Aging Clin Exp Res (2014) 26:161–169
DOI 10.1007/s40520-014-0203-5

ORIGINAL ARTICLE

Glucose impairment and ghrelin gene variants are associated to cognitive dysfunction

M. Mora · M. L. Mansego · M. Serra-Prat · E. Palomera · X. Boquet · JF Chaves · M. Puig-Domingo · The Mataró Ageing Study Group

Received: 14 February 2013 / Accepted: 10 September 2013 / Published online: 12 March 2014

Abstract

Background and aims Cognitive state and brain volume have been related to body mass index, abdominal fat, waist–hip ratio, components of metabolic syndrome (MS) and ghrelin. Genetic variations within the ghrelin gene have been recently associated to MS. The aim of our study was to investigate cognitive state by Mini-Mental State Examination (MMSE) in relation to MS components (ATP-III criteria) and ghrelin gene polymorphisms in dwelling individuals aged ≥70.

Methods 280 subjects (137 men/143 women, age 77.03 ± 5.92) from the Mataró Ageing Study were included. Individuals were phenotypically characterized by anthropometric variables, lipids, glucose, blood pressure and MMSE. SNPs -501AC (rs26802), −994CT (rs26312), −604GA (rs27647), M72L (rs696217) and L90G (rs4684677) of the ghrelin gene were studied. Genotypes were determined by polymerase chain reaction and SNapshot minisequencing.

Results 22.1 % had MMSE <24. MMSE <24 was associated with age ($p < 0.001$), female gender ($p = 0.016$), low education ($p < 0.001$) and glucose impairment or diabetes ($p = 0.040$). MMSE was influenced by obesity, central obesity, MS and glucose impairment. This latter association remained significant after adjustment by gender, age, alcohol, educational level, GDS and ApoE genotype ($p = 0.009$). Ghrelin SNPs were associated to MMSE: M72L C/A genotype showed lower score than C/C ($p = 0.032$, after adjusting for confounders 0.049); L90G A/T genotype showed lower score than A/A ($p = 0.054$, after adjusting 0.005). MMSE <24 was associated to L90G (39.1 % in A/T genotype vs 19.3 % in A/A, $p = 0.026$, after adjusting for confounders $p = 0.002$, OR 6.18 CI 1.93–21.75).

Conclusions Glucose impairment and L90G Ghrelin gene variant influence cognitive function in old dwelling individuals participating in the Mataró Ageing Study.

Keywords Ghrelin polymorphisms · Cognitive state · Glucose impairment · Metabolic syndrome · MMSE

The Mataró Ageing Study Group: Ayllón J, Buquet X, Bosch A, Burdoy E, Cademas I, Dordas J, Espinosa C, Falcón I, Gordillo M, Merino MJ, Mussoll J, Palomera E, Papiol M, Pous E, Pubill M, Puig J, Puig-Domingo M (Project co-director), Sanahuja J, Serra P, Serra-Prat M (Project co-director), Serrano C, Vilardebò A, Villarroya I.

M. Mora
Department of Endocrinology and Nutrition, Hospital Clínic I Universitari of Barcelona, Barcelona, Spain

M. L. Mansego
Department of Nutrition and Food Sciences, Physiology and Toxicology, University of Navarra, Pamplona, Navarra, Spain

M. L. Mansego · J. Chaves
Genotyping and Genetic Diagnosis Unit, Research Foundation, CIBER de Diabetes y Enfermedades Metabólicas, Hospital Clínico Universitario de Valencia, Valencia, Spain

M. Serra-Prat · E. Palomera
Research Unit and Ciberhep, Hospital de Mataró, Mataró, Spain

X. Boquet
Research Unit Biochemistry Service, Hospital de Mataró, Mataró, Spain

M. Puig-Domingo (✉)
Department of Endocrinology and Nutrition, Hospital Universitari Germans Trias i Pujol, Ctra. del Canyet, s/n, 08916 Barcelona, Spain
e-mail: mpuigd@gmail.com

Introduction

Metabolic syndrome (MS) [1] is associated to severe morbidities, and especially when type-2 diabetes is present, cardiovascular and cerebrovascular diseases [2]. Recent studies have found associations between cognitive impairment and MS [3, 4] and in particular with diabetes [5, 6] and obesity [7]. Moreover, obesity has been linked to reduced brain volume [8] and reduced grey matter volume [9]. However, the relationship between MS and cognitive function is still controversial; some studies have found a positive association [10, 11] while in others no association has been demonstrated [12]. What degree of causality could be attributed to the vascular morbidity or to the metabolic scenario or other factors present in these conditions remains to be established.

Other factors may also play a role in cognitive function, and among these factors different hormones may be relevant. Ghrelin is a candidate participant in this interplay, because it is linked to MS as well as it is recognized as a neuroprotective factor. Ghrelin is an orexigenic hormone secreted mainly by the stomach, which stimulates growth hormone (GH) release, appetite and food intake and plays an important role in regulating the energy homeostasis of the organism [13]. Some studies, including one performed by our group [14], have evaluated the relationship of ghrelin with the MS. In one of those, studying middle-aged individuals [15], lower levels of ghrelin in people with MS was found, and in other in elderly people, this association was mostly explained by body weight [16] and waist circumference [17]. Ghrelin polymorphisms have also been previously explored in the context of its association with MS with variable results [18], some showing no relation with metabolic disturbances [19] while others have found a positive association [20, 21]. In particular, an association has been described between M72L and MS [20, 21] as well as with higher fasting glucose, lower high-density lipoprotein and higher triglyceride levels [20]. In addition, the prevalence of MS has been found to be lower among individuals carrying 51Gln allele [20]. In relation to its functionality, different variants in the promoter region of the ghrelin gene have been associated to increased ghrelin levels (M72L, -604T) [22].

Moreover, ghrelin seems to have an extra-hypothalamic role, promoting learning and memory by activating plasticity in the hippocampus, enhancing reward and motivation and having a neuroprotective role [23]. Several studies have demonstrated that increased levels of ghrelin are associated with better memory retention in rats [24, 25] and protect from development of neurodegeneration in mice after intrahippocampal injection of amyloid Aβ1-42 oligomer [26]. Recent studies in Alzheimer disease patients have also shown decreased ghrelin mRNA levels in the brain, and it has been postulated that this may contribute to the cognitive impairment observed in this disease [27].

The purpose of the present study was to investigate the relationship between cognitive function and MS components (ATP-III criteria) and five SNPs of ghrelin gene that have been previously described to be associated with MS, in non-institutionalized individuals aged 70 years or older.

Subjects and methods

Study population

A cross-sectional study was set up including subjects participating in the Mataró Ageing Study, a population-based cohort study designed to identify risk factors for frailty and successful ageing condition among old people living in Mataró and Argentona (Barcelona, Spain), which have been previously described elsewhere [28]. All non-institutionalized residents aged 70 years or older were eligible for the study. Exclusion criteria included severe physical or mental disability that did not allow visiting the study centre and individuals with previous gastric surgery. Sample selection was done on random basis from the municipal census. A total of 824 individuals of both sexes were invited to participate, this was first done by postal contact, followed by a telephone call from May 2002 to June 2003. Of those invited to participate, 176 (21.3 %) were excluded because of non-fulfilment of selection criteria and 87 (10.6 %) because of impossibility to contact after attempting three times by telephone. Of the remaining 561 individuals, 139 (24.8 %) did not accept to participate, 62 (11 %) accepted but did not come to the appointment visit and 47 (8.4 %) declined to participate for other social reasons. Finally, 313 cases participated in the study (inclusion rate of 55.8 %). As more than 95 % of people older than 65 living in Argentona and Cirera-Molins—a neighbourhood of Mataró city—receive medical assistance in the local medical centres of the Consorci Sanitari del Maresme, individuals initially contacted but who did not participate in the study did not differ in age and sex distribution compared to participants. There were no differences in comorbidities (including cardiovascular, rheumatologic, metabolic and mental disturbances, as well as neoplastic diseases) as evaluated in the clinical records of all individuals under medical coverage of the Consorci Sanitari of Maresme, the only health care provider of this geographical area of the Maresme region of Barcelona.

Data collection and biological samples for genetic studies were obtained in 280 individuals (137 men and 143 women). The Ethic Committee of the Consorci Sanitari of Maresme approved the study protocol and all subjects signed an informed consent before entering.

Data collection

The questionnaires used for evaluating nutrition, the physical assessments and procedures were performed at two Primary Health Care Centres by a trained fieldwork team composed of 10 general practitioners.

According to the study protocol, in the first visit a revision of the electronic medical history of the individual and/or a re-evaluation in case of no previous registration at the Consorci Sanitari of Maresme medical histories database was done. Chronic and previous diseases, life style factors, education, a physical examination and a laboratory study for biochemical, hormonal and genetic determinations were recorded in the database. Educational level was classified as no schooling and schooled individuals. Lifestyle variables such as smoking (never smoking/previous smoker/current smoker) and alcohol (never consuming/occasional/current) were also included.

The physical examination included weight (in kg) and height (in cm) measures with subjects wearing light clothes; waist circumference was measured in standing position in a line between the last rib and the iliac crest. Body mass index (BMI) was calculated as weight divided by height (metres) squared. Blood pressure was measured twice with the subject being seated after 5 min of rest, the mean of two reading was used in the analyses.

Functional capacity was assessed by calculation of Barthel score [29] and considered optimal when score = 100. The Mini-Mental State Examination (MMSE) was used to assess cognitive function and subjects with <24 points were considered cognitively impaired [30]. A validated Spanish version of MMSE was used [31]. MMSE score was evaluated according 5 items dimensions: orientation (in time -5 points- and in place -5 points-), memory fixation (3 points), concentration and calculation (5 points), memory (3 points) and language and building (objects identification: 2 point, talking: 1 point, hand manipulation: 3 points, reading: 1 point, writing: 1 point and copying: 1 point). Depressive status was evaluated performing the Geriatric Depression Scale (GDS) [32].

Analytical measurements

Blood samples for all measurements were obtained after a 12 h of fast through the night. Glucose and lipids were analysed by enzymatic techniques. Total plasma ghrelin concentrations were measured with a human radioimmunoassay (RIA) kit (Linco Research Inc, St. Charles, MO, USA). The detection limit was 93 pg/mL with intra- and inter-assay variation coefficients of 11.1 and 14.7 %, respectively. Total IGF-I was measured using a two-side immunoradiometric assay (Immunotech IGF-I kit, Immnunotech-Beckman, Marseille, France; intra-assay variation coefficients (CV): <6.3 %; interassay CV: 6.8 %; sensitivity: 30 ng/ml).

ApoE measurement

Genotyping for the ApoE polymorphisms was performed following the method described by Hixon and Vernier [33] with minor modifications.

Metabolic syndrome (MS) definition

We used the Adult Treatment Panel III (ATP III) definition of MS [1]. Individuals were classified as having MS if the waist circumference was >102 cm in men or >88 cm in women (central obesity) plus two or more of the following: (1) arterial blood pressure >130/85 mmHg or antihypertensive treatment, (2) triglycerides >150 mg/dl or hypertriglyceridaemia treatment, (3) high-density lipoprotein (HDL) ≤40 mg/dl in men or ≤50 mg/dl in women, or (4) fasting glucose ≥100 mg/dl or diabetes (glucose impairment, GI). Additionally, for data analysis in relation to ghrelin polymorphisms, waist circumference was also used as a continuous variable.

Ghrelin gene polymorphisms

Five single-nucleotide polymorphisms (SNPs) of the ghrelin gene (GHRL) were investigated in this cohort: −994CT (rs26312), −604GA (rs27647), −501AC (rs26802), M72L (rs696217) and L90G (rs4684677). DNA was isolated from peripheral blood cells using the Chemagic System (Chemagen; Baesweiler, Germany). Polymerase chain reaction (PCR) amplicons were designed by Primer3 programme [34] to completely traverse the promoter, exon 1, exon 3 and exon 4 of *GHRL*. The size of PCR products was analysed by electrophoresis on 2 % agarose gels. Products were treated with Exonuclease I (Amersham Biosciences) and shrimp alkaline phosphatase (Amersham Biosciences) to remove excess primers and deoxynucleotide triphosphates. For the examination of the six SNPs, extension SNaPshot primers specific to the polymorphic sites were used for the SNapshot minisequencing reaction using the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems). The resulting products were purified by one unit of Calf Intestine Phosphatase (New England Biolabs, Ipswich, MA, USA). Snapshot products were resuspended in 4.5 μL Hi-Di^{TM} Formamide (Applied Biosystems) and 0.5 μL GeneScan Size Standard. Then, they were electrophoretically analysed using a DNA Analyzer 3730 (Applied Biosystems). The results of genotyping were analysed and evaluated by GeneMapper software v. 3.7 (Applied Biosystems).

Table 1 Characteristics of individuals according to MMSE (<24 vs. ≥24)

	Total ($n = 280$)	MMSE <24 ($n = 62$)	MMSE ≥24 ($n = 218$)	p
Age (years)	77.03 (5.92)	80.14 (7.25)	76.17 (5.21)	<0.001
Gender (M/W) (%)	48.9/51.1	35.5/64.5	52.8/47.2	0.016
No schooling (%)	48.9	75.8	41.2	<0.001
Waist circumference (cm)	101.52 (11.56)	101.49 (16.33)	101.53 (9.82)	0.985
Pathologic waist circumference (%)	67.3	80.6	63.4	0.011
Total cholesterol (mg/dl)	201.81 (37.22)	212.77 (44.67)	210.23 (35.05)	0.699
LDL (mg/dl)	129.64 (33.64)	127.01 (44.20)	130.35 (30.25)	0.597
Triglycerides (mg/dl)	126.02 (74.50)	144.64 (115.62)	120.98 (58.05)	0.144
High triglycerides (%)	24.0	23.2	24.2	0.884
HDL (mg/dl)	55.06 (2.61)	54.19 (10.10)	55.29 (13.22)	0.502
Low HDL (%)	19.8	16.1	20.8	0.433
Hypertension (%)	88.1	90.3	87.4	0.537
Fasting glycaemia (mg/dl)	107.23 (27.58)	109.67 (26.50)	105.87 (27.34)	0.355
GI (%)	51.1	63.2	47.8	0.040
BMI (kg/m2)	28.15 (4.12)	29.33 (3.90)	27.81 (4.13)	0.010
Obesity (IMC ≥30) (%)	30.9	43.5	27.3	0.015
Metabolic syndrome (%)	50	58.9	47.5	0.131
Coronary disease (%)	12.9	11.3	13.4	0.659
Stroke (%)	12.2	9.7	13.0	0.484
Albumin (mg/dl)	41.55 (2.89)	41.39 (3.75)	41.60 (2.62)	0.695
Ghrelin (pg/ml)	1,090.75 (404.85)	1,028.89 (342.73)	1,107.11 (418.95)	0.203
IGF-I (ng/ml)	108.87 (36.84)	101.29 (38.58)	110.90 (36.18)	0.083
Hours of walking/day	1.78 (4.93)	1.52 (2.28)	1.86 (5.46)	0.629
Barthel	96.51 (5.83)	94.34 (9.15)	97.12 (4.32)	0.025
MMSE	28.99 (5.54)	18.58 (3.48)	27.86 (1.66)	<0.001
Possible depression by GDS (%)	34.2	48.3	30.3	0.009
Alcohol (never/occasional/current) (%)	21.9/49.3/28.9	41.7/43.3/15	16.2/51/32.9	<0.001
Smoking (never/previous/current) (%)	57/36.5/6.5	73.8/24.6/1.6	52.3/39.8/7.9	0.008

Values are mean (standard deviation); ns $p > 0.05$; *HDL* high-density lipoprotein, *GI* glucose impairment or diabetes (ATP-III criteria), *BMI* body mass index, *MMSE* Mini-Mental State Examination, *GDS* geriatric depression scale

Data analysis

Categorical variables were expressed as percentages, and continuous data as mean (standard deviation). SNPs association to categorical variables was evaluated by Chi-square or Fisher test accordingly. SNPs association with continuous data was analysed by ANOVA/T Student test for data with a normal distribution and the Kruskal–Wallis/U Mann–Whitney test for data without a normal distribution. Analysis of association with a response variable was based on linear or logistic regression depending on quantitative or categorical variables. A crude analysis was performed, and then adjusted for the variables that were significantly associated to MMSE. The final analysis included those that remained significant. We considered statistical significance for association with a p value <0.05. Odds ratio was used to evaluate the association of the different pathological conditions considered with each SNP. In the whole cohort, all observed genotypes distributions were compatible with Hardy–Weinberg equilibrium.

Results

Characteristics of the cohort and correlations with phenotype

The phenotypic characteristics of the individuals according to gender have been described elsewhere [14]; roughly, women presented worse metabolic profile, higher prevalence of obesity, central obesity, and metabolic syndrome,

as well as lower levels of total ghrelin and IGF-I. The cohort was studied according to its cognitive state and was divided into two groups: cognitively impaired (MMSE <24, $n = 62$) and not cognitively impaired (MMSE ≥24, $n = 218$). The general characteristics of the cohort and its description according to cognitive deterioration are shown in Table 1. No association was found between MMSE or cognitive impairment and physical activity (hours of walking/day) or medication.

MMSE score was inversely correlated to BMI ($p = 0.031$, $r = -0.129$), waist circumference ($p < 0.001$, $r = -0.555$) and triglyceridaemia ($p = 0.040$, $r = -0.127$), but not to cholesterol levels, nor to waist–hip ratio and nor to ghrelin and IGF-I levels. However, after adjustment by gender, age and educational level became non significant. MMSE was significantly associated to alcohol and smoking (MMSE values for never alcohol/occasional/current: 25.53 ± 5.43/26.02 ± 4.02/27.13 ± 3.77, $p < 0.001$, after adjustment for age and gender $p = 0.003$; MMSE values for no smoking/previous/current: 25.03 ± 4.88/26.64 ± 3.73/27.94 ± 2.44, $p = 0.002$, after adjustment for age and gender $p = 0.234$). When association between MMSE score and metabolic parameters was analysed, the only association that remained significant after adjustment by gender, age and educational level was GI (MMSE 25.74 (4.75) in individuals with GI vs 26.74 (3.79) in individuals without GI, $p = 0.003$, after adjustment $p = 0.023$; Table 2). No association was found between MMSE and medical treatment such as antihypertensive, antidiabetic, antiplatelet, anticoagulant or anxiolytic agents.

Ghrelin polymorphisms in relation to mental state

Analysis of ghrelin plasma levels as a function of ghrelin polymorphisms was searched for and no association was observed. We found an association between MMSE score as a continuous variable with M72L and L90G SNPs (Table 3). M72L C/A genotype was associated to a lower MMSE in comparison to C/C genotype (C/A 24.53 (5.00) and C/C 26.18 (4.15), $p = 0.032$). L90G A/T genotype was associated to a lower MMSE in comparison to A/A (A/T 24.30 (5.31) and A/A 26.12 (4.18), $p = 0.054$). In both, the association remained significant after adjustments by age, gender, educational level, GI and ApoE ($p = 0.049$ for M72L and $p = 0.005$ for L90G).

When we studied the relationship between ghrelin polymorphisms and cognitive impairment (Table 4) we found an association with L90G (39.1 % of MMSE score <24 in A/T in comparison with 19.3 % in A/A, $p = 0.026$). When this association was adjusted by age, gender, alcohol, GDS, GI, educational level and ApoE, it remained significant ($p = 0.002$; OR 6.18 CI 1.93–21.75), $R^2 = 0.250$).

Table 2 Relationship between MMSE and metabolic parameters, as crude data and after adjusting by age, gender, alcohol and educational level

	MMSE mean (SD)	p	p^a
Obesity			
Yes	24.23 (4.18)	0.022	0.512
	25.32 (4.67)[a]		
No	26.83 (4.90)		
	26.25 (4.17)[a]		
Central obesity			
Yes	25.24 (4.70)	0.001	0.287
	25.43 (4.51)[a]		
No	26.96 (3.66)		
	27.08 (3.73)[a]		
Metabolic Syndrome			
Yes	25.35 (4.52)	0.024	0.263
	25.35 (4.54)[a]		
No	26.57 (4.52)		
	26.56 (4.16)[a]		
GI			
Yes	25.74 (4.75)	0.003	0.023
	25.19 (4.78)[a]		
No	26.74 (3.79)		
	26.70 (3.81)[a]		
Diabetes			
Yes	24.76 (5.10)	0.087	0.342
	24.94 (5.05)[a]		
No	26.05 (4.25)		
	26.23 (4.11)[a]		

Values are mean (standard deviation); ns $p > 0.05$; Obesity: BMI ≥ 30 kg/m2; *BMI* body mass index, *GI* glucose impairment (ATP-III criteria)

[a] Adjusted by age, gender, alcohol, GDS, educational level and ApoE

Discussion

We studied the relationship between MS components, ghrelin polymorphisms and cognitive state in a sample of dwelling old individuals with a well-characterized metabolic phenotype and belonging to a Mediterranean population. We found a positive association of MMSE score with glucose status, M72L and L90G polymorphisms of the ghrelin gene; all the analyses performed were adjusted for gender and phenotype, as well as ApoE genotype, indicating that these ghrelin SNPs do have an independent association with cognitive status in our sample of population of the Mataró Ageing study.

Certain metabolic phenotypes, in particular obesity, and especially glucose impairment, have been linked to cognitive state. Several reports have recently been published in relation to this subject, reinforcing the potential deleterious

Table 3 Association between MMSE and ghrelin polymorphisms

SNP	MMSE mean (SD)	Unadjusted	Adjusted[a]	Adjusted[b]
−994CT	C/C ($n = 213$) 26.06 (4.31)	$p = 0.612$	–	–
	C/T ($n = 51$) 25.47 (4.37)			
−604GA	A/A ($n = 84$) 26.02 (4.50)	$p = 0.768$	–	–
	G/A ($n = 135$) 25.79 (4.25)			
	G/G ($n = 46$) 26.30 (4.31)			
−501AC	A/A ($n = 127$) 26.06 (4.18)	$p = 0.748$	–	–
	C/A ($n = 105$) 25.72 (4.54)			
	C/C ($n = 33$) 26.30 (4.31)			
M72L	C/A ($n = 36$) 24.53 (5.00)	$p = 0.032$	$p = 0.058$	$p = 0.049$
	C/C ($n = 230$) 26.18 (4.15)		$\beta = 0.104$	$\beta = 0.109$
L90G	A/A ($n = 243$) 26.12 (4.18)	$p = 0.054$	$p = 0.005$	$p = 0.005$
	A/T ($n = 23$) 24.30 (5.31)		$\beta = -0.152$	$\beta = -0.153$

MMSE Mini-Mental State Examination

[a] By age, gender, educational level, alcohol, GDS and GI (glucose impairment or diabetes in ATP-III criteria)

[b] By age, gender, educational level, alcohol, GDS, GI and ApoE

Table 4 Association between cognitive impairment and ghrelin polymorphisms

SNP	MMSE <24 (%)	Unadjusted	Adjusted	OR (CI 95 %)
−994CT	C/C 21.1	$p = 0.872$	–	–
	C/T 21.6			
−604GA	A/A 16.7	$p = 0.375$	–	–
	G/A 24.4			
	G/G 19.6			
−501AC	A/A 21.3	$p = 0.357$	–	–
	C/A 23.8			
	C/C 12.1			
M72L	C/A 30.6	$p = 0.133$	–	–
	C/C 19.6			
L90G	A/A 19.3	$p = 0.026$	$p = 0.002$*	6.18 (1.93–21.75)*
	A/T 39.1			

* By age, gender, GDS, alcohol and educational level, GI (glucose impairment or diabetes in ATP-III criteria) and ApoE. R2 model: 0.250

role of metabolic syndrome in cognitive impairment. In relation to glucose state, a systematic review of prospective observational studies [5] supports that diabetic people have a greater rate of cognitive decline. Hippocampus and prefrontal lobe seem to have a reduced volume in diabetic individuals [35], which could cause specific verbal memory impairments. This hippocampal volume loss in diabetics may be due to hyperglycaemia and the formation of toxic advanced glycation end-products; also, the presence of increased pro-inflammatory cytokines such as IL-6 in obesity, and its coexisting endothelial dysfunction condition may contribute to decreased substrate supply to the hippocampus, neuronal loss and finally to cognitive deterioration. In our cohort the crude analysis, as well as the adjustment, showed a strong deleterious effect of GI upon MMSE score. When diabetic subjects were analysed alone, a trend was also observed; as diabetic individuals were older than the whole group of subjects with fasting glucose >100 mg/dl, the important effect of age, as well as the higher statistical power of the former group of GI may explain these differences.

Other components of metabolic syndrome, such as insulin resistance per se, as well as metabolic syndrome as a whole have been postulated as factors or conditions related to cognitive impairment [3, 4, 6]. The association between BMI and dementia is a controversial issue; a study has shown that BMI is associated to a decreased brain volume but with no effect in cognition [36] while another showed that higher BMI is associated to reduced risk of dementia [37]. A more consistent relationship has been described for central obesity and dementia instead of global obesity [38]. In individuals less than 75 years, BMI and adiposity show a U-shaped relationship with dementia risk, by which very low and very high BMI quartiles showed a worse cognitive function; this relationship is difficult to interpret in cross-over studies as most or a very significant proportion of patients with dementia usually lose weight after diagnosis [39]. This dual association of either a protective or a deleterious effect of body weight on cognition in relation to a younger or not so younger age may indicate that an individual predisposition may modulate such a relationship. In those individuals prone to develop cognitive impairment, obesity may act as an accelerator while in those resistant or lacking this susceptibility and having survive obesity after 70 years, such a condition may be no more deleterious but protective.

An indirect effect of ghrelin/obestatin generated in the digestive tract upon metabolic syndrome development and its consequences on cognitive function may also be possible.

Ghrelin has been previously related to different conditions found in elderly people [14, 16, 17, 40, 41]. In a previous study, we found hunger and weight loss to be

influenced by ghrelin levels [41]. A recent study discusses the role for ghrelin in cognitive function in the non-demented population; in one small study including 35 individuals [42] circulating acetylated ghrelin was higher in subjects with lower MMSE. In our subjects, total ghrelin levels did not differ between any particular cognitive state; this may be related to the potentially insufficient value of a single fasting determination of ghrelin, a hormone which fluctuates during the daytime period in relation to prandial physiology, as well as the measurement of total ghrelin rather than the acetylated form. In Alzheimer disease patients, an altered expression of the ghrelin gene has been recently reported, by which a lower expression of the gene in the temporal lobe participates in the development of cognitive deficiency [27]. The data coming from animal models show that intrahippocampal ghrelin administration in mice is followed by an amelioration of cognitive dysfunction produced by beta amyloid injection [26]. The interpretation of all these findings is currently difficult due to the relatively low number of studies that have been performed, although a relationship between ghrelin seems plausible. More data from both clinical and experimental studies are required for its clarification. Whether peripheral ghrelin acts in the central nervous system or just ghrelin synthesized in the brain participates in the modulation of different central functions will require extensive studies. Also, the ghrelin gene product obestatin described in 2005 [43], which is thought to act as a contra-ghrelin factor, may modulate ghrelin gene actions on cognition, but until now data regarding in this issue are scarce. Obestatin action has been implicated in the protection and improvement of cognitive function in rats, as ghrelin does. However, they appear to work in parallel with the metabolic effect. Moreover, L90G polymorphism is related to obestatin expression, and thus, part of L90G Ghrelin genotype effect may be due to obestatin [22, 43]. A recent study in Japanese population has described an association between L90G and Alzheimer's disease [44]. To our knowledge, this is the first time that an association between ghrelin polymorphisms and MMSE is reported, and specifically in relation to M72L and L90G.

The strengths of our study are that the sample is a homogeneous sample obtained from specific geographical with a cultural common background that warrants a similar lifestyle; moreover, the phenotypical characterization of the subjects is quite extensive. However, our study has also several limitations. First, although it is a population-based study where non-institutionalized individuals were selected, the inclusion of a younger sample of individuals would have overcome the problem of the overrepresentation of certain genotypes due to survival bias. The number of included subjects was also restricted to the recruitment possibilities in the specific geographical areas involved in the study; a superior number of participants would have allowed a stronger statistical power. Additionally, the measurement of acylated ghrelin rather than total ghrelin, as well as obestatin, would have helped to explore potential relationships not found with our current design. In our subject's sample, a high prevalence of metabolic syndrome and a high percentage of central obesity according to ATP-III criteria were found, which may have contributed to a bias. Moreover, we have to take into account that MMSE is considered a screening test of cognitive function with great value, although a deeper insight to the characterization of the cognitive function status requires the use of neuropsychological test batteries.

Conclusions

In summary, metabolic factors contribute to different cognitive status assessed by MMSE score; glucose impairment seems to be one of these factors among others such as obesity, in particular central obesity, and metabolic syndrome. M72L Ghrelin polymorphism seems to influence MMSE and L90G A/T is associated to cognitive impairment in elder Spanish community dwelling individuals.

Acknowledgments This work was supported by Fondo Investigaciones Sanitarias Grant 07/0601. CIBERDEM is an initiative of Fondo de Investigaciones Sanitarias (FIS) research grant PI070497 from the Fondo de Investigaciones Sanitarias; ACOMP/2009/201, GRUPOS03/101, 2005/027 and PROMETEO/2009/029 from the Valencian Government. Special thanks to Nicola Van Berckel for her English version of the manuscript. The authors have nothing to declare in relation to this article.

Conflict of interest The authors have nothing to declare in relation to this article.

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