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Original Article

Association study between the *DNMT3A* -448A>G polymorphism and risk of Alzheimer's disease in Caucasians of Italian origin

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Abstract: Increasing evidence points to an epigenetic contribution in Alzheimer's disease (AD) pathogenesis. In this regard, variants and polymorphisms of DNA methyltransferase genes (DNMTs) are being investigated for their contribution to cognitive decline and dementia, but results are still scarce or controversial. In the present study we genotyped 710 Caucasian subjects of Italian descent, including 320 late-onset AD (LOAD) patients, 70 individuals with amnestic Mild Cognitive Impairment (MCI), and 320 matched healthy controls, for the presence of a functional *DNMT3A* -448A>G (rs1550117) polymorphism, searching for association with disease risk. In addition, we searched for correlation between the studied polymorphism and circulating levels of folate, homocysteine (hcy) and vitamin B12, all involved in DNA methylation reactions and available from 189 LOAD patients and 186 matched controls. Both allele and genotype frequencies of rs1550117 were closely similar between MCI, LOAD and control subjects, and no association with dementia or pre-dementia conditions was observed. Plasma hcy levels were significantly higher ($p = 0.04$) and serum folate levels significantly lower ($p = 0.01$) in LOAD than in controls, but no difference in circulating folate, hcy or vitamin B12 levels was seen between carriers and non-carriers of the minor *DNMT3A* -448A allele. Collectively, present results confirmed previous associations of increased hcy and decreased folate with LOAD risk, but do not support an association between the *DNMT3A* -448A>G polymorphism and AD in our population.

Keywords: *DNMT3A* -448A>G polymorphism, rs1550117, Alzheimer's disease, Mild Cognitive Impairment, epigenetics, homocysteine, folate

Introduction

Alzheimer's Disease is a neurodegenerative disorder representing the most common form of dementia in the elderly. The disease is characterized by a progressive decline in cognitive functions resulting from the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles, which ultimately lead to premature neuronal death [1, 2]. Only about 5% of AD cases present with an early-onset (EOAD) before the age of 65 years, whereas >95% of the patients develop the disease after the age of 65 years. They are classified as late-onset AD (LOAD) [3]. The disease is usually preceded by a pre-dementia phase referred as to amnestic Mild Cognitive Impairment (aMCI), a condition that is considered to represent one of the earliest phases of the degenerative process leading to dementia of Alzheimer's type [4].

LOAD is considered a multifactorial disease, resulting from a complex interplay between lifelong stochastic, genetic and environmental factors [5]. A growing body of evidence suggests an epigenetic contribution in LOAD, and global alterations of DNA methylation levels as well as gene-specific methylation changes have been reported in post-mortem AD brains or in blood DNA of living LOAD patients [6].

Table 1. Demographic characteristics of the study population

	Number	Males (%)	Females (%)	Age (Mean ± SD)
AD	320	113 (35.3%)	207 (64.7%)	76.0 ± 7.3
MCI	70	30 (42.8%)	40 (57.2%)	74.0 ± 6.6
Controls	320	118 (36.9%)	202 (63.1%)	74.9 ± 8.7

Table 2. Demographic characteristics of the subgroup with biochemical data

	AD (n = 189)	Controls (n = 186)	*p*-value
Age	77.6 ± 6.9	77.2 ± 9.8	0.73[a]
Gender (F/M)	121/68	112/74	0.46[b]
Homocysteine (μmol/l)	14.7 (11.33-21.8)	13.8 (10.6-17.8)	0.04[c]
Folate (ng/ml)	5.1 (3.8-7.3)	5.9 (4.6-8.2)	0.01[c]
Vitamin B12 (pg/ml)	364.0 (258.8-500.2)	387.5 (290-568.5)	0.16[c]

P-value obtained by means of [a]t-test, [b]Fisher's exact test, [c]Mann-Whitney U test.

DNA methylation consists in the addition of a methyl group to the C5 position of cytosine residues. Usually, it occurs on CpG dinucleotides in the promoter regions and represents a repressive mark for gene expression [7]. DNA methylation reactions require *S*-adenosylmethionine (SAM) as the methyl donor compound. SAM is produced from the remethylation of homocysteine (hcy) to methionine in the one-carbon metabolic pathway, a complex biochemical pathway that requires dietary folates and vitamin B12 as cofactors [8]. DNA methyltransferases (DNMTs) catalyze DNA methylation in a reaction that transfers the methyl group from SAM to the DNA, thus generating a methylated DNA template and *S*-adenosylhomocysteine (SAH), which is then converted back to hcy by SAH hydrolase. The DNMT family comprises several members, including the 'maintenance' methyltransferase DNMT1 that shows a preference for hemimethylated DNA, and the *'de novo'* methyltransferases DNMT3A and DNMT3B [9].

Folates are B-group vitamins and the main nutritional determinants of plasma hcy levels. Epidemiological studies revealed that elevations in plasma hcy levels increase dementia risk and that there is an inverse correlation between plasma hcy concentrations and cognitive performance in aged people [10, 11]. Indeed, AD patients often show decreased serum folate and vitamin B12 levels and increased homocysteinemia with respect to age-matched healthy subjects [12]. Studies on animal and cell models of AD showed that B-vitamin deficiency results in hyperhomocysteinemia and impairment of DNA methyltransferase (DNMT) activities, leading to hypomethylation and increased expression of AD-related genes [13-15].

However, the contribution of *DNMT*s genes to LOAD is still controversial. Di Francesco and coworkers found an increase in global DNA methylation in LOAD blood DNA associated with higher *DNMT1* expression and protein levels [16], but common *DNMT1* polymorphisms were not associated with human intelligence [17], neither with LOAD risk [18]. Association studies aiming at investigating *DNMT3B* promoter polymorphisms and LOAD risk are controversial [19]. Besides these evidences, it is known that among the DNMTs, the DNMT3A appears to have a crucial role in neurogenesis, learning and memory as well as in age-related memory decline [20, 21]. In this regard, a *DNMT3A* -448 A>G promoter polymorphism (rs1550117) was recently proposed as a candidate LOAD risk factor in Chinese individuals [22].

We performed the present study in order to evaluate the contribution of rs1550117 to LOAD risk in Caucasian subjects of Italian origin. Furthermore, we compared allele and genotype frequencies for rs1550117 between amnestic MCI subjects and healthy controls, and investigated the contribution of this polymorphism to circulating biomarkers of one-carbon metabolism (folate, hcy, and vitamin B12 levels) available from 189 LOAD patients and 186 healthy controls.

Materials and methods

Subjects

DNA samples from a total of 320 LOAD patients, 70 subjects with the diagnosis of aMCI, and 320 non-demented healthy controls were collected at the Department of Neuroscience of the Pisa University Hospital. Diagnosis of probable AD was performed according to DSM-IV [23] and NINCDS-ADRDA criteria [24]. Based on

Table 3. Distribution of genotype and allele frequencies in AD patients, MCI individuals and controls

	Controls (%)	AD (%)	MCI (%)	OR[a] (95% CI)	*p*-value[a]	OR[a] (95% CI)	*p*-value[a]	OR[a] (95% CI)	*p*-value[a]
				AD vs Controls		*MCI vs Controls*		*AD + MCI vs Controls*	
Genotypes									
GG	260 (81.3%)	256 (80%)	57 (81.4%)	1.00[b]	--	1.00[b]	--	1.00[b]	--
GA	58 (18.1%)	60(18.8%)	13 (18.6%)	1.01 (0.67-1.54)	0.95	1.20	1.00	1.00 (0.68-1.49)	0.99
AA	2 (0.6%)	4 (1.2%)	0 (0%)	1.18 (0.16-8.51)	0.87	--	--	0.93 (0.13-6.68)	0.94
AA+GA vs. GG	60 (18.7%)	64 (20%)	13 (18.6%)	1.02 (0.68-1.54)	0.92	1.19	1.00	1.00 (0.68-1.48)	0.99
AA vs. GG+GA	2 (0.6%)	4 (1.2%)	0 (0%)	1.17 (0.16-8.4)	0.87	--	--	0.92 (1.13-6.64)	0.94
Alleles									
Allele G	0.90	0.89	0.91	1.00[b]	--	1.00[b]	--	1.00[b]	--
Allele A	0.10	0.11	0.09	1.02 (0.69-1.49)	0.90	1.15	1.00	0.99 (0.69-1.43)	0.99

[a]Adjusted for age and gender. [b]Reference values for OR.

age at onset above 65 years and absence of a family history of dementia, all the subjects were regarded as sporadic LOAD cases. Cognitive dysfunction was measured in all patients using a battery of neuropsychological tests including the Auditory Verbal Learning Test (AVLT)-delayed recall, the Mini-Mental State Examination (MMSE), the Clinical Dementia Rating (CDR), and Activities of Daily Living (ADLs) Scale. All the 70 MCI individuals met the diagnostic criteria proposed by the Mayo Clinic Alzheimer's Disease Research Centre for amnestic MCI [25]. Healthy volunteer subjects, matched with the patients for age and gender, were used as normal controls. Family history of dementia was ascertained by the Neurologists of the Department of Neuroscience of the Pisa University Hospital, excluding all the subjects with even one relative who developed AD or other dementias. All the control subjects were evaluated in order to exclude the presence of cognitive impairment. All the recruited individuals were Caucasians of Italian descent from at least three generations and the demographic characteristics of the case-control cohort are shown in **Table 1**. Each subject gave an informed and written consent for genotype analysis before blood drawing. The study was performed in accordance with the Declaration of Helsinki, following the requirements of the Ethics Committee of the Pisa University Hospital that approved the study (Protocol number 3618/2012).

Genotyping

Genomic DNA was isolated from whole blood by means of the QIAamp Blood Mini Kit (Qiagen, Milan, Italy) following the manufacturer's instructions.

The *DNMT3A* -448A>G polymorphisms was determined by a PCR-RFLP assay adapted from [26]: a 175-bp product was amplified using 1.25 units of Taq DNA polymerase (Euroclone, Milan, Italy), 5 pmol of the forward primer (5'-CTTGGGGCACCTCTGTCTAA-3') and the reverse primer (5'-AGTAGAATTCGGGGTGCAGA-3'), 0.15 mM of each dNTP, 1.5 mM $MgCl_2$, and 50 ng of genomic DNA in a total volume of 25 µL. PCR conditions consisted of an initial denaturation step of 5 minutes at 95°C, followed by 40 cycles of 30 s at 95°C, 45 s at 59°C, and 45 s at 72°C and a final extension of 10 minutes at 72°C.

The PCR product was then digested with TaaI (Fermentas, Milan, Italy) for 2 h at 65°C; the digested products were separated on a 3.0% agarose gel and the RFLP bands visualized under ultraviolet light with ethidium bromide (EB) staining. The major G allele consists of a TaaI restriction site that results in two bands (132 bp and 43 bp, respectively), while the minor A allele is not digested resulting in an uncutted 175 bp fragment. Internal control samples, whose genotypes had been previously assessed, were always included and analyzed on each gel.

Biochemical analyses

Peripheral blood samples from 189 LOAD patients and 186 healthy controls have been collected during the neurological visit for the evaluation of circulating biomarkers of one-carbon metabolism. Those analyses were restricted to individuals not taking drugs or supplements, such as B-vitamin supplements, known or suspected to interfere with one-carbon metabolism. Because of this we collected those

Table 4. Comparison of Hcy, Folate and Vitamin B12 levels in total population (AD + Controls), AD patients and controls

	Total	AD	Controls
Homocysteine (μmol/l)			
GG	13.9 (10.8-19.5)	15.0 (11.4-21.8)	13.3 (10.5-17.7)
GA+AA	13.1 (10.7-19.9)	13.2 (11.3-21.3)	12.1 (10.2-19.1)
p-value[a]	0.69	0.69	0.99
Folate (ng/ml)			
GG	5.3 (4.2-7.4)	5.0 (3.8-7.3)	5.7 (4.5-7.8)
GA+AA	6.1 (4.1-8.5)	5.5 (3.8-7.0)	6.3 (4.0-8.5)
p-value[a]	0.39	0.64	0.81
Vitamin B12 (pg/ml)			
GG	370.0 (272.4-540.0)	357.8 (268.5-491.6)	401.0 (292.0-578.4)
GA+AA	389.0 (241.0-538.0)	359.0 (224.5-550.0)	396.0 (305.6-499.0)
p-value[a]	0.62	0.93	0.47

[a]Data are expressed as median (range) and the *P*-value is obtained by means of Mann-Whitney U test.

data only from a subgroup of LOAD and control subjects that met the inclusion criteria, and have no data from aMCI individuals as most of them reported to take vitamin supplements. **Table 2** shows the demographic characteristics of this subgroup. For what concerns the analysis of circulating markers of one-carbon metabolism, the plasma was immediately separated and stored in freezer at -80°C. All the analyses were performed with standard protocols at the diagnostic laboratory of the Pisa University Hospital as detailed elsewhere [27]. Briefly: plasma hcy was measured by liquid chromatography/tandem mass spectrometry. Serum folate and vitamin B12 were determined by electrochemiluminescence immunoassay analyses.

Statistical analyses

To verify that allele frequencies were in Hardy-Weinberg equilibrium, and to assess differences in allele distributions between groups, we used the Chi-square (χ^2) analysis. The differences in genotype frequencies were analyzed between the case and the control group by 2·2 contingency tables using χ^2 analysis or Fisher's exact test. Odds ratios (ORs) have been calculated by means of logistic regression analysis and given with 95% confidence intervals (CIs). The power of the study was evaluated with the statistical package QUANTO 1.2.4.exe. Plasma hcy, serum folate and vitamin B12 values did not follow a normal distribution in our cohort, so that differences in median homocysteine, folate, and vitamin B12 levels between groups were assessed by means of the Mann-Whitney U test. The same test was used to correlate biochemical data with the studied polymorphism. Statistical analyses were performed with the MedCalc v.12.5 and GraphPad Prism 6 software. A p value <0.05 was considered as statistically significant.

Results

Genotyping

Distribution of *DNMT3A* -448A>G allele and genotype frequencies in LOAD patients, MCI individuals, and healthy controls are shown in **Table 3**. The genotype frequencies conformed to Hardy-Weinberg expectations in controls (p = 0.52). Both allele and genotype frequencies were similar between MCI, LOAD patients and controls, and no statistically significant difference was observed in the comparison of LOAD vs. controls, MCI vs. controls or LOAD+MCI vs. controls (**Table 3**).

Biochemical analyses

As shown in **Table 2** we observed significantly increased median hcy levels (p = 0.04) and significantly decreased median folate values (p = 0.01) in LOAD patients with respect to controls. Vitamin B12 values were lower in the LOAD group than in controls, but this difference did not reach statistical significance (p = 0.16) (**Table 2**).

Correlation between the DNMT3A -448A>G polymorphism and biochemical data

We searched for a correlation between plasma hcy, and serum folate and vitamin B12 values with the *DNMT3A* -448A>G polymorphism. Given the very low frequency of homozygous AA carriers in our cohort (**Table 3**), comparisons were performed in A carriers (AA + GA) vs. non carriers (GG). As shown in **Table 4** none of the studied biochemical markers of one-carbon metabolism (plasma hcy, serum folate or vitamin B12) showed a different distribution between carriers and non-carriers of the *DNMT3A* -448A allele.

Discussion

A very recent case-control study performed in Chinese LOAD patients reported association of the *DNMT3A* -448A>G polymorphism (rs1550117) with disease risk [22]. Replication of those findings in populations other than Asians is required to better address the role of this polymorphism in LOAD, and we performed the present study to investigate the role of the *DNMT3A* -448A>G polymorphism in LOAD risk in a Caucasian cohort of Italian descent. For this purpose we genotyped 320 LOAD patients and 320 healthy controls matched for age and gender. We also addressed the contribution of the study polymorphism to circulating markers of one-carbon metabolism, namely plasma hcy and serum folate and vitamin B12 levels in LOAD and control subjects. Furthermore, we genotyped 70 aMCI subjects and compared allele and genotype frequencies of rs1550117 in that cohort with those observed in healthy controls. The present study revealed very similar allele and genotype frequencies for rs1550117 between MCI, LOAD patients and controls and no association or trend for association with disease risk. Moreover, no difference in serum folate and vitamin B12 or in plasma hcy concentrations was seen between carriers and non-carriers of the minor A allele. Collectively, present data do not support a major role for the study polymorphism in AD risk in our population.

To date, different studies revealed a possible role of epigenetics and DNA methylation in AD [6]. In eukaryotes, DNA methylation consists of the addition of a methyl group from SAM to the C5 position of the cytosine residues in a reaction catalyzed by DNMTs. DNMT1 is the maintenance methyltransferase [28], shows a preference for hemimethylated DNA [29, 30] and is ubiquitously and highly expressed in proliferating cells, representing the major DNMT activity in somatic tissues throughout mammalian development [31]. DNMT3A and DNMT3B are active *de novo* methyltransferases responsible for the establishment of DNA methylation patterns during early development and in germ cells. There are several isoforms of both enzymes, which are differentially expressed at different stages of development and in different tissues [32-34].

Studies on animal models showed that Dnmt1 and Dnmt3a are expressed in post-mitotic neurons and regulate neural plasticity [35, 36]. Particularly, two Dnmt3a isoforms (Dnmt3a1 and Dnmt3a2) seem to be significantly reduced in brains of aged mice, likely contributing to age-related cognitive decline [20]. Similarly, it was suggested that DNMT3A moderates cognitive decline in human subjects with MCI [37].

Several polymorphisms are known in *DNMT* genes and most of them have been associated with increased risk of human cancers [38, 39]. However, genetic association studies addressing the contribution of *DNMT* genes to Alzheimer's disease or cognitive performances are still scarce and controversial [17-19, 37].

Among *DNMT3A* polymorphisms, the *DNMT3A* -448A>G one was associated with increased risk of cancer, particularly colorectal cancer, and a stratified analysis by ethnicity revealed significant increased cancer risk among Asians [39]. It was also demonstrated that the minor A allele increases the promoter activity (>2-fold) in *in vitro* studies [26]. Recently, Ling and colleagues investigated the *DNMT3A* -448A>G promoter polymorphism in 300 Chinese LOAD patients and 320 matched controls observing that this polymorphism was associated with a two-fold increased LOAD risk in their cohort [22]. Particularly, the A allele was more frequent in Chinese LOAD patients (24%) than in healthy controls (20%), resulting in an 8% frequency of *DNMT3A* -448 homozygous AA LOAD carriers [22]. Most of the available literature concerning the association of the study polymorphism with cancer risk was produced in Asian populations, showing minor allele frequencies (MAF) for the *DNMT3A* -448A allele ranging from almost 20 to more than 30% and

association with cancer risk under various genetic models [39]. The present study showed that in our population the frequency of the minor A allele was significantly lower than in Asian cohorts and very similar in MCI, AD patients and healthy controls (about 10%, **Table 3**). Given a MAF of about 10% the present study had 85% statistical power to detect Odds Ratios (ORs) of 2.0 or higher for the minor allele. However, as shown in **Table 3**, both allele and genotype frequencies of rs1550117 were closely similar between MCI, LOAD and control subjects, no significant difference was observed, and none of the genetic models we addressed showed ORs similar to those reported in Asians (**Table 3**). Particularly, the frequency of heterozygous AG subjects was highly similar among the three groups under investigation (**Table 3**), and that of homozygous AA subjects was lower than 1% in our cohort, being this genotype detected only in 6 out of 710 genotyped subjects, and quite different from the 8% detected in Asian LOAD patients (**Table 3**). Collectively, present data do not support a major role for the study polymorphism in Caucasian LOAD patients of Italian descent. The allele and genotype frequencies of rs1550117 detected in the present study are consistent with those previously reported in the literature for European cohorts [40-42]. In this regard, previous studies in Caucasians of European descent investigated the role of *DNMT3A* -448A>G polymorphism in endometriosis, ovarian cancer and systemic lupus erythematosus [40-42]. All these studies reported allele and genotype frequencies similar to those that we found in our population [40-42]. Furthermore, as confirmed by the International HapMap Project and the 1000 Genomes Project, this polymorphism is much more frequent in Asians than Caucasians [43, 44].

We also addressed the contribution of the *DNMT3A* -448A>G polymorphism to circulating biomarkers of one-carbon metabolism. In literature there are several studies that evaluated one-carbon biomarker levels in LOAD patients and healthy controls, overall suggesting that increased plasma hcy levels and decreased serum folate levels could be regarded as LOAD risk factors [8, 45]. According to those data, our population showed a significant increase of hcy levels and a significant decrease of folate values in AD patients with respect to controls (**Table 2**). However, no difference in the circulating values of those biomarkers was seen between carriers and non-carriers of the *DNMT3A* -448A allele (**Table 4**). DNMTs transfer the methyl group from SAM to the DNA in a reaction that generates SAH, which is then converted to hcy by SAH hydrolase. Hcy can then re-enter the trans-methylation pathway being first re-methylated to methionine and then converted to SAM. Hcy re-methylation requires folate derivatives as methyl donors and vitamin B12 as a cofactor [46]. It is therefore likely to hypothesize that functional *DNMT3A* polymorphisms could result in impaired hcy, folate or vitamin B12 levels, but no such effect was seen in the present cohort of LOAD and control subjects, strengthening present results of lack of association between the *DNMT3A* -448A>G polymorphism and LOAD risk.

Ethnic differences for variants in one-carbon metabolic genes and LOAD risk are known in the literature, and are likely to result from differences in allele and genotype frequencies as well as from complex interactions among the studied polymorphisms and dietary or environmental factors [46]. In this regard, the methylenetetrahydrofolate reductase (*MTHFR*) 677C >T polymorphism represents one of the major determinants of circulating hcy levels and the most frequently studied polymorphism of the one-carbon metabolic pathway in LOAD genetic association studies [47]. However, a recent meta-analysis of 40 case-control studies revealed association of the *MTHFR* 677C>T polymorphism with LOAD risk in Asian populations, but not in Caucasians [47].

In conclusion, to the best of our knowledge the present is the first genetic association study addressing the contribution of rs1550117 to LOAD risk in Caucasians of Italian descent. We observed very similar allele and genotype distributions of the study polymorphism among aMCI, LOAD and healthy matched controls and no association with dementia or pre-dementia conditions. Furthermore, no difference in circulating markers of one-carbon metabolism was seen between carriers and non-carriers of the *DNMT3A* -448A>G minor allele. Collectively, present data do not support a major role for this polymorphism in AD risk among Caucasians of Italian descent. Additional studies in other LOAD groups are required to further address the contribution of this polymorphism to LOAD risk in different populations and ethnic groups.

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Disclosure of conflict of interest

None.

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References

[1] Alzheimer's Association. 2015 Alzheimer's disease facts and figures. Alzheimers Dement 2015; 11: 332-384.

[2] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 2011; 1: a006189.

[3] Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol 2014; 88: 640-651.

[4] Petersen RC, Roberts RO, Knopman DS, Boeve BF, Geda YE, Ivnik RJ, Smith GE, Jack CR Jr. Mild cognitive impairment: ten years later. Arch Neurol 2009; 66: 1447-1455.

[5] Van Cauwenberghe C, Van Broeckhoven C, Sleegers K. The genetic landscape of Alzheimer disease: clinical implications and perspectives. Genet Med 2015; [Epub ahead of print].

[6] Bennett DA, Yu L, Yang J, Srivastava GP, Aubin C, De Jager PL. Epigenomics of Alzheimer's disease. Transl Res 2015; 165: 200-220.

[7] Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012; 13: 484-492.

[8] Hinterberger M, Fischer P. Folate and Alzheimer: when time matters. J Neural Transm 2013; 120: 211-224.

[9] Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA methyltransferases. Chembiochem 2011; 12: 206-222.

[10] de Jager CA. Critical levels of brain atrophy associated with homocysteine and cognitive decline. Neurobiol Aging 2014; 35 Suppl 2: S35-39.

[11] Smith AD, Smith SM, de Jager CA, Whitbread P, Johnston C, Agacinski G, Oulhaj A, Bradley KM, Jacoby R, Refsum H. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PLoS One 2010; 5: e12244.

[12] Shen L, Ji HF. Associations between Homocysteine, Folic Acid, Vitamin B12 and Alzheimer's Disease: Insights from Meta-Analyses. J Alzheimers Dis 2015; 46: 777-790.

[13] Fuso A, Nicolia V, Cavallaro RA, Scarpa S. DNA methylase and demethylase activities are modulated by one-carbon metabolism in Alzheimer's disease models. J Nutr Biochem 2011; 22: 242-251.

[14] Fuso A, Seminara L, Cavallaro RA, D'Anselmi F, Scarpa S. S-adenosylmethionine/homocysteine cycle alterations modify DNA methylation status with consequent deregulation of PS1 and BACE and beta-amyloid production. Mol Cell Neurosci 2005; 28: 195-204.

[15] Li W, Liu H, Yu M, Zhang X, Zhang M, Wilson JX, Huang G. Folic acid administration inhibits amyloid beta-peptide accumulation in APP/PS1 transgenic mice. J Nutr Biochem 2015; 26: 883-891.

[16] Di Francesco A, Arosio B, Falconi A, Micioni Di Bonaventura MV, Karimi M, Mari D, Casati M, Maccarrone M, D'Addario C. Global changes in DNA methylation in Alzheimer's disease peripheral blood mononuclear cells. Brain Behav Immun 2015; 45: 139-144.

[17] Haggarty P, Hoad G, Harris SE, Starr JM, Fox HC, Deary IJ, Whalley LJ. Human intelligence and polymorphisms in the DNA methyltransferase genes involved in epigenetic marking. PLoS One 2010; 5: e11329.

[18] Pezzi JC, Ens CM, Borba EM, Schumacher-Schuh AF, de Andrade FM, Chaves ML, Fiegenbaum M, Camozzato AL. DNA methyltransferase haplotype is associated with Alzheimer's disease. Neurosci Lett 2014; 579: 70-74.

[19] Coppedè F, Zitarosa MT, Migheli F, Lo Gerfo A, Bagnoli S, Dardano A, Nacmias B, Mancuso M, Monzani F, Siciliano G, Sorbi S, Migliore L. DNMT3B promoter polymorphisms and risk of late onset Alzheimer's disease. Curr Alzheimer Res 2012; 9: 550-554.

[20] Oliveira AMM, Hemstedt TJ, Bading H. Rescue of aging-associated decline in Dnmt3a2 expression restores cognitive abilities. Nature Neuroscience 2012; 15: 1111-1113.

[21] Morris MJ, Adachi M, Na ES, Monteggia LM. Selective role for DNMT3a in learning and memory. Neurobiol Learn Mem 2014; 115: 30-37.

[22] Ling C, Fangyu D, Wanhua H, Kelong C, Zhimin W, Yuting Z, Rong Z. DNMT3A rs1550117 Poly-

morphism is Associated With Late-Onset Alzheimer's Disease in a Chinese Population. Am J Alzheimers Dis Other Demen 2015; [Epub ahead of print].

[23] American Psychiatric Association: Diagnostic and statistical manual of mental disorders: DSM-IV. Edited by American Psychiatric Association, Washington (DC), 4th ed, 1994.

[24] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7: 263-269.

[25] Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med 2004; 256: 183-194.

[26] Fan H, Liu D, Qiu X, Qiao F, Wu Q, Su X, Zhang F, Song Y, Zhao Z, Xie W. A functional polymorphism in the DNA methyltransferase-3A promoter modifies the susceptibility in gastric cancer but not in esophageal carcinoma. BMC Med 2010; 8: 12.

[27] Coppedè F, Tannorella P, Pezzini I, Migheli F, Ricci G, Caldarazzo Ienco E, Piaceri I, Polini A, Nacmias B, Monzani F, Sorbi S, Siciliano G, Migliore L. Folate, homocysteine, vitamin B12, and polymorphisms of genes participating in one-carbon metabolism in late-onset Alzheimer's disease patients and healthy controls. Antioxid Redox Signal 2012; 17: 195-204.

[28] Bestor T, Laudano A, Mattaliano R, Ingram V. Cloning and sequencing of a cDNA-encoding DNA methyltransferase of mouse cells - the carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. J Mol Biol 1988; 203: 971-983.

[29] Fatemi M, Hermann A, Pradhan S, Jeltsch A. The activity of the murine DNA methyltransferase Dnmt1 is controlled by interaction of the catalytic domain with the N-terminal part of the enzyme leading to an allosteric activation of the enzyme after binding to methylated DNA. J Mol Biol 2001; 309: 1189-1199.

[30] Goyal R, Reinhardt R, Jeltsch A. Accuracy of DNA methylation pattern preservation by the Dnmt1 methyltransferase. Nucleic Acids Res 2006; 34: 1182-1188.

[31] Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, Jones PA. The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Res 1999; 27: 2291-2298.

[32] Weisenberger DJ, Velicescu M, Cheng JC, Gonzales FA, Liang G, Jones PA. Role of the DNA methyltransferase variant DNMT3b3 in DNA methylation. Mol Cancer Res 2004; 2: 62-72.

[33] Gordon CA, Hartono SR, Chedin F. Inactive DNMT3B splice variants modulate de novo DNA methylation. PLoS One 2013; 8: e69486.

[34] Chen T, Ueda Y, Xie S, Li E. A novel Dnmt3a isoform produced from an alternative promoter localizes to euchromatin and its expression correlates with active de novo methylation. J Biol Chem 2002; 277: 38746-38754.

[35] Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, Fan G. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 2010; 13: 423-430.

[36] LaPlant Q, Vialou V, Covington HE 3rd, Dumitriu D, Feng J, Warren BL, Maze I, Dietz DM, Watts EL, Iniguez SD, Koo JW, Mouzon E, Renthal W, Hollis F, Wang H, Noonan MA, Ren Y, Eisch AJ, Bolanos CA, Kabbaj M, Xiao G, Neve RL, Hurd YL, Oosting RS, Fan G, Morrison JH, Nestler EJ. Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. Nat Neurosci 2010; 13: 1137-1143.

[37] Chouliaras L, Kenis G, Visser PJ, Scheltens P, Tsolaki M, Jones RW, Kehoe PG, Graff C, Girtler NG, Wallin AK, Rikkert MO, Spiru L, Elias-Sonnenschein LS, Ramakers IH, Pishva E, van Os J, Steinbusch HW, Verhey FR, van den Hove DL, Rutten BP. DNMT3A moderates cognitive decline in subjects with mild cognitive impairment: replicated evidence from two mild cognitive impairment cohorts. Epigenomics 2015; 7: 533-537.

[38] Zhu J, Du S, Zhang J, Wang Y, Wu Q, Ni J. Polymorphism of DNA methyltransferase 3B -149C/T and cancer risk: a meta-analysis. Med Oncol 2015; 32: 399.

[39] Liu CH, Tao T, Jiang L, Xu B, Zhang L, Lu K, Zhang XW, Chen SQ, Liu DC, Chen M. DNMT3A -448A>G polymorphism and cancer risk: a meta-analysis. Genet Mol Res 2015; 14: 3640-3649.

[40] Mostowska A, Sajdak S, Pawlik P, Lianeri M, Jagodzinski PP. DNMT1, DNMT3A and DNMT3B gene variants in relation to ovarian cancer risk in the Polish population. Mol Biol Rep 2013; 40: 4893-4899.

[41] Szczepanska M, Mostowska A, Wirstlein P, Malejczyk J, Ploski R, Skrzypczak J, Jagodzinski PP. Polymorphic variants of DNMT3A and the risk of endometriosis. Eur J Obstet Gynecol Reprod Biol 2013; 166: 81-85.

[42] Piotrowski P, Grobelna MK, Wudarski M, Olesinska M, Jagodzinski PP. Genetic variants of DNMT3A and systemic lupus erythematosus susceptibility. Mod Rheumatol 2015; 25: 96-9.

[43] Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature 2015; 526: 68-74.

[44] The International HapMap Consortium, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Bonnen PE, de Bakker PI, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA, Nemesh J, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Gonzaga-Jauregui C, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE. Integrating common and rare genetic variation in diverse human populations. Nature 2010; 467: 52-58.

[45] Zhuo JM, Wang H, Pratico D. Is hyperhomocysteinemia an Alzheimer's disease (AD) risk factor, an AD marker, or neither? Trends Pharmacol Sci 2011; 32: 562-571.

[46] Coppedè F. One-carbon metabolism and Alzheimer's disease: focus on epigenetics. Curr Genomics 2010; 11: 246-260.

[47] Peng Q, Lao X, Huang X, Qin X, Li S, Zeng Z. The MTHFR C677T polymorphism contributes to increased risk of Alzheimer's disease: evidence based on 40 case-control studies. Neurosci Lett 2015; 586: 36-42.