

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

journal homepage: <http://www.elsevier.com/locate/pjnns>

## Original research article

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



Kamila Żur-Wyrozumska<sup>\*</sup>, Joanna Pera, Anna Dziubek, Małgorzata Sado, Aleksandra Golenia, Agnieszka Słowik, Tomasz Dziedzic

Department of Neurology, Jagiellonian University Medical College, Kraków, Poland

## ARTICLE INFO

## Article history:

Received 11 May 2016

Accepted 19 January 2017

Available online 3 February 2017

## Keywords:

Genetics

Amyotrophic lateral sclerosis

Polymorphism

5,10-Methylenetetrahydrofolate reductase

## 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 MTHFR 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.

© 2017 Polish Neurological Society. Published by Elsevier Sp. z o.o. All rights reserved.

<sup>\*</sup> Corresponding author at: Department of Neurology, Jagiellonian University Medical College, ul. Botaniczna 3, 31-503 Kraków, Poland. Tel.: +48 793577767.

E-mail addresses: [kamilaanna@op.pl](mailto:kamilaanna@op.pl) (K. Żur-Wyrozumska), [pera@su.krakow.pl](mailto:pera@su.krakow.pl) (J. Pera), [andziubek@wp.pl](mailto:andziubek@wp.pl) (A. Dziubek), [masado@interia.pl](mailto:masado@interia.pl) (M. Sado), [agolenia@gmail.com](mailto:agolenia@gmail.com) (A. Golenia), [agnieszka.slowik@uj.edu.pl](mailto:agnieszka.slowik@uj.edu.pl) (A. Słowik), [tomasz.dziedzic@uj.edu.pl](mailto:tomasz.dziedzic@uj.edu.pl) (T. Dziedzic).

<http://dx.doi.org/10.1016/j.pjnns.2017.01.008>

0028-3843/© 2017 Polish Neurological Society. Published by Elsevier Sp. z o.o. All rights reserved.

## 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  $\beta$ -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 ( $57.7 \pm 12.1$  vs  $57.9 \pm 12.6$  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<sup>®</sup> 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 comprised 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    |
|---------------------------------|---------------|--------------------|------|
| <b>Genotype</b>                 |               |                    |      |
| 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     |               |                    |      |
| <b>Allele</b>                   |               |                    |      |
| 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     |               |                    |      |

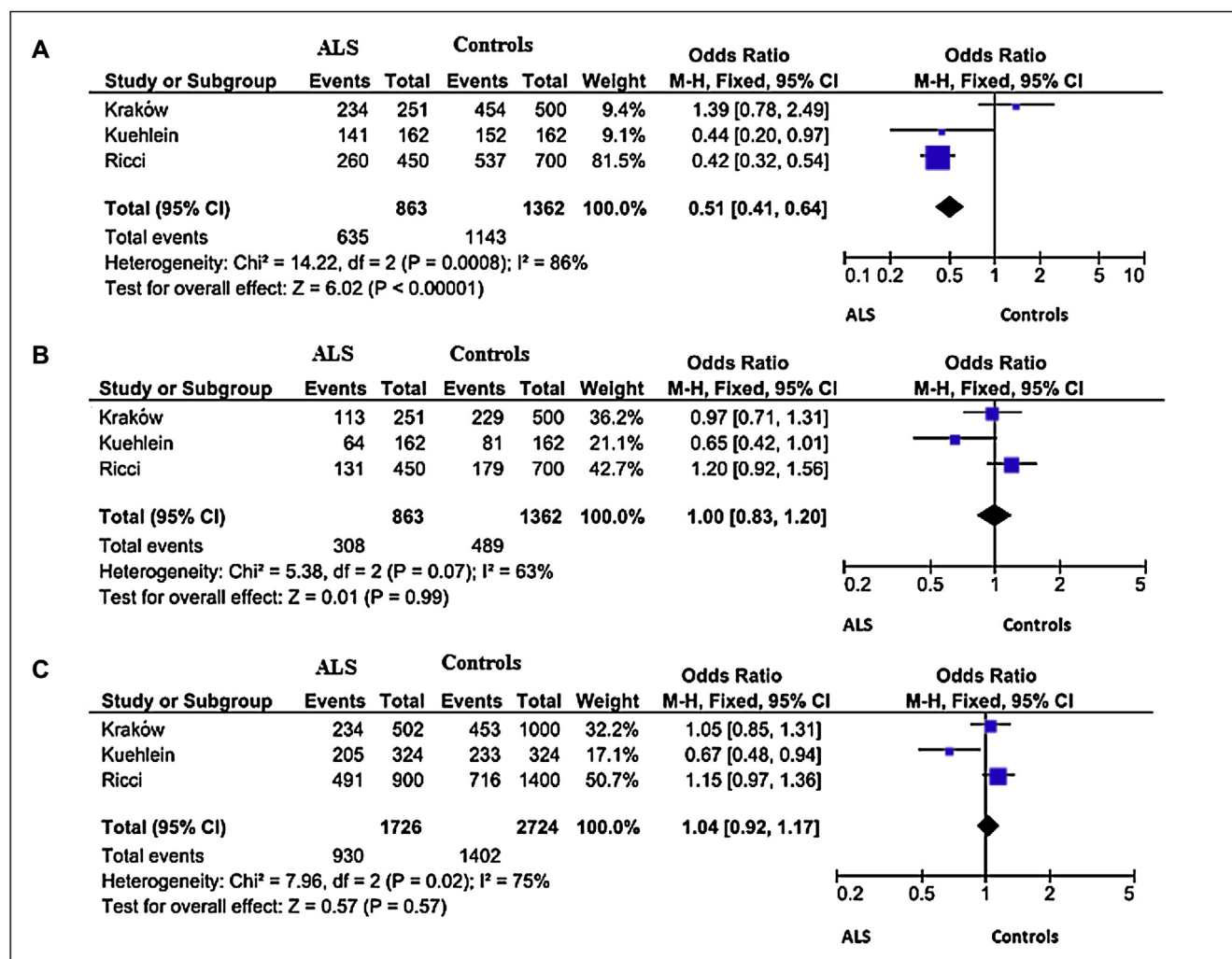


Fig. 1 – The association between MTHFR gene polymorphism and the risk of ALS: (A) CC + TC vs TT genotypes, (B) CC vs TC + TT genotypes and (C) C allele vs T allele.

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 MTHFR gene is associated with reduced enzymatic activity resulting in mild hyperhomocysteinemia [16]. Homocysteine exerts direct neurotoxic effects. In vivo, administration of the glutamate D-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  $\mu\text{mol/L}$  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.

### Acknowledgement and financial support

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.

### REFERENCES

- [1] Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. *N Engl J Med* 2001;344(22):1688–700.
- [2] Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. *Orphanet J Rare Dis* 2009;4:3.
- [3] Ho PI, Ortiz D, Rogers E, Shea TB. Multiple aspects of homocysteine neurotoxicity: glutamate excitotoxicity, kinase hyperactivation and DNA damage. *J Neurosci Res* 2002;70(December (5)):694–702.
- [4] Streck EL, Matté C, Vieira PS, Calcagnotto T, Wannmacher CM, Wajner M, et al. Impairment of energy metabolism in hippocampus of rats subjected to chemically-induced hyperhomocysteinemia. *Biochim Biophys Acta* 2003;1637 (April (3)):187–92.
- [5] Ho PI, Collins SC, Dhitavats S, Ortiz D, Ashline D, Rogers E, et al. Homocysteine potentiates beta-amyloid neurotoxicity: role of oxidative stress. *J Neurochem* 2001;78 (July (2)):249–53.
- [6] Nagele P, Meissner K, Francis A, Födinger M, Saccone NL. Genetic and environmental determinants of plasma total homocysteine levels: impact of population-wide folate fortification. *Pharmacogenet Genomics* 2011;21(July (7)):426–31.
- [7] Gudnason V, Stansbie D, Scott J, Bowron A, Nicaud V, Humphries S. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. *EARS group. Atherosclerosis* 1998;136(February (2)):347–54.
- [8] Waśkiewicz A, Piotrowski W, Broda G, Sobczyk-Kopciół A, Płoski R. Impact of MTHFR C677T gene polymorphism and vitamins intake on homocysteine concentration in the Polish adult population. *Kardiol Pol* 2011;69(12):1259–64.
- [9] Kühnlein P, Jung H, Farkas M, Keskitalo S, Ineichen B, Jelcic I, et al. The thermolabile variant of 5,10-methylenetetrahydrofolate reductase is a possible risk factor for amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2011;12(March (2)):136–9.
- [10] Sayci A, Ozel MD, Emel E, Idrisoglu HA. Gender-specific association of methylenetetrahydrofolate reductase gene polymorphisms with sporadic amyotrophic lateral sclerosis. *Genet Test Mol Biomarkers* 2012;16(July (7)):716–21.
- [11] Ricci C, Penco S, Benigni M, Mosca L, Tarlarini C, Lunetta C, et al. No association of MTHFR c.677C>T variant with sporadic ALS in an Italian population. *Neurobiol Aging* 2012;33(January (1)):208.e7–8.
- [12] Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial “Clinical limits of amyotrophic lateral sclerosis” workshop contributors. *J Neurol Sci* 1994;124(Suppl.):96.
- [13] Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1:293.
- [14] Hua Y, Zhao H, Kong Y, Ye M. Association between the MTHFR gene and Alzheimer's disease: a meta-analysis. *Int J Neurosci* 2011;121(August (8)):462–71.
- [15] de Lau LM, Koudstaal PJ, van Meurs JB, Uitterlinden AG, Hofman A, Breteler MM. Methylenetetrahydrofolate reductase C677T genotype and PD. *Ann Neurol* 2005;57(June (6)):927–30.
- [16] Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996;93:7–9.
- [17] Adalbert R, Engelhardt JI, Siklós L. DL-Homocysteic acid application disrupts calcium homeostasis and induces degeneration of spinal motor neurons in vivo. *Acta Neuropathol* 2002;103(May (5)):428–36.
- [18] Sung JJ, Kim HJ, Choi-Kwon S, Lee J, Kim M, Lee KW. Homocysteine induces oxidative cytotoxicity in Cu,Zn-superoxide dismutase mutant motor neuronal cell. *Neuroreport* 2002;13(March (4)):377–81.
- [19] Zhang X, Chen S, Li L, Wang Q, Le W. Folic acid protects motor neurons against the increased homocysteine, inflammation and apoptosis in SOD1 G93A transgenic mice. *Neuropharmacology* 2008;54(June (7)):1112–9.
- [20] Bukharaeva E, Shakirzyanova A, Khuzakhmetova V, Sitdikova G, Giniatullin R. Homocysteine aggravates ROS-induced depression of transmitter release from motor nerve terminals: potential mechanism of peripheral impairment in motor neuron diseases associated with hyperhomocysteinemia. *Front Cell Neurosci* 2015;9. <http://dx.doi.org/10.3389/fncel.2015.00391> [article 391]

- [21] Zhang CE, Wei W, Liu YH, Peng JH, Tian Q, Liu GP, et al. Hyperhomocysteinemia increases betaamyloid by enhancing expression of gamma-secretase and phosphorylation of amyloid precursor protein in rat brain. *Am J Pathol* 2009;174(4):1481-91.
- [22] Levin J, Bötzel K, Giese A, Vogeser M, Lorenzl S. Elevated levels of methylmalonate and homocysteine in Parkinson's disease, progressive supranuclear palsy and amyotrophic lateral sclerosis. *Dement Geriatr Cogn Disord* 2010;29(6):553-9.
- [23] Valentino F, Bivona G, Butera D, Paladino P, Fazzari M, Piccoli T, et al. Elevated cerebrospinal fluid and plasma homocysteine levels in ALS. *Eur J Neurol* 2010;17(January (1)):84-9.
- [24] Zoccolella S, Bendotti C, Beghi E, Logroscino G. Homocysteine levels and amyotrophic lateral sclerosis: a possible link. *Amyotroph Lateral Scler* 2010;11(1-2):140-7.
- [25] Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. *Am J Clin Nutr* 2005;82(October (4)):806-12.
- [26] Hemendinger RA, Armstrong 3rd EJ, Brooks BR. Methyl vitamin B12 but not methylfolate rescues a motor neuron-like cell line from homocysteine-mediated cell death. *Toxicol Appl Pharmacol* 2011;251(March (3)):217-25.
- [27] Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet* 2011;378(August (9791)):584-94.