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Cholesteryl ester transfer protein (CETP) I405V polymorphism in Italian memory complainers

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ABSTRACT

Recent studies have provided evidence that a functional single-nucleotide polymorphism (SNP) at the codon 405 (isoleucine to valine I405V; SNP rs5882) of exon 14 of cholesteryl ester transfer protein gene (CETP) is associated with lower risk of incident dementia. To analyze a possible implication for CETP I405V polymorphism as a genetic factor for dementia and possible effects on memory tasks, whether or not such effects are dependent on the Apolipoprotein E (ApoE) ε4 allele, we replicated this association study on our dataset. The study group consisted of 282 Italian subjects: 121 patients self-referred to the Department of Neurology, University of Florence, for everyday memory deficits, and a group of 161 non-symptomatic subjects. We performed the analysis of the CETP I405V polymorphism by using the high resolution melting analysis (HRMA). All patients went through an extensive neuropsychological battery consisting of global measurements and tasks exploring short- and long-term memory. The study protocol was approved by the local ethics committee and informed consent for genetic screening was obtained from study participants, or, where appropriate, a relative or legal representative. We found a significant different distribution of the CETP genetic variant polymorphism in the total group of patients with respect to controls. Analyzing the correlation with neuropsychological performance we found that VV carriers showed a higher score on MMSE and on a long term memory test. These reports suggest that CETP I405V can be correlated with memory and memory related disease. Larger studies are needed to confirm this recent hypothesis.

KEYWORDS: Mild Cognitive Impairment, CETP, apolipoprotein E, genetic variation

INTRODUCTION

Mild Cognitive Impairment (MCI) is considered to be a transition state between normal cognition and dementia. The subtype of MCI is highly heterogeneous in terms of etiology, presentation, and prognosis. Patients with the amnestic subtype of MCI (aMCI) are at a high risk of progression to Alzheimer disease (AD), representing the prodromal stage of AD. aMCI subjects could be an interesting population to study physiological memory processes because complaints and deficits are isolated to memory [1]. Considering the importance of gene regulation and protein function for development and plasticity of the CNS, many genes can be considered to play a role in memory abilities and other cognitive processes [2].

The cholesteryl ester transfer protein gene (CETP) is a glycoprotein that mediates the transfer of cholesteryl esters from high density lipoprotein

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(HDL) to very low-density lipoprotein (VLDL) in exchange for triacylglycerols, playing a central role in reverse cholesterol transport [3]. The CETP gene is located on chromosome 16 and presents a biallelic functional polymorphism, rs5882, on exon 14 (I405V) [4]. V Single Nucleotide Polymorphism (SNP) is associated with reduced levels of CETP protein serum concentration and activity, corresponding increases in HDL levels and HDL/LDL particle sizes [4, 5].

Contrasting results on the role of V SNP regarding the implication of CETP polymorphism in higher longevity [6], dementia risk and memory decline [7] have been published. In the light of these findings, we analyzed the correlation between the CETP I405V variant and memory deficits, evaluating the distribution of the I405V polymorphism in individuals reporting memory complaints versus healthy subjects.

MATERIALS AND METHODS

The studied group consisted of 282 Italian subjects: 121 consecutive patients (83 females and 38 males, age at onset mean ± SD 64.5±7.4) self-referred to the Department of Neurology, University of Florence, for everyday memory deficits, and a group of 161 non-symptomatic subjects (67 males, 94 females, mean age 59.1±16.5 years) as controls.

All subjects received a thorough clinical history, neurological and neuropsychological examinations, laboratory tests, MRI and CETP and ApoE polymorphisms. The local ethical committee approved the protocol of the study and written consent for genetic screening was obtained from all subjects. We excluded individuals with medical conditions or history of diseases possibly affecting brain structure or function, i.e., stroke, insulin-dependent diabetes, psychiatric diseases, head trauma, any neurodegenerative disease, MRI evidence of hydrocephalus, intracranial mass, infarcts and moderate to severe non-specific white matter disease. We used Petersen's validated criteria for diagnosis of aMCI [8], while we considered those individuals who reported memory deficits but showed intact cognitive performance, according to appropriate normative values, and who were free of alterations on laboratory and neuroimaging investigations as affected by Subjective Memory Complaints (SMC) [9].

According to test results, the group of patients was divided into 45 subjects diagnosed as MCI (36 females and 9 males, age at onset mean ± SD 66.2±7.2) and 76 SMC (47 females and 29 males, age at onset mean ± SD 61.9±8.27). We evaluated the presence of depression as present or absent from the clinical history and in terms of the score on the Hamilton depression rating scale (HDRS) [10]. Finally, as a measurement of the functional status of a person, we used the Instrumental Activities of Daily Living (IADLs) score [11]. Cognitive functions were evaluated using an extensive neuropsychological battery [12] consisting of global measurements (Mini-Mental State Examination: MMSE), tasks exploring verbal and spatial short- and long-term memory (digit span, Corsi tapping test, five words and paired words acquisition and recall after 10 min and 24 h, short story immediate and delayed recall), language (Token Test and category fluency test) and visuo-motor functions (copying drawings). Visuo-spatial long-term memory was assessed by means of recall on the Rey-Osterrieth Complex Figure Test [13]. Everyday memory was evaluated using the Rivermead Behavioral Memory Test (RBMT) [14]. Attention/executive functions were assessed by means of the Stroop test [15], the dual task [16], the phonemic fluency test [17] and the Trail Making test; the score was expressed as time spent to carry out part B minus time spent to carry out part A (Trail Making B-A) [18]. In order to estimate premorbid verbal intelligence (pvIQ), all cases were given the TIB ("Test di Intelligenza Breve") [19]. All test scores were adjusted for each subject's age and education level. We used the Memory Assessment Clinics Questionnaire (MACQ) [20] to detect and quantify the subjective memory trouble.

DNA from subjects was extracted from peripheral blood samples using the phenol-chloroform procedure. Analysis of CETP rs5882 I405V polymorphism was performed using High Resolution Melting Analysis (HRMA) and the three genotypes were identified through sequencing (310 ABI PRISM Genetic Analyzer, Applied Biosystem) [21]. Genotyping for the

ApoE polymorphism was performed according to standard protocols [22].

Frequencies of CETP and APOE alleles and genotypes were estimated by gene counting. The data were analyzed using SPSS software (Statistical Package for Social Sciences 11 for Windows, Chicago IL, USA, SPSS Inc., 2002). To compare differences between groups, we used the Student's T Test for continuous variables and Chi-square for non-continuous ones. Odds ratio (ORs) and corresponding 95% confidence intervals (CIs) were calculated using logistic regression models adjusted for gender, APOE status and age.

RESULTS

All genotype distributions of CETP I405V were in Hardy-Weinberg equilibrium in all the studied groups.

The distribution of the CETP I405V genotypes frequencies shows a statistically significant difference in both the SMC and MCI groups compared to the group of healthy subjects (VV genotype $p = 0.0071$ in the MCI and $p < 0.0001$ in SMC) (Table1).

After stratification for ApoE ε4 status, our data show a statistically significant association with ApoE ε4 non-carriers in the patient groups (MCI and SMC) compared to the controls, thus suggesting a lack of interaction between CETP and the ApoE genes (Table 2).

Stratifying all cases by CETP genotypes, we did not find any significant difference in clinical and demographic characteristics, such as: age (at evaluation), sex, schooling and score on TIB, IADLs, HDRS and Mac Q Tot., except for the MMSE evaluation in the IV carriers (Table 3). In fact, heterozygotes for the Val allele performed

Table 1. Genotype frequency distribution of the CETP I405V polymorphism in cases and control subjects.

Genotype	SMC (%)	MCI (%)	Controls (%)
II	21 (27.6%)	14 (31.2%)	73 (45.3%)
IV	34 (44.8%)	23 (51.1%)	78 (48.5%)
VV	21 (27.6%)[a]	8 (17.7%)[b]	10 (6.2%)
Total	76	45	161

[a]Frequency of the VV genotype in MCI vs. controls: $\chi^2 = 7.24$; $p = 0.0071$; OR (95%): 4.17 (1.23-14.24).
[b]Frequency of the VV genotype in SMC vs. controls: $\chi^2 = 21{,}54$; $p < 0.0001$; OR (95%): 7.30 (2.74-19.84).

Table 2. Genotype frequency distribution of the CETP I405V polymorphism in cases and control subjects stratified by the presence of ApoE ε4.

Genotypes	APOE ε4+			APOE ε4−		
	SMC (%)	MCI (%)	Controls (%)	SMC (%)	MCI (%)	Controls (%)
II	6 (28.5%)	4 (44.4%)	10 (43.48%)	15 (26.3%)	10 (27.7%)	63 (45.6%)
IV	11 (52.3%)	4 (44.4%)	13 (56.52%)	25 (43.8%)	19 (52.7%)	65 (47.1%)
VV	4 (19.1%)	1 (11.2%)	0	17 (29.8%)[b]	7 (19.4%)[a]	10 (7.2%)
Total	21	9	23	57	36	138

[a]Frequency of the VV genotype in MCI vs. controls: $p = 0.0091$; OR (95%): 4.4 (1.18-16.72).
[b]Frequency of the VV genotype in SMC vs. controls: $p < 0.0001$; OR (95%): 7.14 (2.47-21.11).

Table 3. Correlation between the CETP I405V genotype and some clinical characteristic tasks in sample subjects.

Variables	Isoleucine Homozygote	Heterozygote	Valine Homozygote	P-value
Age (at evaluation)	66.2±7.3	69.6±7.7	65.7±9.1	n.s.
Female sex n. (%)	26 (30.9%)	39 (46.4%)	19 (22.6%)	n.s.
Schooling	10.8±4.9	10.0±4.8	12.8±5.0	n.s.
TIB	108.5±9.7	107.8±10.9	112.1±9.8	n.s.
IADL	7.8±0.4	7.7±0.9	7.7±0.8	n.s.
HRSD	27.09±4.6	26.3±3.4	25.5±3.8	n.s.
Mac Q Tot	26.8±2.6	25.4±3.0	25.7±3.3	n.s.
MMSE	28.27±2.0	27.39±2.1*	28.65±1.9*	0.02*

*Anova Test, Bonferroni's Post-Hoc
TIB: Test di Intelligenza Breve; IADL: Instrumental Activities of Daily Living; HRSD: Hamilton Rating Scale for Depression; Mac Q Tot: Memory Assessment Clinics Questionnaire; MMSE: Mini Mental State Examination
n.s.: not significant.

Table 4. Association of the CETP I405V genotype with neuropsychological tasks (expressed by mean plus standard error) in sample subjects as a function of the CETP I405V genotype.

Variables	II carriers (*n* =35)	IV carriers(57)	VV carriers (*n* = 29)	P value
Five Words 10 min recall	18.9±1.3	18.5±1.9	18.5±1.7	0.51
24-h Delayed Five Words Recall	14.5±2.9	14.4±3.4	14.8±2.2	0.85
Paired Words 10 min recall	21.9±2.5	21.0±3.9	22.9±2.2	0.05
Paired Words 10 min recall	21.3±3.3*		22.9±2.2*	0.02*
24-h Delayed Paired Words Recall	20.3±3.3	19.8±4.3	20.7±3.4	0.64
Short Story Delayed Recall	11.7±4.5	10.9±4.4	13.3±3.8	0.08
Phonemic Fluency	35.8±9.9	35.7±7.9	38.1±9.4	0.52
Trail Making B-A Test	18.0±42.5	33.0±44.4	34.7±59.7	0.31

*Independent-Sample T test.

more poorly compared to VV carriers on MMSE in a significant statistical manner (p = 0.02) (Table 3). As far as single cognitive domains are concerned, no difference was found between carriers of different genotypes. Although scores obtained on cognitive tasks tapping attention/ executive, visuo-spatial and language functions were similar between different carriers, a few tests, especially the ones related to memory, show a p-value close to statistical significance (Table 4). Thus, as suggested by the difference between VV and IV carriers on MMSE scores, we grouped homozygotes cases (VV) with respect to the others (VI+II), finding a statistically significant difference in "Paired-Words 10 min recall" (Table 4).

DISCUSSION

Our data showed a higher frequency of the Valine variant of the CETP I405V polymorphism in all our memory complaint individuals (both in MCI

and SMC) compared to controls. This data may suggest a possible role as a susceptibility factor of the V allele for memory deficits, even if some studies suggest a positive association of the V allele both on healthy longevity [6] and lower risk of developing dementia [7]. Our results seem to head in the direction of our previous findings in which the *CETP VV* genotype was much less frequent in centenarians than in younger subjects [23]. Actually, the role of CETP polymorphism in AD is not clear, some studies show a possible susceptibility role of the V allele for dementia, instead others do not find this association [24, 25]. On the other hand, our clinical results appear to suggest a positive role of the V allele in memory processes. As previously described by Barzilai *et al.* in centenarians [26], our sample shows a correlation between better performance on a global cognitive measure, such as the MMSE, and homozygosis for the V allele, supporting a causal relationship between the CETP genotype and cognitive function not only in healthy subjects but also in memory complainers-V carriers scored best on memory tasks, especially on long-term tests. Our data suggest that the CETP I405V polymorphism could be involved in memory skills, although it does not seem to be a major susceptibility factor.

Like ApoE, the CETP gene is one of the genes involved in the cholesterol shuttle [27]. Synaptic plasticity mechanisms, including those underlying long-term potentiation (LTP) and long-term depression (LTD), provide a neuronal substrate for learning and memory [28] and since brain cholesterol is important mainly in neuronal repair capacity and cell wall integrity, it may have an important role in the memory process. Thus, ApoE, a well known risk for AD, seems to have a specific effect on episodic memory and cognitive performance, neuropsychologically explored, even in normal subjects [29]. It could be argued that CETP SNP could also be relevant. Moreover, the statistically significant difference in the distribution of the VV genotype in memory complainers (MCI and SMC) compared to controls is confirmed after ApoE stratification (Table 2), thus suggesting that the results are ApoE independent.

Although a lot of studies analyzed the role of the CETP gene in correlation with memory and memory related disease, it is not yet clear in which manner and with which trend it could affect memory, both in the physiological and pathological fields. We can speculate that there is a possible interaction between different polymorphisms in the CETP gene [4] and other genes involved in cholesterol homeostasis. Further elucidation of the involved biological mechanisms may provide key insights into therapeutic and preventive intervention for age and memory related disease.

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