Apolipoprotein E polymorphism in type 2 diabetic patients of Talca, Chile

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Abstract

Apolipoprotein E (apo E) modulates the metabolism of atherogenic lipoprotein particles and participates in the process of cellular incorporation of specific lipoproteins. Genetic polymorphism of apo E has been reported as an important dyslipidemia genetic marker associated with coronary artery disease. Type 2 diabetes mellitus is a disease with a high incidence and prevalence in the world. The main cause of death in these patients is myocardial stroke and a high incidence of general cardiovascular complications. The purpose of this work was to characterize the genotype of apo E in diabetic patients from Talca, Chile, in order to describe the allelic frequency of the apo E gene and its correlation to the lipids profile. Type 2 diabetic patients (200) were recruited from the Diabetes Program of Talca Hospital, Chile. Apo E genotype was determined by restriction fragment-length polymorphism analysis. A biochemical characterization was performed in all the subjects. Type 2 diabetic patients had elevated levels of glycemia, lipid profile and BMI compared to the control group. The E3/3 genotype and ε3 allele had a higher frequency in both groups. The E2/3 and E3/4 genotypes had higher levels of triglyceride (TG) and cholesterol respectively; however, there was not any statistical relationship between them. In conclusion, genotype of apo E in diabetic patients did not differ with healthy; E2/3 and E3/4 genotypes tend to have higher levels of triglyceride and cholesterol respectively. We think that these results corroborate that in the etiology of the dyslipidemia, there is more than one associate genetic marker.

Keywords: Diabetes mellitus; Glycemia; Apolipoprotein E; Lipid profile

1. Introduction

Genetic polymorphism of apolipoprotein E (apo E) has been reported as an important dyslipidemia

Abbreviations: apo E, apolipoprotein E; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride

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genetic marker associated with coronary artery disease [1,2] and it is possible that the nephropathy in type 2 diabetes mellitus can be also associated with the polymorphism of the apo E. Apo E is a plasmatic protein that modulates the metabolism of atherogenic lipoprotein particles, mainly chylomicrons and very low-density lipoproteins (VLDL). One of the main apo E functions is to participate in the process of cellular incorporation of specific lipoproteins. Apo E acts as a high affinity ligand for several hepatic lipoprotein receptors, including the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP) [3].

Genes mutations coding for some of the apolipoproteins can result in either the absence or non-functional products, thereby producing genetic forms of dyslipidemia. Genetic polymorphism of apolipoprotein can contribute to the variation in lipoproteins concentration in the population and it can be involved in multifactorial dyslipidemia pathogenesis [4].

The apo E in mammals is synthesized in most of the organs and cells studied, mainly in liver, brain, spleen, kidney and macrophages. Its synthesis is regulated by a complex interaction of development, hormonal and dietary factors in hepatic and steroidogenic cells. However, the role of apo E in the pathogenesis of dyslipidemia is still not totally elucidated and requires further investigation. Studies in patients with different forms of dyslipidemia have demonstrated that the ε2 allele was associated with hypertriglyceridemia and ε4 allele was associated with hypercholesterolemia. The Framingham group reports [1] concluded that apo E alleles are important markers of dyslipidemia and cardiovascular disease. The estimated frequency of diseases associated with the ε4 allele was higher than any other lipid genetic abnormality [5,6].

Type 2 diabetes mellitus is a disease with a high incidence and prevalence throughout the world, and presents important chronic complications like retinopathy, neuropathy and nephropathy. However, the main cause of death in these patients is myocardial stroke and high incidence of cardiovascular complications, which have been associated with all classic factors of risk (hypertension, smoking and dyslipidemia). The characteristic pattern of dyslipidemia in these patients shows high levels of triglyceride (TG), mainly VLDL rich in triglyceride, and diminished levels of high-density lipoprotein (HDL) cholesterol. However, patients with type 2 diabetes mellitus have a higher prevalence of small and dense LDL particles, which are more susceptible to oxidation, therefore increasing atherogenic risk even when there is not a high concentration of LDL cholesterol [7,8].

Diabetes has been established as an independent cardiovascular risk factor, which is independent from atherosclerosis disease. This risk is two- to four-fold higher than in non-diabetic individuals and more than 50% of diabetic patients show evidence of cardiovascular disease even from early diabetes. Also, the risk of myocardial stroke and death by coronary disease in patients with type 2 diabetes mellitus is the same as in non-diabetic patients with a pre-existent coronary disease [9,10]. The identification of factors and/or classic risk markers, as genetic factors, have not significantly diminished cardiovascular prognosis, since other specific problem for diabetic patients is the high cardiovascular risk associated with terminal kidney disease [8]. Around 20–40% of the diabetic patients have diabetic nephropathy, being one of the main causes of morbidity and premature mortality in type 2 diabetes mellitus [1,11,12]. The purpose of this work was to characterize genotypically apo E in type 2 diabetic patients from Talca Regional Hospital of Chile in order to describe the allelic frequency of the apo E gene and its correlation to the lipid profile.

2. Patients and methods

2.1. Patients and controls

A total of 200 adult patients, 96 men and 104 women, aged 27–79 years, were selected from the Diabetes program of Talca Hospital (VII Region, Chile). All subjects chosen for this study had the following criteria: (1) diagnosed with type 2 diabetes mellitus; (2) not receiving hypolipidemic drugs in the 3 months previous to the study; (3) a length of evolution of disease more than 10 years; and (4) provided informed consents. All selected patients were subject to a controlled diet by a nutritionist, and an insulin treatment and/or oral hypoglycemic. A group of 139 healthy controls (aged 27–64 years) was also collected and analyzed for genotyping of apo E polymorphism and biochemical parameters. This latter group was included as comparison group for

apo E polymorphism frequency between diabetic patients and healthy individuals and they did not have important morbid antecedents and were part of a preventive health examination, which included medical checking and biochemical profiles.

2.2. General survey

In all patients and controls, a full general revision of antecedents was carried out, including arterial hypertension and nutritional state. Anthropometry data included measurement of height (using a stadiometer) and weight (using a digital balance). Biochemical characterization included: (1) glycemia, lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride) were measured enzymatically (Roche Laboratories, Mannheim, Germany); (2) glycosylated hemoglobin (HbA_{1c}) was measured according to kit manufacturing (Roche Laboratories, Mannheim, Germany). Samples of venous blood were collected from the patients after 12 h overnight fast. In those patients with severe diabetic decompensation (glycemia higher than 13.88 mmol/L), metabolic control was attempted in order to optimize, before lipid profile evaluation, which was not possible in seven cases and therefore they were excluded from this study.

2.3. Determination of apo E genotype

Genomic DNA was extracted from peripheral blood of each patient according to Lahiri and Nurnberger [13]. DNA samples were frozen to −20 °C until processed. Genotyping of three common alleles (ε2, ε3, ε4) of the apo E gene was performed according to Hixson and Vernier [14]. Briefly, genomic DNA was amplified by polymerase chain reaction (PCR) using the following primers: apo E 15′-TCCAAGGAGCTGCAGGCGGCGCA-3′ and apo E 25′-ACAGAATTCGCCCCGG-CCTGGTACACTGCCA-3′. Amplification mixture consisted of 200 ng of DNA, primer (1 μmol/L each), dNTP mix (0.25 mmol/L), 2.5 μL of 10× buffer (in mmol/L: 100 Tris–HCl, pH 8.0, 500 KCl, 15 $MgCl_2$), 10% DMSO and 1.25 U Taq DNA polymerase. The reaction mixture was subject to 40 cycles of 30 s at 94 °C, 30 s at 65 °C and 1.5 min at 70 °C. The final cycle was 10 min at 72 °C. The PCR product was digested with HhaI [15] at 37 °C for 2 h. The fragments obtained were electrophoresed through a 20% polyacrylamide non-denaturing gel for 6 h in a constant voltage of 150 V. After electrophoresis, the gel was stained with ethidium bromide and visualized by ultraviolet transillumination.

2.4. Statistical analysis

Variables were tested in duplicate. Continuous variables were expressed as the mean ± S.D. Logistic regression analysis (SAS System V8, 1999–2001, Cary, NC), one-way ANOVA and *t*-test were used to test differences in means. Frequencies and percentages described the non-parametric variables and the comparison among these groups was based on the χ^2 tests. A p value of <0.05 was considered to be statistically significant. Values for parameters that were not normally distributed were log-transformed for the ANOVA analysis.

3. Results

3.1. Clinical and laboratory characteristic

The final diabetic patients group consisted of 93 men and 100 women. As expected, body mass index (BMI), systolic blood pressure (SP), diastolic blood pressure (DP), glycemia and lipid profile were different between controls and type 2 diabetes mellitus groups ($p < 0.05$) (Table 1). BMI showed a highly overweight type 2 diabetes mellitus population for which the average was higher in females than for males ($p < 0.001$). HbA_{1c} levels were over the normal range (higher than 6.5%) in all the apo E genotypes. Given the selection criteria, age of controls was significantly lower than the patient group ($p < 0.01$) and the biochemical parameters were normal.

3.2. Genotypes and allelic frequency of apo E

No significant differences were observed in the distribution of apo E genotypes among the control and patient groups (Table 2). The frequency of apo ε3 allele was significantly higher in both group ($p < 0.01$) followed by apo ε4 and apo ε2 alleles, respectively. No E2/2 individuals were found and the presence of E2/3 and E3/4 was observed with higher frequency in controls than in diabetic patients, but they

Table 1
Clinical and laboratory characteristics of the control and diabetic patients

	Controls ($n = 139$)	Diabetic patients ($n = 193$)
Age (years)	37.8 ± 10.5	59.5 ± 10.4[a]
Sex (male/female)	35/104	100/93
BMI (kg/m^2)	22.4 ± 2.1	29.2 ± 4.8[b]
Systolic blood pressure (mm Hg)	122.1 ± 5.6	145.2 ± 20.4[b]
Diastolic blood pressure (mm Hg)	76.5 ± 4.7	82.2 ± 13.9[b]
Glycemia (mmol/L)	4.5 ± 0.5	9.5 ± 3.9[a]
Total cholesterol (mmol/L)	4.2 ± 0.6	5.5 ± 1.3[a]
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.1 ± 0.3[b]
LDL cholesterol (mmol/L)	2.7 ± 0.6	3.9 ± 1.2[a]
TG (mmol/L)	1.4 ± 0.1	2.4 ± 1.7[a]

Values are expressed in means ± S.D.; *t*-test.
[a] $p < 0.01$.
[b] $p < 0.05$.

Table 2
Genotypes and allelic frequency of apo E in type 2 diabetic patients and control group

	n	E2/2	E2/3	E2/4	E3/3	E3/4	E4/4	ε2	ε3	ε4
Diabetic patients (%)	193	0	12	4	133	43	1	16	321	48
		–	(6.2)	(2.0)	(68.9)	(22.1)	(0.5)	(4.1)	(83.4)	(12.5)
Controls (%)	139	0	10	3	87	39	0	13	223	42
		–	(7.2)	(2.2)	(62.6)	(28.0)	–	(4.7)	(80.2)	(15.1)

were not statistically different ($p > 0.05$). The only patient carrying the E4/4 genotype was excluded from the statistical analysis.

3.3. Biochemical characteristics in diabetic patients related to apo E genotype

BMI indicate that 86.5% of the patients were obese, 12.4% overweight and only 1.0% had a normal BMI (Table 3). However, no difference was observed in the BMI among apo E genotypes. Patients carrying the E2/3 genotype had a higher level of TG and 50% of them had a level over 2.29 mmol/L; however, there were no differences between the apo E genotypes ($p > 0.05$). Individuals from E3/4 diabetic group had higher levels of total cholesterol (66% over 2.20 mmol/L) and LDL cholesterol. Logistic regression analysis between ε3 and ε2 or ε4 in all continuous variables (glycemia, lipid profiles, PS, PD, BMI) indicated that the glycemia ($p < 0.01$) and BMI

Table 3
Biochemical characteristics in type 2 diabetic patients related to apo E genotype

	E2/3	E2/4	E3/3	E3/4	E4/4
N (male/female)	12 (3/9)	4 (2/2)	133 (65/68)	43 (22/21)	1 (0/1)
Age (years)	60 ± 9	60 ± 8	59 ± 11	60 ± 9	48
BMI (kg/m^2)	27 ± 3	32 ± 4	30 ± 5	31 ± 5	32
Glycemia (mmol/L)[a]	11.7 ± 5.3	13.6 ± 6.3	8.8 ± 3.6	10.4 ± 4.2	14.8
HbA_{1c} (%)	9.6 ± 2.9	8.6 ± 2.9	9.0 ± 3.0	9.0 ± 3.0	11.2
Total cholesterol (mmol/L)	5.5 ± 1.3	5.0 ± 0.5	5.4 ± 1.4	5.8 ± 1.0	5.7
HDL cholesterol (mmol/L)	1.1 ± 0.4	1.3 ± 0.3	1.1 ± 0.3	1.0 ± 0.3	1.0
LDL cholesterol (mmol/L)	3.2 ± 1.0	3.5 ± 0.7	3.8 ± 1.3	4.2 ± 1.0	3.6
TG (mmol/L)	3.3 ± 3.3	1.7 ± 0.4	2.2 ± 1.6	2.5 ± 1.6	2.6

Means ± 1 S.D. Only one patient presented E4/4 genotype.
[a] ANOVA; $p < 0.05$.

Table 4
Allelic distribution (%) of apo E in type 2 diabetes mellitus, controls and other world populations

	ε2	ε3	ε4
Diabetics	4.1	83.4	12.5
Controls	4.7	80.2	15.1
Finns [4]	2.9	75.0	22.1
Germans [4]	7.7	77.3	15.0
Chinese [4]	12.2	78.2	9.6
Japanese [4]	8.1	84.9	6.7
Japanese [17]	4.6	84.9	10.5

χ^2: (A) p = NS between diabetics patients and controls; (B) p = NS between diabetics patients and other populations.

($p < 0.05$) are the best risk indexes and indicate that their levels are higher in E2 and E4 genotypes.

3.4. Allelic distribution of apo E in type 2 diabetic patients

The allelic distribution was different between ε2, ε3 and ε4 alleles (one-way ANOVA, $p < 0.01$). However, when these frequencies were compared with other world populations, there were no significant differences between them (Table 4).

4. Discussion

Many studies have been carried out in different populations, but none in Latin America or Chile, concerning the genetic polymorphism of apo E and its relation with different pathologies like Alzheimer, cardiovascular disease and diabetes mellitus. In this study, the diabetic patients and controls were mainly Caucasian, with a minimal indigenous influence (absence of one or two mapuche last names and physical characteristics) [16]. In general, Chilean population has healthy nutritional habits, under Mediterranean influences. The population has a regular intake of vegetables, legumes, fish and vegetable oils. In Chile, fast food is very common in big cities; however, this is not the case in small cities like Talca. In order to control the diet effect over dyslipidemia expression, the diabetic patients were under nutritionist supervision, following the recommendations of the Chilean Ministry of Health.

We observed that ε3 allele is the most frequent, followed by ε4 and ε2, in diabetic patients and control groups, which agrees with other studies that have been carried out in Finn, German, Chinese and Japanese population [4]. Considering the genotype of all diabetic and control patients as a whole, we did not found statistical differences between them and populations previously described [4,17].

The genetic polymorphism of apo E has been reported as an important genetic marker of dyslipidemia. In our study, E3/4 genotype was related with hypercholesterolemia and E2/3 genotype with hypertriglyceridemia in diabetic patients. The role of apo E in the regulation of plasma triglyceride levels is well documented [18]. Clinical studies have shown that plasmatic concentrations of apo E account for 20–40% of the variability of triglyceride levels. Apo E-deficient mouse hepatocytes have impaired secretion of VLDL triglycerides, suggesting another physiological role for apo E in the VLDL assembly-secretion process [15]. Eto et al. [19] proposed that an increased level of triglyceride-rich lipoproteins and the accumulation of lipoprotein remnants (alterations that are linked to ε2 allele) contribute to renal damage. Kimura et al. [20] proposed a possible protective effect of ε4 allele, which could be related to the fact that part of the apo E is synthesized in the kidney.

Although our results show that a diabetic population has a marked dyslipidemia, with 51.8% of hypercholesterolemia, 54.0% of hypertriglyceridemia and 37.3% of mixed dyslipidemia, it was not possible to establish any statistical relationship between the dyslipidemia and the genotype of apo E. This study could corroborate that in the etiology of the dyslipidemia, there is more than one associate genetic marker. However, we were able to demonstrate that glycemia levels and BMI are good index risk predictors in type 2 diabetes mellitus patients. Although we did not found statistical differences in

lipid profile and apo E genotype, it is interesting to know the prevalence of apo E polymorphism in Chilean diabetic patients and to correlate in the future these results with the presence of nephropathy in these patients.

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