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A Delta-Sarcoglycan Gene Polymorphism as a Risk Factor for Hypertrophic Cardiomyopathy

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Background: The C allele of c.−94C>G polymorphism of the delta-sarcoglycan gene was associated as a risk factor for coronary spasm in Japanese patients with hypertrophic cardiomyopathy (HCM). *Aim:* We evaluated whether the c.−94C>G polymorphism can be a risk factor for HCM in Mexican patients. *Methods:* The polymorphism was genotyped and the risk was estimated in 35 HCM patients and 145 healthy unrelated individuals. Data of this polymorphism reported in Mexican Amerindian populations were included. *Results:* The C allele frequency in HCM patients was higher with an odds ratio (OR) of 2.37, and the risk for the CC genotype increased to 5.0. The analysis with Mexican Amerindian populations showed that the C allele frequency was significantly higher in HCM patients with an OR of 2.96 and for CC genotype the risk increased to 7.60. *Conclusions:* The C allele of the c.−94C>G polymorphism is a risk factor for HCM, which is increased by the Amerindian component and can play an important role in the etiology and progression of disease in Mexican patients.

Introduction

Hypertrophic cardiomyopathy (HCM) is the most frequent genetic cardiovascular disease; it has an autosomal dominant inheritance and a mortality of 1%–2% per year (Maron *et al.*, 1995). It affects 1 in 500 (0.2%) individuals in the general population, however, this prevalence could be underestimated because many individuals are asymptomatic or do not present the obstructive form and, consequently, are not subject to clinical investigation or referred for an echocardiographic study (Richardson *et al.*, 1996). Therefore, there is a need for studies at the clinical and basic levels to develop new strategies for the diagnosis, treatment, and prevention of this disease.

In HCM, the heart muscle shows an increase in left ventricular mass, which may progress to cardiac dilatation and functional insufficiency and, both myofibrils and cardiomyocytes are disorganized. The consequences of this disorder are the presence of arrhythmias, diastolic dysfunction, myocardial infarction, infective endocarditis, and sudden cardiac death (Maron *et al.*, 2003b; Poliac *et al.*, 2006). Mutations in different genes have been implicated in the etiology of HCM such as those that encode for sarcomeric proteins (50%–70% of cases) and cellular cytoskeleton (Tsubata *et al.*, 2000; Arad *et al.*, 2002); others are involved in energy metabolism (Gollob *et al.*, 2001; Palau, 2001; Antonicka *et al.*, 2003) and mitochondrial bioenergetics (Marín-García and Goldenthal, 2002).

The delta sarcoglycan protein (d-SG) has been associated with limb-girdle muscular dystrophy Type 2F (LGMD2F), dilated cardiomyopathy (DCM), and recently with HCM (Sakamoto *et al.*, 1997; Ozawa *et al.*, 1998; Honda *et al.*, 2007). The d-SG is located in the sarcolemma membrane, assembled into the sarcoglycan complex that is responsible for providing stability to the plasma membrane of the cytoskeleton and to support the association between dystrophin and dystroglycan proteins (Ozawa *et al.*, 2005). Moreover, d-SG is also located in the terminal cistern of the sarcoplasmic reticulum of skeletal muscles independently of dystrophin; thus, it has been suggested that this protein could be involved in calcium regulation (Ueda *et al.*, 2001).

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The polymorphism c.−94C>G of the delta-sarcoglycan gene (SGCD) was associated with a significant risk factor for coronary spasm in Japanese patients with HCM (Honda *et al.*, 2007). A previous report shows that the distribution of the C allele of this polymorphism in Mexican Mestizo and Mexican Amerindian populations was higher than in Asians and lesser than in African and European populations, suggesting that this may play an important role in HCM of Mexican patients (Ordoñez-Razo *et al.*, 2010). In the present study, we evaluated whether c.−94C>G polymorphism may be associated as a risk factor for HCM in Mexican patients.

Material and Methods

Study subjects

The c.−94C>G polymorphism was analyzed in 35 patients with HCM and 145 healthy individuals (controls). The patients were aged 30–70 years and the diagnosis of HCM was corroborated by Doppler echocardiography. Patients were selected if they presented two major criteria or one major and one minor (Maron *et al.*, 2003a) in the absence of other structural alteration. The control group was comprised of 145 healthy unrelated individuals aged 30–70 years. Patients and controls, as well as their parents and grandparents, were born in Mexico. The participants provided written informed consent prior to their inclusion in the study, which was approved by the Ethics Committee in Investigation in Health, National Medical Center XXI Century, Mexican Institute of Social Security.

Data reported by our group on the frequency of the c.−94C>G polymorphism in Mexican Amerindian populations (23 Triquis, 24 Mayas, 25 Zapotecos, 41 Nahuas, and 52 Mixtecos) were included in the analysis.

DNA extraction and c. −94C> G genotyping assays

Genomic DNA was extracted from 5 ml of peripheral blood of all samples using a QuickGene DNA whole blood kit (Fujifilm Life Science, Tokyo, Japan). The c.−94C>G polymorphism was genotyped using real-time polymerase chain reaction (PCR) allelic discrimination by TaqMan assay (C_26840118_10; TaqMan SNP Genotyping Assays, Applied Biosystems, Foster City, CA) on an ABI Prism 7900HT (Applied Biosystems, Foster City, CA). Real-time PCR was initiated for preincubation for 10 min at 95°C followed by 40 cycles of 15 s at 92°C and 1 min at 60°C. Genotyping call rate exceeded 95% and no discordant genotypes were observed in 50 duplicate samples.

Statistical analysis

Continuous variables were compared by 2-tailed unpaired *t*-tests and categorical variables were compared by chi-squared analysis and Fisher's exact probability. The analysis between risk of HCM and c.−94C>G polymorphism was estimated using odds ratio (OR) and their 95% confidence intervals (CIs). Genotype distributions were tested for deviation from Hardy–Weinberg equilibrium in the groups. Allele and genotype frequencies were compared using the χ^2-test; $p < 0.05$ was considered statistically significant.

Results

This study included 35 patients with HCM (13 females and 22 males) with an average age of 50.49 ± 15.5 years and 145 normal controls (95 females and 50 males) with an average age of 47.48 ± 8.78 years (Table 1). The differences in clinical parameters of HCM patients and controls were analyzed and only gender was statistically significant ($p = 0.0039$, Table 1). However, when the gender was compared with the genotypes of the polymorphism no significant differences were found between healthy women and those with HCM ($p = 0.094$; Table 2) and between healthy men and those with HCM ($p = 0.085$; Table 2). Allele and genotype distributions of the c.−94C>G polymorphism were analyzed in both groups by real-time PCR. The risk was estimated using OR (95% CI) and genotype distributions were tested for deviation from Hardy–Weinberg equilibrium (Table 3). Allele frequencies of allele C in the HCM patients were significantly higher than that in controls ($p = 0.00274$) with an OR of 2.37 (Table 3). The analysis of genotype frequencies showed that the CC genotype was more frequent in the cases than in controls (Table 3). Moreover, the CG- and GG genotypes frequencies were less in cases than in controls and when the risk was analyzed, the CC genotype presented the higher risk for HCM (OR = 5.008; $p = 0.00907$) compared with the GG genotype (Table 3).

To analyze whether the Amerindian ancestry of the Mexican population could play an important role in the risk of HCM we compared the allele frequencies for HCM patients with data for this polymorphism previously reported by our group (Ordoñez-Razo *et al.*, 2010). This analysis showed that C allele was more frequent in HCM patients, than that in the Mexican Amerindians with a significant difference ($p = 0.00012$; Table 3). The risk of the C allele was high when HCM patients were compared with Mexican Amerindian populations (OR = 2.96; Table 3). However, the risk was increased to 7.60 when the analysis was performed with the CC genotype.

Discussion

Mutations in SGCD are associated with LGMD-2F, DCM, and HCM; however, the pathogenesis of the HCM is uncertain (Nigro *et al.*, 1996; Politano *et al.*, 2001). In the Japanese

Table 1. Clinical Parameters in Controls and Hypertrophic Cardiomyopathy Patients

Parameter	*Controls* (n = *145*)	*HCM* (n = *35*)	p-*Value*
Age (years)	47.48 ± 8.78	50.49 ± 15.5	0.13
Gender (F/M)	95/50	13/22	**0.0039**
Hypertension (%)	0 (0)	0 (0)	–
Total Cholesterol (mg/dL)	208.90 ± 38.01	206.43 ± 36.41	0.73
HDL-C (mg/dL)	43.32 ± 12.87	41.71 ± 13.23	0.51
LDL-C (mg/dL)	127.41 ± 34.86	122.34 ± 37.16	0.45
Triglycerides (mg/dL)	185.83 ± 119.50	218.31 ± 172.85	0.19
BMI (kg/m^2)	26.65 ± 4.56	27.58 ± 4.65	0.28
DM (%)	0 (0)	0 (0)	–

Bold *p*-values indicate statistically significant results.

HCM, hypertrophic cardiomyopathy; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; BMI, body mass index; DM, diabetes mellitus.

Table 2. Gender and Genotype Frequencies of c.−94C>G Polymorphism in Controls and Hypertrophic Cardiomyopathy Patients

	Controls, n *(%)*				*HCM*, n *(%)*				
	n	*CC*	*CG*	*GG*	n	*CC*	*CG*	*GG*	p-*Value*
Female	95	26 (27)	49 (52)	20 (21)	13	6 (46)	7 (54)	0 (0)	0.094
Male	50	16 (32)	19 (38)	15 (30)	22	13 (59)	6 (27)	3 (14)	0.085

population it was suggested that the C allele of the c.−94C>G polymorphism of the SGCD may play a role in HCM (Honda *et al.*, 2007). The distribution of the C allele in Mexican populations was higher than in Japanese populations, and therefore this may suggest that this is related to HCM in Mexican patients (Ordoñez-Razo *et al.*, 2010).

In this study we analyzed the role of the C allele as a risk factor in Mexican patients with HCM and the results showed that they are at very high risk for HCM when compared with controls (OR = 2.37; $p = 0.00274$) and at even higher risk when compared with Mexican Amerindian populations (OR = 2.96; $p = 0.00012$). A similar risk was shown in Japanese patients with HCM and coronary spasm (OR = 3.1; CI = 1.0–9.5 and $p = 0.045$) when compared with patients with HCM but not coronary spasm (Honda *et al.*, 2007). The high risk of the C allele observed in Amerindian ethnic groups could imply an increased susceptibility of HCM in them. Reports on the genetic admixture of Mexican Mestizos (controls) have estimated a high proportion (69%) of Amerindian component (Juárez-Cedillo *et al.*, 2008), which may suggest that Amerindian ancestry contributes to an increased risk for HCM. Although no differences were found in allele frequency among Amerindian populations, it is important to note that these are highly diverse, because Triquis, Mayas, Zapotecos, Nahuas, and Mixtecos were included in the study. Therefore, it is necessary to perform further studies with the aim to analyze potential biomarkers associated with risk factors for these populations, which have not been sufficiently studied.

Although in this study it was only possible to analyze a small size of patients with HCM, the differences are significant enough to suggest that the polymorphism of the SGCD could be important as a risk factor for HCM in Mexican patients and consequently in the future could be used as a risk marker for this disease. In this sense and due to the fact that the risk for HCM is higher when the CC genotype is present (OR = 5.0 for controls and OR = 7.6 for Amerindians), it is important to make the distinction between heterozygous- and homozygous alleles if the polymorphism is used as a genetic risk marker.

In the animal model, hamster BIO14.6, it has been observed that the deficiency or absence of the protein d-SG due to deletions or mutations in the *SGCD* gene (including exon 1 and the promoter region) appears to be a cause of HCM (Nigro *et al.*, 1997; Sakamoto *et al.*, 1997). The molecular mechanism by which the C allele of the c.−94C>G polymorphism may lead to HCM is unknown; however, we propose that may be due to the change being located in the 5′-UTR region of the gene, and this can affect the post-transcriptional regulation of the d-SG protein causing its deficiency. It is known that 5′-UTR regions have crucial roles in many aspects of post-transcriptional regulation of genes expression such as nuclear-cytoplasm transport, translation efficiency, subcellular localization, and stability of messenger RNA (Sonenberg, 1994; van der Velden and Thomas, 1999; Pesole *et al.*, 2000). The d-SG deficiency provoked in this way can induce disruption of the dystrophin-associated glycoprotein complex and membrane instability, which lead to an increase of cytosolic Ca^{2+} in cardiomyocytes causing their death and generating HCM (Fraysse *et al.*, 2010). These aspects merit further basic and clinical research.

The results suggest that the C allele of the c.−94C>G polymorphism of the SGCD gene is a risk factor for HCM that is increased by Amerindian ancestry. This polymorphism can play an important role in the etiology and progress of HCM in Mexican patients and could be used in the future as a genetic risk marker.

Table 3. Allele and Genotype Frequencies of c.−94C>G Polymorphism in Hypertrophic Cardiomyopathy Patients, Controls, and Amerindians populations

		Genotype frequency, n *(%)*			*Allele frequency*, n *(%)*			
	n	*CC*	*CG*	*GG*	*C*	*G*	*C vs. G*	*CC vs. GG*
Control	145	43 (30)	68 (47)	34 (23)	154 (53)	136 (47)	OR = 2.37 CI = [1.334–4.213] **p=0.00274**	OR = 5.00 CI = [1.367–18.340] **p=0.00907**
HCM	35	19 (54)	13 (37)	3 (9)	51 (73)	19 (27)		
Amerindian	165	40 (24)	77 (47)	48 (29)	157 (48)	173 (52)	OR = 2.96 CI = [1.674–5.227] **p=0.00012**	OR = 7.60 CI = [2.096–27.552] **p=0.00058**

Bold values indicate statistically significant results.
CI, confidence interval (95%); OR, odds ratio.

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Author Disclosure Statement

All the authors declare that they have no conflicts of interest.

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