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The PPAR-gamma Pro12Ala polymorphism and risk of cognitive impairment in a longitudinal study

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Abstract

The Pro12Ala polymorphism in the PPAR- γ gene has been associated with reduced incidence of type 2 diabetes. Although diabetes has been implicated as a risk factor for dementia, the association of Pro12Ala with cognitive impairment is unclear. Dementia and cognitive impairment without dementia (CIND) were determined during six annual follow-up evaluations in a cohort of 929 older Latinos. Among those with diabetes at baseline, there was an increased rate of dementia/CIND for Ala carriers compared to non-carriers (adjusted hazard ratio (HR) = 2.5, 95% confidence interval (CI): 1.3–4.9) but not among non-diabetic participants (adjusted HR=0.94; 95% CI: 0.49–1.8). Among males, there was also an increased rate for Ala carriers (adjusted HR=2.7, 95% CI: 1.4–5.2) but not among female carriers (adjusted HR = 0.88; 95% CI: 0.47–1.6). The rate of dementia/CIND was highest in diabetic male Ala carriers (adjusted HR= 4.2; 95% CI: 1.5–11) compared to non-diabetic male carriers (adjusted HR=2.9; 95% CI: 1.1–7.4), diabetic female carriers (HR=1.6; 95% CI: 0.66–4.1), and non-diabetic female carriers (HR=0.52; 95% CI: 0.21–1.3). These data suggest that although the Ala variant is associated with a reduced risk of type 2 diabetes, it may increase the risk of cognitive impairment in individuals once diabetes has developed. Male Ala carriers may also have a greater risk of dementia/CIND.

Keywords

Pro12Ala genotype; cognitive impairment; dementia

1. Introduction

The $\epsilon 4$ allele of the APOE gene is the only genetic risk factor to date that has been clearly associated with late-onset Alzheimer's disease, a progressive neurodegenerative disorder that accounts for the majority of age-related dementia. Less than 50% of non-familial

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Alzheimer's cases carry the $\epsilon 4$ allele (Corder, et al., 1994), and other genes may be involved in the pathogenesis of cognitive impairment.

Epidemiologic studies have implicated type 2 diabetes as a risk factor for dementia (Haan, 2006; Launer, 2005). A polymorphism in the PPAR- γ gene, Pro12Ala, has been associated with reduced incidence of type 2 diabetes and greater insulin sensitivity (Altshuler, et al., 2000; Buzzetti, et al., 2004; Hara, et al., 2000), suggesting that the Ala allele may be protective for type 2 diabetes. Although type 2 diabetes has been linked to dementia, the role of Pro12Ala in relation to cognitive impairment is unclear; results from the few studies reporting on the association provide conflicting results (Koivisto, et al., 2006; Sauder, et al., 2005; Scacchi, et al., 2007; Yaffe, et al., 2006).

Mexican American populations have a relatively high prevalence of the Ala variant of the Pro12Ala polymorphism (~20%) (Mori, Y., et al., 1998; Yen, et al., 1997), and rates of diabetes are also disproportionately higher among Mexican Americans than among non-Hispanic whites (Cossrow and Falkner, 2004). This study examined the association of the Pro12Ala genotype with the incidence of age-related dementia or cognitive impairment but no dementia (CIND) in a community-based cohort of older Mexican Americans. We hypothesized that the Ala- genotype would be associated with an increased incidence of cognitive impairment compared to the Ala+ genotype. We also evaluated whether the estimated effect of the genotype on cognitive impairment was modified by baseline diabetes status, sex, age, or APOE- $\epsilon 4$ status. Additionally, we investigated whether an association between the Pro12Ala polymorphism and cognitive impairment was mediated by known metabolic and vascular risk factors.

2. Methods

2.1 Study population and methods

The study population was comprised of participants from the Sacramento Area Latino Study on Aging (SALSA), a cohort of community-dwelling Latinos, aged 60–101 at baseline in 1998–99. Participants were residents in the Sacramento Metropolitan Statistical Area and surrounding suburban and rural counties in California. Details of the sampling frame and recruitment have been published in a separate report (Haan, et al., 2003). The study was approved by the University of California Davis and the University of Michigan Institutional Review Boards. Written informed consent was obtained from all participants. All field staff were bilingual in Spanish and English and interviewed participants at their homes in their language of choice. The study population for these analyses was comprised of 929 SALSA participants who had determination of their Pro12Ala genotype at the 4th follow-up visit and were free of dementia or CIND at baseline. The mean age of these study participants at baseline was 69.6 years (range: 60–93) and 59% were female.

2.2 Outcomes

Dementia and CIND status were determined at baseline and at each of six follow-up evaluations. Classifications of cognitive status were based on a multi-stage assessment protocol. At each evaluation, the Modified Mini-Mental State Examination (3MSE) and a verbal delayed word list recall test (DelRec) from the Spanish and English Verbal Learning Test were administered to participants (Gonzalez, et al., 2001; Teng and Chui, 1987). A participant was referred for neuropsychological evaluation if there was a decline from the baseline 3MSE score by more than 8 points and the current 3MSE score was <20th percentile or there was a decline from the baseline DelRec score by more than 3 points and the current DelRec score was <20th percentile. The neuropsychological evaluation consisted of an expanded test battery and the Informant Questionnaire on Cognitive Decline

(IQCODE) (Jorm and Jacomb, 1989). Participants were referred for a neuroclinical examination if they scored below the 10th percentile on one or more of the neuropsychological tests and 3.40 or greater on the IQCODE, scored below the 10th percentile on four or more neuropsychological tests regardless of IQCODE, or scored greater than 4.0 on the IQCODE. Diagnoses of demented, CIND, or normal in these participants were adjudicated by a neurologist, geriatrician, and a neuropsychologist. Dementia classification required clinically significant impairment in two or more cognitive domains and in independent functioning. California Alzheimer's Disease Diagnostic and Treatment Centers criteria were used to diagnose vascular dementia and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorder Association criteria were used to diagnose AD (McKhann, et al., 1984; Tierney, et al., 2001). CIND diagnosis was made if there was significant cognitive impairment but criteria for dementia were not met. Two dementia cases were identified from death certificates and were assigned a dementia diagnosis after case review; for those, date of death was assigned as the diagnosis date. For the present study, prevalent cases of dementia and CIND at baseline were excluded and incident cases of all-cause dementia and CIND were combined.

2.3 PPAR-gamma genotyping

DNA was obtained from a fasting blood sample drawn by venipuncture at the 4th follow-up interview of the study. Samples were returned to the clinic lab within 4 hours, refrigerated at 4° C, and shipped within 2 days after the blood draw to the University of Michigan for DNA extraction. DNA amplification and genotyping was performed by Ellipsis Biotherapeutics Corporation (Toronto, Ontario, Canada), using single-base primer extension technology in a tagged fluorescent assay with a Beckman Coulter GenomeLab SNPstream instrument.

2.4 Covariates

Age, education, sex, and self-report of physician's diagnosis of hypertension, history of stroke, and diabetes were assessed by interview at the baseline home visit. Education was measured as years of formal schooling. The majority (99%) of the study sample was genotyped for APOE from blood samples obtained at the 4th annual follow-up visit; the remaining 1% were genotyped for APOE using DNA derived from buccal cell swabs at baseline. APOE genotype was dichotomized by presence of the ϵ 4 allele.

Fasting blood at baseline was drawn for measurement of glucose, insulin, lipids, folate, homocysteine, and high-sensitivity C-reactive protein. Type 2 diabetes was determined at baseline by a fasting glucose greater than 125 mg/dL, self-report of a physician's diagnosis, or use of a diabetes medication (insulin and/or oral glucose-lowering agents) obtained by a medicine chest inventory at participants' homes at baseline. Duration of diabetes at baseline was ascertained by self-report. A modification of the Type 2 Diabetes Symptom Checklist (Grootenhuys, et al., 1994), a self-report questionnaire used to measure differences in severity of diabetes-related symptoms between individuals, was administered to study participants at baseline. A diabetes symptom summary score was derived from the questionnaire by summing the number of symptoms reported by the participant with scores ranging from 0 to 34. Two sitting blood pressure measurements were averaged and hypertension was classified by a systolic blood pressure $\geq$ 140 mmHg, a diastolic blood pressure $\geq$ 90 mmHg, or a self-report of a physician's diagnosis of high blood pressure. Weight, height, and waist circumference were measured using standard tape, scale and stadiometer and protocol. Body mass index (BMI) was calculated as measured weight in kilograms divided by measured standing height in meters squared. Obesity was determined by a BMI $\geq$ 30. Waist circumference was measured in centimeters and categorized by the sex-specific cutpoints $\leq$ 88 vs $>$ 88 cm for women and $\leq$ 102 vs $>$ 102 cm for men.

2.5 Statistical Analysis

The Pro/Ala and Ala/Ala genotypes were combined into a single category because of the low frequency of the homozygous Ala genotype. Cox proportional hazards regression was used to investigate the association of the Pro12Ala genotype and rate of incident dementia/CIND during the 6-year follow-up period. The Cox models were fit with each participant contributing time at risk starting from baseline until time of dementia/CIND diagnosis or censoring. Heterogeneity of associations with the genotype by baseline diabetes status, sex, age, and APOE-ε4 status were assessed by including product terms in the models and using likelihood ratio tests and by evaluation of the hazard ratios after stratification. Additional analyses were conducted adjusting for standard risk factors for dementia/CIND including age, education, diabetes, history of stroke at baseline, and APOE-ε4.

Subanalysis was conducted to evaluate differences in baseline metabolic and vascular risk factors for dementia/CIND by genotype using chi-square tests and t-tests that would indicate potential pathways that may mediate the relation between the Pro12Ala polymorphism and dementia/CIND. Factors evaluated were fasting glucose, fasting insulin, folate, homocysteine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, high-sensitivity C-reactive protein, systolic blood pressure, diastolic blood pressure, body mass index, waist circumference, obesity, stroke, hypertension, the diabetes symptom summary score, duration of diabetes, and treatment with diabetes medication.

Statistical analyses were performed using PC-SAS (version 9.1), and all reported *p* values are based on two-sided tests.

3. Results

The Ala allele frequency in the study population was 0.11. The percentage for each genotype was 79.1% (*n*=735) for Pro/Pro, 19.9% (*n*=185) for Pro/Ala, and 1.0% (*n*=9) for Ala/Ala. These frequencies did not differ from the expected frequencies under Hardy-Weinberg equilibrium ($\chi^2 = 0.50$, *df* = 1, *p* = 0.48).

During the follow-up period (mean: 6 years, range: 1–9 years), 106 (11.4%) of the study participants eligible for this analysis were diagnosed with either incident dementia (*n*=57) or CIND (*n*=49).

The characteristics of the study sample at baseline by genotype are shown in Table 1. There was little difference in age and education between the genotype groups. Fasting glucose levels were lower in the Ala+ group. Diabetes, history of stroke at baseline, and APOE-ε4 were moderately less prevalent in the Ala+ group. Females were more likely than males to be carriers of an Ala allele.

Table 2 shows the association between Pro12Ala and incident dementia/CIND in the total sample and stratified by baseline diabetes status and by sex. Although there was slight evidence of an interaction between baseline diabetes status and the genotype ($\chi^2 = 2.6$, *df* = 1, *p* = 0.10), the results presented are stratified by diabetes status because of meaningful differences in the estimated effect of the genotype on dementia/CIND rate between these two groups. The test of the interaction term between sex and genotype yielded $\chi^2 = 5.4$, *df* = 1, *p* = 0.02. There was no evidence of an interaction between the Pro12Ala genotype and ApoE-ε4 or age. In the total sample, there was a moderately increased rate of dementia/CIND for Ala+ carriers compared to non-carriers. This result was essentially unchanged after adjustment for education, APOE-ε4, and history of stroke at baseline (HR = 1.5, 95% CI: 0.90–2.4). Among diabetic participants, there was a doubling of the rate of dementia/CIND for Ala+ carriers compared to non-carriers. There was no attenuation of the estimate

after covariate adjustment (HR = 2.5, 95% CI: 1.3–4.9). There was little association of the Pro12Ala genotype with the rate of dementia/CIND among non-diabetic participants. Among males, there was more than a 3-fold increased rate of dementia/CIND for Ala+ males compared to Ala- males (adjusted HR = 3.3, 95% CI: 1.7–6.4). There was little association of the Pro12Ala genotype with the rate of dementia/CIND among females.

Table 3 shows the estimated crude and adjusted hazard ratios for the genotype-dementia/CIND association by the cross-classification of diabetes and sex. Although sample sizes are limited in each category, these results suggest an increased risk of dementia/CIND for diabetic and non-diabetic male carriers of Ala+. There was a moderate inverse relationship between Ala+ and dementia/CIND rate among non-diabetic females.

To investigate possible mechanisms for the strong association among diabetic males, we further examined differences in baseline metabolic and vascular risk factors for dementia/CIND by genotype (Table 4). There were no appreciable differences with respect to fasting glucose, folate, homocysteine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, high-sensitivity C-reactive protein, systolic blood pressure, diastolic blood pressure, body mass index, waist circumference, obesity, stroke, hypertension, or treatment with diabetes medications between the two genotype groups. Baseline fasting insulin levels were 50% higher in the male diabetic Ala carriers than in non-carriers. Addition of fasting insulin level (logged) to the Cox regression model (adjusted for education, APOE-ε4, and history of stroke at baseline) attenuated the hazard ratio for the genotype-dementia/CIND association from 4.2 to 3.1 (95% CI: 1.1–8.7). A one-unit increase in log insulin was associated with a near doubling of the rate of dementia/CIND (HR= 1.8, 95% CI: 0.96–3.6) in diabetic males. Diabetic male Ala carriers reported more diabetes-related symptoms but a shorter duration of diabetes than Ala non-carriers.

4. Discussion

In this cohort study of older Mexican Americans, there was an increased rate of dementia/CIND for carriers of the Ala allele in the Pro12Ala polymorphism among diabetic participants and among male participants but not among females. The inclusion of standard risk factors for cognitive impairment in the models did not result in a reduction in the estimated effect of the Ala allele.

A number of reports show that the Ala allele of the Pro12Ala polymorphism is associated with reduced incidence of type 2 diabetes (Altshuler, et al., 2000; Buzzetti, et al., 2004; Hara, et al., 2000). A moderately lower prevalence of diabetes at baseline was also observed for Ala carriers in this study population. Juxtaposed to this finding was an increased risk of dementia/CIND, higher baseline fasting insulin levels, more self-reported diabetes-related symptoms, and a shorter duration of diabetes in diabetic male carriers of the Ala allele. These data suggest that the Ala variant is associated with a reduced risk of diabetes, but that it may also be a prognostic factor for increased diabetes severity in Ala carriers that develop type 2 diabetes and may increase the risk of cognitive impairment in these individuals once diabetes has developed. These findings suggest that there may be separate mechanisms by which the Pro12Ala genotype affects cognitive impairment in late life: one mechanism operating through changes in glucose metabolism that results in a lower risk of diabetes and thus potentially reducing the risk of cognitive impairment, and another operating independently of glucose metabolism that results in a higher risk of cognitive impairment once diabetes has developed.

Several studies have reported an association between diabetic carriers of an Ala allele in the Pro12Ala polymorphism and increased severity of diabetes-related traits (Mori, H., et al.,

2001; Stefanski, et al., 2006; Zietz, et al., 2002). Diabetic male Ala carriers were reported to have higher total cholesterol and LDL-cholesterol levels compared to diabetic male non-carriers in a population-based sample of type 2 diabetic patients (Zietz, et al., 2002). Our results also show higher baseline total cholesterol and LDL-cholesterol levels in diabetic male Ala carriers although the differences were relatively small. We observed substantially higher baseline fasting insulin levels in diabetic male Ala+ individuals compared to Ala-diabetic males. Abnormalities in insulin metabolism have been reported in patients with Alzheimer's disease including higher plasma insulin levels, lower cerebrospinal fluid insulin levels, and reduced insulin-mediated glucose disposal compared with healthy controls (Craft, et al., 1999; Craft, et al., 1998; Razay and Wilcock, 1994). Hyperinsulinemia and insulin resistance are associated with increased risk for vascular disease (Laakso, 1996), which is a significant risk factor for stroke and closely correlated with cognitive decline and dementia. Other epidemiologic studies report a positive association between hyperinsulinemia and increased risk of cognitive impairment and dementia (Kalmijn, et al., 1995; Kuusisto, et al., 1997; Luchsinger, et al., 2004; Peila, et al., 2004).

The present study has several strengths including a longitudinal assessment of cognitive status. Dementia and CIND cases were diagnosed with a standard battery of cognitive tests and clinical evaluation. Information on known metabolic and vascular risk factors for dementia/CIND allowed for examination of the potential pathways that may mediate the relation between the Pro12Ala polymorphism and cognitive impairment.

A chief limitation of this study is the low frequency of dementia cases in the sample, which limited the examination of the effect of the Pro12Ala polymorphism of specific classifications of dementia such as Alzheimer's disease or vascular dementia, particularly in stratified analyses based on baseline diabetes status and gender. Also, we were unable to examine the association between changes in glucose metabolism over the study period and genotype. Further, these findings may not apply to other ethnic groups with different genetic and cultural influences.

Ala carriers who have developed type 2 diabetes and male Ala carriers may have greater risk of dementia/CIND. Additional study is needed to confirm a relation between the Ala variant of the Pro12Ala polymorphism and risk of dementia/CIND.

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Table 1

Baseline characteristics of the total sample by Pro12Ala genotype

Characteristic	Ala- (n=735)	Ala+ (n=194)	p-value [†]
Age, yrs, mean (SD)	69.5 (6.2)	70.0 (6.6)	0.33
Education, yrs, mean (SD)	8.0 (5.4)	8.4 (5.1)	0.33
Women, % (n)	56.6 (416)	66.0 (128)	0.02
Diabetes, % (n)	30.6 (225)	24.7 (48)	0.11
Stroke, % (n)	8.7 (64)	5.7 (11)	0.17
APOE-ε4, % (n)	18.2 (134)	13.4 (26)	0.11

[†] p-values are from chi-square tests for categorical data and t-tests for continuous variables

Table 2

Estimated crude and adjusted hazard ratios (HR) for carriers of the Ala allele and by baseline diabetes status and by sex from Cox proportional hazards regression models

Subgroups	Number of dementia or CIND cases/N	HR (95% CI)	p-value
Total sample			
Unadjusted	106/929	1.4 (0.90, 2.2)	0.13
Adjusted [*]		1.5 (0.90, 2.4)	0.06
Diabetes			
Unadjusted	46/273	2.2 (1.2, 4.2)	0.01
Adjusted [†]		2.5 (1.3, 4.9)	0.006
No diabetes			
Unadjusted	60/656	1.0 (0.56, 1.9)	0.92
Adjusted [†]		0.94 (0.49, 1.8)	0.86
Females			
Unadjusted	62/544	0.88 (0.47, 1.6)	0.67
Adjusted [‡]		0.90 (0.48, 1.7)	0.75
Males			
Unadjusted	44/385	2.7 (1.4, 5.2)	0.002
Adjusted [‡]		3.3 (1.7, 6.4)	0.0005

* Adjusted for age, education, sex, diabetes, APOE-ε4, and stroke

† Adjusted for age, education, sex, APOE-ε4, and stroke

‡ Adjusted for age, education, diabetes, APOE-ε4, and stroke

Table 3

Hazard ratios for carriers of the Ala allele by baseline diabetes status and sex from Cox proportional hazards regression models

	Number of dementia or CIND cases/N	HR (95% CI)	p-value
Subgroups			
Non-diabetic females			
Unadjusted	36/390	0.60 (0.25, 1.5)	0.26
Adjusted*		0.52 (0.21, 1.3)	0.17
Diabetic females			
Unadjusted	26/154	1.5 (0.63, 3.6)	0.36
Adjusted*		1.6 (0.66, 4.1)	0.28
Non-diabetic males			
Unadjusted	24/266	2.3 (0.92, 5.5)	0.07
Adjusted*		2.9 (1.1, 7.4)	0.02
Diabetic males			
Unadjusted	20/119	3.6 (1.4, 9.0)	0.008
Adjusted*		4.2 (1.5, 11)	0.005

* Adjusted for age, education, APOE-ε4, and stroke

Table 4

Baseline vascular and metabolic parameters in the subgroup of diabetic males at baseline by Pro12Ala genotype.

Characteristic	Ala- (n=101)	Ala+ (n=18)	p
Fasting glucose, mg/dL [†]	146	149	0.77
Fasting insulin, μ IU/mL [†]	9.3	13.8	0.03
Folate, ng/mL	517	521	0.92
Homocysteine, μ mol/L [†]	10.0	10.2	0.70
Cholesterol, mg/dL	199	210	0.32
HDL-cholesterol, mg/dL [†]	42	44	0.49
LDL-cholesterol, mg/dL	114	121	0.49
Triglycerides, mg/dL [†]	171	187	0.57
High-sensitivity C-reactive protein, mg/L [†]	2.3	2.7	0.68
Systolic blood pressure, mm Hg	142	138	0.52
Diastolic blood pressure, mm Hg	79	74	0.06
Body mass index, kg/m ²	29.6	30.7	0.28
Large waist circumference, % (n)	42 (40)	44 (8)	0.83
Obese, % (n)	40 (38)	41 (7)	0.90
Stroke, % (n)	14 (14)	11 (2)	0.75
Hypertension, % (n)	72 (73)	67 (12)	0.63
Treatment with diabetes medications, % (n)	61 (62)	61 (7)	0.98
Duration of diabetes, yrs	11.0	8.9	0.50
Diabetes symptom score [†]	4.7	5.5	0.53

Data are means unless otherwise stated

[†]Geometric mean reported due to skewness of variable

p-values are from chi-square tests for categorical data and t-tests for continuous variables