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Fogh, I., Lin, K., Tiloca, C. et al. (46 more authors) (2016) Association of a Locus in the CAMTA1 Gene With Survival in Patients With Sporadic Amyotrophic Lateral Sclerosis. JAMA Neurology, 73 (7). pp. 812-820. ISSN 2168-6149

https://doi.org/10.1001/jamaneurol.2016.1114

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A locus in the CAMTA1 gene is associated with survival in patients with sporadic amyotrophic lateral sclerosis

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Keywords
Amyotrophic lateral sclerosis, Genome wide association studies, survival, Cox proportional hazards regression analysis.
Summary (300 words)

Background
Amyotrophic lateral sclerosis (ALS) has a poor prognosis with a median survival of 3 years. However, a significant proportion of patients survive more than 10 years from symptom onset. Identification of gene variants influencing survival is crucial, as ALS is a rare disease and prevention is not feasible, leaving treatment that modifies survival the only realistic strategy. We performed a genome-wide association study (GWAS) to identify genetic modifiers of ALS survival.

Methods
We analysed survival in a large international GWAS data collection of 4255 patients (73.4% deceased) with genotype data extended to 7174392 variants by imputation analysis. We employed Cox proportional hazards regression under an additive model with adjustment for age of onset, sex and the first four principal components of ancestry, followed by meta-analysis. In a subset of 3439 patients Cox proportional hazards regression model was additionally adjusted by site of onset. Survival distribution for the most associated variants was assessed by Kaplan-Meier analysis.

Findings
We identified two novel loci significantly associated with ALS survival at 10q23 (rs139550538, p = 1.87x10^-9) and in the CAMTA1 gene at 1p36 (rs2412208, p = 3.53 x 10^-8). At locus 10q23 the adjusted hazard ratio for patients with the rs139550538 AA or AT genotype was 1.61 (95%CI: 1.38-1.89), corresponding to a 7 month reduction in survival compared to TT carriers. For rs2412208-CAMTA1 the adjusted hazard ratio for patients with the GG or GT genotype was 1.17 (95%CI 1.11-1.24), corresponding to a 4 month reduction in survival compared to TT carriers.

Interpretation
We have identified two loci that influence survival in ALS. Identification of the underlying mechanisms may suggest new therapeutic targets for ALS treatment.

Funding
This project was funded by the European Union FP7 and JPND programmes, the UK NIHR, the Motor Neurone Disease Association.
Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of motor neurons in which relentlessly progressive weakness of voluntary muscles leads to death from respiratory failure on average within 3 years of symptom onset. ALS is a heterogeneous disease with a poorly understood aetiology. Phenotypic variability in ALS is remarkable, comprising heterogeneity in disease duration, age and site of onset, and type of motor neuron affected. Several ALS genes have been identified. Of these, a massive hexanucleotide repeat expansion in the C9orf72 gene is the most common mutation in both familial and sporadic patients. Large genome wide association studies (GWAS) have identified a number of susceptibility genes including UNC13A and SARM1.

Despite the poor prognosis of ALS, about 5% of patients may survive more than 10 years. Long survivors are more likely to have primary lateral sclerosis, but all phenotypic patterns are represented. Younger age at onset correlates with longer survival, and other prognostic factors include disease progression rate at diagnosis, site of involvement at onset, certain phenotypic patterns (flail limb variants), cognitive impairment and respiratory involvement.

Previous studies have reported association of survival with single nucleotide polymorphisms (SNPs) in the KIFAP3 and UNC13A genes, although the KIFAP3 finding has not been replicated. Identification of gene variants influencing survival is crucial. ALS is a rare disease and prevention is not feasible, leaving treatment that modifies survival the only realistic strategy. An approach that leads to improved understanding of the biological basis of survival in ALS could lead to the development of a rational treatment. Therefore, to identify modifier genes that might influence ALS survival, we performed a GWAS using Cox proportional hazards regression model including age and site of onset as covariates followed by meta-analysis.

Methods

Samples and data

Genotypes were obtained from published GWAS of ALS sporadic patients from Italy, USA, UK, Ireland, Sweden, Belgium and France, including the Italian Consortium for the Genetics of ALS (SLAGEN) collected after ethnically approved informed consent (webappendix p 2, Table 1). Participating patients fulfilled the El Escorial revised criteria for ALS without a reported family history of ALS. Individuals included were of European ancestry by self-declaration. Clinical information was collected from medical notes, including date of last consultation, and survival data from death certificates, hospital or public records. Symptom onset was defined as the date of first weakness, speech or swallowing disturbances. Survival duration was defined as the difference between date of death/tracheostomy and date of symptom onset and, for those still alive, as the difference between censor date and symptom onset. The censor date was taken as date of last follow-up. Site of onset was defined as bulbar for those with first weakness affecting speech or swallowing, or spinal for those with limb or respiratory symptoms at onset.

Genotyping and quality control

Genotyping was performed on Illumina DNA microarrays as previously described. Standard GWAS quality control measures were used to exclude samples or SNP assays of poor quality (webappendix p 2, webappendix tables 2 and 3). Imputation was performed using 1000 Genomes Project Phase I version 3 (NCBI build 37, hg19 coordinates, August 2012) as the reference panel and IMPUTE2 software. Imputed genotypes were filtered for uncertainty of inferred genotype and minor allele frequency (MAF).

Statistical analysis

Multivariate Cox proportional hazards regression was modelled to estimate crude hazard ratios (HRs) and build by backward elimination (Wald test), estimation of hazard ratio, and 95% confidence interval (CI). Cox proportional hazards baseline model included age at onset (as continuous variable), gender and site at onset (bulbar versus spinal) as factor variables (webappendix table 6).
The proportional hazard assumption was tested by comparing the hazard curves stratified by sex, age and site at onset. All tests were two-tailed and significance was assessed at p <0·05 and performed in SPSS (version 22, IBM Corporation, Chicago, IL, USA).

The Cox proportional hazards model was applied genome-wide to filtered imputed data in each population, with the following independent variables: SNP genotype under a log-additive model, four principal components of ancestry, sex and age of onset. To maximise power in the exploratory analysis, site at onset was omitted in the final model due to the smaller numbers of patients (81%) with this information.

The model was built by backward elimination using the pacoxph program in the ProbABEL toolset to estimate for each SNP the hazard ratio with 95% CIs, model and covariate p-values. Statistical significance was assessed at the genome-wide level (p = 5 x 10^{-8}).

Summary statistics for 7174392 overlapping SNPs were combined in meta-analysis using METAL weighted by $\beta$-coefficients and the inverse of the corresponding standard errors; fixed-effects model was applied to adjust data from the seven independent studies. Genomic inflation was tested by Q-Q plots and factor lambda estimate ($\lambda_{BG}$ = 1·05) (webappendix p 2, fig.1).

The most associated variants were tested for heterogeneity of allele frequencies between studies by Cochran’s Q test (Q) (webappendix p 3, Table 7). SNPs achieving genome-wide significance in the meta-analysis were tested by Kaplan-Meier analysis and a log rank test. Kaplan–Meier curves for additive and dominant models were compared by $\chi^2$ likelihood ratio tests.

Role of the funding source

The funders had no role in study design, data collection, analysis or interpretation, nor writing of the report. All authors had full access to all the data and the corresponding authors had final responsibility for deciding to submit for publication.

Results

The international ALS cohort analysed in the present study included 4255 patients, 2591 male and 1664 female, of whom 3125 (73·4%) had died with median survival of 33 (interquartile range (IQR): 27) months. The mean age of onset including censored individuals was 59 (standard deviation (SD)±12) years (webappendix Table 4).

Site of onset data were available on a subset of 3439 (81%) patients (2066 male, 1372 female), 27% had bulbar onset, with a mean age of onset of 60·3 (SD±11·3), compared with spinal onset mean age 56·6 (SD±12·3) while median survival was 26·3 (IQR: 19-2) and 33·8 (IQR: 21-2-54) months in bulbar and spinal patients, respectively. Full details are reported in the webappendix Table 5.

There were 7174392 SNPs with genotypes passing quality control. There were two loci exceeding the genome-wide significance threshold, one on chromosome 10q23 and one on chromosome 1p36 (figure 1, table 1). At locus 10q23, the top ranked SNP was rs139550538, hazard ratio 1·61 (95% CI: 1·38, 1·89), p = 1·87x10^{-9}. This is a moderately rare variant (MAF 0·03), intronic within the insulin-degrading enzyme gene, IDE (figure 2).

At the 1p36 locus there were four SNPs exceeding genome wide significance, with the top ranked SNP being rs2412208, hazard ratio 1·17 (95% CI: 1·11, 1·24), p = 3·53 x 10^{-7} followed by 87 SNPs in strong linkage disequilibrium with rs2412208. All these SNPs fell within a 90 Kb region encompassing intron 3-4 of the calmodulin binding transcription activator 1 gene, CAMTA1 (figure 2 and table 1). Cox proportional hazard regression analyses conditioning upon the most associated SNPs in both loci showed no evidence of residual association.

Because rs139550538 is rare, Kaplan-Meier analysis was performed under a dominant model (226 patients (5·6%) carried at least one A allele). AA or AT genotype was associated with ALS survival (log rank p = 1·3 x 10^{-7}) and a median survival of 30·7 months compared with 36·7 months for the TT homozygotes (figure 3, table 2). Kaplan-Meier analysis of SNP rs2412208 under an additive model showed that carrying a G allele (45% of patients) was significantly associated with a decreased survival (log-rank p = 5·6x10^{-7}) with median survivals of 36-0 months (GG), 36-8 months (GT) and 40-8 months (TT) (Figure 4, Table2). The hazard ratio estimates were consistent across the seven datasets.
analysed (figure 5). Under a dominant model the results were similar (Figure 4) and a $\chi^2$ likelihood ratio test comparing the two models was not significant ($p = 0.12$), showing either could be valid.

We tested whether observed effect sizes ($\beta$) of the most associated SNPs from the combined Cox proportional hazard analysis were homogeneous across cases. There was some evidence for heterogeneity across the different datasets ($rs2412208 I^2 = 59.1\%$, $P = 0.02$) (webappendix table 7).

In the subset of 3439 ALS patients with clinical data information including site of onset information, Cox proportional hazards regression was modelled with this variable as additional covariate. The top rank SNP was rs2412208 at 1p36 with the combined hazard ratio of 1.19 (95% CI: 1.27-1.12; $p=5.11\times10^{-8}$) (webappendix Fig.2, Table 8) confirming association of CAMTA1 locus with ALS survival identified by the larger sample size when this covariate was excluded from the model. The SNP rs139550538 in IDE gene was less significant (HR 1.51; 95% CI: 1.27-1.78; $p=2.24\times10^{-6}$) possible because the lower frequency of this SNP in a reduced sample. Additionally, in linear regression analysis testing bulbar versus spinal phenotypes, this variant was not significantly associated ($p = 0.5$) (data not shown) indicating that the inclusion of site at onset as covariate in the Cox proportional hazard model was sufficient to decrease the strength of locus 10q23 association with survival.

Kaplan-Meier distribution of rs2412208 genotypes indicated that risk allele G was associated (log-rank $p=5\times10^{-6}$) with a shorter survival of 3-5 months corresponding to 19% increased rate of mortality compared to the TT homozygotes (webappendix Fig.3).

We examined previously reported candidate genes for ALS survival. SNP rs1541160 in the KIFAP3 gene was not significantly associated with survival in this study (HR 1.04; 95% CI: 0.98, 1.1; $p = 0.423$) (webappendix Fig 4), confirming previous findings. SNP rs12608932 in the UNC13A gene showed suggestive association with HR 1.17; 95% CI: 1.1, 1.24; $p = 0.003$ but coverage for this SNP was limited to a reduced subset of patients ($n = 3574$) (webappendix Fig. 5) and further studies on a larger scale are needed to validate the genetic effect of UNC13A as survival modifier. Of 105 SNPs tested in the DAO gene, the top ranked was rs4623951, with a hazard ratio of 1.07 (95%CI: 1.02-1.13, $p = 0.0053$).

**Discussion**

We have identified two loci associated with survival in patients with ALS at genome wide significance in a large meta-analysis using Cox proportional hazards regression analysis. The discovery of gene variants within IDE and CAMTA1 genes as survival modifiers in ALS is important both because of improved understanding of the disease process, and because the genes and associated pathways might become a target for therapy development. Furthermore, if gene variants have a large effect on survival, it is important to account for this in the design and analysis of clinical trials.

The effect size of the variants found is comparable to that of Riluzole, a drug shown to improve survival in ALS, for which the hazard ratio for those not taking Riluzole compared with those taking the drug is 1.14. A weakness of this study is that the extent of Riluzole use was not available to include in the analysis. Generally, rates of prescription are higher in countries in which access to healthcare is free or reimbursed than in those where private insurance is required, and if such differences correlate with allele frequency differences, a spurious association might arise. We have mitigated against this both by accounting for differences in allele frequency by ancestry using principal components, and by accounting for differences in Riluzole prescription rates by performing a meta-analysis stratified by country.

The most associated polymorphism at the 10q23 locus was a low frequency variant within the IDE gene, a zinc metallopeptidase that degrades intracellular insulin and other peptides such as beta-amyloid. Tagged proxies for this polymorphism were in weak ($r^2 < 0.4$) linkage disequilibrium and located in a neighbouring gene, KIF11, a motor kinesin-like protein involved in the spindle function during cell mitosis (figure 2A). The biological basis of this association is therefore unclear.

The most associated 87 variants in the CAMTA1 gene ($p \leq 10^{-5}$) map to a small 90 kb region within intron 3-4 (figure 2B) encompassing the CG-1 DNA-binding domain. The CG-1 motif is a functional domain with a nuclear localisation signal and transcriptional regulation properties that extends from exon 3 to exon 7 (6825092 to 7640553 base pair; GRCh37/hg19 Assembly) within CAMTA1. Intragenic CAMTA1 micro-rearrangements disrupting a CG-1 DNA binding domain have been reported to cosegregate with non-progressive congenital cerebellar ataxia (NPCA) and gait instability in several unrelated families. Common
variants within CAMTA1 have been reported to be associated with variation in human episodic memory\cite{26}. Mutant CAMTA1 knock-out mice, disrupted in the CG-1 domain, show severe ataxia and neuronal atrophy approximating the phenotype of haploinsufficiency observed in NPCA patients\cite{27}. Furthermore, the identification of the consensus sequences of the DNA-binding site of CG-1 domain combined with expression analyses in CAMTA1 knock-out mice have shown more than 80 neural related genes regulated by CAMTA1.\cite{1}

The finding of a gene involved in cerebellar disease in ALS is not surprising, given that ATXN2 trinucleotide repeat expansion causes spinocerebellar ataxia or ALS\cite{22}, the finding of C9orf72 pathology in the cerebellum of ALS patients\cite{23}, and the discovery of abnormal eye gaze in ALS patients\cite{24}. Thus there is increasing evidence of a relationship between ALS and cerebellar degeneration that is currently under-recognised, in the same way as the relationship between ALS and frontotemporal dementia remained undetected until recently.

Strength of this study is the large sample size. Use of the Cox proportional hazard model allowed us to include the nearly 27% of patients still alive (n = 1131) and means the analysis is not biased by restricting the analysis to a linear regression in those who have died. Weaknesses of our study include the difficulty of imputing low frequency variants; several significant or suggestive findings were of rare alleles. Better imputation panels and disease specific imputation panels will improve imputation of rare genetic variation.

In conclusion, we have identified genetic variants statistically significantly associated with survival. The promise of this research is not only to improve our understanding of the biology of the disease and suggest biological targets for pharmaceutical intervention to extend the survival time, but also to use genetic risk scores as an adjunct to clinical trials to account for the genetic contribution to survival.

References


