TP53 Codon 72 Polymorphism Is Associated with Coronary Artery Disease in Chilean Subjects

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Key Words

Coronary artery disease • Gene polymorphism • TP53

Abstract

Objective: To investigate the possible association between the codon 72 polymorphism (Pro72Arg, rs1042522) of the tumor suppressor gene (*TP53*) and the presence of coronary artery disease (CAD) in Chilean subjects. **Subjects and Methods:** A total of 209 unrelated patients with a diagnosis of CAD confirmed by angiography (33–74 years old) and 216 healthy controls (30–68 years old) were included in this study. The Pro72Arg polymorphism of the *TP53* gene was evaluated by PCR-RFLP. **Results:** The genotype distribution for the Pro72Arg variant of the *TP53* gene in CAD patients (PP: n = 13, 6.2%; PR: n = 61, 29.4%; RR: n = 135, 64.6%) and controls (PP: n = 18, 8.3%; PR: n = 94, 43.5%; RR: n = 104, 48.1%) was significantly different (p = 0.003). Similarly, the allelic frequency was also different (p = 0.003). The odds ratio for CAD related to the 72Arg allele was 2.0 (95% CI = 1.33–2.90), confirming the presence of an association. **Conclusion:** These findings suggest that the Pro72Arg polymorphism of the *TP53* gene is associated with CAD in Chilean individuals.

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Introduction

The molecular basis of coronary artery disease (CAD) is currently being investigated. Different genes have been implicated in the etiology of CAD [1, 2], including the *TP53* gene [3]. The principal function of the *TP53* gene is to guard cells against malignant transformation, prevent the proliferation of cells containing damaged DNA, and if repair is not possible, activate the mechanism by which tissues remove unwanted, aged, or damaged cells through the apoptotic pathway [4]. This mechanism has been related to the etiology of atherosclerotic lesion [5]; however, the role of *TP53* is not completely understood.

The *TP53* gene functions as an antioncogene, associated with upregulation of apoptosis [3]. *TP53* wild type accumulates in atherosclerotic tissue and may mediate the apoptosis of vascular cells [6], and hence could regulate cell division and apoptosis within atherosclerotic plaque depending on the cell type and level of *TP53* expression induced by DNA damage. Mutations that affect the *TP53* gene can induce dysfunction of p53 and inhibit apoptosis and it has been hypothesized that loss of *TP53* gene activity could play a relevant role in the pathogenesis of atherosclerosis [5]. A common polymorphism in

the *TP53* gene, Pro72Arg (rs1042522), results in the occurrence of arginine (Arg) or proline (Pro) at codon 72 in the aminoacidic sequence of the protein. This polymorphism has been associated with various types of cancer [7–9] and lupus erythematosus [10]. Recently, the possible association with cardiovascular disease was also investigated [11–13]. However, the results are contradictory. In Chile, CAD is one of the main causes of death [14], but information related to the genetic basis of CAD in Chile is insufficient [15, 16]. Thus, the aim of the present study was to evaluate the possible association between Pro72Arg gene polymorphism of the *TP53* gene and CAD in Chilean subjects.

Subjects and Methods

Subjects

The Pro72Arg polymorphism was studied in 209 unrelated Chilean patients, aged 33–74 years, admitted to the Cardiology Service of the Hernán Henríquez Hospital of Temuco City, Chile, with angiographically determined coronary artery stenosis >70%. The control group consisted of 216 unrelated individuals, aged 30–68 years, from Temuco City. We used a structured questionnaire to identify disease-free controls and to exclude subjects who were suspected of having any form of vascular disease. Controls with a familial history of CAD, determined by interviewing, were excluded from the study. Demographic data and history of hypertension, diabetes mellitus, cigarette smoking, and hypercholesterolemia were assessed in each subject. In both groups, there was no preselection of serum lipid concentrations. Subjects with a history of diabetes or basal glycemia ≥ 126 mg/dl were defined as diabetic. The study protocol was approved by the Ethics Committee of our university, and all subjects gave written informed consents.

Serum Measurements

Biochemical measurements were determined from blood samples collected by venipuncture after overnight (>12 h) fast. Serum glucose, uric acid, triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic methods previously described [15]. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation when the triglyceride concentrations did not exceed 400 mg/dl.

DNA Analysis

Genomic DNA was extracted from blood leukocytes using the salting-out method optimized by Salazar et al. [17]. The Pro72Arg polymorphism of the *TP53* gene was detected using polymerase chain reaction followed by enzymatic restriction according to conditions described by Smith et al. [11]. The correct assessment of genotypes for the Pro72Arg polymorphism of the *TP53* gene was evaluated using a homozygous sample for the restriction site as a positive control. In addition, all gels were reread blindly by 2 persons (J.C. and L.S.) without any change, and 10% of the analyses were repeated randomly.

Table 1. Demographic and clinical characteristics of subjects with CAD (cases) and controls

	Cases (n = 209)	Controls (n = 216)	p
Age, years	62 $\pm$ 10	42 $\pm$ 8	<0.001
Male, %	64	56	0.109
Diabetes, %	32	5	<0.001
Systolic blood pressure, mm Hg	140 $\pm$ 26	125 $\pm$ 23	<0.001
Diastolic blood pressure, mm Hg	80 $\pm$ 18	75 $\pm$ 9	0.020
Body mass index, kg/m ²	28.2 $\pm$ 4.5	25.8 $\pm$ 4.5	<0.001
Smoking, %	64	41	<0.001
Hypercholesterolemia, %	80	20	<0.001
Hypertension, %	79	26	<0.001
Angina, %	77	0	<0.001
AMI, %	66	0	<0.001
Familial history of CAD, %	32	0	<0.001
Number of diseased vessels, %			
Single vessel	31	0	–
Double vessel	28	0	–
Triple vessel	41	0	–
Total cholesterol, mg/dl	214 $\pm$ 51	175 $\pm$ 33	<0.001
LDL-C, mg/dl	127 $\pm$ 42	95 $\pm$ 27	<0.001
HDL-C, mg/dl	32 $\pm$ 8	49 $\pm$ 12	<0.001
Triglycerides, mg/dl	181 $\pm$ 92	114 $\pm$ 86	<0.001
Fasting glucose, mg/dl	113 $\pm$ 42	94 $\pm$ 37	<0.001
Uric acid, mg/dl	5.6 $\pm$ 1.6	4.5 $\pm$ 1.5	<0.001

AMI = Acute myocardial infarction. p values from Student t test or χ^2 test.

Statistical Analysis

Statistical analysis was carried out using the Sigma Stat Software, version 2.0 (Jandel Sci., San Rafael, Calif., USA). Data are presented as mean $\pm$ SD. Differences between the means of continuous variables were evaluated by Student t test or one-way ANOVA. The allelic frequencies and genotype distribution were estimated by gene counting. Differences between noncontinuous variables and Hardy-Weinberg equilibrium were tested by χ^2 analysis. The odds ratio (OR) and 95% confidence interval (CI) associated with the mutated 72R allele were also calculated. Statistical significance was set at $p < 0.05$.

Results

Clinical Variables

The clinical, anthropometric and laboratory characteristics of the study subjects are given in table 1. The CAD subjects had elevated values of body mass index and a higher prevalence of traditional risk factors, including smoking, diabetes, hypertension, hypercholesterolemia and familial history of CAD ($p < 0.001$). The baseline se-

Table 2. Genotype distribution and relative allele frequencies of the Pro72Arg polymorphism of *TP53* in Chilean subjects with CAD (cases) and controls

	Genotype			Allele	
	PP	PR	RR	P	R
Cases (n = 209)	13 (6.2%)	61 (29.2%)	135 (64.6%)	0.208	0.792
Controls (n = 216)	18 (8.3%)	94 (43.5%)	104 (48.1%)	0.301	0.699
	$\chi^2 = 11.74$; 2 d.f.; p = 0.003 ^a			$\chi^2 = 9.14$; 1 d.f.; p = 0.003	
d.f. = Degree of freedom. ^a Power of performed test with $\alpha = 0.050$: 0.888.					

rum concentrations of triglycerides, total cholesterol, LDL-C, glucose and uric acid were higher in CAD patients than controls ($p < 0.05$). Similarly, the CAD subjects exhibited elevated values of systolic and diastolic blood pressure ($p < 0.05$). In addition, CAD subjects presented lower HDL-C concentrations ($p < 0.001$).

Allele Frequencies

The genotype distribution and the relative allele frequency of the Pro72Arg polymorphism of the *TP53* gene in CAD patients and controls are given in table 2. The genotype distribution was as expected from the Hardy-Weinberg equilibrium for CAD and control subjects (CAD, $\chi^2 = 2.743$, $p = \text{NS}$ and controls, $\chi^2 = 0.255$, $p = \text{NS}$), similar to previous data observed in a Chilean adult population sample [8, 9]. The genotype distribution for the Pro72Arg polymorphism in CAD patients and controls was significantly different ($p = 0.003$). Moreover, the relative allelic frequency was different in both groups ($p = 0.003$). The OR for CAD related to the 72Arg allele was 2.0 (95% CI = 1.33–2.90) confirming the presence of association. However, we did not find any association between traditional risk factors for CAD and the different genotypes of the Pro72Arg polymorphism of the *TP53* gene (table 3). In addition, a comparison of studies involving different populations with our study is given in table 4.

Discussion

Several genes have been related to the etiology of CAD [1, 2], including the tumor suppressor gene *TP53* [18]. A common polymorphism in *TP53*, Pro72Arg, has been the focus of analysis in CAD; nevertheless, the results were discordant [11, 12]. Thus, in the present study,

Table 3. Clinical and demographic characteristics of subjects with CAD according to Pro72Arg genotypes of the *TP53* gene

	PP (n = 13)	PR (n = 61)	RR (n = 135)	p
Age, years	60 ± 13	62 ± 12	63 ± 11	0.425
Body mass index, kg/m ²	27 ± 4.6	28 ± 5.1	28 ± 4.4	0.937
SBP, mm Hg	127 ± 23	138 ± 24	142 ± 27	0.421
DBP, mm Hg	75 ± 13	74 ± 11	74 ± 9	0.976
Diabetes, %	32	35	28	0.674
Hypercholesterolemia, %	80	79	81	0.911
Hypertension, %	79	80	78	0.913
Number of diseased vessels, %				
Single vessel	30	34	30	
Double vessel	30	34	19	0.090
Triple vessel	40	32	51	
Total cholesterol, mg/dl	199 ± 45	200 ± 41	206 ± 28	0.937
LDL-C, mg/dl	128 ± 23	130 ± 34	135 ± 15	0.915
HDL-C, mg/dl	29 ± 9	32 ± 8	33 ± 8	0.520
Triglycerides, mg/dl	216 ± 100	184 ± 169	181 ± 49	0.809
Fasting glucose, mg/dl	111 ± 49	108 ± 30	115 ± 47	0.785
Uric acid, mg/dl	5.6 ± 1.2	5.8 ± 1.9	5.5 ± 1.5	0.782

DBP = Diastolic blood pressure; SBP = systolic blood pressure. p values from one-way ANOVA or χ^2 test.

we have investigated the possible association between the Pro72Arg polymorphism and CAD in Chilean individuals. The analysis of clinical and laboratory parameters in both groups showed that serum concentrations of total cholesterol, LDL-C, triglycerides, glucose and uric acid were higher in CAD than control subjects. In addition, the HDL-C concentrations were lower in the CAD group. These results show a significant association between these traditional risk factors and CAD as previously reported [19].

Table 4. Comparison of different studies of the Pro72Arg polymorphism of the *TP53* gene

Reference	Study design	Cases/ controls	Ethnicity	CVD diagnosis	Frequency of 72R, cases/controls	Association with CVD
Smith et al. [11]	case-control ¹	383/56	diverse ²	diverse ³	0.693/0.759	no
Alkhalaf et al. [12]	case-control ⁴	158/110	Kuwaiti	CAD ⁵	0.478/0.532	no
Manfredi et al. [13]	case-control ⁶	180/70	Italian	CAD ⁷	0.722/0.742	no
Our study	case-control ⁸	209/216	Chilean	CAD ⁹	0.792/0.699	yes

CVD = Cardiovascular disease.

¹ Matched by age.

² The cases group was composed of individuals of European (89.2%), Japanese (3.3%), Middle Eastern (1.81%), and mixed and/or other origin (5.70%). The mean age of this population cohort was 79.80 ± 5.32 years (range: 66–97 years). The elderly control sample was composed of 89.3% European, 5.4% Japanese, 1.8% Afro-Brazilian, and 3.5% mixed origin subjects.

³ Subjects were considered positive for cardiovascular disease when they self-reported previous myocardial infarction and/or

coronary heart disease, cerebrovascular disease and/or transitory ischemic attacks and were also taking specific medication prescribed by physicians.

⁴ Matched by age and gender.

⁵ CAD was confirmed by cardiac catheterization.

⁶ Not matched by gender.

⁷ CAD documented by angiography.

⁸ Matched by gender.

⁹ CAD documented by angiography (coronary artery stenosis >70%).

Our findings also exhibit a significant association between CAD and the 72R allele (Arg72 variant) of the Pro72Arg polymorphism of the *TP53* gene (OR = 2.0, 95% CI = 1.33–2.90). The frequency of the R allele was higher in CAD subjects than controls (0.79 vs. 0.69, $p = 0.003$) similar to the study by Kojima et al. [20], in which there was a significant association between the homozygous Arg/Arg genotype and coronary luminal narrowing. Furthermore, Bonafé et al. [21] showed that individuals carrying the Arg variant exhibited higher levels of CK-MB and troponin I, both biochemical markers of myocardial injury as they had previously shown that the 72Arg variant is associated with restenosis after angioplasty [22]. Accordingly, the *TP53* codon 72 polymorphism seems to contribute to a genetically determined variability in atherosclerosis. However, Manfredi et al. [13], Smith et al. [11] and Alkhalaf et al. [12] did not find any association between the Pro72Arg polymorphism and the occurrence of CAD in Italian, Brazilian and Kuwaiti subjects, respectively, but Manfredi et al. [13] and Smith et al. [11] detected an association between the 72Arg allele and lower levels of HDL-C. The reasons for these discrepancies include the differences in the study design, the definition of inclusion and exclusion criteria, the number of participants, and the different ethnicities of subjects as show in table 4.

In relation to the possible mechanisms that can explain our findings, it is important to mention that the Pro72Arg polymorphism affects the proline-rich domain

of p53, a sequence necessary for the transmission of anti-proliferative signals, and it has been suggested that the 2 alleles may produce functionally distinct proteins with differences in their capacity to activate gene expression [23]. Dumont et al. [24] established that the 72Arg variant is more efficient in inducing apoptosis, probably caused by a better interaction with the p53 binding protein homolog (MDM2), facilitating the nuclear exportation and mitochondrial localization of p53. In addition, the Pro72Arg polymorphism has been associated with interindividual variation in apoptotic response. In atherosclerotic plaque, reactive oxygen species are responsible for DNA damage [5], and a single rupture in a double-stranded DNA molecule may be sufficient to prompt a rise in levels of p53 [25]. Apoptosis in endothelial cells have been related to plaque instability and thrombus formation [26]. A disturbance in the apoptotic response may lead to accumulation of intimal cells through atherogenesis [5]. The functional consequence of the Pro72Arg polymorphism has been related to inhibition of p73 function, a member of the p53 family of nuclear transcription factors, implicated in tumor suppression [27]. The Arg polymorphic allele is more efficient in binding to p73, blocking its action and facilitating the proliferation of vascular smooth muscle cells [27, 28].

Based on the above mechanism, it is probable that CAD individuals carrying the Arg variant of the *TP53* Pro72Arg polymorphism are more susceptible to suffer deregulation of apoptosis during atherosclerosis progres-

sion, and consequently, promote the development of CAD. This explanation is similar to that offered for the Pro72Arg polymorphism in the different types of cancers, particularly cervical and lung cancers [8, 9, 29]. Interestingly, the allelic frequency for the Pro72Arg polymorphism of *TP53* observed in 60 Chilean patients with cervical cancer [8] and in 111 patients with lung cancer [9] was similar to that in patients with CAD evaluated in our study. According to Ojeda et al. [8], the world distribution of this polymorphism reflects ancient human migration routes, more specifically the dispersal of modern *Homo sapiens* out of Africa some 100,000–150,000 years ago. The high frequency of the 72Arg allele in Central and South America contradicts the hypothesis of Beckman et al. [30] that the world distribution of this polymorphism reflects an adaptation to ultraviolet radiation. Thus, the high frequency of this polymorphism in Chile can be explained, at least in part, by the present results and why no such associations were found in other countries.

Conclusion

The present study showed an association between CAD and *TP53* codon 72 polymorphism in Chilean subjects indicating that this genetic variation could be a genetic marker for CAD in Chile. However, this study is restricted by the use of controls not matched by age. In addition, this observation needs to be replicated in other population groups.

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