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Genetic polymorphisms of the folate pathway in amyotrophic lateral sclerosis and multiple sclerosis: a systematic review and meta-analysis

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ABSTRACT. The folate cycle is a biochemical pathway that plays an important role in the development and maintenance of the nervous system. Biocompounds synthesized in this cycle must be carefully regulated, since the accumulation of some substances can be neurotoxic and increase susceptibility to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). The methylenetetrahydrofolate reductase (*MTHFR*), 5-methyltetrahydrofolate-homocysteine S-methyltransferase (*MTR*), and solute carrier family 19 member 1 (*SLC19A1*) genes encode important proteins for this regulation. In this systematic review and meta-analysis, we investigated the association of some polymorphisms in the *MTHFR*, *MTR*, and *SLC19A1* genes and their associations with ALS and MS. The protocol of this systematic review is registered in the PROSPERO platform (CRD42021232352). We performed a search in EMBASE, Pubmed/NCBI, Scopus, Virtual Health Library (BVS), and Web of

Science databases for studies that described polymorphisms in these genes, regardless of statistical association. Thirteen studies were included, and four polymorphisms were identified: C677T (rs1801133) and A1298C (rs1801131) in the *MTHFR* gene, A2756G (rs1805087) in the *MTR* gene, and A80G in the *SLC19A1* gene. In the meta-analysis, the allelic and genotypic comparison for the C677T polymorphism showed a 1.5-fold increased risk for MS. Despite this significant result, we found a lack of association of most polymorphisms in the *MTR*, *SLC19A1* and *MTHFR* genes and susceptibility for developing ALS and MS. Further studies are needed to clarify the role of polymorphisms in folate pathway genes in the susceptibility for developing these neurodegenerative diseases.

Key words: One-carbon metabolism; Neurodegenerative diseases; Polymorphisms; Systematic review; Meta-analysis

INTRODUCTION

Neurodegenerative diseases are pathologies characterized by the progressive and irreversible loss of neurons (Kovacs, 2017). These diseases show an increased incidence, once it is directly associated with aging (Hou et al., 2019). Individuals over 65 years of age are expected to represent approximately 16% of the world population in 2050 (Oskarsson et al., 2018). Thus, a substantial increase in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS), is projected (Hou et al., 2019).

ALS is a neurodegenerative disease characterized by progressive degeneration of motor neurons in the cerebral cortex, brainstem, and spinal cord, impairing body motor ability. The death of nerve cells results in muscular paralysis and atrophy (Hardiman et al., 2017). In Brazil, ALS incidence is approximately 0.6-2.6/100,000 inhabitants/year (Prado et al., 2016). The disease can be classified, according to its etiology, as sporadic (sALS) or familiar (fALS). The sALS form represents 90% of the cases with a multifactorial background, while the fALS form shows a strong genetic association and, frequently, a dominant inheritance pattern (Hardiman et al., 2017). ALS is more frequent in Caucasian men, between 50 and 75 years, causing progressive and irreversible deficits to the motor system (Chia et al., 2018).

MS is an autoimmune neurodegenerative disease characterized by demyelination mediated by inflammation. As a consequence, MS can prove to be a highly debilitating disease, once the loss of myelin sheath can affect vision, motor coordination, and muscular tone (Filippi et al., 2018). MS prevalence in Brazil is approximately 15 cases in 100,000 inhabitants/per year (Pereira et al., 2015). As for staging, MS is characterized by three forms: Relapsing-remitting, Secondary-Progressive, and Primary-Progressive, with different symptoms for each stage (Filippi et al., 2018). The disease affects mainly females (Voskuhl and Gold, 2012) between 20 and 50 years (Vaughn et al., 2019), debilitating several systems.

Both diseases have an unclear etiology (Hardiman et al., 2017; Filippi et al., 2018), however, several pathophysiological mechanisms have been described. In this context, alterations in the folate cycle were related to the etiology of the diseases (Zoccolella et al.,

2010). The folate cycle is a biochemical cycle responsible for folate metabolism and for controlling the levels of homocysteine (Hcy) in the blood plasma. The homeostasis of these compounds is crucial for several processes, such as DNA methylation, nitrogen bases synthesis, cell division, and growth, erythrocyte formation, maturation, and maintenance of the nervous system (CNS) (Crider et al., 2012).

The genes Methylenetetrahydrofolate Reductase (*MTHFR),* 5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase (*MTR),* and Solute Carrier Family 19 Member 1 (*SLC19A1* or *RFC1*) code enzymes responsible for several key steps in the intracellular maintenance of folate, Hcy, and methionine (Zoccolella et al., 2010). Genetic polymorphisms can increase the susceptibility for neurodegenerative diseases (Wang et al., 2015; Mahmuda et al., 2016) due to the impairment in the enzymatic action or structural enzymatic alterations. These mechanisms can lead to the accumulation of Hcy, generating hyperhomocysteinemia (HHcy), a neurotoxic condition that can lead to damage to motor neurons (Zoccolella et al., 2010). Thus, this systematic review and meta-analysis aimed to analyze the polymorphisms reported in ALS and MS in *MTHFR, MTR,* and *SLC19A1* (*RFC1*) genes, relating them to the diseases, regardless of statistical association.

MATERIAL AND METHODS

Registration

This systematic review was performed since it provides a highly relevant and reliable source of information in the scientific literature and aimed to examine all the polymorphisms in the genes *MTHFR, MTR,* and/or *SLC19A1* (*RFC1*) already reported in ALS and/or MS. The study was registered in the PROSPERO platform on April 23rd, 2021, under number CRD42021232352, and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009).

Search strategy

To formulate the search strategy, we applied the PEO acronym: Population = patients with ALS or MS; Exposure = polymorphisms in the genes *MTHFR, MTR,* and/or *SLC19A1* (*RFC1*); Outcome = association or not with the diseases. The guide question for this systematic review was: "Does the presence of polymorphisms in the *MTHFR, MTR,* and *SLC19A1* genes have any influence on the development of degenerative sclerosis such as Amyotrophic Lateral Sclerosis and Multiple Sclerosis?".

The search strategy was structured using terms indexed in Medical Subject Headings (MeSH) and Descriptors in Health Sciences (DeCS) for “amyotrophic lateral sclerosis”, “multiple sclerosis”, “5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase”, “Methylenetetrahydrofolate Reductase (NADPH2)”, “Reduced Folate Carrier Protein”, combined with boolean operators AND/OR. The search was carried out between December 2020, and January 2021, in PubMed/NCBI, Virtual Health Library (BVS), Embase, Web of Science, and Scopus databases. We also

performed a search in the grey literature. The search strategy adapted for each database is listed in Table 1.

Table 1. Search strategy applied in each database included in this systematic review of genetic polymorphisms of the folate pathway that could be related to amyotrophic lateral sclerosis and multiple sclerosis. Five databases were used, with a search strategy adapted to each database. All search strategies and databases used are reported here.

Database	Search strategy
BVS	tw: (("Polymorphism, Genetic" OR polymorphism*) AND ("5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase" OR "MTR" OR "methionine synthase") OR ("Methylenetetrahydrofolate Reductase (NADPH2)" OR "Methylenetetrahydrofolate Reductase" OR "Methylene-THF Reductase (NADPH)") ("Reduced Folate Carrier Protein" OR "RFC1" OR "SLC19A1" OR "Solute Carrier Family 19 Member 1")) 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	'amyotrophic lateral sclerosis'/exp OR 'multiple sclerosis'/exp AND 'mthfr gene'/exp OR 'slc19a1 gene'/exp OR 'mtr gene'/exp
Pubmed/ NCBI	((("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 ("5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase" OR "MTR" OR "methionine synthase") OR ("Methylenetetrahydrofolate Reductase (NADPH2)" OR "Methylenetetrahydrofolate Reductase" OR "Methylene-THF Reductase (NADPH)") ("Reduced Folate Carrier Protein" OR "RFC1" OR "SLC19A1" OR "Solute Carrier Family 19 Member 1"))
Scopus	TITLE-ABS-KEY (('polymorphism' AND 'mtr' OR 'rfc1' OR 'mthfr') AND 'amyotrophic AND lateral AND sclerosis' OR 'als' OR 'multiple AND sclerosis')
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 ("5-Methyltetrahydrofolate-Homocysteine S-Methyltransferase" OR "MTR" OR "methionine synthase") OR ("Methylenetetrahydrofolate Reductase (NADPH2)" OR "Methylenetetrahydrofolate Reductase" OR "Methylene-THF Reductase (NADPH)") ("Reduced Folate Carrier Protein" OR "RFC1" OR "SLC19A1" OR "Solute Carrier Family 19 Member 1")))

Inclusion and exclusion criteria

We included only observational studies that report polymorphisms in the *MTHFR*, *MTR*, and/or *SLC19A1* (*RFC1*) genes, associated or not with ALS and/or MS, with no time or language restrictions. We excluded studies in non-humans, reviews, articles that do not answer the guide question, and duplicate data.

Selection process

The results of the searches were exported to the Rayyan QCRI software (https://rayyan.qcri.org/) (Ouzzani et al., 2016), to improve the duplicate removal process and make the selection step more reliable through of the reviewers. In all stages, two

independent reviewers carried out the selection of the studies. Discrepancies were solved by a third reviewer. The studies were selected through the reading of the title, abstract, and posteriorly, the full text. After this stage, the selected articles were assessed for risk of bias.

Methodological Quality Analysis

The selected studies were assessed for risk of bias using the Joanna Briggs Institute (JBI) (Moola et al., 2020) critical appraisal tools, according to the study design, by two independent reviewers. Disagreements were solved by a third reviewer. The JBI critical appraisal tools consisted of questions answered with "yes", "no", "unclear" or "not applicable". A study that answered "yes" to all criteria, is classified as low risk of bias. The results are demonstrated through a graphical frequency and the ratings were not used as criteria for study inclusion or exclusion.

Data synthesis

For the descriptive analysis we extracted the following data: (1) first author and year of publication (study); (2) study design; (3) population; (4) disease; (5) sample size; (6) sex of participants; (7) mean age of participants; (7) gene; (8) polymorphism; (9) genotypic, allele and haplotypic frequencies for case and control groups; (10) comparison performed; (11) chi-square value; (12) odds ratio (OR) - 95% confidence interval (95% CI); and (13) P-value. The data extraction was performed by two independent reviewers.

Statistical analysis

The association of each single nucleotide polymorphism (SNP) with ALS or MS was performed by calculating the OR and 95% CI. The OR was obtained through the comparisons of allelic (wild vs mutant) and dominant (heterozygous + mutant vs wild) genetic models. The heterogeneity of the studies was assessed using the I^2 test.

The meta-analytical model was applied according to the results of heterogeneity in the studies. An I^2 = 25-50%, 50-70%, and >70 is represented as low, moderate, and high heterogeneity, respectively (Higgins and Green 2009). Thus, the fixed effect model (Mantel-Haenszel method) was applied when $I^2 < 25\%$, attributing that the differences between the effect estimates are merely random. On the other hand, when I^2 is between 25-75%, we applied the random effect model (DerSimonian-Laird method).

Sensitivity analysis (exclusion of outliers) was performed to confirm and explain possible sources of heterogeneity found 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 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.

The possibility of publication bias in the studies was investigated by funnel plot (Egger et al., 1997) and calculated by Egger's test (Egger and Smith, 1997). A P-value of Egger's test <0.05 suggests a strong probability of publication bias. Statistical analyses were carried out using RStudio software (version 4.1.0).

RESULTS

Found results

The search strategy identified 199 studies. We excluded 16 duplicate data sets, leaving 183 unique studies, and only five studies were considered eligible for this systematic review. Due to the small number of studies selected for inclusion, we also performed a search in the reference lists of these articles, and we included eight additional studies. Thus, a total of 13 studies were included in our systematic review. The flowchart for the selection process is demonstrated in Figure 1.

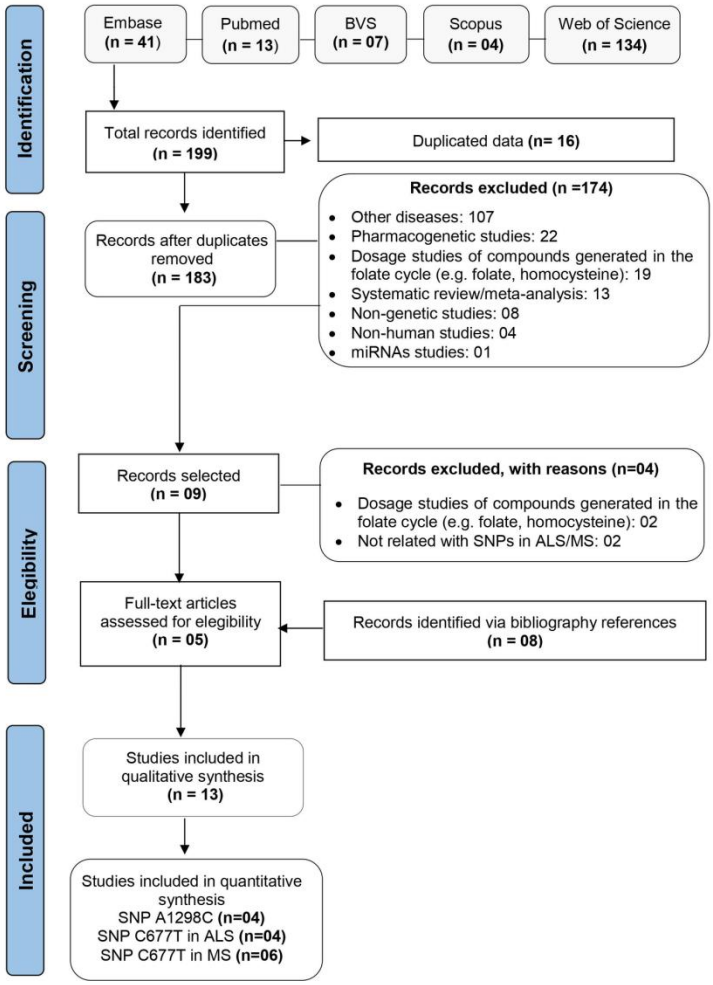

Figure 1. PRISMA flowchart detailing the study selection process for systematic review and meta-analysis of genetic polymorphisms of folate pathway in degenerativesclerosis: MS and ALS. n: number of studies; ALS: amyotrophic lateral sclerosis; MS: multiple sclerosis; SNP: single nucleotide polymorphism. Adapted from The PRISMA Group, 2009.

Characteristics of included studies

The selected studies were published between 2006 and 2019. Of the thirteen studies, twelve were of the case-control type and only one of the cohort type. Nine studies described polymorphisms in MS and four in ALS. Three studies were conducted in Germany, two in Turkey, Australia, and Iran, and one in Switzerland, Tunisia, Italy, and Poland. We identified four polymorphisms: C677T (rs1801133) and A1298C (rs1801131) in *MTHFR* gene, A2756G (rs1805087) in *MTR* gene, and A80G in *SLC19A1* (*RFC1*) gene. Polymorphisms in the *MTHFR* gene were most studied, showing results in 12 publications, while the *MTR* gene and *SLC19A1* (*RFC1*) were found in three and one study, respectively.

Assessment of methodological quality

The studies were heterogeneous for methodological quality assessment. Five criteria were completely fulfilled in all studies (Q1 - groups comparable to each other; Q2 - cases and controls matched appropriately; Q8 - outcomes assessed in a standard, valid and reliable way; Q10 for case-control studies and Q11 for cohort studies - appropriate statistical analysis). Questions 9, in case-control studies (exposure period), and 10, for cohort studies (strategies to address incomplete follow-up) were not applicable for our quality analyses. The quality assessment of the included studies is shown in Figure 2.

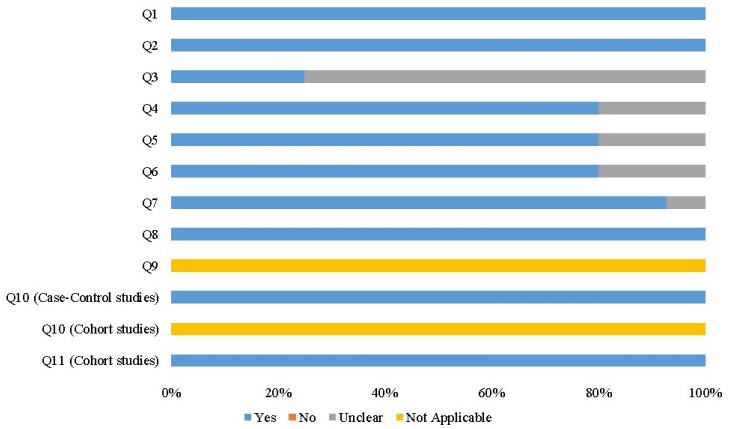

Figure 2. Risk of bias analysis in included studies. Analysis based on questionnaires provided by the Joanna Briggs Institute. Most studies were evaluated using the tool developed for case-control studies and showed homogeneity in the results. Questions 09 and 10 were considered not applicable for case-control and cohort studies, respectively. Q: question.

Synthesis of the results

For the *MTHFR* gene, the SNP C677T showed association with MS in the studies of, Alatab et al. (2011), Cevik et al. (2014), Naghibalhossaini et al. (2015) and Cakina et al. (2019), with risks between 1.32 and 6.23 (95% CI = 0.48-3.66 and 308-1259, respectively). Alatab et al. (2011) demonstrated that individuals with TT genotype have a 18-fold increased risk for MS (95% CI=0.80-4.2). Cakina et al. (2019) found risks of 3,16 (95% CI=1.23-817; p=0.04) for the disease among homozygous individuals (TT). In Cevik et al. (2014) study, the comparison CT + TT vs CC showed a 2.35-fold increased risk for MS (95% CI=1.45-3.82; p= 0.0005).

Naghibalhossaini et al. (2015) found similar OR values for CT genotype (OR = 2,9; 95% CI = 1.88-4.49), while the TT genotype demonstrated a strong association for the MS susceptibility (OR = 6.23; 95% CI = 3.08-12.59). However, Tajouri et al. (2006), Klotz et al. (2010) and Mrissa et al. (2013) failed to find an association between the C677T genotypes and MS (Table1).

Regarding the SNP C677T in the *MTHFR* gene and ALS susceptibility, the study of Kühnlein et al. (2011) was the only one to find an association with the disease. Individuals with CT and TT genotypes showed a 2.26-fold increased risk for ALS when compared to the CC genotype (95% CI=1.03-4.97; p = 0.02). Nevertheless, the studiesof Ricci et al. (2012), Sazci et al. (2012) and Żur-Wyrozumska et al. (2017) showed no association between C677T polymorphism and ALS (Table1).

The SNP A1298C in the *MTHFR* gene showed association with MS in Klotz et al. (2010), Mrissa et al. (2013), Naghibalhossaini et al. (2015) and Cakina et al. (2019) studies, with risks between 0.67 and 4.34 (95% CI=0.26-1.75 and 2.71-6.9, respectively). However, Szvetko et al. (2007) do not find an association between A1298C SNP and MS. For ALS, none study had success in demonstrating the association of this SNP with the disease (Kühnlein et al., 2011; Sazci et al., 2012) (Table1).

For the polymorphism A2756G in the *MTR* gene, no studies have found an association with MS (Ineichen et al., 1997; Cakina et al., 2019) or ALS (Kühnlein et al., 2011). Similarly, only one study addressed the potential association between *SLC19A1* (RFC1) polymorphism and MS. However, Ineichen et al. (2014) found no association between A80G SNP and the disease. Moreover, no study evaluated the possible association between *SLC19A1* polymorphisms and ALS susceptibility (Supplementary 1).

Meta-analysis

The meta-analysis was performed for two SNPs in the *MTHFR* gene: A1298C with MS, and C677T in both diseases. Some studies potentially available for the statistical analysis were not included due to the missing data. In addition, several authors were contacted in an attempt to obtain more data. However, most of these did not respond to emails or no longer had the data available.

Meta-analysis for SNP A1298C in *MTHFR* gene and MS included four studies with 470 patients and 597 controls. The forest plot showed no association between the polymorphism and the disease in both genotypic (AC + CC vs AA) and allelic comparisons (A vs C) (Figure 3).

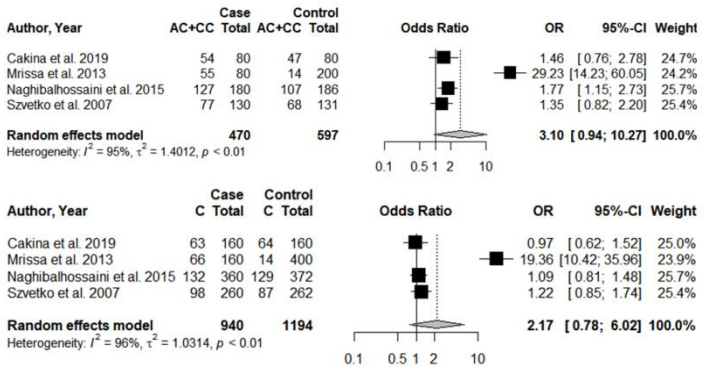

Figure 3. Forest plot for the genotypic and allelic comparison of SNP A1298C in *MTHFR* gene and MS (AC + CC vs AA and A vs C). Odds ratio (OR) and 95% confidence interval (95% CI) were calculated with the random-effect model, due to the value found in the heterogeneity test (I^2).

The analysis for the SNP C677T in the *MTHFR* gene and ALS included four studies with 1,300 patients and 1,801 controls. Similarly, the forest plot also demonstrated no association between the SNP and ALS in genotypic (CT + TT vs CC) and allelic comparisons (C vs T) (Figure 4).

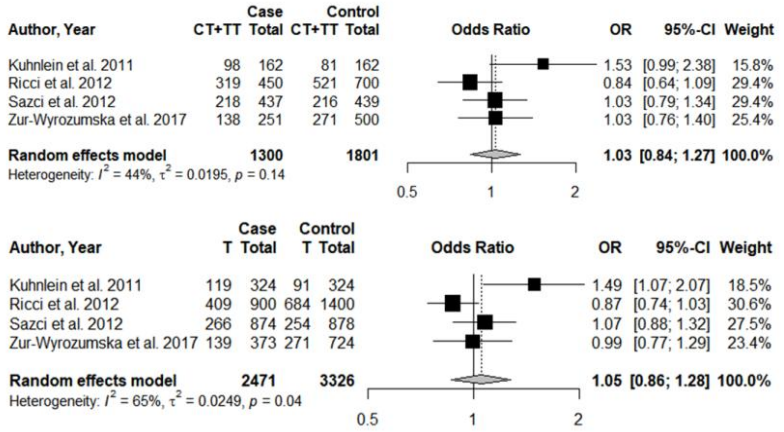

Figure 4. Forest plot for the genotypic and allelic comparison of SNP C677T in *MTHFR* gene and ALS (CT + TT vs CC and C vs T). Odds ratio (OR) and 95% confidence interval (95% CI) were calculated with the random-effect model, due to the value found in the heterogeneity test (I^2).

Regarding the SNP C677T and MS, there were included six studies with a total of 765 patients and 911 controls. Our meta-analysis revealed a 1,5-fold higher risk for MS development in the allelic comparison (C vs T) (95% CI = 1,04-2,16). The results for genotypic comparison (CT + TT vs CC) also showed association with the disease (95% CI = 1,01-1,90) (Figure 5).

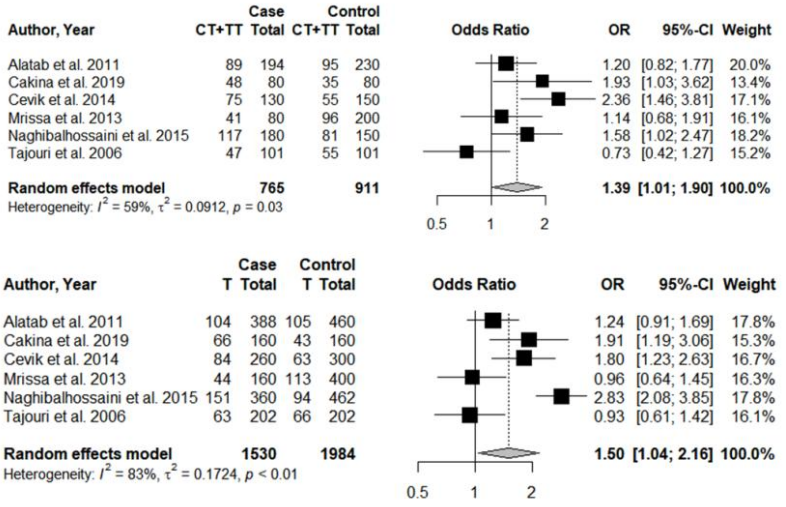

Figure 5. Forest plot for the genotypic and allelic comparison of SNP C677T in *MTHFR* gene and MS (CT + TT vs CC and C vs T). Odds ratio (OR) and 95% confidence interval (95% CI) were calculated with the random-effect model, due to the value found in the heterogeneity test (I^2).

According to the funnel plot and Egger's test, the SNP C677T demonstrated no significant publication bias in studies with ALS (genotypic: P = 0,1139; allelic: P = 0,1247) (Figure 6A and 6B) and MS (genotypic: P = 0,9807; allelic: P = 0,4411) (Figure 6C and 6D). Analysis for the SNP A1298C in MS also showed no significant publication bias for genotypic and allelic comparisons (P = 0,3207 and P = 0,1903, respectively) (Figure 6E and 6F).

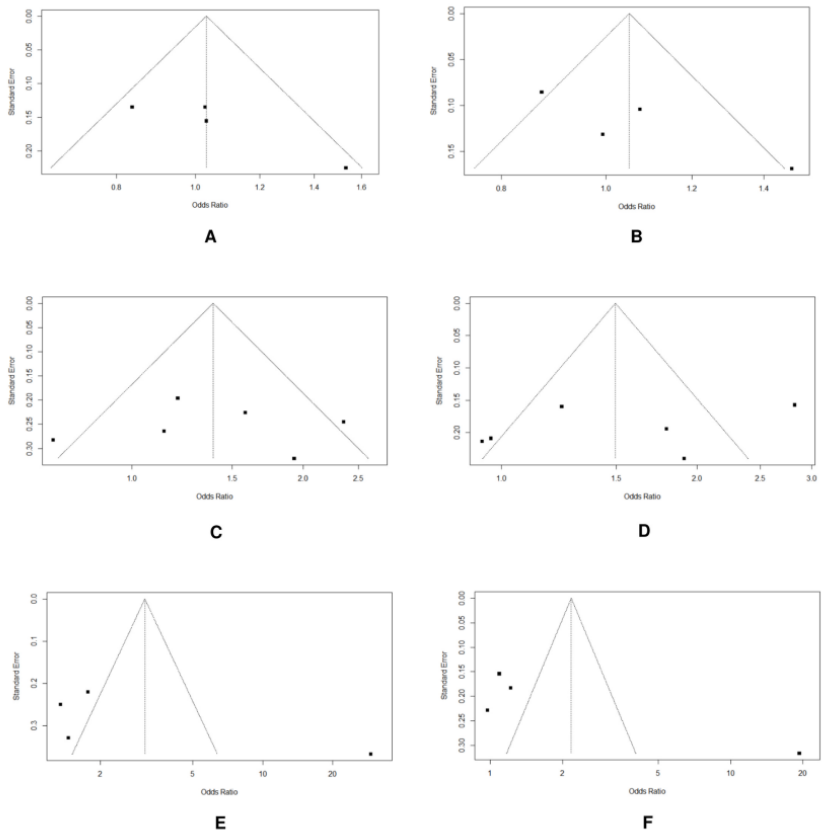

Figure 6. Funnel plots for the publication bias of the studies included in the meta-analysis. A: genotypic comparison by the dominant model (CT+TT vs. CC) for SNP C677T in *MTHFR* gene and ALS, B: allelic comparison (C vs. T) for SNP C677T in *MTHFR* gene and ALS; C: genotypic comparison by the dominant model (CT+TT vs. CC) for SNP C677T in *MTHFR* gene and MS, D: allelic comparison (C vs. T) for SNP C677T in *MTHFR* gene and MS, E: genotypic comparison by the dominant model (AC+CC vs. AA) for SNP A1298C in *MTHFR* gene and MS, F: allelic comparison (A vs. C) for SNP A1298C in *MTHFR* gene and MS.

In the sensitivity analysis, new meta-analyses were performed excluding studies to confirm and explain the heterogeneity rates found. For the A1298C SNP in the *MTHFR* gene in MS, the article by Mrissa et al. (2013) was excluded in the new analysis, due to the large difference in the proportion of cases vs. controls (80 and 200, respectively). Figure 7 shows that in the new meta-analysis the heterogeneity rate found was 0% for genotypic and allelic analysis.

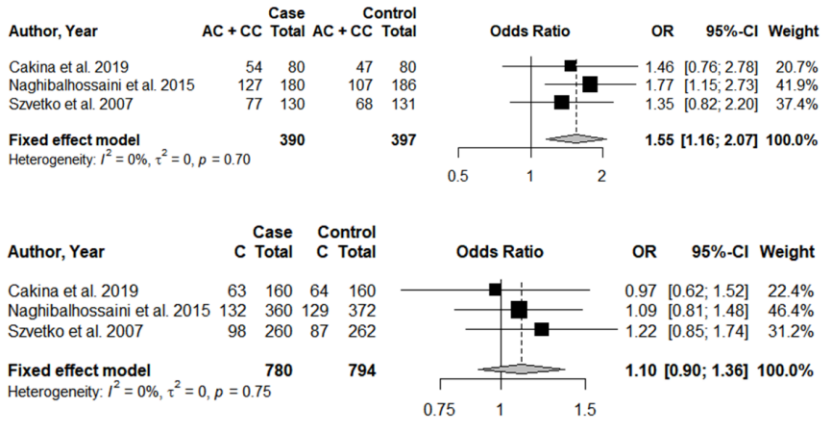

Figure 7. Forest plot for new meta-analysis (sensitivity analysis) of the A1298C SNP in the *MTHFR* gene and MS (AC + CC vs AA and A vs C).

In the new meta-analysis for the C677T SNP in the *MTHFR* gene in ALS, the article by Kühnlein et al. (2011) was excluded, because this is the only study that associated the SNP with the disease. Figure 8 shows that in the new meta-analysis, the heterogeneity rate found was 0% for genotypic analysis and 20% for allelic analysis.

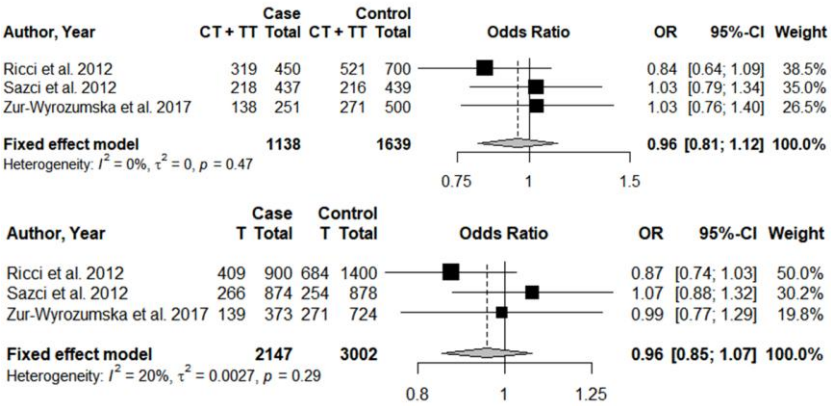

Figure 8. Forest plot for new meta-analysis (sensitivity analysis) of the C677T SNP in the *MTHFR* gene and ALS (CT + TT vs CC and C vs T).

Finally, in the new meta-analysis for the C677T SNP in the *MTHFR* gene in MS, the articles by Tajouri et al. (2006) and Mrissa et al. (2013) were excluded because they did not find an association between the SNP and MS. Figure 9 shows that in the new meta-analysis, the heterogeneity rate found was lower than in the original analysis (39% for genotypic analysis and 78% for allelic analysis).

Furthermore, the distribution of genotypes in the control groups of each study included in the meta-analysis was in accordance with the HWE (P > 0,05) (Supplementary 2).

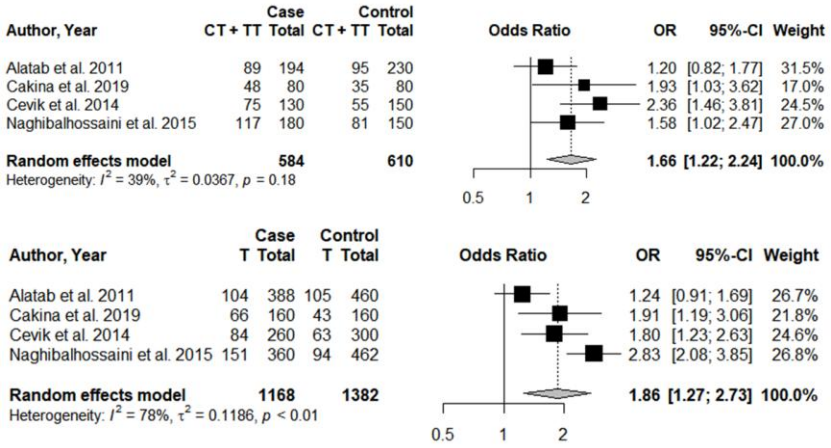

Figure 9. Forest plot for new meta-analysis (sensitivity analysis) of the C677T SNP in the *MTHFR* gene and MS (CT + TT vs CC and C vs T).

DISCUSSION

The etiology of degenerative sclerosis (ALS and MS) remains unclear (Hardiman et al., 2017; Filippi et al., 2018), and the investigation of several risk factors, such as environmental or genetics, is still necessary. Moreover, genetic polymorphisms may increase susceptibility to these diseases (Szvetko et al., 2007; Klotz et al., 2010; Ricci et al., 2012). Thus, this systematic review and meta-analysis aimed to explore the role of polymorphisms in *MTHFR*, *MTR,* and *SLC19A1* genes and the susceptibility to ALS and MS.

The folate cycle is a metabolic pathway involved in vital functions, like nucleic acid synthesis, shuttling reactions, methylation of biomolecules like DNA, RNA, and proteins, also being responsible for the conversion of Hcy into methionine (Ineichen et al., 2014; Nazki et al., 2014; Klemann et al.,2018; Cakina et al., 2019). This conversion is crucial for human metabolism since elevated plasma Hcy levels cause neurotoxicity (Sazci et al., 2012), which is frequently associated with several neurological disorders (e.g., Alzheimer's and Parkinson's diseases) (Morris, 2002; Irizarry et al., 2005; Ansari et al., 2014; Klemann et al.,2018). DNA methylation may also play an important role in the development of neurodegenerative diseases. Andlauer et al. (2010), performed a Genetic Wide Association Study (GWAS), study and identified several susceptibility loci to MS, among them the *SHMT1* gene, which encodes a methyltransferase that is important for the methylation of DNA which is responsible for the transfer of the methyl group within the cycle of a carbon.

The enzymatic protein products MTHFR, MTR, and RFC1 are encoded by homonymous genes and are the most relevant ones in the folate cycle, performing important functions. MTHFR is a key enzyme in folate metabolism, which reduces 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), the major circulating form of this compound (Nazki et al., 2014). The SNPs C677T and A1298C can modify MTHFR activity, which results in reduced enzymatic activity and higher Hcy levels

(Naghibalhossaini et al., 2015; Kühnlein et al., 2011). The MTR enzyme catalyzes the remethylation of Hcy to methionine through a donation of a methyl group of 5-MTHF, an important reaction for the binding of methionine to the production of S-adenosylmethionine (SAM), which is the enzyme responsible for the methylation reactions of biomolecules in the body (Nazki et al., 2014). The RFC1 is a transmembrane protein responsible for the intracellular transport of 5-MTHF, integrating this compound in several functions in the metabolic pathway of a carbon (Bi et al., 2009; Mahmuda et al., 2016).

The role of C677T and A1298C polymorphisms in the *MTHFR* gene in the susceptibility to MS is still not clear. The studies of, Alatab et al. (2011), Cevik et al. (2014), Naghibalhossaini et al. (2015) and Cakina et al. (2019) showed an association of the TT genotype for C677T SNP and the risk of MS development. The study of Naghibalhossaini et al. (2015) demonstrated a strong association between the CT and TT genotypes and MS risk. The CT genotype increased 2,9-fold the susceptibility to the disease (95% CI = 1,88 – 4,49; p=0,00), while the TT genotype increased 6,23-fold the risk for MS development (95% CI= 3,08-12,59; p=0,00). On the other hand, the studies of Tajouri et al. (2006); Klotz et al. (2010) and Mrissa et al. (2013) found no significant association between the polymorphism and the disease.

For the SNP A1298C, the studies from Klotz et al. (2010), Mrissa et al. (2013), Naghibalhossaini et al. (2015) and Cakina et al. (2019) found an association between the polymorphism and MS risk. Naghibalhossaini et al. (2015) showed that the AC genotype increases 2,14-fold the MS susceptibility (95% CI= 1,37-3,34; p=0,001). However, Szvetko et al. (2007) found no association between this polymorphism and the disease.

The SNPs A2576G in the *MTR* gene and A80G in the *SLC19A1* gene were poorly evaluated in both MS and ALS. Cakina et al. (2019) is the only study to evaluate the relationship between the A2756G polymorphism and MS. However, no association was found. Similarly, Ineichen et al. (2014) is the only study to evaluate the relationship between the A80G polymorphism and the MS risk, finding an elevated risk for the G allele and MS early development (p=0,030).

For ALS, the studies on the association of polymorphisms in the *MTHFR*, *MTR*, and *SLC19A1* genes with the disease are even more limited. The studies of Ricci et al. (2012) and Żur-Wyrozumska et al. (2017) found no association between genetic variants and ALS. On the other hand, the study by Kühnlein et al. (2011) showed a significant association between the SNP C677T and the risk of ALS (OR= 2,26 (1,03-4,97); p=0,020). Similarly, Sazci et al. (2012) correlated the genotypes C677C/A1298A and T677T/A1298A with the disease, however only for females.

MS and ALS are marked by inflammation. The study of Alatab et al. (2011) demonstrated that MS patients with TT genotype for the SNP C677T in the *MTHFR* gene showed increased levels of proinflammatory biomarkers, such as TNF-α, hs-CRP, e IL-1 β, when compared with CC or CT genotypes. This result can reflect an uncharacterized mechanism that connects MTHFR pathways to the high expression of these cytokines. Moreover, these proinflammatory biomarkers can be associated with the inflammation through the accumulation of Hcy, not metabolized due to the inefficiency of the protein encoded from these *MTHFR* gene variants. This SNP can modify the function of the MTHFR enzyme, promoting HHcy, which can play a role in the enhancement of inflammation through the release of proinflammatory biomarkers, like IL-6 and VEGF-A (Maeda et al., 2006; Zhang et al., 2006; Lisboa et al., 2020). Moreover, HHcy can activate

nuclear factor-kappa Beta (NF-KB) and induce the transcription of several cytokines (Au-Yeung et al., 2003; Zhang et al., 2006).

HHcy is related in patients with both MS (Besler and Comoğlu, 2003; Vrethen et al., 2003) and ALS (Zoccolella et al., 2008; Zoccolella et al., 2010). The elevation of this neurotoxic compound is also described in other neurodegenerative diseases, such as Parkinson's (Postuma and Lang, 2004) and Alzheimer's (Ng et al., 2018), and the main responsible for this condition is the low enzymatic activity of MTHFR (Brustolin et al., 2010).

Furthermore, the reduction in MTHFR activity is also related to low levels of SAM, which is required for remyelination in the CNS, an important mechanism for patients with MS, since demyelination is a pathological characteristic of the disease. Thus, the HHcy-mediated neurotoxicity is increased and the remyelination of neurons decreased (Mrissa et al., 2013).

HHcy also promotes an increase in the production of reactive oxygen species (ROS) (Bukharaeva et al., 2015). In ALS, there is a decrease in antioxidant enzymes (Sharma et al., 2015) and high levels of ROS can lead to an enhancement in the loss of motor neurons. In addition, it can also promote calcium accumulation in the cytosol, mitochondrial dysfunction, activation of apoptotic mechanisms, and increased damage mediated by excitatory neurotransmitters, such as glutamate (Sazci et al., 2012). Moreover, high levels of ROS synergistically impair the presynaptic compartment of the neuromuscular junction in mammals (Bukharaeva et al., 2015).

Another polymorphism related to an increase in Hcy levels is the SNP A80G in the *SLC19A1* gene, a variant that can impact intracellular folate transport in the body and, consequently, also affecting folate levels in the CNS. Folate levels are inversely correlated with Hcy levels, and due to the small amount of circulating folate, Hcy levels tend to be higher. Thus, this polymorphism can lead to the accumulation of this substance, a neurotoxic condition (Bi et al., 2009).

The MTR enzyme also plays a role in maintaining the levels of biocompounds that originated in the folate cycle. MTR enzyme uses vitamin B12 as a cofactor for its reactions, deficits in this vitamin can affect its enzymatic activity and collaborate with the demyelination of neurons in the CNS (Miller et al., 2005; Kocer et al., 2009). The SNP A2756G in the *MTR* gene is associated with structural alterations in the enzyme, which affects the stability of the secondary structure of the MTR and its enzymatic function. These alterations promote an intracellular homeostatic imbalance of compounds from the folate cycle, such as folate, Hcy, and methionine levels (Ma et al., 1999). Although it plays a fundamental role in the maintenance of the nervous system, a study performed by de Lima et al. (2022), with Brazilian individuals, found no significant association between polymorphisms in *MTR* and *SLC19A1* genes and ALS.

The C677T, A1298C, A80G, and A2756G polymorphisms are also implicated in other degenerative diseases or neurological conditions. Cheng et al. (2010) associated the SNP C677T in the *MTHFR* gene with cognitive disorders due to elevated serum total homocysteine (tHcy) levels, also reporting a higher frequency of CT and TT genotypes in individuals with HHcy in a population from northern China. Almaguer-Mederos et al. (2020) associated the SNP A1298C also in the *MTHFR* gene with the saccadic movements (saccade latency) in Cuban patients with spinocerebellar ataxia type 2. Bi et al. (2009) correlated the SNP A80G in the *SLC19A1* gene with Alzheimer's susceptibility. Fong et al.

(2011) showed a significant association between Parkinson's susceptibility and the additive effect of SNPs C1783T in the *MTRR* gene and A2756G in the *MTR* gene in individuals of Chinese origin in Taiwan.

Although some studies show significant association among SNPs in *MTHFR*, *MTR*, and *SLC19A1* and neurodegenerative diseases, the relationship between dysregulation in compounds of the folate cycle, such as folate and Hcy with ALS and MS remains unclear. Furthermore, different factors can infer different results in genetic association studies, such as statistical analyses, ethnicity, study designs, sample sizes, and genotyping methods. In addition, the lack of definition in the etiological mechanisms of these diseases makes it difficult to determine other factors that could alter the results and/or susceptibility to the disease.

Our results showed that the T allele of C677T polymorphism was significantly associated with MS. Moreover, genotypic comparison also showed an increased risk for the disease regarding CT+TT genotypes. Nevertheless, our data need to be interpreted with caution due to heterogeneity values. In this condition, the ideal is to perform analyzes by subgroups (e.g., males and females) or meta-regression. Achieving these analyzes is difficult due to insufficient published data on specific characteristics of the participants, such as the exact number of males and females individuals genotyped in each group. Furthermore, we had no responses to most emails sent to authors. Considering meta-regression, its application is not recommended when there are less than ten studies in a meta-analysis (Higgins and Green, 2009).

According to the Cochrane manual (Higgins and Green, 2009), investigations of heterogeneity when there are few studies are of questionable value. We decided to incorporate heterogeneity across studies, using random effects meta-analysis where applicable. In addition, we explored possible sources of heterogeneity through outlier exclusion, which consists of removing studies that are inconsistent with a clinical or methodological justification. Additional analyzes are needed to explain the heterogeneity found, mainly in the analysis of the C677T SNP in the *MTHFR* gene and MS, however, we suggest that the high heterogeneity found between studies is directly related to the small number of studies included in the meta-analysis or the high variation in sample size between studies.

In conclusion, this systematic review and meta-analysis revealed a 1,5-fold higher risk for MS considering the 677T allele, and a 1,39-fold risk for the CT+TT genotypes of C677T polymorphism in the *MTHFR* gene. Our results indicate that SNPs in folate pathway genes can become strong susceptibility factors for degenerative sclerosis. This review provides an important basis for understanding the genetic factors involved in the susceptibility to neurodegenerative diseases. Our results can assist in the development of therapeutic technologies for ALS and MS as well as contribute to genetic research in the area.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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