5,10-Methylenetetrahydrofolate Reductase C677T Gene Polymorphism Can Influence Age at Onset of Parkinson's Disease

Juei-Jueng Lin[1,2,3], Kuo-Chu Yueh[1], Chin-San Liu[4], Jung-Tung Liu[5], and Shinn-Zong Lin[6]

Abstract- A case-control study was designed to investigate a possible genetic susceptibility of the *MTHFR* C677T polymorphism and assess whether the genetic polymorphism could be a predictor of levodopa-induced adverse effects in patients with Parkinson's disease (PD) of Chinese descent living in Taiwan. There were 94 sporadic PD patients with levodopa therapy at least for five years and 146 control subjects, matched by sex and gender, in this study. Results revealed that there were no differences of the allelic and genotypic frequencies of the *MTHFR* C677T polymorphism between PD patients and the controls. Analysis of age at onset stratified by *MTHFR* C677T polymorphism showed a trend of early age at onset in the PD patients carrying with *T* allele. The genetic influence was particularly significant in late-onset PD (onset age at or older than 60 years) with an early age at onset for 3.4 years. However, the *MTHFR* C677T polymorphism was not associated with the risk to develop dyskinesia, motor fluctuation and psychosis induced by levodopa in PD patients. In conclusion, results of the study revealed that the *MTHFR* C677T polymorphism could significantly influence age at onset of PD in Chinese population, but neither as a genetic susceptibility nor as a predictor of levodopa-induced adverse effects in PD.

Key Words: Case-control study, Genetic polymorphism, Levodopa-induced adverse effect, 5,10-Methylenetetrahydrofolate reductase gene, Parkinson's disease

Acta Neurol Taiwan 2007;16:150-157

INTRODUCTION

The combined effect of environmental precipitating factors and the presence of a genetic susceptibility may contribute to the pathogenesis of Parkinson's disease (PD)[(1)]. Increased evidence suggests that a high plasma level of homocysteine (Hcy) might contribute to these processes through direct neurotoxic effects[(2,3)]. In animal

From the Departments of Neurology, [1]Chushang Show-Chwan Hospital, Nantou, Taiwan; [2]Chung-Shan Hospital, Taipei, Taiwan; [3]Chung-Shan Medical University Hospital, Taichung, Taiwan; [4]Changhua Christian Hospital, Changhua, Taiwan; [5]Department of Neurosurgery, Chung-Shan Medical University Hospital, Taichung, Taiwan; [6]Neuro-Medical Scientific Center, Tzu-Chi Buddhist General Hospital, Hualian, Taiwan.
Received January 23, 2007. Revised February 16, 2007.
Accepted April 30, 2007.

Reprint requests and correspondence to: Juei-Jueng Lin, MD, MPH. Department of Neurology, Chushang Show-Chwan Hospital, No. 75, Sec. 2, Chi-Shang Rd, Nantou, Taiwan.
E-mail: jjlinn@tcts.seed.net.tw

models of PD, injection of Hcy in the brain exacerbated 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine-induced motor dysfunction and loss of dopaminergic neurons[2]. Hcy has been also observed to cause DNA strand breaks and to enhance oxidative stress, mitochondrial dysfunction, and apoptosis induced by rotenone and iron in cultured human dopaminergic cells[3].

Clinically, an increased plasma level of Hcy was initially identified as a risk factor for vascular diseases[4,5] and was subsequently shown to be a risk factor for Alzheimer's disease[6], cortical and hipocampal atrophy[7], depression[8], and nondemented elderly people with decreased cognitive performance[9]. Total plasma Hcy was also significantly higher among PD patients compared to nonparkinsonian controls[10,11]. The hyperhomocysteinemia in PD patients were principally the result of levodopa administration and from methylation of levodopa and dopamine by catechol O-methyltransferase[12-14]. The 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*) is a folate-dependent enzyme that catalyzes remethylation of Hcy. Individuals with homozygous thermolabile (*TT*) genotype for the *MTHFR* C677T polymorphism display a reduced enzymatic activity, resulting in mild hyperhomocystinemia[15]. A study by Yasui et al.[16] demonstrated a higher plasma Hcy level in PD patients carrying *TT* genotype than those carrying with other genotypes. Therefore, the *MTHFR* could be a candidate gene responsible for the risk of PD through affecting Hcy level by *MTHFR* polymorphism. A recent study by de Lau et al.[17] in a Caucasian population has shown that *TT* genotype of the *MTHFR* C677T polymorphism was associated with an increased risk for PD, particularly in smoker. To reproduce the genetic susceptibility of *MTHFR* polymorphism for PD in different populations, the present study investigated a possible association between *MTHFR* polymorphism and PD patients in a Chinese population living in Taiwan. In addition, this study also investigated the role of *MTHFR* polymorphism in the risk of developing levodopa-induced adverse effects in chronically treated PD patients, because genetic factors may contribute to the pathogenesis of adverse drug reaction[18-20].

PATIENTS AND METHODS

The enrolled PD patients were recruited from the Movement Disorders Clinic of Chushang Show-Chwan Hospital in Nantou, Taiwan from January 2004 to December 2005. They have received treatment with levodopa at least for five years. Idiopathic PD was diagnosed according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria[21]. Clinical data were collected during the last office visit through physical examination, detailed history, and review of patient charts and outside documents. The precise duration of PD and duration of levodopa treatment had been given from the beginning of parkinsonian symptoms and levodopa therapy until blood sampling during the last office visit for genetic analysis, respectively. Dyskinesia was defined as drug induced hyperkinetic or dystonic movements or postures or both[22] or if patients had a score of ≥ 1 on items 32 to 39 of the Unified Parkinson's Disease Rating Scale (UPDRS) part 4[23]. "On-off" phenomenon denoted sudden, unpredictable fluctuation of motor symptoms, and wearing-off effects were defined as reemergence of parkinsonian symptoms after a dose of levodopa[24]. However, we recorded these two symptoms as one item (motor fluctuation) and did not differentiate between "on-off" and "wearing off" in the analysis. Finally, levodopa-induced psychosis was defined as PD patients having symptoms of disorder of perception, usually consisting of visual hallucinations with or without insight, and/or disordered thought with clear sensorium[25]. Patients with previous psychiatric illness or whose hallucinations began before commencing antiparkinsonian medication were excluded. The control volunteers were selected by cluster sampling from the same community over the same period of time. They were all unrelated and received detailed interviews which consisted of a personal health history and family history of PD. A simple physical examination was performed to make sure that there were no parkinsonian symptoms. Participants who were aborigines or having a positive family history of PD were excluded. Therefore, PD patients and control subjects in this study came from the same ethnic origin of Chinese descent. Informed consent

was obtained from the PD patients and control subjects, according to a protocol approved by the Human Subjects Research Ethics Committee of the Hospital.

Genomic DNA was extracted from peripheral whole blood using the IsoQuick Nucleic acid extraction kit (ORCA Research Inc., Bothell, WA, USA), according to the manufacturer's protocol. The primers designed and procedure of the conventional polymerase chain reaction (PCR) and fragment restriction length polymorphism (FRLP) used for amplification of *MTHFR* C677T polymorphism was described by Frosst et al.[26], resulting in three genotypes (CC/TT homozygotes and CT heterozygote). Each blood specimen was tested in duplicate to ensure reproducibility of the results.

Statistical analysis was performed using the SPSS for Windows release 12.0, run on an IBM-compatible computer. Tests for the allelic and genotypic frequencies were performed using the chi-square (X^2) tests. Differences between the mean age at onset of PD and the genotypes were compared using one-way ANOVA. The demographic data of PD patients with and without levodopa-induced adverse effects were expressed as mean ±S.D. and were compared by unpaired Student's *t* test. Tests for the difference in the *MTHFR* C677T polymorphism between PD patients with and without levodopa-induced adverse effects were also performed using the X^2 tests. A *p*-value of < 0.05 was considered significant.

RESULTS

This study included 94 sporadic PD patients treated with levodopa and 146 control subjects matched by sex and gender. The demographic data of both study groups are shown in Table 1. The genotype frequencies of *MTHFR* C677T polymorphism in the controls were consistent with the Hardy-Weinberg equilibrium. Although the frequencies of both the homozygote TT genotype and T allele of the polymorphism in the PD patients were higher than those of the controls, these differences did not reach statistical significance ($X^2 = 2.848$, $p = 0.241$ and $X^2 = 2.48$, $p = 0.120$, respectively) (Table 1). Further analysis of the *MTHFR* C677T polymorphism between the PD patients and controls stratified by age at onset and gender did not reveal any significant differences.

The mean age at onset of PD patients in this study was 61.2±10.0 years (mean±standard deviation, ranging from 32 to 90 years). When the mean age at onset of PD was stratified by the *MTHFR* genotype, age at onset of PD patients with *T* allele bearers (either *MTHFR-TT*

Table 1. *Demographic characteristics and MTHFR C677T polymorphism for the study groups*

	PD patients	Controls	*P*-value
Number of subjects	94	146	
Mean age (years)	69.5 ± 11.0	68.5 ± 6.1	
Gender (male/female)	1.12	1.11	
Mean age at onset of PD (years)	61.2 ± 10.0	-	
Duration of PD (years)	8.3 ± 2.7	-	
Duration of levodopa treatment (years)	7.0 ± 2.3	-	
Mean levodopa dosage (mg/day)	627.5 ± 322.7	-	
MTHFR C677T polymorphism			
Genotype frequency			
CC	55.3%	63.7%	0.241
CT	58.3%	33.6%	
TT	6.4%	2.7%	
CC	55.3%	63.7%	0.195
CT + *TT*	44.7%	36.3%	
Allele frequency			
C	74.5%	80.5%	0.120
T	25.5%	19.5%	

MTHFR: 5,10-methylenetetrahydrofolate reductase gene, PD: Parkinson's disease

Table 2. *Correlation between C677T polymorphism of MTHFR and age at onset of PD*

MTHFR polymorphism	No.	Mean age at onset of PD
Total PD patients	94	61.2 ± 10.0
CT + *TT*	54	59.5 ± 11.0[a]
CC	52	63.3 ± 13.0
Early-onset PD (onset age < 60 years)	37	
CT + *TT*	14	49.0 ± 7.0[b]
CC	23	47.3 ± 5.4
Late-onset PD (onset age > 60 years)	57	
CT + *TT*	28	67.9 ± 4.5[c*]
CC	29	71.3 ± 6.6

Abbreviation as in Table 1; * p < 0.05.

a: No difference of mean age at onset between patients carrying *T* allele *MTHFR* and *CC* genotypes (t = -1.53, 95% CI = -8.71~1.121, p = 0.129); b: No difference of mean age at onset between early-onset PD patients carrying *T* allele *MTHFR* and *CC* genotypes (t = 0.77, 95% CI = -2.76 ~ 6.13, p = 0.447); c: Significant difference of mean age at onset between late-onset PD patients carrying *T* allele *MTHFR* and *CC* genotypes (t = -2.332, 95% CI = -6.43 ~ -0.48, p = 0.023)

Table 3. *Frequencies of the MTHFR C677T polymorphism of the PD stratified by levodopa-induced adverse effects*

		Genotype frequency			Allele frequency		
Variable	No. (%)	*CC* (%)	*CT* + *TT* (%)	*p*-value	*T* (%)	*C* (%)	*p*-value
Total PD	94 (100%)	52 (55.3%)	42 (44.7%)		48 (25.5%)	140 (74.5%)	
Dyskinesia							
with	15 (16.0%)	7 (46.7%)	8 (53.3%)	0.462	8 (26.7%)	22 (73.3%)	0.876
without	79 (84.0%)	45 (57.0%)	34 (43.0%)		40 (25.3%)	118 (74.7%)	
Motor fluctuation							
with	39 (41.5%)	26 (66.7%)	13 (33.3%)	0.062	16 (20.5%)	62 (79.5%)	0.184
without	55 (58.5%)	26 (47.3%)	29 (52.7%)		32 (29.1%)	78 (70.9%)	
Psychosis							
with	21 (22.3%)	12 (57.1%)	9 (42.9%)	0.849	9 (21.4%)	33 (78.6%)	0.489
without	73 (77.7%)	40 (54.8%)	33 (45.2%)		39 (26.7%)	107 (73.3%)	

Abbreviation as in Table 1.

or *MTHFR-CT*, 59.5 years of mean age) was 3.8 years earlier than that with *T* allele nonbearers (*MTHFR-CC*, 63.3 years of mean age), despite that the difference did not reach statitical significance (95% CI=-8.71~1.12, p=0.129). When PD patients were divided into two subgroups, early-onset (onset age < 60 years) and late-onset (onset age ≥ 60 years), further analysis revealed a statistical significance of 3.4 years early age at onset of PD in the late-onset group with *T* allele bearers (95% CI=-6.43 ~ -0.48, p = 0.023) (Table 2). There was no statistical difference in the mean age at onset of PD between *T* allele bearers and nonbearers in the early-onset group. The cumulative percentage of the *MTHFR* genotype according to the age at onset of PD patients is shown in Fig.

In the study, mean duration of treatment with levodopa in those 94 sporadic PD patients was 7.1±2.0 years (ranging from 5 to 18 years) and mean dosage of levodopa was 627.5±322.7 mg/day. Fifteen PD patients (16.0%) developed dyskinesia, 39 PD patients (41.5%) showed motor fluctuation, and 21 PD patients (23.3%) had levodopa-induced psychosis. Patients with dyskinesia, motor fluctuation, or psychosis had a significantly longer duration of the disease, and had been treated longer with levodopa than all other patients. Patients with levodopa-induced dyskinesia or motor fluctuation had been younger at the age at onset than that in patients without these adverse effects, but patients with levodopa-induced psychosis had been older at the age at

onset. Analysis of the difference in the *MTHFR* C677T polymorphism in PD patients stratified by levodopa-induced adverse effects revealed that the genetic polymorphism was not associated with the risk to develop dyskinesia, motor fluctuation and psychosis induced by levodopa (Table 3).

DISCUSSION

Previous studies have shown associations of the *MTHFR* C677T polymorphism with a number of disorders, including vascular disorders (such as ischemic stroke, coronary arterial disease and peripheral arterial disease), psychiatric diseases (such as schizophrenia and depression), cancers (such as breast, gastric and colorectal cancer), and neurodegeneration disorders (such as Alzheimer's disease)[4-11]. Theoretically, *TT* genotype of the *MTHFR* C677T polymorphism might be associated with a greater risk for PD, because the genotype would reduce enzymatic activity and result in hyperhomocysteinema[2,3,15,16]. However, the former studies did not support the hypothesis and revealed an insignificant association between the *MTHFR* C677T polymorphism and the risk of PD in the English[27], Germans[28] and Polacks[29]. Results of our study also revealed non-insignificance of the genetic polymorphism in the risk of PD in a Chinese population living in Taiwan. A recent study by de Lau et al.[17] in Hollanders demonstrated that *TT* genotype of the *MTHFR* C677T polymorphism was associated with a borderlinely increased risk for PD and their further analysis showed a strong and significant increase in risk for PD with *TT* genotype in smoker (RR=3.74; 95% CI=1.78-7.85). The explanations for the significance restricted in smoker were that smoking could increase plasma Hcy levels and the *TT* genotype could interact with smoking to increase plasma Hcy level, particularly in smoker[30,31]. Unfortunately, information on smoking status in our present study was not available, therefore, multiplicative effects could not be analyzed as was done by de Lau et al.[17]

Plasma Hcy levels are kept low by remethylation to methionine, which require folate and vitamin B_{12}, and by conversion to cysteine, for which vitamin B_6 is an essential cofactor[32]. Increased plasma Hcy levels are associated with low plasma levels as well as low dietary intakes of folate, vitamin B_{12}, and vitamin B_6[33,34]. Considering the potential neurotoxicity of Hcy[2,3], it is theoretically possible that higher intakes of folate and vitamin B_{12} and B6 might decrease the risk of PD by decrease of plasma Hcy levels. However, a retrospective study by Chen at al.[35] showed no significant association for either these vitamins. A prospective, population-based cohort study by de Lau et al.[36] revealed that a higher intake of either vitamin B_{12} or folate did not significantly decrease risk of PD, but the association was found in the high vitamin B6 intake. Although a potential effect of folate and vitamin is not completely ruled out, these findings may point toward a controversy of the hyperhomocysteinemia in the risk of PD and the *TT* genotype in the genetic susceptibility of PD. An alternative explanation for the non-association by Harmon et al.[27] indicated that brain could be protected in the situation of hyperhomocysteinemia by the preferential accumulation of folate in the CNS where its concentration was higher than that in serum.

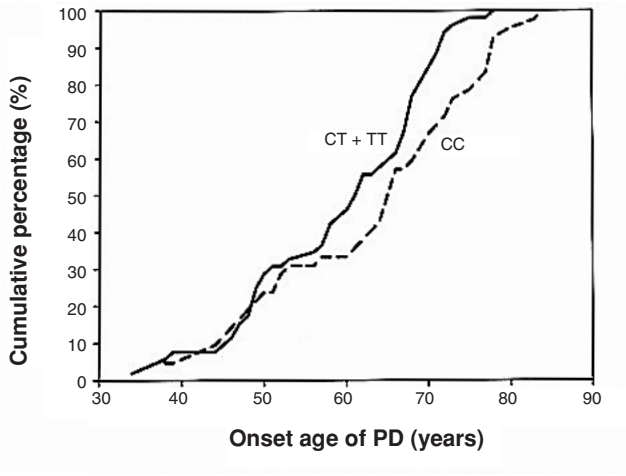

Figure. Cumulative percentage of the *MTHFR* genotype according to the age at onset of PD patients (n = 94)

Methodologic strengths of our study include the virtually complete long-term follow up at least five years, adequate stratification and control selection from the same geographic area. We attempted to minimize poten-

tial confounding variables by carefully matching controls to PD patients individually. Anyway, ethnic origin is an import issue for the genetic association study because there are significant discrepancies of genetic polymorphism among populations of different genetic backgrounds[37,38]. As a summation from the previous studies in the literature, the frequency of the *TT* genotype of *MTHFR* polymorphism in the Caucasian populations of healthy subjects was 7-13%[17,27-29]. Our study in Chinese showed 2.7% of the *TT* genotype frequency which was much lower in comparison with the Caucasian populations. Therefore, the discrepancy of genetic association studies in the different genetic background may result from the different *TT* genotype frequency. Another reason was that the result might be a false negative because there was a limited number of patients (94 of total cases studied) and a lack of statistical power in our study[39].

In this case-control study, we found a significant effect of the *MTHFR* C677T polymorphism on the age at onset in Chinese PD population. The *T* allele of the gene was associated with a trend of early age at onset of PD and the trend was particularly noted in late-onset PD patients. A study by Müller's et al.[28] also revealed the CC genotype of *MTHFR* A1298C was associated with the latest onset of disease in the German population. Generally, an interaction exists between genetic susceptibility and environmental exposure may contribute to the pathogenesis in sporadic PD and older onset PD[1]. Because the enrolled patients in our study were all sporadic PD, we therefore proposed that the older *T*-allele carriers would be susceptible to other environmental factors, e.g. hyperhomocysteinemia, which is responsible to an early onset of PD. A study by Kalina and Czeizel[40,4] showed that the *T* allele carriers of the *MTHFR* C677T polymorphism with congenital heart disease (CHD) died earlier due to myocardial infarction and the C allele carrier with a lower Hcy level may provide protection against fatal coronary artery occlusion.

To date, there have been only a few studies investigating the relationship between the genetic polymorphism and L-dopa-induced adverse effects in PD patients, but their results were in consistent[18-20,41,42]. In this study, genetic variation analysis of the *MTHFR* C677T polymorphism in PD patients with and without levodopa-induced adverse effects revealed the genetic marker was not associated with the risk to develop adverse effects. This finding may advise that, at least among Chinese, the *MTHFR* C677T polymorphism is unlikely to be a useful predictor for the occurrence of adverse effects in levodopa-treated PD patients.

In conclusion, the present case-control study demonstrated that *MTHFR* C677T polymorphism did not confer genetic susceptibility contributing the risk of PD among ethnic Chinese living in Taiwan, but the genetic polymorphism could influence onset age of PD. There may be other genetic risks or more than one susceptible gene contributing to the pathogenesis of PD in Chinese populations. Analysis of the underlying mechanism is required for prophylactic and therapeutic strategies.

REFERENCES

1. Foltynie T, Sawcer S, Brayne C, et al. The genetic basis of Parkinson's disease. J Neurol Neurosurg Psychiatry 2000; 73:363-70.
2. Duan W, Ladenheim B, Cutler RG, et al. Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. J Neurochem 2002;80:101-10.
3. Kruman II, Culmsee C, Chan P, et al. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci 2000; 20:6920-6.
4. Bots ML, Launer LJ, Lindemans J, et al. Homocysteine, atherosclerosis and prevalent cardiovascular disease in the elderly: the Rotherdam Study. J Intern Med 1997;242:339-47.
5. Bostom AG, Rosenberg IH, Silbershatz H, et al. Nonfasting plasma total homocysteine levels and stroke incidence in elderly person: the Framingham study. Ann Intern Med 1999;131:352-5.
6. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-83.
7. Den Heijer T, Vermeer SE, Clarke R, et al. Plasma homo-

cysteine and brain atrophy on MRI of non-dementia elderly. Brain 2003;126(Pt 1):170-5.
8. Tiemeier H, van Tuijl HR, Hofman A, et al. Vitamin B12, folate and homocysteine in depression: the Rotherdam Study. Am J Psychiatry 2002;159:2099-101.
9. Prins ND, Den Heijer T, Hofman A, et al. Homocysteine and cognitive function in the elderly: the Rotherdam Study. Neurology 2002;59:1375-80.
10. Kuhn W, Roebroek R, Blom H, et al. Elevate plasma levels of homocysteine in Parkinson's disease. Eur Neurol 1998;40:225-7.
11. Blandini F, Facellu R, Martignoni E, et al. Plasma homocysteine and L-dopa metabolism in patients with Parkinson's disease. Clin Chem 2001;47:1102-4.
12. Yasui K, Nakaso K, KowaH, et al. Levodopa-induced hyperhomocysteinaemia in Parkinson's disease. Acta Neurol Scand 2003;108:66-7.
13. O'Suilleabhain PE, Bottiglieri T, Dewey RB, et al. Modest increase in plasma homocysteine follows levodopa initiation in Parkinson's disease. Mov Disord 2004;19:1403-8
14. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci 2003;26:137-46.
15. Woitalla D, Kuhn W, Muller T. *MTHFR* 677T polymorphism, folic acid and hyperhomocysteinemia in levodopa treated patients with Parkinson's disease. J Neural Trans 2004;68:15-20.
16. Yasui K, Kowa H, Nakaso K, et al. Plasma homocysteine and *MTHFR* C677T genotype in levodopa-treated patients with PD. Neurology 2000;55:437-40.
17. de Lau LML, Koudstaal PJ, van Meurs JB, et al. Methylenetetrahydrofolate reductase C677T genotype and PD. Ann Neurol 2005;57:927-30.
18. Kaiser R, Hofer A, Grapengiesser A, et al. L-dopa-induced adverse effects in PD and dopamine transporter gene polymorphism. Neurology 2003;60:1750-5.
19. Goetz CG, Burke PF, Leurgans S, et al. Genetic variation analysis in Parkinons's disease patients with and without hallucinations: case-control study. Arch Neurol 2001;58: 209-13.
20. Wang J, Liu ZL, Chen B. Association study of dopamine D2, D3 receptor gene polymorphism with motor fluctuation in PD. Neurology 2001;56:1757-9.
21. Hughes AJ, Daniel SE, Kilford L, et al. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 case. J Neurol Neurosurg Psychiatry 1995;55:181-4.
22. Hagell P, Widner H. Clinical rating of dyskinesias in Parkinson's disease: use and reliability of a new rating scale. Mov Disord 1999;14:448-55.
23. Defer GL, Widner H, Marie RM, et al. Core assessment program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD). Mov Disord 1999;14:572-84.
24. Poewe H. Clinical aspects of motor fluctuations in Parkinson's disease. Neurology 1994;44:6-9
25. Lin JJ, Chang DC, Yueh KC. Improvement of levodopa induced psychosis and dyskinesia in Parkinson's disease patients with low dose olanzapine: an open-label trial. Acta Neurol Taiwan 2002;11:128-34.
26. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111-3.
27. Harmon DL, Ramsbottom D, Whitehead AS, et al. The thermolabile variant of 5,10-methylenetetrahydrofolate reductase is not associated with Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;62:671.
28. Müllner M, Kolsch H, Linnebank M. Methylenetetrahydrofolate reductase in Parkinson's disease. Ann Neurol 2005;58:972-3.
29. Religa D, Czyzewskin K, Styczynska M, et al. Hyperhomocysteinemia and methylenetetrahydrofolate reductase polymorphism in patients with Parkinson's disease. Neurosci Lett 2006;404:56-60.
30. Brown KS, Kluijtmans LA, Young IS. The 5,10-methylenetetrahydrofolate reductase C677T polymorphism interacts with smoking to increase homocysteine. Atherosclerosis 2004;174:315-22.
31. Husemoen LL, Thomsen TF, Fenger M, et al. Effect of lifestyle factors on plasma total homocysteine concentrations in relation to *MTHFR* (C677T) genotype. Eur J Clin Nutr 2004;58:1142-50.
32. Refsum HM, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3-32.
33. Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an

elderly population. JAMA 1993;270:2693-8.

34. Selhub J, Jacques PF, Rosenberg IH, et al. Serum total homocysteine concentrations in the Third National Health and Nutrition Examination Survey: 1991-1994 population references ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med 1999;131:331-9.
35. Chen H, Zhang SM, Schwarzchild MA, et al. Folate intake and risk of Parkinson's disease. Am J Epidemiol 2004;160: 368-75.
36. de Lau LML, Koudstaal PJ, Witteman JCM, et al. Dietary folate, vitamin B12, and vitamin B6 and the risk of Parkinson's disease. Neurology 2006;67:315-8.
37. Lin JJ, Yeuh KC, Chang DC, et al. The homozygote 10/10-copy genotype of variable number tandem repeat dopamine transporter gene confers protection against Parkinson's disease. J Neurol Sci 2003;209:87-92.
38. Lin JJ, Yueh KC, Chang DC, et al. The homozygote AA genotype of the α1- antichymotrypsin gene (ACT-AA) may confer protection against early-onset Parkinson's disease for female. Parkinsonism Rel Disord 2004;10:468-73.
39. Kidd KK. Associations of disease with genetic markers: deja vu all over again. Am J Med Genet (Neuropsychiatr Genet) 1993;48:71-3.
40. Kalina A, Czeizel AE. The methylenetetrahydrofolate reductase gene polymorphism (C677T) is associated with increased cardiovascular mortality in Hungary. Int J Cardiol 2004;97:333-4.
41. Makoff AJ, Graham JM, Arranz MJ, et al. Association study of dopamine receptor gene polymorphism with drug induced hallucination in patients with idiopathic Parkinson's disease. Pharmacogenetics 2000;10:43-8.
42. Wang J, Zhao C, Chen B, et al. Polymorphisms of dopamine receptor and transporter genes and hallucinations in Parkinson's disease. Neurosci Lett 2004;355:193-6.