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Association of the rs1801133 variant in the *MTHFR* gene and sporadic Parkinson's disease

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Abstract

The MTHFR gene has been reported as a susceptibility locus for sporadic Parkinson's disease (sPD). The functional variant rs1801133 has been linked to hyperhomocysteinemia and dopaminergic cell death. Among different populations, Mexican-Mestizos (most present-day Mexicans) have the highest frequency of this variant. Therefore, we sought to determine a possible association of rs1801133 with SPD. In total, 356 individuals were included: 140 patients with PD, diagnosed according to the Queen Square Brain Bank criteria, and 216 neurologically healthy controls. Genotyping was performed using TaqMan probes for rs1801133 and real-time PCR. Logistic regression analysis with adjustment for smoking and gender was used to test for an association between genotype and SPD. The CC genotype was associated with SPD; exp(β) = 2.06; 95% CI: 1.101-3.873, p = 0.024. No association with age at onset, cognitive impairment or gender was found in our study group. Our data suggest an important role of MTHFR gene variants in SPD.

Key words: *rs1801133, MTHFR, Parkinson's disease, common variants, C677T, Mexico.*

Introduction

Parkinson's disease (PD) is a multifactorial neurodegenerative disease that affects about 1-2% of people older than 65 years [23], sporadic cases (sPD) being more frequent than familial ones. Among genetic factors influencing PD, rare variants in *PARK* genes such as *LRRK2* (PARK8) and *SNCA* (PARK1) [2,5] are known to play a major role in PD pathogenesis (rare variant common disease hypothesis) [3,14]. However, it is possible that common variants in other genes account for part of the unrevealed heritability of PD (the common variant common disease hypothesis) [16]. In this regard, the *MTHFR* gene has been

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recently proposed as a candidate risk gene for PD by two independent meta-analyses [24,25].

The enzyme MTHFR (EC 1.5.1.20) catalyzes the transformation of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. The T allele of the functional rs1801133 variant in this gene generates a thermolabile enzyme with reduced activity. The homozygous state of this variant (TT) has been linked to elevated plasma homocysteine (Hcy) levels [9], although optimal folate and vitamin B_{12} intake can counteract the effect of genotype [12]. Elevated Hcy may hasten dopaminergic cell death through oxidative stress and excitotoxicity [7,18].

Variants in the *MTHFR* gene may also influence the response to treatment, since impaired transmethylation potential has been detected in hyperhomocysteinemic L-dopa-treated PD patients [6].

Among different populations, Mexican-Mestizos (most present-day Mexicans) have the highest frequency of the T allele [20]; therefore we sought to determine whether rs1801133 is associated with SPD in our population.

Material and methods

Patients and controls

We conducted a case-control study that included 140 SPD patients and 216 neurologically healthy controls. Institutional Committees approved the study and informed written consent was obtained from participants. Patients were recruited from February 2009 to June 2010, from four 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 [15]. The threshold for early-onset Parkinson's disease (EOPD) was considered as onset earlier than 40 years old. Cognitive impairment was assessed using the Folstein Mini Mental State Examination Test. We did not measure plasma Hcy levels because most PD patients could show elevated levels derived from pharmacological therapy with L-dopa. Controls were healthy blood bank donors or patients' spouses who agreed to participate in an additional neurological evaluation; they were Mexican-Mestizos without family history of neurodegenerative disorders.

DNA isolation and genotyping

DNA was extracted from peripheral blood samples by the DTAB CTAB method [13]. Genotyping was performed by real-time PCR using TaqMan probes (Hydrolysis probes) using the C_1202883_20 assay (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed on a LightCycler 480 II (Roche Diagnostics GmbH, Switzerland); PCR reactions were conducted according to the manufacturer's instructions. Random samples were confirmed by high resolution melting curves (Fig. 1). The samples were previously screened for common variants in six *PARK* genes, including A30P of *SNCA* and G2019S and G2385R of *LRRK2*; the prevalence of DNA changes was low [10].

Statistical analysis

Statistical analysis was performed using SPSS software v. 18.0 (SPSS Inc., Chicago, IL, USA) for the χ^2 test, logistic regression and ANOVA. Hardy-Weinberg equilibrium (HWE) was estimated in both groups using the χ^2 test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl [20/08/2013]). Statistical power was calculated *a posteriori* using Quanto Software Version 1.2.

Results

In total, 356 individuals were genotyped, 140 patients with SPD (95 males and 45 females, aged:

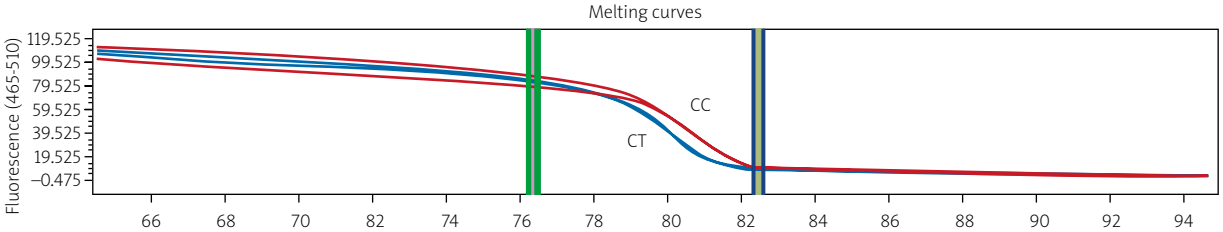

Fig. 1. Melting curves for the MTHFR rs1801133 showing CC and CT genotypes.

Table I. Genotype frequencies of rs1801133 in cases and controls

Genotypes	Controls n = 216	Patients n = 140	OR (95% CI)	p-value*
CC	37 (0.17)	38 (0.27)	2.02 (1.081-3.779)	0.026
TC	118 (0.55)	71 (0.51)		
TT	61 (0.28)	31 (0.22)		

65.46 ± 11.5 [mean ± standard deviation] years old), age at onset 58 ± 13.66), whereas 216 healthy individuals constituted the control group (140 males and 76 females, aged: 63.68 ± 8.8 years old). Hardy-Weinberg equilibrium test showed that alleles were distributed according to expected frequencies in both groups (corrected p-values, controls p = 0.13, cases p = 1.00). Distribution of genotypes between groups is shown in Table I.

The association test showed that the C allele was associated with PD only under the recessive model (OR = 2.02, CI: 1.08-3.77, p = 0.02); after logistic regression for known confounding factors, the association remained significant (exp(β) = 2.06, CI: 1.101-3.873, p = 0.024). There was no association of any allele and cognitive impairment (p = 0.33). One-way ANOVA showed no differences between genotypes and age at onset (p > 0.05). Additionally, when age at onset was categorized (EOPD), the χ^2 test did not show an association of any allele or genotype and EOPD (p > 0.05) (TT group n = 31).

Discussion

In contrast to the hypothesis that the TT genotype of rs1801133 in the *MTHFR* gene leads to elevated levels of Hcy, cell death and therefore a higher risk of neurodegeneration [25], our data suggest that the CC genotype is associated with PD. This may be explained by the fact that the TT genotype has the greatest influence on Hcy levels in populations with low folate and high B_{12} vitamin plasma concentration such as Africans but not Mexican-Mestizos, in whom folate and B_{12} levels were high and moderate respectively [11]. Thus the TT genotype does not always imply high Hcy levels; in fact, a protective effect of the TT genotype against preeclampsia was reported in Maya-Mestizo women [1].

Others have also suggested that homozygosis of the T allele may confer a survival advantage in populations with sufficient dietary folate consumption [11,17,19]. Therefore, it is expected that in some populations such as Mexican-Mestizos, the T allele even when linked to an impaired biochemical function (elevated Hcy) may represent the wild-type allele, since an advantage may fix an allele within a population under particular environmental conditions. A presumptive advantage may exist, as described previously in an intervention where folate sources and dosages where controlled [4]. In contrast to its counterpart, the TT genotype showed only a slight decrease in global DNA methylation after folate depletion; conversely, under low folate basal levels, the TT genotype has shown significantly diminished global DNA methylation [8]. Thus, the finding that the derived CC genotype has the greatest decrease in DNA methylation after folate depletion may represent a different mechanism linked to neurodegeneration, besides the known effect of hyperhomocysteinemia on neuronal cytotoxicity [4].

The ancestral C allele may be considered the risk variant for PD in the Mexican-Mestizo population. Although it is speculative, hypomethylation as observed with the CC genotype under folate depletion may potentially impact epigenetic regulation of other genes such as *LRRK2* and *SNCA* [21]. SNCA protein is also involved in the arrest of DNMT1 (a major element in epigenetic regulation) in post-mortem brains of PD patients, worsening in this way the hypomethylation phenomenon [21]. Interestingly, another study found this same genotype (CC of rs1801133) related to earlier age at onset of PD [22]. Our data do not replicate the observation, probably because in our group patients with EOPD were uncommon. Larger studies documenting age at onset and MTHFR genotype may confirm this finding. To our knowledge this is the first report on association of the CC genotype of MTHFR and SPD. Other studies in different populations with larger samples may add support to our hypothesis in which convergent pathways between common and rare variants may potentially affect complex neurodegenerative disorders such as PD.

Limitations of the study

Some of the limitations of the present study were that since a considerable proportion of patients were treated with L-dopa among other anti-parkinsonian drugs, homocysteine levels or global methylation were not measured and therefore the genotype-phenotype correlation could not be explored to support our hypothesis. The presence of essential hypertension could not be assessed accurately, since the study was not intended to do so. The statistical power reached was 72% (lower than the expected 80%).

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Disclosure

Authors report no conflict of interest.

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