Frequency of APOE, ACE, MTHFR an CCR5 Polymorphisms in Patients with Mild Cognitive Impairment in Costa Rican Population

Norbel Román1,2*, Carolina Boza3, Leonardo Calvo1 and Andrés Flores3

1Memory and Aging Clinic, Hospital San Juan de Dios, USA
2Neurology Department, Hospital San Juan de Dios, USA
3Hematology Research Center (CIHATA), University of Costa Rica, USA

Abstract

Background: This is a descriptive cross-sectional epidemiological study describing the prevalence of polymorphisms within the Apolipoprotein E (ApoE), Methylene tetrahydrofolate reductase (MTHFR), Angiotensin converting enzyme (ACE), and Chemokine receptor 5 (CCR5) genes in patients with mild-cognitive impairment (MCI).

Methods: The study analyzed 84 blood samples from patients diagnosed with MCI at the Memory and Aging Clinic at the Hospital San Juan de Dios in Costa Rica. The authors performed a genetic analysis to determine and compare the genotypic and allelic frequencies in MCI patients versus those reported for the Costa Rican population.

Results: Genotypic and allelic frequencies obtained were compared to reports in the Costa Rican population, and a gender-based analysis. There was significant difference in the APOE and MTHFR polymorphism (p=0.007446 and p=0.003329, respectively).

Discussion: The study found a statistical difference in prevalence of the ApoE (ε2, ε3, ε4 alleles) and MTHFR (C677T) polymorphisms in the MCI patients. The study lacks a cohort of age-matched control subjects that do not have MCI. However, this study is very relevant to our understanding of the role played by these genes in the etiopathogenesis of MCI.

Keywords: Mild cognitive impairment; Alzheimer's disease; ApoE; MTHFR; ACE; CCR5; Polymorphisms

Introduction

Mild cognitive impairment and Alzheimer’s disease

Alzheimer’s disease (AD) is the most common cause of dementia. It is a multifactorial disease in which genetic and environmental conditions interact to present a clinical manifestation [1]. It is characterized by progressive cognitive impairment and memory damage. Patients meeting criteria for Mild Cognitive Impairment (MCI) are at an increased risk for developing to diagnosable AD [2] and are considered to be a transitional phase between healthy cognitive aging and dementia up to 60% of the cases within a ten year period [3].

MCI is defined as a subtle but measurable memory disorder, and represent an important step forward in diagnosing AD in its earliest stage. Diagnoses based mainly on cognitive performance present limitations, associated to the variability in methods and tests employed in the evaluation and how they are interpreted. Recent advances in understanding of imaging and biochemical changes in early stages of the disease have improved the possibility to diagnose AD in earlier stages [4].

Genetic polymorphisms such as Apolipoprotein E (ApoE), Methylene tetrahydrofolate reductase (MTHFR), Angiotensin I-converting enzyme (ACE) and Chemokine receptor 5 (CCR5) have been associated with age-related disorders, due to their implications in various complex disorders such as cerebrovascular disease (CVD), coronary artery disease (CAD) and AD [5].

Apolipoprotein E (rs429358 and rs7412)

The ε4 genotype for the ApoE is a risk factor for developing AD. ApoE-ε4 presents 15-16% of the general population [6], with greater presence in Caucasian populations [7] and nearly in 50% of subjects with AD. The presence of ApoE-ε4 genotype increases the risk of developing AD 3 to 8 times higher and decreases age onset between 7 to 15 years [8]. In homozygous form, the risk increases 33 times [9]. In late-onset AD is found in 65% of the cases [10,11] and the percentage rises to 80% in presence of a family member with EA [12]. ApoE remains the biomarker for predicting and diagnosing AD [13].

Methylenetetrahydrofolate reductase (rs1801133)

Several studies support that the polymorphism C677T in the MTHFR promotes brain atrophy associated with cognitive impairment [14]. MTHFR is an enzyme responsible for intracellular folate homeostasis and metabolism. The most common functional variant in the MTHFR gene is the polymorphism C677T (rs1801133). It has been shown that de homozygous (T/T) and heterozygous (T/C) variants of the C677T polymorphism are responsible for 30% and 65%, respectively, of the activity of the MTHFR enzyme, in comparison to subjects with the homozygous wild type variant. As a result, variants T/T and C/T have been associated with lower levels of serum folate and higher homocysteine levels. These levels can cause neurotoxic effects.
such as damage at DNA level (synthesis transmethylation), imbalance in neurotransmitters, and alteration in the basal ganglia, among others. This relates to the onset of neurodegenerative disorders such as AD [14]. In Costa Rica, previous research estimated that the prevalence of the C677T polymorphism have a similar trend reported for Latin American populations with a frequency of 677 genotype C/T of 45.9\%, genotype T/T of 26.8\% and for wild type (677 C/C) of 27.3\% [15].

**Angiogenin I-converting enzyme (rs1799752)**

The ACE (dipeptidyl carboxypeptidase, EC 3.4.15.1) is a membrane-bound ectoenzyme. ACE is a very important component of the renin-angiotensin system (RAS) promoting the formation of angiogenin II from angiogenin I and inactivating the vasodilator bradykinin [16]. In addition, a local RAS in the brain plays an important role in the central nervous system. In addition, the angiogenin present in astrocytes is required for the functional maintenance of the blood brain barrier [17], which is affected in AD [18]. The activity of the ACE in the brain varies significantly between individuals with AD or in patients with MCI and healthy controls subjects [19] and has been demonstrated that ACE inhibit amyloid-beta peptide aggregation and plaque formation in vitro [20]. The I/D polymorphism in intron 16 of the ACE gene on chromosome 17q23 [21] is associated with lower ACE level, mainly those subjects who carry the I allele have a lower ACE level than subjects bearing the D allele [22]. Arregui et al. [23] observed an increase in ACE activity in patients with AD.

**Chemokine receptor 5 (rs333)**

Chemokines are produced due to the activation of a wide spectrum of inflammatory cells including the astrocytes [24]. It has been found that some chemokines, their receptors and ligands are found in brain with AD. The monocyte chemotactic protein 1 (MCP-1) and ligand of CC chemokine receptor 2 (CCR2) are found in senile plaque, and the reactive microglia promotes de activation of the astrocytes, suggesting neuroinflammation [25]. It has been found that CCR5 deficiency activated astrocytes and Aβ accumulation via upregulation of CCR2 [26]. These findings suggest that chemokines and their receptors and ligands may contribute to the development and/or the progression of AD through modification of astrocyte activation.

**Memory and aging clinic**

In Costa Rica, the first prevalence study conducted in a community (Santo Domingo, Heredia) with a sample of 400 subjects, showed a 4.2\% prevalence of probable dementia (in any of its subtypes). Among the subjects evaluated 41 were diagnosed with AD (n=14) and MCI (n=27) [27].

At the Memory and Aging Clinic of the Hospital San Juan de Dios (CMEC) interdisciplinary diagnosis by consensus assessments have been conducted for the past 7 years, using a protocol established by the our team of neurologists, geriatricians and clinical psychologists. In 2012 the CMEC reports a first analysis of the prevalence of MCI in the population served by the clinic (n=128), during 2010-2011. The most frequent diagnosis was MCI (44.5\%), while dementia was found in 30.5\% of cases, where the AD (43.6\%) and vascular dementia (25.6\%) predominated.

These results showed that the CMEC is attracting patients at early stages, so our efforts should focus on this population. This highlights the importance of providing follow-up to patients and reinforces the need to implement the detection of biomarkers and the presence of other genetic mutations considered as risk factors for dementia (APOE), as a part of the diagnostic process. Thus, during 2012 there was a national campaign for early diagnosis, promoted by the Alzheimer and Other Dementias Association (ASCADA), which gave the first donation of the ApoE detection kits. In this study, we aimed to establish the prevalence of ApoE, MTHFR C677T, ACE I/D and CCR5 Δ32 in DNA samples of patients with MCI in Costa Rican population, evaluated and diagnosed by the CMEC and compare them with previous reports in control subjects in this country.

**Methods and Materials**

**Sample collection**

This is a descriptive and transversal study. Patients were previously evaluated in the Memory Clinic-Hospital San Juan de Dios using a protocol established by our team of neurologists, geriatricians and clinical psychologists, which includes a battery of tests for assessing cognitive and functional performance (screening, medical history, neuropsychological assessment, neurological examination, review of the patient's records, molecular biology studies and neuroimaging). Subjects gave written informed consent and the University of Costa Rica's institutional Bioethics review board approved the study. Patients diagnosed with MCI were randomly selected.

We collected blood samples with EDTA and DNA isolation was obtained following the standard NaCl precipitation method [28] and analyzed 84 anonymous samples for the presence of ApoE, ECA, CCR5 and MTHFR in patients diagnosed with MCI during 2012-2014.

**Genetic analysis**

ApoE polymorphism was amplified by RFLP-PCR method [29]. Amplification of ApoE products that were suitable for Hhal digestion proved successful for most DNA samples, with the exception of samples that were extensively degraded prior to amplification. The products were detected in 8\% polyacrylamide gel by electrophoresis. Fragments of 72 bp and 48 bp were produced in ApoE ε4, fragments of 91 bp and 83 bp are produced in ApoE ε2 in 91 bp and 48 bp are generated in ApoE ε3.

MTHFR polymorphism was performed by RFLP-PCR method was used [30]. After amplification using HinfI restriction enzyme to identify the mutation. The mutant allele generated two fragments: 175 bp and 23bp, while the wild-type is not cleaved and is identified by a 198 bp fragment.

The primers and PCR conditions for the ACE polymorphism were based on those described by Rigat et al. [31], obtaining a 190 bp for DD genotype and a fragment of 490 bp in the presence of the corresponding insertion genotype II; heterozygous individuals have both bands (I/D).

To avoid false-positive DD genotype, a second amplification [32] was performed, attempting to obtain a band of 300 bp for the heterozygous genotype (I/D) and the homozygous deletion allele (D/D) a band of 200 bp. The bands obtained were analyzed in 2% agarose gels by electrophoresis.

CCR5 polymorphism was performed by PCR which flank the deletion of 32 bp [33]. The products were detected in 4% agarose gel by electrophoresis. The genotyping was performed according to the size of the amplification products (CCR5 1/1) a band of 184 bp was observed for the homozygous, deletion of 32 bp (Δ32/ Δ32); the expected product was 152 bp and heterozygotes (1/Δ32) 2 bands: 184 and 152 bp.

**Statistical Analysis**

Chi square test was used to compare the frequencies between...
populations. This test takes large (>30), independent samples, and categorical variables and random sampling. Confidence intervals were calculated for the difference of proportions that turned out significant using Tukey multiple comparison method, with a global confidence of 95%.

**Results**

A description of the demographic and genotypic characteristics in patients with mild cognitive impairment diagnosed at the Memory Clinic of San Juan de Dios Hospital is presented in Table 1. The age average of the population (n=84) is 62.8 years (SD: 13.2), with a mean of education of 7.3 years (SD: 5.4). The gender distribution consisted in 67% women and 34% men.

For the ApoE analysis the ε2/ε2 genotype was absent in our sample. The ε4 allele frequency is 0.14, where the genotype ε3/ε4 predominates (22.62%). The most frequent genotype was ε3/ε3 (67.86%). The ε3 allele of ApoE polymorphism was the most frequent (0.83), while the ε2 allele had a frequency of 0.04.

The C allele of the MTHFR C677T polymorphism was similar to the T allele with a frequency of 0.46 and 0.54, respectively. The most frequent genotype was the C677T (64.29%) and the wild type CC 677 the less frequent (14.29%).

For the ACE polymorphism, the D allele was also similar to I allele with a frequency of 0.46 and 0.54, respectively. The most frequent genotype was I/D (53.57%), and the less frequent was the D/D genotype (19.05%). For both MTHFR and ACE polymorphisms, it is important to notice that they present a similar behavior, having frequencies near 50%.

The distribution of the CCR5 allele frequencies was 0.96 for the I allele and 0.04 for the ∆32 allele, which is not usual taking into account the sample size (n=84). The CCR5 I/I genotype was the most frequent (91.67%) and the ∆32/∆32 genotype was absent in our sample.

Significant differences were found when comparing the control population versus population with MCI in the ApoE and MTHFR polymorphism, assuming an alpha =0.05 (maximum error allowed type I), with p-values of 0.007446 and 0.003329, respectively.

Confidence intervals (CI) were calculated. For the ε3 polymorphism of ApoE, the difference between the proportions of the two samples is 0.0821429 (CI=[0.1601294 to -0.0079776]), which means that the presence of the ε3 allele is 0.16% to 0.07% less than in the control sample. Note that this interval is very close to 0, implying that it is not significant.

For the ε4 polymorphism, the difference is 0.09404762 (CI=0.02618271 to 0.1659556), implying that in the sample evaluated in comparison to the control sample, the presence of ε4 allele is between 2.6% to 16.6% higher than in the general population.

Regarding MTHFR, the difference in ratio for the allele with the C variant for T is 0.1388071, (CI=[0.04852785 to 0.2271886]). This indicates that in the sample evaluated, in comparison to the control sample, this variant is 4.8% to 22.7% more prevalent.

In gender-based analysis, Table 2 presents the distribution of the allelic and genotypic frequencies of the ApoE, ACE, MTHFR, CCR5 polymorphisms by gender. Allelic distribution is very homogeneous in most polymorphisms. In the genotypic frequency, is important to highlight that the ApoE ε3/ε4 genotype is more frequent in males.

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**Table 1:** Demographic characteristics, genotypic and allele frequencies of the polymorphisms studied in patients with MCI and controls in Costa Rican population reported in other studies.
Table 2: Gender frequencies of the ApoE, ACE, MTHFR, CCR5 polymorphism in patients with mild cognitive impairment in Costa Rica population.

| Polymorphism | Genotypic frequencies n (f) | Allelic Frequencies n (f) |
|--------------|----------------------------|--------------------------|
|              | Male          | Female      | Male          | Female      | P          |
| ApoE         |               |             |               |             |            |
| E2/E2        | 0             | 0           | E2            | 2 (0.04)    | 4 (0.04)   |            |
| E2/E3        | 2 (7.14)      | 4 (7.14)    | E3            | 44 (0.79)   | 95 (0.85)  | 0.5375     |
| E2/E4        | 0             | 0           |               |             |            |            |
| E3/E3        | 16 (57.14)    | 41 (73.21)  |               |             |            |            |
| E3/E4        | 10 (35.71)    | 9 (16.07)   |               |             |            |            |
| E4/E4        | 0             | 2 (3.57)    |               |             |            |            |
| ACE          |               |             |               |             |            |
| I/I          | 7 (25.00)     | 16 (28.57)  | I             | 30 (0.54)   | 61 (0.54)  |            |
| I/D          | 16 (57.14)    | 29 (51.79)  | D             | 26 (0.46)   | 51 (0.46)  | 0.9998     |
| D/D          | 5 (17.86)     | 11 (19.64)  |               |             |            |            |
| MTHFR        |               |             |               |             |            |
| CC677        | 3 (10.71)     | 9 (16.07)   | C             | 23 (0.41)   | 55 (0.49)  | 0.412      |
| C677T        | 17 (60.71)    | 37 (66.07)  |               | 33 (0.59)   | 57 (0.51)  |            |
| 677TT        | 8 (28.57)     | 10 (17.86)  |               |             |            |            |
| CCR5         |               |             |               |             |            |
| 01-Jan       | 25 (89.29)    | 52 (92.86)  | 1             | 53 (0.95)   | 108 (0.96) | 0.8914     |
| 1/∆32        | 3 (10.71)     | 4 (7.14)    |               | 3 (0.05)    | 4 (0.04)   |            |
| ∆32/∆32      | 0             | 0           |               |             |            |            |

The presence of the ApoE ε4 genotype is associated with its double structural conformation of domains interacting with each other by salt bridges between N terminal and C terminal contributing to neurodegeneration including mitochondrial dysfunction. It has been linked to deposition of cholesterol in areas previously damaged or complexing with beta-amyloid protein which reduces the capacity of synaptic plasticity contributing to cognitive failure [42,43].

The statistical significant differences indicates that the control group reported in other studies in Costa Rica and the MCI population analyzed, behave differently with respect to the general allele frequency for ApoE and MTHFR genes. Further analysis is important to determine which of those alleles differ from a control and a case. It is known that the presence of a matched control is necessary to limit bias in the variables. Currently the CMEC is selecting a control group matched to analyze these polymorphisms and thus carry out a comparative study directly with our population.

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References
1. Román N, Boza C (2012) Revisión sobre la relación del genotipo para APOE-E4 y el desarrollo de demencia tipo Alzheimer. Rev CI EMed UCR.
2. Petersen RC, Stevens JC, Gangul M, Tangalos EG, Cummings JL, et al. (2001) Practice parameter: Early detection of dementia: mild cognitive impairment (an evidence-based review). Neurology 56: 1133-1142.
3. Albert MS, Moss MB, Tanzi R, Jones K (2001) Preclinical prediction of AD using neuropsychological tests. J Int Neuropsychol Soc 7: 631-639.
4. Ringman JM, Medina LD, Rodriguez Y, Chavey M, Lu P, et al. (2009) Current concepts of mild cognitive impairment and their applicability to persons at-risk for familial Alzheimer’s disease. Current Alzheimer Research 6: 341-346.
5. Masharip A, Mwila HM, Patrice NM, Shabir L, Peter M, et al. (2014) Frequency of APOE, MTHFR and ACE polymorphisms in the Zambian population. BMC Research Notes 7: 194.
6. Salvia N, Clarimon J (2010) Genética en la Enfermedad de Alzheimer. Rev Neurol 50: 360-364.
7. Crean S, Ward A, Mercald C, Collins JM, Cook MN, et al. (2010) Apolipoprotein
E. ε4 Prevalence in Alzheimer’s disease patients varies across global population: A systematic literature review and meta-analysis. Dement Geriatr Cogn Disord 31: 20-30.

8. Thambisetty M, Beason-Held, An Y, Kraut MA, Resnik SM (2010) ApoE 4 genotype and longitudinal changes in cerebral blood flow in normal aging. Arch Neurol 67: 93-98.

9. Anoop S, Mirsa A, Meena K, Luthra K (2010) Apolipoprotein E polymorphism in cerebrovascular and coronary heart disease. Indian J Med Res 132: 363-378.

10. Reiman EM, Long B, Jessa BS, Tariot PN (2010) Alzheimer’s prevention initiative: A proposal to evaluate pre-symptomatic treatment as quickly as possible. Biomark Med 4: 3-14.

11. Cruchaga C, Kuwe J, Mayo K, Spiegel N, Bertelsen S, et al. (2010) SNPs associated with cerebrospinal fluid phospho-Tau levels influence rate of decline in Alzheimer’s disease. PLoS Genet 6: e1001101.

12. Dickstein D, Walsh J, Broutingham H, Stockton S, Gandy S, et al. (2010) Role of vascular risk factors and vascular dysfunction in Alzheimer disease. Mount Sinai Journal of Medicine 77: 82-102.

13. Thambisetty M, Lovestone S (2010) Blood based biomarkers of Alzheimer’s disease: Challenging but feasible. Biomark Med 4: 36-42.

14. Peng Q, Lao X, Huang X, Qin X, Li S, et al. (2015) The MTHFR C677T polymorphism contributes to increased risk of Alzheimer’s disease: Evidence based on 40 case-control studies. Neuroscience Letters 586: 36-42.

15. Salazar L, Chaves L, Cartín M, Schuster G, Wulf K, et al. (2006) Common polymorphisms and cardiovascular factors in patients with myocardial infarction of Costa Rica. Rev biol trop 54: 1-11.

16. Erdös E, Skögel R (1987) The angiotensin I-converting enzyme. Lab Invest 56: 345-348.

17. Kakunuma Y, Hama H, Sugiyama F, Nagayoshi K, Goto K, et al. (1998) Impaired blood-brain barrier function in angiotensinogen-deficient mice. Nat Med 4: 1078-1080.

18. Skoog I, Wallin A, Fredman P (1998) A population study on blood-brain barrier function in 85 years old. Neurology 50: 966-971.

19. He M, Chrui T, Manuyama M, Tomita M, Nakayama K, et al. (2006) ACE activity in CSF of patients with mild cognitive impairment and Alzheimer’s disease. Neurology 67: 1309-1310.

20. Hu J, Igarashi A, Kamata M, Nagawa H (2001) Angiotensin-converting enzyme degrades Alzheimer amyloid β-peptide (AP), retards AP aggregation, deposition, fibril formation and inhibits cytotoxicity. J Biol Chem 276: 47863-47868.

21. Arbustini E, Grasso M, Fasani R, Kersy C, Diegoli M, et al. (1995) Angiotensin-converting enzyme gene deletion allele is independently and strongly associated with coronary atherosclerosis and myocardial infarction. Br Heart J 74: 584-591.

22. Tiet L, Rigat B, Visvikis S, Breda C, Corvol P, et al. (1992) Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet 51: 197-205.

23. Arregui A, Perry E, Rosser M, Tomlinson B (1982) Angiotensin-converting enzyme in Alzheimer’s disease: Increased activity in caudate nucleus and cortical areas. J Neurochem 38: 1490-1492.

24. Dorf M, Berman M, Tanabe S, Heesen M, Luo Y (2000) Astrocytes express functional chemokine receptors. Journal of Neuroimmunology 111: 109-121.

25. Simard AR, Soulet D, Gowing G, Julien JP, Rivest S (2006) Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer’s disease. Neuron 49: 489-502.

26. Lee YK, Kwak DH, Oh KW, Nam SY, Lee BJ, et al. (2009) CCR5 deficiency induces astrocyte activation, Aβeta deposit and impaired memory function. Neurobiology of learning and memory 92: 356-363.

27. Wesseling, C, Román N, Quiroz I, Pérez L, Garcia V, et al. (2013) Parkinson’s and Alzheimer’s diseases in Costa Rica: A feasibility study toward a national screening program. Global Health Action 6: 23606.

28. Miller S, Dykes D, Polesky H (1998) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16: 1215.

29. James E, Hisson D, Vernier T (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. Journal of Lipid Research 31: 545-548.

30. Froiss P, Blom H, Milos R, Gyotte P (1995) A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase gene. Nat Genet 10: 111-113.

31. Rigat B, Hubert C, Ahenc F, Cambien F (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86: 1343-1346.

32. Odawara M, Matsunami A, Yamashita K (1997) Misting frequency of the angiotensin-converting enzyme gene polymorphism and an improved method for its avoidance. Hum Genet 100: 163-166.

33. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, et al. (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. Science 2739: 1856-1862.

34. Marca V, Acosta O, Cornejo M, Ortega O, Huerta D, et al. (2011) Polimorfismo genético de la apolipoproteína E en una población peruana. Rev Peru Med Exp Salud Pública 28: 589-594.

35. Salazar L, Hidalgo A, Arauz J, Méndez M, Cartín M, et al. (2009) Polimorfismos del gen de la enzima convertidora de angiotensina (Inserción/Deleción) y factores de riesgo asociados en pacientes con infarto agudo del miocardio. Rev Costarr Cardiol 11: 7-12.

36. Herrmann F, Salazar L, Schröder W, Grimm R, Schuster G, et al. (2001) Prevalence of molecular risk factors FV Leiden, FV II, FII 20210G>A and MTHFR 677C>T in different populations and ethnic groups of Germany, Costa Rica and India. IJHG 1: 33-39.

37. Alberca R, Montes E, Gil E, Mir P, Lozano P (2002) Enfermedad de Alzheimer y mujer. Rev Neurol 35: 571-579.

38. Manly JJ, Tang MX, Schupf N, Stern Y, Vonsattel JP, et al. (2008) Frequency and course of mild cognitive impairment in a multiethnic community. Ann Neurol 63: 494-506.

39. Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, et al. (2012) Prevalence of apolipoprotein e4 genotype and homozygotes (ApoE e4/e4) among patients diagnosed with Alzheimer’s disease: A systematic review and meta-analysis. Neuroepidemiology 38: 1-17.

40. Hua Y, Zhao H, Kong Y, Ye M (2011) Association between the MTHFR gene and Alzheimer’s disease: A meta-analysis. International Journal of Neuroscience 121: 462-471.

41. Wang B, Jin F, Kan R, Ji S, Zhang C, et al. (2005) Association of MTHFR gene polymorphism C677T with susceptibility to late-onset Alzheimer’s Disease. Journal of Molecular Neuroscience 27: 23-28.

42. Rajagopalan P, Jahanhash N, Stein JL, Hua X, Madsen SK, et al. (2012) Common folate gene variant MTHFR C677T is associated with brain structure in two dependent cohort of people with mild cognitive impairment. Neuroimage Clin 4: 179-187.

43. Luo M, Huihui J, Xiaozhi H, Lianq J, Ting Z (2015) Correlation of homocysteine metabolic enzymes gene polymorphism and mild cognitive impairment in the Xinjiang Uyghur population. Med Sci Monit 21: 326-332.