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RAPID COMMUNICATIONS

Association of Single Nucleotide Polymorphism at rs2275294 in the *ZNF512B* Gene with Prognosis in Amyotrophic Lateral Sclerosis

Haixia Jiang[1] · Baiyuan Yang[2] · Fang Wang[3] · Kelu Li[3] · Yongyun Zhu[3] · Bin Liu[3] · Hui Ren[3] · Sijia Tian[4] · Yanming Xu[4] · Ailan Pang[5] · Xinglong Yang[3]

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Abstract

The aim of this study is to explore whether the single nucleotide polymorphism rs2275294 in the *ZNF512B* gene is related to the length of survival of patients with amyotrophic lateral sclerosis (ALS). This prospective study examined 212 patients with ALS, who were genotyped at the rs2275294 locus in *ZNF512B* using the ligase method. Genotype was compared with clinical data and survival. Kaplan–Meier survival analysis and Cox hazard regression were used to identify risk factors of shorter survival. Our results were meta-analyzed together with previous work in order to examine the potential association between the rs2275294-C allele and survival. Of the 212 patients, 166 carried the CC + CT genotype at the rs2275294 locus, while 46 carried the TT genotype. Patients with the C allele showed significantly shorter survival than those without it (34.13 ± 1.9 vs. 45.32 ± 5.7 months, $p = 0.036$). Cox analysis identified the C allele and time from symptom onset to diagnosis as risk factors for shorter survival. Meta-analysis of 447 patients in China and Japan confirmed the rs2275294-C allele to be an independent risk factor of shorter survival in ALS patients. The C allele at the rs2275294 locus in *ZNF512B* is a risk factor for shorter survival in patients with ALS.

Keywords Amyotrophic lateral sclerosis · *ZNF512B* gene · rs2275294

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that involves both upper and lower motor neurons. The main symptom is progressive development of muscle weakness in bulbar and the limbs. Patients may require a ventilator to aid in breathing and they may die (Nowicka et al. 2019; Masrori and Damme 2020). The average survival time of ALS is only 3–4 years after diagnosis. The pathogenesis of ALS has not yet been completely elucidated, and genetic and environmental factors appear to play an important role (Mejzini et al. 2019). About 10% of patients have familial ALS, while the remaining cases of ALS are sporadic. Familial ALS has been associated with mutations in several genes, including *SOD1, OPN, C9orf72, TARDBP, FUS*, and *VCP* (Chia et al. 2018). Several genes have also been associated with risk of sporadic ALS (Es et al. 2007, 2009). For example, the C allele at locus rs2275294 in the *ZNF512B* gene has been associated with increased risk of ALS in the Japanese population (Iida et al. 2011). Our own work has associated polymorphism at the same locus to increased ALS risk in southwest China (Yang et al.

Haixia Jiang, Baiyuan Yang and Fang Wang contribute equally.

✉ Ailan Pang
879384106@qq.com

✉ Xinglong Yang
yxldoc11@163.com

1 Department of Anesthesia, First Affiliated Hospital of Kunming Medical University, Kunming 650032, Yunnan, People's Republic of China

2 Department of Neurology, Seventh People's Hospital of Chengdu, Chengdu 690041, Sichuan, People's Republic of China

3 Department of Geriatric Neurology, First Affiliated Hospital of Kunming Medical University, Kunming 650032, Yunnan, People's Republic of China

4 Department of Neurology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China

5 Department of Neurology, First Affiliated Hospital of Kunming Medical University, Kunming 650032, Yunnan, People's Republic of China

2015) and meta-analysis of studies also came to a similar conclusion (Ning et al. 2018). Another study in Japan has associated the rs2275294-C allele with significantly shorter survival among ALS patients (Tetsuka et al. 2013). Here, we examined whether the same might be true of ALS patients in southwest China.

Methods

Patient Enrollment

Patients older than 18 years diagnosed between 1 January 2010 and 31 December 2016 with ALS by at least two neurologists in the Department of Neurology of West China Hospital (Sichuan University) based on El Escorial criteria (Brooks et al. 2000) were prospectively enrolled in this study. This study was approved by the Ethics Committee of West China Hospital, and participants signed informed consent for their medical data to be analyzed and published in an anonymized format for research purposes.

Patients were excluded if they also suffered other acute or chronic diseases or immune system disorders, malignancy, muscle atrophy, or frontotemporal lobar dementia. Patients were also excluded if they were lost to follow-up.

Follow-Up and Clinicodemographic Data Collection

Clinical information was collected, including name, sex, age at disease onset, time of symptom onset, time from onset to diagnosis, and time from onset to last follow-up or death. Patients were followed up until 31 December 2019.

Genotyping

Genomic DNA was extracted from peripheral leukocytes of all patients using phenol–chloroform DNA extraction, and the genotype at the rs2275294 locus was genotyped using DNA ligation (Thomas et al. 2004). Data analysts were blinded to patient prognosis. For quality control, 20% of samples were re-analyzed by different researchers and got a replicate rate of 100%.

Statistical Analysis

SPSS 25.0 (IBM, Chicago, IL, USA) was used for statistical analysis. Inter-group differences were assessed for significance using the χ^2 or t test, and differences were considered significant when $p < 0.05$. Effects of risk genotypes on survival time were assessed using the Kaplan–Meier survival curve method and the log-rank test. Cox regression was performed to identify which of the following factors was associated with survival of ALS patients: risk genotype, sex, age at symptom onset, site of symptom onset, interval between symptom onset and diagnosis, and using NIPPV. The threshold for significance was defined as a two-tailed $p < 0.05$.

The results from the present study were meta-analyzed together with results from previous work using Stata 12.0 (StataCorp, College Station, TX, USA). Heterogeneity across the studies was assessed based on I^2 (Higgins et al. 2003).

Results

Characteristics of Patients with Different rs2275294 Genotypes

Of the 260 patients originally recruited, 48 were lost to follow-up, so the final analysis included 212 patients (148 men) with an average age at onset of 49.57 ± 11.44 years (Table 1). Most patients (172) showed muscle weakness in limbs as the first symptom. The distribution of rs2275294 genotype was in accordance with Hardy–Weinberg equilibrium ($p = 0.475$).

Table 1 Clinical characteristics of patients, stratified by genotype at rs2275294

Characteristic	Genotype		*p*
	CC + CT ($n = 166$)	TT ($n = 46$)	
Male	118 (71.1)	30 (65.21)	0.443
Age at onset, year	49.25 ± 11.48	50.70 ± 11.33	0.45
Bulbar as first symptom	31 (18.7%)	9 (19.6)	0.89
Survival, month since diagnosis	34.13 ± 1.9	45.32 ± 5.7	**0.036**
Time from onset to diagnosis, month	13.59 ± 12.39	18.73 ± 15.98	**0.020**

Values are *n* (%) or mean ± SD

The bold indicate statistical significance

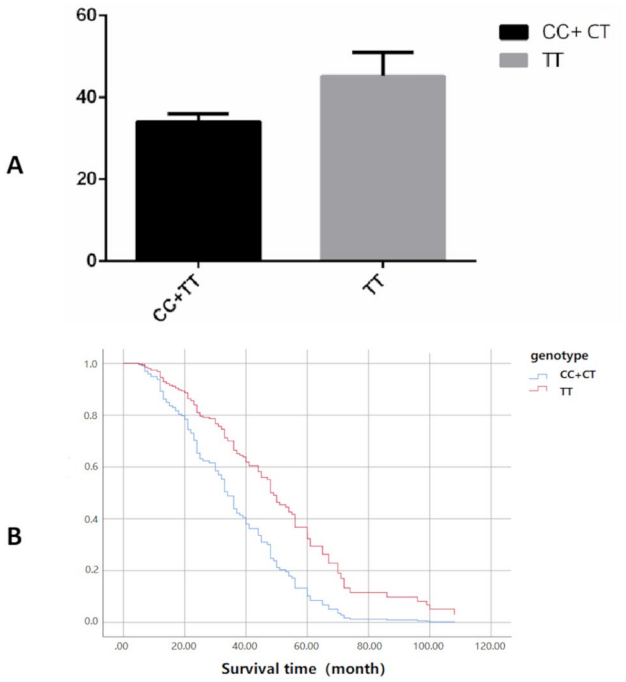

Fig. 1 Comparison of survival between ALS patients carrying the CC + CT or TT genotype at rs2275294. **a** Average survival time. **b** Kaplan–Meier survival curves

Survival of Patients with Different rs2275294 Genotypes

Patients carrying the C allele showed significantly shorter survival than those carrying the T allele (34.13 ± 1.9 vs 45.32 ± 5.7 months, $p = 0.036$; Fig. 1a), and Kaplan–Meier curves confirmed that the C allele was associated with shorter survival (Fig. 1b). To identify risk factors of shorter survival, Cox analysis was performed with the following variables: rs2275294 genotype, sex, age at onset, first symptoms, and time from onset to diagnosis. The C allele and time from onset to diagnosis were risk factors for poor prognosis (Fig. 2).

Next we combined our results from patients in southwest China with results from a study of patients from northeast China (Yu et al. 2018) and a study of patients in Japan (Tetsuka et al. 2013). Since the results showed no detectable heterogeneity ($I^2 = 0.0\%$, $p = 0.722$), they were meta-analyzed using a fixed-effect model. The rs2275294-C allele was confirmed to be an independent risk factor of poor prognosis in these Asian populations (hazard ratio 2.01, 95% confidence interval 1.43–2.82, $p < 0.001$; Fig. 3). No strong indication of publication bias was observed in funnel plots or in Egger's and Begg's tests (both $p > 0.05$) (data not shown).

Discussion

Our study found that among ALS patients from southwest China the C allele at the rs2275294 locus in an enhancer region of the *ZNF512B* gene is associated with shorter survival. We identified the C allele as an independent risk factor of shorter survival in this cohort, and we confirmed this finding through meta-analysis with another Chinese cohort and a Japanese cohort. We also identified time from onset to diagnosis as a risk factor of poor prognosis.

The *ZNF512B* gene (also known as *KIAA1196*), which encodes a protein of 893 residues, is expressed in the brain and spinal cord. Upregulation of ZNF512B activates TGF-β/Smad signaling, while downregulation inhibits this pathway (Houi et al. 2002). Activation of TGF-β signaling can improve motor symptoms in a transgenic mouse model of ALS based on mutations in the *SOD1* gene (Duque et al. 2020), and it can inhibit the cytoplasmic aggregation of TAR DNA-binding protein (TDP43), a hallmark of ALS (Schober et al. 2007). Therefore, TGF-β may exert a neuroprotective effect in ALS (Day et al. 2005). The C allele at rs2275294 appears to downregulate ZNF512B and weaken its binding to nuclear proteins (Iida et al. 2011). Therefore, rs2275294-C may reduce neuroprotective TGF-β/Smad signaling. This may explain why the allele is associated with increased risk of ALS (Yang et al. 2015), progression of the disease (Yu

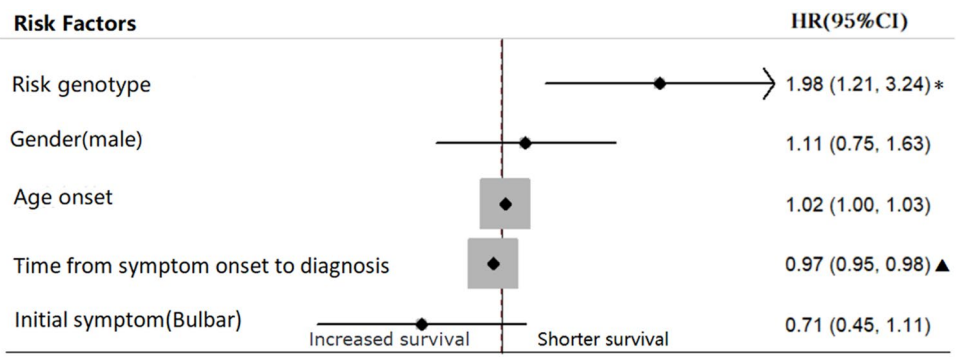

Fig. 2 Cox analysis to identify risk factors associated with shorter survival in ALS patients

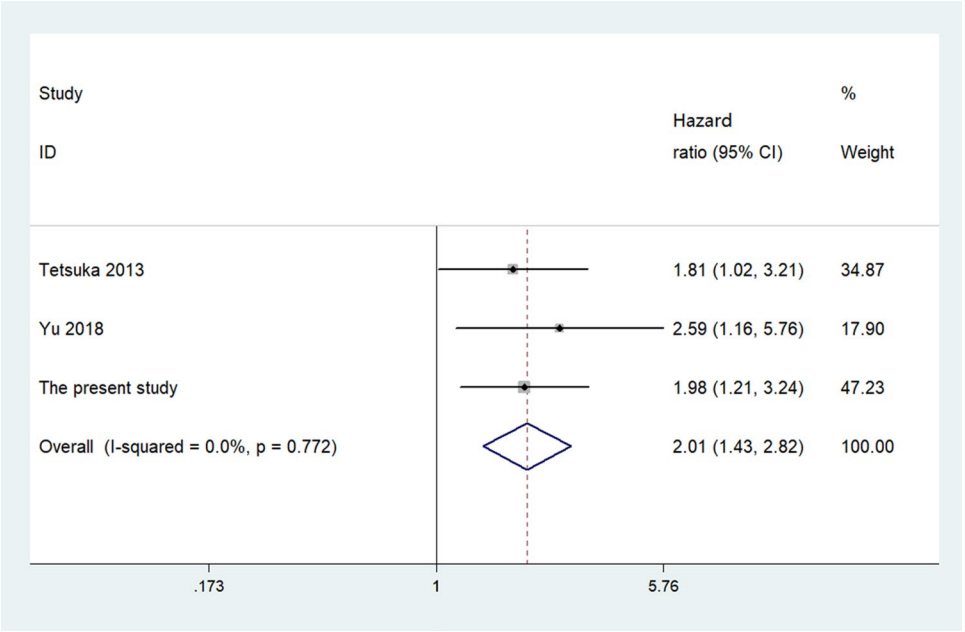

Fig. 3 Meta-analysis of survival in patients with different genotypes at rs2275294. Results were taken from the present study in southwest China, a study in Northeast China (Yu et al. 2018), and a study in Japan (Tetsuka et al. 2013)

et al. 2018), and shorter survival time [(Tetsuka et al. 2013) and the present study].

These considerations suggest that rs2275294-C is a prognostic marker in ALS, extending the list of polymorphisms that may help predict patient outcomes. Missense mutations in the *FUS* gene and increased copy number of the *C9ORF72* gene have been associated with worse prognosis (Westeneng et al. 2018). Our previous work also associated the AA genotype at the rs9268856 locus in the HLA-DRA/HLA-DR5 gene (Yang et al. 2017) and the CC genotype at the rs12608932 locus of the *UNC13A* gene (Yang et al. 2019) with shorter survival time. These results raise hope for identifying one or more single nucleotide polymorphic sites that may allow reliable prediction of prognosis in ALS.

Our observation of an association between shorter time from onset of ALS to diagnosis and shorter survival may reflect that early diagnosis is more likely to occur when the disease is rapidly progressing, such that clinical symptoms meet the diagnostic criteria even in early stages. We failed to observe a significant difference in survival between patients who were older or younger at disease onset; this negative result may reflect our small sample. Similarly, we did not observe a significant dependence of prognosis on the type of first symptom; this may reflect the low proportion of patients whose first symptoms involved the bulbar. Larger studies are needed to clarify the risk factors of ALS.

Our findings should be interpreted with caution, given some limitations. Firstly, the sample size was small, which may affect the result of the present study to some extent. Meanwhile, we didn't sequence the ZNF512B gene entirely; thus, some other valuable variants may also be neglected. In addition, whether 18.5% loss to follow-up in the present study confers a negative effect on our study was still unknown.

All in all, our results support an association of rs2275294 polymorphism with survival in ALS, which may be generalizable to other patient populations, at least within Asia, based on our meta-analysis. Future studies, preferably from multiple sites and involving different ethnicities, should examine this and other polymorphic loci in *ZNF512B* as well as loci in other genes in order to identify a reliable marker system for predicting prognosis.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

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