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Prediction of Coronary Heart Disease Risk in a South European Population: A Case-Control Study

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1. Introduction

1.1 Coronary heart disease etiology

Coronary heart disease (CHD) is the most common cause of death in the industrialized countries. Although the past two decades have brought considerable advances in its detection and treatment, it remains a leading cause of death and disability in Western countries (Mackay & Mensah, 2004). CHD is a complex disease, whose primary cause is atherosclerosis. It is a progressive chronic disease process with contributions from environment, lifestyle and genetic factors. This complex disease clusters in families, suggesting a substantial genetic predisposition (Marenberg et al., 1994) and we know that its susceptibility can be mediated by both genetic and environmental factors (Talmud, 2007).

The influence of a family history of CHD, particularly of early onset, while universally recognized as important, has proved difficult to clarify fully. Habits and behaviors tend to persist in families, and familial aspects of the disease are partially explained by associations of behavioral risk factors and others in which behavior is important, including obesity, smoking, hypertension, dyslipidemia and diabetes. Having a parent with a history of myocardial infarction (MI) nearly doubles a person´s own risk of future MI, and the risk increases if both parents have a history risk of MI (Chow, 2011).

Furthermore, family history is a significant risk factor for atherosclerosis, and the contribution of family history cannot be fully accounted for by known cardiac risk factors (Colditz et al., 1986; Slack & Evans, 1966; Snowden et al., 1982). Genetic factors also contribute significantly to most of the major risk factors for CHD [diabetes, hypertension, elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) and low levels of plasma high-density lipoprotein cholesterol (HDL)], and yet the contribution of family history is not fully explained by known cardiac risk factors, suggesting that other yet-to-be-determined genetic factors also contribute to cardiovascular risk (Cohen, 2006; Slack et al.,1966).

However no common major genetic alterations have been found that can explain CHD, and the scientific evidence accumulated over recent years on the pathophisiology and genetics of

this complex disease indicates that there is unlikely to be a single gene that is responsible for its genetic component. Genetic predisposition for cardiovascular disease appears to be the result of the cumulative effects of various genetic polymorphisms and allele combinations, which in isolation would only confer moderately elevated risk, but the risk can be increased by various gene-gene and gene-environment interactions. (Lanktree & Hegele, 2009). Despite extensive exploration of many genes, strong evidence of a molecular genetics association with coronary artery disease or myocardial infarction remains to be obtained. The existence of these multiple predisposing genes with modest individual effect, gene–gene and gene–environment interactions, and interpopulation heterogeneity of both genetic and environmental disease have made its molecular detection and replication very difficult (Hunter, 2005).

Interactions between genes belonging to different physiological and enzyme systems have been investigated in recent years, involving adipocyte differentiation, lipid metabolism and glucose homeostasis, all of which affect the development of atherosclerosis and CHD. One of these studies (Peng et al., 2004) suggests that the association of the ε4 allele of the apolipoprotein E (apo E) gene and the 151C/T variant of the peroxisome proliferator-activated receptor gamma (PPARγ) gene reduces CHD risk. The ε4 carrier's had significant higher LDL-C levels than other apo E carriers and this tendency could be modified by PPAR gamma C/T genotype. In the Peng study, the ε 4 allele was an independent risk factor for CHD (OR=4.29, 95%CI: 1.6-11.48, P=0.004). A significant interaction between ε 4 allele and PPAR gamma C/T variant, was detected on CHD risk (P=0.045), and the interaction effect of the two genes on serum cholesterol level, attenuated de risk of CHD.

The concept of gene-gene interaction can thus be extended to the existence of protective and/or suppressive genetic variants, which when identified could make an important contribution to preventing further development of CHD or improving its clinical course.

Gene-environment interaction implies that, in combination, the effect of the genotype and the environmental factor is greater than the additive effect of each. At the molecular level the environment modifies the molecular function of the gene product, because in the population there is a range of genetic risk profiles under the influence of the environmental spectrum of risk and lifestyle choices they made (e.g. smoking). Smoking alone is known to approximately double the life time risk of CHD (1.94; 1.25-3.01), but the male smoker´s with the ε4 genotype had a hazard ratio of 3.17; 1.82-5.50, even after adjusting for the traditional risk factors (including plasma lipids) the risk remained high at 2.79 (1.59-4.91) (Humphries & Donati, 2002; Humphries et al., 2007).

Diseases such as CHD may be thought of as resulting from failure of adequate homeostasis within a physiological system. This may occur as a result of failure at the genetic level (gene transcription), due to an environmental exposure (smoking, alcohol, diet, etc.) or due to an imbalance between the two [Stephans & Humphries, 2003].

Therefore, CHD is the terminal manifestation of multiple intermediate disease processes, which have genetic, environmental determinants and their interactions (Hunter, 2005; Manolio et al., 2006).

1.2 Personalized medicine and possibility to predict coronary heart disease risk

Although there has been considerable success in identifying genetic variants that influence well-known risk factors, such as cholesterol levels, the progress done in new genes which

might influence the early occurrence of CHD, has been slow. Recent genetic approaches involving genomic associations in large scale (GWA) can identify novel susceptibility genes and genetic variants involved in the pathophysilogy of CHD (vasculogenesis, inflammation, immunity, new apolipoprotein and some genes with pathophysiological role still unknown (Watkins & Farrall, 2006). It is also expected that genetic profiling, that is, the simultaneous testing of multiple genetic variants, can eventually be used to predict CHD risk in individuals. This may lead to personalized medicine in which preventive and therapeutic interventions will be targeted at genetic profiles rather than at conventional risk factors (Van der Net et al., 2009).

One of the major promises is that this advance will lead to personalized medicine, in which preventive and therapeutic interventions for complex diseases are tailored to individuals based on their genetic profiles (Shiffman et al., 2006). Personalized medicine already exists for monogenetic disorders such as hereditary forms of cancer, in which genetic testing is the basis for informing individuals about their future health status and for deciding upon specific, often radical interventions. Because the etiology of complex diseases is essentially different from that of monogenic diseases, new emerging genomic knowledge that may be applied to primary care and public health will be one of the major challenges for the next decades (Janssens & Van Duijn, 2008). The existence of a predictive test or a prediction model that can discriminate between individuals who will develop coronary disease and those who will not is important for personalized medicine. However, current risk assessment protocols are imperfect, particularly in assessing the risk of early onset CHD (Akosah, 2003).

Data from Framingham study population enabled prediction of CHD during a follow-up interval of several years, based on blood pressure, smoking history, total cholesterol and HDL-cholesterol levels, diabetes, and left ventricular hypertrophy on the ECG. These prediction algorithms have been adapted to simplify score sheets that allow physicians to estimate multivariable CHD risk in middle-aged patients (Wilson et al., 1998).

Diamond and Forester understood that different results, obtained from different tests with substantial imperfections, must be integrated into a diagnostic about the probability for coronary disease, in a given patient. This approach estimates the pretest likelihood of coronary disease (defined by age, sex and symptoms) and the sensitivity and specificity of four diagnostic tests: stress electrocardiography, cardiokymography, thallium scintigraphy and cardiac fluoroscopy. The probability for coronary artery disease was estimated by Bayes´ theorem from each patient´s age, sex and symptoms classification, and from the observed test responses (Diamond & Forester, 1979).

For estimating the likelihood of severe coronary heart disease, investigators from Duke University elaborated a risk score based on clinical symptomatic variables (Pryor et al., 1983, 1991, from Duke University). A similar score also based in the patient´s history was investigated by the Stanford University (Sox et al., 1990).

Another score denominated ARIC score following the study with the same name, Atherosclerosis Risk in Communities (ARIC) (Chambless et al., 2003), was also developed. At European level, the Score risk algorithm led by Ian Graham, based on a European population of 250,000 people, allows prediction of the risk of atherosclerotic manifestations other than cardiovascular diseases, such as stroke and peripheral vascular disease,

according to age sex, smoking habit, systolic blood pressure and total cholesterol level (Graham, 2004).

However, these protocols may be improved by the inclusion of genetic information which is independent of traditional risk factors (Zdravkovic et al., 2002). Recent studies have attempted to assess whether the addition of new emerging risk factors, such as C reactive protein, homocysteine and genetic polymorphisms, can improve CHD risk prediction in addition to traditional risk factors (Folsom et al., 2006; Koenig et al, 2004; Morrison et al., 2007; Humphries et al., 2007) This is the goal to achieve through the new millennium genetic epidemiology: the development of tests, based on DNA, to determine the genetic predisposition to CHD. However, the contribution of an isolated genetic variant is small considering the polygenic nature of the disease. Several genetic markers, relevant in the pathophisiology of CHD, have been studied, such as the candidate genes variants involving several enzymatic pro-atherosclerotic systems, anti-oxidants, inflammatory, pro thrombotic and other involving the lipid and carbohydrates metabolism. More recently research has centered on genetic variants with strong associations with CHD, identified through genome wide association studies (GWA), some with pathophysiological roles that are still unknown, such as the single nucleotide polymorphism (SNP), situated at the 9p21 genetic locus (McPherson et al., 2007; Ripatti et al., 2010).

To validate, in our population setting (Madeira Island), the risk conferred by these recently discovered genetic factors and to improve assessment information about the magnitude of CHD risk associated to traditional risk factors, biochemical risk marker and genetic factors, we aimed to obtain a new model of risk score useful in the discrimination of total coronary disease risk in this population.

We therefore performed an epidemiological study, based on 7 genetic variants, some of them with a consistent association with CHD. These variants had already been investigated in previous works by our research group (Mendonça et al., 2004a, 2004b, 2008a, 2008b).

2. Combined model of risk score, including genetic and environmental information for CHD risk prediction (case-control study)

2.1 Methodology

2.1.1 Study design and population

Case-control study with a total of 1406 Caucasian subjects, average 53.5±9.7 years, 77.0% male, native and resident in Madeira Island (Portugal).

The cases (n=723), mean age 53.7±8.9 years and 79.9% male, were selected consecutively following hospital discharge from patients admitted with myocardial infarction or coronary artery disease confirmed by coronary angiography showing at least 75% obstruction of at least one coronary artery. Myocardial infarction was defined by a positive troponin blood test in the setting of symptoms and electrocardiogram changes (both ST-segment elevation and non–ST-segment elevation changes) consistent with MI (*Joint European Society of Cardiology/American College of Cardiology Committee criteria, 2000*). The control group was comprised of 683 healthy volunteers, mean age 53.3±10.5 years and 73.9% male, selected randomly from the electoral register from individuals with no history or suggestion of CHD.

The use of the electoral register to select the controls was intended to ensure that they did not differ significantly from the cases in terms of gender and age. For each control, a clinical observation with reference to the classical risk factors and ECG was registered, but in doubtful cases complementary exams such as a stress test, and echocardiogram or an angiography, were requested. After the subject's inclusion, the clinical history of each one was recorded, with reference to demographic and other data including age, place of birth and residence, family history of CHD, personal history of hypertension or diabetes, smoking and alcohol habits, dyslipidemia and quantity and type of physical exercise. Their weight, height, waist and hip circumference, heart rate, blood pressure were measured. Subjects were considered to have a family history of CHD if it had been diagnosed in their father or a brother before the age of 50 or mother or a sister before the age of 60 (Sesso et al., 2001). Subjects were classified as having hypertension if they reported the fact, were taking antihypertensive medication or had systolic BP of ≥140 mmHg and/or diastolic BP of ≥90 mmHg, based on the mean of three measurements (Chobanian et al., 2003). They were classified as having diabetes if they were taking oral anti-diabetic medication or insulin or if their fasting plasma glucose was higher than 7.0 mmol/l or 126 mg/dl (Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Waist measurements were taken at the level of the umbilicus and we classified men as having a large waist if it was greater than 102 cm (40 inches) and women as having a large waist if it was greater than 88cm (Xavier F. Pi-Sunyer, 1998). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, with obesity defined as a BMI of >30 (Veja, 2002; Pi-Sunyer, 1998). Subjects were considered to be smokers if they smoked or had ceased less than five years previously. Dyslipidemia was defined as total fasting cholesterol of >5.2 mmol/l or 200 mg/dl, or LDL cholesterol of ≥3.4 mmol/l or 130 mg/dl, HDL ≤ 40 mg/dL, triglycerides ≥ 1.5 mmol/L or 150 mg/dL, as well as being considered present in individuals taking lipid-lowering medication (NCEP, 2001)). We evaluated physical activity by asking subjects if they exercise or play sports in their leisure time and their responses were classified as either never/seldom, sometimes, or often/very often (Mainous et al., 2007).

Blood samples were collected from cases and controls for biochemical and genetic analysis.

The study protocol was approved by the hospital's Ethics Committee and all participants gave their informed consent.

2.1.2 Biochemical and genetic analysis

The blood for routine tests, biochemical risk markers: Apo B 100, Homocysteine, Total Cholesterol, HDL, LDL, Triglycerides, Lipoprotein (a), CRP (hs) and genetic analysis, were collected in all subjects.

Biochemical analysis: To determine serum glucose, total, HDL and LDL cholesterol and triglycerides, blood samples were extracted after 14-16 hours' fasting, placed in dry tubes and centrifuged half an hour later at 3500 g. Serum levels of total, LDL, HDL cholesterol and triglycerides were quantified by an enzymatic technique using a Hitachi 911 auto-analyser. A direct enzymatic technique was used for HDL and LDL cholesterol. Biochemical risk markers lipoprotein (a), apolipoprotein B100, and high-sensitivity C-reactive protein (hs-

CRP) were quantified by nephelometry using a Behring BN 100 automatic system. Homocysteine was measured by fluorescence polarized immunoassay using an Abbot IMX automatic device. To measure fibrinogen, samples were also collected with the patient fasting and placed in a tube containing sodium citrate, and measurements were taken with a Behring BSC automatic analyser.

Genetic analysis: For both patients and controls, genomic DNA was extracted from an 80 µL aliquot of whole blood collected in tubes containing EDTA using standard phenol/chloroform methodologies with ethanol precipitation. The genotypes chosen for this study, were screened for their association with CHD, already investigated in previous studies (ACE I/D, AGT 235 M/T, ATIR 1166 A/C, MTHFR 677 C/T, PON 192 Q/R, Apo E (ε4), rs1333049 G/C).

The genotypic analysis was effectuated using probes oligonucleotydes marked with specific fluorescence for each one of the alleles in a test that combines the stipulated PCR technique and TaqMan (7300 SDS Software, Applied Biosystems).

2.1.3 Statistical analysis

Allele frequencies were deduced from the genotype distribution. To test deviation of the Hardy Weinberg Equilibrium (HWE), was performed by Pearson's χ^2 tests, using the observed genotype frequencies and the expected genotype frequencies. The student T test and *one way* ANOVA procedures were used for means comparison between groups. Multiple comparisons were performed by the adequate post hoc (Tukey). Genotypes distributions and allele frequencies were compared between the cases and the controls using a χ^2 test. Strength of the association was expressed by odds ratio (OR) as well as their 95% confidence intervals (CI).

We constructed a Genetic Risk Score (GRS) using a coding value of "0" for original non risk homozygote, "1" for heterozygote and "2" for mutated risk homozygote, and summing these values for each single nucleotide polymorphism (SNP). The obtained score was divided into five quintiles (0-4; 4-5; 5-6; 6-7; ≥7) and these was compared in patient and control population (using independent T-Student test) and odds ratio and 95% Confidence Intervals, were determined. To investigate independent variables associated with coronary heart disease, a multivariate logistic regression model (forward Wald), containing the traditional risk factors, the biochemical markers and GRS), was constructed. Finally, a receiver operating characteristic (ROC) curve, based on sensitivity and specificity of the multivariable model, was computed, to estimate CHD susceptibility and Hosmer-Lemeshow goodness-of-fit test, estimated the model calibration.

3. Results

3.1 Basal characteristics of the population

As expected, the cases were more likely to be smokers, diabetics and hypertensives. They had a higher alcohol intake, higher values for glycemia, triglycerides, dyslipidemia, body mass index, high-sensitivity C-reactive protein (hs-CRP), lipoprotein (a) and lower values for HDL cholesterol (Table 1).

	Total (n=1406)	Cases (n=723)	Controls (n=683)	p-value
Age (Years)	53.5 ± 9.7	53.7 ± 8.9	53.3 ± 10.5	0.387
Sex M (%)	1083(77.0%)	578 (79.9%)	505 (73.9%)	0.007
Hypertension (%)	704 (50.1%)	416 (57.5%)	288 (42.2%)	<0.0001
Diabetes (%)	324 (23.0%)	240 (33.2%)	324 (23.0%)	<0.0001
Obesity (%)	397(28.2%)	231(32.0%)	166 (24.3%)	<0.0001
Smoking (%)	484 (34.4%)	325 (45.0%)	159 (23.3%)	<0.0001
Alcohol gr/ day	0.8	1.6	0.0	<0.0001
Dyslipidemia (%)	1246 (88.6%)	694 (96.0%)	552 (80.8%)	<0.0001
Glycemia mg/dl	102.0	107.0	98.0	<0.0001
Triglycerides mg/dl	132.0	152.0	115.0	<0.0001
HDL mg/dl	44.6 ± 14.6	38.5 ± 10.3	51.2 ± 15.7	<0.0001
Systolic BP (mm Hg)	135.5 ± 20.4	136.4 ± 20.2	134.4 ± 20.6	0.040
Diastolic BP (mm Hg)	81.2 ± 11.3	81.7 ± 11.2	80.8 ± 11.5	0.206
Fibrinogen (mm Hg)	348.9 ± 94.1	344.9 ± 96.6	341.1 ± 96.7	0.397
Lp(a (mg/dl))	16.5	20.8	15.2	0.010
(hs) CRP mg/dl	2.0	3.0	1.6	<0.0001
Genetic Risk Score	5.03 ± 1.84	5.4 ± 1.7	4.6 ± 1.9	<0.0001

Table 1. Basal characteristics of the population.

3.2 Selection of the used polymorphisms and genetic risk score

The genotypes chosen for this study, were screened for their association with CHD, already investigated in previous studies (ACE I/D, AGT 235 M/T, ATIR 1166 A/C, MTHFR 677 C/T, PON 192 Q/R, Apo E (ε4) and rs1333049 G/C).

The genetic risk score based on 7 SNPs variants (ACE I/D, AGT 235 M/T, ATIR 1166 A/C, MTHFR 677 C/T, PON 192 Q/R, Apo E (ε4), rs1333049 G/C), was calculated for 1406 individuals and ranged from 11 and 0 in the whole population. The GRS average was 5.03±1.84 in the whole population. CHD patients had higher GRS than control population (5.41±1.73 vs. 4.63±1.87, p<0.0001).

In the last GRS quintile (score ≥7) including more than a quarter of the patients, the CHD risk was three times higher than in the reference group.

3.3 Independent variable associated with CHD and risk prediction models of CHD

The association of the GRS with CHD was tested in a multivariate analysis containing the traditional risk factors and comprising the GRS. After logistic regression model the GRS stayed in the equation as an independent risk marker associated with increased risk of CHD; (OR=1.333; p<0.0001). Diabetes type 2, dyslipidemia, arterial hypertension and alcohol, also remained in the equation as independent risk factors of CHD (Table 3).

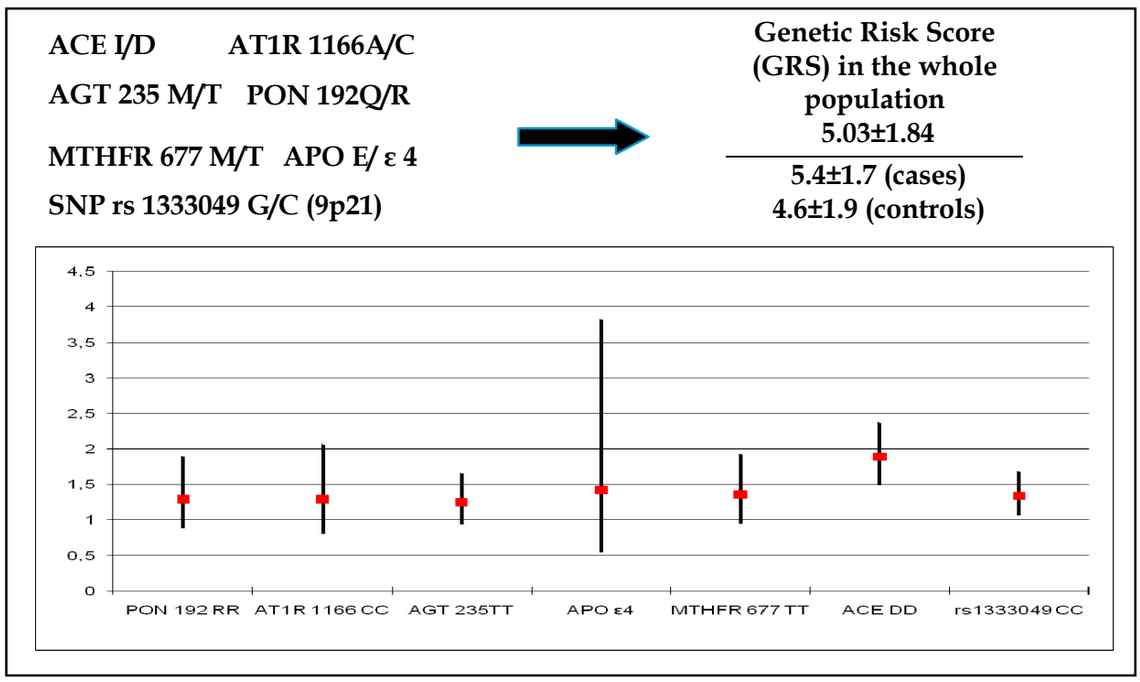


Fig. 1. CHD genetic risk (OR) conferred by each isolated polymorphism and the GRS obtained by the addition of all the 7 risk alleles of the studied variants.

GRS by Quintiles	Cases (n=723)	Controls (n=682)	Odds ratio (CI 95%)	P value
1º - [0-4[	90 (12.4%)	184 (27.0%)	Reference	-------
2º - [4-5[	127 (17.6%)	118 (17.3%)	2.20 (1.52 – 3.19)	<0.0001
3º - [5-6[	172 (23.8%)	151 (22.1%)	2.33 (1.65 – 3.30)	<0.0001
4º - [6-7[	151 (20.9%)	121 (17.7%)	2.55 (1.78 – 3.67)	<0.0001
5º - ≥7	183 (25.3%)	108 (15.8%)	3.46 (2.41 – 4.98)	<0.0001

Table 2. Association between the *SGR* by quintiles and CHD risk (OR) in the studied population.

Variables	**B**	**S.E.**	**Wald**	**df**	**Odds Ratio (IC 95%)**	**Valor-p**
Diabetes 2	1.072	0.153	49.310	1	2.922 (2.166-3.941)	<0.0001
Dyslipidemia	1.713	0.231	54.946	1	5.545 (3.526-8.723)	<0.0001
AHT	0.265	0.123	4.652	1	1.304 (1.025-1.659)	0.031
Alcohol	0.010	0.002	40.435	1	1.010 (1.007-1.014)	<0.0001
GRS	0.287	0.034	70.725	1	1.333 (1.246-1.425)	<0.0001
Constant	-3.499	0.301	135.090	1	0.030	<0.0001

Table 3. Independent variables associated with coronary heart disease risk.

Afterwards, two predictive models of CHD risk were constructed and the area under the receiver operating characteristic (AUC) curves for the constructed model which included both genetic and traditional risk factors was compared with the AUC for the model, which included only the traditional risk factors. The top chart including the GRS increased the AUC over the bottom chart which was only based on traditional risk factors (Figure 2 and 3).

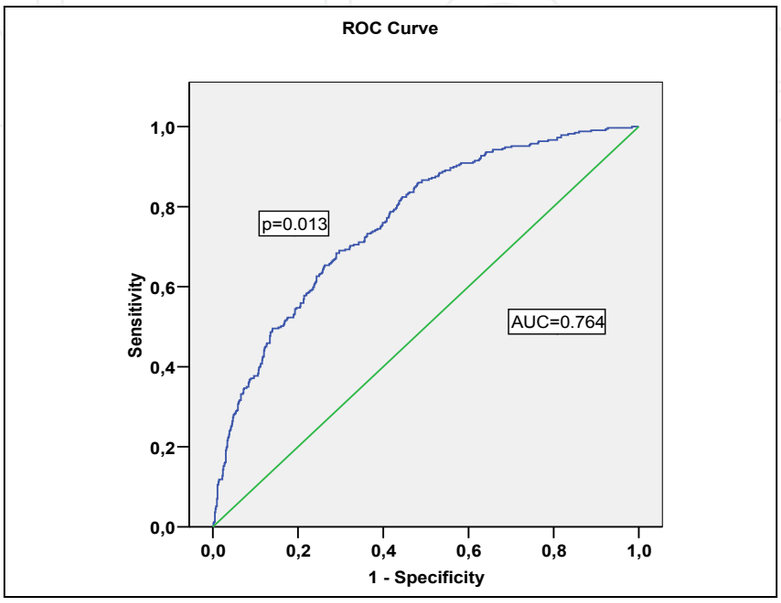


Fig. 2. ROC curve, based on sensitivity and specificity of the multivariable model, with traditional risk factors and GRS.

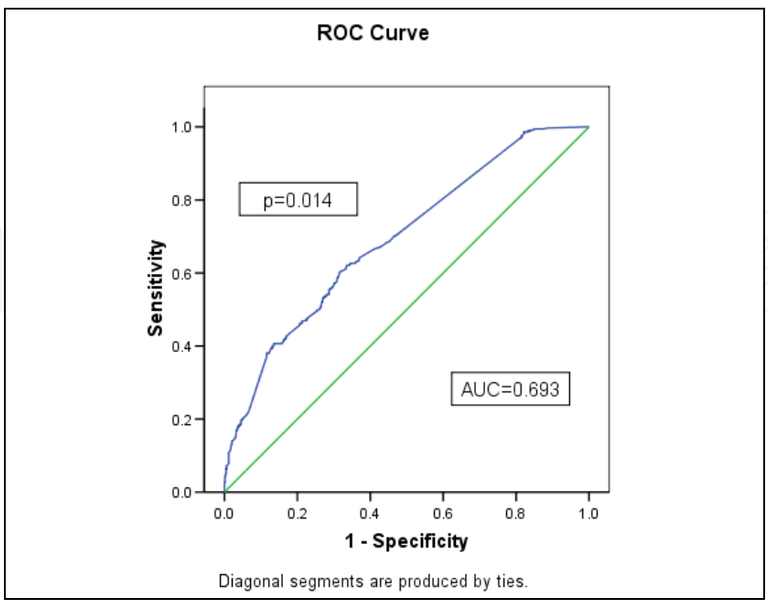


Fig. 3. ROC curve, based on sensitivity and specificity of the multivariable model, with traditional risk factors and without GRS.

In the model with GRS (Figure 2), the AUC was 75%, indicating good CHD discrimination and the goodness-of-fit statistic test value was 6.186; p=0.626, indicating a good calibration of the model. In the model without the GRS (Figure 3) the AUC was 69.3%.

4. Discussion

Risk prediction is an important part of cardiovascular disease prevention and the ability to identify high risk individuals who do not present with the traditional risk factors enable clinicians to personalize strategies for prevention and treatment.

Risk prediction is based on the knowledge of the conventional risk factors (high blood pressure, smoking, blood cholesterol level) and in the family history. However the fact that some individuals with coronary disease are of low risk when considering the classic risk factors forced the scientific community to go further in the predictive models. The GRS used in this work comprises SNPs selected from candidate genes and others identified through large-scale genomic association studies, and introduced the concept that the addition of a GRS can improve the prediction of CHD beyond that supplied by traditional risk factors, especially in the population fringe with few classic risk factors.

It was based essentially in variants which had shown association with coronary disease not only in the published literature in general but also in previous studies in our Funchal research group, already mentioned. The fact that these genes have shown, an association with CHD in our population makes them of great value in CHD risk prediction, in our population.

In the present work the risk associated with each single polymorphism is small with the exception of angiotensin convertion enzyme (ACE DD) and the SNP rs1333049, (OR=1.8 $p<0.0001$ and 1.4 $p<0.0001$, respectively). However, although the risk associated to each isolated variant was modest, the sum of the risk alleles, represented by GRS, was associated with a significant elevation of the risk. To illustrate this finding when the GRS, was codified in quintiles, the highest quintile (6 or more risk alleles) presented a 3.5 times higher risk than the reference group (OR=3.46 [2.41-4.98]; $p<0.0001$).

The availability of clinical genetic testing for cardiovascular disease has only recently emerged. In large part, this has stemmed from technological advances in molecular sequencing, which have substantially decreased testing costs. While a decade ago the sequencing of a single gene might have cost tens of thousands of Euros and taken months of effort, the same result can now be obtained with much less time and cost. Sequencing technology is now undergoing a period of extremely rapid development that portends revolutionary advances in the field. When validated and implemented in clinical testing, these advancements could greatly improve our ability to provide genetic diagnoses for many conditions.

In our Human Genetic Laboratory the genotypic analysis was done using oligonucleotides probes marked with specific fluorescence for each one of the alleles in a test that combines the stipulated PCR technique and TacMan (Applied Biosystems).

The genotypes were determined by 7300 System SDS Software (Applied Biosystems). The calculated material cost is 1.1 Euros by individual sample and by each SNP. This value includes all required material and reagents to perform the whole analysis.

To establish an appropriate coronary risk score, with reduced costs, in spite of the large sample size, we used only SNPs that already had shown functional impact in coronary pathophisiology and large prevalence in our population.

Outsourcing might seem another way to reduce costs and these operating processes are gaining acceptance in many hospitals.

Genome-wide association studies suggest that common genetic variants explain only a modest fraction of heritable risk for common diseases, raising the question of whether rare variants account for a significant fraction of unexplained heritability. Although DNA sequencing costs have fallen markedly, they remain far from what is necessary for rare and novel variants to be routinely identified at a genome-wide scale in large cohorts (Sarah Ng et al., 2009).

Meanwhile, if we are evaluating not a single SNP, but a group of SNPs, creating a genetic score based on a small number of genes with reasonable costs, if this score can improve our prediction of the coronary artery disease risk, this genetic score could potentially be useful in the clinical practice.

In an epidemiologic study conducted in 2004, with the use of new risk prediction models which included genetic information, Khoury et al predicted that the prevention measures aiming at a small fraction (about 15%) of the high risk population with few classical risk factors, will be performed with the resources currently dedicated to the prevention, used in a more judicious way, but will also allow to significantly reduce the global load of coronary disease (Khoury et al., 2004).

The success of a model including a GRS should be measured by its ability to help individuals and physicians in making decisions regarding lifestyle advice or pharmacological treatment in order to decrease CHD risk and disability.

In this study, after multivariate logistic regression, the independent predictors of CHD were dyslipidemia, type 2 diabetes, hypertension, daily intake of alcohol and GRS. Although after multivariate logistic regression the risk of CHD determined by the GRS did not exceed that of conventional risk factors, this independent predictor based on genetic risk variants has some advantages.

The genetic analysis is easy to perform and is highly reliable in respect to certain biomarkers, most of which require lengthy methods for their determination, more prone to biological variation and error.

One of its greatest benefits will be its usefulness in identifying the 15% of high risk individuals who are masked as borderline or apparently low risk and that would not be identified in clinical surveys based on current protocols (Framingham, National Cholesterol Education Program Adult Treatment Panel III guidelines). In 2003, Akosah et al reported that among individuals who have suffered premature myocardial infarction (age <55 years male and <65 years female) 70% were considered to be of low risk, and of these 87% had not been eligible for treatment with statins (Akosah et al., 2003).

In the present study the inclusion of the genetic profile represented by GRS greatly increased the predictive capacity of the model over the observed in the model which included the traditional risk factors only (69.3% to 74.6%), showing the value of the genetic profile in the prediction of coronary heart disease risk. It introduces the concept of a GRS that aggregates information from multiple genetic variants into a single score and indicates

that a GRS can improve the ability to predict incident CHD beyond that afforded by traditional non genetic risk factors (Morrison et al., 2007).

A potential advantage of genetic profiling over conventional risk factors in the prediction of CHD risk is that genetic information is already present at birth. Therefore, genetic profiles could theoretically predict CHD risk before conventional risk factors become deleterious (Van der Net et al., 2009).

However, the dream of personalized medicine and predictive indexes may yet be achieved, but it seems that will take far more than these first steps on the road to gene discovery. This is a topic that surely should be revisited once efforts toward gene discovery, identification of functional variants and pathways, and searches for less common variants allowing for heterogeneity have progressed (Ioannidis, 2009).

5. Conclusions

The result of this study has demonstrated that a GRS, based on the presence of some susceptibility gene variants for coronary heart disease, allow the identification of sub-groups with a significantly higher risk of coronary disease.

The risk model that includes the GRS, the classical risk factors and some new risk markers, increases the GRS predictive power and provides a good estimation of the CHD risk. It introduces the concept that a risk model that aggregates information from several genetic variants (GRS), the traditional risk factors and some new risk markers, can improve the ability to predict incident CHD beyond that supplied by the classical risk factors.

The proposed model can become a simple and useful tool in the CHD risk stratification, giving us an early estimate of the disease susceptibility and prevention.

Key message

- Coronary Heart Disease is a complex multifactorial disease.
- Genetic profile, lifestyle and environmental factors play an important role in its development.
- Its genetic predisposition appears to be the result of the cumulative effects of various genetic polymorphisms or allelic combinations that in isolation would only confer a light or moderate risk. However, this risk will be modulated (increased or decreased) by various gene-gene and gene-environment interactions.
- Risk prediction of incident CHD is based on the knowledge of the conventional risk factors (high blood pressure, smoking, blood cholesterol level) and in the genetic profile, which can improve the prediction of incident CHD beyond that supplied by traditional risk factors, especially in the population fringe with few classical risk factors.
- The GRS aggregates information from some genetic variants into a single score and the present work indicates that the addition of this GRS to the information obtained by the classical risk factors can improve the ability to predict CHD risk
- With these mixed risk assessment tools, we can identify patients at high risk who would get the most benefit from the correction of modifiable risk factors through lifestyle interventions or specific medications.
- In the future, these new technologies and risk algorithms may change the strategies followed nowadays by Health Systems, and will permit to significantly reduce the load and the global burden of coronary disease.

6. Summary

Coronary heart disease (CHD) is the most common cause of death in the industrialized countries. As these populations are aging, the prevalence of CHD is arising, and it is expected that this tendency will increase, in the future, due to the obesity epidemic that is rising all over the world and this will place a considerable burden on healthcare systems. The knowledge of new risk models, can improve the diagnosis capacity and prevention as well as the early treatment of this condition.

The aim of this chapter is to obtain a risk model, which includes the classical risk factors and the genetic profile through the genetic risk score (GRS), in which the risk of CHD was estimated in a South European population. For that purpose a total of 1406 individuals, 723 consecutive patients with documented CHD in the coronary angiogram (53.7±8.9 years, 79.9% male) and 683 controls without any clinical manifestation of CHD (53.3±10.5 years, 73.9% male), randomly selected from electoral rolls, were evaluated. Cases and controls were selected with no significantly difference in terms of sex and age. Seven genetic polymorphisms, ACE I/D, AGT 235T/M , AT1R1166 A/C, PON Q192R, MTHFR M677C/T, Apo E (Ɛ2, Ɛ3, Ɛ4) and 9p21 locus (rs1333049), were determined, using the combined PCR stipulated technique with TaqMan (Applied Biosystems). A genetic risk score (GRS) was obtained, for each individual, using a coding value of 0 for the original non mutated homozygote, 1 for the heterozygote, and 2 for the mutated homozygote. The GRS was created by summing these values for each individual. The obtained score was divided into five quintiles (0 and 4; 4 and 5; 5-6; 6-7; ≥7) and was compared in patient and control population, using independent T-Student test. Case control Odds ratios and 95% Cornfield Confidence Intervals were determined. A forward Wald logistic regression model was constructed, adjusted for age and gender, having entered all conventional risk factors. Finally, a ROC curve was computed, to evaluate the capacity of this model to predict coronary disease susceptibility and *Hosmer Lemeshow goodness-of-fit* test estimated model calibration.

The mean GRS was 5.03±1.84, in the total population (maximum 11 and minimum 0). CHD patients had higher GRS than the control population (5.41±1.73 vs. 4.63±1.87, $p<0.0001$). In the last GRS quintile (score≥7) including more than a quarter of the patients, the CHD risk was three times higher than in the reference group. After logistic regression the GRS stayed in the equation as an independent risk marker associated with increased risk of CHD; (OR=1.333; $p<0.0001$). Diabetes type 2, dyslipidemia, arterial hypertension and alcohol intake, also remained in the equation as independent risk factors of CHD.

Two receiver operating characteristic (ROC) curve models were constructed and the model including both genetic and traditional risk factors was compared with the model which included only the traditional risk factors. The first presented increased AUC (75%) over that observed when the receiver operating characteristic curve was based only on traditional risk factors (69.3%).

Conclusion: This risk model including the GRS, the classic risk factors and the new risk markers, provided a good estimation of the CHD risk. It introduces the concept that a GRS that aggregates information from multiple genetic variants can improve the ability to predict incident CHD beyond that afforded by traditional non genetic risk factors. The proposed model can become a simple and useful tool in the CHD risk stratification, supplying us with an early estimation of disease susceptibility and prevention.

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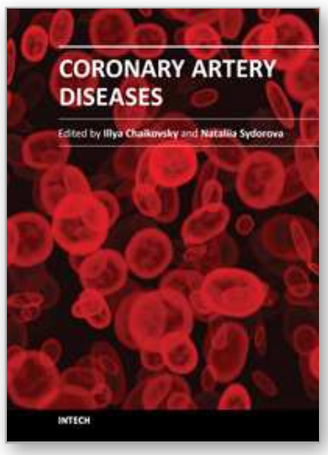
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This book has "wide geography" both literally and figuratively. First of all, this book brings together contributions from around the world, both from post-industrial countries and developing world. This is natural, because coronary artery disease is becoming pandemic worldwide. CAD is the single most frequent cause of death in developed countries, causes about 1 in every 5 deaths. Mortality from cardiovascular disease is predicted to reach 23.4 million in 2030. Moreover, in the developing world, cardiovascular disease tends to affect people at a younger age and thus could negatively affect the workforce and economic productivity. The morbidity, mortality, and socioeconomic importance of CAD make its diagnosis and management fundamental for all practicing physicians. On another hand, the book widely represents "geography" of CAD itself, i.e. many various aspects of its pathophysiology, epidemiology, diagnosis, treatment are touched in this book. This book does not pretend on complete and integral description of the Coronary artery disease. Rather, it contains selected issues on this complex multifactorial disease. Nevertheless, we hope that readers will find Coronary Artery Disease useful for clinical practice and further research.

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