ELSEVIER

Atherosclerosis 148 (2000) 125–129

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Genetic polymorphism of heparan sulfate proteoglycan (Perlecan, HSPG2), lipid profiles and coronary artery disease in the Australian population

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Received 21 January 1999; received in revised form 5 April 1999; accepted 2 June 1999

Abstract

Perlecan is one of the three major classes of heparan sulfate proteoglycans (HSPGs) within the cardiovascular system; it interacts with lipid metabolism by binding to lipoprotein lipase (LpL) and apolipoprotein B (apo B) and may be related to vascular disease. We explored interactions between an HSPG2 polymorphism (BamHI marker), and apo B and coronary artery disease (CAD) in patients undergoing coronary angiography. The frequencies of the HSPG2 BamHI $+/+$, $+/-$, and $-/-$ genotypes were 4.7, 31.7 and 63.6%, respectively, with a '+' allele frequency of 20.6%. The genotype distribution was in Hardy–Weinberg equilibrium ($\chi^2 = 0.669$, $P > 0.05$). The $+/+$ homozygotes had the lowest apo B levels (0.74 ± 0.06 g/l, $n = 36$) compared to $+/-$ (0.89 ± 0.03 g/l, $n = 241$) and $-/-$ (0.93 ± 0.02 g/l, $n = 480$) genotypes. Although plasma apo B concentration was the strongest lipid risk factor for significant CAD, the HSPG2 genotypes were not independently associated with the presence of CAD ($P = 0.640$ in males; $P = 0.224$ in females), with significant CAD ($P = 0.764$; $P = 0.110$) or with the number of significantly stenosed coronary arteries ($P = 0.945$; $P = 0.335$). In Australian Caucasians undergoing coronary angiography the HSPG2 BamHI polymorphism is associated with lower circulating apo B but not with the occurrence or severity of CAD. This may be due to HSPG2-mediated alterations in the HSPG2-apo B-LpL system and requires further exploration.

Keywords: Heparan sulfate proteoglycan; Polymorphism; Lipids; Coronary artery disease

1. Introduction

Heparan sulfate proteoglycans (HSPGs) are a family of macromolecules characterized by the presence of long and unbranched, highly charged, and differently monosaccharide structured, sulfated polysaccharide chains (heparan sulfates [HSs]) [1–3]. HSs are covalently attached to different core proteins and serve as protein ligands mediating many biological processes [1–3]. HSPGs are essential components of cell surfaces and extracellular matrix in the cardiovascular system and are important for angiogenesis, blood coagulation, growth factor/cytokine action, cell adhesion, and for lipid metabolism by binding to lipoprotein lipase (LpL) and apolipoprotein B (apo B) [3,4]. Immunohistochemical studies show that HSPGs are overexpressed in human coronary arteriosclerotic lesions, findings consistent with their possible involvement in atherogenesis [5].

A variety of HSPG core proteins have been cloned and the three major classes of HSPGs within the cardiovascular system are sydecan, glypican and perlecan [4]. Perlecan (HSPG2) is abundant in extracellular matrix and also appears on cell surfaces. Altered expression of perlecan has been identified in proliferating vascular smooth muscle cells (VSMC) and has an association with the severity of atherosclerotic lesions [6,7]. The gene coding for perlecan is located at chromosome 1p36.1 [8,9]. In European insulin-dependent diabetes mellitus (IDDM) patients an intron 6 BamHI polymorphism at the putative HS attachment site is reported to

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0021-9150/00/$ - see front matter
PII: S0021-9150(99)00213-0

be associated with reduced risk of albuminuria [10], itself a risk factor for vascular disease in diabetic patients [11–13]. Because apo B binds to HSPGs in the vessel wall we explored associations between this HSPG2 BamHI polymorphism, apo B and coronary artery disease (CAD) in Australian Caucasians undergoing coronary angiography.

2. Patients and methods

2.1. The patient population

Seven hundred and fifty seven Australian Caucasians aged 65 years or less, both men (555) and women (202), consecutively referred to the Eastern Heart Clinic at Prince of Wales Hospital for coronary angiography were studied. The severity of CAD was determined by the number of significantly stenosed coronary arteries. Each angiogram was classified as revealing either normal coronary arteries or having no coronary lesion with more than 50% luminal stenosis or as having one, two, or three major epicardial coronary arteries with more than 50% luminal obstructions. Each patient's medical history was obtained using a questionnaire with standardised choices of answers to be ticked during the interview and DNA samples were collected for each patient as described previously [14].

2.2. Measurements of the plasma lipids

Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by the Clinical Chemistry Department of Prince of Wales Hospital using standard enzymatic methods. Low density lipoprotein cholesterol (LDL-C) levels were calculated with the Friedewald formula. Levels of apolipoprotein AI (apo AI), apolipoprotein B (apo B) and lipoprotein (a) (Lp(a)) were measured in the laboratory using in-house enzyme-linked immunosorbent assay (ELISA) methodology as described previously [14].

2.3. Determination of the HSPG2 BamHI polymorphism

We detected the HSPG BamHI polymorphism using the polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) with the primers described by Hansen et al. [10]. The 250 bp PCR products were digested with a BamHI restriction enzyme and this resulted in two fragments (150 and 100 bp) when the recognition site was present. The genotypes were identified as $+/+$, $+/-$ and $-/-$ according to the presence or absence of the recognition site by SDS-PAGE (Fig. 1).

2.4. Statistical analysis

A computer package of SPSS Advanced Statistics 8.0 for PC Windows 95 was used to analyze the data. All continuous variables are presented as mean $\pm$ S.E.M. Hardy–Weinberg equilibrium for the HSPG2 BamHI genotype distribution was assessed by a χ^2 analysis as described by Emery [15]. A one-way analysis of variance (ANOVA) was used to estimate relationships between the HSPG2 genotypes and quantitative variables, and a general factorial ANOVA to estimate the independent effects of the polymorphism on lipid profiles (TC, TG, HDL-C, TC/HDL-C, LDL-C, Lp(a), apo AI, apo B) after adjusting for age, gender and significant CAD. A contingency table χ^2 analysis was employed to assess the contribution of the polymorphism to CAD, and to evaluate relationships between the genotypes and other medical conditions including past history of myocardial infarction (MI), diabetes mellitus, hypertension and family history of CAD. To assess the independent effect of the polymorphism on CAD, established risk factors were entered into a stepwise logistic regression model as covariates. Statistical significance was defined as $P < 0.05$ and two-tailed P values are reported.

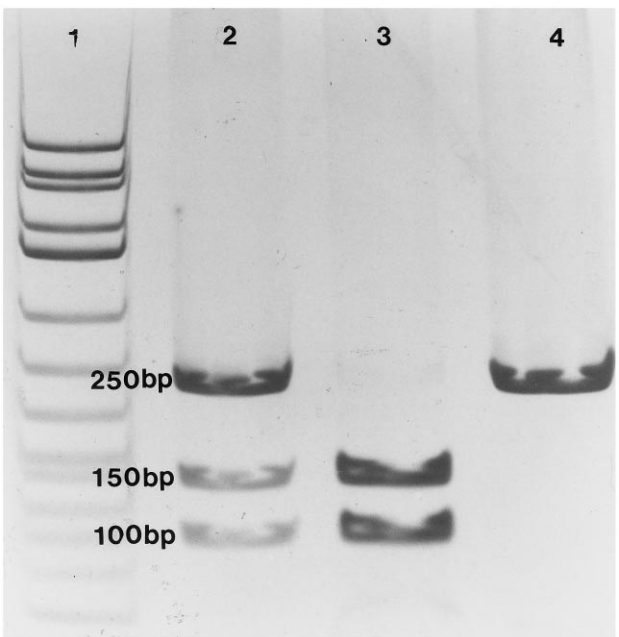

Fig. 1. The SDS-PAGE gel shows the polymerase chain reaction (PCR) products from the amplification of the polymorphism region of the HSPG2 gene. The polymorphism creates a BamHI recognition site which digests the 250 bp fragment into 150 and 100 bp fragments. Lane 1 shows a molecular size marker (Promega, ϕx174 DNA/*Hin*fI), lane 2 shows a $+/-$ heterozygote and lane 3 is a $+/+$ homozygote while lane 4 is a $-/-$ homozygote.

3. Results

3.1. Genetic characteristics of the patients

The frequencies of the HSPG2 BamHI genotypes were 4.7, 31.7 and 63.6% for $+/+$, $+/-$ and $-/-$ respectively and the '+' allele frequency was 20.6%. The genotype distribution was in Hardy–Weinberg equilibrium ($\chi^2 = 0.669$, $P > 0.05$) and was not different between men and women ($\chi^2 = 0.493$, $P = 0.781$).

3.2. The HSPG2 BamHI polymorphism and lipid profiles

The demographic information in relation to the HSPG BamHI genotypes in men and women is shown in Table 1(a,b). Males with $+/-$ and $+/+$ genotypes had lower TG levels compared to $-/-$ homozygotes ($F = 3.198$, $P = 0.042$) (Table 1(a,b)). As lipid profiles differed between patients with and without significant CAD, we assessed the independent effects on the lipid profiles of the HSPG2 polymorphism in a general factorial ANOVA model in which gender and significant CAD were entered as cofactors and age was entered as a covariate. The HSPG2 genotypes were significantly associated with the plasma concentration of apo B after adjustment for age, gender and significant CAD ($F = 4.052$, $P = 0.018$) (Table 2). Patients with $+/+$ genotypes had the lowest adjusted apo B levels (0.74 ± 0.06 g/l, $n = 36$) compared to $+/-$ (0.91 ± 0.02 g/l, $n = 241$) and $-/-$ (0.92 ± 0.02 g/l, $n = 480$) genotypes. It was also true when the analysis was conducted in males and females separately. After adjustment for age and the presence of significant CAD, apo B levels in males were 0.80 ± 0.07 vs 0.88 ± 0.02 vs 0.93 ± 0.02 g/l for $+/+$ vs $+/-$ vs $-/-$ ($F = 3.490$, $P = 0.031$) and in females 0.68 ± 0.10 vs 0.94 ± 0.04 vs 0.91 ± 0.02 g/l for $+/+$ vs $+/-$ vs $-/-$ ($F = 3.101$, $P = 0.047$). As shown in Table 3, the TC, TG and LDL-C levels in $+/+$ homozygotes were not different from the levels in those with $+/-$ and $-/-$ genotypes after adjusting for age, gender and the presence of significant CAD; this was also true for the TC/HDL-C ratio. Among the patients studied, 30% were receiving lipid-lowering drugs. However, adjusting for this did not alter any of the above findings.

3.3. The HSPG2 BamHI polymorphism and CAD

The frequencies for the HSPG2 BamHI $+/+$, $+/-$ and $-/-$ genotypes in patients with and without angiographically defined significant CAD are shown in Table 4. The HSPG2 genotypes were not associated with the presence of significant CAD ($\chi^2 = 0.538$, $P = 0.764$ in males; $\chi^2 = 4.208$, $P = 0.122$ in females) by simple χ^2 comparisons. In a stepwise logistic regression analysis, in which significant CAD was entered as dependent variable and age, BMI, waist/hip ratio, smoking status, lipid levels, diabetes and/or hypertension and family history of CAD were entered as independent variables, the HSPG2 genotypes were not predictive of the occurrence of significant CAD after controlling for these confounding risk factors in both men ($P = 0.640$) and women ($P = 0.224$). Yet, in a stepwise logistic regression model in which all the above lipid variables were entered independently, the plasma concentration of apo B was the strongest predictor of significant CAD in both men ($P = 0.0004$) and women ($P = 0.0101$). Furthermore, the polymorphism was not associated with a past history of MI ($\chi^2 = 4.045$, $P = 0.132$ in males; $\chi^2 = 0.667$, $P = 0.717$ in females) or with the number of significantly stenosed vessels ($\chi^2 = 1.700$, $P = 0.945$; $\chi^2 = 6.646$, $P = 0.355$), a family history of CAD ($P = 0.891$; $P = 0.858$), diabetes ($P = 0.224$; $P = 0.180$) or hypertension ($P = 0.610$; $P = 0.734$).

Table 1
Characteristics of (a) the 555 male patients and (b) the 202 female patients undergoing coronary angiography according to HSPG2 BamHI genotypes (mean ± S.E.M.)[a]

	+/+ (28)	+/− (178)	−/− (349)	*P* value
(a) *Male patients*				
Age (years)	54.0 ± 2.1	56.0 ± 0.5	56.2 ± 0.4	0.350
BMI (kg/m^2)	27.3 ± 0.8	27.9 ± 0.3	28.2 ± 0.2	0.339
Waist/Hip	0.97 ± 0.007	0.97 ± 0.005	0.98 ± 0.003	0.132
TC (mmol/l)	5.19 ± 0.22	5.27 ± 0.07	5.37 ± 0.05	0.434
Triglyceride (mmol/l)	1.99 ± 0.29	1.82 ± 0.08	2.08 ± 0.06	0.042
HDL-C (mmol/l)	0.95 ± 0.05	1.04 ± 0.02	0.98 ± 0.01	0.086
LDL-C (mmol/l)	3.31 ± 0.17	3.40 ± 0.07	3.44 ± 0.05	0.718
TC/HDL	5.77 ± 0.40	5.49 ± 0.15	5.82 ± 0.10	0.182
Apo AI (g/l)	1.05 ± 0.10	0.97 ± 0.03	0.95 ± 0.02	0.306
Apo B (g/l)	0.89 ± 0.05	0.92 ± 0.02	0.96 ± 0.01	0.158
Lp(a) (mg/l)	390 ± 86	258 ± 21	299 ± 17	0.094
Smoking Dose (packyrs)	24.6 ± 6.0	28.7 ± 2.4	27.3 ± 1.6	0.766
	+/+(8)	+/− (63)	−/− (131)	*P* value
(b) *Female patients*				
Age (years)	56.6 ± 2.6	57.6 ± 1.0	58.0 ± 0.7	0.825
BMI (kg/m^2)	28.0 ± 1.6	29.1 ± 0.7	27.6 ± 0.4	0.136
Waist/Hip	0.89 ± 0.022	0.85 ± 0.012	0.87 ± 0.008	0.447
TC (mmol/l)	5.10 ± 0.22	5.71 ± 0.14	5.44 ± 0.10	0.151
Triglycerides (mmol/l)	1.33 ± 0.24	1.83 ± 0.13	1.65 ± 0.08	0.268
HDL-C (mmol/l)	1.31 ± 0.07	1.26 ± 0.04	1.33 ± 0.04	0.532
LDL-C (mmol/l)	3.19 ± 0.29	3.62 ± 0.13	3.37 ± 0.09	0.208
TC/HDL	4.02 ± 0.38	4.84 ± 0.20	4.43 ± 0.13	0.111
Apo AI (g/l)	0.88 ± 0.08	1.14 ± 0.05	1.14 ± 0.04	0.202
Apo B (g/l)	0.73 ± 0.08	0.95 ± 0.04	0.90 ± 0.02	0.069
Lp(a) (mg/l)	280 ± 83	328 ± 42	330 ± 30	0.920
Smoking Dose (packyrs)	7.7 ± 7.7	14.0 ± 2.8	12.8 ± 2.0	0.762

[a] *P* values were obtained by one-way analysis of variance (ANOVA).

Table 2
Association between plasma apolipoprotein B (apo B) levels (mean ± S.E.M.) and HSPG2 BamHI polymorphism in patients with and without angiographically defined significant coronary artery disease (CAD) (>50% luminal obstruction)

HSPG2 genotypes	Without significant CAD	With significant CAD
+/+	0.75 ± 0.06	0.88 ± 0.05
+/−	0.86 ± 0.03	0.96 ± 0.02
−/−	0.87 ± 0.02	0.97 ± 0.01

4. Discussion

As far as we are aware, this is the first study to explore the distribution of the HSPG2 BamHI polymorphism in relation to circulating apo B and to relationships with lipid variables and other risk factors in patients undergoing coronary angiography. The genotype distributions of the polymorphism were compared in patients with and without angiographically defined significant CAD, and in patients with different numbers of significantly stenosed arteries but failed to identify any associations between the polymorphism and CAD. However, there was a significant association between the HSPG2 genotypes and plasma apo B levels after controlling for age, gender, use of lipid-lowering drugs and significant CAD. Patients with +/+ genotypes had the lowest apo B levels; and increased apo B plasma concentration was the strongest lipid risk factor for significant CAD in this population. And also the plasma TC, TG, TC/HDL-C and LDL-C levels were consistently lower in the HSPG2 +/+ homozygotes compared to +/− and −/− genotypes although the differences were not statistically significant (Table 3).

Table 3
Lipid profiles among HSPG2 BamHI genotypes after adjusting for age, gender, use of lipid-lowering drugs and the presence of significant coronary artery disease (CAD) (mean ± S.E.M.)[a]

	+/+ (36)	+/− (241)	−/− (480)	*P* value
TC (mmol/l)	4.89 ± 0.23	5.47 ± 0.08	5.38 ± 0.06	0.057
Triglyceride (mmol/l)	1.63 ± 0.24	1.74 ± 0.08	1.85 ± 0.06	0.081
HDL-C (mmol/l)	1.13 ± 0.07	1.18 ± 0.02	1.16 ± 0.02	0.766
LDL-C (mmol/l)	3.01 ± 0.21	3.49 ± 0.07	3.38 ± 0.05	0.079
TC/HDL (mmol/l)	4.72 ± 0.41	5.00 ± 0.14	5.06 ± 0.10	0.703
Apo AI (g/l)	0.97 ± 0.08	1.07 ± 0.03	1.04 ± 0.02	0.537
Apo B (g/l)	0.74 ± 0.06	0.89 ± 0.03	0.93 ± 0.02	0.018
Lp(a) (mg/l)	303 ± 73	278 ± 25	296 ± 18	0.831

[a] *P* values were obtained by general factorial analysis of variance (ANOVA).

Table 4
The distribution of HSPG2 BamHI polymorphism in patients with and without angiographically defined significant coronary artery disease (CAD)[a]

HSPG2 genotypes	Male patients		Female patients	
	Significant CAD		Significant CAD	
	Yes*	No	Yes**	No
+/+	23 (5.3%)	5 (4.2%)	4 (3.9%)	4 (4.0%)
+/−	137 (31.4%)	41 (34.5%)	39 (37.9%)	24 (24.2%)
−/−	276 (63.3%)	73 (61.3%)	60 (58.3%)	71 (71.8%)
Total	436	119	103	99

[a] The genotype distributions were not different among patients with and without significant CAD.
* $\chi^2 = 0.538$, $P = 0.764$ in males;
** $\chi^2 = 4.208$, $P = 0.122$ in females.

The metabolism of lipoproteins is partially regulated by HSPGs through interactions with lipoprotein lipase (LpL) and apolipoprotein B (apo B) [16]. LpL is the key enzyme catalyzing the hydrolysis of TG in TG-rich lipoproteins [16]. It is synthesised predominantly in myocytes and adipocytes and is transferred and anchored to the luminal surface of endothelial cells via extracellular matrix and cell surface HSPGs [4,16]. The binding of LpL to HSPGs makes it possible for LpL to interact with circulating lipoproteins and mediate the degradation and retention of lipoproteins within the subendothelial matrix [4,16]. Apo B has separate binding domains for LpL and HS and is involved in the localization of LpL to the endothelial cell surface [4,17]. Alterations in apo B expression could affect the interactions of LpL with HSPGs or apo B containing lipoproteins [4,17]. Thus, the decreased plasma apo B level in the HSPG2 +/+ genotypes underscores the relevance of HSPG to apo B-lipoprotein metabolism as discussed by Mahley and Ji [18], and could be a consequence of polymorphism-related enhanced binding of apo B to perlecan HS chains on cell surfaces. The increased binding of apo B-HSPG2 may therefore enhance the localization of LpL to the endothelial cell surface and the degrading of circulating TG-rich lipoproteins. This could explain why the HSPG2 +/+ genotypes also had lower levels of TC, TG, TC/HDL-C ratio and LDL-C. The retention of lipoproteins within the subendothelial matrix upregulated by this HSPG2-apo B-LpL system could also increase atherogenic risk and explain why the polymorphism was not protective in relation to CAD although it was associated with a less atherogenic lipid profile.

The frequencies of the HSPG2 BamHI genotypes in the 757 Australian white patients we studied (4.7, 31.7 and 63.6% for +/+, +/− and −/−, respectively) are similar to those reported by Hansen et al. in Danish

IDDM patients (7 vs 31 vs 62%) [10]. Hansen et al. also showed that in diabetic patients the BamHI polymorphism +/+ genotypes had less risk of albuminuria [10] which is an independent risk factor for CAD in diabetic patients [11–13]. However we did not observe a similar relationship between +/+ genotypes and reduced CAD risk.

In conclusion, a novel HSPG2 (perlecan) BamHI polymorphism in Australian Caucasians undergoing coronary angiography was examined. We found that the polymorphism was associated with lower apo B levels but that it was unrelated to the presence or absence of significant CAD in this population. The mechanism(s) responsible for these changes in HSPG2-apo B-LpL system needs further exploration.

Acknowledgements

The project is funded by research grants from National Health and Medical Research Council of Australia and Eastern Heart Clinic, Prince of Wales Hospital, Sydney. We wish to thank Dr Roger Allan, Dr Greg Cranney, Ms Lily Fenech, Ms Shirley Brown, Mr Steven Brouwer and nurses in the Eastern Heart Clinic for their assistance in clinical data collection. We are also most grateful to all the cardiologists in the Department for allowing us to study their patients. This work was supported by grants from National Health and Medical Research Council of Australia, and Eastern Heart Clinic, Prince of Wales Hospital.

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