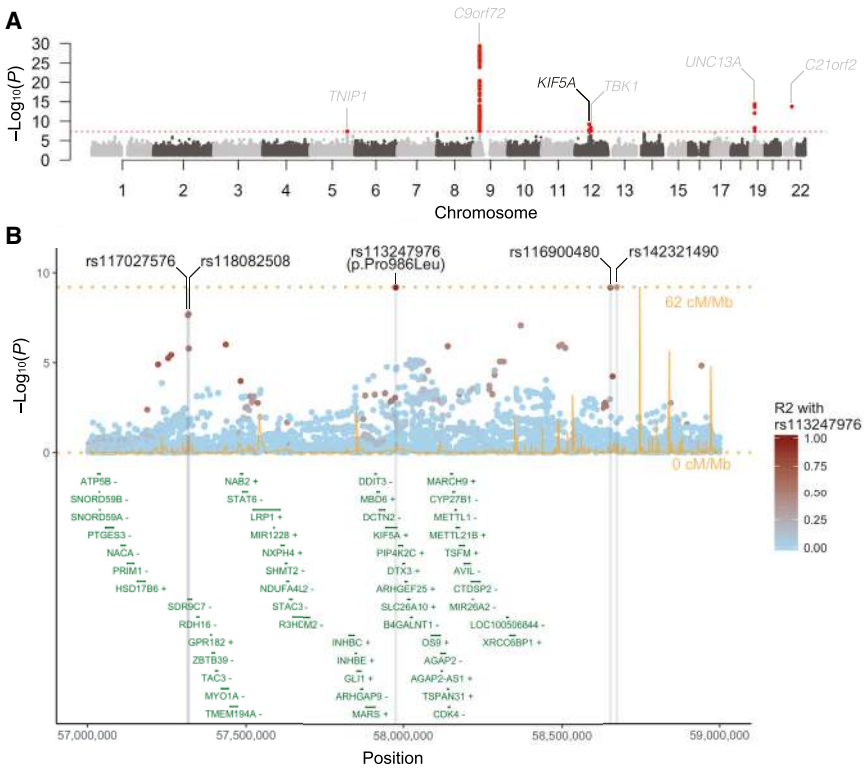

Figure 1. Identification of Association between *KIF5A* Locus and ALS Risk through GWAS

(A) Manhattan plot showing p values from the discovery set GWAS. Analysis of a combined set of 20,806 cases and 59,804 controls is shown. The dashed red line denotes the threshold for genome-wide significance after multiple test correction ($p < 5.0 \times 10^{-8}$). Five previously reported ALS-associated loci are labeled in gray and one novel loci, containing the *KIF5A* gene, is labeled in black. (B) Regional association plot of the *KIF5A* locus. Recombination rates are from HapMap phase 2 European ancestry samples. The R^2 pattern is based on the rs113247976 SNP using 85 European ancestry samples (CEU) from the November 2010 release of the 1000 Genomes Project dataset. R^2 of the p.Pro986Leu (rs113247976) with additional SNPs achieving genome-wide significance was 0.544 (rs117027576), 0.544 (rs118082508), 0.741 (rs116900480), and 0.347 (rs142321490).

hexanucleotide repeat expansions located within the first intron of the *C9orf72* gene on chromosome 9p21 were identified as the most common cause of both FALS (~40%) and SALS (~7%) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Interestingly, this repeat expansion contributes to ~10% of all frontotemporal dementia (FTD) cases, thus genetically explaining much of the overlap between these clinical syndromes (Majounie et al., 2012). As a result of these major discoveries, there are several ongoing efforts toward directed silencing of these mutant genes, which could result in a therapeutic treatment for up to 10% of all ALS cases and for a similar portion of FTD cases.

In addition to the insights provided by each novel ALS gene, the collective knowledge gained from genetic factors provides a more comprehensive understanding of the interacting pathways underlying motor neuron degeneration. For example, the identification of ALS genes has revealed at least three pathways believed to contribute to the development of ALS: (1) RNA metabolism (based on the observation of mutations in *C9orf72*, *TDP-43*, *FUS*, *HNRNPA1*, and *MATR3*), (2) protein homeostasis (*UBQLN2*, *VCP*, *OPTN*, and *VAPB*), and (3) cytoskeletal dynamics (*PFN1*, *TUBA4A*, and *DCTN1*) (Chia et al., 2018; Robberecht and Eykens, 2015; Taylor et al., 2016). Understanding the mechanisms leading to disease pathogenesis again provides targets for therapeutic intervention that may be applicable to all forms of ALS.

Due to the decreased accessibility of multiple affected family members with unknown genetic etiology, there has been an increased focus on the identification of ALS-associated genes with moderate to low impact. Despite their lower effect, such genes continue to provide valuable insight into ALS pathogenesis. For example, the product of the risk factor *TBK1* is known to interact with the product of ALS-associated gene *OPTN*, further solidifying the role of autophagy and protein homeostasis in disease development (Cirulli et al., 2015; Freischmidt et al., 2015; Maruyama et al., 2010; Morton et al., 2008). Similarly, the risk factor *NEK1*, identified through a rare variant burden analysis (RVB) of index FALS (i.e., one affected sample per family), is a known binding partner of *C21orf2*, an ALS risk factor found through genome-wide association studies (GWASs) (Cirulli et al., 2015; Kenna et al., 2016; Malovannaya et al., 2011; van Rheenen et al., 2016). The interaction of these two proteins is required for efficient DNA damage repair (Fang et al., 2015), a pathway that is becoming increasingly implicated as a contributing factor in ALS and other neurodegenerative diseases (Coppedè and Migliore, 2015; Lopez-Gonzalez et al., 2016; Madabhushi et al., 2014; Wang et al., 2013).

RESULTS

GWASs Identify *KIF5A* as a Novel ALS-Associated Gene

To identify new susceptibility loci operating in ALS, we undertook a large-scale GWAS involving 12,663 patients diagnosed with ALS and 53,439 control subjects (Tables S1 and S2). Our data were then incorporated into a meta-analysis with a recently published GWAS involving 12,577 ALS cases and 23,475 control subjects (van Rheenen et al., 2016). After imputation and quality-control measures (see STAR Methods and Figure S1 for the workflow and Figure S2 for the multidimensional scaling plot), 10,031,630 genotyped and imputed variants from 20,806 ALS cases and 59,804 control samples were available for association analysis (Figure 1A). Quantile-quantile plots did not show evidence of significant population stratification (λ_{1000} = 1.001;

Figure S3). SNPs achieving genome-wide significance ($p < 5.0 \times 10^{-8}$) are listed in Tables 1 and S3 and suggestive loci with SNPs associated at $p < 5.0 \times 10^{-7}$ are listed in Table S4.

Our analysis revealed five previously identified loci that achieved genome-wide significance (loci including *TNIP1*, *C9orf72*, *TBK1*, *UNC13A*, and *C21orf2*) (Benyamin et al., 2017; Laaksovirta et al., 2010; Shatunov et al., 2010; van Es et al., 2009; van Rheenen et al., 2016). In addition, we observed a strong association signal for five SNPs in linkage disequilibrium on chromosome 12q14.1 that reached genome-wide statistical significance spanning a region of several hundred kilobases (Table 1; Figure 1B). Of the five SNPs, two of them resided in close proximity to each other within a large intergenic region and two in proximity to *short-chain dehydrogenase/reductase family 9C member 7* (*SDR9C7*), a gene expressed primarily in skin. However, one SNP (rs113247976) results in a p.Pro986Leu coding change within the *kinesin family member 5A* (*KIF5A*) gene ($p = 6.4 \times 10^{-10}$, odds ratio [OR] = 1.38, 95% CI = 1.24–1.53; Figure 2). The case:control allele frequencies for the combined discovery cohort were 2.07%:1.55% and genotype counts were 5: 529: 12,043 to 7: 786: 22,682 (homozygotes alternative allele: heterozygotes: homozygous reference allele; Figure 2). Calculations based on our cohort size as well as the OR and allele frequency of rs113247976 result in an ~99.5% power to detect this as an ALS-associated SNP.

Rare Variant Burden Analysis Identifies *KIF5A* as an ALS Gene

In an independent line of investigation, we attempted to identify novel ALS genes through exome-wide RVB. In brief, RVB compares the frequency of variants within each gene below a user-defined frequency threshold in a case-control cohort. As the last two ALS-associated genes identified by this methodology (*TBK1* and *NEK1*) displayed an increased frequency of loss-of-function (LOF) variants, we focused our initial analysis on such variants (consisting of nonsense and predicted splice altering) (Cirulli et al., 2015; Freischmidt et al., 2015; Kenna et al., 2016).

Toward this end, we performed RVB testing for association of LOF variants in a cohort of 1,138 index FALS cases and 19,494 controls, after applying quality-control filters (Experimental Model and Subject Details; Figure S4; Tables S5 and S6). Genes displaying $p < 5.0 \times 10^{-4}$ are shown in Table 2. The previously identified ALS genes, *TBK1* ($p = 5.58 \times 10^{-7}$, OR = 15.11, 95% CI = 5.81–38.69) and *NEK1* ($p = 1.68 \times 10^{-6}$, OR = 6.64, 95% CI = 3.32–12.51), yielded strong associations with ALS, reaching exome-wide significance (Figure 3). In addition, we observed a single novel gene reaching exome-wide significance, *KIF5A* ($p = 5.55 \times 10^{-7}$; OR = 32.07, 95% CI = 9.05–135.27). Within this gene, we observed 6 LOF variants in our 1,138 cases (0.53%) compared to 3 such variants in our comparison cohort of 19,494 controls (0.015%; Table 2). There was no evidence of genomic inflation ($\lambda = 0.93$; Figure S5), sequencing center or other sub-cohort bias (Figure S6), or call rate bias (Figure S7) in our analysis. Of the index FALS cases carrying *KIF5A* LOF mutations, we obtained DNA from two siblings of the proband carrying a c.2993-3C>T, exon 27 - 5′ splice junction variant and from a sibling of a different proband carrying a c.3020+2T>A, exon 27 - 3′ splice junction variant. These variants segregated with disease within each of these families. Sanger sequencing validated all identified LOF variants containing samples in the discovery set and affected relatives.

Interestingly, when we investigated the location of the six ALS-associated variants present in *KIF5A*, all occurred within a 34 bp stretch of DNA and were predicted to affect splicing of exon 27, which encodes amino acids 998–1007 (Table 3; Figure 4A). Five of the six variants were located on sequential base pairs on the 3′ end of the exon, whereas one was located on the 5′ end of the exon. We used the application ASSEDA (Automated Splice Site and Exon Definition Analyses) to predict any mutant mRNA splice isoforms resulting from these variants (Tompson et al., 2007). This algorithm was chosen as it is known to have high performance in splice prediction (Caminsky et al., 2014). ASSEDA predicted a complete skipping of exon 27 for all variants, yielding a transcript with a frameshift at coding amino acid 998, the deletion of the normal C-terminal 34 amino acids of the cargo-binding domain, and the extension of an aberrant 39 amino acids to the C terminus (Table 3; Figures 4B and 4C). The presence of transcripts with skipped exon 27 was demonstrated by performing RT-PCR in two patients carrying exon 27 - 3′ splice junction variants (c.3020+2T>A and c.3020+1G>A) using RNA from lymphoblasts and peripheral blood mononuclear cells, respectively. This splice form was not detected in four control lines (Figure 4D). Sequence analysis of the smaller RT-PCR products obtained from the patient cells confirmed the exon 26–28 splicing event. Material for RT-PCR was not available for any other patient carrying a *KIF5A* LOF variant.

Our initial RVB was restricted to single nucleotide variants due to the limited sensitivity and comparatively high false-positive rates associated with identifying small insertions and deletions (indels) within exome sequencing data (Fang et al., 2014). Based on our discovery of increased LOF variants within *KIF5A*, we re-evaluated this region for the presence of indels. Our analysis revealed two (0.026%) indels within our cohort of 1,138 FALS cases, compared to zero (0%) indels among 19,494 control samples. Both of these indels (p.Asp996fs and p.Asn999fs) resulted in a frameshift of the KIF5A protein coding sequence, and were located close to the splice junction variants that we previously observed to cause skipping of exon 27, resulting in a frameshift at amino acid 998 (Table 3). Sanger sequencing confirmed the presence of both indels. Combining the results of the single nucleotide and indel variant analysis yielded a highly significant p of 3.8×10^{-9} (OR = 41.16, 95% CI = 12.61–167.57). We failed to detect any signals of RVB association for rare missense variants across *KIF5A* or within any sub-domain of the gene (Table S8).

Replication Analysis of rs113247976 and LOF Variants in *KIF5A*

Given the strong signal of the missense variant identified by our GWAS (p.Pro986Leu, rs113247976) and its close proximity to the LOF variants identified by our RVB (amino acids 996–999), we attempted to replicate its association with ALS by analyzing additional cohorts. To accomplish this, we evaluated this variant in a cohort of 4,159 ALS cases and 18,650 controls that were non-overlapping with our GWAS discovery analysis

Table 1. SNPs Achieving Genome-wide Significance in the Discovery GWAS

SNP Information				Present Study (8,229 Cases/36,329 Controls)				Van Rheenen et al. (12,577 Cases/23,475 Controls)				Combined Discovery Set (20,806 Cases/59,804 Controls)			
SNP	Chr	Position	Gene	Case MAF	Control MAF	OR [95% CI]	p	Case MAF	Control MAF	OR [95% CI]	p	Case MAF	Control MAF	OR [95% CI]	p
Novel Loci															
rs117027576	12	57,316,603	*KIF5A*	1.55%	1.27%	1.45 [1.20–1.76]	1.1×10^{-4}	1.98%	1.59%	1.33 [1.16–1.53]	4.3×10^{-5}	1.81%	1.40%	1.37 [1.23–1.54]	2.3×10^{-8}
rs118082508	12	57,318,819	*KIF5A*	1.56%	1.28%	1.45 [1.20–1.76]	1.0×10^{-4}	1.98%	1.60%	1.33 [1.16–1.53]	3.8×10^{-5}	1.81%	1.41%	1.37 [1.23–1.54]	2.0×10^{-8}
rs113247976*	12	57,975,700	*KIF5A*	1.83%	1.42%	1.46 [1.23–1.74]	9.2×10^{-6}	2.14%	1.70%	1.33 [1.17–1.52]	1.1×10^{-5}	2.02%	1.53%	1.38 [1.24–1.53]	6.4×10^{-10}
rs116900480	12	58,656,105	*KIF5A*	1.75%	1.46%	1.42 [1.21–1.68]	1.9×10^{-5}	2.08%	1.66%	1.34 [1.18–1.53]	7.1×10^{-6}	1.95%	1.54%	1.37 [1.24–1.52]	6.6×10^{-10}
rs142321490	12	58,676,132	*KIF5A*	1.79%	1.48%	1.43 [1.21–1.68]	1.5×10^{-5}	2.08%	1.66%	1.34 [1.18–1.53]	8.0×10^{-6}	1.97%	1.55%	1.37 [1.24–1.52]	6.1×10^{-10}
Previously Published Loci															
rs10463311	5	150,410,835	*TNIP1*	73.19%	74.84%	0.94 [0.89–0.98]	7.8×10^{-3}	73.34%	75.79%	0.91 [0.87–0.94]	8.5×10^{-7}	73.28%	75.21%	0.92 [0.89–0.95]	4.0×10^{-8}
rs3849943	9	27,543,382	*C9orf72*	71.79%	76.31%	0.84 [0.80–0.88]	1.4×10^{-12}	72.78%	76.5%	0.83 [0.80–0.87]	4.0×10^{-19}	72.39%	76.38%	0.84 [0.81–0.86]	3.8×10^{-30}
rs74654358	12	64,881,967	*TBK1*	3.77%	4.01%	1.20 [1.07–1.34]	1.6×10^{-3}	5.12%	4.61%	1.23 [1.13–1.34]	7.7×10^{-7}	4.59%	4.25%	1.22 [1.14–1.30]	4.7×10^{-9}
rs12973192	19	17,753,239	*UNC13A*	67.62%	69.37%	0.86 [0.82–0.91]	1.3×10^{-8}	64.52%	66.00%	0.9 [0.87–0.93]	2.4×10^{-8}	65.75%	68.05%	0.89 [0.86–0.91]	3.9×10^{-15}
rs75087725	21	45,753,117	*C21orf2*	0.70%	0.46%	1.99 [1.44–2.75]	2.2×10^{-5}	1.83%	1.27%	1.61 [1.39–1.87]	8.7×10^{-11}	1.38%	0.78%	1.67 [1.46–1.91]	1.8×10^{-14}

Position is based on Human Genome Assembly build 37. Nearest gene or previously published gene names are included. Chr, chromosome; MAF, minor allele frequency; OR, odds ratio; 95% CI, confidence interval; *rs113247976 represents the p.Pro986Leu variant in *KIF5A* (GenBank: NM_004984.2).

Cohort	Cases	Controls	Case MAF	Control MAF	OR (95% CI)	P
Italy	2,853	2,143	1.28%	0.82%	1.45 (0.90 - 2.34)	
United Kingdom	449	226	3.56%	1.33%	2.07 (0.85 - 5.07)	
Belgium / France	1,150	595	1.91%	1.26%	1.42 (0.75 - 2.68)	
USA	3,777	33,365	2.01%	1.46%	1.45 (1.19 - 1.75)	
Present Study	**8,229**	**36,329**	**1.83%**	**1.42%**	**1.46 (1.24 - 1.73)**	**9.23×10^{-6}**
Van Rheenen Study	12,577	23,475	2.23%	1.75%	1.33 (1.17 - 1.52)	1.13×10^{-5}
Combined Discovery Set	**20,806**	**59,804**	**2.07%**	**1.55%**	**1.38 (1.25 - 1.53)**	**6.43×10^{-10}**
Replication	**4,159**	**18,650**	**1.78%**	**1.32%**	**1.42 (1.17 - 1.70)**	**3.82×10^{-4}**
Joint	**24,965**	**78,454**	**2.02%**	**1.49%**	**1.39 (1.33 - 1.45)**	**7.09×10^{-13}**

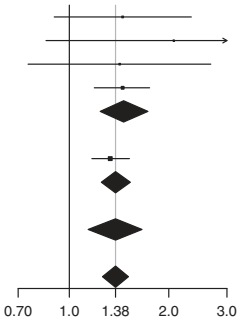

Figure 2. Discovery and Replication of the Association of the *KIF5A* p.Pro986Leu (rs113247976) Variant with ALS

Analysis of the p.Pro986Leu (rs113247976) variant within each of the described cohorts is shown. Allelic association for all subcohorts was analyzed by logistic regression followed by a fixed-effects meta-analysis. The Forest plot (right) displays the distribution of OR estimates across study cohorts with the vertical gray line denoting the OR estimated under the meta-analysis, and with the width of the horizontal lines and the diamonds corresponding to 95% confidence intervals.

(STAR Methods; Figure S8). This included non-overlapping samples from our RVB (673 FALS and 17,696 controls). Analysis of the cohort revealed an allele frequency of 1.78% in cases and 1.32% in controls (rs113247976, $p = 3.82 \times 10^{-4}$, OR = 1.42, 95% CI = 1.17–1.70), thereby replicating the association of the original GWAS. A meta-analysis of the GWAS and replication cohort (n = 24,965 cases and 78,454 controls) yielded a highly significant p of 7.09×10^{-13} (OR = 1.39, 95% CI = 1.33–1.45; Figure 2). These results support the association of *KIF5A* p.Pro986Leu with ALS. However, at this point we cannot definitely state that the missense variant is the primary risk factor, as we cannot rule out other variants in linkage disequilibrium.

We next performed mutational screening of *KIF5A* in an additional cohort of 9,046 ALS cases that had not been included in our original RVB. This revealed three additional carriers of C-terminal variants. One sporadic patient harbored an exon 26 frameshift mutation (p.Asn997fs) and a second sporadic patient harbored an exon 27 splice-altering mutation (c.2993-1G>A; Table 3). The third patient carried a p.Arg1007Lys (c.3020G>A) mutation and had a familial history of ALS. This mutation was also observed in FALS patients from our RVB; however, a comparison of 240,715 common variant sites between the two patients failed to reveal a familial relationship (Experimental Model and Subject Details). Additionally, one patient was observed to carry a predicted splice-altering variant proximal to exon 3 (c.291+5G>A). However, this variant was not supported as creating an aberrant transcript by ASSEDA. The cohort used for this analysis was comprised mainly of sporadic ALS cases. LOF variants were not observed in a follow-up panel of 1,955 controls. Comparison of the LOF variants in sporadic patients (2/9,046 cases, 0.022%) with either the 1,955 replication controls or all controls analyzed in this study (21,449 controls) yielded insignificant p values (0.868 and 0.423, respectively). Interestingly, the frequency of LOF variants in sporadic cases is lower than that observed in our original FALS cohort (0.703%), suggesting that *KIF5A* LOF variants display a high penetrance. Furthermore, the rate of LOF variants reported in the Exome Aggregation Consortium (ExAC) database is lower than we observed in the control samples used in our discovery cohort (0.007% versus 0.015%).

ALS-Associated Mutations in *KIF5A* Are Distinct from SPG10/CMT2 Mutations

Missense mutations within *KIF5A* are a known cause of hereditary spastic paraparesis (spastic paraplegia type 10, autosomal dominant; OMIM: 604187) and of Charcot-Marie-Tooth disease type 2 (CMT2) (Crimella et al., 2012; Jennings et al., 2017; Liu et al., 2014; Reid et al., 2002). Although SPG10 and CMT2 share

Table 2. Top ALS Associations Identified through RVB of FALS and Control Exome Sequencing Results

Gene	FALS	Control	OR	p
KIF5A	6 (0.53%)	3 (0.02%)	32.07 (9.05–135.27)	5.55×10^{-7}
TBK1	8 (0.70%)	9 (0.05%)	15.11 (5.81–38.69)	5.58×10^{-7}
NEK1	12 (1.05%)	32 (0.16%)	6.64 (3.32–12.51)	1.68×10^{-6}
CALHM2	7 (0.62%)	9 (0.05%)	12.13 (4.47–31.79)	9.19×10^{-6}
COL14A1	8 (0.70%)	16 (0.08%)	8.04 (3.32–18.08)	2.72×10^{-5}
AK1	10 (0.88%)	34 (0.17%)	5.37 (2.55–10.41)	5.62×10^{-5}
ATRN	5 (0.44%)	9 (0.05%)	11.06 (3.57–31.02)	1.66×10^{-4}
VLDLR	5 (0.44%)	9 (0.05%)	10.87 (3.51–30.43)	1.79×10^{-4}
FUS	4 (0.35%)	4 (0.02%)	16.53 (4.25–64.33)	2.08×10^{-4}
ZMYND12	6 (0.53%)	12 (0.06%)	7.92 (2.86–19.96)	2.61×10^{-4}

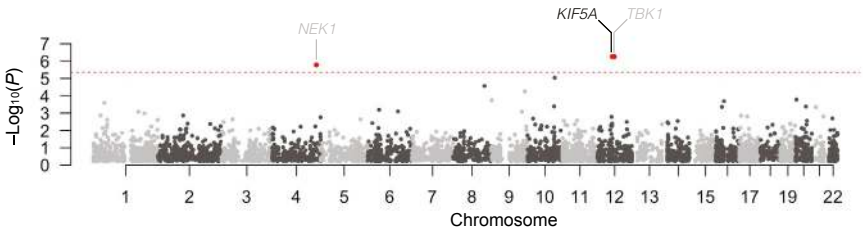

Figure 3. Identification of Association between *KIF5A* and ALS Risk through Rare Variant Burden Analysis of Exome Sequencing

Manhattan plot showing gene-level p values from an exome-wide rare variant burden analysis. Analyses of 1,138 index FALS cases versus 19,494 controls were restricted to rare LOF variants (splice altering/nonsense, MAF < 0.001). A minimum of three LOF variant carriers were required for analysis. The dashed red line denotes the threshold for exome-wide significance after correction for 11,472 genes (4.36×10^{-6}). Previously reported (gray) and novel (black) genes exhibiting a significant excess of rare LOF variants in patients are shown.

clinical features with ALS, a careful examination of the clinical records of the ALS cases with LOF mutations in *KIF5A* ruled out misdiagnosis. Furthermore, we detected no variants previously associated with SPG10 or CMT2 in our FALS cohort (Liu et al., 2014).

To further elucidate genotype-phenotype relationships, we evaluated the location of mutations within *KIF5A*. Interestingly, mutations contributing to SPG10 and to CMT2 are almost exclusively missense changes and are located in the N-terminal motor domain (amino acids 9–327) of KIF5A (Figure 5). In contrast, the mutations identified as contributing to ALS are found predominantly in the C-terminal cargo-binding region of KIF5A (amino acids 907–1032) with the highly penetrant FALS mutations showing LOF. These results indicate that the functional domain mutated in KIF5A dictates the clinical phenotype, resulting in distinct yet overlapping neurodegenerative diseases.

Patients with *KIF5A* LOF Mutations Display Younger Age at Onset and Longer Survival

To establish the existence of any commonalities between patients with LOF mutations in the C-terminal region of KIF5A, we evaluated their clinical phenotype. Cases with LOF mutations exhibited a median age of onset at 46.5 years (n = 19; Table S7). This is lower than the age of onset reported for ALS in epidemiological studies (65.2 years, interquartile range 56.0–72.2) (Ahmeti et al., 2013). Interestingly, we also observed an increased disease duration (survival) in patients harboring these LOF mutations. The median survival time of ALS patients is 20–36 months (Ahmeti et al., 2013). In contrast, cases with LOF mutations exhibited a median survival of nearly 10 years (117 months, n = 17; Table S7). ALS patients with symptom onset before 40 years of age have been shown to have longer survival, often exceeding 10 years (Chiò et al., 2009). In contrast,

Table 3. LOF Variants within *KIF5A* Identified in Probands

Position	Variant	Exon	cDNA	Description	Predicted Exon Skipping	Gender	Age of Onset (Years)	Site of Onset	Survival (Months)	Alive (Yes/No)
Control Variants										
57,963,470	A>G	11	c.1117+4A>G	3′ splice junction	P	M	n/a	n/a	n/a	n/a
57,966,423	C>T	15	c.1630C>T	p.Arg544*	–	F	n/a	n/a	n/a	n/a
57,976,884	G>C	28	c.3021G>C	5′ splice junction	N	F	n/a	n/a	n/a	n/a
FALS Variants										
57,975,729	GA>A	26	c.2987delA	p.Asp996fs	–	M	45	n/a	n/a	n/a
57,976,382	C>T	27	c.2993-3C>T	5′ splice junction	Y	M	29	L	>264	Y
57,976,385	GA>G	27	c.2996delA	p.Asn999fs	–	M	42	L	>12	Y
57,976,411	A>G	27	c.3019A>G	p.Arg1007Gly	Y	F	53	L	45	N
57,976,412	G>A	27	c.3020G>A	p.Arg1007Lys	Y	M	50	L	>108	Y
57,976,412	G>A	27	c.3020G>A	p.Arg1007Lys	Y	F	50	n/a	>240	Y
57,976,413	G>A	27	c.3020+1G>A	3′ splice junction	Y	M	45	B	>220	Y
57,976,414	T>A	27	c.3020+2T>A	3′ splice junction	Y	M	46	B	124	N
57,976,415	A>G	27	c.3020+3A>G	3′ splice junction	Y	M	50	B	54	N
SALS Variants										
57,957,481	G>A	3	c.291+5G>A	3′ splice junction	N	n/a	n/a	n/a	n/a	n/a
57,975,731	CA>C	26	c.2989delA	p.Asn997fs	–	F	50	L	>96	Y
57,976,384	G>A	27	c.2993-1G>A	5′ splice junction	Y	n/a	52	B	n/a	n/a

P, possible; Y, yes; N, no; M, male; F, female; L, limb onset; B, bulbar onset, n/a, not available or applicable. Note that ASSEDA does not predict exon skipping based on frameshifts or nonsense mutations (Tompson et al., 2007).

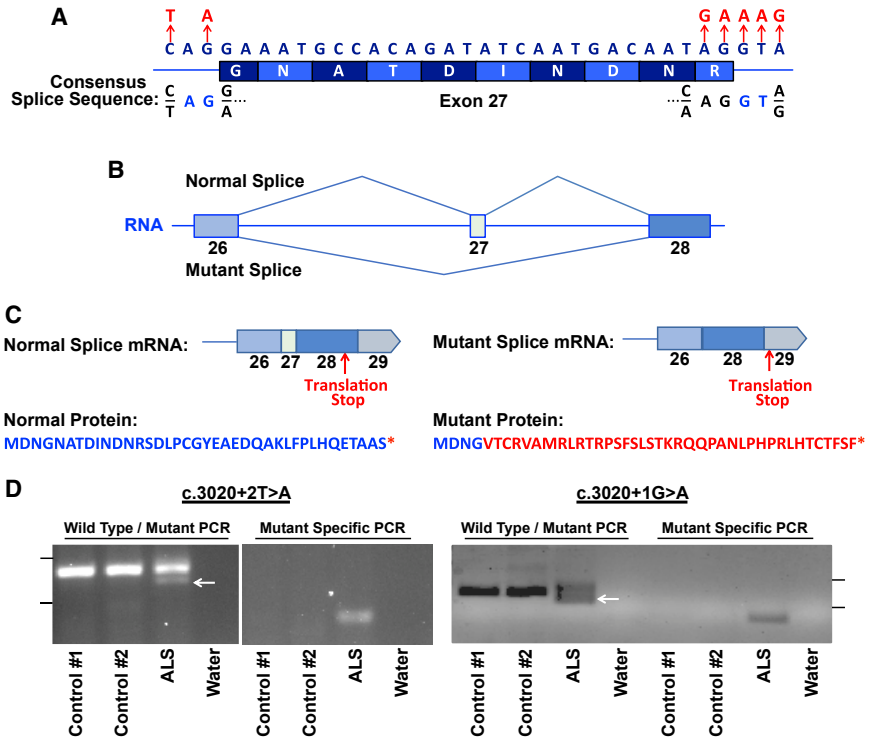

Figure 4. ALS-Associated LOF Variants of *KIF5A* Disrupt C-Terminal Sequence by Inducing Skipping of Exon 27

(A) Single nucleotide variants (SNVs) within *KIF5A* identified in ALS patients are clustered at the 5′ and 3′ splice junctions of exon 27. The consensus splice sequence is shown.

(B) ALS-associated SNVs are predicted to induce skipping of exon 27 and result in an aberrant mRNA transcript.

(C) The skipping of exon 27 of *KIF5A* yields an out-of-frame and extended disrupted C-terminal peptide sequence. The amino acids in red signify the divergence from the normal protein.

(D) RT-PCR was performed using RNA derived from ALS patients with the indicated LOF variant or without (controls) using primers to amplify either both wild-type (155 bp) and mutant (127 bp) splice forms or specifically the mutant splice form (80 bp, right panel). The arrow represents the position of the mutant-specific product. The tick marks represent 200 bp (upper) and 100 bp (lower) markers.

patients with uncomplicated types of hereditary spastic paraparesis and CMT2 display a normal life expectancy (Patzkó and Shy, 2011).

DISCUSSION

We previously identified *KIF5A* as a candidate gene for ALS in our prior study that lacked the power to draw a definitive conclusion (Kenna et al., 2016). *KIF5A* was also a candidate ALS gene in a previous GWAS, though it similarly failed to reach genome-wide significance (McLaughlin et al., 2017; van Rheenen et al., 2016), as well as a single gene study selected based on the *a priori* knowledge of its role in HSP/CMT2 and cytoskeletal function (Brenner et al., 2018). Here, we have confirmed *KIF5A* as an ALS-associated gene with genome-wide significance through two independent approaches. By performing a GWAS involving ~80,000 samples, in addition to replicating five previously published loci, as well as the previously reported locus *SCFD1* using a linear mixed model analysis (data not shown), we identified a missense variant within the *KIF5A* gene that reached genome-wide significance for association with ALS risk. It should be stated though, that as with all GWASs, we cannot rule out that other variants in linkage disequilibrium represent the primary risk factor. In an independent line of investigation, we applied RVB to exome sequencing of ~21,000 samples and identified an exome-wide significant association between FALS risk and rare *KIF5A* LOF variants. Analyses of *KIF5A* in independent replication cohorts both confirmed our initial finding for the p.Pro986Leu variant and revealed three additional carriers of LOF variants in 9,046 ALS cases. Taken together, our results indicate that the p.Pro986Leu *KIF5A* variant may represent a relatively common, but low penetrance, risk allele for ALS, while LOF variants constitute rare, but high penetrance, risk factors.

Kinesins are microtubule-based motor proteins involved in intracellular transport of organelles within eukaryotic cells. In mammals, there are three heavy-chain isoforms of KIF5: KIF5A, KIF5B, and KIF5C (Miki et al., 2001). The three proteins homo- and heterodimerize through their coiled-coiled stalk domain, and create a complex with two kinesin light chains via binding to the tail domain (Hirokawa et al., 1989). All three KIF5 genes are expressed in neurons (Kanai et al., 2000) and function to transport many cargos by binding to distinct adaptor proteins.

The central role of kinesins in axonal transport leads us to speculate that mutations in *KIF5A* cause disease by disrupting this process. Indeed, defects in axonal transport are a common observation in ALS patients and are already known to directly contribute to motor neuron degeneration pathogenesis (Chevalier-Larsen and Holzbaur, 2006; Hirokawa et al., 2010; Millecamps and Julien, 2013). KIF5 mediates the transport of granules containing both RNA and RNA-binding proteins within neuronal dendrites and axons (Kanai et al., 2004). Among these cargos are the ALS-associated proteins FUS and hnRNPA1 (Guo et al., 2017; Kim et al., 2013; Kwiatkowski et al., 2009; Vance et al., 2009). Similarly, KIF5 mediates the transport of VAPB through the adaptor protein protrudin (Matsuzaki et al., 2011), and mutations in the *VAPB* gene have been identified in ALS and late-onset spinal muscular atrophy (Nishimura et al., 2004, 2005). KIF5 is responsible for the axonal transport of neurofilaments (Wang and Brown, 2010) and KIF5A knockout mice display abnormal transport of neurofilaments (Xia et al., 2003). Abnormal accumulation of neurofilaments is a pathological hallmark of ALS and rare mutations in neurofilament heavy polypeptide (NEFH) are associated with ALS (Al-Chalabi et al., 1999).

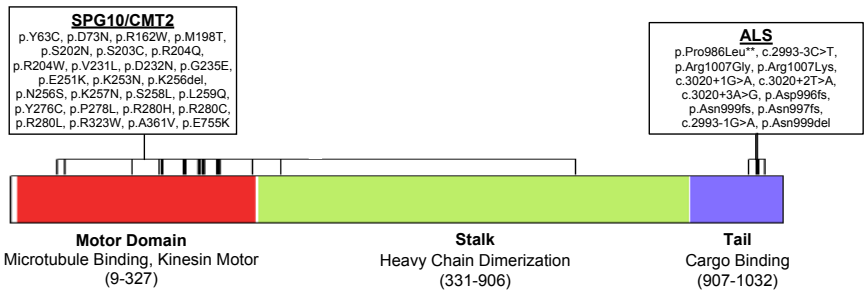

Figure 5. *KIF5A* ALS Mutations Show Distinct Localization from Missense Mutations Previously Associated with SPG10 and CMT2

Causative mutations for SPG10 and CMT2 described within the literature (Crimella et al., 2012; Jennings et al., 2017; Liu et al., 2014; Reid et al., 2002) and ALS-associated mutations identified within this study are shown. As illustrated, mutations causative for SPG10/CMT2 are predominantly missense changes located in the N-terminal motor domain. In contrast, ALS mutations are primarily located at the C-terminal motor domain and are LOF. The double asterisk denotes the risk variant identified through the GWAS.

KIF5 also contributes to the transport of mitochondria (Kanai et al., 2000; Tanaka et al., 1998) and motor neurons derived from *KIF5A*$^{-/-}$ mice display transport deficits and reduced survival (Karle et al., 2012). Impaired transport and dysfunction of mitochondria represent another common hallmark observed in ALS patients (Chevalier-Larsen and Holzbaur, 2006; Guo et al., 2017; Palomo and Manfredi, 2015; Smith et al., 2017). KIF5 also contributes to the transport of AMPA-type (Heisler et al., 2014; Setou et al., 2002) and $GABA_A$ receptors (Nakajima et al., 2012). In keeping with reported ALS genes such as *NEK1* (Thiel et al., 2011) and *PFN1* (Wu et al., 2012), modulation of *KIF5A* expression has been shown to influence the formation of neurite-like membrane protrusions (Matsuzaki et al., 2011). Given its critical interactions with the cytoskeleton, the identification of *KIF5A* mutations further extends the list of cytoskeletal-related proteins implicated in ALS pathogenesis, such as PFN1, TUBA4A, NEFH, and peripherin (Al-Chalabi et al., 1999; Gros-Louis et al., 2004; Smith et al., 2014; Wu et al., 2012).

An important question raised by the current study is why variation within the C-terminal cargo-binding domain is associated with ALS, while missense variations of the N-terminal motor domain are associated with hereditary spastic paraparesis and CMT2. Missense mutations within this latter domain have been shown to affect microtubule binding and/or ATP hydrolysis, resulting in a defective KIF5A-mediated anterograde transport of cargo along dendrites and axons. This, in turn, leads to the axonal retrograde degeneration observed both in hereditary spastic paraparesis and CMT2, two length-dependent axonopathies (Ebbing et al., 2008). In contrast, the primary cellular lesion in ALS is believed to occur within motor neuron cell bodies, where cytoplasmic protein aggregates are consistently observed, and to propagate anterogradely along neurites. We anticipate that LOF variants within the C-terminal domain of KIF5A will disrupt binding with specific cargo proteins. This is supported by a study in zebrafish in which truncation of the C terminus resulted in a dramatic disruption of axonal localization of mitochondria (Campbell et al., 2014). One possible mechanism is that disruption of binding to cargo may possibly lead to their accumulation and seed aggregation within the cell body, resulting in a deficiency at neurite terminals. Deficiency in KIF5A expression and cargo binding has been associated with accumulation of phosphorylated neurofilaments and amyloid precursor protein within neuronal cell bodies, and subsequent neurodegeneration, in patients with multiple sclerosis (Hares et al., 2017). While differences in KIF5A kinetics and KIF5A interactions constitute one possibility to explain the phenotypic heterogeneity, it is also possible C-terminal and N-terminal variants act through a common mechanism, but that a difference in the relative extent of loss- or gain-of-function toxicities leads to milder (i.e., hereditary spastic paraplegia or CMT2) or more severe (i.e., ALS) phenotypes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes eight figures, eight tables, and consortia memberships and can be found with this article online at https://doi.org/10.1016/j.neuron.2018.02.027.

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DECLARATION OF INTERESTS

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Editor-in-Chief of *Amyotrophic Lateral Sclerosis and Frontotemporal Dementia*. L.H.v.d.B. serves on scientific advisory boards for the Prinses Beatrix Spierfonds, Thierry Latran Foundation, Biogen, and Cytokinetics and serves on the editorial board of *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* and the *Journal of Neurology, Neurosurgery, and Psychiatry*. J.H.V. reports that his institute received consultancy fees from Vertex Pharmaceuticals. A. Chiò serves on scientific advisory boards for Biogen Idec, Cytokinetics, Italfarmaco, and Neuraltus. P.M.A. serves on advisory board panels for Biogen and Orphazyme. V.S. serves as a consultant for Cytokinetics.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
TRIzol Reagent	Thermo Fisher Scientific	Cat# 15596026
RNA-to-cDNA Kit	Applied Biosystems	Cat# 4368814
RNase Inhibitor	Applied Biosystems	Cat# 4374966
OneTaq Hot Start DNA Polymerase	New England BioLabs	Cat# M0481S
Critical Commercial Assays		
HumanOmniExpress-24 DNA Analysis Kit	Illumina	Cat# WG-312-3007
TruSeq Exome Enrichment Kit	Illumina	Cat# FC-121-1096
TruSeq PE Cluster Kit	Illumina	Cat# PE-401-3001
TruSeq SBS Kit	Illumina	Cat# FC-401-3001
TrueSeq DNA PCR-free Kit	Illumina	Cat# 20000902
HiSeq X Ten Reagent Kit	Illumina	Cat# FC-501-2501
Oligonucleotides		
F1 primer sequence: CAGTGGAGCCACATCTTCTG	Operon Technologies	N/A
R1 primer sequence: TCTCTTGGTGGAGAGGGAAA	Operon Technologies	N/A
F2 primer sequence: CCAACATGGACAATGGAGTGA	Operon Technologies	N/A
Software and Algorithms		
ASSEDA	Tompson et al., 2007	http://www.cytognomix.com/?post_type=duka&p=2670
BWA	Wellcome Trust Sanger Institute	http://bio-bwa.sourceforge.net
GenABEL	The GenABEL Project	http://www.genabel.org
GATK	Broad Institute	https://software.broadinstitute.org/gatk/
id_geno_checksum	Broad Institute	https://personal.broadinstitute.org/sripke/share_links/checksums_download/
KING	Manichaikul et al., 2010	http://people.virginia.edu/~wc9c/KING/
LASER	University of Michigan	http://csg.sph.umich.edu/chaolong/LASER/
Mach2dat	Marchini and Howie, 2010	https://genome.sph.umich.edu/wiki/Mach2dat:_Association_with_MACH_output
METAL	Willer et al., 2010	http://csg.sph.umich.edu/abecasis/metal/index.html
Minimac3	Das et al., 2016	https://genome.sph.umich.edu/wiki/Minimac3
PLINK	Chang et al., 2015	http://zzz.bwh.harvard.edu/plink/
R	R Core Team	https://www.r-project.org
SHAPEIT	Delaneau et al., 2013	https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html
dbNSFP	Liu et al., 2013	http://varianttools.sourceforge.net/Annotation/DbNSFP
Other		
Genotyped data	Current paper	https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000101.v4.p1

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, John Landers (john.landers@umassmed.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study cohorts

GWAS cohort I

We undertook a GWAS of patients diagnosed with ALS (case cohort) and neurologically normal control individuals (control cohort). DNA was extracted from either whole blood or frozen brain tissue samples using standard procedures. All 12,663 patients included in the case cohort had been diagnosed with ALS according to the El Escorial criteria (Brooks, 1994) by a neurologist specializing in ALS, had onset of symptoms after age 18 years, and were of non-Hispanic white race/ethnicity. Both patients with familial ALS and patients with sporadic ALS were included in the analysis.

For the control cohort, we used genotype data obtained from (a) the database of Genotypes and Phenotypes (dbGaP) web repository (n = 44,017 US samples); (b) the HYPERGENES Project (n = 887 Italian samples) (Salvi et al., 2012); and (c) the Wellcome Trust Case Control Consortium (n = 5,663 British samples). An additional 2,112 US and Italian control samples were genotyped in the Laboratory of Neurogenetics, National Institute on Aging. The control cohort was matched to the case cohort for race and ethnicity, but not for age or sex. A detailed description of the cohorts is available in Tables S1 and S2.

Written consent was obtained from all individuals enrolled in this study, and the study was approved by the institutional review board approval of the National Institute on Aging (protocol number 03-AG-N329).

GWAS cohort II

Summary statistics from a recently published GWAS based on logistic regression analysis involving 12,577 cases and 23,475 controls were downloaded from the Project MinE Variant Browser. Additional details of the cohorts used in this study are available in van Rheenen et al. (2016).

FALS discovery cohort

A total of 1,463 FALS patients were included in the initial cohort (pre-QC). Patients were recruited at specialist clinics in Australia (n = 92), Belgium (n = 13), Canada (n = 34), Germany (n = 228), Ireland (n = 18), Israel (n = 26), Italy (n = 230), Netherlands (n = 50), Spain (n = 60), Turkey (n = 72), UK (n = 223), and USA (n = 417). All samples were exome sequenced except those from the Netherlands which were whole genome sequenced. Familial history was considered positive for ALS if the proband had at least one affected relative within three degrees of relatedness.

Control discovery cohort

Read level sequencing data were obtained from dbGAP and the European Genome-Phenome Archive (EGA) and are listed in Table S5.

ALS WXS/WGS replication cohort

Replication analyses included sequencing data for a further 9,046 ALS cases and 1,955 non-ALS controls that were not also represented in the FALS discovery set. These samples included 2,742 cases subjected to WXS by the ALS Sequencing Consortium, as described previously (Cirulli et al., 2015); 719 cases subjected to WXS by the Laboratory of Neurogenetics, National Institute on Aging; 307 cases and 296 controls subjected to WGS by the Laboratory of Neurogenetics, National Institute on Aging; 155 cases subjected to WGS by the CReATe Consortium; 1,017 cases subjected to WGS by the NYGC ALS Consortium, Genomic Translation for ALS Care (GTAC) Consortium and Answer ALS Foundation; and 4,100 cases and 1,659 controls subjected to WGS by the Project MinE Sequencing Consortium.

All samples included in the case cohort had been diagnosed with ALS according to the El Escorial criteria (Brooks, 1994) by a neurologist specializing in ALS. We received approval for this study from the institutional review boards of the participating centers, and written informed consent was obtained from all patients (consent for research).

METHOD DETAILS

Data generation and pre-processing

Generation of SNP array callset

The case cohort (n = 12,663 samples) and part of the control cohort (n = 2,112) were genotyped in the Laboratory of Neurogenetics, National Institute on Aging, using HumanOmniExpress BeadChips (version 1.0, Illumina, San Diego, CA) according to the manufacturer's protocol. These SNP genotyping arrays assay 716,503 SNPs across the genome. Individual-level genotypes for these samples are available on the dbGaP web portal (accession number phs000101.v4.p1). The remainder of the control cohort had been previously genotyped on HumanOmni BeadChips (Illumina) as part of other GWAS efforts (Table S2). Analyses were confined to the 595,692 autosomal SNPs that were common across the SNP genotyping arrays.

Generation of FALS case-control callset for exome-wide RVB discovery analysis

Exome sequencing of cases was performed as previously described (Kenna et al., 2016). Control exome sequences were generated as described under the relevant dbGAP and EGA project accessions. Sequence reads were aligned to human reference GRCh37 using BWA (Burrows-Wheeler Aligner) and processed according to recommended Genome Analysis Toolkit's (GATK) best practices (https://software.broadinstitute.org/gatk/best-practices/). Joint variant detection and genotyping of all samples were performed using the GATK HaplotypeCaller. Variant quality control was performed using the GATK variant quality score recalibration method with default filters. A minimum variant quality by depth (QD) score of 2 was also imposed and all genotypes associated with genotype quality (GQ)< 20 were reset to missing. Variants were also excluded in the event of case or control call rates < 70% (post genotype QC). Identified variants can be viewed through our web based ALS Variant Server (see link below).

Generation of ALS case-control callset for KIF5A replication analysis

Data for the *KIF5A* locus was extracted from all independently generated sequencing datasets and remapped to GRCh37. Variant calling was performed using the GATK haplotype caller as described above. In addition to the *KIF5A* locus, data was also extracted for a panel of 240,715 common variant sites and used to perform a single unified sample QC as described below.

Functional annotation of variants identified by WXS/WGS

Variant calls were assigned predicted functional consequences using snpEFF (Single Nucleotide Polymorphism Effect) (Cingolani et al., 2012), dbNSFP (A Database of Human Non-synonymous SNVs and Their Functional Predictions and Annotations) (Liu et al., 2013) and dbscSNV (database of splice site consequences of Single Nucleotide Variants) (Jian et al., 2014), which is incorporated into dbNSFP. Variants were classified as "loss of function" (LOF) where the sequence change was predicted to encode a premature stop codon, a frameshift causing insertion-deletion or a splice site disrupting SNV. Variants were classified as potentially splice altering if assigned an "ada" or "rf" score > 0.7 by dbscSNV. Splice variants of potential interest were further assessed for putative effects on exon skipping using a secondary algorithm - automated splice site and exon definition server (ASSEDA) (Tompson et al., 2007).

RT-PCR Analysis

Total RNA was prepared from lymphoblast lines using Trizol reagent. Reverse transcription using Applied Biosystems RNA to cDNA kit (# 4368814) was performed with 0.5 μg with RNase inhibitor in a 20 μl reaction according to the manufacturer's protocol. PCR was carried out using New England Biolabs One Taq Hot Start DNA Polymerase (# M0481S), 2 μl RT reaction (representing 50 ng input RNA) and forward and reverse primer (0.15 μM each) in a 20 μl reaction volume. Amplification conditions were as follows: 94°C for 30 s, {94°C for 20 s, 58°C for 20 s, 68°C for 1 minute} x 35 cycles, followed by an extension stage of 68°C for 5 minutes and a 4°C hold. Amplification of both normal and mutant splice forms used primers F1 (CAGTGGAGCCACATCTTCTG) and R1 (TCTCTTGGTGGA GAGGGAAA). Primers used for the specific amplification of the mutant splice form were F2 (CCAACATGGACAATGGAGTGA), which spans exons 26 and 28, and R1.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses

Analysis of SNP array genotypes

Standard quality-control procedures were applied to our genotype data using PLINK software package (version 1.9) (Chang et al., 2015), and a summary of the workflow is shown in Figure S1. We excluded samples that demonstrated: call rates of less than 97.5%; non-European ancestry; abnormal *F* inbreeding coefficient; mismatch between phenotypic and genotypic gender; or, cryptic relatedness defined as identity-by-descent proportion of inheritance (pi_hat from PLINK) greater than 0.125. Samples in common between our study and van Rheenen's study were identified using the checksum program id_geno_checksum and were removed from our analyses. We excluded palindromic SNPs, as well as SNPs with: call rates less than 95% in the US and Italian cohorts or less than 99% in the UK, French and Belgium cohorts; minor allele frequency less than 0.05 in the control cohorts; Hardy-Weinberg equilibrium *P* less than 10^{-7} in the US and Italian control cohorts and less than 10^{-5} in the UK, French and Belgium cohorts; missingness by case-control status *P* less than 10^{-5}; or SNPs associated between the UK and French control cohorts with *P* less than $5.0x10^{-8}$. After quality control, 8,229 case and 36,329 control samples were included in the analysis, and 436,746 SNPs were available for imputation in the USA and Italy cohorts, and 420,131 SNPs were available in the UK, French and Belgium cohorts.

Estimation of the haplotypes was performed with SHAPEIT (version 2.r790) (Delaneau et al., 2013). Imputation was performed for individual batches based on ethnicity using the 1000 Genomes Project dataset (phase 3, version 5a, release 2013-05-02, http://www.internationalgenome.org/) as reference and using Minimac3 software (version 1.0.11) (Das et al., 2016) with default settings. After imputation, principal components were calculated using PLINK software after removing known hypervariable regions and the 1 MB surrounding the *C9orf72* region. After analysis of the Scree plots, 2 to 4 principal components were retained per cohort as covariates in the association analyses to compensate for any residual population stratification.

Logistic regression was performed per batch using mach2dat software (version 1.0.24) (Marchini and Howie, 2010) incorporating 2 to 4 principal components, age and gender as covariates, with dosage of imputed SNPs selected based on a Minimac3 R^2 value of imputation accuracy greater than 0.3. SNPs with an absolute beta coefficient value above 5 or with a minor allele frequency less

than 0.01 were excluded from meta-analysis. Meta-analysis was then performed combining the association results of the 13 batches of our individual-level studies with van Rheenen's study summary statistics using METAL software (version 2011-03-25) (Willer et al., 2010) under an inverse-weighted, fixed effect model. A threshold P of 5.0×10^{-8} was set for genome-wide significance after Bonferroni correction for multiple testing in the GWAS (Pe'er et al., 2008).

The programming code used to analyze these data is freely available on GitHub (https://github.com/AudeDN/ALS_GWAS_1000G_mach2dat_2017), and GWAS summary statistics results for all tested SNPs are available from https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000101.v4.p1.

Analysis of WXS/WGS genotypes

For both the discovery and replication phases, samples were excluded from the study in the event of failing to meet standard genotype call rate, heterozygosity, duplication, relatedness or population stratification filters as summarized in Table S6. Each of these filters was performed using a set of autosomal markers meeting all of the following criteria: call rate > 0.95, minor allele frequency (MAF) > 0.01, p > 0.001 for deviation from Hardy-Weinberg equilibrium, linkage disequilibrium pruning ($R^2 < 0.5$, window size = 50, step = 5). Filtering of autosomal markers, sample call rate assessments and sample heterozygosity assessments were performed using PLINK software. Study duplicates and sample relatedness within the WXS/WGS cohorts was identified using KING software (Manichaikul et al., 2010). Study duplicates between WXS/WGS cohorts and GWAS datasets were identified using the checksum program id_geno_checksum. LASER was used to generate PCA coordinates for samples from the Human Genome Diversity Panel (HGDP). Samples from the FALS discovery cohort were then mapped to this reference co-ordinate space. The discovery cohort was restricted to cases and controls occurring within 3 standard deviations of the mean for European HGDP samples along principal components 1-4.

RVB analyses were performed by penalized logistic regression of case-control status with respect to number of minor alleles observed per sample per gene with and MAF < 0.001. Analyses were only performed where the dataset contained more than 3 variant allele occurrences. Replication analyses of rs113247976 were performed using the same logistic regression protocol as used for RVB analyses. All analyses were conditioned on the first 4 eigenvectors generated by principal components analysis of common variant profiles. Genomic inflation factors were calculated using genome-wide association analysis for quantitative, binary and time-till-event traits using GenABEL software. Candidate associations were tested for signs of call-rate or subcohort biases as outlined in Figures S6 and S7. Meta-analysis of rs113247976 association results between sequencing and GWAS was performed using METAL. Unless otherwise indicated, all statistical analyses were performed using R (version 3.2.0).

Control-control analyses

To identify genes potentially subject to confounding biases in FALS RVB analyses and to assess the potential impact of batch effects with non-ALS-related data, population or phenotypic stratifiers, the control sample cohort was divided into 28 pseudo case-control groups based on the sequencing center or associated dbGaP / EGA project (Table S5). Genes shown in gray achieve for possible confounder association. Loci achieving a minimum $p < 1 \times 10^{-3}$ were deemed as displaying possible association with non-ALS related batch effects.

DATA AND SOFTWARE AVAILABILITY

Datasets

The programming code used to analyze the GWAS data including the imputation with SHAPEIT and Minimac3, individual-based association analysis using Mach2dat and a meta-analysis using METAL is freely available on GitHub: https://github.com/AudeDN/ALS_GWAS_1000G_mach2dat_2017). GWAS summary statistics results for all tested SNPs and identified SNVs from our 1,138 FALS cohort used for the RVB analysis can be viewed through our web based ALS Variant Server (http://als.umassmed.edu). For each variant, information on over 50 annotation fields and the results can be downloaded directly into Excel. The accession number for the genotyped data reported in this paper is dbGaP: phs000101.v4 (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000101.v4.p1).

Data Resources and Databases

1000 Genomes Project dataset: http://www.internationalgenome.org
Database of Genotypes and Phenotypes (dbGaP): https://www.ncbi.nlm.nih.gov/gap
dbNSFP: https://sites.google.com/site/jpopgen/dbNSFP
dbscSNV: incorporated into dbNSFP (see previous link).
European Genome-phenome Archive (EGA): https://ega-archive.org
HapMap project: http://www.sanger.ac.uk/resources/downloads/human/hapmap3.html.
Human Genome Diversity Panel (HGDP): http://www.hagsc.org/hgdp/
HYPERGENES Project: https://cordis.europa.eu/project/rcn/86758_en.html
Project MinE Variant Browser: http://databrowser.projectmine.com
snpEFF: http://snpeff.sourceforge.net/SnpEff.html
Wellcome Trust Case Control Consortium: https://www.wtccc.org.uk/

Software

ASSEDA: http://www.cytognomix.com/?post_type=duka&p=2670
BWA: http://bio-bwa.sourceforge.net
GenABEL: http://www.genabel.org
GATK: https://software.broadinstitute.org/gatk/
id_geno_checksum: https://personal.broadinstitute.org/sripke/share_links/checksums_download/
KING: http://people.virginia.edu/~wc9c/KING/
LASER: http://csg.sph.umich.edu/chaolong/LASER/
Mach2dat: https://genome.sph.umich.edu/wiki/Mach2dat:_Association_with_MACH_output
METAL: http://csg.sph.umich.edu/abecasis/metal/index.html
Minimac3: https://genome.sph.umich.edu/wiki/Minimac3
PLINK: http://zzz.bwh.harvard.edu/plink/
R: https://www.r-project.org
SHAPEIT: https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html

Neuron, Volume *97*

Supplemental Information

Genome-wide Analyses Identify KIF5A as a Novel ALS Gene

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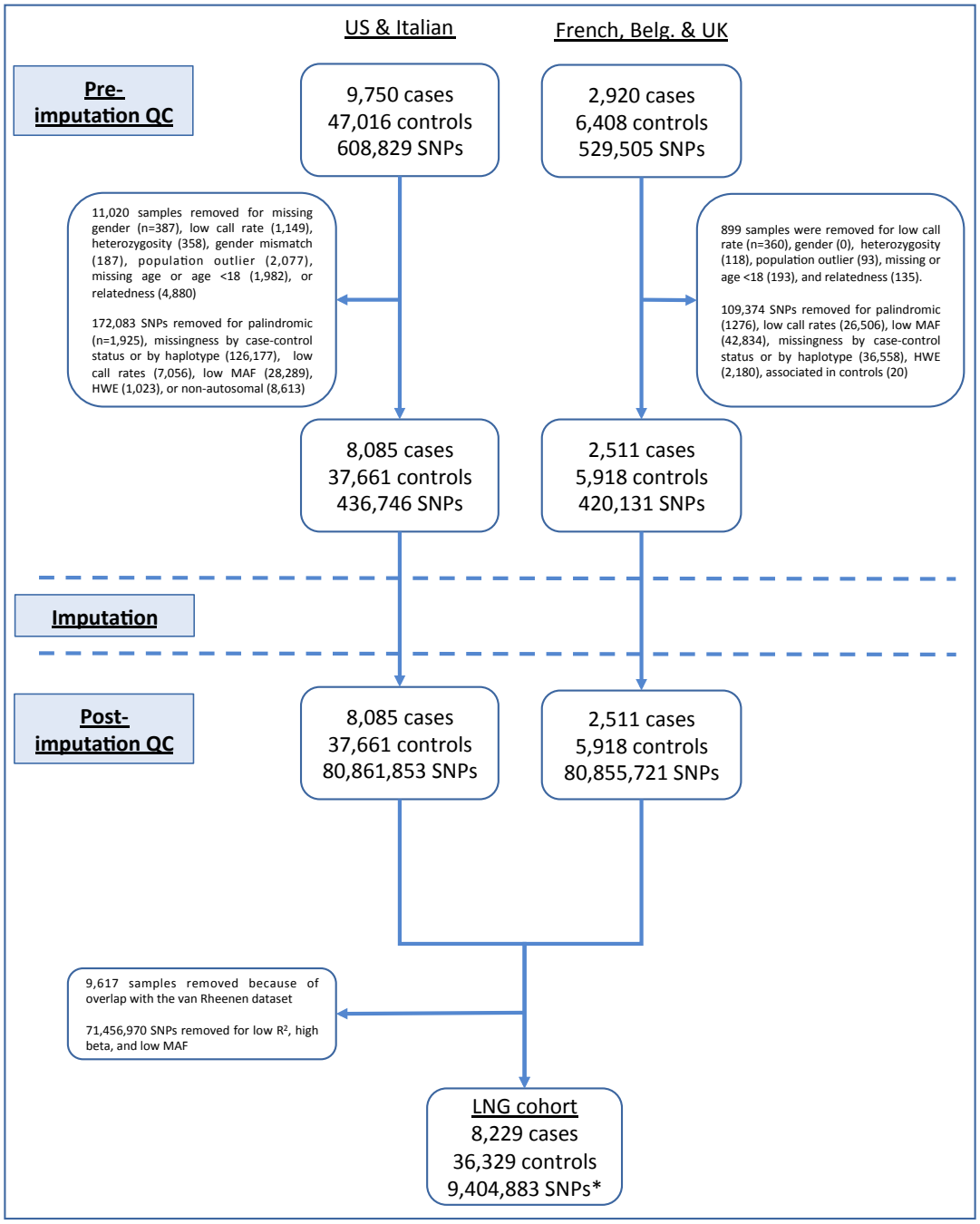

Figure S1. Related to Figure 1; Workflow showing the quality control procedures applied to the present study. *increased to 10,031,630 when merged with the Van Rheenen et al dataset; Belg., Belgium; SNP, single nucleotide polymorphism; MAF, minor allele frequency, HWE, Hardy-Weinberg equilibrium; R^2, R-square value representing imputation precision; LNG, Laboratory of Neurogenetics.

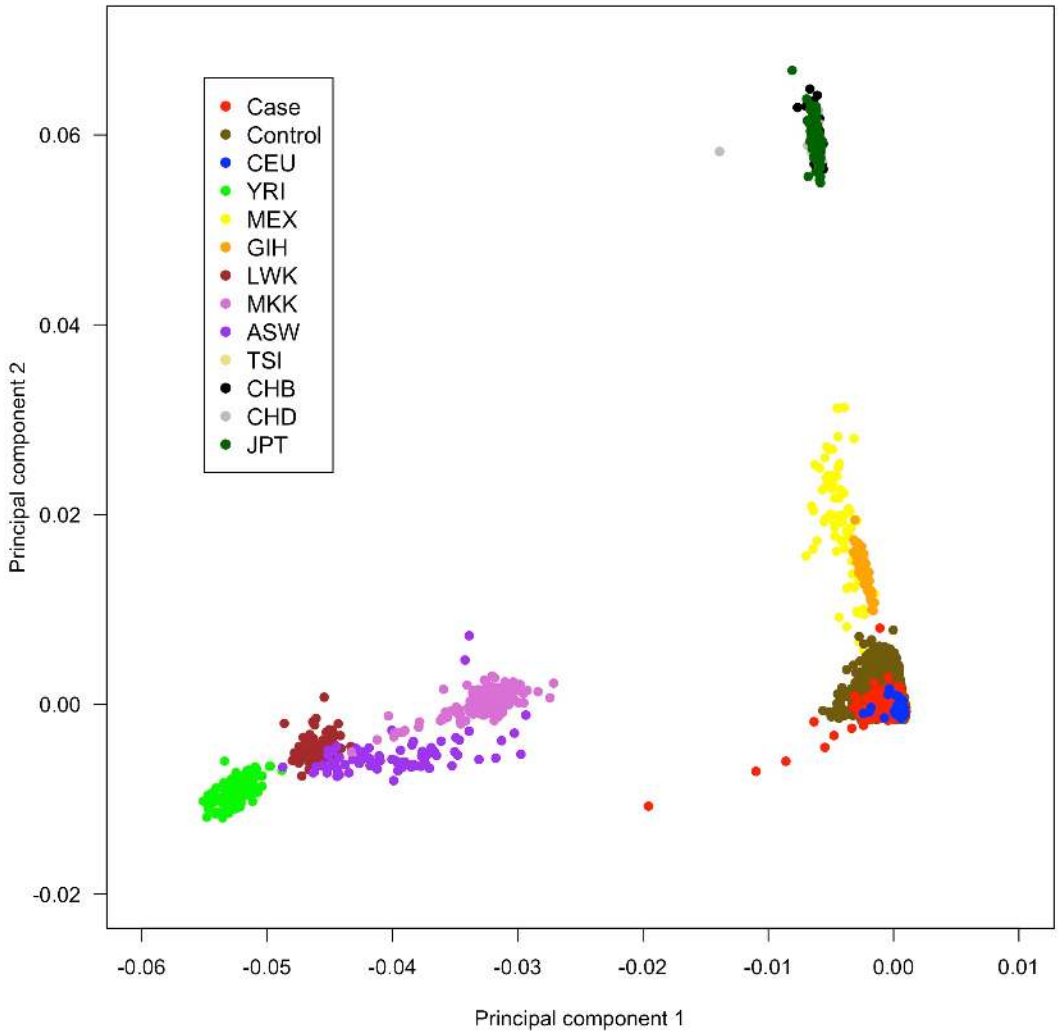

Figure S2. Related to Figure 1; Multi-dimensional scaling plot of the 44,558 genotyped samples included in analysis compared to the HapMap populations.

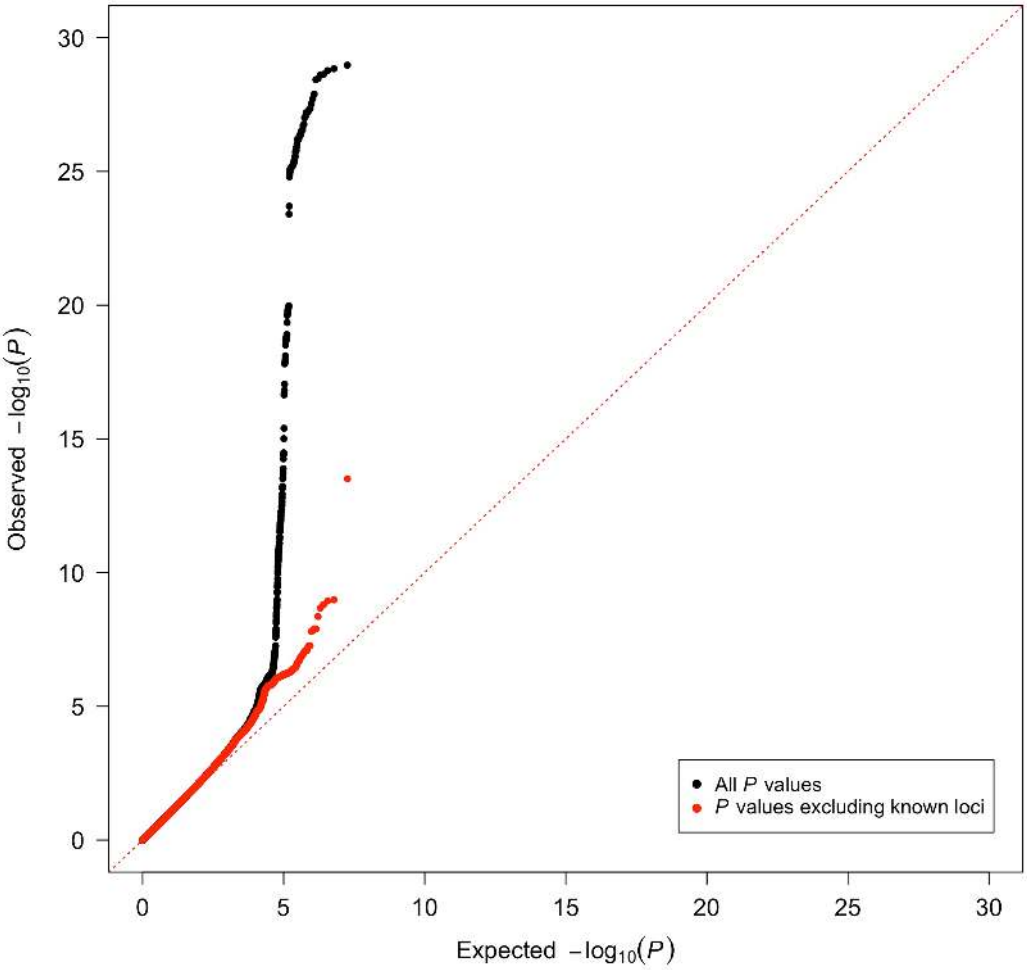

Figure S3. Related to Figure 1; Quartile-Quartile plot of *P*-values from the meta-analysis based on logistic regression analysis. The black curve represents all SNPs, and the red curve represent SNPs after excluding variants within +/- 500 kilobases of the *C9orf72* and the *UNC13A* loci. Raw genome inflation factor (λ) was 1.042 and adjusted λ scaled to 1,000 cases and 1,000 controls was 1.001 based on the entire SNP dataset.

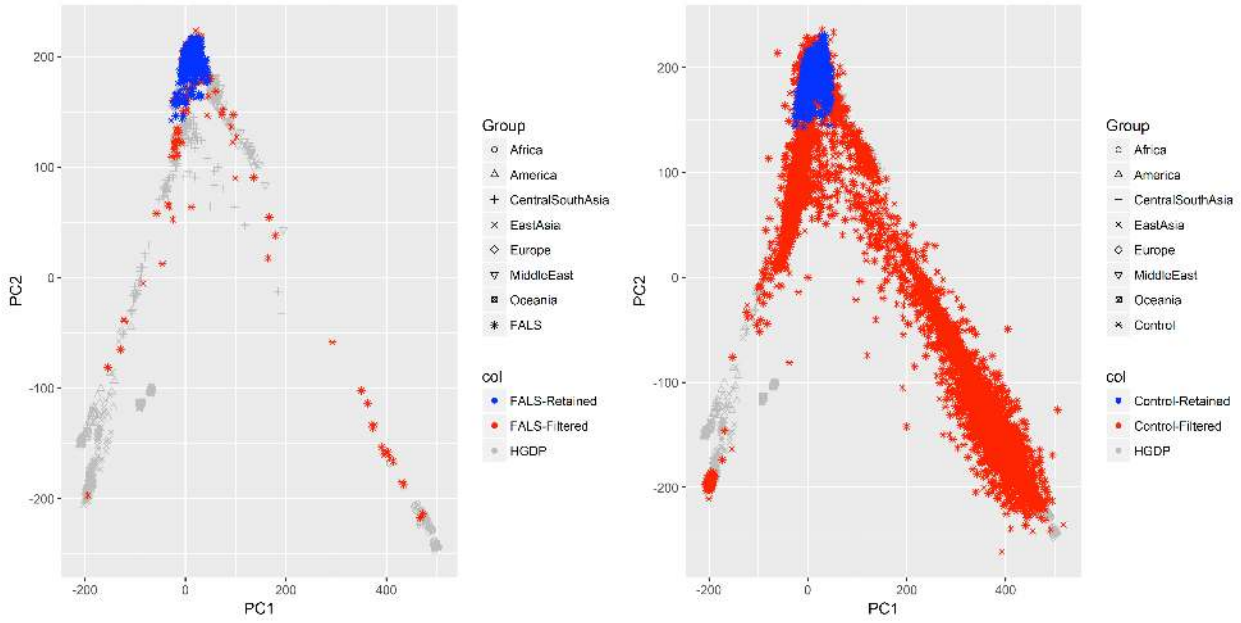

Figure S4. Related to Figure 3; Principal components analysis of samples included in the RVB analysis compared to the Human Diversity Panel. Ancestry filtering of the FALS discovery cohort was performed as follows: LASER was used to generate PCA coordinates for samples from the Human genome diversity panel (HGDP). Samples from the FALS discovery cohort were then mapped to this reference co-ordinate space. The discovery cohort was restricted to cases and controls occurring within 3 standard deviations of the mean for European HGDP samples along principal components 1-4.

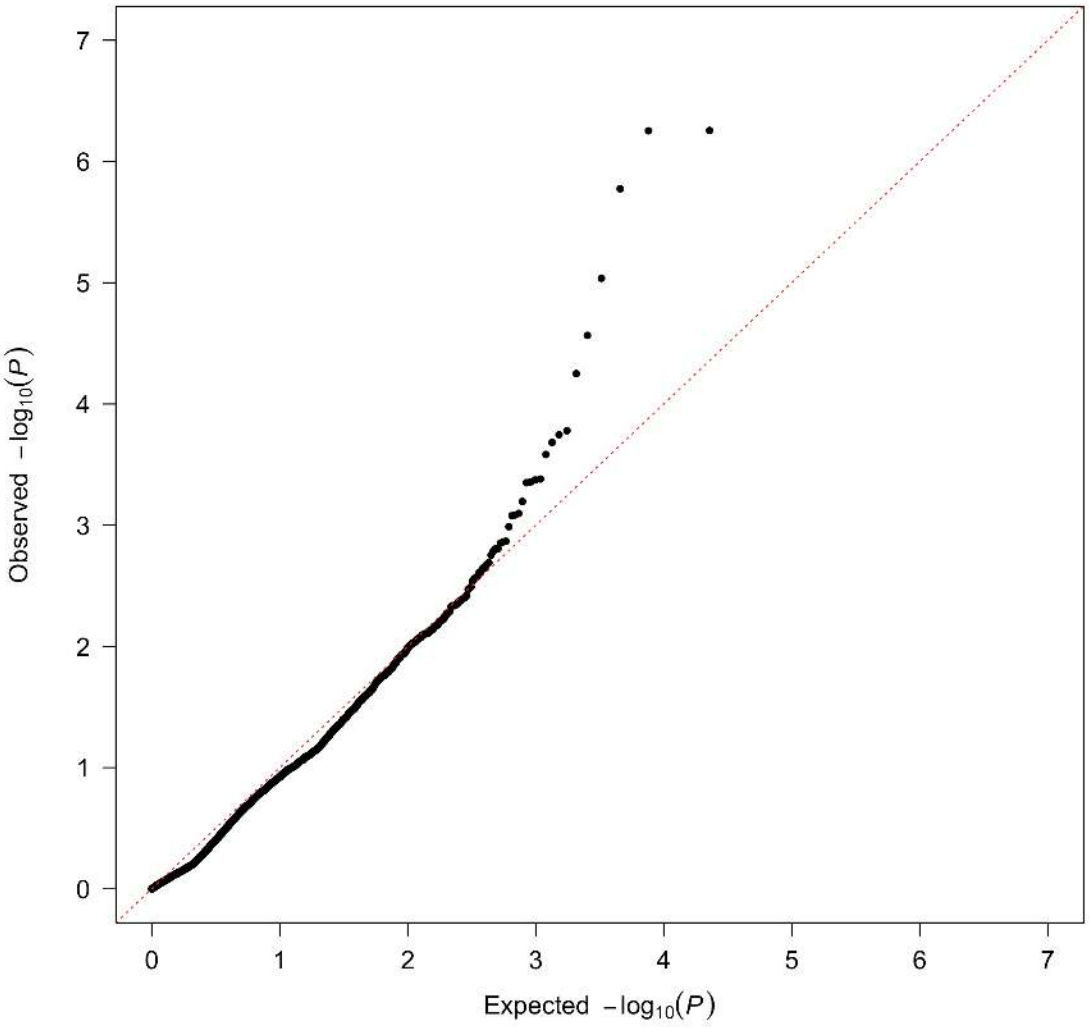

Figure S5. Related to Figure 3; Quartile-Quartile plot of P values from the gene-based rare variant burden analysis of exome data. The genomic inflation factor ($\lambda = 0.93$) was calculated based on the entire gene dataset.

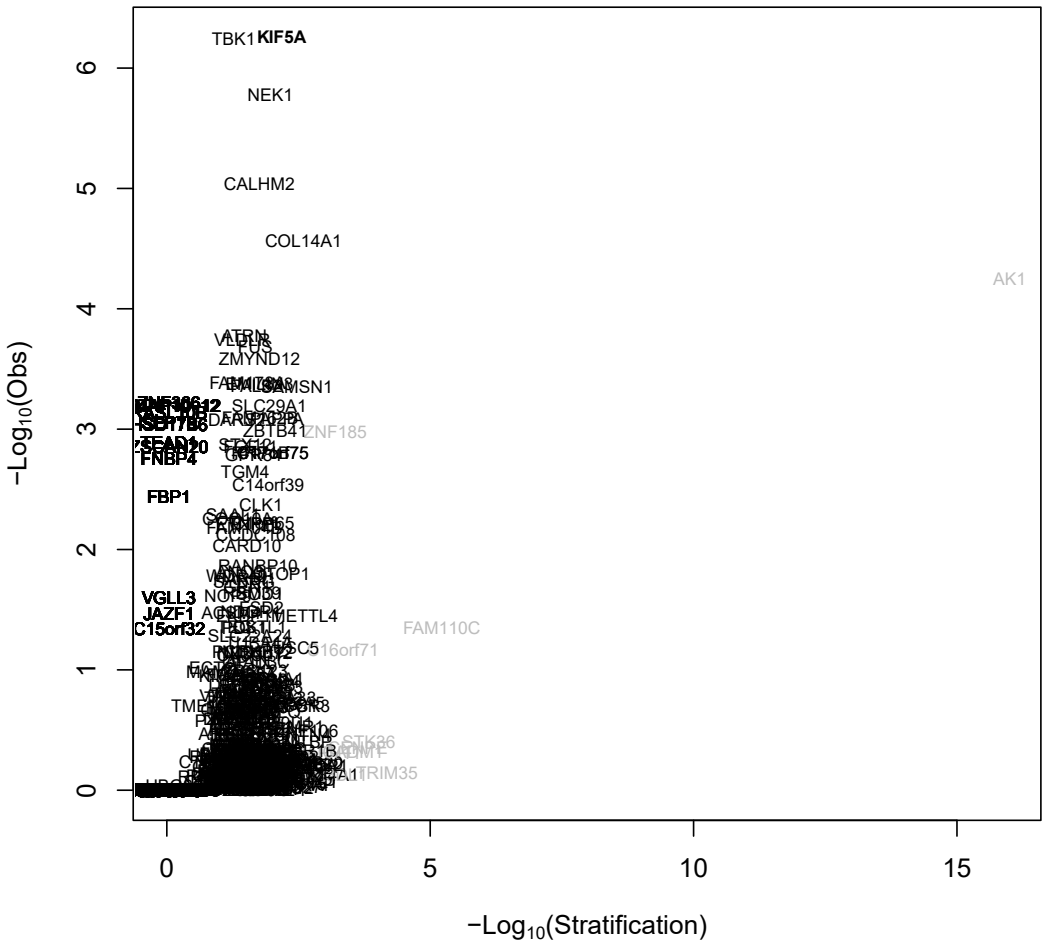

Figure S6. Related to Figure 3; Control-control analyses. *P* values from RVB analysis of FALS cases versus controls (y-axis) are plotted against minimum *P* values from RVB analyses of candidate batch effects (x-axis). To assess the potential impact of batch effects, the sample cohort was divided into 28 pseudo case-control groups based on the sequencing center or associated dbGaP project. Loci showing possible association with non-ALS related batch effects are coloured light grey. No evidence of confounder bias was observed for *KIF5A* or previously reported ALS genes.

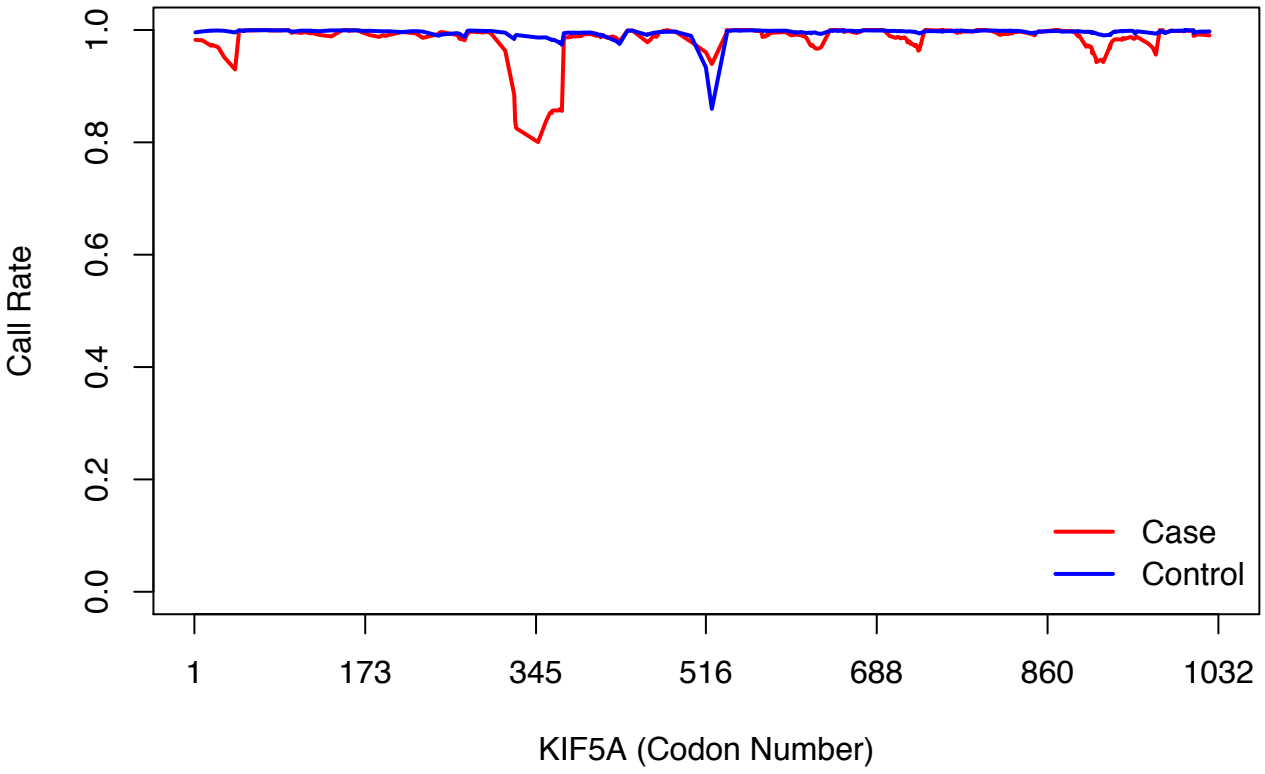

Figure S7. Related to Fgure 3; Plot of variant call rates across the KIF5A protein-coding region in FALS versus controls analyzed by RVB testing.

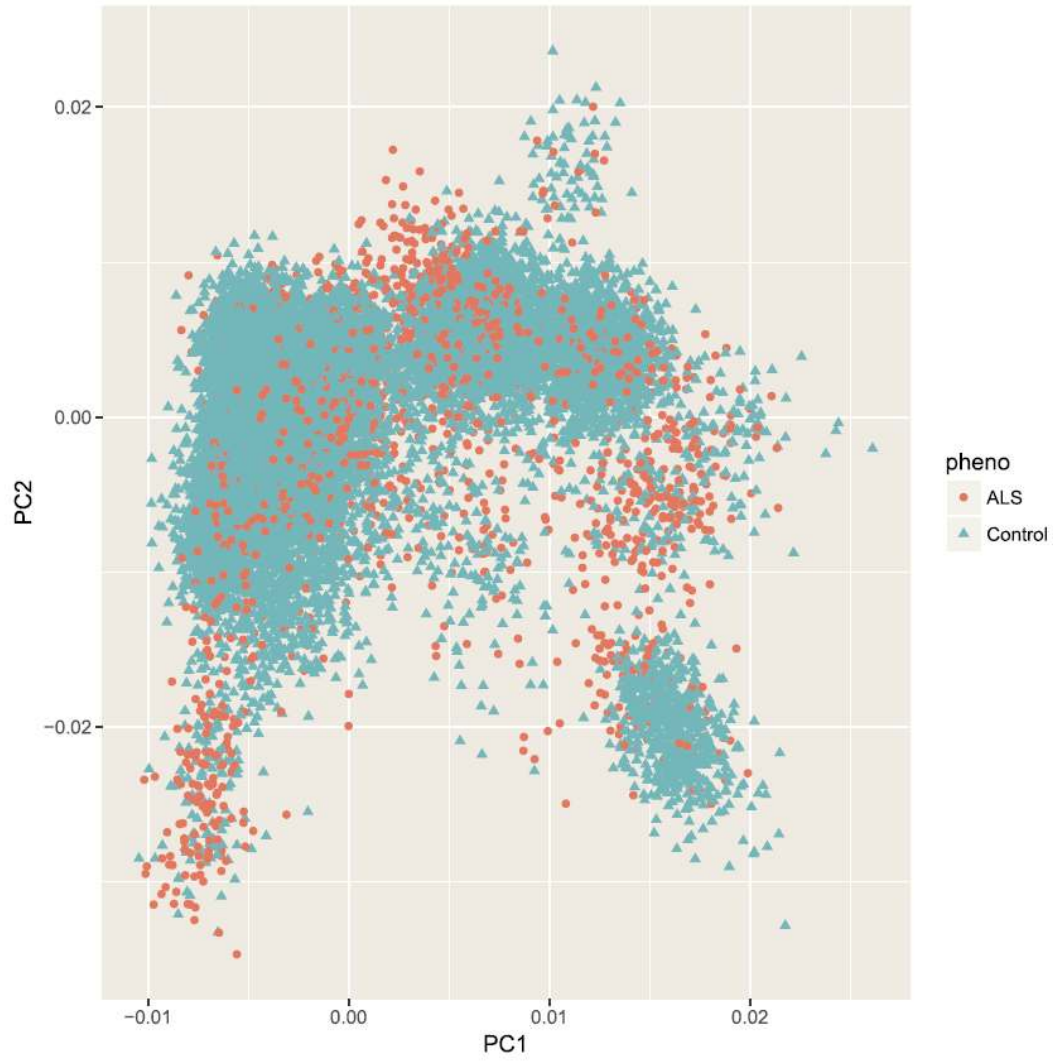

Figure S8. Related to Figure 2; Principal components analysis of samples included in *KIF5A* replication cohort.

Table S1. Related to Figure 1; Demographics and baseline characteristics of patients diagnosed with ALS and control individuals included in the GWAS analysis.

	US		Italian		UK		French & Belgian		Total cohort	
	cases	controls	cases	controls	cases	controls	cases	controls	cases	controls
Sample number	3,777	33,365	2,853	2,143	449	226	1,150	595	8,229	36,329
Female (%)	1,515 (40.1)	23,870 (71.5)	1,239 (43.4)	896 (41.8)	193 (43.0)	109 (48.2)	486 (42.3)	422 (70.9)	3,433 (41.7)	25,297 (69.6)
Age (SD)	58.1 (12.3)	64.2 (13.3)	61.8 (11.8)	50.6 (17.4)	60.3 (12.8)	57.0 (0.0)	60.5 (12.6)	66.9 (16.8)	59.8 (12.3)	63.4 (13.9)
Bulbar-onset* (%)	963 (25.5)	-	741 (26.0)	-	141 (31.4)	-	357 (31)	-	2,202 (26.8)	-
Family history† (%)	458 (12.1)	-	248 (8.7)	-	54 (12.0)	-	195 (17.0)	-	955 (11.6)	-

SD, standard deviation. *Data not available for site of symptom onset for 199 patients. †Data not available for familial history of 154 patients.

Table S2. Related to Figure 1; DbGaP studies contributing to the GWAS analysis.

Accession Number	Study	Sample number	Females (%)	Average age (SD)	Genotyping platform	Ascertainment criteria
phs000001	NEI Age-Related Eye Disease Study (AREDS)	1,644	959 (58.3)	68.2 (4.8)	HumanOmni2.5	Population controls
phs000007	Framingham Cohort	1,298	718 (55.3)	75.7 (8.6)	HumanOmni5	Population controls
phs000187	High Density SNP Association Analysis of Melanoma	1,027	414 (40.3)	51.3 (12.6)	HumanOmniExpress	Population controls
phs000196	CIDR: The NeuroGenetics Research Consortium Parkinson's Disease Study	10	6 (60)	74.3 (18.6)	HumanOmni1	Population controls
phs000292	GENEVA Genetics of Early Onset Stroke (GEOS) Study	89	0 (0)	41.5 (6.4)	HumanOmni1	Population controls
phs000304	Genes and Blood Clotting Study (GABC)	403	259 (64.3)	21.6 (3.3)	HumanOmni1	Population controls
phs000315	Woman's Health Initiative (WHI GARNET)	4,206	4206 (100)	65.7 (6.9)	HumanOmni1	Population controls
phs000368	Polycystic Ovary Syndrome Genetics (POLYGEN)	2,974	2973 (100)	46.8 (15.2)	HumanOmniExpress	Population controls
phs000372	Alzheimer's Disease Genetics Consortium Genome Wide Association Study	533	335 (62.9)	75.8 (9)	HumanOmniExpress	Population controls
phs000394	Autopsy-Confirmed Parkinson Disease GWAS Consortium (APDGC)	299	152 (50.8)	82.1 (12.6)	HumanOmni1	Population controls
phs000397	NIA Long Life Family Study (LLFS)	1,804	957 (53)	65.9 (12.3)	HumanOmni2.5	Population controls
phs000404	The Genetic Architecture of Smoking and Smoking Cessation	81	50 (61.7)	36.6 (5.9)	HumanOmni2.5	Population controls
phs000421	A Genome-Wide Association Study of Fuchs' Endothelial Corneal Dystrophy	497	294 (59.2)	70.4 (10.2)	HumanOmni2.5	Population controls
phs000428	Health and Retirement Study (HRS)	9,394	5437 (57.9)	68.4 (9.4)	HumanOmni2.5	Population controls
phs000615	NINDS Stroke Genetics Network (SiGN)	743	416 (56)	56 (16.1)	HumanOmni5	Population controls
phs000675	GWAS on Selected WHI Hormone Trial European Americans	5,626	5626 (100)	68 (5.9)	HumanOmni1	Population controls
phs000801	NCI Non-Hodgkin Lymphoma GWAS	1,544	790 (51.2)	58.4 (11.6)	HumanOmniExpress	Population controls
phs000869	Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study (BEAGESS)	1,174	271 (23.1)	61.3 (10.9)	HumanOmni1	Population controls

Table S3. Related to Figure 1; SNPs achieving genome-wide significance in the GWAS analysis.

SNP Information				Present Study (8,229 Cases / 36,329 Controls)			Van Rheenen *et al.* (12,577 Cases / 23,475 Controls)			Combined Discovery Set (20,806 Cases / 59,804 Controls)			
SNP	**Chr**	**Position**	**Gene**	**Beta [SE]**	**OR [95% CI]**	***P***	**Beta [SE]**	**OR [95% CI]**	***P***	**I^2**	**Beta [SE]**	**OR [95% CI]**	***P***
Novel Loci													
rs117027576	12	57,316,603	*KIF5A*	0.373 [0.096]	1.45 [1.20-1.76]	$1.1x10^{-4}$	0.286 [0.070]	1.33 [1.16-1.53]	$4.3x10^{-5}$	25.6	0.316 [0.057]	1.37 [1.23-1.54]	$2.3x10^{-8}$
rs118082508	12	57,318,819	*KIF5A*	0.374 [0.096]	1.45 [1.20-1.76]	$1.0x10^{-4}$	0.288 [0.070]	1.33 [1.16-1.53]	$3.8x10^{-5}$	25.8	0.317 [0.051]	1.37 [1.23-1.54]	$2.0x10^{-8}$
rs113247976*	12	57,975,700	*KIF5A*	0.381 [0.086]	1.46 [1.23-1.74]	$9.2x10^{-6}$	0.288 [0.066]	1.33 [1.17-1.52]	$1.1x10^{-5}$	0.0	0.322 [0.052]	1.38 [1.24-1.53]	$6.4x10^{-10}$
rs116900480	12	58,656,105	*KIF5A*	0.354 [0.083]	1.42 [1.21-1.68]	$1.9x10^{-5}$	0.294 [0.065]	1.34 [1.18-1.53]	$7.1x10^{-6}$	0.0	0.317 [0.051]	1.37 [1.24-1.52]	$6.6x10^{-10}$
rs142321490	12	58,676,132	*KIF5A*	0.357 [0.082]	1.43 [1.21-1.68]	$1.5x10^{-5}$	0.292 [0.066]	1.34 [1.18-1.53]	$8.0x10^{-6}$	0.0	0.317 [0.056]	1.37 [1.24-1.52]	$6.1x10^{-10}$
Previously Published Loci													
rs10463311	5	150,410,835	*TNIP1*	-0.065 [0.024]	0.94 [0.89-0.98]	$7.8x10^{-3}$	-0.100 [0.020]	0.91 [0.87-0.94]	$8.5x10^{-7}$	0.0	-0.085 [0.016]	0.92 [0.89-0.95]	$4.0x10^{-8}$
rs3849943	9	27,543,382	*C9orf72*	-0.17 [0.024]	0.84 [0.80-0.88]	$1.4x10^{-12}$	-0.181 [0.020]	0.83 [0.80-0.87]	$4.0x10^{-19}$	0.0	-0.176 [0.016]	0.84 [0.81-0.86]	$3.8x10^{-30}$
rs74654358	12	64,881,967	*TBK1*	0.182 [0.058]	1.20 [1.07-1.34]	$1.6x10^{-3}$	0.206 [0.042]	1.23 [1.13-1.34]	$7.7x10^{-7}$	0.0	0.198 [0.034]	1.22 [1.14-1.30]	$4.7x10^{-9}$
rs12973192	19	17,753,239	*UNC13A*	-0.149 [0.026]	0.86 [0.82-0.91]	$1.3x10^{-8}$	-0.106 [0.019]	0.9 [0.87-0.93]	$2.4x10^{-8}$	38.6	-0.121 [0.015]	0.89 [0.86-0.91]	$3.9x10^{-15}$
rs75087725	21	45,753,117	*C21orf2*	0.687 [0.162]	1.99 [1.44-2.75]	$2.2x10^{-5}$	0.479 [0.074]	1.61 [1.39-1.87]	$8.7x10^{-11}$	31.1	0.515 [0.067]	1.67 [1.46-1.91]	$1.8x10^{-14}$

Position is based on Human Genome Assembly build 37. Nearest gene or previously published gene names are included. Chr, chromosome; SE, standard error; OR, odds ratio; 95% CI, 95% confidence interval; *, rs113247976 represents the p.Pro986Leu variant in *KIF5A* (NM_004984.2).

Table S4. Related to Figure 1; Suggestive SNPs with *P* values less than 5.0x10-7 in the GWAS analyses.

SNP Information				Present Study (8,229 Cases / 36,329 Controls)				Van Rheenen *et al.* (12,577 Cases / 23,475 Controls)				Combined Discovery Set (20,806 Cases / 59,804 Controls)			
SNP	**Chr**	**Position**	**Gene**	**Case MAF**	**Control MAF**	**OR [95% CI]**	***P***	**Case MAF**	**Control MAF**	**OR [95% CI]**	***P***	**Case MAF**	**Control MAF**	**OR [95% CI]**	***P***
rs17070492	8	2,420,855	*LOC101927815*	10.01%	9.76%	1.10 [1.02-1.18]	$1.3x10^{-2}$	9.17%	10.09%	1.16 [1.09-1.23]	$1.3x10^{-6}$	9.50%	9.89%	1.13 [1.08-1.19]	$1.0 x10^{-7}$
rs10139154	14	31,147,498	*SCFD1*	34.10%	31.30%	1.07 [1.03-1.12]	$2.1x10^{-3}$	33.76%	31.17%	1.08 [1.04-1.12]	$1.9x10^{-5}$	33.90%	31.25%	1.08 [1.05-1.11]	$1.4 x10^{-7}$
rs10143310	14	92,540,381	*ATXN3*	24.85%	24.36%	1.09 [1.04-1.015]	$3.3x10^{-4}$	24.04%	22.95%	1.08 [1.04-1.13]	$2.6x10^{-4}$	24.36%	23.81%	1.09 [1.05-1.12]	$3.2 x10^{-7}$
rs9901522	17	14,673,934	*PMP22*	7.08%	6.31%	1.16 [1.06-1.26]	$5.2x10^{-4}$	6.87%	5.97%	1.16 [1.08-1.24]	$4.6x10^{-5}$	6.95%	6.18%	1.16 [1.10-1.22]	$8.6 x10^{-8}$

Table S5. Related to Figure 3; DbGaP/EGA studies contributing to the RVB analysis.

Accession Number	Study	Sample number	Females (%)
phs000179	Genetic Epidemiology of COPD (COPDGene)	2	100%
phs000254	NHLBI GO-ESP: Lung Cohorts Exome Sequencing Project (Cystic Fibrosis)	238	49.6%
phs000281	NHLBI GO-ESP: Women's Health Initiative Exome Sequencing Project (WHI) - WHISP	1904	100%
phs000290	NHLBI GO-ESP: Lung Cohorts Exome Sequencing Project (Pulmonary Arterial Hypertension)	73	82.2%
phs000291	NHLBI GO-ESP: Lung Cohorts Exome Sequencing Project (Lung Health Study of COPD)	332	37%
phs000296	NHLBI GO-ESP: Lung Cohorts Exome Sequencing Project (COPDGene)	285	52.6%
phs000307	NHLBI Framingham Heart Study Allelic Spectrum Project	1317	51.6%
phs000347	NHLBI GO-ESP: Family Studies (Aortic Disease)	29	34.5%
phs000354	NHLBI GO-ESP Family Studies: Pulmonary Arterial Hypertension	9	88.9%
phs000362	NHLBI GO-ESP: Family Studies: (Familial Atrial Fibrillation)	12	16.7%
phs000398	NHLBI GO-ESP: Heart Cohorts Exome Sequencing Project (ARIC)	800	54.6%
phs000400	NHLBI GO-ESP: Heart Cohorts Exome Sequencing Project (CHS)	186	28%
phs000401	NHLBI GO-ESP: Heart Cohorts Exome Sequencing Project (FHS)	348	36.8%
phs000402	NHLBI GO-ESP: Heart Cohorts Exome Sequencing Project (JHS)	296	58.8%
phs000403	NHLBI GO-ESP: Heart Cohorts Exome Sequencing Project (MESA)	259	45.2%
phs000422	NHLBI GO-ESP: Lung Cohorts Exome Sequencing Project (Asthma)	189	65.1%
phs000498	Jackson Heart Study Allelic Spectrum Project	1629	63.8%
phs000518	NHLBI GO-ESP Family Studies: Idiopathic Bronchiectasis	24	70.8%
phs000572	Alzheimer's Disease Sequencing Project (ADSP)	4655	58.8%
phs000632	NHLBI GO-ESP: Family Studies (Hematological Cancers)	19	36.8%
phs000651	Building on GWAS: the U.S. CHARGE consortium - Sequencing (CHARGE-S): FHS	550	61.5%
phs000667	Building on GWAS for NHLBI-Diseases: The U.S. CHARGE Consortium - Sequencing (CHARGE-S): CHS	1209	52.9%
phs000668	Building on GWAS: the U.S. CHARGE consortium - Sequencing (CHARGE-S): ARIC	5497	58.5%
phs000744	Yale Center for Mendelian Genomics (Y CMG)	1944	44.7%
phs000806	MIGen_ExS: Ottawa Heart Study	1966	33.1%
phs000814	MIGen_ExS: Italian Atherosclerosis Thrombosis and Vascular Biology	3591	11.3%
phs000908	Identification of Rare Variants in PD through Whole Exome Sequencing	105	66.7%
phs000917	MIGen_ExS: PROMIS	7298	17.9%
phs001000	MIGen_ExS: U. of Leicester	1081	0%
phs001101	MIGen_ExS: MDC	1075	44.7%
EGAO00000000079	UK10K	4062	65%
phs000101	NIH Exome Sequencing of Familial Amyotrophic Lateral Sclerosis Project	201	45%

Table S6. Related to Figure 2, 3; Quality control filtering of the FALS discovery and *KIF5A* replication cohorts.

FALS discovery cohort

Cohort	Cases	Controls
Initial Sample Set	1,463	41,410
Post HGDP Continental Ancestry Filter	1,397	24,563
Post Call Rate Filter	1,331	20,789
Post Heterozygosity Filter	1,319	20,664
Post Relatedness Filter	1,138	19,494

rs113247976 replication cohort (FALS discovery + ALS WXS/WGS replication cohort)

Cohort	Cases	Controls
Initial Sample Set	12,180*	21,533**
Post Call Rate Filter	11,916	21,050
Post Heterozygosity Filter	11,721	21,028
Post Ancestry Filter (PCA)	11,373	21,009
Post Relatedness & GWAS Checksum Filter	4,160	18,650

* All 1,138 FALS passing QC in FALS discovery cohort + 11,042 additional ALS WXS/WGS cases

** All 19,494 controls passing QC in FALS discovery cohort + 2,039 additional WXS/WGS controls

LOF screen (ALS WXS/WGS replication cohort)

Cohort	Cases	Controls
Initial Sample Set	11,042*	2,039**
Post Call Rate Filter	10,741	2,039
Post Heterozygosity Filter	10,549	2,026
Post Ancestry Filter (PCA)	10,201	2,008
Post Relatedness	9,046	1,955

* 11,042 additional ALS WXS/WGS cases not included in FALS discovery cohort

** 2,039 additional WXS/WGS controls not included in FALS discovery cohort

See Experimental Procedures for further details on filtering parameters.

Table S7. Related to Figure 3; RVB analysis according to mutation type across KIF5A and within gene sub-domains.

Analysis	FALS	Control	OR (95% CI)	*P*
Missense - Full CDS	9 (0.79%)	80 (0.41%)	1.93 (0.915-3.60)	$8.09x10^{-2}$
Missense - Motor Domain	3 (0.26%)	18 (0.09%)	3.27 (0.86-9.25)	$7.74x10^{-2}$
Missense - Microtubule Binding Domain	2 (0.18%)	8 (0.04%)	5.07 (0.95-18.52)	$5.57x10^{-2}$
Missense - Coiled-Coil Domain	3 (0.26%)	55 (0.28%)	1.01 (0.28-2.60)	$9.83x10^{-1}$
Missense - C-Terminal Domain	3 (0.26%)	7 (0.04%)	7.23 (1.74-24.55)	$9.41x10^{-3}$
Loss of Function	6 (0.53%)	3 (0.02%)	32.07 (9.05-135.27)	$5.55x10^{-7}$
Loss of Function (including frameshifts)	8 (0.70%)	3 (0.02%)	41.16 (12.61-167.57)	$3.77x10^{-9}$

FALS, familial ALS; OR, odds ratio; 95% CI, 95% confidence interval; CDS, coding sequence

Table S8. Related to Figure 3; Clinical information of probands and relatives carrying *KIF5A* LOF variants.

Position	Variant	Relation to Proband	DNA Available	Exon	cDNA	Description	Gender	Age of Onset (years)	Site of Onset	Survival (months)	Alive
57,975,729	GA>A	Proband	Y	26	c.2987delA	p.Asp996fs	M	45	n/a	n/a	n/a
57,976,382	C>T	Proband	Y	27	c.2993-3C>T	5' Splice Junction	M	29	L	>264	Y
57,976,382	C>T	Sister	Y	27	c.2993-3C>T	5' Splice Junction	F	52	L	84	N
57,976,382	C>T	Brother	Y	27	c.2993-3C>T	5' Splice Junction	M	18	L	324	N
		Brother	N				M	n/a	L	n/a	N
57,975,731	CA>C	Sporadic	Y	26	c.2989delA	p.Asn997fs	F	50	L	>96	Y
57,976,384	G>A	Sporadic	N	27	c.2993-1G>A	5' Splice Junction	n/a	52	B	n/a	n/a
57,976,385	GA>G	Proband	Y	27	c.2996delA	p.Asn999fs	M	42	L	>12	Y
		Brother	N				M	38	n/a	24	N
57,976,411	A>G	Proband	Y	27	c.3019A>G	p.Arg1007Gly	F	53	L	45	N
57,976,412	G>A	Proband	Y	27	c.3020G>A	p.Arg1007Lys	M	50	L	>108	Y
57,976,412	G>A	Proband	Y	27	c.3020G>A	p.Arg1007Lys	F	50	n/a	>240	Y
57,976,413	G>A	Proband	Y	27	c.3020+1G>A	3' Splice Junction	M	45	B	>220	Y
		Parent	N				n/a	47	n/a	156	N
		Uncle/Aunt	N				n/a	57	n/a	144	N
		Uncle/Aunt	N				n/a	55	n/a	121	N
		Uncle/Aunt	N				n/a	46	n/a	24	N
57,976,414	T>A	Proband	Y	27	c.3020+2T>A	3' Splice Junction	M	46	B	124	N
57,976,414	T>A	Brother	Y	27	c.3020+2T>A	3' Splice Junction	M	48	L	117	N
		Mother	N				F	35	L	144	N
57,976,415	A>G	Proband	Y	27	c.3020+3A>G	3' Splice Junction	M	50	B	54	N

All mutations were heterozygous; Genomic coordinates are based on Human Genome Assembly build 37; Protein change is based on transcript NM_004984.3; n/a, not applicable or not available

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