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ORIGINAL ARTICLE

ERCC6L2 rs591486 polymorphism and risk for amyotrophic lateral sclerosis in Greek population

Efthimios Dardiotis[1] · Emmanouil Karampinis[1] · Vasileios Siokas[1] · Athina-Maria Aloizou[1] · Dimitrios Rikos[1] · Styliani Ralli[1] · Dimitra Papadimitriou[2] · Dimitrios P. Bogdanos[3,4] · Georgios M. Hadjigeorgiou[5]

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Abstract

Background A number of genetic variants have been associated with amyotrophic lateral sclerosis (ALS). A recent study supports that rs591486 across the ERCC6L2 gene and exposure to pesticides seem to have a joint effect on the development of Parkinson's disease, a disease which shares a few common characteristics with ALS.

Objective To detect a possible contribution of rs591486 ERCC6L2 to ALS.

Methods A total of 155 patients with ALS and 155 healthy controls were included in the study and genotyped for rs591486. Using logistic regression analyses (crude and adjusted for age and sex), rs591486 was tested for association with ALS risk. Subgroup analysis based on ALS site of onset was also performed. Cox regression analysis was applied in order for the effect of ERCC6L2 rs591486 on ALS age of onset to be tested.

Results Adjusted analysis showed that ERCC6L2 rs591486 was associated with an increased risk of ALS development, in dominant [odds ratio, OR (95% confidence interval, CI) 2.15 (1.04–4.46), $p = 0.037$] and over-dominant [OR (95%CI) = 1.91 (1.01–3.60), $p = 0.043$], modes. Subgroup analysis based on ALS site of onset revealed an association between ERCC6L2 rs591486 and ALS with limb onset. Results for Cox regression analysis indicated that G/A carriers had a lower age of ALS limb onset when compared to G/G carriers.

Conclusions The current study provides preliminary indication for an implication of ERCC6L2 rs591486 in ALS development, as a possible genetic risk factor. These results possibly suggest that oxidative stress may be the main contributor in the pathophysiology of ALS.

Keywords ALS · Oxidative stress · Pesticides · Polymorphism · ERCC6L2

Emmanouil Karampinis and Vasileios Siokas contributed equally to this work.

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✉ Efthimios Dardiotis
edar@med.uth.gr

[1] Department of Neurology, University Hospital of Larissa, Laboratory of Neurogenetics, Faculty of Medicine, University of Thessaly, Biopolis, Mezourlo Hill, 41100 Larissa, Greece

[2] Neurological Department, Henry Dunant Hospital Center, 107 Mesogeion Avenue, 11526 Athens, Greece

[3] Department of Rheumatology and Clinical Immunology, University General Hospital of Larissa, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 40500 Larissa, Greece

[4] Cellular Immunotherapy & Molecular Immunodiagnostics, Biomedical Section, Centre for Research and Technology-Hellas (CERTH)- Institute for Research and Technology-Thessaly (IRETETH), 41222 Larissa, Greece

[5] Department of Neurology, Medical School, University of Cyprus, Nicosia, Cyprus

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive adult-onset disease that primarily affects the upper and lower motor neurons, as well as the frontotemporal and other regions of the brain [1, 2]. It is characterized by increasing muscular atrophy, loss of strength, paralysis, and death, particularly due to respiratory failure [3, 4]. The etiology of ALS still largely remains unknown; however, a few genetic and environmental factors seem to be implicated in its development [5–9].

ALS has two forms; a familial and a sporadic one, both of which are caused by the accumulation of abnormal proteinaceous aggregations in motor neurons and glial cells [10]. Excitotoxicity, oxidative stress, mitochondrial dysfunction, neuroinflammation, altered energy metabolism, and RNA misprocessing are the main pathological factors responsible for the impaired homeostasis which creates these inclusions of the abnormal proteins [11]. CNS is susceptible to oxidative stress [12]. This is the consequent of the fact that the neuronal membrane presents a high abundance of polyunsaturated fatty acids as well as oxygen, and a relatively low component of antioxidants [13]. Therefore, oxidative stress is considered to be the most commonly identified contributor to ALS-associated degeneration [14].

The familial form of ALS accounts for 10% of the ALS cases [15] and is inherited following dominant traits, frequently with high penetration [16]. Advances In technology have permitted broad DNA sequencing in sporadic ALS and revealed that genetic polymorphisms in establishing genes are not rare; for example, SOD1 mutations occur in 2% of sporadic ALS [16].

Apart from genetics, exogenous and environmental factors, such as environmental toxicants, smoking, infections, and traumatic brain injury among others, have been incriminated for possible contribution to ALS development as well [17]. However, it should be mentioned that neither genetic architecture nor environmental factors seem to be sufficient for the development of ALS on their own [5, 18].

ERCC6L2 (excision repair cross-complementing rodent repair deficiency complementation group 6-like 2) is a gene located in chromosome 9, implicated in DNA repair and mitochondrial function [19]. The ERCC6L2 gene product belongs to the snf2 family of helicase-related proteins [19]. Snf2 is the catalytic subunit of the chromatin remodeling complex [20]. The ERCC6L2 protein is involved in transcription regulation, DNA repair, DNA translation, and chromatin unwinding [21]. It consists of 712 amino acids [19] and has the characteristics of an early DNA damage response protein that traffics to mitochondria and the nucleus after genotoxic stress in a reactive oxygen species (ROS)-dependent fashion [19]. Its parallel increase with ROS indicates that this protein can be associated with alterations in cellular homeostasis [19], as well as oxidative stress, which holds crucial pathological significance in neurodegenerative diseases.

Recent results from genome-wide gene-environment interaction analysis of pesticide exposure and risk of PD support that SNPs across the ERCC6L2 gene (namely rs67383717 and rs591486) and exposure to pesticides seem to have a joint effect on the development of PD [22]. Considering the similarities of the two diseases (ALS and PD) in terms of causative risk factors, as discussed above [23], as well as the impact of ERCC6L2 and especially of the rs591486 SNP on PD (due to the strong association that was found), we deemed that it would be useful to study whether there is an association between ALS susceptibility and the rs591486 ERCC6L2 gene variant. Therefore, the aim of this study is to examine the effect of the ERCC6L2 rs591486 to ALS development.

Methods

Participants

ALS was diagnosed by a specialist neurologist following the El Escorial criteria [24]. Only patients with definite ALS were included. The study protocol was approved by the local ethics committees and written informed consent was obtained from all participants included in the study.

DNA isolation and genotyping procedure

Peripheral blood leucocytes were used for DNA extraction with the method of salting out [25, 26]. Using TaqMan allele specific discrimination assays on an ABI PRISM 7900 Sequence Detection System, tag SNPs were genotyped, and with the SDS software (Applied Biosystems, Foster City, CA, USA), they were analyzed. The personnel that performed the experimental work, was unaware of information regarding the participants [25]. Ten percent of the samples (randomly selected) was regenotyped, without revealing any conflict. The genotype call rate was 99.03%.

Statistical analysis

The exact test, with a p value of ≤ 0.05, was indicative for deviation from the Hardy-Weinberg equilibrium (HWE) [27]. The study's statistical power [28] was calculated using the CaTS (http://www.sph.umich.edu/csg/abecasis/cats) Power Calculator for Genetic Studies (Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA) [29].

Using binary logistic regression models (univariate and after adjustment for age and sex), odds ratios (ORs) and 95% confidence intervals (CIs) were calculated in order for possible associations between rs591486 and ALS risk to be estimated. Analysis was performed with the SNPStats

software (http://bioinfo.iconcologia.net/SNPstats/) [30], assuming the co-dominant (genotypic), dominant, recessive, over-dominant, and additive models of inheritance. We further performed subgroups analyses based on ALS site of onset. More precisely, we evaluated the effect of the rs591486 on patients with ALS with bulbar onset and on patients with limb onset by comparing them with controls.

The effect of the SNP genotype on the age of ALS onset, compared to the homozygosity for the wild allele, was evaluated using Cox proportional hazard regression models [31]. Subgroup cox analyses based on site of onset was also performed, as previously described.

A value smaller than 0.05 was set as the statistically significant threshold. With SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) statistical analysis was carried out.

Results

In total, 310 individuals were recruited for this study; 155 patients with definite ALS [77 (49.7%) female, mean age ± standard deviation (SD) = 63.74 ± 11.30 years], and 155 matched for age and sex healthy controls. The majority of the ALS patients mentioned alcohol consumption (67.1%) or smoking (68.4%). The most common site of onset was the lower limbs (34.8%), followed by the bulbar type (32.3%).The main demographic characteristics of the ALS cohort participants are displayed in Table 1.

Table 1 Demographic characteristics of the ALS cohort

Characteristics ($n = 155$)	Mean (SD)
Age (years)	63.74 (11.30)
	Frequency (%)
Female ($n = 77$)	49.7
Left-headed ($n = 19$)	12.2
Years of education	
≤ 6 ($n = 114$)	73.6
> 6 ($n = 41$)	26.4
Alcohol consumption	
Yes ($n = 104$)	67.1
No ($n = 51$)	32.9
Smoking	
Yes ($n = 106$)	68.4
No ($n = 49$)	31.6
Site of onset	
Mainly bulbar ($n = 50$)	32.3
Bulbar and limbs ($n = 8$)	5.2
Lower limbs ($n = 54$)	34.8
Upper limbs ($n = 34$)	21.9
Lower and upper limbs ($n = 9$)	5.8

SD, standard deviation

Fischer's exact test revealed that rs591486 was in HWE in both ALS cases and healthy controls group, with p values equal to 0.26 and 0.078 respectively. Based on the power analysis, our study had a power of 80.0% to detect an association of a variant with a genetic relative risk of 1.51, assuming the multiplicative model, MAF equal to 48%, and type I error level of 0.05.

The present study included data from 310 individuals; 155 healthy controls and 155 individuals with ALS. Analysis performed to assess the genotypic frequencies for ERCC6L2 rs591486 showed that genotypes G/G, G/A, and A/A were found in 48 (31%), 66 (43%), and 40 (26%) of the healthy controls. Concerning the individuals with ALS, the results for G/G, G/A, and A/A were 31 (20%), 84 (55%), and 38 (25%), as well. The allelic frequencies in healthy individuals were 53% (162 individuals) for the G allele and 47% (146 individuals) for the A allele, whereas the results for ALS patients were 48% (146 individuals) for the G allele, and 52% (160 individuals) for the A allele. Allele and genotype frequencies in ALS cases and in healthy controls are shown in Supplementary Table 1.

According to univariate single locus logistic regression analysis, ERCC6L2 rs591486 was significantly associated with ALS, particularly in dominant and over-dominant mode. For the dominant mode specifically, ERCC6L2 rs591486 was associated with an increased risk of ALS development [odds ratio, OR (95% confidence interval, CI) 1.78 (1.06–3.00), p = 0.028]. Additionally, heterozygosity (G/A) of the SNP studied in over-dominant mode was found to significantly contribute to ALS [OR (95%CI) = 1.62 (1.03–2.55), $p = 0.035$]. A non-statistically significant trend for association was also found in the co-dominant mode for the G/A genotype [OR (95%CI) = 1.97 (1.13–3.43), $p = 0.052$]. The statistically significant results were maintained after adjustment for age and sex in both dominant [OR (95%CI) = 2.15 (1.04–4.46), $p = 0.037$] and over-dominant mode [OR (95%CI) = 1.91 (1.01–3.60), p = 0.043]. ORs, CIs, and p values for all modes for the comparison between the whole ALS sample and the controls are presented in Table 2.

Subgroup adjusted for age and sex analysis, based on the ALS site of onset, revealed an association between ERCC6L2 rs591486 and ALS with limb onset in dominant [OR (95%CI) = 3.90 (1.46–10.38), $p = 0.0038$], log-additive [OR (95%CI) = 1.78 (1.04–3.06), $p = 0.033$], and co-dominant [$p = 0.015$, with OR (95%CI) = 4.06 (1.46–11.30) for the G/A genotype and OR (95%CI) = 3.57 (1.13–11.27) for the A/A genotype] modes. ORs, CIs, and p values for all modes for the comparison between the ALS subgroups based on the site of onset and the controls are presented in Table 3.

Cox proportional hazard regression analyses (Tables 4 and 5) showed that the rs591486 had a significant effect on the age of onset of ALS with limb onset. In particular, individuals carrying the G/A genotype of the rs591486 had a significantly

Table 2 Single locus analysis (crude and adjusted for age and sex) for association between ERCC6L2 rs591486 and ALS, in co-dominant, dominant, recessive, over-dominant, and log-additive modes

Mode	Genotype	Univariate		Multivariate	
		OR (95%CI)	*p* value	OR (95%CI)	*p* value
Co-dominant	G/G	1.00	0.052	1.00	0.07
	G/A	1.97 (1.13–3.43)		2.45 (1.13–5.31)	
	A/A	1.47 (0.78–2.77)		1.66 (0.68–4.05)	
Dominant	G/G	1.00	*0.028*	1.00	*0.037*
	G/A-A/A	*1.78 (1.06–3.00)*		*2.15 (1.04–4.46)*	
Recessive	G/G-G/A	1.00	0.82	1.00	0.82
	A/A	0.94 (0.56–1.57)		0.92 (0.45–1.90)	
Over-dominant	G/G-A/A	1.00	*0.035*	1.00	*0.043*
	G/A	*1.62 (1.03–2.55)*		*1.91 (1.01–3.60)*	
Log-additive	–	1.21 (0.88–1.66)	0.23	1.29 (0.83–2.01)	0.25

ERCC6L2, excision repair cross-complementing rodent repair deficiency, complementation group 6-like 2; *ALS*, amyotrophic lateral sclerosis; *CI*, confidence interval; *OR*, odds ratio. Statistical significant values are given in italic

younger age of onset of ALS with limb onset compared to the wildtype G/G genotype [hazard ratio (95% CI) 2.180 (1.091–4.357), $p = 0.027$, and mean age of limb onset ALS onset: 60.20 years for G/A and 66.73 years for G/G carriers].

Discussion

In the current study, we performed a single locus analysis in order to detect a possible association between rs591486 ERCC6L2 and ALS risk in a Greek cohort. We found that rs591486 was associated with an increased risk of ALS. Moreover, we found that rs591486 ERCC6L2 influences the age of onset of ALS with limb onset. Therefore, our results provide preliminary indication for a potential link between ERCC6L2 genetic variability and the development of ALS.

ERCC6L2 is a helicase implicated in DNA repair and mitochondrial function [19]. It is an early DNA damage response protein that traffics to mitochondria and the nucleus after genotoxic stress, and also its presence is related to an increase of ROS [19]. Cellular augmentation of ROS significantly contributes to processes that damage tissues via oxidation, through mechanisms such as apoptosis and autophagy [32]. Consequently, ERCC6L2 could be implicated in changes in cellular homeostasis causing oxidative stress. Since CNS is rather susceptible to oxidation due to the conformation of its

Table 3 Single locus analysis (crude and adjusted for age and sex) for association between ERCC6L2 rs591486 and ALS based on site of disease onset (bulbar or limbs), in co-dominant, dominant, recessive, over-dominant, and log-additive modes

Mode	Genotype	BULBAR ONSET				LIMB ONSET			
		Univariate		Multivariate		Univariate		Multivariate	
		OR (95%CI)	*p* value	OR (95%CI)	*p* value	OR (95%CI)	*p* value	OR (95%CI)	*p* value
Co-dominant	G/G	1.00	0.17	1.00	0.19	1.00	0.087	1.00	*0.015*
	G/A	2.33 (0.80–6.79)		3.23 (0.81–12.91)		2.31 (1.07–5.01)		*4.06 (1.46–11.30)*	
	A/A	2.64 (0.85–8.23)		3.06 (0.65–14.38)		1.85 (0.78–4.41)		*3.57 (1.13–11.27)*	
Dominant	G/G	1.00	0.063	1.00	0.069	1.00	*0.034*	1.00	*0.0038*
	G/A-A/A	2.45 (0.89–6.74)		3.17 (0.86–11.69)		*2.14 (1.03–4.46)*		*3.90 (1.46–10.38)*	
Recessive	G/G-G/A	1.00	0.34	1.00	0.56	1.00	0.88	1.00	0.51
	A/A	1.49 (0.66–3.37)		1.44 (0.43–4.81)		1.05 (0.54–2.04)		1.33 (0.57–3.09)	
Over-dominant	G/G-A/A	1.00	0.46	1.00	0.26	1.00	0.089	1.00	0.062
	G/A	1.33 (0.62–2.86)		1.84 (0.62–5.44)		1.67 (0.92–3.01)		2.03 (0.96–4.31)	
Log-additive	–	1.55 (0.92–2.60)	0.098	1.71 (0.82–3.59)	0.15	1.32 (0.88–1.98)	0.18	*1.78 (1.04–3.06)*	*0.033*

ERCC6L2, excision repair cross-complementing rodent repair deficiency, complementation group 6-like 2; *ALS*, amyotrophic lateral sclerosis; *CI*, confidence interval; *OR*, odds ratio. Statistical significant values are given in italic

Table 4 Cox regression analysis for single locus association of the ERCC6L2 rs591486 with age at onset of ALS (crude and adjusted for sex)

Genotype	Univariate		Multivariate	
	HR (95%CI)	*p* value	HR (95%CI)	*p* value
G/G	1.00	–	1.00	–
G/A	1.257 (0.832–1.900)	0.277	1.275 (0.843–1.929)	0.249
A/A	1.039 (0.645–1.675)	0.874	0.988 (0.610–1.599)	0.960

ERCC6L2, excision repair cross-complementing rodent repair deficiency, complementation group 6-like 2; *ALS*, amyotrophic lateral sclerosis; *CI*, confidence interval; *HR*, hazard ratio

key proteins, the aforementioned change could possibly lead to the accumulation of abnormal proteins and, consequently, to cell death [33, 34].

Rs591486 is present within intron 2 of the ERCC6L2 gene. Therefore, our results should be interpreted with caution as rs591486 may or may not affect the function of the ERCC6L2 gene itself. It could also represent a regulatory element for a different gene, or even be in LD with a distant SNP. Therefore, a tagging SNP selection approach could have possibly revealed the effect of the entire ERCC6L2 gene on ALS and not only of variants in high proximity to rs591486. Moreover, a supportive functional analysis could have let us extract the strongest conclusions regarding the role of the rs591486 on the ERCC6L2 and ALS.

The lowest *p* value for HWE in the control group compared to the case group might be related to the small sample size of the study [35]. It is possible that this difference in *p* values between the two groups would have been eliminated, along with an increase in the study's power, if additional participants had been recruited. [36].

Genetic variability seems to play a major role in neurodegenerative diseases, as the coexistence of polymorphisms with exogenous factors can affect their development, and variants have been shown to modify the risk of ALS through candidate gene association studies (CGASs) and genome-wide association studies (GWASs) [15, 37, 38]. For instance, SNPs of around 30 genes appear to modify the effect of environmental exposures in ALS etiology [5]. Additionally, SNPs of ERCC6L2 such as rs591486 have been found to have a joint effect with exposure to pesticides in the development of PD [22]. Concerning the aforementioned ERCC6L2, through its suggested contribution to oxidative stress, it may also be involved in the pathology of ALS.

Quite a few studies regarding neurodegeneration and ALS genetic architecture published so far have primarily focused on genes and their protein products that are implicated in oxidative stress. The main protein studied in ALS is the SOD1. SOD1 is an antioxidant enzyme that decomposes the harmful superoxide radical into O2 and H_2O_2, which is less harmful to cells [39]. SOD1 mutations occur in 2% of sporadic ALS [16] and in 20% of the familial cases [40]. Conditions in which neuronal cells are not able to cope with mitochondrial and oxidative stress seem to contribute or lead to neurodegenerative diseases including ALS, at least in cases with the most highly linked to SOD1 genetic variant [41].

Other ALS-linked genes that code proteins which induce oxidative stress or impair protein degradation machinery are RNA-binding proteins such as TDP-43, FUS, and hnRNPA1. They participate in different facets of the oxidation process, such as unfolding, aggregation, and proteolytic cleavage [42]. Regarding ALS genetics, impairment in autophagy adaptors such as optineurin, ubiquilin 2, and p62 results in disturbances in protein quality control, and therefore to oxidative stress [16]. Our study has concluded that ERCC6L2 can be possibly added to the list of genetic risk factors of ALS. However, due to the fact that our findings are preliminary, further research towards its contribution to oxidative stress and its interaction with other oxidative agents is strongly recommended.

Our study has several strong points that must also be noted. It is characterized by the selection of the gene on a biological basis and by the sample homogeneity [43], as all the participants in each cohort were recruited from the same hospital and had a Greek origin. However, there are a few limitations in the

Table 5 Cox regression analysis for single locus association of the ERCC6L2 rs591486 with age at onset of ALS (crude and adjusted for sex) based on site of onset (bulbar of limb)

Genotype	BULBAR ONSET				LIMB ONSET			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95%CI)	*p* value	HR (95%CI)	*p* value	HR (95%CI)	*p* value	HR (95%CI)	*p* value
G/G	1.00	–	1.00	–	1.00	–	1.00	–
G/A	1.140 (0.402–3.231)	0.805	1.244 (0.439–3.528)	0.681	*2.287 (1.152–4.538)*	*0.018*	*2.180 (1.091–4.357)*	*0.027*
A/A	1.126 (0.371–3.415)	0.834	0.761 (0.238–2.428)	0.644	1.933 (0.892–4.191)	0.095	1.896 (0.874–4.113)	0.106

ERCC6L2, excision repair cross-complementing rodent repair deficiency, complementation group 6-like 2; *ALS*, amyotrophic lateral sclerosis; *CI*, confidence interval; *HR*, hazard ratio. Statistical significant values are given in italic

current study that must also be recognized. Firstly, as a case-control study, it carries the inherent limitations of its type, and secondly, our results would have presented more robustness, if additional clinical outcome measures [44–46] and environmental covariates [5, 47], such as pesticide exposure [48, 49], had been included in regression models. Finally, we included patients without screening for known causative fALS mutations [16], such as mutations of SOD1, TARDBP, and FUS genes and the C9orf72 mutation [50].

In conclusion, despite the fact that the exact mechanism of ALS development is yet to be discovered, the current study provides preliminary indication regarding the potential role of the ERCC6L2 rs591486 as a possible genetic risk factor for the development of ALS. Our findings should be replicated in other ethnicities and experimental models to study the exact role of ERCC6L2 rs591486 in oxidative stress, which appears to be a main contributor in the pathophysiology of ALS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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