Am J Cardiovasc Dis 2012;2(2):123-132
www.AJCD.us /ISSN:2160-200X/AJCD1203003

Original Article

Relationship of PON1 192 and 55 gene polymorphisms to calcific valvular aortic stenosis

Luis M Moura[1,6], Susana Faria[2], Miguel Brito[3], Fausto J Pinto[4], Steen D Kristensen[5], Isabel M Barros[6], Nalini Rajamannan[7], Francisco Rocha-Gonçalves[1]

[1]Department of Medicine, Oporto School of Medicine, University of Oporto, Portugal; [2]CMAT; Mathematical Research Centre, Department of Mathematics and Applications, University of Minho, Campus de Azurém, Portugal; [3]Department of Biology, Technological School of Health, Lisbon Polytechnic Institute, Portugal; [4]Department of Cardiology, Lisbon School of Medicine, University of Lisbon, Portugal; [5]Department of Cardiology, Aarhus University Hospital, Skejby, Denmark; [6]Pedro Hispano Hospital, Matosinhos, Portugal; [7]Northwestern School of Medicine, Chicago, IL, USA

Received March 8, 2012; accepted April 22, 2012; Epub May 15, 2012; Published June 15, 2012

Abstract: Introduction and Objectives: Paraoxonases may exert anti-atherogenic action by reducing lipid peroxidation. Previous studies examined associations between polymorphisms in the paraoxonase 1 (PON1) gene and development of coronary artery disease (CAD), with inconsistent results. Given the similarities in clinical and pathophysiological risk factors of CAD and calcific aortic valve stenosis (CAVS), we postulated a link between PON1 alleles and CAVS progression. Methods: We investigated the association between PON1 55 and 192 single nucleotide polymorphisms (SNPs), their enzyme activity, and CAVS progression assessed by aortic valve area and transvalvular peak velocity in 67 consecutive patients with moderate CAVS and 251 healthy controls. Results: PON1 paraoxonase activity was higher in CAVS patients (P<0.001). The PON1 genotype Q192R SNP (P=0.03) and variant allele (R192) (P=0.01) frequencies differed between CAVS patients and controls. Significant association existed between PON1 enzyme activity, phenotypic effects of PON1 192 genotype polymorphisms, and CAVS progression, but not between PON1 55 and high-density lipoprotein (P=0.44) or low-density lipoprotein cholesterol (P=0.12), between 192 genotype and high -density lipoprotein (P=0.24) or low-density lipoprotein cholesterol (P=0.52). Conclusion: The PON1 genotype Q192R SNP has an important effect on CAVS disease progression. This study helps outline a genotype-phenotype relationship for PON1 in this unique population.

Keywords: Calcific aortic stenosis, polymorphism, paraoxonase, atherosclerosis, genetics, association

Introduction

Congenital bicuspid aortic valve and advanced age are leading risk factors for calcific aortic valve stenosis (CAVS), the third leading cause of heart disease in adults [1-3]. However, epidemiological databases have helped to define other clinical risk factors, including smoking, male sex, hypertension, hypercholesterolemia, excess body mass index, and diabetes [4, 5].

Genetic predisposition has also been suggested as a mechanism for CAVS development [6], although few studies have elucidated the genes and genetic variants involved. Probst et al [7] demonstrated a familial aggregation for CAVS in western France. Polymorphisms in the vitamin D receptor are more common in patients with CAVS [8], and loss of function through mutation of a polymorphism in the NOTCH1 receptor accelerates calcification of the valve in patients with CAVS and other congenital heart abnormalities [9, 10].

Two studies have correlated genetic lipoprotein abnormalities in patients predisposed to CAVS development [11, 12] and Nordstrom et al [13] demonstrated that PvuII polymorphisms in the estrogen receptor alpha gene are related to presence of aortic stenosis (AS) in postmenopausal women and to lipid levels in adolescent females, suggesting that this polymorphism may influence CAVS risk by affecting lipid levels.

The importance of oxidative modification of low-density lipoprotein (LDL) in the pathogenesis of

atherosclerosis is well recognized [14, 15]. As an anti-atherogenic mediator, high-density lipoprotein (HDL), besides its key role in reverse cholesterol transport [16], protects itself and LDL against oxidation and reduces lipoprotein-associated peroxides[17-19]. The antioxidant property of HDL has been attributed, in part, to the paraoxonase 1 (PON1) enzyme [15, 18-20]. PON1 is an aryldialkylphosphatase synthesized in the liver and transported in systemic circulation exclusively in association with an HDL sub fraction containing apolipoproteins (apo) A-I and apoJ [21-23]. Serum PON1 activity is inversely related to coronary artery disease (CAD), hypercholesterolemia, and diabetes [23, 24].

Previous studies [23] reported two protein polymorphisms in PON1 Q192R and L55M that exert significant effects on PON1 activity. We typed these two polymorphisms in all subjects and determined allele frequencies. Individually, both polymorphisms exert significant effects on all substrate activities. The previous studies showed that QQ individuals had lower PON1 activity compared to RR, whereas LL individuals had higher activity for all substrates compared to MM. The M55 variant is associated with lower PON1 activity levels, probably as a result of a large imbalance with the Q192 PON1 variants [24, 25]. The variability of PON1 activity towards paraoxon is partly determined by polymorphisms in the paraoxonase gene located on chromosome 7 between q21.3 and 22.1. Of these, the codon 192 polymorphism produces two alloenzymes, with low and high activity, respectively [26, 27]. In the high-activity alloenzyme, glutamine (Q allele) is replaced by arginine (R allele) at codon 192. In several but not all studies, the R192 allele was associated with CAD risk [28-33] and adverse lipoprotein profile [28, 32, 34-36]. PON1 has other common polymorphisms at codon 55 [Leu (L)/Met (M)] that correlate with enzyme activity [26, 37]. Increased enzyme activity has been associated with higher HDL levels in some populations, but not in all studies [38]. The PON 192 and 55 polymorphisms also have been associated with risk of ischemic stroke [39].

In the past decade, several studies have investigated the association between the single nucleotide polymorphisms (SNPs) of the PON gene cluster and CAD susceptibility, but have yielded apparently conflicting results. The PON1 genotype Gln192Arg (Q192R) SNP was significantly associated with stroke in CARE trial patients [25] and PON1 activity is lower in patients with CAVS and inversely correlated with CAVS severity [26]. Inconsistent results could be due to insufficient power, the small effect of the polymorphism on CHD risk, and/or false-positive results. A recent meta-analysis suggested a weak association, overall, between Q192R SNP and CHD risk [27]. Altogether, these clinical studies focused on Q192R PON1 SNP suggest that a certain genetic background may be related to higher risk of developing CAVS. We chose PON1 as a candidate marker to test the hypothesis that common PON1 genetic variants could influence aortic valve calcification and CAVS progression. The study aimed to evaluate (1) the allele frequency of Q192R and L55M SNPs, (2) the effects of this polymorphism on serum lipoprotein variables, and (3) the relationship between Q192R and L55M PON1 gene SNPs and PON1 enzyme activity in CAVS progression in our patient population.

Methods

Research ethics

The study complied with the Helsinki declaration and the Pedro Hispano Hospital Institutional Review Board (Matosinhos, Oporto, Portugal) approved the protocol (IRB-22352). All participants were fully informed and provided signed consent before enrollment.

Study population

Participants in the prospective Rosuvastatin Affecting Aortic Valve Endothelium (RAAVE) study were asymptomatic patients with moderate AS, defined as an aortic valvular area (AVA) of 1.0 to 1.5 cm^2. We selected 255 consecutive, statin-naïve, new AS referrals to our inpatient and outpatient cardiology clinic [28] and excluded those with history of CAD (myocardial infarction and/or angiographically demonstrated coronary artery stenosis), aortic valve surgery, congenital cardiac disease (bicuspid aortic valve), statin therapy, active or chronic liver disease, or currently taking an angiotensin-converting enzyme inhibitor. No other medications were contraindicated, including other antihypertensives, oral hypoglycemics, or insulin. Other exclusion criteria were echocardiographic evidence of rheumatic mitral valve disease, aortic regurgitation, subaortic obstruction, and creatinine concentration ≥2.0 mg/dL (to minimize the risk of hypercalcemia as a potential

confounding factor).

Of the remaining 135 eligible participants, 14 were unsuitable due to technical problems with initial echocardiography or difficulties in obtaining medical history. Sufficient DNA was available for genetic analysis of 67 of the 121 asymptomatic AS patients finally included, before randomization to treatment. To estimate 10-year cardiovascular risk of death, we included a control group of 251 healthy volunteers (age >45 years, without CAD or AS, with low cardiovascular risk based on sex, age, smoking status, blood pressure, and total cholesterol). Data obtained for all participants included age, sex, smoking history, and presence of hypercholesterolemia, arterial hypertension (>140/90 Hg), and diabetes. At inclusion, no participant had detectable evidence of inflammatory, neoplastic, metabolic, or vascular disease in an initial history, examination, or routine tests. Patients received standard clinical care throughout the study.

Biochemistry

Blood was drawn after 12-hour fasting for routine clinical biochemistry in the Pedro Hispano Hospital laboratory, following standard institutional protocols. In-hospital audit had previously demonstrated sample result variability between 5% and 15% for these assays (data not shown).

PON1 activity

Serum PON1 paraoxonase (PON1-para) activity was determined using paraoxon (Aldrich Chemical Co, St. Louis, MO) as previously described [29]. Briefly, the assay buffer was prepared from 0.132 M Tris–HCl, pH 8.5, and 1.32 mM CaCl2. Each set of assays used 6 mM freshly prepared paraoxon substrate solution (120 mM paraoxon in acetone diluted with 0.132 mM Tris –HCl). The assay tube contained 152 µl Tris buffer, 8 µl serum (1:2 diluted with water), and 40 µl 6-mM paraoxon. The reaction was initiated at 37.8°C by adding the substrate solution; absorbance was continuously monitored at 405 nm. A molar extinction coefficient of 18.05 x 10^3 was obtained and used to calculate activity, and units were expressed as U/L.

PON1 genotyping

Genetic samples were taken following 10 minutes of supine rest and immediately prior to echocardiography. Whole blood samples were collected, stored, and frozen at -80°C. Extraction was done using phenol-chloroform purification, and DNA was eluted in 50 µl TE. Polymorphisms were genotyped using a TaqMan allelic discrimination assay. Q192R (rs662) and L55M (rs854580) PON1 polymorphisms were typed using primers and probes from the Applied Biosystems Predesigned Drug Metabolism Genotyping Assays program (Applied Biosystems, Foster City, CA). Genotyping was done with the ABI Prism 7900HT Sequence Detection System and typing errors were reduced using Simwalk2 (Sobel and Lange, 1996). The Q192R genotype was 97% concordant with previous assessments based on PON1 activity status [30] and, in a subset of unrelated individuals, both polymorphisms were in Hardy-Weiberg equilibrium.

Echocardiography

Comprehensive transthoracic echocardiograms were performed and reported in a single central echocardiography laboratory (Acuson Sequoia C512, Siemens Healthcare, Erlangen, Germany) by either of two experienced cardiologists specialized in echocardiography (LM and IB). Immediate inter-observer review determined which studies should be repeated to maintain quality control. Everyone involved in performing and interpreting echocardiograms was blinded to patient treatment status. Hemodynamic progression was assessed using serial echocardiographic studies at 6-month intervals.

Standard Doppler recordings were made of the left ventricular outflow tract and aortic valve from multiple views to obtain peak transvalvular jet velocity (V_{max}), mean and peak gradients, and AVA in accordance with international guidelines [31, 32]. Left atrial volume relative to body surface area was estimated from end-systolic measurements [33-37]. Reproducibility of the echocardiography observations by the two cardiologists was assessed in a 30-patient subset. All echocardiography parameters examined showed intra-class coefficient correlation (ICC) between 0.962 and 0.989 (intra-observer) and between 0.955 and 0.992 (inter-observer). Conventional Doppler and tissue Doppler imaging (TDI) parameters [38, 39] had good intra- and inter-observer coefficients of reproducibility (1.56-9.02). Coefficients of variation and reproducibility were, for intra-observer variability, 1.88% and 0.16 m/sec for V_{max} and 3.89% and 0.06 cm^2 for AVA, respectively, for observer 1

and 2.01% and 0.14 m/sec for Vmax, and 3.25% and 0.08 cm^2 for AVA, respectively, for observer 2, and for inter-observer variability, 2.0% and 0.14 m/sec for V_{max} and 2.91% and 0.04 cm^2 for AVA, respectively.

Statistical analysis

Patient characteristics are presented as mean±standard deviation (SD) for normally distributed continuous variables; categorical variables are expressed as frequencies (percentages) and non-normally distributed continuous variables as the median (1st-3rd interquartile ranges). Continuous variables were assessed usingKolmogorov-Smirnov test to verify the assumption of normality.

Reproducibility was assessed with the Bland and Altman method [40, 41] and results expressed as a coefficient of reproducibility (twice the SD of the differences). Differences in baseline characteristics between CAVS patients and controls were compared with independent t-tests for normally distributed continuous variables, Mann-whitney U tests for non-normally distributed continuous variables, and chi-square for categorical variables.

Differences between PON1 genotypes groups and lipid profile were compared using one-way ANOVA. The relationship between enzyme activity and alleles was studied using one-way ANOVA followed by Tukey HSD multiple comparison test. Differences between AS patients and controls in the distribution of genotype and allele frequencies and estimates of Hardy-Weinberg equilibrium were tested using chi-squared. Logistic regression modeling determined the association between the independent effect of the Q192R SNP and the effect of interaction between genotype, PON1 activity, and other covariates on lipids and CAVS progression. All statistical analysis was performed using SPSS, version 17.0.1 for Windows SPSS Inc, Chicago, IL). Significance level was .05 for all analyses.

Results

Population characteristics

Patients and controls were chosen from consecutive referrals to our inpatient and outpatient cardiology clinic. Social and clinical differences between the two groups have been published in detail elsewhere [28]; a summary is provided in **Table 1**. In addition to having similar serum lipid levels, the groups did not differ with respect to sex (male: AS group 62.7% vs. controls 49.0%, *P*=0.320) or other established cardiovascular risk factors. The AS group was more likely to have elevated total cholesterol (227.0±52.0 md/dL vs. 189.1±44.1 mg/dL in controls; *P*=0.004), elevated HDL-cholesterol (53.4±10.7 md/dL vs. 43.7±11.4. mg/dL, respectively; *P*=0.001), and diabetes mellitus (44.8% vs. 11.8%, respectively; *P*=0.001).

Baseline cardiac function

Both groups had similar normal left ventricular systolic function (*P*=0.067), with ejection fraction 54.3±2.1% in AS patients and 55.6±4.4% in controls (**Table 1**). End-diastolic long axis diameter was 51.4±5.7 mm in the AS group vs. 52.3±4.2 mm in controls (*P*=0.311). End-systolic long-axis diameter was 33.4±4.8 mm in the AS group vs. 34.6±3.9 mm in controls (*P*=0.165). There was no difference between groups in left ventricular mass (*P*=0.091) or left atrial volume (*P*=0.625).

Paraoxonase1 55 and 192 polymorphism and the presence of CAVS

The PON1 55 and 192 polymorphisms had allele frequencies of 51.8% for L and 83.2% for R in AS patients, and 42% and 72.9%, respectively, in controls (**Table 2**). Analysis of Q192R distributions in the different PON1 genotypes showed significant prevalence in the frequency of R alleles in the AS patients (*P*=0.01); in addition, 62.7% were carriers of RR variant allele vs. 45.0% of controls (*P*=0.03). With respect to PON1 L55M, there were no significant differences in allelic frequency and allelic variants (LL, MM and LM) between groups (*P*>0.05) (**Table 2**). For both PON1 55 and 192 polymorphisms, good agreement was found between the observed and expected genotype frequencies according to the Hardy-Weinberg equilibrium (data not shown).

Linkage disequilibrium

The analysis of linkage disequilibrium between PON1 polymorphisms is presented in **Table 3**. We observed, as have other studies, a strong (D>90%) genetic linkage disequilibrium

Table 1. Baseline Clinical Characteristics

Characteristic	*All patients n=318*	*CAVS patients n=67*	*Controls n=251*	*P*
Clinical				
Age (years)	73.7 ±9.2	73.4±8.9	73.8±9.1	0.831
Male	165(51.9%)	42(62.7%)	123(49.0%)	0.070
BMI (kg/m^2)	28.3 ±5.0	29.2 ±4.5	28.1±4.9	0.210
Arterial hypertension	118(37.1%)	20(29.9%)	98(39.1 %)	0.088
Diastolic BP (mmHg)	74.9±12.9	77.5±13.0	74.2±12.8	0.192
Systolic BP(mmHg)	148.8±23.7	153.1±21.9	147.6±24.1	0.171
Diabetes	60 (18.9%)	30(44.8%)	30(12.0%)	0.001*
Sinus rhythm	271(85.2%)	60(89.6%)	211(84.0 %)	0.284
Heart rate (beats/min)	71.5±12.8	74.3±13.1	70.7±12.6	0.138
Biochemical				
Total Cholesterol (mg/dL)	197.1±48.3	227.0±52.0	189.1±44.1	0.004
HDL (mg/dL)	45.8±11.9	53.4±10.7	43.7±11.4	0.001
LDL (mg/dL)	126.5±42.0	145.7±42.2	121.3±40.5	0.078
BNP (pg/mL)	39.9 (15.9-91.8)	37.9 (15.9-87.3)	45.3 (23.4-91.8)	0.405
PON1 activity (μmol/min/mL)	155.0±39.6	230.8±1.20	134.7±6.48	<0.001
Echocardiography				
End-diastolic long-axis diameter (mm)	52.1±4.6	51.4±5.7	52.3±4.2	0.311
End-systolic long-axis diameter (mm)	34.3±4.1	33.4±4.8	34.6±3.9	0.165
Ejection fraction (%)	55.3±4.1	54.3±2.1	55.6±4.4	0.067
Left ventricular mass index (g/m^2)	78.4±22.4	81.9±29.1	77.5±20.2	0.091
Left atrial volume/SA (mL/m^2)	36.0±11.4	36.3±10.4	35.9±11.7	0.625

Values are means±standard deviations for normally distributed continuous variables, medians (interquartile range) for non-normally distributed continuous variables, and frequencies (percentages) for categorical variables. Abbreviations: CAVS, calcific aortic valvular stenosis; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BNP, *brain natriuretic peptide*; PON1, Paraoxonase 1; SA, *surface area*.

Table 2. Frequency of Paraoxonase 1 (PON1) 55 and 192 Polymorphisms

Polymorphism (frequencies and percentages)	Genotype	CAVS patients n=67	%	Controls n=251	%	*P*
PON1: Q192R						0.03
	RR	42	62.7	113	45.0	
	QR	20	29.9	98	39.1	
	QQ	5	7.5	40	15.9	
	Allele Frequency					0.01
	R	124	83.2	344	72.9	
	Q	25	16.8	128	27.1	
PON1: L55M						>0.05
	LL	20	29.9	54	21.6	
	MM	20	29.9	94	37.3	
	LM	27	40.3	103	41.1	
	Allele Frequency					>0.05
	L	67	50.8	211	42.0	
	M	65	49.2	291	58.0	

between PON1 polymorphisms at the promoter and coding regions.

Paraoxonase1 55 and 192 polymorphisms and lipid profile

Mean levels of serum lipoproteins for Q192R and L55M are presented in **Table 4**. After adjusting for age, sex, and blood pressure, the Q and R alleles were not associated with significantly increased HDL cholesterol (*P*=0.44); the

Table 3. Linkage disequilibrium among Paraoxonase 1(PON1) polymorphisms

	PON1 192		*PON1 55*	
	D%	*P*	*D%*	*P*
PON1 192				
	98.9	0.008	99.4	0.029
PON1 55				
	92.5	<0.0001		

D%=percentage of linkage disequilibrium. *P* values from chi-square analysis.

Table 4. Relationship between paraoxonase 1(PON1) 55 and 192 genotype and lipid serum levels

Lipid Profile	*RR*	*QQ*	*QR*	*P*
Total Cholesterol (mg/dL)	210.9±52.3	240.0±49.2	226.6±52.8	0.25
HDL Cholesterol	51.8±11.4	45.3±8.0	51.2±124	0.44
LDL Cholesterol	134.7±42.4	161.8±42.5	152.7±41.1	0.12
	LL	*MM*	*LM*	*P*
Total Cholesterol (mg/dL)	244.1±61.3	260.2±79.2	246.7±75.6	0.34
HDL Cholesterol	79.2±9.8	55.8±7.8	51.2±159	0.24
LDL Cholesterol	144.9±53.4	161.8±42.5	172.3±63.3	0.52

Values are means±standard deviations. Statistically significant *(P* <0.05) between compared groups. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 5. Correlation of enzyme activity with PON1 192 and 55 genotype and CAVS progression

Q192R Activity (U/L)	*RR*	*QQ*	*QR*	*P*
	168.74±4.16	103.52±2.13	145.45±3.17	<0.001
	LL	*MM*	*LM*	*P*
L55 M Activity (U/L)	136.56±2.45	116.93±7.23	129.77±2.92	0.12
Echo Parameters	*RR*	*QQ*	*QR*	*P*
AVA Reduction (cm^2)	-0.14±0.06	-0.07±0.06[a]	-0.04±0.07[a]	0.029
V_{max} Increase (m/s)	0.19±0.16[b]	0.17±0.16[b]	0.05±0.16	0.041
	LL	*MM*	*LM*	*P*
AVA Reduction (cm^2)	-0.17±0.06	-0.14±0.08	-0.12±0.10	0.62
V_{max} Increase (m/s)	0.17±0.19	0.19±0.13	0.15±0.11	0.84

Values are means±standard deviations . Statistically significant *(P* <0.05) between compared groups. PON activity in U/L=micromole of substrate hydrolysed/min. Statistically significant (*P* <0.05) between compared groups. (a) significant differences compared to RR allele, (b) significant differences compared to QR allele Abbreviations: AVA: aortic valvular area e VMAX:transvalvular jet velocity

same was true for L and M alleles (*P*=0.24). Neither Q nor R allele genotypes were associated with serum levels of total cholesterol (*P*=0.25 and *P*=0.34, respectively) or LDL cholesterol (*P*=0.12 and *P*=0.52, respectively).

Paraoxonase 1 55 and 192 polymorphisms and PON1 enzyme activity

The carriers of RR variant allele demonstrated a significantly higher AVA reduction than the carriers of QQ variant allele (one-way ANOVA, Tukey's HSD test p=0.04) and QR variant allele (one-way ANOVA, Tukey's HSD test p=0.02). Moreover the carriers of QR variant allele have showed a significantly lower V_{max} increase than QQ allele (one-way ANOVA, Tukey's HSD test p=0.04) and RR variant allele (one-way ANOVA, Tukey's HSD test p=0.04) **(Table 5).**

Moreover, individually, both polymorphisms exerted significant effects on all substrate activi-

Table 6. Predictors of CAVS progression by stepwise multiple logistic regression

PON1 activity	*OR*	*95% CI*	*P*
	1.35	1.1-1.7	0.003
R192 allele			
	1.23	1.1-14	0.002

Statistically significant (P <0.05) between compared groups. Abbreviations CI: confidence interval; OR: odds ratio

ties. In addition, RR individuals had higher activity than QQ or QR individuals, and LL individuals, compared to MM and LM, had even lower PON1 substrate activities.

Paraoxonase 1 55 and 192 polymorphisms and CAVS progression

To test the genotype effect on CAVS progression we evaluated the independent association between PON1 activity and genotypes as markers of disease progression using a multiple logistic regression analysis, having adjusted the model for predictors such as baseline AVA, sex, age, hypertension, diabetes mellitus, LDL cholesterol, degree of valve calcification, LV hypertrophy, MR, LV end-diastolic volume, statin use, and baseline CAVS severity. Disease progression was considered a variable of independent outcome and was classified according to the criteria presented above. Results of the stepwise multiple logistic regression are shown in Table 6. The odds ratio and associated 95% confidence intervals were estimated to assess the magnitude of the association between the various factors and disease progression. PON1 activity was strongly associated with CAVS disease progression. In comparison to Q192 allele, the presence of R192 allele was associated with a 23% greater increase in CAVS disease progression.

Discussion

The present study demonstrates that (1) the relative allele frequency of the Q192R SNP of the PON1 gene differs markedly between CAVS patients and controls, (2) genotype has no effect on lipid profile, and (3) the variation of PON1 activity could explain the variation in phenotypic results, particularly CAVS progression.

PON1 is considered anti-atherogenic because of its ability to destroy the inflammatory lipid peroxides formed by LDL oxidation [15, 42], which promotes many of the steps in CAVS progression. Because PON1 protects the aortic valve against degenerative processes by inhibiting LDL oxidation, decreased PON1 activity will increase oxidative stress and inflammation, while excessive inflammation will accelerate valve degeneration and stenosis. To date, however, no specific genetic marker has been identified that predicts CAVS development. Whether or not there is a genetic contribution, in addition to multiple clinical atherosclerotic risk factors, to the development and progression of degenerative CAVS remains controversial: some in vitro studies have shown increased protection of the Q 192 allele against LDL oxidation compared to the R192 allele [19, 42], while other studies have found equal [43] or decreased protection [44]. Because it has been suggested that polymorphisms negatively influence PON1 transcription or activity (increasing the rate of atherosclerosis), we analyzed the influence of Q192R and L55M SNPs in patients with CAVS.

Previous studies associated the R192R variant with increased levels of serum LDL cholesterol, HDL cholesterol, and triglycerides [45, 46]; recent studies have found either no adverse genotypic effect on lipoprotein variables [47-49] or a positive association between the Q192 allele and levels of HDL cholesterol and apo-I [50]. In addition, the physical relationship of PON1 with HDL and the existence of cholesterol regulatory elements at the PON1 locus suggest a further relationship of PON1 with lipoproteins, which may contribute to its role in vascular disease [51]. The biological basis for these divergent observations, including ours, is not clear. Our study suggests that Q192 PON1 bearers were better protected from oxidative damage caused by serum LDL cholesterol than were bearers of R192 PON1 and confirms recent in vitro findings [35]. In our study, PON1 genotype Q192R SNP and its activity were also significantly correlated with CAVS progression evaluated by AVA and V_{max}. These results are consistent with earlier findings on CAD progression and stroke [25, 52].

The main limitation of this study is the small number of patients. Although our patient population was homogeneous in terms of demographic characteristics, our results could have been influenced at least in part, as in many observational studies, by differences other than

genetics. As a genetic association study, there are certain limitations in that it cannot provide evidence of causative relationship between the Q192R genotype and outcome phenotypes. Although the observed associations are consistent with biological plausibility, it is likely that the genotypic effect may be due to linkage disequilibrium with another closely linked functional mutation in the PON1 gene or a nearby gene in the chromosome. Further, because this is a non-randomized and observational study, our results are necessarily limited by the population frequencies of individual alleles, and gene -gene and/or gene-environment interaction might have uniquely influenced the outcome measures in this study cohort. For these reasons additional studies with a larger sample size are needed to confirm our observations.

In conclusion, we show that the Q192R polymorphism of the PON1 gene and PON1 activity influence the risk of CAVS progression. However, knowledge of a simple aspect of the PON1 phenotype may not provide a complete picture of the role PON1 may play in metabolism and even in an individual's susceptibility to cardiovascular disease. PON1 is controlled primarily by variation at the structural gene, but also by variation at several other unidentified genes. Nevertheless, the approach we used holds potential for future experimental and clinical trials designed to shed light on the role of polygenic factors in the CAVS progression.

Acknowledgements

We thank E Lilly, PhD, for improving the use of English in the manuscript.

Address correspondence to: Prof. Dr. Luis M Moura, Serviço de Medicina A, Faculdade de Medicina da Universidade do Porto, Alameda Prof. Hernani Monteiro; 4200-319, Porto, Portugal. Tel: +351 225513659; Fax: +351226092318; E-mail: luismoura@med.up.pt

References

[1] Freeman RV, Crittenden G and Otto C. Acquired aortic stenosis. Expert Rev Cardiovasc Ther 2004; 2: 107-116.

[2] Sverdlov AL, Ngo DT, Chapman MJ, Ali OA, Chirkov YY and Horowitz JD. Pathogenesis of aortic stenosis: not just a matter of wear and tear. Am J Cardiovasc Dis 2011; 1: 185-199.

[3] Sverdlov AL, Ngo DT and Horowitz JD. Pathogenesis of aortic sclerosis: association with low BMI, tissue nitric oxide resistance, but not systemic inflammatory activation. Am J Cardiovasc Dis 2012; 2: 43-49.

[4] Aronow WS, Ahn C, Kronzon I and Goldman ME. Association of coronary risk factors and use of statins with progression of mild valvular aortic stenosis in older persons. Am J Cardiol 2001; 88: 693-695.

[5] Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, Kitzman DW and Otto CM. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. J Am Coll Cardiol 1997; 29: 630-634.

[6] Rajamannan NM. Calcific aortic stenosis: lessons learned from experimental and clinical studies. Arterioscler Thromb Vasc Biol 2009; 29: 162-168.

[7] Probst V, Le Scouarnec S, Legendre A, Jousseaume V, Jaafar P, Nguyen JM, Chaventre A, Le Marec H and Schott JJ. Familial aggregation of calcific aortic valve stenosis in the western part of France. Circulation 2006; 113: 856-860.

[8] Ortlepp JR, Hoffmann R, Ohme F, Lauscher J, Bleckmann F and Hanrath P. The vitamin D receptor genotype predisposes to the development of calcific aortic valve stenosis. Heart 2001; 85: 635-638.

[9] Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD and Srivastava D. Mutations in NOTCH1 cause aortic valve disease. Nature 2005; 437: 270-274.

[10] Garg V. Molecular genetics of aortic valve disease. Curr Opin Cardiol 2006; 21: 180-184.

[11] Avakian SD, Annicchino-Bizzacchi JM, Grinberg M, Ramires JA and Mansura AP. Apolipoproteins AI, B, and E polymorphisms in severe aortic valve stenosis. Clin Genet 2001; 60: 381-384.

[12] Novaro GM, Sachar R, Pearce GL, Sprecher DL and Griffin BP. Association between apolipoprotein E alleles and calcific valvular heart disease. Circulation 2003; 108: 1804-1808.

[13] Nordstrom P, Glader CA, Dahlen G, Birgander LS, Lorentzon R, Waldenstrom A and Lorentzon M. Oestrogen receptor alpha gene polymorphism is related to aortic valve sclerosis in postmenopausal women. J Intern Med 2003; 254: 140-146.

[14] Steinberg D, Parthasarathy S, Carew TE, Khoo JC and Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915-924.

[15] Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, Edwards PA and Fogelman AM. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler Thromb Vasc Biol 1996; 16: 831-842.

[16] Fielding CJ and Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995; 36: 211-228.
[17] Mackness MI, Arrol S, Abbott C and Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 1993; 104: 129-135.
[18] Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM and Navab M. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest 1995; 96: 2882-2891.
[19] Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL and La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998; 101: 1581-1590.
[20] Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, Roberts C, Durrington PN and Mackness MI. Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? Arterioscler Thromb Vasc Biol 2001; 21: 1451-1457.
[21] Blatter MC, James RW, Messmer S, Barja F and Pometta D. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. Eur J Biochem 1993; 211: 871-879.
[22] Kelso GJ, Stuart WD, Richter RJ, Furlong CE, Jordan-Starck TC and Harmony JA. Apolipoprotein J is associated with paraoxonase in human plasma. Biochemistry 1994; 33: 832-839.
[23] McElveen J, Mackness MI, Colley CM, Peard T, Warner S and Walker CH. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clin Chem 1986; 32: 671-673.
[24] Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M and Durrington PN. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis 1991; 86: 193-199.
[25] Ranade K, Kirchgessner TG, Iakoubova OA, Devlin JJ, DelMonte T, Vishnupad P, Hui L, Tsuchihashi Z, Sacks FM, Sabatine MS, Braunwald E, White TJ, Shaw PM and Dracopoli NC. Evaluation of the paraoxonases as candidate genes for stroke: Gln192Arg polymorphism in the paraoxonase 1 gene is associated with increased risk of stroke. Stroke 2005; 36: 2346-2350.
[26] Maganti K, Rigolin VH, Sarano ME and Bonow RO. Valvular heart disease: diagnosis and management. Mayo Clinic proceedings. Mayo Clinic 2010; 85: 483-500.
[27] Wang M, Lang X, Zou L, Huang S and Xu Z. Four genetic polymorphisms of paraoxonase gene and risk of coronary heart disease: a meta-analysis based on 88 case-control studies. Atherosclerosis 2011; 214: 377-385.
[28] Moura LM, Ramos SF, Zamorano JL, Barros IM, Azevedo LF, Rocha-Goncalves F and Rajamannan NM. Rosuvastatin affecting aortic valve endothelium to slow the progression of aortic stenosis. J Am Coll Cardiol 2007; 49: 554-561.
[29] Rainwater DL, Mahaney MC, Wang XL, Rogers J, Cox LA and Vandeberg JL. Determinants of variation in serum paraoxonase enzyme activity in baboons. J Lipid Res 2005; 46: 1450-1456.
[30] Richter RJ, Jampsa RL, Jarvik GP, Costa LG and Furlong CE. Determination of paraoxonase 1 status and genotypes at specific polymorphic sites. Curr Protoc Toxicol 2004; Chapter 4: Unit4 12.
[31] Bonow RO, Carabello BA, Chatterjee K, de Leon AC, Faxon DP, Freed MD, Gaasch WH, Lytle BW, Nishimura RA, O'Gara PT, O'Rourke RA, Otto CM, Shah PM, Shanewise JS, Smith SC, Jacobs AK, Adams CD, Anderson JL, Antman EM, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Lytle BW, Nishimura R, Page RL and Riegel B. ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (writing Committee to Revise the 1998 guidelines for the manage. Journal of the American College of Cardiology 2006; 48: e1-148.
[32] Galderisi M, Henein MY, D'Hooge J, Sicari R, Badano LP, Zamorano JL and Roelandt JR. Recommendations of the European Association of Echocardiography: how to use echo-Doppler in clinical trials: different modalities for different purposes. Eur J Echocardiogr 2011; 12: 339-353.
[33] Rodriguez L, Garcia M, Ares M, Griffin BP, Nakatani S and Thomas JD. Assessment of mitral annular dynamics during diastole by Doppler tissue imaging: comparison with mitral Doppler inflow in subjects without heart disease and in patients with left ventricular hypertrophy. Am Heart J 1996; 131: 982-987.
[34] Nishimura RA and Tajik AJ. Evaluation of diastolic filling of left ventricle in health and disease: Doppler echocardiography is the clinician's Rosetta Stone. J Am Coll Cardiol 1997; 30: 8-18.
[35] Nagueh SF, Middleton KJ, Kopelen Ha, Zoghbi Wa and Quiñones Ma. Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. Journal of the American College of Cardiology 1997; 30: 1527-1533.
[36] Jassal DS, Tam JW, Dumesnil JG, Giannoccaro PJ, Jue J, Pandey AS, Joyner CD, Teo KK and Chan KL. Clinical usefulness of tissue Doppler imaging in patients with mild to moderate aortic stenosis: a substudy of the aortic stenosis progression observation measuring effects of rosu-

vastatin study. J Am Soc Echocardiogr 2008; 21: 1023-1027.
[37] Steine K, Rossebo AB, Stugaard M and Pedersen TR. Left ventricular systolic and diastolic function in asymptomatic patients with moderate aortic stenosis. Am J Cardiol 2008; 102: 897-901.
[38] Lund O, Flo C, Jensen FT, Emmertsen K, Nielsen TT, Rasmussen BS, Hansen OK, Pilegaard HK and Kristensen LH. Left ventricular systolic and diastolic function in aortic stenosis. Prognostic value after valve replacement and underlying mechanisms. Eur Heart J 1997; 18: 1977-1987.
[39] Weber M, Arnold R, Rau M, Elsaesser A, Brandt R, Mitrovic V and Hamm C. Relation of N-terminal pro B-type natriuretic peptide to progression of aortic valve disease. Eur Heart J 2005; 26: 1023-1030.
[40] Bland JM and Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1: 307-310.
[41] Bland JM and Altman DJ. Regression analysis. Lancet 1986; 1: 908-909.
[42] Durrington PN, Mackness B and Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 2001; 21: 473-480.
[43] Cao H, Girard-Globa A, Berthezene F and Moulin P. Paraoxonase protection of LDL against peroxidation is independent of its esterase activity towards paraoxon and is unaffected by the Q-->R genetic polymorphism. J Lipid Res 1999; 40: 133-139.
[44] Kuremoto K, Watanabe Y, Ohmura H, Shimada K, Mokuno H and Daida H. R/R genotype of human paraoxonase (PON1) is more protective against lipoprotein oxidation and coronary artery disease in Japanese subjects. J Atheroscler Thromb 2003; 10: 85-92.
[45] Saha N, Roy AC, Teo SH, Tay JS and Ratnam SS. Influence of serum paraoxonase polymorphism on serum lipids and apolipoproteins. Clin Genet 1991; 40: 277-282.
[46] Hegele RA, Brunt JH and Connelly PW. Multiple genetic determinants of variation of plasma lipoproteins in Alberta Hutterites. Arterioscler Thromb Vasc Biol 1995; 15: 861-871.
[47] Antikainen M, Murtomaki S, Syvanne M, Pahlman R, Tahvanainen E, Jauhiainen M, Frick MH and Ehnholm C. The Gln-Arg191 polymorphism of the human paraoxonase gene (HUMPONA) is not associated with the risk of coronary artery disease in Finns. J Clin Invest 1996; 98: 883-885.
[48] Herrmann SM, Blanc H, Poirier O, Arveiler D, Luc G, Evans A, Marques-Vidal P, Bard JM and Cambien F. The Gln/Arg polymorphism of human paraoxonase (PON 192) is not related to myocardial infarction in the ECTIM Study. Atherosclerosis 1996; 126: 299-303.
[49] Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, Miller JE, Boulton AJ and Durrington PN. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis 1998; 139: 341-349.
[50] Ruiz J, Blanche H, James RW, Garin MC, Vaisse C, Charpentier G, Cohen N, Morabia A, Passa P and Froguel P. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet 1995; 346: 869-872.
[51] Deakin S, Leviev I, Guernier S and James RW. Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. Arterioscler Thromb Vasc Biol 2003; 23: 2083-2089.
[52] Wheeler JG, Keavney BD, Watkins H, Collins R and Danesh J. Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. Lancet 2004; 363: 689-695.