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Enhanced Genetic Vulnerability to Amyotrophic Lateral Sclerosis: Insights from a Case–Control Study on the *MTHFR C677T* Variant in a Brazilian Population

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Abstract

Background/Objectives: Amyotrophic Lateral Sclerosis (ALS) is a rare, neurodegenerative disease with complex genetic and environmental determinants. The *MTHFR C677T* (rs1801133) variant, known for reducing enzymatic activity in the folate cycle, has been implicated in ALS risk, though findings remain inconsistent across diverse populations. **Methods**: A population-based case–control study was conducted in 248 age-matched individuals to investigate the *MTHFR C677T* (rs1801133) and ALS susceptibility. Molecular analysis was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Genetic associations were evaluated under multiple inheritance models, while survival analysis utilized the Kaplan–Meier method to assess the relationship between *MTHFR* genotypes and patient prognosis. **Results**: The C677T variant showed a significant association under the codominant and recessive models, suggesting involvement in ALS risk (OR = 4.63; $p = 0.01$ and OR = 3.92; $p = 0.02$), respectively. However, stratification by sex demonstrated an association predominantly in women (OR = 7.10, $p = 0.02$; OR = 5.87, $p = 0.04$). Additionally, Kaplan–Meier analysis revealed a numerically shorter mean survival time for the mutant genotype compared with wild-type and heterozygous carriers, without statistical significance. **Conclusions**: Notably, we identified a significant association between the *MTHFR C677T* (rs1801133) variant and ALS risk, particularly among women. These findings suggest that the mutant (T/T) genotype showed a stronger association, potentially reflecting postmenopausal hormonal influences on one-carbon metabolism and related susceptibility pathways.

Keywords: amyotrophic lateral sclerosis; *MTHFR C677T*; one-carbon metabolism; hyperhomocysteinemia; genetic association study; sex-specific effects

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and lethal neurodegenerative disease (ND) affecting upper and lower motor neurons, causing irreversible damage that leads to neuronal apoptosis [1–3]. The disease has an annual incidence of ~2/100,000

and a prevalence of ~5.4/100,000, predominantly affecting males, Caucasian populations, and individuals aged between 55 and 75 years [4].

ALS has a complex etiology in which genetic factors, such as mutations and variants, play a critical role [5–7]. Additionally, disruptions in cellular mechanisms, including oxidative stress, glutamatergic excitotoxicity, and hyperhomocysteinemia (HHcy), have been identified as key contributors to disease pathology [8–10].

Within the context of cellular biochemistry, the folate metabolic cycle (one-carbon metabolism) stands out as a significant factor in ALS pathophysiology [10–12]. Variants affecting this pathway may promote neurodegeneration, highlighting the relevance of genes involved in one-carbon metabolism. In this context, the *methylenetetrahydrofolate reductase* (*MTHFR*) gene and its functional single-nucleotide variant C677T (rs1801133) have been directly linked to mechanisms intrinsic to ALS, largely due to their impact on folate metabolism and homocysteine homeostasis [7,8].

Dysregulation of folate metabolism, often associated with genetic polymorphisms, disrupts cellular homeostasis and contributes to neurodegeneration. For instance, reduced enzymatic activity of MTHFR promotes the accumulation of homocysteine (Hcy), a sulfur-containing amino acid that exerts neurotoxic effects on cellular pathways [10,11].

Despite growing evidence implicating one-carbon metabolism in ALS pathophysiology, the contribution of common functional variants in folate-pathway genes, particularly the *MTHFR C677T* (rs1801133) variant, to ALS susceptibility remains inconsistent across studies and populations.

Importantly, data from highly admixed populations, such as the Brazilian population, are scarce, and potential sex-specific effects have not been systematically explored. Accordingly, this study was designed to investigate the potential association between the *MTHFR C677T* (rs1801133) variant and ALS susceptibility and prognosis in a Brazilian population.

2. Materials and Methods

This case–control study consists of 101 patients diagnosed with ALS (case group) who underwent medical follow-up at the referral hospital for neuromuscular diseases in Goiânia, GO, Brazil, Centro de Reabilitação e Readaptação Dr. Henrique Santillo (CRER). According to the Instituto Brasileiro de Geografia e Estatística (IBGE, 2024) [13], the estimated population of the state of Goiás for 2024 is 7,474,621 individuals. Thus, considering the worldwide incidence of ALS, it is estimated that our state has approximately 149 patients with ALS. Thus, our sample corresponds to ~68% of the expected number of cases in the state, considered a significant sample of cases of this rare disease, considering our population size.

The control group consisted of 147 individuals with no diagnosis of ALS or other ND, who were recruited from the Laboratório de Análises Clínicas e Educação em Saúde at the Universidade Federal de Goiás, Brazil (LACES-UFG). The groups were matched for age according to the STrengthening the Reporting of OBservational Studies in Epidemiology (STROBE) and STrengthening the REporting of Genetic Association Studies (STREGA) guidelines [14]. Age matching is the most rigorous and frequent criterion, as age is the primary confounding factor in ND such as ALS. The study was approved by the UFG Ethics Committee, under number CAAE 79593117.7.0000.5083, and conducted following the Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association Declaration of Helsinki.

The inclusion criteria were patients aged between 18 and 90 years, regardless of the stage of disease progression, who followed the laboratory and imaging exam protocol defined for diagnosing ALS, standardized by CRER. Patients unable to provide venous blood samples were excluded. The clinical and sociodemographic data of the patients were obtained through previously applied questionnaires, and the remaining data were obtained through medical records. After explanations about the objective and design of the research, the individuals signed the informed consent form and peripheral blood samples were collected for subsequent molecular analysis.

Molecular analysis of the *MTHFR C677T* (rs1801133) variant was performed according to our previously published protocol (available at: https://dx.doi.org/10.17504/protocols.io.brrkm54w (accessed on 14 May 2026)), using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. Statistical analyses were performed using R software (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria) within the RStudio environment version 4.5.1 (RStudio, PBC, Boston, MA, USA). The general characterization of the groups was performed using descriptive statistics, with absolute (*n*) and relative (%) frequencies reported, and mean and standard deviation applied to continuous variables. To assess sociodemographic characteristics and lifestyle habits, Student's *t*-test was used for quantitative variables and Fisher's exact test for qualitative variables.

For genetic analysis, the genotypes were classified, according to the inheritance models, as codominant, dominant, recessive and overdominant for application of binomial logistic regression. Then, the Odds Ratio (OR) and the Confidence Intervals (CI) with a 95% confidence level were calculated. Hardy–Weinberg equilibrium (HWE) was evaluated using the exact test (two-sided) with mid-p adjustment when indicated; expected counts were computed from sample-based allele frequencies [15].

Subsequently, the data were stratified by gender and submitted to genetic models with logistic regression. Allelic analyses were performed using Fisher's exact test. Additionally, the association of genotypes with the survival of ALS patients was assessed using the Kaplan–Meier curve. For all tests, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Sociodemographic and Lifestyle Data

The sociodemographic analysis (Table 1) revealed that the groups were well-matched regarding age, with a mean of 57.68 ± 11.24 years ($p = 0.700$). However, significant differences were observed in gender distribution and alcohol intake. The ALS group showed a higher prevalence of males (55.45%) compared to the control group (34.69%; $p = 0.001$), consistent with the known epidemiological profile of the disease. Furthermore, alcohol consumption was significantly higher in ALS patients (45.54%) than in controls (29.25%; $p = 0.012$). Smoking habits did not differ significantly between the groups ($p = 0.713$).

Table 1. Comparison of sociodemographic variables and lifestyle habits between the groups.

Variables	Control, *n* (%)	Case, *n* (%)	Total	*p*-Value
Age (Mean ± SD)	57.92 (9.99)	57.34 (12.89)	57.68 (11.24)	0.700 [a]
Gender				
Female Male	96 (65.31%) 51 (34.69%)	45 (44.55%) 56 (55.45%)	141 (56.85%) 107 (43.15%)	0.001 [b,*]

Table 1. *Cont.*

Variables	Control, *n* (%)	Case, *n* (%)	Total	*p*-Value
Alcohol Intake				
No	104 (70.75%)	55 (54.46%)	159 (64.11%)	0.012 [b,*]
Yes	43 (29.25%)	46 (45.54%)	89 (35.89%)	
Smoking				
No	90 (61.22%)	65 (64.36%)	155 (62.50%)	0.713 [b]
Yes	57 (38.78%)	36 (35.64%)	93 (37.50%)	

[a] Student *t*-test; [b] Pearson's Chi-square test. *n* = absolute frequency; % = relative frequency; SD = standard deviation. * = $p \leq 0.05$.

3.2. Genetic Analysis

The distribution of *MTHFR C677T* (rs1801133) genotypes and the respective association models are detailed in Table 2. The analysis of the inheritance models revealed a significant correlation between the variant and the risk of developing ALS. The mutant (T/T) genotype was identified as a significant risk factor for the disease under the codominant model (T/T vs. C/C: OR = 4.63, 95% CI: 1.45–17.74; $p = 0.01$), with a similar association observed in the recessive model (T/T vs. C/C + C/T: OR = 3.92, 95% CI: 1.27–14.66; $p = 0.02$). In contrast, no significant associations were observed under the dominant or overdominant models. Additionally, we observed a higher T-allele frequency in cases vs. controls ($p = 0.03$), and genotype distributions were in HWE among cases (p-exact = 0.509; mid-p = 0.449)—Table S1.

Table 2. Association between genotypes and ALS, using genetic inheritance models.

Models	Genotype	Control, *n* (%)	Case, *n* (%)	OR (95% CI)	*p*-Value
Codominant	C/C	76 (51.70%)	41 (40.59%)	Ref	Ref
	C/T	67 (45.57%)	50 (49.50%)	1.38 (0.81–2.35)	0.22
	T/T	4 (2.72%)	10 (9.90%)	4.63 (1.45–17.74)	0.01 *
Dominant	C/C	76 (51.70%)	41 (40.59%)	Ref	Ref
	C/T + T/T	71 (48.29%)	60 (59.40%)	1.56 (0.94–2.62)	0.08
Recessive	C/C + C/T	143 (97.27%)	91 (90.09%)	Ref	Ref
	T/T	4 (2.72%)	10 (9.90%)	3.92 (1.27–14.66)	0.02 *
Overdominant	C/C + T/T	80 (54.42%)	51 (50.49%)	Ref	Ref
	C/T	67 (45.57%)	50 (49.50%)	1.17 (0.70–1.94)	0.54
Alleles					
	C	219	132	-	0.03 *
	T	75	70	-	

n = absolute frequency; % = relative frequency; CI = confidence interval; OR = odds ratio; Ref = reference; C = cytosine; T = thymine. * = $p \leq 0.05$.

However, when the analysis was stratified by sex, the association between the *MTHFR C677T* (rs1801133) and ALS remained significant in the female subgroup (Table 3). In women, T/T genotype was associated with increased ALS risk in both codominant and recessive models (OR = 7.10; 95% CI: 1.40–52.59; $p = 0.02$; OR = 5.87; 95% CI: 1.21–42.22; $p = 0.04$). Conversely, no significant associations were detected under the dominant and overdominant models. Moreover, a borderline-significant difference in allelic frequencies was observed between cases and controls ($p = 0.05$). In the male subgroup, multivariate logistic regression analysis adjusted for age showed no statistically significant association

between the variant and ALS risk across all inheritance models (Table 3). These findings suggest that in this population, the mutant (T/T) genotype may be associated with ALS susceptibility among women.

Table 3. Association between genotypes and ALS stratified by sex, using genetic inheritance models.

Female					
Models	**Genotype**	**Control, *n* (%)**	**Case, *n* (%)**	**OR (95% CI)**	***p*-Value**
Codominant	C/C	54 (56.25%)	19 (42.22%)	Ref	Ref
	C/T	40 (41.66%)	21 (46.66%)	1.49 (0.71–3.15)	0.29
	T/T	2 (2.08%)	5 (11.11%)	7.10 (1.40–52.59)	0.02 *
Dominant	C/C	54 (56.25%)	19 (42.22%)	Ref	Ref
	C/T + T/T	42 (43.75%)	26 (57.77%)	1.75 (0.86–3.63)	0.12
Recessive	C/C + C/T	94 (97.91%)	40 (88.88%)	Ref	Ref
	T/T	2 (2.08%)	5 (11.11%)	5.87 (1.21–42.22)	0.04 *
Overdominant	C/C + T/T	56 (58.33%)	24 (53.33%)	Ref	Ref
	C/T	40 (41.66%)	21 (46.66%)	1.22 (0.59–2.50)	0.57
Alleles					
	C	148	59	-	0.05
	T	44	31	-	
Male					
Models	**Genotype**	**Control, *n* (%)**	**Case, *n* (%)**	**OR (95% CI)**	***p*-Value**
Codominant	C/C	22 (43.13%)	22 (39.28%)	Ref	Ref
	C/T	27 (52.94%)	29 (51.78%)	1.07 (0.48–2.37)	0.85
	T/T	2 (3.92%)	5 (8.92%)	2.50 (0.48–18.77)	0.30
Dominant	C/C	22 (43.13%)	22 (39.28%)	Ref	Ref
	C/T + T/T	29 (56.86%)	34 (60.71%)	1.17 (0.54–2.54)	0.68
Recessive	C/C + C/T	49 (96.07%)	51 (91.07%)	Ref	Ref
	T/T	2 (3.92%)	5 (8.92%)	2.40 (0.49–17.32)	0.30
Overdominant	C/C + T/T	24 (47.05%)	27 (48.21%)	Ref	Ref
	C/T	27 (52.94%)	29 (51.78%)	0.955 (0.44–2.04)	0.90
Alleles					
	C	71	73	-	0.58
	T	31	39	-	

n = absolute frequency; % = relative frequency; CI = confidence interval; OR = odds ratio; Ref = reference; C = cytosine; T = thymine. * = $p \leq 0.05$.

Additionally, analysis of the sociodemographic and clinical characteristics of ALS cases according to *MTHFR C677T* genotypes (Table 4) revealed significant associations with self-reported ethnicity and physical activity. An association between the C677T variant and the variables ethnicity and physical activity was observed. Interestingly, individuals who self-identified as white predominantly carried the wild-type (C/C) or heterozygous (C/T) genotypes, with only one white individual presenting the mutant (T/T) genotype ($p = 0.005$). In contrast, 90% of individuals with the mutant (T/T) genotype were brown ($p = 0.001$). Additionally, all individuals carrying the mutant (T/T) genotype reported

participation in some form of physical activity, a finding that was statistically significant ($p = 0.006$) (Table 4).

Table 4. Association between genotypes of the *MTHFR C677T* (rs1801133) variant and the sociodemographic and clinical profile of ALS patients.

	MTHFR, n (%)			*p* [a]
	Wild (C/C)	**Heterozygous (C/T)**	**Mutant (T/T)**	
Gender				
Female	19 (46.3)	21 (42.0)	5 (50.0)	0.85
Male	22 (53.7)	29 (58.0)	5 (50.0)	
Ethnicity				
White	20 (48.8)	28 (56.0)	1 (10.0)	0.005 *
Black	6 (14.6)	1 (2.0)	0 (0.0)	0.23
Brown	15 (36.6)	21 (42.0)	9 (90.0)	0.001 *
Physical Activity				
No	23 (56.1)	24 (48.0)	0 (0.0)	0.006 *
Yes	18 (43.9)	26 (52.0)	10 (100.0)	
Alcohol Intake				
No	27 (65.9)	23 (46.0)	5 (50.0)	0.16
Yes	14 (34.1)	27 (54.0)	5 (50.0)	
Smoking				
No	29 (70.7)	29 (58.0)	7 (70.0)	0.42
Yes	12 (29.3)	21 (42.0)	3 (30.0)	
Classification				
Sporadic	39 (95.1)	46 (92.0)	10 (100.0)	0.57
Familial	2 (4.9)	4 (8.0)	0 (0.0)	
Neurological disease in the Family				
No	24 (58.5)	30 (60.0)	6 (60.0)	0.99
Yes	17 (41.5)	20 (40.0)	4 (40.0)	
Previous pathologies				
No	23 (56.1)	26 (52.0)	5 (50.0)	0.90
Yes	18 (43.9)	24 (48.0)	5 (50.0)	

[a] Post hoc Chi-square; *n* = absolute frequency; % = relative frequency. C = cytosine; T = thymine. * = $p \leq 0.05$.

3.3. Survival Analysis According to MTHFR C677T (rs1801133) Genotypes

Survival analysis was performed using the Kaplan–Meier method to evaluate the association between the *MTHFR C677T* (rs1801133) genotypes and ALS prognosis (Figure 1). Patients carrying the mutant (T/T) genotype showed a trend toward shorter survival compared with wild-type (C/C) and heterozygous (C/T) carriers. However, this difference was not statistically significant according to the log-rank test ($p = 0.58$). The considerable overlap among the survival curves suggests that the numerical differences observed may be attributable to limited statistical power rather than a true prognostic effect.

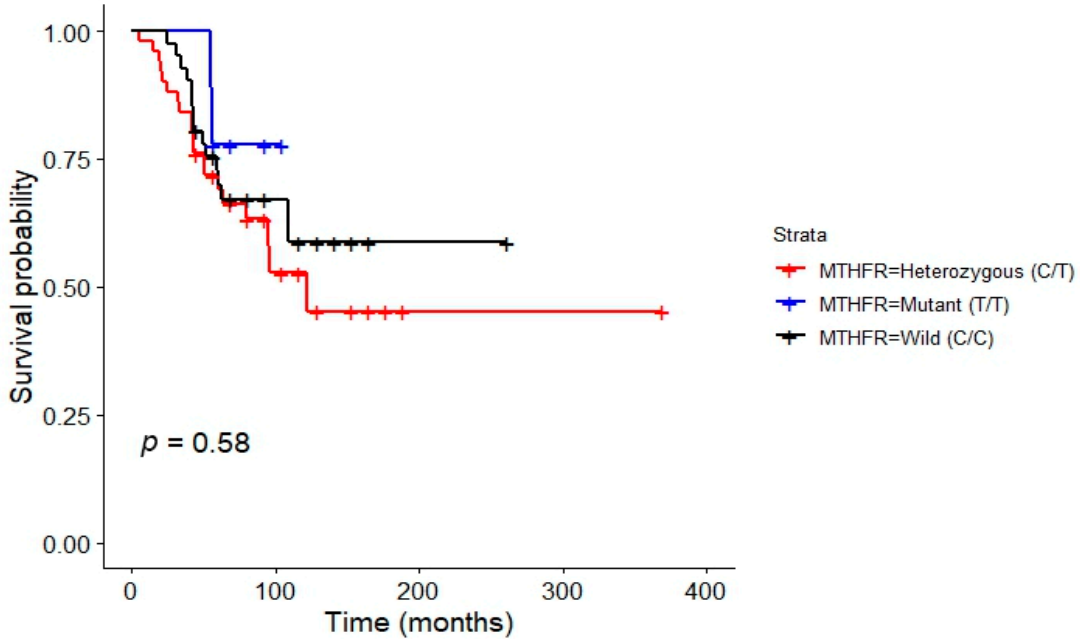


Figure 1. Kaplan–Meier survival curves of ALS patients stratified by *MTHFR C677T* (rs1801133) genotypes (C/C, C/T, and T/T). Patients carrying the homozygous mutant genotype (T/T) exhibited a numerically shorter survival compared to wild-type (C/C) and heterozygous (C/T) carriers; however, no statistically significant differences were observed among groups according to the log-rank test ($p = 0.58$).

4. Discussion

This observational case–control study was designed to explore the association between the *MTHFR C677T* (rs1801133) variant and ALS susceptibility and prognosis in a Brazilian population. Although ALS pathogenesis remains complex, the role of specific genetic variants in disease risk and progression is still not fully elucidated, and the literature regarding these associations remains limited and inconsistent [16].

ALS represents a profound clinical challenge, characterized as a relentlessly progressive and fatal ND. While ALS etiology is widely accepted to be multifactorial, involving a complex interplay of genetic, epigenetic, and environmental factors, the specific contribution of individual lifestyle and modifiable risk factors remains largely elusive. Currently, advanced age and male sex remain the only universally established non-genetic risk factors for ALS [4]. Our findings are consistent with this paradigm, as evidenced by the marked male predominance observed in our ALS cohort, while also highlighting the need to explore factors beyond these traditional markers to better understand disease susceptibility.

Epidemiologically, ALS consistently exhibits a male-to-female ratio of approximately 1.5:1. This observation is often attributed to differential occupational and environmental exposures among men, which may act as significant non-genetic contributors to neurodegeneration [4]. Furthermore, recent global projections indicate a progressive increase in both the incidence and prevalence of ALS over the next two decades [16]. Given this demographic profile and the persistent sex-related disparity in disease burden, there is a need to examine sex-specific genetic factors [9,17–19]. Variants such as *MTHFR C677T* (rs1801133), investigated in the present study, may act as modifiers of susceptibility and help explain these epidemiological trends.

Another critical factor identified in our study was the association between alcohol consumption and ALS susceptibility. The role of alcohol in ALS pathogenesis remains debated. While some studies suggest that high alcohol intake may adversely affect neuronal integrity, others report neutral or even protective associations, indicating substantial

heterogeneity across cohorts and exposure patterns [1,20–22]. Notably, some studies have paradoxically described a protective association, potentially linked to the antioxidant properties of specific beverages, such as wine [21,23], or to the modulation of glutamatergic neurotransmission [1].

From a mechanistic perspective, excessive alcohol consumption is known to disrupt neurotransmitter homeostasis and exacerbate core ALS-related pathogenic processes, specifically excitotoxicity and oxidative stress. Furthermore, alcohol metabolism is documented to interfere with folate homeostasis, directly inhibiting the enzymatic activities critical to the one-carbon metabolism cycle [1]. This interaction is particularly relevant given our findings, as alcohol-induced interference with folate pathways may exacerbate the metabolic stress already present in carriers of the variant.

Notably, under the codominant model, we observed a robust association between the mutant (T/T) genotype and ALS susceptibility (OR = 4.63; $p = 0.014$), while the recessive model indicated an approximately four-fold increase in risk among mutant (T/T) carriers (OR = 3.92; $p = 0.02$). These findings suggest that an additive allelic effect may be a critical determinant of disease risk, further highlighting the importance of evaluating genetic models beyond the simple recessive or dominant approaches. The hypothesis that the variant contributes to ALS pathogenesis is supported by evidence indicating that this alteration impairs the intracellular folate pathway [24].

This association suggests that the homozygous state may represent a critical threshold for metabolic dysfunction. Given that the mutant (T/T) genotype exhibits the lowest MTHFR enzymatic activity, it is plausible that only a pronounced disturbance of one-carbon metabolism is sufficient to produce neurotoxic effects on motor neurons, potentially via systemic HHcy [11]. Accordingly, susceptibility may not be purely additive but contingent on a genotype configuration that exceeds a critical boundary for folate homeostasis.

Elevated homocysteine levels are inherently neurotoxic, promoting neuronal damage through mechanisms such as DNA hypomethylation, impaired nucleotide synthesis, and exacerbated oxidative stress [25,26]. These metabolic disturbances are well-documented in the progression of ND and may represent a critical link between the *MTHFR C677T* (rs1801133) variant and the clinical manifestation of ALS [8,10].

Additionally, given the well-established sexual dimorphism in ALS incidence and the potential influence of hormonal status on disease susceptibility [20], we performed sex-stratified analyses to assess whether the effect of the variant differs between men and women. Elucidating sex-specific genetic influences is essential to refine our understanding of ALS risk, as traditional models often overlook the interplay between sex-related neuroprotective mechanisms and underlying metabolic vulnerabilities [9,19]. The female-specific signal observed may reflect an interaction between reduced MTHFR activity and postmenopausal hormonal changes, which modulate one-carbon metabolism, homocysteine burden, and methylation capacity, thereby warranting replication in biomarker-based studies.

In women, the postmenopausal decline in estrogen levels is believed to reduce the neuroprotective effects that contribute to the lower incidence of ALS in younger females. As these hormonal levels decline with age, intrinsic neuroprotective mechanisms are diminished, potentially unmasking the susceptibility conferred by specific genetic variants [9]. Our findings support this paradigm, demonstrating a clear association between the mutant (T/T) genotype and ALS susceptibility in women. This suggests that the combined effects of diminished estrogen-mediated neuroprotection and metabolic stress induced by MTHFR dysfunction may synergistically increase disease risk, reinforcing the hypothesis that genotype–environment interactions are highly sex-specific [19,20].

In accordance with HWE, this study identifies the *MTHFR C677T* variant as significantly associated with ALS susceptibility in the Brazilian population. Notably, the observed sex-specific effect suggests a distinct biological vulnerability that needs further investigation. The adherence of case genotype distributions to HWE supports the robustness of our findings and reinforces the reliability of genotyping procedures and the representativeness of the study population [14,15,27].

Regarding sociodemographic composition, individuals who self-identified as white represented the majority of our sample. This pattern is consistent with previous reports indicating a higher prevalence of ALS in white populations, which has often been attributed to population-specific genetic risk architectures [6,28,29]. However, these findings should be interpreted with caution, as they may also reflect longstanding disparities in healthcare access. Marginalized groups, including Black and mixed-race (brown) individuals, often face barriers to specialized neurological care, which may contribute to underdiagnosis [30,31].

In the Brazilian context, the high degree of ethnic admixture poses a significant challenge for genetic epidemiological studies. The classification of individuals as "brown" encompasses substantial genetic heterogeneity, which complicates the identification of distinct etiological mechanisms [32,33]. Consequently, understanding how ancestry-specific genetic backgrounds influence ALS susceptibility remains a complex yet essential area of investigation in highly admixed populations.

When examining lifestyle-related factors, we identified physical activity as a contributor, reported by 53.46% of ALS patients. The role of physical activity as a risk factor for ALS remains a subject of considerable debate. Although regular exercise is traditionally regarded as neuroprotective, accumulating evidence suggests that strenuous or high-intensity physical activity may paradoxically increase ALS risk [34]. Several mechanistic pathways have been proposed, including heightened metabolic demands that amplify systemic oxidative stress, as well as exercise-induced upregulation of pro-inflammatory cytokines, which may initiate or exacerbate neuroinflammatory cascades detrimental to motor neurons [35–37].

Our findings align with those reported by Huisman et al. (2013) [38] and Beghi (2013) [35], highlighting the need to consider not only engagement in physical activity but also its intensity, frequency, and cumulative lifetime burden as potential metabolic stressors influencing ALS susceptibility. In this context, it is important to note that ALS is a rapidly progressive neurodegenerative disease, with a mean survival of approximately three to five years [39,40].

In the present study, we investigated whether *MTHFR C677T* (rs1801133) genotypes were associated with survival outcomes in ALS patients; however, no significant genotype–survival associations were identified. One plausible explanation resides in the biochemical complexity of the one-carbon metabolic network, which depends on the coordinated activity of multiple enzymes [1]. It is therefore conceivable that compensatory pathways involving other folate-dependent enzymes may mitigate the functional consequences of reduced MTHFR activity, thereby attenuating or obscuring genotype-specific differences in survival among affected individuals.

This study has some limitations that should be considered when interpreting the findings. Although our sample represents approximately 68% of the estimated ALS cases in the state of Goiás, the overall number of participants remains limited for a genetic association study involving a low-frequency genotype such as the homozygous T/T genotype. Consequently, the small number of individuals carrying this genotype may have reduced the statistical power to detect associations of smaller effect sizes, particularly in the survival analysis. In addition, the case–control design does not allow for the assessment of longitudinal changes in folate-cycle biomarkers (e.g., homocysteine, folate, SAM/SAH ratio),

which could have provided additional mechanistic insight into how *MTHFR* genotypes influence disease susceptibility and progression.

Although direct assessments of MTHFR enzymatic activity and circulating homocysteine concentrations would provide additional mechanistic resolution, the present study was specifically designed as a population-based genetic association analysis. Consequently, biochemical measurements were not available for this cohort. This limitation does not diminish the relevance of the observed genetic associations but rather delineates the scope of the current investigation. Accordingly, we underscore the importance of future studies integrating genetic variation with metabolic biomarkers to validate and extend these findings, particularly in the context of sex-specific susceptibility mechanisms.

Additionally, the Brazilian population is highly admixed, and self-reported ethnicity may not accurately reflect genomic ancestry. This may introduce residual confounding in the observed associations, particularly given the heterogeneous distribution of folate-pathway genotypes across ancestral groups. Another limitation relates to lifestyle variables, such as alcohol intake and physical activity, which were obtained through self-report and are therefore subject to recall bias. Moreover, the lack of standardized quantification (e.g., type, frequency, or intensity of exposure) limits the ability to explore dose–response relationships.

Finally, although Kaplan–Meier survival curves suggested numerical differences among C677T groups, the lack of statistical significance may reflect the limited sample size, the relatively short follow-up period for some patients, or the presence of compensatory metabolic pathways within one-carbon metabolism that buffer the effects of reduced MTHFR enzymatic activity. Accordingly, larger multicenter studies incorporating biochemical profiling, genomic ancestry assessment, and longitudinal designs are needed to validate these findings and further clarify the role of this genetic variation in ALS susceptibility and prognosis.

5. Conclusions

Our data implicate the *MTHFR C677T* locus in ALS susceptibility and suggest that postmenopausal, metabolism-dependent mechanisms may amplify risk among women. While survival was not impacted, the pattern observed is compatible with limited statistical power and homeostatic buffering within the folate network. Priorities now include replication in larger, multi-center cohorts and harmonized biomarker panels coupled to genomic ancestry, to translate sex-aware, metabolism-informed risk stratification into the ALS clinic.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/sclerosis4020012/s1. Molecular analysis of the *MTHFR C677T* (rs1801133) variant was performed according to our previously published protocol (available at: https://doi.org/10.17504/protocols.io.brrkm54w), using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. Table S1. Hardy–Weinberg equilibrium (HWE) analysis for *MTHFR C677T* (rs1801133) in case and control.

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Abbreviations

ALS	Amyotrophic Lateral Sclerosis
ND	Neurodegenerative disease
MTHFR	Methylenetetrahydrofolate reductase
SNV	Single-nucleotide variant
Hcy	Homocysteine
HHcy	Hyperhomocysteinemia
HWE	Hardy–Weinberg equilibrium
OR	Odds ratio
CI	Confidence interval
PCR-RFLP	Polymerase chain reaction–restriction fragment length polymorphism
SAM/SAH	S-adenosylmethionine/S-adenosylhomocysteine
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
STREGA	STrengthening the REporting of Genetic Association studies
CRER	Centro de Reabilitação e Readaptação Dr. Henrique Santillo (Goiânia, Brazil)
IBGE	Instituto Brasileiro de Geografia e Estatística
LACES-UFG	Laboratório de Análises Clínicas e Educação em Saúde, Universidade Federal de Goiás

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