Journal of Integrative Neuroscience

J. Integr. Neurosci. **2023**; 22(4): 106
https://doi.org/10.31083/j.jin2204106

Original Research

Lack of Association between *CD33 rs3865444* and Amyotrophic Lateral Sclerosis: A Case-Control Study

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Academic Editor: Gernot Riedel
Submitted: 21 March 2023 Revised: 26 May 2023 Accepted: 29 May 2023 Published: 26 July 2023

Abstract

Background: Microglial activation is considered to assume a role in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). To date, the relationship between ALS and the *rs3865444* polymorphism of the cluster of differentiation 33 (CD33) has not been explored. The current report aimed to investigate the potential connection between *CD33 rs3865444* and ALS. **Methods**: Patients diagnosed with sporadic ALS according to the revised El Escorial criteria, as well as age and sex matched community controls, were enrolled. Two evenly numbered, age and sex matched groups of 155 participants each were genotyped. **Results**: No association was found between *rs3865444* and ALS [log-additive odds ratio (OR) = 0.83 (0.57, 1.22), over-dominant OR = 0.86 (0.55, 1.36), recessive OR = 0.73 (0.25, 2.17), dominant OR = 0.82 (0.52, 1.29), co-dominant OR1 = 0.68 (0.23, 2.05) and co-dominant OR2 = 0.84 (0.53, 1.33)]. Moreover, no relationship was established between rs3865444 and the age of ALS onset based on both unadjusted and sex adjusted Cox-proportional hazards models. Finally, no association between rs3865444 and ALS was found in subgroup analyses based on the site of ALS onset (bulbar or spinal) and sex. **Conclusions**: The current analysis is the first to report that rs3865444 is not linked to ALS. Larger multi-racial studies are required to confirm these findings.

Keywords: motor neuron disease; amyotrophic lateral sclerosis; CD33; cluster of differentiation 33; rs3865444

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an inexorable neurodegenerative disorder characterized by concomitant degeneration of upper and lower motor neurons [1]. Typically, ALS initially manifests with focal signs and symptoms that progressively deteriorate and expand to involve additional body regions, but generalized weakness and muscle wasting may be evident from its onset [2]. Cognitive and behavioral disorders may coexist; however, they are not uniformly present [3]. Despite its low worldwide prevalence (about 4–5 cases per 100,000 people) conservative projections predict a forthcoming "growth spurt" that will accompany the constant ageing of the world population [4,5]. Therefore, ALS devastating nature, along with its ominous epidemiological trajectory increases the urgency for a better understanding of its pathogenesis that will pave the way for advances in its management.

To date, the primary event that instigates the pathophysiological cascade leading to progressive and incessant degeneration of motor neurons remains elusive [6]. Regardless, multiple pathophysiological mechanisms have been implicated in the neurodegenerative processes and are considered to be set in motion by complex interactions among numerous important genetic and environmental factors [7]. In brief, glutamate-mediated excitotoxicity, oxidative (reactive oxygen species) and metabolic (mitochondrial dysfunction) stress, protein misfolding, neurofilament accumulation, formation of intracellular aggregates, disruption of axonal transport and activation of microglia are consid-

ered to underlie neurodegenerative processes in ALS [8]. Whether these mechanisms are uniformly implicated in all patients or specific mechanisms, tend to assume predominant roles in distinct subgroups of ALS cases remains to be determined [6,7].

The cluster of differentiation 33 (CD33) is a transmembrane sialic-acid-binding immunoglobulin-like lectin (siglec) which is principally found on leucocyte subsets and is suspected to regulate immune and inflammatory responses [9,10]. The *CD33* gene is located in chromosome 19p13.33 [11]. *CD33 rs3865444* polymorphism constitutes a sequence variant situated within a regulatory region of the *CD33* gene that is considered to alternate the expression of CD33, ultimately controlling microglial activation [12]. Its pivotal role in neurodegeneration has been primarily demonstrated by previous studies suggesting a protective effect of the minor allele against the development of Alzheimer's disease (AD) [13,14]. Lately, limited research has provided evidence on the protective role of rs3865444 against Parkinson's disease (PD), as well as its implication in the pathogenesis of multiple sclerosis (MS) [15–17]. Considering the extensive pathophysiologic and genetic overlap across the spectrum of neurodegeneration, *rs3865444* could assume a role in ALS related neurodegeneration, as well [18].

To date, no published article has focused on the relationship between *CD33 rs3865444* and ALS. Therefore, the current analysis was undertaken to look into this association. For this purpose, a genetic association study involving individuals diagnosed with ALS and healthy controls of Greek ancestry was performed. The associations between (1) *rs3865444* and ALS and (2) *rs3865444* and age of disease onset were separately investigated.

2. Materials and Methods

2.1 Participants and Settings

The relationship between *CD33 rs3865444* and ALS was explored using a case control design. All procedures were approved by the Institutional Ethics Review Board of the University of Thessaly (59295/23-01-2017) approved the study protocol before its initiation. All participants provided written informed consent prior to participation.

The participant sets of the present study have been previously described [8,19–21]. Individuals with ALS were prospectively and consecutively enrolled from the University Neurology Department of the General University Hospital of Larissa. ALS was diagnosed by senior neurologists using the El Escorial diagnostic criteria [22]. Healthy controls were derived from the same community using individual matching (1:1) based on sex and age ($\pm$ 2 years).

The following prerequisites were considered for the inclusion of patients with ALS: they were above 18 years of age, of Greek ancestry, and diagnosed with sporadic ALS using the revised El Escorial criteria. Candidates with ALS were excluded based on the following: they were diagnosed with any neurodegenerative entity or had a first-degree relative with Motor Neuron Disease (MND) and/or Frontotemporal Dementia (FTD).

Table 1. Patient characteristics.

Demographics		n = 155
Age	(mean years $\pm$ SD)	63.74 $\pm$ 11.30
	median (IQR)	65 (57, 72)
Sex (Female/Male)		77/78
Handedness (Right/Left)		136/19
Years of education ($\leq$6, >6 years)		114/41
Alcohol consumption (Yes/No)		104/51
Smoking (Yes/No)		106/49
Site of disease onset (Spinal/Bulbar/Mixed)		97/50/8

Age is presented in mean $\pm$ SD, median (IQR); SD, standard deviation; IQR, interquartile range.

The following prerequisites were considered for the inclusion of healthy controls: they were above 18 years of age, of Greek ancestry, and belonged to the same community as the ALS cases. Healthy controls were not considered for enrollment when: they were diagnosed with any neurodegenerative entity or had a first-degree relative with MND and/or FTD.

2.2 DNA Isolation and Genotyping

DNA was isolated from peripheral blood leucocytes via the method of salting out [23,24]. Primers and probes for the genotyping of *CD33 rs3865444*, as well as amplification conditions, have been previously described in great detail [17]. Genotyping was performed by applying TaqMan allele-specific discrimination assays using an ABI PRISM 7900 Sequence Detection System (SDS), and analysis of the results was conducted with the SDS software (SDS 2.4, Applied Biosystems, Foster City, CA, USA).

2.3 Statistical Analysis and Outcome Measures

The main focus of the present article was the potential association between *CD33 rs3865444* and ALS. A secondary outcome was the investigation of the relationship between the aforementioned single nucleotide polymorphism (SNP) and the age of ALS onset.

Quality assessment was performed by establishing that the group of healthy controls was in Hardy-Weinberg equilibrium (HWE). The power of our sample was determined based on a minor allele frequency (MAF) of 24%, an estimated prevalence of about 5 ALS cases in a 100,000 population, and a relative risk increase of approximately 40% [25]. SNPStats software (https://www.snpstats.net/) was used for the investigation of the association between *rs3865444* and ALS [26]. The dominant, recessive, overdominant, log-additive and co-dominant models of inheritance were assessed. Both unadjusted and age-sex adjusted models were analyzed. Cox proportional hazards models were performed to explore the potential association be-

Table 2. Allelic and genotypic frequencies for *CD33 rs3865444*.

SNP	Genotypes/alleles	Healthy controls (n = 155)	ALS (n = 155)	Whole sample (n = 310)
rs3865444		n (%)	n (%)	n (%)
Genotype	C/C	82 (53)	90 (58)	172 (56)
	C/A	64 (42)	59 (38)	123 (40)
	A/A	8 (5)	6 (4)	14 (5)
Missing data		1	0	1
Allele	C	228 (74)	239 (77)	467 (76)
	A	80 (26)	71 (23)	151 (24)

SNP, single nucleotide polymorphism; ALS, amyotrophic lateral sclerosis.

Table 3. Single locus unadjusted and age-sex adjusted analysis of the association between *CD33 rs3865444* and ALS.

		Unadjusted analysis		Adjusted analysis	
Mode	Genotype	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Co-dominant	C/C	1.00	0.65	1.00	0.5
	C/A	0.84 (0.53–1.33)		0.79 (0.41–1.51)	
	A/A	0.68 (0.23–2.05)		0.44 (0.09–2.05)	
Dominant	C/C	1.00	0.39	1.00	0.35
	C/A-A/A	0.82 (0.52–1.29)		0.74 (0.40–1.39)	
Recessive	C/C-C/A	1.00	0.58	1.00	0.35
	A/A	0.73 (0.25–2.17)		0.49 (0.11–2.20)	
Over-dominant	C/C-A/A	1.00	0.53	1.00	0.59
	A/C	0.86 (0.55–1.36)		0.84 (0.44–1.58)	
Log-additive	—	0.83 (0.57–1.22)	0.35	0.74 (0.43–1.26)	0.26

ALS, amyotrophic lateral sclerosis; CI, confidence interval; OR, odds ratio.

tween *rs3865444* and the age of ALS onset, using the SPSS statistical package (version 26.0, IBM Corp., Chicago, IL, USA) Both unadjusted and sex adjusted models were tested.

Moreover, we performed subgroup analyses based on the site of ALS onset by comparing: (1) bulbar onset ALS patients with healthy controls, (2) spinal onset ALS patients with healthy controls and (3) bulbar *vs*. spinal onset ALS patients. A second subgroup analysis was performed after stratification for sex. For both subgroups analyses, single locus association analyses and cox proportional hazards models were performed as previously described. Both crude and age-sex adjusted analyses were performed (Cox models featured age as the time-to-event variable and were only adjusted for sex, models stratified for sex did not require adjustment for sex).

All analyses were evaluated according to the $\alpha = 0.05$ threshold of statistical significance.

3. Results

3.1 Sample Characteristics

A group of 155 participants diagnosed with definite sporadic ALS and an evenly numbered group of age-sex matched community controls were formed. The genotype call rate was 99.68%, with genetic data available from 155 patients with ALS and 154 healthy controls. Our sample size possessed a power above 80% to detect a valid association between *CD33 rs3865444* and ALS. *CD33 rs3865444* followed the HWE principle in the group of healthy participants ($p = 0.4$). Patient characteristics are displayed in Table 1.

3.2 Genotyping Results

Genotypic and allelic frequencies are illustrated in Table 2. The MAFs were 26% and 23% for the healthy subjects and ALS cases, respectively.

The unadjusted analysis did not reveal any relationship between *CD33 rs3865444* and ALS (primary outcome measure) regarding any inheritance pattern; log-additive odds ratio (OR) = 0.83 (0.57, 1.22), over-dominant OR = 0.86 (0.55, 1.36), recessive OR = 0.73 (0.25, 2.17), dominant OR = 0.82 (0.52, 1.29), co-dominant OR1 = 0.84 (0.53, 1.33) and co-dominant OR2 = 0.68 (0.23, 2.05) (Table 3). Similarly, age and sex adjusted analyses failed to demonstrate any significant association (Table 3).

The association between *rs3865444* and the age of ALS onset was additionally explored (Table 4). Neither unadjusted (C/C *vs*. C/A; hazard ratio (HR) = 0.88 (0.64, 1.23), C/C *vs*. A/A; HR = 0.72 (0.31, 1.64)) nor sex-adjusted (C/C *vs*. C/A; HR = 0.84 (0.60, 1.17), C/C *vs*. A/A; HR = 0.73 (0.32, 1.67)) Cox-proportional hazards models were consistent with no association between the two.

Table 4. Cox regression for the association between *CD33 rs3865444* and age of disease onset.

	Crude Model		Sex-Adjusted Model	
Genotype	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
C/C	Ref	NA	Ref	NA
C/A	0.88 (0.64, 1.23)	0.46	0.84 (0.60, 1.17)	0.30
A/A	0.72 (0.31, 1.64)	0.43	0.73 (0.32, 1.67)	0.46

Ref, reference group; HR, hazard ratio; CI, confidence interval; NA, not applicable.

Table 5. Single locus unadjusted and age-sex adjusted analysis of the association between *CD33 rs3865444* and bulbar onset ALS.

Bulbar ALS onset *vs*. healthy controls					
		Unadjusted analysis		Adjusted analysis	
Mode	Genotype	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Co-dominant	C/C	1.00	0.55	1.00	0.11
	C/A	0.88 (0.46–1.70)		0.67 (0.25–1.80)	
	A/A	0.35 (0.04–2.95)		0.09 (0.01–1.08)	
Dominant	C/C	1.00	0.56	1.00	0.21
	C/A-A/A	0.82 (0.43–1.57)		0.55 (0.21–1.43)	
Recessive	C/C-C/A	1.00	0.3	1.00	0.053
	A/A	0.37 (0.05–3.05)		0.10 (0.01–1.22)	
Over-dominant	C/C-A/A	1.00	0.85	1.00	0.65
	A/C	0.94 (0.49–1.80)		0.80 (0.31–2.07)	
Log-additive	—	0.79 (0.45–1.38)	0.4	0.49 (0.21–1.11)	0.082
Spinal ALS onset *vs*. healthy controls					
		Unadjusted analysis		Adjusted analysis	
Mode	Genotype	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Co-dominant	C/C	1.00	0.77	1.00	0.76
	C/A	0.82 (0.48–1.40)		0.82 (0.40–1.68)	
	A/A	0.92 (0.28–2.94)		0.61 (0.12–3.03)	
Dominant	C/C	1.00	0.49	1.00	0.52
	C/A-A/A	0.83 (0.50–1.39)		0.80 (0.40–1.58)	
Recessive	C/C-C/A	1.00	0.99	1.00	0.61
	A/A	0.99 (0.31–3.12)		0.66 (0.14–3.19)	
Over-dominant	C/C-A/A	1.00	0.48	1.00	0.67
	A/C	0.83 (0.49–1.40)		0.86 (0.43–1.72)	
Log-additive	—	0.88 (0.57–1.35)	0.56	0.81 (0.45–1.44)	0.46
Bulbar ALS onset *vs*. spinal ALS onset					
		Unadjusted analysis		Adjusted analysis	
Mode	Genotype	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Co-dominant	C/C	1.00	0.61	1.00	0.61
	C/A	0.93 (0.46–1.89)		0.99 (0.46–2.13)	
	A/A	2.59 (0.29–23.21)		2.83 (0.29–27.33)	
Dominant	C/C	1.00	0.98	1.00	0.84
	C/A-A/A	1.01 (0.51–2.02)		1.08 (0.51–2.28)	
Recessive	C/C-C/A	1.00	0.33	1.00	0.32
	A/A	2.66 (0.30–23.44)		2.84 (0.30–26.92)	
Over-dominant	C/C-A/A	1.00	0.73	1.00	0.85
	A/C	0.89 (0.44–1.78)		0.93 (0.44–1.99)	
Log-additive	—	1.11 (0.61–2.02)	0.73	1.18 (0.62–2.25)	0.61

ALS, amyotrophic lateral sclerosis; CI, confidence interval; OR, odds ratio.

Table 6. Subgroup crude cox analysis for the association between *CD33 rs3865444* and age of disease onset, based on site of ALS onset.

Bulbar ALS onset				
	Crude Model		Sex-Adjusted Model	
Genotype	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
C/C	Ref	NA	Ref	NA
C/A	0.70 (0.39, 1.27)	0.24	0.59 (0.32, 1.08)	0.09
A/A	0.34 (0.04, 2.52)	0.29	0.34 (0.05, 2.56)	0.29
Spinal ALS onset				
	Crude Model		Sex-Adjusted Model	
Genotype	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
C/C	Ref	NA	Ref	NA
C/A	1.07 (0.70, 1.64)	0.74	1.07 (0.70, 1.64)	0.75
A/A	0.70 (0.28, 1.76)	0.45	0.71 (0.28, 1.79)	0.47

Ref, reference group; HR, hazard ratio; CI, confidence interval; NA, not applicable.

Table 7. Single locus unadjusted and age adjusted analysis of the association between *CD33 rs3865444* and ALS stratified by sex.

Subgroup of male participants					
		Unadjusted analysis		Adjusted analysis	
Mode	Genotype	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Co-dominant	C/C	1.00	0.88	1.00	0.71
	C/A	0.89 (0.47–1.72)		1.14 (0.47–2.73)	
	A/A	0.72 (0.15–3.39)		0.45 (0.05–3.94)	
Dominant	C/C	1.00	0.68	1.00	0.92
	C/A-A/A	0.87 (0.46–1.65)		1.04 (0.45–2.43)	
Recessive	C/C-C/A	1.00	0.71	1.00	0.44
	A/A	0.75 (0.16–3.47)		0.43 (0.05–3.64)	
Over-dominant	C/C-A/A	1.00	0.79	1.00	0.68
	A/C	0.92 (0.48–1.75)		1.20 (0.51–2.85)	
Log-additive	—	0.88 (0.51–1.50)	0.63	0.93 (0.45–1.93)	0.85
Subgroup of female participants					
		Unadjusted analysis		Adjusted analysis	
Mode	Genotype	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Co-dominant	C/C	1.00	0.7	1.00	0.32
	C/A	0.79 (0.41–1.52)		0.50 (0.19–1.33)	
	A/A	0.65 (0.14–3.08)		0.41 (0.04–3.84)	
Dominant	C/C	1.00	0.42	1.00	0.13
	C/A-A/A	0.77 (0.41–1.46)		0.49 (0.19–1.27)	
Recessive	C/C-C/A	1.00	0.67	1.00	0.58
	A/A	0.72 (0.16–3.33)		0.54 (0.06–4.82)	
Over-dominant	C/C-A/A	1.00	0.53	1.00	0.2
	A/C	0.81 (0.43–1.55)		0.54 (0.20–1.40)	
Log-additive	—	0.79 (0.46–1.37)	0.4	0.55 (0.25–1.24)	0.14

ALS, amyotrophic lateral sclerosis; CI, confidence interval; OR, odds ratio.

3.3 Subgroup Analyses

3.3.1 Site of ALS Onset

The unadjusted analysis did not reveal any relationship between *CD33 rs3865444* and bulbar onset ALS regarding any inheritance pattern; log-additive OR = 0.79 (0.45, 1.38), over-dominant OR = 0.94 (0.49, 1.80), recessive OR = 0.37 (0.05, 3.05), dominant OR = 0.82 (0.43, 1.57), co-dominant OR1 = 0.88 (0.46, 1.70) and co-dominant OR2 = 0.35 (0.04–2.95) (Table 5). Similarly, the unadjusted analysis did not reveal any relationship between *CD33 rs3865444* and spinal onset ALS regarding any inheritance pattern; log-additive OR = 0.88 (0.57, 1.35), over-dominant OR = 0.83 (0.49, 1.40), recessive OR = 0.99 (0.31, 3.12), dominant OR = 0.83 (0.50, 1.39), co-dominant OR1 = 0.82 (0.48–1.40) and co-dominant OR2 =

Table 8. Subgroup crude cox analysis for the association between *CD33 rs3865444* and age of ALS onset, based on sex.

The subgroup of male participants				
	Male participants		Female participants	
Genotype	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
C/C	Ref	NA	Ref	NA
C/A	1.14 (0.72, 1.82)	0.57	0.64 (0.39, 1.02)	0.06
A/A	0.64 (0.19, 2.06)	0.44	0.93 (0.29, 3.04)	0.91

Ref, reference group; HR, hazard ratio; CI, confidence interval; NA, not applicable.

0.92 (0.28–2.94) (Table 5). Finally, no association was estimated in the comparison between bulbar *vs*. spinal ALS onset. Similarly, age and sex adjusted analyses failed to demonstrate any significant association (Table 5).

In the cox models, no association was found between the *rs3865444* and age of ALS onset neither in cases with bulbar (C/C *vs*. C/A; HR = 0.70 (0.39, 1.27), C/C *vs*. A/A; HR = 0.34 (0.04, 2.52)) nor in those with spinal onset (C/C *vs*. C/A; HR = 1.07 (0.70, 1.64), C/C *vs*. A/A; HR = 0.70 (0.28, 1.76)). Similarly, sex adjusted analyses failed to demonstrate any significant association (Table 6).

3.3.2 Subgroup Analyses Based on Sex

The unadjusted analysis did not reveal any relationship between *CD33 rs3865444* and ALS in male participants regarding any inheritance pattern; log-additive OR = 0.88 (0.51, 1.50), over-dominant OR = 0.92 (0.48, 1.75), recessive OR = 0.75 (0.16, 3.47), dominant OR = 0.87 (0.46, 1.65), co-dominant OR1 = 0.89 (0.47, 1.72) and co-dominant OR2 = 0.72 (0.15, 3.39) (Table 7). Similarly, the unadjusted analysis did not reveal any relationship between *CD33 rs3865444* and ALS in female participants regarding any inheritance pattern; log-additive OR = 0.79 (0.46, 1.37), over-dominant OR = 0.81 (0.43, 1.55), recessive OR = 0.72 (0.16, 3.33), dominant OR = 0.77 (0.41, 1.46), co-dominant OR1 = 0.79 (0.41, 1.52) and co-dominant OR2 = 0.65 (0.14–3.08) (Table 7). Similarly, age adjusted analyses failed to demonstrate any significant association (Table 7).

In the cox models, no association was estimated between the *rs3865444* and the age of ALS onset neither in the male (C/C *vs*. C/A; HR = 1.14 (0.72, 1.82), C/C *vs*. A/A; HR = 0.64 (0.19, 2.06)) nor in the female subgroup (C/C *vs*. C/A; HR = 0.64 (0.39, 1.02), C/C *vs*. A/A; HR = 0.93 (0.29, 3.04)). Results can be found in Table 8.

4. Discussion

The current report was the first to focus on *CD33 rs3865444* in patients with ALS and did not establish any associations between the two. Although there is no direct evidence to assess the reproducibility of our results, there is indirect congruent evidence originating from genome-wide association studies (GWASs), which have not revealed a link between *rs3865444* and ALS [27,28]. In rare entities such as ALS, however, the sample size which is required by GWASs to identify SNPs conferring small-sized risks and low-frequency risk-variants may not be feasible to achieve [29]. Of note, although heritability is estimated to explain as much as 50% of the variability of sporadic ALS, the ensemble of risk-loci identified by GWASs occurs in $\leq$10% of patients with sporadic ALS [28]. Therefore, the absence of GWAS-based evidence on an association between *rs3865444* and ALS is not definitive. In any case, despite the negative results of the present article (and the indirect concurring findings of previous GWASs), additional research is warranted since linkage disequilibrium may vary among ethnic groups, and our findings may not be applicable across individuals with different racial backgrounds.

CD33 rs3865444 polymorphism is considered to alternate the expression of *CD33*, ultimately controlling microglial activation [12]. To date, most evidence of its implication in neurodegeneration stems from AD research [13,14]. Relevant studies have shown that individuals with *rs3865444* A/A alleles exhibit decreased expression of *CD33* in peripheral monocytes and brain tissue compared to those with wild type alleles [30]. Reduced micro-glia expression of *CD33* appears to lead to increased phagocytosis of amyloid beta (Aβ) peptide since CD33-lacking microglia phagocytize considerably greater quantities of Aβ peptide [31]. Thus, individuals with minor alleles probably carry a protective effect against the accumulation of Aβ peptide. Although the pathophysiological underpinnings of ALS are heterogeneous from those of AD, microglial activation and neuroinflammation, as well as the balance between homeostatic and deleterious inflammatory processes, appear to be of pivotal importance in the pathogenesis of the disease. The presumed mechanism of *CD33 rs3865444* action is presented at Fig. 1.

Of note, microglia are speculated to assume a dual role in the pathophysiology of ALS, exhibiting both neurotoxic and neuroprotective properties [32]. Several researchers have even reported the co-existence of detrimental and protective roles in the same disease models [33,34]. It has been argued that neuroinflammation and microglia activation may assume different roles in the temporal course of ALS or, more intriguingly, that different subsets of ALS cases could demonstrate heterogeneous underlying pathophysiologic mechanisms leading to the common "net outcome" (degeneration of the upper and lower motor neurons) [35]. Based on the aforementioned considerations, it

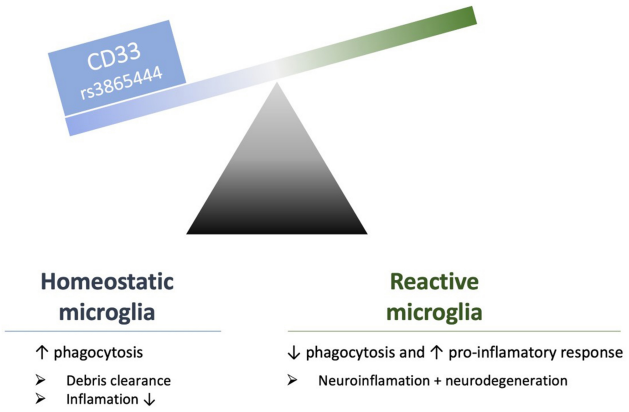

Fig. 1. Microglia, the immune cells of the central nervous system, assume a pivotal role in the dynamic regulation of inflammatory responses. Although microglia-mediated inflammation aims at the protection of the brain parenchyma from various insults, the inflammatory milieu might have detrimental effects on the neurons leading to dysfunction and death. Regulatory processes are pivotal in maintaining the balance between inflammatory and homeostatic processes, promoting the resolution of cerebral inflammation and inducing reparatory processes whenever appropriate.

is clear that different patient subsets could exhibit different associations with *CD33 rs3865444*, as well as other markers of microglial activation. Therefore, current analytic approaches assessing cases with ALS as a whole may fail to detect any association between the two due to systemic bias.

Our study has several limitations. First, despite achieving a power above 80%, there is an almost 20% chance that our analyses failed to detect non-trivial associations between *rs3865444* and ALS. More so, there is an even greater probability that the size of our sample was insufficient to reveal truly significant, smaller-sized associations (compared to the relative risk of 1.42 that was used in our power-estimation statistics). Furthermore, study participants originated from a restricted area, geographically located in Central Greece. The formation of such a geographically restricted sample has introduced several advantages by the formation of two comparable participant groups with respect to several unmeasured exposures to numerous environmental factors that may be implicated in ALS (e.g., soil and water metal and pesticide concentrations [36]) but has probably introduced an overmatching bias in genetics. Moreover, our cohort has not been screened for the main genes that have been linked to ALS, such as superoxide dismutase type 1 (*SOD1*), chromosome 9 open reading frame 72 (*C9orf72*), Transactive response DNA-binding protein 43 (*TDP-43*) or fused in sarcoma/translocated in liposarcoma (*FUS/TLS*) [37,38]. Finally, the con-duction of larger, cross-ethnic studies is warranted to confirm the extrapolation of our findings in populations with different racial backgrounds.

5. Conclusions

Additional research is required to extract definitive conclusions about the connection between *CD33 rs3865444* and ALS, while multinational studies are required to capture differential associations across different racial groups. The meticulous documentation of additional data, along with the imperative transparent reporting of future articles, will unravel potential associations between *rs3865444* and other important clinical parameters, such as treatment-response and prognosis.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

VS: Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft preparation, writing—review and editing, supervision. IL: Methodology, software, validation, formal analysis, investigation, data curatimon, writing—original draft preparation, writing—review and editing. AMA, CB, AN, DP, DPB: Validation, investigation, data curation, writing—review and editing. ZT: Software, validation, investigation, data curation, writing—review and editing. GN, EL, PL: Validation, writing—review and editing. GMH: Conceptualization, methodology, resources, writing—review and editing, supervision, project administration. ED: Conceptualization, methodology, resources, writing—review and editing, supervision, project administration, funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures were approved by the Institutional Ethics Review Board of the University of Thessaly (59295/23-01-2017) approved the study protocol before its initiation. All participants provided written informed consent prior to participation.

Acknowledgment

Not applicable.

Funding

This study was supported in part by a research grant from the Research Committee of the University of Thessaly, Greece (code: 5287).

Conflict of Interest

The authors declare no conflict of interest. Vasileios Siokas, Christos Bakirtzis, Efthimios Dardiotis, and Ioannis Liampas are serving as one of the Guest editors of this journal. We declare that Vasileios Siokas, Christos Bakirtzis, Efthimios Dardiotis, and Ioannis Liampas had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Gernot Riedel.

References

[1] Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. Journal of the Neurological Sciences. 1994; 124: 96–107.

[2] Shefner JM, Al-Chalabi A, Baker MR, Cui LY, de Carvalho M, Eisen A, *et al*. A proposal for new diagnostic criteria for ALS. Clinical Neurophysiology. 2020; 131: 1975–1978.

[3] Beeldman E, Govaarts R, de Visser M, Klein Twennaar M, van der Kooi AJ, van den Berg LH, *et al*. Progression of cognitive and behavioural impairment in early amyotrophic lateral sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry. 2020; 91: 779–780.

[4] Xu L, Liu T, Liu L, Yao X, Chen L, Fan D, *et al*. Global variation in prevalence and incidence of amyotrophic lateral sclerosis: a systematic review and meta-analysis. Journal of Neurology. 2020; 267: 944–953.

[5] Arthur KC, Calvo A, Price TR, Geiger JT, Chiò A, Traynor BJ. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. Nature Communications. 2016; 7: 12408.

[6] Vucic S, Rothstein JD, Kiernan MC. Advances in treating amyotrophic lateral sclerosis: insights from pathophysiological studies. Trends in Neurosciences. 2014; 37: 433–442.

[7] Geevasinga N, Menon P, Özdinler PH, Kiernan MC, Vucic S. Pathophysiological and diagnostic implications of cortical dysfunction in ALS. Nature Reviews Neurology. 2016; 12: 651–661.

[8] Liampas I, Siokas V, Aloizou AM, Bakirtzis C, Tsouris Z, Nousia A, *et al*. MOBP rs616147 polymorphism and risk of amyotrophic lateral sclerosis in a greek population: a case-control study. Medicina. 2021; 57: 1337.

[9] Cao H, Crocker PR. Evolution of CD33-related siglecs: regulating host immune functions and escaping pathogen exploitation? Immunology. 2011; 132: 18–26.

[10] Siokas V, Aslanidou P, Aloizou AM, Peristeri E, Stamati P, Liampas I, *et al*. Does the CD33 rs3865444 Polymorphism Confer Susceptibility to Alzheimer's Disease? Journal of Molecular Neuroscience. 2020; 70: 851–860.

[11] Reference SNP (rs) Report. 2022. Available at: https://www.ncbi.nlm.nih.gov/snp/rs3865444#publications (Accessed: 4 December 2022).

[12] Crocker PR, McMillan SJ, Richards HE. CD33-related siglecs as potential modulators of inflammatory responses. Annals of the New York Academy of Sciences. 2012; 1253: 102–111.

[13] Walker DG, Whetzel AM, Serrano G, Sue LI, Beach TG, Lue LF. Association of CD33 polymorphism rs3865444 with Alzheimer's disease pathology and CD33 expression in human cerebral cortex. Neurobiology of Aging. 2015; 36: 571–582.

[14] Javor J, Ďurmanová V, Párnická Z, Minárik G, Králová M, Pečeňák J, *et al*. Association of CD33 rs3865444:C>A polymorphism with a reduced risk of late-onset alzheimer's disease in Slovaks is limited to subjects carrying the APOE ε4 allele. International Journal of Immunogenetics. 2020; 47: 397–405.

[15] Javor J, Bucová M, Ďurmanová V, Radošinská D, Párnická Z, Čierny D, *et al*. Alzheimer's Disease Risk Variant rs3865444 in the CD33 Gene: A possible role in susceptibility to multiple sclerosis. Life. 2022; 12: 1094.

[16] Siokas V, Arseniou S, Aloizou AM, Tsouris Z, Liampas I, Sgantzos M, *et al*. CD33 rs3865444 as a risk factor for Parkinson's disease. Neuroscience Letters. 2021; 748: 135709.

[17] Siokas V, Tsouris Z, Aloizou AM, Bakirtzis C, Liampas I, Koutsis G, *et al*. Multiple sclerosis: Shall we target CD33? Genes. 2020; 11: 1334.

[18] Arneson D, Zhang Y, Yang X, Narayanan M. Shared mechanisms among neurodegenerative diseases: from genetic factors to gene networks. Journal of Genetics. 2018; 97: 795–806.

[19] Siokas V, Aloizou AM, Liampas I, Bakirtzis C, Nasios G, Paterakis K, *et al*. Lack of an association between SCFD1 rs10139154 polymorphism and amyotrophic lateral sclerosis. Molecular Medicine Reports. 2022; 25: 146.

[20] Siokas V, Aloizou AM, Liampas I, Tsouris Z, Mentis AFA, Nasios G, *et al*. Lack of association between TREM2 rs75932628 variant and amyotrophic lateral sclerosis. Molecular Biology Reports. 2021; 48: 2601–2610.

[21] Siokas V, Karampinis E, Aloizou AM, Mentis AFA, Liakos P, Papadimitriou D, *et al*. CYP1A2 rs762551 polymorphism and risk for amyotrophic lateral sclerosis. Neurological Sciences. 2021; 42: 175–182.

[22] Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders. 2000; 1: 293–299.

[23] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 1988; 16: 1215.

[24] Dardiotis E, Siokas V, Zafeiridis T, Paterakis K, Tsivgoulis G, Dardioti M, *et al*. Integrins AV and B8 gene polymorphisms and risk for intracerebral hemorrhage in Greek and Polish populations. Neuromolecular Medicine. 2017; 19: 69–80.

[25] GAS Power Calculator. 2006. Available at: https://csg.sph.umich.edu/abecasis/gas_power_calculator/ (Accessed: 4 December 2022).

[26] Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006; 22: 1928–1929.

[27] van Rheenen W, van der Spek RAA, Bakker MK, van Vugt JJFA, Hop PJ, Zwamborn RAJ, *et al*. Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology. Nature Genetics. 2021; 53: 1636–1648.

[28] Ryan M, Heverin M, McLaughlin RL, Hardiman O. Lifetime Risk and Heritability of Amyotrophic Lateral Sclerosis. JAMA Neurology. 2019; 76: 1367–1374.

[29] Rich KA, Roggenbuck J, Kolb SJ. Searching far and genome-wide: the relevance of association studies in amyotrophic lateral sclerosis. Frontiers in Neuroscience. 2021; 14: 603023.

[30] Malik M, Simpson JF, Parikh I, Wilfred BR, Fardo DW, Nelson PT, *et al*. CD33 Alzheimer's risk-altering polymorphism, CD33 expression, and exon 2 splicing. Journal of Neuroscience. 2013; 33: 13320–13325.

[31] Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, *et al*. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron. 2013; 78: 631–643.

[32] Geloso MC, Corvino V, Marchese E, Serrano A, Michetti F, D'Ambrosi N. The dual role of microglia in ALS: mechanisms and therapeutic approaches. Frontiers in Aging Neuroscience. 2017; 9: 242.

[33] Chiu IM, Morimoto ETA, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP, *et al*. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. Cell Reports. 2013; 4: 385–401.

[34] Liao B, Zhao W, Beers DR, Henkel JS, Appel SH. Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. Experimental Neurology. 2012; 237: 147–152.

[35] Clarke BE, Patani R. The microglial component of amyotrophic lateral sclerosis. Brain. 2020; 143: 3526–3539.

[36] Siokas V, Aloizou AM, Pateraki G, Liampas I, Mitsias PD, Bogdanos DP, *et al*. Toxicology of neurodegenerative diseases. In Tsatsakis AM (ed.) Toxicological risk assessment and multi-system health impacts from exposure (pp. 247–258). Academic Press: Cambridge, MA, USA. 2021.

[37] Mathis S, Goizet C, Soulages A, Vallat JM, Masson GL. Genetics of amyotrophic lateral sclerosis: A review. Journal of the Neurological Sciences. 2019; 399: 217–226.

[38] Conlon EG, Fagegaltier D, Agius P, Davis-Porada J, Gregory J, Hubbard I, *et al*. Unexpected similarities between C9ORF72 and sporadic forms of ALS/FTD suggest a common disease mechanism. eLife. 2018; 7: e37754.