Candidate Gene Polymorphism in Cardiovascular Disease: The BIP Cohort

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Abstract

Background: Cardiovascular disease is now well established as a multifactorial disease. In a given individual, the level of cardiovascular risk is due to the interaction between genetic and environmental components. The BIP cohort comprises 3000 patients with cardiovascular disease who were tested for the benefits of bezafibrate treatment. This cohort has the data for the lipid profile of each individual, fibrinogen, insulin, as well as clinical, demographic and lifestyle parameters

Objectives: To genotype up to 64 variable sites in 36 genes in the BIP cohort. The genes tested in this assay are involved in pathways implicated in the development and progression of atherosclerotic plaques, lipid and homocystein metabolism, blood pressure regulation, thrombosis, rennin-angiotensin system, platelet aggregation, and leukocyte adhesion.

Methods: DNA was extracted from 1000 Israeli patients from the BIP cohort. A multilocus assay, developed by the Roche Molecular System, was used for genotyping. Allele frequencies for some of the markers were compared to the published frequencies in a healthy population (the French Stanislas cohort, n=1480).

Results: Among the 26 comparable alleles checked in the two cohorts, 16 allele frequencies were significantly different from the healthy French population: *ApoE* (E3, E2, E4), *ApoB* (71ile), *ApoC* (3482T, 455C, 1100T, 3175G, 3206G), *CETP* (405val), *ACE* (Del), *AGT* (235thr), *ELAM* (128arg); $P < 0001$ and *LPL* (93G, 291Ser, 447ter); $P < 005$.

Conclusions: Although a comparable healthy Israeli population study is needed for more precise interpretation of these results, frequency differences in these polymorphic alleles – associated with lipid metabolism, renin-angiotensin system and leukocyte adhesion mechanism – between CVD patients and healthy individuals nevertheless implicate these candidate genes as predisposing for CVD.

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Cardiovascular disease is now well established as a multifactorial disease. In a given individual, the level of cardiovascular risk is due to the interaction between genetic and environmental components. Moreover, the genetic and environmental parameters are themselves composites and their component parts interact among themselves to change risk.

As more candidate genes associated with CVD are identified there is increasing need to perform multilocus genotyping in CVD patients compared to healthy controls in order to identify common genetic polymorphisms that may confer only a modest risk independently but which may collectively comprise a high CVD risk profile. We applied the Roche Molecular System multilocus genotyping assay [1,2] to the BIP cohort (Bezafibrate Infarction Prevention), which was a multicenter, placebo-controlled, randomized, double-blinded trial in 3000 patients with established CVD [3]. The BIP study has detailed lipid profile data for each individual together with important clinical, demographic and lifestyle information [4].

In the present study we report for the first time the allele frequencies in the BIP cohort and compare some of the alleles to the published data on the Stanislas cohort, a healthy French population [5]. This report and others to follow will analyze phenotype-genotype correlation and combinations between polymorphic alleles and environmental factors that might predispose or protect against CVD.

Materials and Methods

Population

The details of design and methods of the BIP study have been presented elsewhere [4]. The current study enrolled 1000 individuals. Genomic DNA was prepared from peripheral blood samples by the Gentra kit as recommended by the manufacturers.

The multilocus assay

The complete assay has been described previously [1,2]. Briefly, each sample was amplified by two 33-cycle polymerase chain reaction (25 ng of genomic DNA each) The 31 biotinylated primer pairs in the multiplex A were designed to amplify the genes: *LPA, ApoA4, ApoB, ApoC3, ApoE, ADRB3, PPARG, LIPC, LPL, PON1, PON2, LDLR, CETP*, and *LTA*. The 33 primer pairs pooled in multiplex B were designed to amplify the genes: *MTHFR, NOS3, DCP1, AGTR1, AGT, NPPA, ADD1, SCNN1A, GNB3, ADRB2, MMP3, F2, F5, F7, PAI1, FGB, ITGA2, ITGB3, SELE, ICAM* and *TNF*. Each PCR product was then hybridized to the corresponding panel of oligonucleotide probes that had been immobilized in a linear array on backed nylon membrane strips. The calorimetric detection was based upon steptavidin-horseradish peroxidase conjugate and substrates.

CVD = cardiovascular disease
PCR = polymerase chain reaction

Statistical analysis

Allele frequencies were estimated by gene counting and were recorded and stored on spreadsheet using Microsoft Excel 1997 (Seattle WA, USA, 1985-2000). Frequencies in the BIP population were compared to the Stanislas population, using the chi-square test with Yates correction as appropriate. Tests were considered significant at $P < 005$.

Results

Observed genotype frequencies did not deviate from those predicted by the Hardey-Weinberg equation. Of the 64 polymorphic alleles analyzed in this study, 26 were also checked in the Stanislas cohort. These allelic frequencies are shown in Table 1.

The BIP population differed significantly from the Stanislas population in the frequency of the following alleles: *ApoE* (E3, E2, E4), *ApoB* (71ile), *ApoC* (3482T, 455C, 1100T, 3175G, 3206G), *CETP* (405val), *ACE* (Del), *AGT* (235thr), *ELAM* (128arg); $P <$ 0001, and *LPL* (93G, 291Ser, 447ter); $P < 005$.

The observed allele frequencies for the 10 other genetic markers were not significantly different between the two populations. In both populations there were no homozygotes for the following alleles: *LPL*; 9asn and 291ser. In the Stanislas cohort there was no homozygote for the *LPL*; 93G, while in the BIP there was.. In both populations there was no detectable variant for the *CETP* 442gly allele; in the Stanislas population there was also undetectable *ApoB* 3500gln while in the BIP there were two heterozygotes. In the Stanislas population there was no homozygote for the F5; 506gln, while in the BIP there were three.

The remaining 37 polymorphic alleles explored in this study will be compared to the Israeli healthy population study currently under investigation.

Table 1. Frequencies of alleles in the BIP cohort and the Stanislas population

Gene	Locus	Genotype	BIP	Stanislas
ApoE	19q	E3	0.84	0.77*
		E2	0.057	0.1*
		E4	0.093	0.13*
ApoB	2p23-24	71ile	0.183	0.31*
ApoC3	11q23	482T	0.317	0.27*
		3500gln	0.001	0
		455C	0.434	0.37*a
		1100T	0.332	0.25*
		3175G	0.187	0.08*
		3206G	0.477	0.36*
CETP	16q	405val	0.395	0.31*
		442gly	0	0
LPL	8q22	93G	0.032	0.02*
		9asn	0.015	0.02
		291ser	0.0055	0.02**
		447ter	0.093	0.12**
PON	7q21-22	192arg	0.287	0.28
ACE	17q23	Del	0.649	0.53*
ATIR	3q21-25	1166C	0.276	0.29
AGT	1q42-43	235thr	0.471	0.4*
MTHFR	1p36	677T	0.396	0.38
ITGB3	17q	33pro	0.165	0.16
Fibrinogen	4q	455A	0.228	0.2
F5	1q21-25	506gln	0.037	0.03
SELE	1q	128arg	0.128	0.09*
		554phe	0.063	0.05

* $P < 0.001$

** $P < 0.05$

Discussion

In the present study we used the CVD-multilocus kit developed by Roche Molecular Systems for analyzing 65 polymorphic alleles in 35 candidate genes involved in lipid metabolism, the development and progression of atherosclerotic plaques, homocystein metabolism, blood pressure regulation, thrombosis, the renin-angiotensin system, platelet aggregation, and leukocyte adhesion.

Our population comprised individuals from the BIP cohort who are all Israeli CVD patients tested previously to assess the benefits of bezafibrate treatment [3]. This cohort has the data for the lipid profile of each individual (cholesterol, triglycerides, HDL, LDL, Lpa, ApoA1, ApoB, ApoE, fibrinogen, insulin, HDL2, HDL3, particle A1, A2), as well as clinical, demographic and lifestyle parameters. The purpose of the study was to compare allele frequencies in CVD patients and healthy controls and correlate genotype-phenotype, and to find a predicted combination of polymorphic alleles and environmental factors that predispose to CVD. In this first report 35 allele frequencies were compared to the Stanislas cohort (a healthy French population) [5]. Sixteen allele frequencies were significantly different from the healthy French population: *ApoE* (E3, E2, E4), *ApoB* (71ile), *ApoC* (3482T, 455C, 1100T, 3175G, 3206G), *CETP* (405val), *ACE* (Del), AGT (235thr), *ELAM* (128arg); $P <$ 0001 and *LPL* (93G, 291Ser, 447ter); $P < 005$.

In other studies on healthy European populations [6] there were differences in several allele frequencies, which might indicate population specificity, but those studies were performed on very small samples (the Italian sample size was 162) so the difference might not reflect changes in population. We decided to compare our population to the Stanislas cohort because of the similarity in sample size, which permitted adequate power to compare frequencies. Nevertheless, we are aware that the best comparison should be done on a similar population and this study in now being designed. We decided to report on our findings as compared to the Stanislas cohort because of the differences between healthy versus CVD populations, which are probably not explained by general allelic differences between populations. Our next report will concern the phenotype-genotype relationship and the effect of environmental factors on the development of CVD.

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