Cortisol, HDL-c, VLDL-c, and APOE Polymorphisms as Laboratorial Parameters Associated to Cognitive Impairment No Dementia (CIND) and Dementia

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Background: Population aging is a global phenomenon whose main consequence is the increase of chronic degenerative diseases, including dementia. The aim of this case–control study was to evaluate the laboratorial parameters lipid profile, cortisol, and apolipoprotein E (APOE) gene genotype, comparing cognitively healthy controls and subjects with cognitive impairment no dementia (CIND) and dementia in a group of elderly people. **Methods:** Three hundred and nine individuals enrolled in the Pietà Study (Brazil) were divided into three groups: control ($n = 158$), CIND ($n = 92$), and dementia ($n = 59$). Participants were interviewed, went through examina-

tion, and had blood samples taken. **Results:** Age and APOE showed significant differences among the groups, while sex and lipid profile did not. According to multivariate regression logistic analyses, higher cortisol levels, lower high-density lipoprotein (HDL-c) and very low-density lipoprotein (VLDL-c), presence of $\epsilon 4$ allele of APOE, and aging were associated with CIND and dementia. **Conclusion:** These laboratorial parameters are risk factors associated to CIND and dementia in the elderly people and should be investigated in order to develop strategies to prevent or delay the onset of dementia in the oldest-old populations. J. Clin. Lab. Anal. 30:374–380, 2016. © 2015 Wiley Periodicals, Inc.

Key words: APOE polymorphisms; cognitive impairment no dementia; cortisol; dementia; lipid profile

INTRODUCTION

Population aging is nowadays a global phenomenon (1). One of the consequences of aging is the increase of chronic degenerative diseases, including dementia (2). Dementia is a syndrome characterized by progressive and global decline of cognitive functions, including memory impairment and at least one of the following changes—aphasia, apraxia, agnosia, and disturbance in executive functioning, causing decline in social and occupational activities (3). The global prevalence of dementia in the elderly (≥ 65 years) in South America was estimated as 7.1%, showing an increase with age: 7.0% for individuals aged 75–79 years old, 11.9% for the group of 80–84, 20.2% for 85–89 years old, and 33.1% for 90 years old or more (4).

Alzheimer's disease (AD) is the most frequent age-related neurodegenerative pathology, being responsible for 50–70% of the cases (5–7). Besides cognitive changes, the disease is associated with behavioral and psychological symptoms that promote progressive disability and possibly, incapacitation (8). The incidence of AD is followed by vascular dementia (VaD) in 30–40% of the cases

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Received 1 May 2014; Accepted 27 June 2015

DOI 10.1002/jcla.21865

Published online in Wiley Online Library (wileyonlinelibrary.com).

and mixed dementia, characterized by simultaneous occurrence of both AD and cerebrovascular disease, corresponding to 15–20% of the cases (7, 9).

The intermediate category between normal cognition and dementia in aged people is designed as cognitive impairment no dementia (CIND) (10). CIND is defined as impairments in memory and/or other cognitive domains not sufficiently severe to be classified as dementia. Individuals with this condition can remain stable or return to normal cognition, but the majority progresses to dementia (11). The term was first described in the Canadian Study of Health and Aging (12) and has been suggested to evaluate cognitive impairment in large cohort studies (13). A recent systematic review, including studies from different countries, estimated that the prevalence of CIND was 5.1–35.9%, showing increased rates among older people (14).

Since individuals with CIND are at an increased risk of developing dementia, it is important to investigate and better comprehend the characteristics of both groups and also the risk factors related to dementia, in order to develop strategies to prevent or delay the onset of this syndrome (15, 16). The known risk factors for dementia and AD include aging (17, 18), female gender (19–21), obesity (22–24), and the allele $\epsilon 4$ of apolipoprotein E gene (*APOE*) (25, 26). In addition, high levels of cortisol have been observed in individuals with hippocampal atrophy, a major structural hallmark of AD, and cognitive decline (27). The objectives of this study were to evaluate laboratorial parameters cortisol levels, lipid profile, and *APOE* genotype by comparing healthy brain aging, CIND, and dementia in a group of elderly people from Brazil.

MATERIALS AND METHODS

Study Design

This case–control cross-sectional study is based on the Pietà project, an epidemiological investigation on successful brain aging in an oldest-old population (≥ 75 years). This community-based survey was conducted in Caeté, Minas Gerais, southeast Brazil, from 2008 to 2009. Methods and baseline characteristics of the participants were previously reported in detail elsewhere (2).

Participants, Interview, and Clinical Evaluation

In this study, 309 patients were included: 158 cognitively healthy controls (G1), 92 individuals with CIND (G2), and 59 with dementia (G3). Age, sex, and the use of medication were informed by the patients or their representatives during interview.

Participants went through comprehensive clinical and neurological examination, including cognitive and functional assessments. The ones with suspected cogni-

tive impairment and a subset of cognitively healthy individuals were referred to complementary neuropsychological and functional evaluations and the presence of cognitive impairment was based on group discussions among the physicians involved in the collection of clinical data. Detailed information about the design and the methods of the study have been published elsewhere (2). Dementia was classified according to *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria (3). The individuals with CIND followed the consensus agreement but not sufficient to be classified as dementia. Subjects without any evidence of cognitive and functional impairment were classified as controls. All participants in use of statins or other hypolipidemic drugs were excluded in this analysis. The study protocol was approved by the Ethics Committee of the Federal University of Minas Gerais and all the participants or their legally representatives signed the written informed consent.

Laboratory Measurements

All the participants of the study were invited to provide a blood sample for laboratory tests, including lipid profile (total cholesterol (TC), triglycerides (TGs), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), very low-density lipoprotein-cholesterol (VLDL-c)), cortisol, and *APOE* genotyping. Blood samples were collected in the morning after an overnight fast of 12 h.

Lipid profile was determined by enzymatic method using Labtest kit, according to the manufacturer's protocol and analyzed in Super Class – Labtest equipment. Cortisol levels were determined by chemiluminescence using DPC Bayer kit, according to the manufacturer's protocol and analysed in Advia Centaur equipment.

Genomic DNA for *APOE* genotyping (rs429358 and rs7412) was extracted from total blood samples in ethylenediaminetetraacetic acid (EDTA). DNA samples were then amplified by polymerase chain reaction (PCR), followed by digestion with *HhaI* and restriction fragment length polymorphism analysis, as previously described by Hixson and Vernier (28). *APOE* genotypes were analyzed blinded to clinical diagnosis.

Statistical Analysis

Analyses were performed with SPSS package (v. 13.0 for Windows; Chicago, IL). Normal distribution of data was checked by Shapiro–Wilk test. Lipid profile (except LDL that was estimated by ANOVA and LSD tests) and age were analyzed by Kruskal–Wallis test with Bonferroni correction. Age, sex, lipid profile, and *APOE* genotypes were also analyzed by asymptotic Pearson chi-square test with residuals test. A forward stepwise multivariate

TABLE 1. Baseline Characteristics of the Population ($n = 309$) by Group

Categories		Control (G1) $n = 158$ (%)	CIND (G2) $n = 92$ (%)	Dementia (G3) $n = 59$ (%)	Total n (100%)	P -value
Gender	Male	64 (53.3)	33 (27.5)	23 (19.2)	120	0.768
	Female	94 (49.7)	59 (31.2)	36 (19.0)	189	
Age	≤ 79 years	96 (58.9) ^b	43 (26.4)	24 (14.7) ^a	163	0.012
	> 79 years	62 (42.5) ^a	49 (33.6)	35 (24.0) ^b	146	

Asymptotic Pearson chi-square test.

^aSignificant by residuals test: less frequent.^bSignificant by residuals test: more frequent.

logistic regression analysis was applied to identify the independent predictors of dementia and CIND among the variables with a P -value < 0.2 in the univariate analysis. Hosmer–Lemeshow Test was applied to evaluate the adequacy of the multivariate model. Hardy–Weinberg equilibrium (HWE) and allelic and genotypic frequencies for the *APOE* gene were evaluated by Genepop program version 4.0.10 (genepop.curtin.edu.au). HWE test was performed in controls to assess possible genotyping error. Level of statistical significance was considered a P -value < 0.05 .

RESULTS

Sociodemographic Parameters

Among the 309 individuals who participated in the present study, 120 (38.8%) were male and 189 (61.2%) were female. When analyzing gender by group, there was no difference among them (Table 1).

Median of age (years) was significantly different among the groups ($P = 0.009$): 78.0 (Interquartile range: IQ 5.0) for G1, 80.0 (IQ 6.0) for G2, and 82.0 (IQ 7.0) for G3. When analyzing groups in pairs, a difference was observed between G1 versus G2 ($P = 0.009$) and G1 versus G3 ($P < 0.001$), but not between G2 and G3 ($P = 0.062$). When the variable age was classified in two categories, according to the median of the participants (79 years), differences were observed among the groups ($P = 0.012$), in which individuals aged 79 years or less were more frequent in G1 and less frequent in G3. The opposite was observed for subjects above 79 years (Table 1).

Laboratory Parameters

APOE gene

Results of allelic and genotypic frequencies for *APOE* are shown in Table 2. All the groups were in HWE ($P = 0.878$ for G1, $P = 0.489$ for G2, and $P = 0.143$ for G3).

The $\epsilon 4$ allele showed positive association with dementia, as compared with the control group (OR 2.64, 95% CI

TABLE 2. Allelic and Genotypic Frequencies of *APOE*

<i>APOE</i>		Control (G1)	CIND (G2)	Dementia (G3)	Total
Allele	$\epsilon 2$	40 (0.127)	19 (0.103)	10 (0.085)	69 (0.112)
	$\epsilon 3$	234 (0.741)	137 (0.745)	74 (0.627)	445 (0.720)
	$\epsilon 4$	42 (0.133)	28 (0.152)	34 (0.288)	104 (0.168)
TOTAL		316	184	118	618
Genotype	$\epsilon 2\epsilon 2$	2 (0.013)	2 (0.022)	2 (0.034)	6 (0.019)
	$\epsilon 2\epsilon 3$	29 (0.183)	12 (0.130)	4 (0.068)	45 (0.146)
	$\epsilon 3\epsilon 3$	87 (0.551)	51 (0.554)	24 (0.407)	162 (0.524)
	$\epsilon 2\epsilon 4$	7 (0.044)	3 (0.033)	2 (0.034)	12 (0.039)
	$\epsilon 3\epsilon 4$	31 (0.196)	23 (0.250)	22 (0.373)	76 (0.246)
	$\epsilon 4\epsilon 4$	2 (0.013)	1 (0.011)	5 (0.085)	8 (0.026)
TOTAL		158	92	59	309

Frequencies by Genepop program.

1.53–4.56, $P < 0.001$) and those with CIND (OR 2.22, 95% CI 1.23–4.13, $P = 0.004$). No significant difference was observed when comparing frequencies for $\epsilon 4$ between CIND and control groups ($P = 0.549$).

When the genotypes were grouped in four categories (Table 3) according to the probability of having AD [$\epsilon 2\epsilon 4$: neutral (A1), $\epsilon 2\epsilon 2$ or $\epsilon 2\epsilon 3$ (A2): protection, $\epsilon 3\epsilon 3$ (A3): reference, $\epsilon 3\epsilon 4$ or $\epsilon 4\epsilon 4$ (A4): risk], significant associations were observed ($P = 0.025$). A3 was less frequent in G3 and A4 was less frequent in G1 and more frequent in G3.

Lipid profile

None of the parameters related to the lipid profile showed statistical significance ($P > 0.05$, Table 4). However, when the lipid profile was analyzed according to the *APOE* genotype (Table 5), LDL-c was significantly different ($P = 0.006$) between groups A2 and A3 ($P = 0.003$) and between A2 and A4 ($P = 0.005$).

Multivariate Model

In our previous study, we showed that cortisol levels were higher in dementia and CIND groups, when compared to controls (29). In this study, cortisol levels

TABLE 3. Frequencies of the *APOE* Genotypes Grouped

	Group/ genotype	Control (G1, %)	CIND (G2, %)	Dementia (G3, %)	Total
<i>n</i>		7	3	2	12
Percentage <i>APOE</i>	ε2ε4	(58.3)	(25.0)	(16.7)	(100.0)
Percentage group	(A1)	(4.4)	(3.3)	(3.4)	(3.9)
<i>n</i>		31	14	6	51
Percentage <i>APOE</i>	ε2ε2, ε2ε3	(60.8)	(27.5)	(11.8)	(100.0)
Percentage group	(A2)	(19.6)	(15.2)	(10.2)	(16.5)
<i>n</i>		87	51	24 ^a	162
Percentage <i>APOE</i>	ε3ε3	(53.7)	(31.5)	(14.8)	(100.0)
Percentage group	(A3)	(55.1)	(55.4)	(40.7)	(52.4)
<i>n</i>		33 ^a	24	27 ^b	84
Percentage <i>APOE</i>	ε3ε4, ε4ε4	(39.3)	(28.6)	(32.1)	(100.0)
Percentage group	(A4)	(20.9)	(26.1)	(45.8)	(27.2)
<i>n</i>		158	92	59	309
Percentage <i>APOE</i>	Total	(51.1)	(29.8)	(19.1)	(100.0)
Percentage group		(100.0)	(100.0)	(100.0)	(100.0)

Asymptotic Pearson chi-square test.

^aSignificant by residuals test: less frequent.^bSignificant by residuals test: more frequent.

TABLE 4. Lipid Profile by Group

	Control (G1)	CIND (G2)	Dementia (G3)	<i>P</i> -value
TC (mg/dl)	211.0 (55.0)	216.0 (52.0)	200.0 (48.0)	0.099
TG (mg/dl)	115.0 (81.0)	111.0 (72.0)	118.0 (74.0)	0.812
VLDL-c (mg/dl)	23.0 (17.0)	22.0 (15.0)	24.0 (15.0)	0.665
LDL-c ^a (mg/dl)	132.5 ± 36.0	138.2 ± 33.9	126.1 ± 36.2	0.141
HDL-c (mg/dl)	52.0 (15.0)	52.0 (21.0)	50.0 (23.0)	0.824

Kruskal–Wallis test.

^aANOVA test.TABLE 5. Lipid Profile According to *APOE* Genotypes

	ε2ε4 (A1)	ε2ε2, ε2ε3 (A2)	ε3ε3 (A3)	ε3ε4, ε4ε4 (A4)	<i>P</i> -value
TG (mg/dl)	126.0 (94.0)	115.0 (80.0)	113.0 (67.0)	112.0 (86.0)	0.993
TC (mg/dl)	191.5 (60.0)	199.0 (56.0)	216.0 (51.0)	213.5 (59.0)	0.062
VLDL-c (mg/dl)	25.0 (18.0)	23.0 (16.0)	22.5 (13.0)	22.0 (17.0)	0.996
LDL-c ^c (mg/dl)	114.4 ± 26.7	119.1 ± 33.2	136.3 ± 35.3	137.3 ± 36.0	0.006 0.003 ^a 0.005 ^b
HDL-c (mg/dl)	54.0 (17.0)	56.0 (18.0)	50.0 (18.0)	49.5 (19.0)	0.443

Kruskal–Wallis test.

^aLSD test: A2 × A3.^bLSD test: A2 × A4.^cANOVA test.

TABLE 6. Multivariate Model of Independent Predictors of CIND and Dementia

Variables	Categories	OR	95% CI	<i>P</i> -value
Cortisol	>15.56 μg/dl	1.77	1.06–2.97	0.030
HDL	<40.0 mg/dl	2.44	1.08–5.52	0.031
VLDL	<40.0 mg/dl	2.94	1.28–6.76	0.011
<i>APOE</i>	ε2ε2, ε2ε3	0.77	0.39–1.51	0.442
	ε2ε4	1.30	0.38–4.51	0.678
	ε3ε4, ε4ε4	2.15	1.22–3.80	0.008
Age	≥79 years	1.87	1.15–3.04	0.011

Hosmer–Lemeshow test (*P* = 0.412).

were analyzed in a multivariate model including other laboratorial parameters. Higher cortisol levels (>75th percentile of control group: 15.56 μg/dl), as well as lower HDL-c (<40.0 mg/dl) and VLDL-c (<40.0 mg/dl), ε3ε4/ε4ε4 *APOE* genotypes, and more advanced age were pointed out as independent predictors of the diagnoses of CIND and dementia (Table 6).

DISCUSSION

This study evaluated laboratorial parameters as risk factors for CIND and dementia in a specific elderly group from a small city in Brazil. This population stands out as being composed of elderly people with similar life habits and exposed to similar environmental factors, decreasing some confounding effects.

The allele ε4 of the *APOE* gene has been considered a significant risk factor for age-related cognitive decline in nonclinical populations (30) and an independent risk factor for AD, mainly in the sporadic form of the disease (31, 32). This allele is more frequently found in patients with AD compared with controls and has been associated with a higher risk of the disease, with a younger age for the symptoms and with greater cognitive decline (32, 33). In fact, we found a higher frequency of the allele ε4 (28.8%) in the individuals with dementia as compared to the other

groups, which indicate that the allele $\epsilon 4$ is associated with dementia in Brazilian subjects. Also, 49.1% of the population with dementia was carrier of the allele $\epsilon 4$ and 8.5% was homozygote for it. Bahia et al. (34) found a prevalence of 50.8% of $\epsilon 4$ carriers in Brazilian patients with probable AD and of 10.8% of patients with $\epsilon 4\epsilon 4$ genotype. Our results are also in accordance with the systematic review of WARD et al. (14), who found a prevalence of 51.3% in patients with AD $\epsilon 4/-$ (95% CI 44.7–57.9) and of 11.1% in patients $\epsilon 4\epsilon 4$ (95% CI 6.3–15.9) in South America.

In a longitudinal study, Hendrie et al. (35) showed that *APOE* $\epsilon 4$ had a significant effect on incidence of AD and cognitive decline in both Yoruba and African-American populations. Nevertheless, this effect was weaker in the first than in the latter and the reasons for these differences remain unclear. In another recent study, Gui et al. (36) confirmed that the homozygous *APOE* $\epsilon 4$ significantly accelerated cognitive decline in elderly Chinese individuals from a cohort study, but this effect did not survive after multiple testing corrections. Moreover, they found no significant association when the allele $\epsilon 4$ was tested in individual variant analysis. Their results suggest that this gene is not a powerful predictor of cognitive decline in Chinese individuals.

Some studies suggest a neuroprotective role of $\epsilon 2$ allele of the *APOE* gene (34,37–39). For instance, Serrano-Pozo et al. (39) found that *APOE* $\epsilon 2$ is associated with milder AD pathology and less-severe antemortem cognitive impairment. Our results showed that the allele $\epsilon 2$ was more frequent in the control group; however, this association was not significant.

None of the parameters of lipid profile (TC, TG, HDL-c, LDL-c, and VLDL-c) showed an association with dementia, in agreement with Caramelli et al. (40), Reitz et al. (41), and Mielke et al. (42). However, other studies observed lower levels of TC (41–43), higher levels of LDL-c (44,45), lower levels of TG (46), and lower levels of HDL-c (47–49) in patients with either dementia or AD. This last finding is associated with atherosclerosis, stroke, and an inflammatory state, which are all related to dementia (49).

Regarding the lipid profile and *APOE* genotypes, the only parameter that showed a significant association was LDL-c. Lower levels were observed in individuals presenting $\epsilon 2\epsilon 2$ or $\epsilon 2\epsilon 3$ compared with the ones that were $\epsilon 3\epsilon 3$ or $\epsilon 4$ carriers, showing the importance of the *APOE* polymorphisms in the interaction of this apolipoprotein and its receptors. This result is in accordance with Notkola et al. (50) and Jasinska-Myga et al. (51), who observed higher levels of LDL-c among the allele $\epsilon 4$ carriers. This allele presents a higher affinity for the receptor governing the uptake of VLDL remnants and downregulates the LDL-c receptors. Furthermore, it increases the absorption of fat intake.

In our previous study (29), we showed that levels of morning serum cortisol were higher in individuals with dementia, intermediate in individuals with CIND, and lower in controls, independently of *APOE* genotype and in agreement with other studies (27,52). Multivariate analyses in the present study confirm these results, given that higher levels of the hormone were independently associated with CIND and dementia. This elevation could be a consequence of hippocampal damage, related to the loss of hypothalamic-pituitary-adrenal axis (HPA) axis inhibition, since the hippocampus regulates this axis. On the other hand, previous elevation of cortisol throughout life could contribute to hippocampal damage and possibly triggers cognitive decline. Yet, other studies found no correlation between cortisol and cognition (53,54). A longitudinal study based on a large sample of community-dwelling adults showed that variability in HPA function is not a strong contributor to cognitive aging (54). Further investigation should be carried out to clarify this subject.

In conclusion and according to the multivariate regression logistic analyses, the laboratorial parameters cortisol, HDL-c and VLDL-c levels, and *APOE* genotypes are factors associated to CIND and dementia in a Brazilian population. These findings constitute additional evidence for the multifactorial characteristic of CIND and dementia, which should be investigated in elder people in order to evaluate their risk of developing these diseases and also to adopt actions to prevent them.

ACKNOWLEDGMENTS

The authors thank to Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their support.

CONFLICT OF INTEREST

The authors disclose no conflict of interest.

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