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ORIGINAL ARTICLE

Analysis of the rs13306560 functional variant in the promoter region of the MTHFR gene in sporadic Parkinson´s disease

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Abstract

OBJECTIVE: Among susceptibility genes for Sporadic Parkinson´s Disease (SPD), the MTHFR gene has been suggested as candidate. The A allele of the functional variant rs13306560 in its promoter region has been liked to decreased transactivation capacity. Therefore, we sought to determine a possible association of the rs13306560 and SPD.

METHODS: In total, 237 individuals were genotyped, 113 patients with SPD diagnosed according to the Queen Square Brain Bank criteria and 124 neurologically healthy controls. Genotyping was performed using TaqMan probes for the rs13306560 and real-time PCR.

RESULTS: The A allelle was associated to protection in SPD, under the dominant model, (OR=0.22, C.I.=[0.048–1.080], p=0.04), nevertheless, after logistic regression analysis with adjustment for gender, resulted only in a trend (Exp (β)=0.211, [I.C. 95.0%, 0.042–1.057], p=0.058).

CONCLUSION: Although further studies are needed, our data suggest an important role of the MTHFR gene variants in the fine-tuning regulation of one-carbon metabolism in the brain.

Abbreviations:

Hcy	- Homocysteine	PCR	- Polymerase Chain Reaction
HWE	- Hardy-Weinberg Equilibrium	SPD	- Sporadic Parkinson´s Disease
MTHFR	- Methylenetetrahydrofolate reductase	SNCA	- Alpha synuclein
Met	- Methionine	SAM	- S-adenosylmethionine

INTRODUCTION

Among neurodegenerative disorders Parkinson´s disease (PD) is the second most prevalent cause of physical disability and affects life quality of about 1–2% of people older than 65 years (Wickremaratchi *et al.* 2009). Sporadic cases of PD (SPD) with late-onset presentation are more frequent than familial ones, or than those with early-onset beginning of the disease. Therefore in order to gain insights in to the genetic predisposition factors involved in SPD; delineation of such phenotypes is of utmost importance. Some variants in the PARKIN (PARK2)(Garcia *et al.* 2014) and SNCA (PARK1) genes have been linked to early-onset and familial PD respectively(Wang *et al.* 2013). Nevertheless, variants in other genes that are not exclusively related to PD may also be part of the genetic susceptibility of this complex neurodegenerative disease; as is the case of MTHFR gene that was recently proposed as candidate gene for PD susceptibility by two independent meta-analyses (Wu *et al.* 2013; Zhu *et al.* 2013). The methylenetetrahydrofolate reductase enzyme (MTHFR) (EC 1.5.1.20) transforms 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine re-methylation to methionine (Met); an essential amino acid obtained from diet.

The most widely studied genetic factor affecting MTHFR and one carbon metabolism is the TT genotype of the functional rs1801133 variant in this gene, which shows reduced activity (30%), and therefore elevated plasma homocysteine (Hcy) levels, which may in turn be toxic (Frosst *et al.* 1995). Nevertheless, optimal folate and vitamin B12 intake can counteract the effected the genotype (Gueant *et al.* 2007).

Recently, Yuan and colleagues found association of the TT genotype of rs1801133 as protective factor for PD (OR=0.78, 95% C.I.=0.65–0.93 p<0.01)(Yuan *et al.* 2016). Interestingly, our group found that TT genotype is more prevalent in control individuals than PD patients (Garcia *et al.* 2015), which is concordant with the findings of Yuan and colleagues; in addition another study found this same genotype (CC of rs1801133) related to earlier age at onset of PD (Vallelunga *et al.* 2014). Conversely, these findings suggest that genotype TT with reduced activity of MTHFR may be protective for PD. Thus we aimed to search for association of the rs13306560 variant of MTHFR and PD; this variant is located in the promoter region of the gene; the A allele is related to reduced activation of the promoter than the G allele (Perez-Razo *et al.* 2015), therefore decreased expression of MTHFR is expected with having the homozygous AA genotype.

MATERIALS AND METHODS

Patients and controls

We performed a case-control study that included 237 individuals, 113 SPD patients and 124 neurologically healthy controls. Institutional Committees approved the study and informed written consent was obtained from participants. Patients were recruited from February 2009-June 2010, from four different tertiary-care level hospitals in Mexico (Neurology Departments from Centro Médico Nacional "20 de Noviembre"-ISSSTE, Centro Médico Nacional Siglo XXI-IMSS, Instituto de Ciencias Médicas y de la Nutrición "Salvador Zubirán", Mexico City; and División de Genética, Centro de Investigación Biomédica de Occidente-IMSS, Jalisco, Mexico). Diagnosis was performed according to Queen Square Brain Bank criteria(Hughes *et al.* 2002). Cognitive impairment was assessed using the Folstein Mini Mental State Examination Test. Controls were healthy donors or patient´s spouses that agreed to participate in an additional neurological evaluation. All participants were Mexican-mestizos (Most present-day Mexicans) (Rubi-Castellanos *et al.* 2009) in order to have groups ethnically matched and without family history of neurodegenerative disorders to assure recruitment of sporadic cases.

DNA isolation and Genotyping

Isolation of DNA was carried out from peripheral blood samples by the DTAB/CTAB method (Gustincich *et al.* 1991). Genotyping was performed by real time PCR using TaqMan probes (Hydrolysis probes) using the C__30914969_10 (Applied Biosystems, Foster City, CA, USA) NM_001256959.1. Real-time PCR was performed on a LightCycler 480 II (Roche Diagnostics GmbH, Switzerland); PCR reactions were done according to manufacturer´s instructions. The samples were previously screened for common variants in six PARK genes, among we can mention A30P of *SNCA* and G2019S and G2385R of *LRRK2*; the prevalence of DNA changes was low(Garcia *et al.* 2014).

Statistical Analysis

Statistical analysis was performed using SPSS software v. 18.0 (SPSS Inc., Chicago, IL, USA) for $\chi 2$ test and logistic regression. Hardy-Weinberg equilibrium (HWE) was estimated in both groups using $\chi 2$ test available in the following link (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl [03/06/2016]).

RESULTS

In total 237 individuals were genotyped, 113 patients with SPD and 124 healthy individuals that constituted the control group; the healthy individuals were selected to be older than patients. Hardy-Weinberg equilibrium test showed that alleles were distributed according to expected frequencies in both groups. Distribution of genotypes between groups and association test are shown in Table 1. The median values of age were 66 in the control group and 62 in the PD group (p<0.001). After logistic regression adjusted by gender the A allele under the dominant model showed Exp (B)=0.211,

[I.C. 95.0%, 0.042–1.057], p=0.058, whereas differences in gender remained significant Exp (B)=5.9 [I.C. 95.0%, Exp (B)=3.24–11.024], p<0.001.

Tab. 1. Association test of the rs13306560 variant of MTHFR gene and PD.

Category	Patients	Controls	*p*-value
Gender	19 females, 94 males	68 females, 56 males	<0.001
Age *	62 (29-94)	66 (40-88)	<0.001
Smoking	65 (no), 48 (yes)	66 (no), 56 (yes)	>0.05
Genotypes	AA = 0 AG = 2 GG = 111	AA = 1 AG = 9 GG = 114	patients, p>0.05 HWE controls, p>0.05 X2 Association + OR=0.22, C.I.=[0.048-1.080], p=0.04

*(median, minimum-maximum) C.I. = Confidence Interval 95%, Odds ratio = OR, + dominant model

DISCUSSION

Several studies have searched for association of the TT genotype of rs1801133 in the MTHFR gene (formerly C677T polymorphism) and susceptibility to PD, based on the finding that this genotype has a reduced enzymatic activity (30%) and therefore elevated levels of Hcy (Zhu *et al.* 2013) that may hasten dopaminergic cell death through oxidative stress and excitotoxicity (Duan *et al.* 2002; Miller 2002). In addition, variants in the MTHFR gene may also influence response to treatment, since impaired of transmethylation potential has been detected in hyperhomocysteinemic L-Dopa-treated PD patients (De Bonis *et al.* 2010). Nevertheless, some of the caveats of such studies are: 1) Under optimal folate and B12 vitamin consumption, the effect of that genotype in homocysteine elevation is null (Gueant-Rodriguez *et al.* 2006), 2) Most studies do not measure plasma Hcy or MTHFR enzymatic activity to confirm the effect of genotypes. 3) The one-carbon metabolism in the brain seems to have a fine-tuning regulation for folate absorption, DNA Methylation and antioxidant production (Kronenberg *et al.* 2009). Thus, it could be inferred that high levels of homocysteine in the brain should not be assumed from only genotype data because other mechanisms different from Hcy toxicity as cause of neurodegeneration ought to be explored (Garcia *et al.* 2015). Such mechanisms may include altered DNA methylation and impaired antioxidant production and abnormal metabolite production (Perez-Razo *et al.* 2015).

Interestingly, implications of high levels of MTHFR are largely unknown. In an original work from Celtikci and colleagues a transgenic mouse model overexpressing MTHFR was produced (Celtikci *et al.* 2008). The transgenic mice (Tg-MTHFR mouse) did not show changes in folate distribution in plasma, where most of the folate is in the form of 5-methylTHF, the MTHFR product. On the other hand, it had an increase of methionine in brain, an increase of glutathione in liver, and a decrease of cysteine in duodenum (Celtikci *et al.* 2008), showing that high levels of MTHFR cause altered distributions of folate and thiols in a tissue-specific manner. Among those alterations, high methionine levels in the brain are of particular interest; these may be generated by the enhanced homocysteine re-methylation due to the increased MTHFR. Two primary pathways participate in the methionine metabolism: transmethylation and transsulfuration, the first generates S-adenosylmethionine (SAM), an important methyl donor for the methylation of lipids, proteins, and nucleotides, whereas the transsulfuration pathway implies the synthesis of glutathione from methionine, SAM activates cystathionine-β-synthase the first enzyme in the transsulfuration pathway. It has been questioned the existence of a complete transsulfuration pathway in the brain (Celtikci *et al.* 2008; Vitvitsky *et al.* 2006), because methionine accumulates in the Tg-MTHFR mouse which is concordant with the low baseline levels of cysteine found in its brain (Celtikci *et al.* 2008). Nevertheless, it was demonstrated that a functional transsulfuration pathway exists in human neurons, astrocytes and mouse brain organotypic preparations by incubating the cells with [35S] methionine and measuring radiolabeled glutathione. Interestingly 40% of glutathione was depleted due to cystathionine-γ-lyase (CγL) inhibition and this correlated with reduced cell viability under oxidative stress (Vitvitsky *et al.* 2006).

Herein we found a trend of the A allele of the rs13306560 variant of the MTHFR to be protective against MTHFR, A allele is thought to generate less MTHFR RNA due to reduced transactivation potential compared to its counterpart the G allele(Perez-Razo *et al.* 2015). Although more evidence is needed to confirm our findings, it is possible to hypothesize that reduced activity (TT genotype of rs1801133) or concentration of MTHFR enzyme (AA genotype of rs13306560) may be protective in the brain when methionine levels are high, transsulfuration pathway is impaired and glutathione generation is compromised. This particular scenario may imply the inhibition of cystathionine-γ-lyase which is expressed in low levels in the brain and is sensitive to oxidative stress (Diwakar & Ravindranath 2007). Together our data suggest that in order to avoid neurodegeneration, a fine-tuning homeostasis of the one-carbon metabolism should prevail within the brain.

LIMITATIONS OF THE STUDY

A considerable proportion of patients were treated with L-dopa among other anti-parkinsonic drugs, therefore plasma homocysteine levels would reflect effects of

treatment instead of genotype-phenotype correlation; therefore, these parameters could not be explored. Since the novel rs13306560 variant is scarce in our population a larger group of study is required to confirm our findings. Serum samples were unavailable therefore MTHFR concentrations were not measured for genotype-phenotype correlations.

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Disclosure statement

No potential conflicts of interest were reported by the authors.

REFERENCES

1 Celtikci B, Leclerc D, Lawrance AK, Deng L, Friedman HC, Krupenko NI, Krupenko SA, Melnyk S, *et al.* (2008). Altered expression of methylenetetrahydrofolate reductase modifies response to methotrexate in mice. Pharmacogenet Genomics **18**: 577–589.
2 De Bonis ML, Tessitore A, Pellecchia MT, Longo K, Salvatore A, Russo A, Ingrosso D, Zappia V, *et al.* (2010). Impaired transmethylation potential in Parkinson's disease patients treated with L-Dopa. Neurosci Lett **468**: 287–291.
3 Diwakar L, Ravindranath V (2007). Inhibition of cystathionine-gamma-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. Neurochem Int **50**: 418–426.
4 Duan W, Ladenheim B, Cutler RG, Kruman, Ii, Cadet JL, Mattson MP (2002). Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. J Neurochem **80**: 101–110.
5 Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, Den Heijer M, *et al.* (1995). A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet **10**: 111–113.
6 Garcia S, Coral-Vazquez R, Gallegos-Arreola MP, Montes-Almanza LA, Canto P, Garcia-Martinez FA, Chavira-Hernandez G, Palma-Flores C, *et al.* (2015). Association of the rs1801133 variant in the MTHFR gene and sporadic Parkinson's disease. Folia Neuropathol **53**: 24–28.
7 Garcia S, Lopez-Hernandez LB, Suarez-Cuenca JA, Solano-Rojas M, Gallegos-Arreola MP, Gama-Moreno O, Valdez-Anguiano P, Canto P, *et al.* (2014). Low prevalence of most frequent pathogenic variants of six PARK genes in sporadic Parkinson's disease. Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences **52**: 22–29.
8 Gueant-Rodriguez RM, Gueant JL, Debard R, Thirion S, Hong LX, Bronowicki JP, Namour F, Chabi NW, *et al.* (2006). Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. The American journal of clinical nutrition **83**: 701–707.
9 Gueant JL, Chabi NW, Gueant-Rodriguez RM, Mutchinick OM, Debard R, Payet C, Lu X, Villaume C, *et al.* (2007). Environmental influence on the worldwide prevalence of a 776C->G variant in the transcobalamin gene (TCN2). Journal of medical genetics **44**: 363–367.
10 Gustincich S, Manfioletti G, Del Sal G, Schneider C, Carninci P (1991). A fast method for high-quality genomic DNA extraction from whole human blood. Biotechniques **11**: 298–300, 302.
11 Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ (2002). The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain **125**: 861–870.
12 Kronenberg G, Colla M, Endres M (2009). Folic acid, neurodegenerative and neuropsychiatric disease. Curr Mol Med **9**: 315–323.
13 Miller JW (2002). Homocysteine, folate deficiency, and Parkinson's disease. Nutr Rev **60**: 410–413.
14 Perez-Razo JC, Cano-Martinez LJ, Vargas Alarcon G, Canizales-Quinteros S, Martinez-Rodriguez N, Canto P, Roque-Ramirez B, Palma-Flores C, *et al.* (2015). Functional Polymorphism rs13306560 of the MTHFR Gene Is Associated With Essential Hypertension in a Mexican-Mestizo Population. Circ Cardiovasc Genet **8**: 603–609.
15 Rubi-Castellanos R, Martinez-Cortes G, Munoz-Valle JF, Gonzalez-Martin A, Cerda-Flores RM, Anaya-Palafox M, Rangel-Villalobos H (2009). Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. Am J Phys Anthropol **139**: 284–294.
16 Vallelunga A, Pegoraro V, Pilleri M, Biundo R, De Iuliis A, Marchetti M, Facchini S, Formento Dojot P, *et al.* (2014). The MTHFR C677T polymorphism modifies age at onset in Parkinson's disease. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology **35**: 73–77.
17 Vitvitsky V, Thomas M, Ghorpade A, Gendelman HE, Banerjee R (2006). A functional transsulfuration pathway in the brain links to glutathione homeostasis. J Biol Chem **281**: 35785–35793.
18 Wang L, Nuytemans K, Bademci G, Jauregui C, Martin ER, Scott WK, Vance JM, Zuchner S (2013). High-resolution survey in familial Parkinson disease genes reveals multiple independent copy number variation events in PARK2. Hum Mutat **34**: 1071–1074.
19 Wickremaratchi MM, Perera D, O'loghlen C, Sastry D, Morgan E, Jones A, Edwards P, Robertson NP, *et al.* (2009). Prevalence and age of onset of Parkinson's disease in Cardiff: a community based cross sectional study and meta-analysis. J Neurol Neurosurg Psychiatry **80**: 805–807.
20 Wu YL, Ding XX, Sun YH, Yang HY, Sun L (2013). Methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms and susceptibility to Parkinson's disease: a meta-analysis. Journal of the neurological sciences **335**: 14–21.
21 Yuan L, Song Z, Deng X, Xiong W, Yang Z, Deng H (2016). Association of the MTHFR rs1801131 and rs1801133 variants in sporadic Parkinson's disease patients. Neurosci Lett **616**: 26–31.
22 Zhu ZG, Ai QL, Wang WM, Xiao ZC (2013). Meta-analysis supports association of a functional SNP (rs1801133) in the MTHFR gene with Parkinson's disease. Gene **531**: 78–83.