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Association between C677T polymorphism of MTHFR gene and risk of amyotrophic lateral sclerosis: Polish population study and a meta-analysis

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ABSTRACT

Objective: Genetic factors play a role in pathogenesis of amyotrophic lateral sclerosis (ALS). A few studies demonstrated that the TT genotype of C677T polymorphism of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene can increase the risk of sporadic ALS. The aim of our study was to determine the relationship between C677T polymorphism of MTHFR gene and the risk of sporadic ALS in Polish population and to perform the meta-analysis assessing the significance this polymorphism for the risk of ALS in Caucasian population.

Methods: We included 251 patients with ALS and 500 control subjects recruited from Polish population and performed the meta-analysis of published data from Caucasian population. MTHFR C677T polymorphism was genotyped using a TaqMan assay and 7900HT Fast real Time PCR System.

Results: The frequency of genotypes did not differ significantly between Polish ALS patients and control subjects (CC: 45.0 vs 45.8%, CT: 48.2 vs 45.0%, TT: 6.8 vs 9.2%, $P = 0.46$). The meta-analysis including 863 ALS patients and 1362 controls revealed that TT genotype increases the risk of sporadic ALS in Caucasian population.

Conclusion: Although we did not find the association between C677T polymorphism of MTHRF gene and risk of ALS in Polish population, the results of meta-analysis suggest that the TT genotype can be a genetic risk factor for ALS in Caucasian population.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease of unknown etiology [1]. Both the genetic and the environmental factors play a role in pathogenesis of this disease [2].

The elevated homocysteine plasma level can lead to neurodegeneration due to the impairment of DNA repairing ability, excitotoxicity, oxidative stress, energy metabolism disturbances and enhancement of the β-amyloid toxicity [3–5].

The plasma homocysteine level depends on the genetic and environmental factors [6,7]. The most common genetic risk factor for hyperhomocysteinemia is the C677T polymorphism of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene [6,8]. The TT variant of this gene is associated with elevated plasma homocysteine levels [7,8].

The results of the genetic studies assessing the association between the MTHFR gene polymorphisms and the risk of ALS are not consistent. The TT genotype of the MTHFR gene was associated with the increased risk of ALS in German and Swiss population (OR: 2.26, 95% CI: 1.03–4.97) [9]. Sayci et al. have found that C677T polymorphism is a genetic risk factor for sporadic ALS in women (OR: 2.56, 95% CI: 1.14–5.74) [10]. On contrary, the study conducted in the Italian population did not show the association between C677T polymorphism and the risk of the sporadic form of ALS (OR: 0.87, 95% CI: 0.73–1.03) [11].

The aim of our study was to determine the relationship between C677T polymorphism of MTHFR gene and the risk of sporadic ALS in Polish population and to perform the meta-analysis assessing the significance this polymorphism for the risk of ALS in Caucasian population.

2. Material and methods

The patients to this study were recruited from ALS patients diagnosed at the Department of Neurology, University Hospital, Kraków, Poland, between 1999 and 2011. All included patients fulfilled the El Escorial criteria of definite or probable sporadic ALS [12,13]. Control subjects were recruited from relatives of the hospital staff, spouses of the patients, and patients hospitalized at the University Hospital for the non-neurological disorders. We included 251 patients with ALS and 500 control subjects. Eighty-four (33.5%) ALS patients had bulbar onset and 167 (66.5%) had limb onset. The mean age did not differ between ALS patients and controls (57.7 ± 12.1 vs 57.9 ± 12.6 years respectively, $P = 0.87$). The proportion of woman was similar in both groups (50.2% of ALS patients vs 50.6% controls, $P = 0.92$). All participants were of Caucasian origin. Informed consent was given by all participants before the inclusion to the study. The Bioethics Committee approved the study protocol.

From all participants 5 ml of peripheral blood was collected. Genomic DNA was immediately extracted from blood using a commercially available kit from QIAGEN QIAamp® DNA Mini. MTHFR C677T polymorphism was genotyped using a TaqMan assay (Applied Biosystems, Carlsbad, CA, USA) and 7900HT Fast Real Time PCR System (Applied Biosystems).

Differences between groups were determined using the unpaired Student's *t*-test (continuous variables) or chi-squared test (categorical variables). Hardy-Weinberg equilibrium was tested using the chi-squared test. The association of the MTHFR genotypes with ALS was tested using logistic regression analysis under assumption of recessive (CC vs CT + TT) or dominant (CC + CT vs TT) effect for the T allele. *P* value <0.05 was considered statistically significant.

To identify the relevant studies for the meta-analysis, we searched the Medline until August 2014 using the following key words: MTHFR, C677T, polymorphism, amyotrophic lateral sclerosis, and we checked references from retrieved articles. We include only studies conducted in Caucasian population and published in English.

The meta-analysis and heterogeneity analysis were performed using Review Manager version 5.0 (Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration). A pooled odds ratio (OR) and 95% confidence intervals were calculated using fixed-effect models.

3. Results

Genotypes distribution in studied groups is shown in Table 1.

Genotypes distribution in control group was in Hardy-Weinberg equilibrium ($\chi^2 = 0.77$; $P = 0.38$), but it deviates from Hardy-Weinberg equilibrium in ALS patients ($\chi^2 = 4.2$; $P = 0.06$).

The frequency of alleles and genotypes did not differ significantly between ALS patients and control subjects. The sub-analysis including only men or only patients with bulbar onset did not reveal differences in genotypes distribution between groups.

For meta-analysis we identified two studies investigated the association between the MTHFR C677T gene polymorphism and risk of ALS in Caucasian population. The German-Swiss study compromised 162 ALS patients (33% female, 67% limb onset, mean age: 60 years) and 162 controls (39% female, mean age: 59 years) [10]. The Italian study included 450 ALS patients (53% female, 69.9% spinal onset, mean age: 61.8 years) and 700 controls (55.6% female, mean age: 60.9 years) [11].

Table 1 – Distribution of genotypes and allele-carriers of *MTHFR C677T* polymorphism in sporadic ALS cases and controls.

	ALS (*n* = 251)	Controls (*n* = 500)	*P*
Genotype			
CC	113 (45.0%)	229 (45.8%)	0.46
TC	121 (48.2%)	225 (45.0%)	
TT	17 (6.8%)	46 (9.2%)	
Recessive model (TT vs TC + CC)			0.26
OR: 1.39, 95% CI: 0.78–2.50			
Dominant model (TT + TC vs CC)			0.85
OR: 0.97, 95% CI: 0.70–1.35			
Allele			
C	234 (93.2%)	453 (90.6%)	0.22
OR: 0.70, 95% CI: 0.39–1.28			
T	139 (55.4%)	271 (54.2%)	0.75
OR: 0.95, 95% CI: 0.71–1.28			

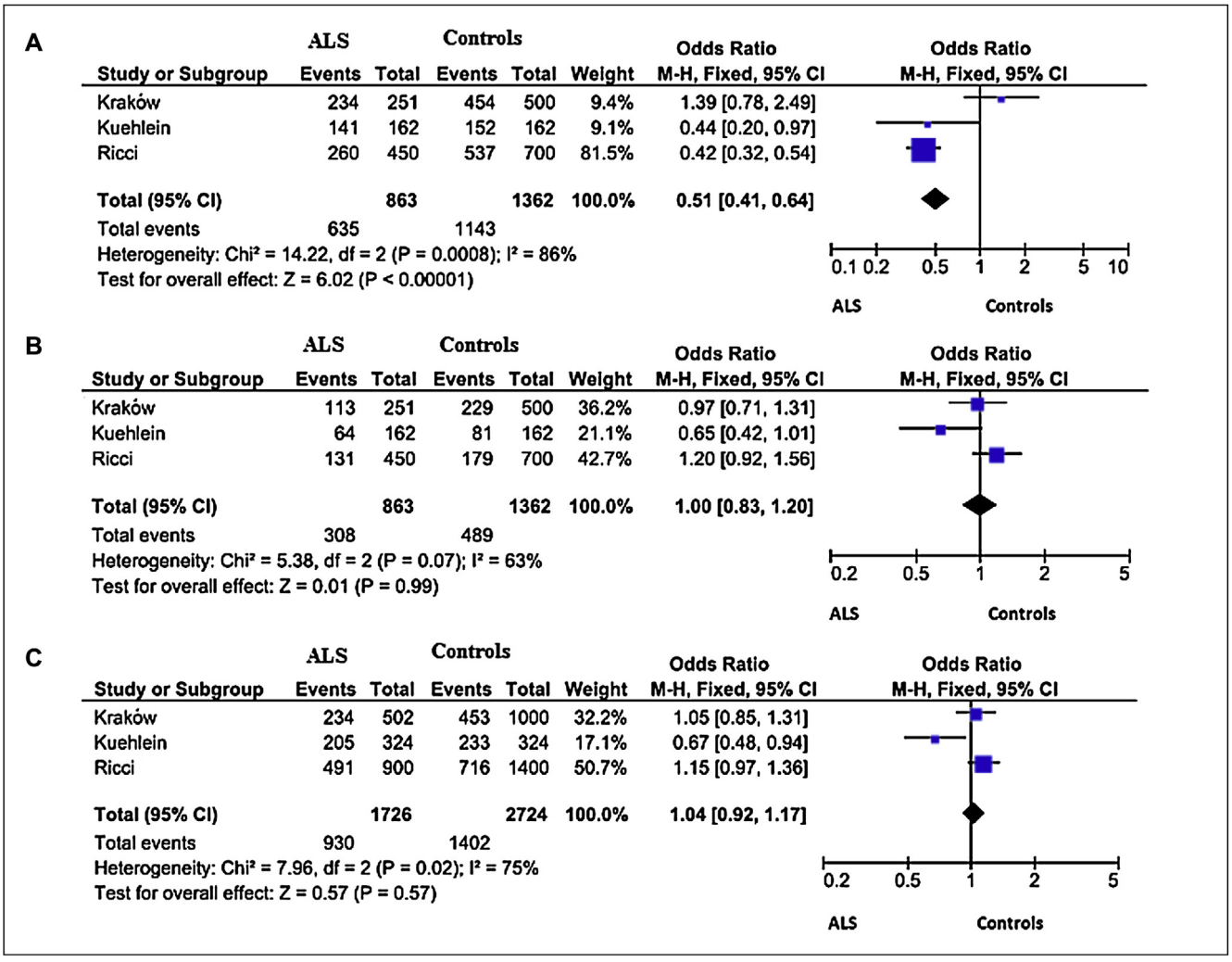

Fig. 1 – The association between MTHFR gene polymorphism and the risk of ALS: (A) CC + TC vs TT genotypes, (B) CC vs TC + TT genotypes and (C) C allele vs T allele.

The meta-analysis revealed that TT genotype increases the risk of sporadic ALS in Caucasian population (Fig. 1).

4. Discussion

We did not find the association between the MTHFR C677T polymorphism and the risk of ALS in Polish population. The obtained negative results can be due to low statistical power of our study and rare frequency of TT genotype in the Polish population.

The meta-analysis including our data and the results of two other studies conducted on Caucasian population revealed that the TT genotype could be the risk factor for sporadic ALS, although significant heterogeneity was observed between the studies. Previous studies demonstrated that the TT genotype of the MTHFR gene could be the genetic risk factor not only for ALS [9,11], but also for other neurodegenerative disorders such as Alzheimer disease or Parkinson disease [14,15]. It suggests that there is a common pathomechanism linking MTHFR C677T polymorphism with neurodegeneration.

MTHFR is a folate-dependent enzyme that catalyzes remethylation of homocysteine. The TT genotype of MTHRF gene is associated with reduced enzymatic activity resulting in mild hyperhomocysteinemia [16]. Homocysteine exerts direct neurotoxic effects. In vivo, administration of the glutamate DL-homocysteic acid to rat spinal cord disrupts calcium homeostasis and leads to degeneration of motor neurons [17]. Homocysteine-induced cytotoxicity was observed in motor neuronal cell line transfected with mutant form (A4V) of Cu, Zn-superoxide dismutase (SOD1) [18]. In this model neurotoxic effect of homocysteine was inhibited by the antioxidant trolox and copper chelator bathocuproinedisulfonate. Using SOD1 (G93A) mice model of ALS, Zhang et al. demonstrated that mutation in SOD1 causes a significant increase in plasma homocysteine level and folic acid treatment significantly delays the disease onset and reduces motor neuron loss [19]. Homocysteine activate N-methyl D-aspartate receptors increasing the inhibitory effect of oxidative stress [20]. Hyperhomocysteinemia increases also beta-amyloid production in rat brain by enhancing gamma-secretase and phosphorylation of amyloid precursor protein [21].

If the association between homocysteine level and ALS turns out to be causal, then lowering homocysteine level should reduce ALS incidence. It was shown that in ALS patients homocysteine plasma and cerebrospinal fluid level is elevated [22–24]. Reduction of 3–4 μmol/L in total homocysteine levels can be achieved by treatment with folic acid and vitamin B12 [25]. Vitamin B12 decreased homocysteine-induced motor neuron death in vitro via reduction in caspase activation [26]. It was also shown that dietary folate modification effect between MTHFR genotype, homocysteine and stroke risk was absented of benefit in populations with increasing folate intake [27], suggesting that similar impact may be seen in ALS patients.

To conclude, the results of meta-analysis suggest that the TT polymorphism of MTHFR gene could be the risk factor for sporadic ALS in Caucasian population. This association requires confirmation in large genetic study with high statistical power.

Conflict of interest

None declared.

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None declared.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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