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EPCR Ser219Gly: Elevated sEPCR, prothrombin F1+2, risk for coronary heart disease, and increased sEPCR shedding in vitro

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Abstract

We have progressively analysed three studies of coronary heart disease (CHD) for a variant in EPCR (Ser219Gly). Initially, in a prospective study, NPHSII, while no overall CHD-risk was identified in heterozygotes, homozygotes for 219Gly exhibited a three-fold elevated risk (HR 3.3, CI 1.22–8.96). In diabetics within NPHSII, there was a suggestion that 219Gly+ was associated with elevated CHD-risk (HR 1.89, CI 0.39–9.06) although numbers were small. To further assess the effect of the variant in diabetes, a case–control study of MI, HIFMECH, was used, in which previous analysis had defined a group with metabolic syndrome, by factor analysis. A significant CHD-risk interaction was identified between genotype and the 'metabolic syndrome' factor (interaction $p = 0.009$). To further assess CHD-risk for this variant in type-2 diabetes and to assess the effect of the variant upon thrombin generation and plasma levels of soluble EPCR, a cross-sectional study of type-2 diabetes was used. A significant CHD-risk was identified for European Whites (OR 2.84, CI 1.38–5.85) and Indian Asians in this study (OR 1.6, CI 1.00–2.57) and the frequency of 219Gly was two-fold higher in Indian Asians. Soluble EPCR levels were strongly associated with genotype, with homozygotes for 219Gly having four-fold higher levels ($p < 0.0001$). In vitro studies of EPCR-transfected cells suggested increased basal release of sEPCR from cells expressing the 219Gly EPCR phenotype. Furthermore, in base-line samples from NPHSII and in the diabetic study, a significant increase in prothrombin F1+2 level was observed for 219Gly. The increased CHD-risk and thrombin generation appears to be acting through increased shedding of the Gly allele from the cell surface.
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1. Introduction

It has been suggested that inflammatory processes form a common underlying pathological mechanism leading to both type-2 diabetes and coronary heart disease (CHD) [1–4]. Mechanisms that protect against inflammation may be par-

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ticularly important for protection against CHD in type-2 diabetes as well as protection against CHD per se. Indian Asians (IA) have an increased risk of type-2 diabetes and CHD compared to White populations that is may not be fully explained by known risk factors [5,6]. The protein C (PC) anticoagulant pathway is an important mechanism for limiting coagulation response to injury by down-regulation of the thrombin feedback loop, and is also important for regulating response to inflammation [7,8]. Key proteins in this pathway are the circulating PC and protein S, and the integral endothelial cell membrane proteins, thrombomodulin (Tm) and endothelial PC receptor (EPCR) [9,10]. The specificity of thrombin, once bound to Tm, changes from procoagulant, since it can no longer cleave fibrinogen or activate platelets, to anticoagulant, by cleavage of PC, to yield activated PC (APC). APC down-regulates the thrombin feedback loop by inactivation of coagulation factors Va and VIIIa [10–12].

We have previously reported a CHD-risk interaction between a Tm haplotype and increased body mass index (BMI) in a case–control study [13] and triglyceride levels in a prospective study [14], suggesting that variants in the pathway may decrease protection against the clinical sequelae of the metabolic syndrome or inflammatory pathways. EPCR and Tm co-localise at the endothelial surface in caveolae; in vitro co-expression studies of EPCR with Tm have shown an increase in PC activation rates compared to Tm alone [15]. Experimental data has suggested that EPCR augments PC activation by binding to, and thus increasing the local concentration of PC [16,17]. EPCR may also contribute to anti-inflammatory mechanisms involving protease activated receptor-1 on the surface of endothelial cells [18,19].

Thrombin indirectly causes shedding of EPCR from the surface of endothelium. Levels in plasma of this soluble form, sEPCR, may be increased in clinical conditions where thrombin is generated, such as CHD [20]. While the physiological role of EPCR at the endothelial surface seems clearly to be antithrombotic and anti-inflamatory, its role, if any, as a circulating protein is less clear. However, sEPCR is almost identical in size to the extracellular domain of full-length EPCR and retains its affinity for PC and APC [21]. When APC is bound to sEPCR, its anticoagulant activity is decreased but it retains ability to bind its inhibitors PC inhibitor and α1-antitrypsin [22]. The sEPCR–PC complex is a poor substrate for the thrombin–Tm complex on endothelium expressing EPCR [23]. Together, these in vitro data suggest that increased sEPCR may be prothrombotic.

Dysfunctional variants of EPCR, or those causing reduced expression or increased shedding, may reduce antithrombotic or anti-inflammatory effects of the pathway and may potentiate development of atherosclerosis and thrombosis. Eighty-four SNP's are listed for the EPCR gene (SNPPER database) although only 48 have been validated and frequencies reported on 13. Only one amino acid change has been identified (Ser219Gly) and lies within the short membrane-spanning region of the protein. Ser219Gly is thus a good candidate to study in terms of potential altered function of the protein. The first risk analysis of this variant was a study of venous thrombosis in which risk was associated with a haplotype containing the Ser219Gly in men [24] We hypothesised that the Gly allele would be associated with higher CHD-risk and we have also determined its effect upon thrombin generation, association with sEPCR levels, and subsequently analysed the variant in vitro studies.

2. Patients and methods

2.1. Northwick Park Heart Study (NPHS II)

NPHSII is a prospective study of men aged 50–64 years at base-line, who were clinically free of cardiovascular disease at that time. The cohort is well documented in peer-reviewed literature [25,26]. The study commenced in 1989 and is based within nine general medical practices in England and Scotland. Of 4600 men, 4141 were eligible for study and 3052 were recruited. Blood was taken in the non-fasting state for haemostatic factors and other biochemical markers. DNA was obtained from 2775 men. For the current analysis, non-Caucasian subjects were excluded, leaving 2735 of whom 2480 individuals were successfully genotyped in the current study. The characterisation of this group did not differ in any significant way from the whole sample (not shown). BMI, serum lipids, smoking habit, systolic and diastolic blood pressure (SBP, DBP) have been recorded. All coagulation parameters were measured in base-line samples. The Office for National Statistics, hospitals, coroners and general practices supplied details of morbidity and mortality. CHD was defined as those who had a myocardial infarction (MI, silent, determined by ECG, or clinical), or those who had coronary intervention procedures. All men gave written informed consent.

2.2. Pan-European Study of Myocardial Infarction (HIFMECH)

Male Caucasian patients who survived a first MI event below 60 years of age and population-based individuals of the same age were recruited from four European centres: Stockholm, Sweden; London, England (Northern Europe), and Marseille, France; San Giovanni Rotondo, Italy (Southern Europe). Patients with familial hypercholesterolemia and insulin-dependent diabetes mellitus were excluded from the study. Consecutive patients, along with randomly selected healthy individuals from the same area, were invited to participate. A total of 598 MI survivors and 653 healthy control subjects were included in the study. Matching of controls to cases was done on the basis of centre and age. While the study design set out to recruit one to one matching, a few more controls to patients were recruited in some centres. One thousand seventy-nine individuals were successfully genotyped for the current study (controls $n = 556$; cases

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