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[Original article]

Prevalence of seven cardiovascular-related genetic polymorphisms in a Chilean mestizo healthy population

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Objective Among the genetic factors associated with cardiovascular disease (CVD), determining polymorphic genotypes could help to understand the appearance of the illness. Ethnic differences in these polymorphisms could explain population variability in susceptibility to CVD.

The main goal of this research is to study the presence of more relevant genetic variants of *ApoE, CETP, ACE, PAI-1, MTHFR, FII* and *FVL* of the coagulation cascade, to describe the presence of cardiovascular-related variants in a mestizo group of the Chilean people.

Methods and results The studied population comprised 146 unrelated subjects from the general population, diagnosed as healthy, who were genotyped through conventional and/or real-time PCR.

The allele frequencies for the Chilean population were: *Apo E*, ε2: 0.036, ε3: 0.875 and ε4: 0.089; *CETP, B1*: 0.51 and *B2*: 0.49; *MTHFR, C*: 0.52 and *T*: 0.48; *ACE, I*: 0.603 and *D*: 0.397; *PAI-1, 4G*: 0.381 and *5G*: 0.619; *FII, G*: 0.97 and *A*: 0.03, and *FV Leiden, G*: 0.97 and *A:* 0.03.

Conclusions This study contributes to establish a first picture in the Chilean mestizo population about the frequencies of these variants, which could act as single or complementary risk factors to trigger CVD. The obtained allele frequencies show great differences in relation to other South American populations.

Keywords *Polymorphism – cardiovascular disease – Apo E – MTHFR – ACE – PAI-1 – FII – FVL.*

INTRODUCTION

Cardiovascular diseases (CVDs) are the first cause of death worldwide representing 30% of all global deaths in 2008. Of these deaths, an estimated 7.3 million were caused by coronary heart disease and 6.2 million were due to stroke. Over 80% of CVD deaths take place in low- and middle-income countries and occur almost equally in men and women. By 2030, almost 25 million people will die from CVDs, mainly from heart disease and stroke[1].

In Chile, cardiovascular disease mortality represents the first cause of death (27.7%) with a mortality rate of 158.9/100,000 inhabitants, a mortality rate of 162.5 × 100,000 inhabitants in men and a mortality rate of 155.3 × 100,000 habitants (2010) in women, and is still increasing[2].

Nowadays, the Chilean public health goal for the decade 2011-2020 is to increase cardiovascular disease survival and expand the proportion of people with controlled arterial hypertension[3].

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The suggested responsible factors for cardiovascular diseases include modifiable and non-modifiable risk factors. Non-modifiable risk factors include, age, gender and several genes[4]. The sum of several unfavourable polymorphisms in these genes can facilitate the appearance of this polygenic disease, whose manifestation needs often the presence of an environmental propitious frame (multifactorial disease). In this respect atherosclerosis is one of the most classic examples of a polygenic disease and a great number of studies have reported polymorphisms that can be involved in its aetiology[5].

One of the most studied polymorphisms associated with cardiovascular risk is *apolipoprotein E* (*ApoE*). *ApoE* genotypes have been related with both LDL-C levels and coronary risk. Compared with individuals with the *ε3/ε3* genotype, *ε2* carriers have a 20% lower risk of coronary heart disease and *ε4* carriers have a slightly higher risk[6]. Similarly, cholesteryl ester transfer protein (CETP) *Taq*1B polymorphism provide some evidence about its effect on the likelihood of having a first event of acute coronary syndrome in normal-weight persons[7,8]. Genetic variations on other enzymes, as for example, 5, 10-methylenetetrahydrofolatereductase (MTHFR), can lead to an increase in risk of cardiovascular events. It has been established that the production of the homocysteine metabolite is decreased when the enzyme is defective[9], nevertheless, MTHFR polymorphisms (C677T and A1298C) appear not to be related to the onset of ischaemic stroke or hypertension[10]. On the other hand, angiotensin-converting enzyme (ACE) D/D genotype which produces an increase of levels of circulating ACE, with consequent increase of angiotensin II, appears to be an independent risk factor for cardiovascular effects[11,12]. Polymorphism in ACE alters the fibrinolytic balance, since a rapid increase induces a dose-dependent effect on the plasmatic levels of the plasminogen activating inhibitor (PAI-1). Thus, polymorphisms in the plasminogen inhibitor activator 1 (PAI-1), also could be important, particularly the 4G/4G variant, which increases the probability of thrombus and the trend to develop thrombosis in veins or arteries[13]. Patients carrying the 4G allele of the PAI-1 4G/5G gene might be predisposed to coronary artery disease[14]. Finally, genetic variations in coagulation factor activities, such as FII and FV Leiden, could have a synergistic effect on CVD by generation of thrombus due to decreased activity, especially in people with mutated alleles[15]. In spite of the low prevalence of these polymorphisms it is suggested that screening for thrombophilia might be justifiable in cases of stent thrombosis[16].

In Chile some studies have shown frequencies of several of these polymorphisms and their association to others pathologies[17-22], however, no studies have been reported for potential synergistic relationship among these factors and cardiovascular events.

Therefore, as interethnic differences could influence the susceptibility to cardiovascular disease, our main goal was to study the presence of the more relevant genetic variants of the ApoE, CETP, ACE, PAI-1, MTHFR, factor II and FVL in a subgroup of the Chilean mestizo people, in order to describe the cardiovascular-related gene variants and to compare the frequencies with other populations of South America.

METHODS

Study subjects

The population studied comprised 146 unrelated subjects from the general population of Santiago de Chile. All individuals were screened for suitability as healthy controls by physical examination, blood pressure, electrocardiograms and interpretation of standard biochemical analyses (i.e., lipid profile, glucose, urea nitrogen, creatinine, total protein, albumin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, drugs of abuse). Participants gave their written consent prior to participation between March 23th and August 2nd, 2010. The research was authorized by the Ethics Committee of the Faculty of Medicine of the University of Chile (N° 08-2008 and 072-2011)[23] and the Ethics Committee of the Ministry of Health of Chile (SSMOc N° 0874). Subjects were informed about the main goal of the study and the implications of genotype determinations, including the usefulness of several of the variants for any other diseases besides cardiovascular (e.g. ApoE genotyping as biomarker for Alzheimer's disease). Anthropometric characteristics and lipid profile for all subjects are reflected in table 1.

Table 1 Anthropometric and biochemical characteristics of the study population

CHARACTERISTICS	
Total (N)	146
Male (%)	98 (67.1%)
Female (%)	48 (32.9%)
Age (y) (mean ± SD)	35.7 ± 14.5
BMI ± SD (< 25 Kg/m^2)	24.5 ± 3.4
Cholesterol (mean ± SD) (RV: 50-200 mg/dL)	136 ± 47
Triglycerides (mean ± SD) (RV: 50-150 mg/dL)	96 ± 77
HDL-cholesterol (mean ± SD) (RV: 30-95 mg/dL)	35 ± 16
LDL-cholesterol (mean ± SD (RV: < 130 mg/dL)	81 ± 32
Cholesterol/HDL-cholesterol (mean ± SD (RV: < 5.0)	4.3 ± 1.7
LDL-cholesterol/ HDL-cholesterol (mean ± SD) (RV: < 2.0)	2.6 ± 1.2

SD: standard deviations, BMI: body mass index. RV: reference value.

Exclusion criteria included predisposing factors for CVD, like family history, hypertension (systolic blood pressure/diastolic blood pressure ≥ 140/90 mm Hg), diabetes, smoking (current smoking, ≥10 cigarettes a day), and alcohol use (≥200 mL/day for at least 6 days in a week), overweight (BMI > 25 Kg/m^2). Subjects taking hormone replacement therapy (HRT) and other hormonal drugs, and subjects who had renal and hepatic insufficiency were also excluded.

Unfortunately, until now there are no characterization studies about Chilean admixture neither validated ancestry biomarkers to use. Based on this characterization, only mestizo people were invited to participate. No pure aboriginal Indians and Caucasians living in Chile were included in this study.

Blood samples and DNA extraction

Blood samples were obtained from all (volunteer) participants in the study. Blood was collected in two 10 mL EDTA-containing vaccutainer™ tubes and kept at 4°C until DNA extraction (within 24 hrs). DNA was extracted with a high pure PCR template preparation kit from Roche Diagnostic (Roche Diagnostics GmbH, Mannheim, Germany). The purity of DNA was evaluated at 260/280 nm determination.

Genotyping

Real-time PCR for the detection of polymorphisms were carried out using the Light Cycler 1.5 system and kits from Roche Diagnostics GmbH, Mannheim, Germany. The genotypes were identified through curves of melting temperature (Tm) as follows: **ApoE**, E2 62.5 ± 2.5 °C , E3 56 ± 2.5 °C, E4 57.5 ± 2.5 °C; **PAI-1**, 4G 54 ± 2.5 °C, 5G 61 ± 2.5 °C; **ACE**, I 62 ± 2.5 °C, D 53.5 ± 2.5 °C; **FII (G20210A),** A 49 ± 2.5 °C, G 59 ± 2.5 °C; **FV (G1691A),** A 57 ± 2.5 °C, G 65 ± 2.5 °C; **MTHFR (C677T),** T 65 ± 2.5 °C and C 62.5 ± 2.5 °C. Genotyping of **CETP** was carried out through conventional PCR as previously described, with modifications[24].

Statistical analyses

Clinical data were expressed as mean ± SD. Allele frequencies were estimated by the gene-counting method. Chi-square and Fisher's exact test were used to investigate expected genotype frequencies assuming Hardy-Weinberg equilibrium. Student *t*-test and Wilcoxon test (Mann-Whitney) were used for comparison of mean differences in cholesterol levels among the genotypes. Analyses were performed with Stata 10.2 (Texas, USA) software.

RESULTS

A total of 146 samples were collected for genotype analysis. The genotype distributions are consistent with the Hardy-Weinberg equilibrium model. In table 2 we can observe genotypes obtained in this study. For *Apo E* alleles only ε2/ε2 genotype was absent in the group studied, and the most frequent genotype was ε3/ε3 (76.6%), followed by ε3/ε4 with a frequency of 16.1%. We detected only one subject with genotype ε4/ε4. The frequency of polymorphisms *TaqIB Cholesteryl ester transferase protein (CETP)* in the group studied was 51% for the wild-type allele (*B1*) and 49% for the mutant allele (*B2*) (table 3). The frequency distributions of

Table 2 Genotype frequencies for *ApoE*, *CETP*, *MTHFR*, *PAI-1*, *ACE*, *FVL* and *FII* polymorphisms in the studied population of Chileans (n = 146).

APO E (rs429358, rs7412) (C112R. R158C)	Genotype frequency (%)
ε2ε2	0 (0%)
ε2ε3	7 (4.8%)
ε2ε4	1 (0.7%)
ε3ε3	112 (76.7%)
ε3ε4	25 (17.1%)
ε4ε4	1 (0.7%)
CETP (rs708272) (G279A)	**Genotype N (%)**
B1B1	38 (26.0%)
B1B2	74 (50.7%)
B2B2	34 (23.3%)
MTHFR (rs1801133) (C677T)	**Genotype N (%)**
CC	37 (25.3%)
CT	78 (53.4%)
TT	31 (21.2%)
ACE (rs1799752) (I/D)	**Genotype N (%)**
II	56 (38.4%)
ID	64 (43.8%)
DD	26 (17.8%)
PAI-1 (rs1799889) (4G/5G)	**Genotype N (%)**
4G4G	24 (16.4%)
4G5G	64 (43.8%)
5G5G	58 (39.7%)
F II (rs1799963) (G20210A)	**Genotype N (%)**
GG	136 (93.2%)
GA	10 (6.8%)
AA	0
FVL (rs6025) (G1691A)	**Genotype N (%)**
GG	138 (94.5%)
GA	8 (5.5%)
AA	0

MTHFR were 52% for the wild-type (*C*) and 48% for the mutant (*T*) allele. ACE genotypes were 60.3% for the wild-type (*I*) and 39.7% for the mutant (*D*) allele. The frequencies of PAI-1 genotypes were 38.1% for the wild-type allele (*4G*) and 61.9% for the mutant allele (*5G*). As expected, we did not find homozygote 5G genotypes and the frequency of heterozygote genotype was very low in both cases (3.0%), similar to results previously reported by Palomo *et al.*[17].

Additionally, we studied the potential association between cholesterol levels and *CETP* and *ApoE* polymorphisms (table 3). Our results show a statistically significant association between *CETP B2B2* and both, total cholesterol ($P = 0.046$) and HDL-cholesterol ($P = 0.003$). Conversely, no statistically significant association between *Apo E* genotypes and cholesterol levels was observed.

The allele frequencies obtained were compared with Caucasians (Spain) and Asians (Japan), who are thought to be representative of the main ancestors of the Chilean population[25] and also with other South American populations. These data are shown in table 4. Interestingly,

Table 3 Levels of total cholesterol, LDL and HDL cholesterol in relation to ApoE and CETP polymorphisms in healthy volunteers (n = 146)

Genotype	Genotype frequencies	Average (SD) mg/mL	*P* value*
A) Total cholesterol			
ApoE			
ε3ε3	76.7	134.4 (46.4)	Ref
ε3ε2	4.8	114.6 (44.5)	0.365
ε3ε4	17.1	145.4 (56.8)	0.881
ε4ε2	0.7	187	0.169
ε4ε4	0.7	192	0.163
CETP			
B1B1	26.0	141.7 (43.5)	Ref
B1B2	50.7	134.8 (46.2)	0.454
B2B2	23.3	118.5 (46.8)	0.046
B) LDL-cholesterol			
ApoE			
ε3ε3	76.7	82.3 (32.4)	Ref
ε3ε2	4.8	65.6 (31.6)	0.257
ε3ε4	17.1	84.8 (34.9)	0.970
ε4ε2	0.7	108	0.291
ε4ε4	0.7	114	0.178
CETP			
B1B1	26.0	80.6 (26.9)	Ref
B1B2	50.7	84.7 (34.5)	0.779
B2B2	23.3	72.2 (31.7)	0.168
C) HDL-cholesterol			
ApoE			
ε3ε3	76.7	34.6 (15.5)	Ref
ε3ε2	4.8	34.8 (15.9)	0.657
ε3ε4	17.1	37.9 (18.1)	0.405
ε4ε2	0.7	17	0.175
ε4ε4	0.7	15	0.168
CETP			
B1B1	26.0	42.3 (18.7)	Ref
B1B2	50.7	33.8 (14.2)	0.052
B2B2	23.3	27.7 (13.3)	0.003

*Wilcoxon's test (Mann-Whitney).
No ε2ε2 subjects were identified.

Table 4 Allele frequencies for ApoE, CETP, MTHFR, ACE, PAI-1, FII and FV polymorphisms in Chileans and other South American ethnicities in comparison with Spanish Caucasians and Japanese populations

Polymorphism	Chile	Argentina	Brazil	Colombia	Venezuela	Mexico	Spain	Japan
Apo E (C112R, R158C)	This study n = 146	Ref 32 n = 216	Ref 33 n = 181	Ref 34 n = 691	Ref 35 n = 215	Ref 36 n = 278	Ref 37 n = 660	Ref 38 n = 2,172
f ε2	0.03	0.067	0.08	0.04	0.06	0.1	0.04	0.052
f ε3	0.88	0.847	0.77	0.86	0.83	0.83	0.86	0.855
f εE4	0.09	0.085	0.15	0.08	0.11	0.07	0.1	0.093
CETP (G279A)	This study n = 146	Ref 39 n = 43	Ref 40 n = 498	Ref 41 n = 500	ND	ND	Ref 42 n = 514	Ref 43 n = 264
f B1	0.51	0.535	0.643	0.5	ND	ND	0.649	0.585
f B2	0.49	0.465	0.357	0.5	ND	ND	0.351	0.415
MTHFR (C677T)	This study n = 146	Ref 44 n = 112	Ref 45 n = 843	Ref 46 n = 206	Ref 47 n = 50	Ref 48 n = 444	Ref 49 n = 200	Ref 50 n = 164
f C	0.52	0.652	0.77	0.65	0.77	0.34	0.59	0.58
f T	0.48	0.348	0.23	0.35	0.33	0.66	0.41	0.42
ACE (I/D)	This study n = 146	Ref 51 n = 75	Ref 52 n = 71	Ref 53 n = 231	Ref 54 n = 125	Ref 55 n = 220	Ref 56 n = 245	Ref 57 n = 95
f I	0.60	0.49	0.35	0.42	0.47	0.52	0.36	0.4
f D	0.40	0.51	0.65	0.58	0.53	0.48	0.64	0.6
PAI-1 (4G/5G)	This study n = 146	Ref 58 n = 40	Ref 59 n = 144	ND	ND	Ref 60 n = 590	Ref 61 n = 127	Ref 62 n = 94
f 4G	0.38	0.425	0.46	ND	ND	0.33	0.28	0.63
f 5G	0.62	0.575	0.54	ND	ND	0.67	0.72	0.37
F II (G20210A)	This study n = 146	Ref 63 n = 418	Ref 64 n = 275	Ref 65 n = 114	Ref 66 n = 51	Ref 55 n = 216	Ref 63 n = 493	Ref 67 n = 93
f G	0.97	0.987	0.982	1.00	1.00	0.980	0.974	1.00
f A	0.03	0.013	0.018	0.00	0.00	0.020	0.026	0.00
FV (G1691A)	This study n = 146	Ref 63 n = 418	Ref 64 n = 275	Ref 65 n = 114	Ref 66 n = 51	Ref 55 n = 216	Ref 63 n = 493	Ref 67 n = 93
f G	0.97	0.985	0.995	0.996	0.992	0.980	0.990	1.00
f A	0.03	0.015	0.006	0.004	0.008	0.020	0.010	0.00

ND: no data available.

Japanese people do not possess the *FII* or *FVL* mutated alleles. These alleles were only found in Spanish, Argentinean, Brazilian and Mexican populations for *FII* and *FVL*, similar to Chileans.

DISCUSSION

There are few investigations available in Chile and South America about the prevalence of the *APOE, ACE, PAI-1, CETP, MTHFR, FII* and *FVL*. Thus, this study contributes to establish a comparative picture of these genotype and allele frequencies in South America which has a complex ethnicity definition given the high degree of interracial mixture. Therefore, this research, which has been developed in a mestizo sub-population of the Chileans, corresponding with about 60% of the population[26], is an approach to the general population of this South American ethnicity.

In this study no statistically significant associations between *Apo E* polymorphisms and levels of cholesterol were found. However, both total cholesterol and HDL-cholesterol are associated with *B2B2* genotype of *CETP* ($P = 0.046$ and 0.003, respectively). As CETP enzyme is mainly related to HDL-cholesterol, probably the observed decrease in total cholesterol is due to the decrease in HDL levels. These results add some evidence to the controversial results obtained in several ethnic groups about the influence of *CETP* polymorphism and HDL-cholesterol levels[27,28].

As shown in table 4, *Apo E* ε3 in Chileans is the most frequent genotype reported (87.5%) of the studied

countries. The Brazilian population shows the lowest reported ε3 frequency (77%), the Japanese population shows the major percentage of the risk allele ε4 (9.3%). For *CETP* polymorphisms the frequency of *B2* in Chile (49%), Argentina (46.5%) and Colombia (50%) shows the major percentage of the risk allele that has been described in the Spanish (35.7%) and Japanese population (41.5%). Brazil presents a frequency of *B2* (35.7%) similar to the Spanish (35%). For *MTHFR* polymorphism the frequency of the *T* allele in the Chilean population (48%) is higher than in the populations of Argentina, Brazil, Colombia, Venezuela, Spain and Japan, and is only lower than the Mexican population (66%). For the *ACE D* allele the obtained frequency (39.7%) is lower than all other analysed populations. The Japanese population (63%) has the highest percentage of the *PAI*-1 *4G* allele, considered a high risk factor, and the frequency in Chile (38.1%) is similar to Mexico (33%). The Spanish population shows the lowest reported frequency (28%). The frequency of mutations in *FII* and *FVL* in the Chilean population shows the major percentage of the risk allele (3%) in the analysed populations.

Overall, when we compare population frequencies, great differences appear in South American populations. This could be relevant for the analysis of population susceptibilities to cardiovascular disease[29,30]. The question whether the seven polymorphisms could act synergistically to produce cardiovascular events remains to be answered.

Some limitations of this study should be noted. In view of the total Chilean population (about 16 million inhabitants) with about 9.5 million of mestizo people, our study had a relatively small sample size (146) which cannot be representative enough of this group. On the other hand, as ethnicity may be an important factor affecting the extrapolation of our results, the comparison with other countries of very different origin (table 4) should be considered merely descriptive at this point. Considering the proposed role on CVD of the variants studied in this research, the association of the studied gene variants with the pathology should be confirmed through case-control studies, in order to establish their usefulness as susceptibility biomarkers.

CONCLUSIONS

Our results show differences in polymorphism in South American populations compared with Asian and Caucasian populations which could be explained by the aboriginal admixture in our region originated primarily from migrations from Siberia 15,000 years ago through Beringia[31]. Thus, the data obtained might help to explain, as a first genomic approach, differences in susceptibility to cardiovascular events in this South American "mestizo" population.

We suggest that studies of these cardiogenes in Chilean CVD patients will help to develop cardiovascular risk biomarkers for a better management of the pathology in this specific population, rather than extrapolating results obtained to other populations.

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CONFLICT OF INTEREST: none.

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