

Single-nucleotide Polymorphism rs2275294 in *ZNF512B* is not Associated with Susceptibility to Amyotrophic Lateral Sclerosis in a Large Chinese Cohort

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that primarily affects motor neurons and has no effective treatment. Recently, Iida *et al.* identified a single-nucleotide polymorphism (SNP) rs2275294 in the *ZNF512B* gene that is significantly associated with susceptibility to ALS in the Japanese population. Here, we performed a case-control study examining the possible association of rs2275294 with risk of sporadic ALS (SALS) in a large Chinese cohort.

Methods: To assess this association, we performed a replication study in 953 SALS patients and 1039 age- and gender-matched healthy control subjects, who were recruited from Peking University Third Hospital and the First Affiliated Hospital of Anhui Medical University from January 2004 to December 2013 throughout China. We genotyped the rs2275294 SNP using polymerase chain reaction and direct sequencing.

Results: The allele frequency of rs2275294 in *ZNF512B* was different between Japanese and Chinese. The association in Chinese between ALS patients and controls did not reach statistical significance ($P = 0.54$; odds ratio = 0.94; 95% confidence interval = 0.76–1.15).

Conclusions: The SNP rs2275294 in *ZNF512B* is not considered to be associated with ALS susceptibility in the Chinese population. Our study highlights genetic heterogeneity in ALS susceptibility in different population. Given our negative results, further replication study involving larger and more homogeneous samples in different ethnicities should be performed in the future.

Key words: Amyotrophic Lateral Sclerosis; Genome Association Study; Single-nucleotide Polymorphism; *ZNF512B*

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder caused by selective loss of motor neurons in the spinal cord, brainstem, and motor cortex, which is characterized by progressive wasting and weaknesses of the limb, bulbar, and respiratory muscles. In the absence of effective treatment, most patients die of respiratory failure within 3–5 years after onset.^[1] Worldwide, familial ALS (FALS) accounts for 10% of all ALS cases with the remainder comprising sporadic ALS (SALS), which may result from combinations of genetic and environmental factors.^[2] Eighteen different genes associated with FALS have been identified to date, including the superoxide

dismutase 1 (*SOD1*), TAR DNA-binding protein 43, fused in sarcoma/translocated in liposarcoma (*FUS/TLS*), and hexanucleotide repeat expansion in C9ORF72.^[3–5] Increasing numbers of ALS susceptibility and modifier loci have been suggested, and there has been a great deal of research progress regarding the genes and molecular mechanisms

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related to ALS.^[3,6,7] However, many remain unknown about the causes and mechanisms of ALS.

Although dozens of genes involved in FALS have been identified, little is known about the genetic contributions to SALS. Candidate gene studies have reported possible ALS risk genes, but they have been difficult to replicate. Some genome-wide association studies (GWASs) identified single-nucleotide polymorphisms (SNPs) in the *FGGY*, *ITPR2*, *DPP6*, *KIFAP3*, and *UNC13A* genes associated with SALS susceptibility.^[8-13] These results are promising, but remain slightly controversial.^[14-21] The association of the 9p21.2 locus has been independently replicated in four studies,^[19-23] but is not found in all population, including those from Japan to China.^[22,23] Our GWAS study identified two new susceptibility loci and suggested new pathogenic mechanisms of ALS.^[24] Therefore, more studies are necessary to evaluate and confirm these previously reported ALS susceptibility genes in the Chinese population.

Recently, Iida *et al.* performed a large-scale case-control association study using gene-based tag (SNPs) and identified a new SNP (rs2275294) in the *ZNF512B* gene that is significantly associated with an increased risk of SALS in the Japanese population.^[25] *ZNF512B* was originally identified as KIAA1196 in the Kazusa human cDNA sequencing project and was shown to be involved in cell differentiation and embryonic development. Similar to other transcription factors, *ZNF512B* was expressed in many tissues, including the brain and spinal cord.^[26,27] Tetsuka *et al.* reported that the *ZNF512B* gene is a prognostic factor in patients with ALS.^[28] In addition, *ZNF512B* was suggested to be an important positive regulator of transforming growth factor β (TGF- β) signaling with decreased *ZNF512B* expression related to increased susceptibility to ALS. However, our previous GWAS suggested genetic heterogeneity in ALS susceptibility in Han Chinese and European population.^[24] In this study, we checked the replication of the association by examining the rs2275294 SNP in 953 SALS patients and 1039 age- and sex-matched healthy controls in Han Chinese population.

METHODS

Participants

ALS patients diagnosed as having probable or definite ALS according to the El Escorial revised criteria and without family history (sporadic) were included in the study.^[29] A total of 953 ALS patients and 1039 healthy control subjects were recruited from Peking University Third Hospital and the First Affiliated Hospital of Anhui Medical University between January 2004 and December 2013 throughout China. All ALS and control subjects provided written informed consent to participate in the study in accordance with the process approved by the Institutional Ethics Committee of Peking University Third Hospital. Patients with mutations in *SOD1*, *TARDBP*, and *FUS* determined by sequencing of their genomic DNA were not included in the study. A total of 1039 age- and sex-matched healthy

control subjects with no previous personal or family history of neurodegenerative disease were also included as controls.

Genotyping

Genomic DNA was extracted from whole blood using standard protocols (Qiagen, Valencia, CA, USA). We genotyped the rs2275294 SNP using polymerase chain reaction (PCR) and direct sequencing.^[26] PCR was performed using 20 ng genomic DNA-derived from ALS and control subjects, and amplification was performed in the GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA, USA) under the following conditions: Initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 96°C for 30 s, annealing at 60–65°C for 30 s, extension at 72°C for 2 min, and postextension at 72°C for 7 min. The PCR products served as templates for direct sequencing using the fluorescent dye-terminator cycle sequencing method. Meta-analysis was performed by an inverse variance method.

Statistical analysis

We used exact methods to assess cases and controls separately for Hardy-Weinberg equilibrium (HWE). Values were expressed as *n* or mean \pm standard deviation (SD). Differences in genotype frequencies between the patients and controls were compared through a chi-squared test. Minor allele frequencies and odds ratios (ORs) with 95% confidence interval (CI) were estimated to determine the role of the SNP. Ages between the two groups were analyzed using Student's *t*-test. A two-tailed $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using the SPSS version 16.0 software package (SPSS Inc., Chicago, Illinois, USA).

RESULTS

The demographic information of the samples (953 SALS and 1039 controls) is shown in Table 1. The study population consisted of 953 patients, including 617 male patients (64.74%) and 336 female patients (35.26%). The male-to-female ratio for the cohort was 1.84:1. Limb onset occurred in 820 cases, while bulbar onset was observed in the others. The mean age at onset of the ALS patients was 50.91 ± 12.00 years, similar to that of the controls (50.24 ± 14.96 years) [Table 1].

The respective genotype distributions in ALS patients and controls did not significantly deviate from HWE. Table 2 summarizes the association of rs2275294 with

Table 1: Demographic description of SALS patients and control subjects in Han Chinese

Items	SALS (<i>n</i> = 953)	Control (<i>n</i> = 1039)
Male, <i>n</i> (%)	617 (64.74)	659 (63.43)
Female, <i>n</i> (%)	336 (35.26)	380 (36.57)
Age on set (years), mean \pm SD	50.91 \pm 12.00	50.24 \pm 14.96
Spinal onset, <i>n</i> (%)	820 (86.04)	–
Bulbar onset, <i>n</i> (%)	133 (13.96)	–

–: Data not applicable; SALS: Sporadic amyotrophic lateral sclerosis; SD: Standard deviation.

Table 2: Association of rs2275294 with ALS in Chinese compared to Japanese

Population	Samples (n)		Risk allele frequency		P	OR (95% CI)
	ALS	Controls	ALS	Controls		
Japanese (by Iida <i>et al.</i>)						
Discovery	454	958	0.49	0.42	6.3×10^{-4}	1.32 (1.13–1.55)
Set 1	249	1030	0.51	0.43	1.80×10^{-3}	1.37 (1.12–1.66)
Set 2	602	2256	0.48	0.42	5.60×10^{-5}	1.30 (1.14–1.48)
Combined	1305	4244			9.30×10^{-10}	1.32 (1.21–1.44)
Chinese (by Yang <i>et al.</i>)						
Replication	301	457	0.52	0.46	0.035	1.25 (1.02–1.53)
Chinese (in our study)						
Replication	953	1039	0.50	0.51	0.54	0.94 (0.76–1.15)

ALS: Amyotrophic lateral sclerosis; OR: Odds ratio; CI: Confidence interval.

ALS in Chinese compared to Japanese. However, the allele frequency of rs2275294 in *ZNF512B* was different between Chinese and Japanese population. There is no significant difference in the prevalence of this SNP (rs2275294) between ALS patients and controls ($P = 0.54$; $OR = 0.94$; $95\% CI = 0.76–1.15$). Based on the negative result of this replication study, we considered no association to exist between the rs2275294 SNP in *ZNF512B* and SALS susceptibility in the Chinese population.

DISCUSSION

The current report is a large-scale study of the genetic susceptibility to ALS, which we presented an SNP analysis of the *ZNF512B* gene in a Chinese cohort comprising 953 ALS cases and 1039 controls. This study confirms that the rs2275294 SNP in *ZNF512B* is negatively associated with susceptibility to ALS in Chinese ($P = 0.54$; $OR = 0.94$; $95\% CI = 0.76–1.15$). Meanwhile, we could not confirm whether the *ZNF512B* gene is a prognostic factor in Chinese ALS patients. However, our results help improve knowledge regarding *ZNF512B* in the Chinese population.

A previous study by Iida *et al.*^[25] suggested that a new SNP rs2275294 is significantly associated with an increased risk of ALS in Japanese subjects. This locus is located in an enhancer region of *ZNF512B*, which is a transcription factor regulating the TGF- β signaling pathway. This risk allele can decrease *ZNF512B* expression and consequently increase susceptibility to ALS. In addition, it was reported that *ZNF512B* is widely expressed in the spinal cord of ALS patients, and *ZNF512B* showed significantly increased expression in the motor neurons of ALS patients compared with controls. Iida *et al.*^[25] performed luciferase assays and electrophoretic mobility shift assays to determine allelic differences in the *ZNF512B* expression level and showed that *ZNF512B* expression level was lower in patients with susceptibility alleles compared with those with nonsusceptibility alleles. The expression of susceptibility alleles led to reduced *ZNF512B* enhancer activity, which in turn led to increased *ZNF512B* insufficiency, eventually resulting in insufficient TGF- β signaling activation and in reduced potential for motor neuron survival and functional recovery.

In the absence of effective treatments, most ALS patients die of respiratory failure within 3–5 years after onset. Tetsuka *et al.*^[28] used Kaplan–Meier survival curves to evaluate the prognosis of ALS in comparison with the survival time of patients without the risk allele. The results indicated that the mean survival time of patients without the risk allele was approximately 12 months longer than that of patients with the risk allele. In addition, Tetsuka *et al.*^[28] found that the *ZNF512B* gene was a new prognostic factor for ALS, independent of gender, age, time from onset to diagnosis, site of symptom onset (bulbar/spinal cord), and treatment with riluzole. However, the study was based on a small sample of 176 patients, and they obtained different results from the original research of Iida *et al.* included 1305 ALS patients. The small sample size might have influenced the results. Although we also conducted a study in 953 patients and performed statistical analyses and related methods, we obtained a negative result regarding the association between rs2275294 and SALS susceptibility in Chinese subjects. Therefore, our results were not similar to studies of Iida *et al.*^[25] and Tetsuka *et al.*^[28]

During our manuscript under revised, Yang *et al.* reported that the CC genotype and C allele at rs2275294 in *ZNF512B* are associated with increased risk of ALS in Han Chinese, particularly females, while no significant differences were identified in patients with PD.^[30] The study included 301 patients with ALS and 457 controls, as well as 555 patients with PD and 473 controls. The results agree with similar studies in Japan, suggesting that rs2275294 affects risk of ALS in East Asian population.

However, our study did not show any evidence of an association between SALS and the rs2275294 variant in the Chinese population. Similar to other controversial causative SNPs of SALS,^[14–23] there are conflicting results regarding rs2275294. There were several possible reasons for the negative association found in the replication analysis. First, ALS may be a more genetically and clinically heterogeneous disease among different population than previously recognized.^[24] Second, due to reciprocities of unknown specific gene–gene or gene–environment interactions in the different onset age, the

influences of some risk alleles identified by GWAS may be population-specific. Due to the different environmental and occupational factors, the age of onset of ALS patients from Guangzhou is younger than those from Shanghai.^[31] Third, sample size is very important, and a small sample size can result in insufficient power, thus bringing about type 1 errors in the results.^[3,32,33] Our sample size including 953 ALS and 1039 controls is much higher than the study conducted by Yang *et al.* (including 301 ALS and 457 controls). Therefore, different investigation may yield different results, and reliable identification of the risk alleles of ALS will require studies using much larger sample sizes. This emphasizes the importance of identifying and replicating risk loci of SALS in independent cohorts. Hence, the public availability of raw genotype data will be helpful for increasing the reliability of future study.

In summary, our replication findings did not provide evidence of an association between the variant rs2275294 in the *ZNF512B* gene and an increased risk of SALS in a Chinese cohort. Our study also highlights the genetic heterogeneity in ALS susceptibility in different population. Given our negative results, future research would benefit from increased sample sizes in different ethnicities and to understand the more genetic heterogeneity fully.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Andersen PM, Al-Chalabi A. Clinical genetics of amyotrophic lateral sclerosis: What do we really know? *Nat Rev Neurol* 2011;7:603-15.
- Al-Chalabi A, Hardiman O. The epidemiology of ALS: A conspiracy of genes, environment and time. *Nat Rev Neurol* 2013;9:617-28.
- Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci* 2014;17:17-23.
- Wroe R, Wai-Ling Butler A, Andersen PM, Powell JF, Al-Chalabi A. ALSOD: The amyotrophic lateral sclerosis online database. *Amyotroph Lateral Scler* 2008;9:249-50.
- Yoshida M, Takahashi Y, Koike A, Fukuda Y, Goto J, Tsuji S. A mutation database for amyotrophic lateral sclerosis. *Hum Mutat* 2010;31:1003-10.
- Ingre C, Roos PM, Piehl F, Kamel F, Fang F. Risk factors for amyotrophic lateral sclerosis. *Clin Epidemiol* 2015;7:181-93.
- Leblond CS, Kaneb HM, Dion PA, Rouleau GA. Dissection of genetic factors associated with amyotrophic lateral sclerosis. *Exp Neurol* 2014;262(Pt B):91-101.
- Schymick JC, Scholz SW, Fung HC, Britton A, Arepalli S, Gibbs JR, *et al.* Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls: First stage analysis and public release of data. *Lancet Neurol* 2007;6:322-8.
- Dunckley T, Huentelman MJ, Craig DW, Pearson JV, Szelinger S, Joshipura K, *et al.* Whole-genome analysis of sporadic amyotrophic lateral sclerosis. *N Engl J Med* 2007;357:775-88.
- van Es MA, Van Vught PW, Blauw HM, Franke L, Saris CG, Andersen PM, *et al.* ITPR2 as a susceptibility gene in sporadic amyotrophic lateral sclerosis: A genome-wide association study. *Lancet Neurol* 2007;6:869-77.
- van Es MA, van Vught PW, Blauw HM, Franke L, Saris CG, Van den Bosch L, *et al.* Genetic variation in DPP6 is associated with susceptibility to amyotrophic lateral sclerosis. *Nat Genet* 2008;40:29-31.
- van Es MA, Veldink JH, Saris CG, Blauw HM, van Vught PW, Birve A, *et al.* Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat Genet* 2009;41:1083-7.
- Landers JE, Melki J, Meisinger V, Glass JD, van den Berg LH, van Es MA, *et al.* Reduced expression of the Kinesin-Associated Protein 3 (KIFAP3) gene increases survival in sporadic amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 2009;106:9004-9.
- Fernández-Santiago R, Sharma M, Berg D, Illig T, Anneser J, Meyer T, *et al.* No evidence of association of FLJ10986 and ITPR2 with ALS in a large German cohort. *Neurobiol Aging* 2011;32:551.e1-4.
- Fogh I, D'Alfonso S, Gellera C, Ratti A, Cereda C, Penco S, *et al.* No association of DPP6 with amyotrophic lateral sclerosis in an Italian population. *Neurobiol Aging* 2011;32:966-7.
- Chiò A, Schymick JC, Restagno G, Scholz SW, Lombardo F, Lai SL, *et al.* A two-stage genome-wide association study of sporadic amyotrophic lateral sclerosis. *Hum Mol Genet* 2009;18:1524-32.
- Daoud H, Belzil V, Desjarlais A, Camu W, Dion PA, Rouleau GA. Analysis of the UNC13A gene as a risk factor for sporadic amyotrophic lateral sclerosis. *Arch Neurol* 2010;67:516-7.
- Laaksovirta H, Peuralinna T, Schymick JC, Scholz SW, Lai SL, Myllykangas L, *et al.* Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: A genome-wide association study. *Lancet Neurol* 2010;9:978-85.
- Shatunov A, Mok K, Newhouse S, Weale ME, Smith B, Vance C, *et al.* Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: A genome-wide association study. *Lancet Neurol* 2010;9:986-94.
- Chen Y, Zeng Y, Huang R, Yang Y, Chen K, Song W, *et al.* No association of five candidate genetic variants with amyotrophic lateral sclerosis in a Chinese population. *Neurobiol Aging* 2012;33:2721.e3-5.
- Chen X, Huang R, Chen Y, Zheng Z, Chen K, Song W, *et al.* Association analysis of four candidate genetic variants with sporadic amyotrophic lateral sclerosis in a Chinese population. *Neurol Sci* 2014;35:1089-95.
- Chen X, Chen Y, Guo X, Cao B, Wei Q, Ou R, *et al.* Replication analysis of genetic variants on 17q11.2 and 9p21.2 with sporadic amyotrophic lateral sclerosis and Parkinson's disease in a Chinese population. *Neurobiol Aging* 2015. pii: S0197-458000392-9.
- Iida A, Takahashi A, Deng M, Zhang Y, Wang J, Atsuta N, *et al.* Replication analysis of SNPs on 9p21.2 and 19p13.3 with amyotrophic lateral sclerosis in East Asians. *Neurobiol Aging* 2011;32:757.e13-4.
- Deng M, Wei L, Zuo X, Tian Y, Xie F, Hu P, *et al.* Genome-wide association analyses in Han Chinese identify two new susceptibility loci for amyotrophic lateral sclerosis. *Nat Genet* 2013;45:697-700.
- Iida A, Takahashi A, Kubo M, Saito S, Hosono N, Ohnishi Y, *et al.* A functional variant in ZNF512B is associated with susceptibility to amyotrophic lateral sclerosis in Japanese. *Hum Mol Genet* 2011;20:3684-92.
- Nagase T, Ishikawa K, Suyama M, Kikuno R, Hirose M, Miyajima N, *et al.* Prediction of the coding sequences of unidentified human genes. XIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. *DNA Res* 1999;6:63-70.
- Tili E, Michaille JJ, Liu CG, Alder H, Taccioli C, Volinia S, *et al.* GAM/ZFp/ZNF512B is central to a gene sensor circuitry involving cell-cycle regulators, TGF {beta} effectors, Drosha and microRNAs with opposite oncogenic potentials. *Nucleic Acids Res* 2010;38:7673-88.
- Tetsuka S, Morita M, Iida A, Uehara R, Ikegawa S, Nakano I.

- ZNF512B gene is a prognostic factor in patients with amyotrophic lateral sclerosis. *J Neurol Sci* 2013;324:163-6.
29. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1:293-9.
 30. Yang X, Zhao Q, An R, Zheng J, Tian S, Xu Y. Association of the functional SNP rs2275294 in ZNF512B with risk of amyotrophic lateral sclerosis and Parkinson's disease in Han Chinese. *Amyotroph Lateral Scler Frontotemporal Degener* 2015:1-6.
 31. Liu MS, Cui LY, Fan DS; Chinese ALS Association. Age at onset of amyotrophic lateral sclerosis in China. *Acta Neurol Scand* 2014;129:163-7.
 32. Pearson TA, Manolio TA. How to interpret a genome-wide association study. *JAMA* 2008;299:1335-44.
 33. Marangi G, Traynor BJ. Genetic causes of amyotrophic lateral sclerosis: New genetic analysis methodologies entailing new opportunities and challenges. *Brain Res* 2015;1607:75-93.