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Lack of association of VEGF-A polymorphisms and susceptibility to amyotrophic lateral sclerosis and multiple sclerosis: A systematic review and meta-analysis

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ABSTRACT. Amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) are degenerative scleroses with unclear etiology. Vascular endothelial growth factor A (*VEGF-A*) is a growth factor that plays multiple roles in the central nervous system. Previous studies indicated a potential association between polymorphisms in this gene and the susceptibility of ALS and MS; however, the results have been inconclusive. Here, we conducted a systematic review and meta-analysis to elucidate the relationship between polymorphisms in the *VEGF-A* gene and these degenerative scleroses. We searched for observational studies in PubMed, Web of Science, EMBASE, Virtual Health Library (BVS) and SCOPUS, without temporal and language restrictions and 12 studies were included in the systematic review. Six polymorphisms were identified: C-1558T, A-1190G, G-1154A (rs1570360), C-2578A (rs699947), C-634G (rs2010963), and C936T (rs3025039). After a systematic literature search, a pooled odds ratio

(OR) and 95% confidence interval (CI) were used to evaluate the association of C-2578A, G-1154A, and G-634C polymorphisms and ALS. Due to the small number of articles found in this review for MS, it was not possible to perform a meta-analysis for this disease. The meta-analysis for ALS included 1441 patients and 1978 controls for C-2578A, and 1134 patients and 1629 controls for G-1154A and G-634C polymorphisms. No SNP was significantly associated with risk for ALS. We conclude that polymorphisms in the *VEGF-A* gene may not be a major risk factor for the development of ALS and MS; However, associated with specific factors, such as sex or haplotype combinations, they may become a strong susceptibility factor. Although we found a lack of association in most *VEGF-A* polymorphisms and the susceptibility for developing ALS and MS, this review provides a comprehensive understanding for the potential role of this gene in degenerative sclerosis.

Key words: *VEGF-A*; Neurodegenerative diseases; Polymorphisms; Systematic review; Meta-analysis

INTRODUCTION

Neurodegenerative diseases are a group of disorders characterized by progressive dysfunction and neuronal loss. Physiopathologically, these diseases can compromise motricity, memory, cognitive, speech, and breathing (Gitler et al., 2017). Amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS), are rare diseases, sharing differences and similarities (Liu et al., 2016; Hardiman et al., 2017).

ALS involves the degeneration of upper and lower motor neurons, resulting in progressive muscle weakness and paralysis. Affect 1.7/100,000 individuals/year (Oskarsson et al., 2018), however, in Brazil, the incidence is approximately 0.4 cases per 100,000 individuals (Prado et al., 2016). ALS is most frequent in men between 55 and 65 years. Etiologically, it can be classified as sporadic (SALS) in 90% of cases, considered multifactorial, or familial (FALS), with genetic origin and autosomal dominant inheritance pattern (Ingre et al., 2015).

Contrastingly, MS is an autoimmune, inflammatory, and demyelinating disease and most patients relate a gradual loss of visual, motor, and sensory functions (Hunter, 2016), but symptoms vary according to the stage of the disease (remitting-recurring MS, secondary-progressive MS, and primary-progressive MS) (Ransohoff et al., 2015). Affects individuals with age between 20 and 40 years and is typically more common in women, on a 2:1 ratio (Ransohoff et al., 2015). MS worldwide incidence is estimated in 3.6/100,000 individuals/year (Hunter, 2016). There are no epidemiological studies reporting incidence in Brazil, however, the prevalence was found to range from 0.75 to 30.7/100,000 inhabitants (Ribeiro, 2019).

Both diseases have complex interactions between genetics and environmental factors (Ransohoff et al., 2015; Hardiman et al., 2017). Vascular alterations and neuroinflammation also play a role in the pathophysiology and clinical progression of

ALS and MS (Evans et al., 2013; Spencer et al., 2018). In this context, the Vascular Endothelial Growth Factor A is a glycoprotein coded by *VEGF-A* homonyms gene (or only *VEGF*), a regulatory key factor for angiogenesis and vascular permeability, with neuroprotective effects in the CNS (Ferrara, 2004).

Polymorphisms in this gene have been previously associated with other neurodegenerative diseases, such as Alzheimer's (Liu et al., 2013) and Parkinson's (Wu et al., 2016). Studies have tried to demonstrate an association between *VEGF-A* gene polymorphisms and degenerative sclerosis; however, the data are scarce and conflicting (Lambrechts et al., 2009; Saravani et al., 2019). In this systematic review, we made a compilation of all the polymorphisms in the *VEGF-A* gene, related to ALS and MS, independent of association, in order to determine the most relevant for each disease.

MATERIAL AND METHODS

Registration

To avoid duplication of systematic reviews on this theme, this study had the protocol registered in the International Prospective Register of Ongoing Systematic Reviews (PROSPERO) on January 6th, 2021, under the number CRD42021222484. For a better report of the findings, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Search strategy

We performed a literature search to identify studies that relate *VEGF-A* gene polymorphisms in ALS and/or MS using PubMed/Medline, Virtual Health Library (BVS), Web of Science, SCOPUS, and EMBASE databases, between November 2020 and March 2021. To achieve more studies with potential eligibility, we also searched for records in gray literature.

Our guiding question for this study was structured according to the PEO acronym (Population, Exposure, Outcome), which better fits in systematic reviews of association (etiology) (Moola et al., 2015): P = population: MS and ALS patients; E = exposure: *VEGF-A* polymorphisms; O = outcome: associated or not with pathology. Thus, we formulate the question: “What are the polymorphisms in the *VEGF-A* gene reported in the literature related to ALS and MS?”

For the search strategy, we used combined terms indexed in Medical Subject Headings (MeSH) for “Vascular Endothelial Growth Factor A”, “Polymorphism, genetic”, “Amyotrophic lateral sclerosis” and “Multiple Sclerosis”. To improve our search strategy, we followed the Peer Review of Electronic Search Strategies (PRESS) for systematic reviews guidelines (McGowan et al., 2016). The search strategy was adapted for every database and is listed in Table 1.

Table 1. Search strategy for VEGF-A polymorphisms and susceptibility to amyotrophic lateral sclerosis and multiple sclerosis applied in each database.

Database	Search strategy
Pubmed	((("Amyotrophic lateral sclerosis" OR "Charcot Disease" OR "Motor Neuron Disease" OR "Amyotrophic Lateral Sclerosis" OR "Lou Gehrig's Disease") OR ("Multiple Sclerosis" OR "Sclerosis Multiple" OR "sclerosis Disseminated" OR "Disseminated Sclerosis" OR MS) OR ("Amyotrophic lateral sclerosis" AND "Multiple Sclerosis"))) AND (("Polymorphism, Genetic" OR polymorphism*) AND ("Vascular Endothelial Growth Factor A" OR "VEGF-A" OR "VEGF" or "Vascular Endothelial Growth Factor")))
Web of Science	(((("Amyotrophic lateral sclerosis" OR "Charcot Disease" OR "Motor Neuron Disease" OR "Amyotrophic Lateral Sclerosis" OR "Lou Gehrig's Disease") OR ("Multiple Sclerosis" OR "Sclerosis Multiple" OR "sclerosis Disseminated" OR "Disseminated Sclerosis" OR MS) OR ("Amyotrophic lateral sclerosis" AND "Multiple Sclerosis"))) AND (("Polymorphism, Genetic" OR polymorphism*) AND ("Vascular Endothelial Growth Factor A" OR "VEGF-A" OR "VEGF" or "Vascular Endothelial Growth Factor")))
BVS	tw: (("Polymorphism, Genetic" OR polymorphism*) AND ("Vascular Endothelial Growth Factor A" OR "VEGF-A" OR vegf or "Vascular Endothelial Growth Factor")) AND ((("Amyotrophic lateral sclerosis" OR "Charcot Disease" OR "Motor Neuron Disease" OR "Amyotrophic Lateral Sclerosis" OR "Lou Gehrig's Disease") OR ("Multiple Sclerosis" OR "Sclerosis Multiple" OR "sclerosis Disseminated" OR "Disseminated Sclerosis" OR MS) OR ("Amyotrophic lateral sclerosis" AND "Multiple Sclerosis")))
EMBASE	(('polymorphism, genetic' OR polymorphism*) AND 'vascular endothelial growth factor a' OR 'vegf-a') AND ('amyotrophic lateral sclerosis' OR 'motor neuron disease' OR 'multiple sclerosis')
SCOPUS	(polymorphism AND vascular endothelial growth factor a OR vegf-a OR vegf AND amyotrophic lateral sclerosis OR multiple sclerosis)

Selection criteria

To be eligible, studies must meet the following criteria:

Inclusion: observational studies, performed only on humans, reporting polymorphisms in *VEGF–A* gene relating it with ALS and MS, which may or may not be associated with the diseases. No language or time restrictions were applied.

Exclusion: Studies with other designs; in non-humans; duplicate data; articles that do not answer the guiding question of the review.

Study selection

All articles resulting from the search were imported to the Rayyan platform (Ouzzani et al., 2016). The initial screening was carried out by reading titles and abstracts. Subsequently, we read the full studies. In both stages, articles that did not meet the established criteria were excluded. At all stages of study selection, two independent reviewers assessed the paper's eligibility. Discrepancies were solved by a third independent reviewer. In articles with potential eligibility, but unavailable, the respective authors were contacted at least 3 times. However, no contact was returned.

Assessment of methodological quality

The risk of bias was evaluated by The Joanna Briggs Institute (JBI) (Institute TJB, 2022) Critical Appraisal tools, applied according to each study design. This tool allows

reviewers to determine the criteria for their inclusion. Therefore, only studies that answered at least 70% of the questions were considered to have a low risk of bias.

Data analysis

For the qualitative and descriptive approach, the following data were extracted: (1) authors and year of publication; (2) study design; (3) population; (4) sample size; (5) sex of participants; (6) mean age of participants; (7) disease; (8) polymorphism; (9) genotypic, allele and haplotypic frequencies; (10) comparison made; (11) chi-square value; (12) odds ratio - 95% confidence interval; and (13) P-value. Data extraction was carried out independently by two reviewers on all included studies.

Statistical analysis

The association of the SNPs and the disease was estimated by calculating a odds ratio (OR) and 95% confidence interval (CI). The ORs were evaluated in dominant (wild vs. heterozygous + mutant genotypes) and allelic (wild vs. mutant) genetic models for each SNP. The heterogeneity between the studies was assessed by the Higgins inconsistency test (I^2).

The choice of the meta-analytical model depended on the result of the between-study heterogeneity test. Thus, the fixed-effect model (Mantel-Haenszel method) was applied when $I^2 < 25\%$, assuming that the differences between the effect estimates are attributed merely to chance. Otherwise, when I^2 25-75% the random-effect model (DerSimonian-Laird method) was applied. I^2 values of 25-75% and > 75% are defined as moderate and high heterogeneity, respectively. Sensitivity analysis was performed to confirm and explore possible sources of heterogeneity between studies.

Hardy-Weinberg equilibrium (HWE) was calculated using Fisher's exact test. In meta-analyses of genetic association studies, it is highly recommended that control groups be evaluated for HWE. Deviations from the HWE in controls have been related to problems in the design and conduct of these studies, mainly due to population stratification, genotyping error or selection bias.

Publication bias was evaluated by funnel plot (Egger et al., 1997), which asymmetry was estimated using linear regression (Egger's test) (Egger and Smith, 1997). A p-value of Egger's test <0.05 suggests a strong probability of publication bias. All statistical tests were performed in RStudio® software (version 4.1.0).

RESULTS

Study selection

We identified 227 articles in the initial search and 90 duplicates were removed. In total, 125 articles failed to satisfy the eligibility criteria. Our search in the gray literature also did not obtain data that meet our objectives. Non-genetic association studies and non-ALS subjects were the primary reasons for excluding search results. Only 12 studies were included in this systematic review, with publication dates between 2004 and 2019. The flow chart of study selection is illustrated in Figure 1.

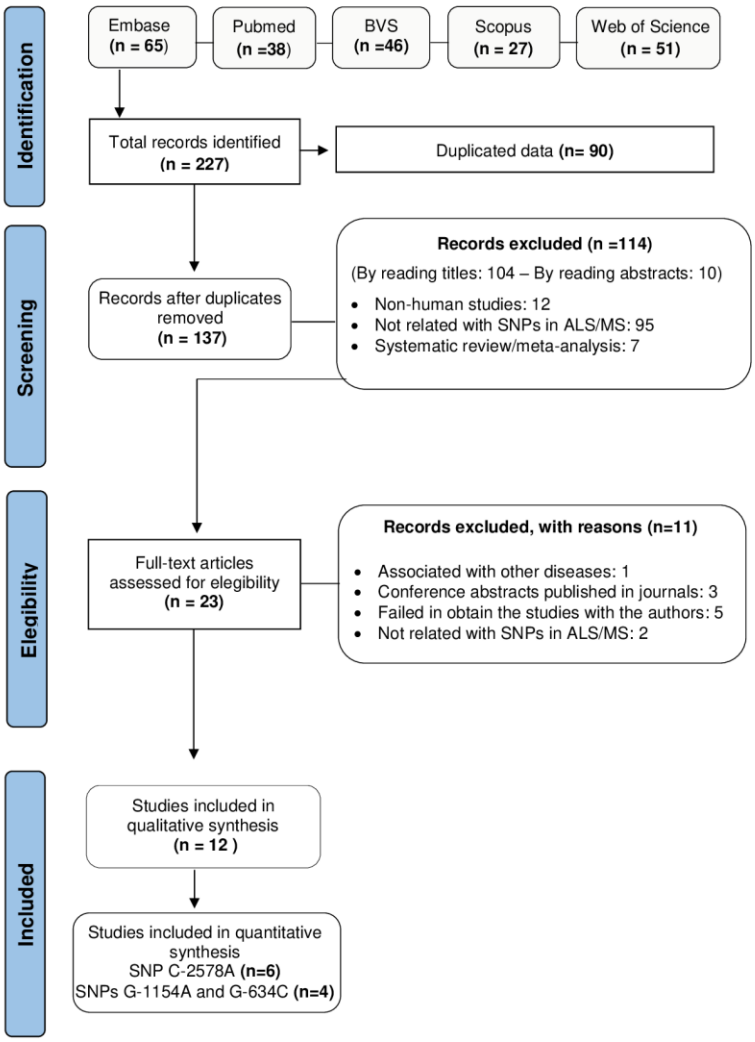

Figure 1. PRISMA flowchart demonstrating the process of including studies in this systematic review and meta-analysis. ALS: amyotrophic lateral sclerosis; VHL: Virtual Health Library; MS: multiple sclerosis; SNP: single nucleotide polymorphism. Adapted from The PRISMA Group, 2009.

Study characteristics

Of the included studies, eight were case-control studies, three were cohort studies, and one was cohort/case-control study. Considering the population, two studies were performed in China, two in Iran, two in Russia, and one in North America, Germany, Italy, Poland, New England, and The Netherlands. Of these, ten studies were about ALS and only two about MS. The mean age for ALS patients ranged between 47.8 and 59.2 years, whereas for MS individuals ranged between 37.4 and 52.3 years. A total of six polymorphisms were cited: C-1558T, A-1190G, G-1154A (rs1570360), C-2578A (rs699947), C-634G (rs2010963), and C936T (rs3025039) (Supplementary 1).

Risk of bias within studies

The studies were homogeneous in the quality assessment and, individually, achieved at least 70% of the answers to the JBI critical appraisal tool. For case-control studies, nine parameters were evaluated, while for the cohorts were eight. Only two studies reported unclear information for the questions Q2 (cases and controls matched appropriately), Q6 (confounding factors identified), and Q7 (strategies to deal with confounding factors stated). However, the 12 studies were classified as low risk of bias (Figure 2).

Figure 2. Risk of bias synthesis regarding the JBI critical appraisal tool parameters for the studies included in this systematic review. Q: question.

Results of individual studies

In Supplementary 1, we can observe that most studies showed no association between *VEGF-A* polymorphisms and ALS. Van Vught et al. (2005), Chen et al. (2006), Zhang et al. (2006), Del Bo et al. (2008), and Golenia et al. (2010) found no association between these polymorphisms with the disease, considering comparisons by genotypes, alleles, or haplotypes. In addition, Golenia et al. (2010) also analyzed plasma levels of VEGF-A and found no significant differences between the sALS and control groups. However, some studies showed a positive association between SNPs in the *VEGF-A* gene and disease susceptibility. Terry et al. (2004) demonstrated a 3-fold increased risk among individuals homozygous for the -2578A/-1154A/-634G or -2578A/-1154G/-634G haplotypes (95% CI=0.7-13.4) (Supplementary 1).

Chen et al. (2007) found an association of A-1154G SNP with the ALS development in the dominant model (AA vs AG+GG) (P = 0.021) (Supplementary 1). In addition, the -1190A/G and -1190G/G genotypes were associated with a 4.1 and 9.4 years earlier onset of ALS, when compared with the -1190A/A genotype. A similar pattern was found for A-1154G SNP: 7.9 and 11.7 years for A/G and G/G genotypes, respectively. On

the other hand, the C-1558T showed a positive effect (7.0 and 9.6 years for C/T and T/T genotypes, respectively). The haplotype analyses did not demonstrate an association with ALS.

Furthermore, some studies showed a possible sex-dependent association. Fernandez-Santiago et al. (2006) found an association between the -1154G allele and women (OR=1.361; P = 0.036). Lysogorskaia et al. (2012) associated the -2578A/A genotype with an increased risk of ALS (OR=1.66; 95% CI=1.03-2.29; P = 0.004) (Supplementary 1). The risk was even greater in male individuals (OR=2.18; 95% CI=1.90-2.47). The -2578A/A genotype was also associated with the earlier disease onset and rapid progression. An association of the -2578A allele was also found (P = 0.036) (Supplementary 1).

Likewise, in another study, Lysogorskaia et al. (2015) found the same genotype (-2578A/A) associated with a 1.7-fold increased risk for ALS (95% CI=1.05-2.93; P = 0.027). In the male group, this chance increased to 2.1-fold (95% CI=1.06-4.17; P = 0.03). A significant difference was also found in the -2578A allele between the case and control groups (P = 0.008) (Supplementary 1).

For MS, both two studies showed an association between the SNPs in the *VEGF-A* gene and the disease. Mazdeh et al. (2017) reported a 2.95-fold increased risk for MS with the C936T SNP in the recessive model (TT vs CT + CC) (95% CI =1.23–7.07; P = 0.015) and with the allele 936T (OR = 1.41; 95% CI 1.08-1.84; P = 0.01). While, Saravani et al. (2019) related an increased susceptibility for MS with the C-2578A SNP in the dominant model (AA vs CA+CC) (OR=2.9; 95% CI=1.4–6.1; P = 0.005) and with the allele -2578C (OR=1.6; 95% IC=1.1-2.2; P = 0.009). All these results showed the possible different roles of *VEGF-A* in ALS and MS.

Meta-analysis

It was possible to perform a meta-analysis with only three of the six polymorphisms identified in our study: C-2578A, G-1154A, and G-634C, applied only for ALS. For the other polymorphisms, we did not reach the minimum number of two studies or failed to obtain the necessary data for the analysis. The total number of subjects enrolled in each SNP was 1441 patients and 1978 controls for C-2578A, and 1134 patients and 1629 controls for G-1154A and G-634C polymorphisms. No SNP was associated with the risk of ALS (Figures 3 to 5).

Associations between the SNPs and the ALS

For the analysis of SNP C-2578A, six studies were included. As shown in Figure 3, the results indicated that there was no association between this SNP and the risk of ALS in the dominant model (CA+AA vs. CC) (OR = 0.88; 95% CI = 0.75-1.02; P = 0.09; I^2 = 0%), and in the allelic comparison (C vs. A) (OR = 1.00; 95% CI = 0.91-1.11; P = 0.94; I^2 = 3%).

Four studies were included for the analysis of SNPs G-1154A and G-634C. Likewise, as shown in Figures 4 and 5, the results indicated that there was no association between these SNPs and the risk of ALS. In the dominant model (GA+AA vs. GG), the SNP G-1154A showed: OR = 0.98; 95% CI = 0.84-1.14; P = 0.77; I^2 = 0%; and in the allelic comparison (G vs. A) it presented OR = 1.01; 95% CI = 0.90-1.13; P = 0.93; I^2 = 0%

(Figure 4). For the SNP G-634C, the genotypic comparison in the dominant model (GC+CC vs. GG) demonstrated: OR = 0.91; 95% CI = 0.78-1.06; P = 0.24; I^2 = 0%; and the allelic comparison (G vs. C) showed: OR = 0.96; 95% CI = 0.85-1.08; P = 0.46; I^2 = 0% (Figure 5).

The analysis of publication bias for the SNP C-2578A no found significant publication bias according to funnel plot (Figure 6A and 6B) and Egger's test for genotypic and allelic comparisons (P = 0.6886 and P = 0.9223, respectively). As shown in figure 6C, for the SNP G-1154A was found a slightly significant risk in the genotypic comparison (Egger's test P = 0.0483). However, the allelic comparison showed no publication bias (Egger's test P = 0.3801) (Figure 6D). For the SNP G-634C, there was no significant publication bias according to funnel plot (Figure 6E and 6F) and Egger's test (genotypic comparison P-value = 0.8971; allelic comparison P-value = 0.0914).

In the sensitivity analysis, new meta-analyses were performed excluding studies to confirm and explore the heterogeneity rates found. However, the results of these new meta-analyses remained similar to the original meta-analysis containing all studies. In addition, the distribution of genotypes in the control groups of each study included in the meta-analysis was in agreement with the HWE (P > 0.05) (Supplementary 2).

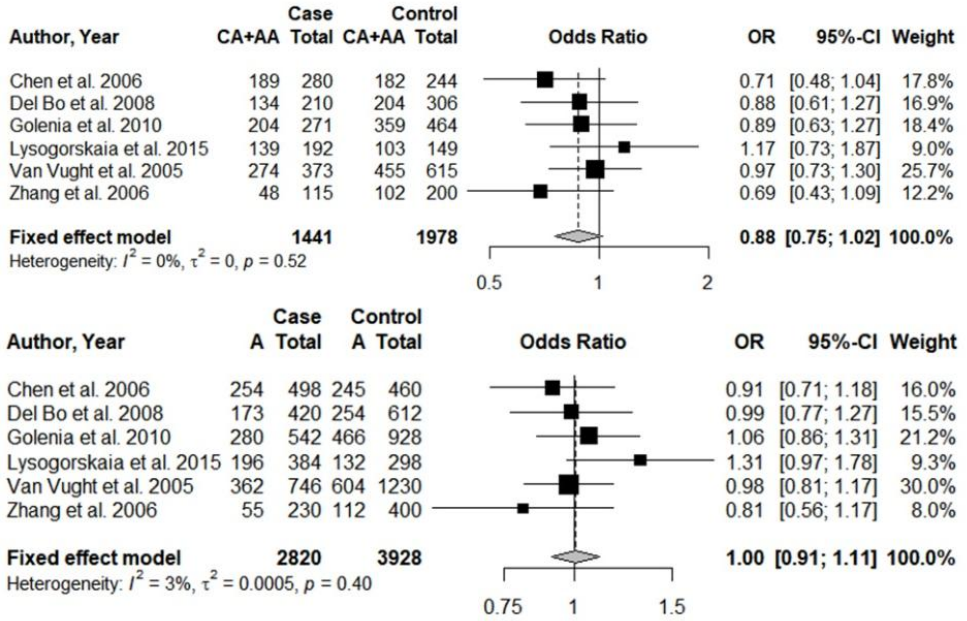

Figure 3. Forest plot for the genotypic and allelic comparison of SNP C-2578A (CA+AA vs CC and C vs A). Odds ratio (OR) and 95% confidence interval (95% CI) calculated with the Mantel-Haenszel test (fixed effect model), due to the value found in the heterogeneity test (I^2).

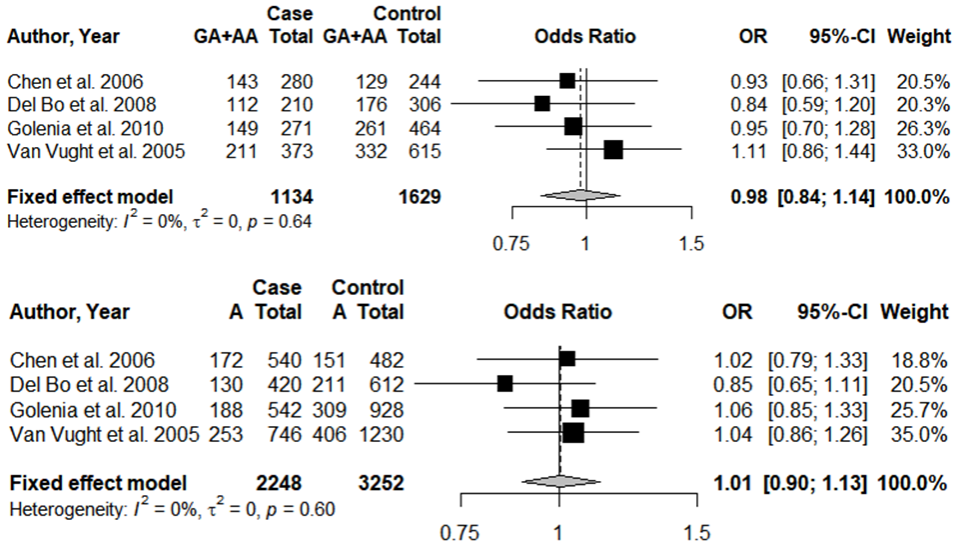

Figure 4. Forest plot for the genotypic and allelic comparison of SNP G-1154A (GA+AA vs GG and G vs A). Odds ratio (OR) and 95% confidence interval (95% CI) calculated with the Mantel-Haenszel test (fixed effect model), due to the value found in the heterogeneity test (I^2).

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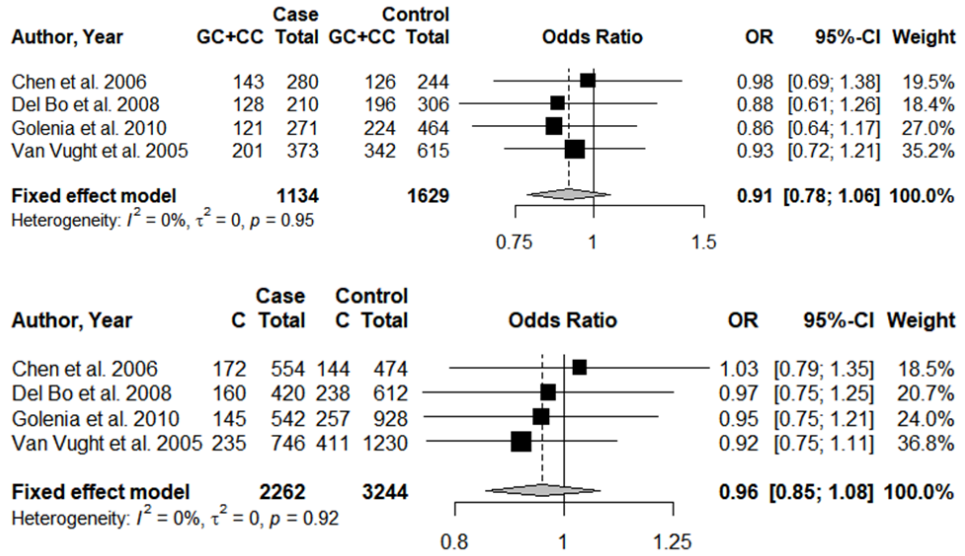

Figure 5. Forest plot for the genotypic and allelic comparison of SNP G-634C (GC+CC vs GG and G vs C). Odds ratio (OR) and 95% confidence interval (95% CI) calculated with the Mantel-Haenszel test (fixed effect model), due to the value found in the heterogeneity test (I^2).

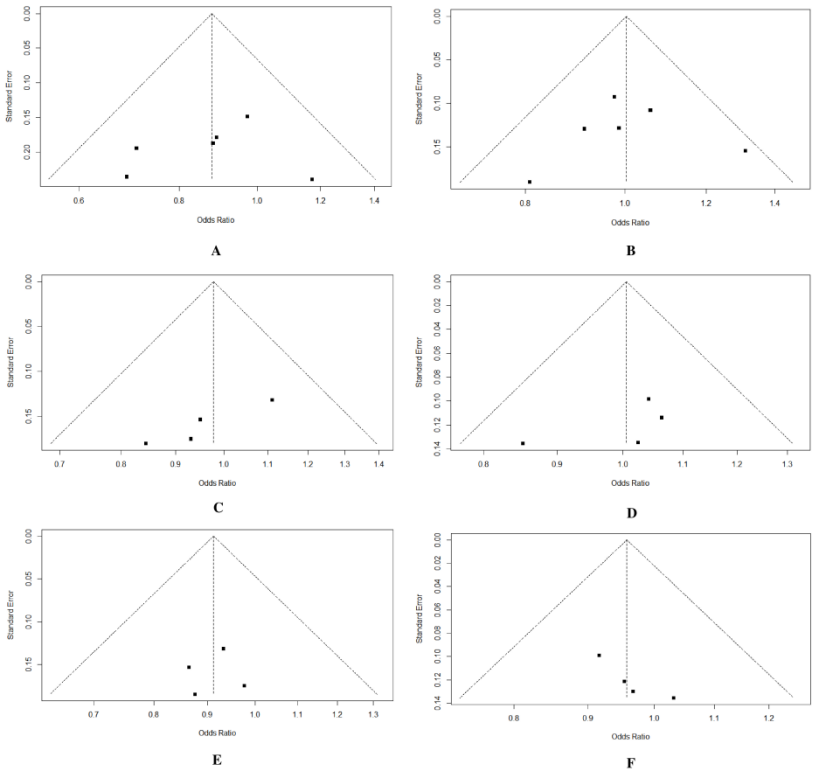

Figure 6. Funnel plots for the publication bias of the studies included in the meta-analysis. A: genotypic comparison by dominant model (CA+AA vs. CC) for SNP C-2578A, B: allelic comparison (C vs. A) for SNP C-2578A, C: genotypic comparison by dominant model (GA+AA vs. GG) for SNP G-1154A, D: allelic comparison (G vs. A) for SNP G-1154A, E: genotypic comparison by dominant model (GC+CC vs. GG) for SNP G-634C, F: allelic comparison (G vs. C) for SNP G-634C.

DISCUSSION

The objective of our systematic review was to compile all the polymorphisms in the *VEGF-A* gene, described in observational studies, related in ALS and/or MS, independent of significant statistical association. Only 12 articles meet the proposed objectives. The few articles found, especially for MS, could reflect the fact that *VEGF-A* does not play a crucial role in triggering mechanisms for susceptibility to the disease. Once MS is an autoimmune pathology, other genes related to the immune system may be related to its development, such as the human leukocyte antigen (HLA) system (Al-Nashml et al., 2018).

However, *VEGF-A* shows an association with MS pathogenesis through angiogenesis. The hypoxia-inducible factor 1 (HIF-1) is an important transcription factor that regulates the response to low oxygen concentrations. Subunits HIF-1α and HIF-1β play a role in several cellular mechanisms, such as inducing inflammatory processes, apoptosis, or inhibiting cell death (Heger et al., 2019; Zhu et al., 2020).

The *VEGF-A* gene is the main target for HIF-1 and induces angiogenesis (Gong et al., 2019). Several studies indicate the role of the response to hypoxia and the HIF-1

signaling pathway in neurological disorders (Palazon et al., 2014; Yang et al., 2016; Guan et al., 2017). Hypoxia is the major cause of periventricular lesions and of the deep white matter, one characteristic of MS (Juurlink, 2013).

Many cells expressing *VEGF-A* have been observed in active lesions for MS, reporting the correlation between the level of this protein and the development of spinal cord injuries, as well as the occurrence of clinical signs of MS. Saravani et al. (2019) found a significant association between the *VEGF-A* C-2578A polymorphism (rs699947) and the disease in the dominant model (P = 0.005) and a higher frequency of the C allele in the MS group, with a 1.6-fold increased risk for the disease susceptibility (P = 0.009).

The high expression of VEGF-A in astrocytic cells is correlated as a key driver of blood-brain barrier (BBB) permeability in mice (Argaw et al., 2012). Moreover, the hyperpermeability of BBB promotes leukocyte infiltration, which can also express VEGF-A and cause CNS damage (Tham et al., 2006). The inactivation of this factor in astrocytes can decrease lymphocytic diapedesis, demyelinating lesions and reduce paralysis in mouse model of MS (Argaw et al., 2012).

Inflammation plays a key role in the development of neurodegenerative diseases. The demyelinating lesions are another characteristic of the MS pathophysiology, resulting from the release of pro-inflammatory molecules, like VEGF-A. Increased levels of this protein can aggravate the inflammatory process (Proescholdt et al., 2002; Su et al., 2006). By contrast, *VEGF-A* is also a neurotrophic factor, and reports of its decreased expression in patients with MS indicate deficits in neurorregenerative attempts (Iacobaeus et al. 2011; Rasol et al., 2016).

The C936T polymorphism (rs3025039) also plays an important role in the etiology of MS, modifying *VEGF-A* functions, such as inflammation, which can lead to changes in the regulation of MS-related pathways. Mazdeh et al. (2017) reported an association between the C936T polymorphism and MS development. The T allele was significantly more frequent among patients with MS when compared to the control group (P = 0.01). In addition, the homozygous TT genotype was associated with an increased risk of developing MS in the recessive model (P = 0.015).

The relationship between *VEGF-A* and ALS susceptibility remains unclear, with controversial studies. Several factors influence these results, as ALS may be strongly related to patients' ancestry and ethnic origin. European populations are more susceptible to the development of ALS than Asians or Africans, becoming an important factor in linking *VEGF-A* with the disease susceptibility (Ioannidis et al., 2001; Marin et al., 2017). However, a recent Brazilian case-control study, performed in a mixed genetic population showed a strong association of VEGF-A rs28357093 with ALS, with a 9-fold increased risk for A/C - C/C genotypes (95%CI = 3.70-21.88; P < 0.001) (da Costa et al., 2022). This result corroborates the complex interaction between genetic and ethnic factors in ALS patients.

Among the selected articles, a significant association was found between polymorphisms A-1154G and C-2578A and ALS development. Chen et al. (2007) reported an association of the genotypes -1154A/G and -1154G/G (P = 0.021) with ALS susceptibility. Lysogorskaia et al. (2012, 2015) found an increased risk for ALS between 1.6-1.7-fold with the -2578A/A genotype (P = 0.004 and P = 0.027, respectively), as well as the association of the A allele with the ALS development (P = 0.036 and P = 0.008, respectively).

Similar to MS, both up or down expression of *VEGF-A* can be a risk for ALS pathophysiology. Considering the direct effects of *VEGF-A* in the neural cells, it plays a role as a neurotrophic and neuroprotective factor, promoting the maintenance and survival of nerve cells (Ekestern, 2004; Storkebaum et al., 2004). Reduced levels of this protein can cause a deficit in neuroprotection and neural hypoperfusion, mechanisms that are harmful to the central nervous system (Holmes and Zachary, 2005; Takahashi and Shibuya, 2005). The reduction of neural blood perfusion promotes ischemia and produces reactive oxygen species, resulting in motor neuron degeneration. Low levels of this factor have been found in the cerebrospinal fluid of patients with early stages of ALS (Iłżecka, 2004).

However, the increased expression of *VEGF-A* is associated with the worsening of the neuroinflammatory process, favoring the BBB dysfunction (Prado et al., 2018). As a cytokine, reactive astrocytes secrete VEGF-A, contributing to increased BBB permeability and peripheral leukocyte migration, enhancing the inflammation (Park et al., 2015).

Most studies of *VEGF-A* polymorphisms do not find a significant association with ALS (Van Vught et al., 2005; Chen et al., 2006; Zhang et al., 2006; Del Bo et al., 2008; Golenia et al., 2010), however, haplotype analysis also shows an association with susceptibility to disease (Lambrechts et al., 2003; Terry et al., 2004). For the polymorphisms C-2578A, A-1154G, and C-634G, the haplotypes AAG or AGG demonstrated risks between 1.8-3.0-fold increased for ALS development, due to decrease of circulating levels of VEGF-A by reduction of gene transcription (Lambrechts et al., 2003; Terry et al., 2004).

In addition, some polymorphisms may express sex-dependent effects. Fernandez-Santiago et al. (2006) found a significant association between the A-1154G polymorphism and ALS susceptibility in women. For the C-2578A polymorphism, studies that perform stratified analyzes for sex found significant associations, with risk 2.1-fold higher for men, due to reduced VEGF-A protein expression (Lambrecths, 2009; Lysogorskaia et al., 2012, 2015). It is suggested that women have naturally higher levels of this factor than men, due to hormonal modulation. Thus, this polymorphism can lead to critical levels of this protein in men (Mueller et al., 2000).

Considering the age of onset of symptoms, the G allele of A-1190G and A-1154G polymorphisms were associated with ALS early-onset. For C-1558T polymorphism, carriers of the T allele presented an advanced age of onset of ALS when compared to patients with the C allele (Chen et al., 2007).

ALS has varied phenotypes among patients and may present, for example, different start locations and severity of symptoms (Connolly et al., 2020). Considering the complexity of the disease, the effects of polymorphisms in the *VEGF-A* gene under ALS phenotypes were investigated, highlighting findings related to sex and age at onset of symptoms.

Our meta-analysis did not find a significant association between the SNPs C-2578A, G-1154A, and G-634C with the risk of ALS. Despite this, some individual studies with these polymorphisms found an association with the disease (Lysogorskaia et al., 2012, 2015). Our findings are in agreement with a meta-analysis performed by Lambrechts et al. (2009) with the same polymorphisms, which found no significant association between these SNPs and ALS. However, stratified analysis by sex found an increased susceptibility for male carriers of the -2578AA genotype.

Therefore, although our systematic review did not find many studies demonstrating the association of *VEGF-A* polymorphisms with the MS and ALS development, a possible role of the expression of this gene with the pathogenesis of neurodegenerative diseases is noted. Both pathologies have not been completely elucidated, thus, further investigations are needed, aiming at elucidating possible mechanisms related to the MS and ALS pathogenesis and their implication in the diagnosis and treatment of these diseases, in order to alleviate the damage caused to patients, providing an improvement in quality of life.

This study found some limitations. First, both ALS and MS are complex diseases with an unclear etiology, involving interaction between environmental and genetic factors. The discrepancies in the results among the studies included in this systematic review can be explained by differences in the sample size, genetic background, the low statistical power, or the difficulty in reproducing genetic studies. For the meta-analysis, it is important to consider the small number of studies included, which may explain the slightly significant risk of bias found. Thus, the Cochrane Handbook (Higgins and Green, 2009) recommends that no bias test be performed in reviews with less than ten studies. Furthermore, some data were insufficient, and it was not possible to obtain eligible studies and additional information to perform the statistical analysis. Thus, further studies involving a large number of patients are necessary.

In conclusion, this systematic review and meta-analysis revealed that polymorphisms in the *VEGF-A* gene may not be the main risk factor for the development of ALS and MS, but associated with specific factors, such as sex or haplotype combinations, may become a strong susceptibility factor. Nevertheless, this study provides a comprehensive understanding of the association between polymorphisms in *VEGF-A* and ALS and MS. The identification of genes and polymorphisms involved in the susceptibility of diseases with unclear neuronal degeneration pathophysiology can assist in the development of effective treatments for affected individuals.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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