

PROCR vascular disease locus: elucidating mechanisms of genetic cross-disease correlations is a priority

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ABSTRACT

Numerous genetic risk loci have been linked to a variety of prevalent human diseases. However, the molecular basis of pleiotropy is frequently unknown. The PROCR locus is linked to a lower risk of Coronary Artery Disease (CAD) but a higher risk of venous thromboembolism (VTE). We provide an integrative method to uncover the molecular mechanism behind the PROCR locus. PROCR-p. Ser219Gly is identified as the likely causing

mutation at the locus, while protein C is identified as a causal component. We show that PROCR-219Gly increases plasma levels of (activated) protein C through endothelial protein C receptor (EPCR) ectodomain shedding in endothelial cells, reducing leukocyte-endothelial cell adhesion and vascular inflammation, using genetic analyses, human recall-by-genotype, and in vitro experimentation. PROCR-219Gly is also linked to an enhanced pro-thrombotic state via coagulation factor VII, an EPCR ligand. Our findings, which link PROCR-219Gly to both CAD and VTE via anti-inflammatory and pro-thrombotic processes, provide a framework for unravelling the mechanisms behind similar cross-phenotype correlations.

Key Words: Coronary Artery Disease; Vascular Smooth Muscle Cells

INTRODUCTION

Pleiotropy of disease-associated genetic variations has been discovered in Genome-wide Association Studies (GWAS). According to a recent research of cross-phenotype genetic association data from the UK Biobank, 96 percent of trait-associated variations (minor allele frequency (MAF) 1%) are linked to more than one ICD-10 code, with some exhibiting relationships with more than 50 codes. The vast majority of pleiotropic variations were shown to have a directionally consistent impact on the risk of numerous diseases, but 1.9 percent of loci (excluding the major histocompatibility complex) exhibited evidence of both higher and lower risk effects owing to the same allele. p.Ser219Gly (rs867186 A>G) in the PROCR gene, which encodes the Endothelial Protein C Receptor (EPCR), a critical regulator of the protein C (PC) pathway, is another example of a pleiotropic variant [1]. This variant's minor G allele has been linked to a lower incidence of coronary artery disease and myocardial infarction, but a higher risk of Venous Thromboembolism (VTE). Although some conventional cardiovascular risk variables (e.g., measures of obesity) show directionally concordant relationships for CAD and VTE, this pattern of conflicting associations appears perplexing.

Furthermore, rs867186-G has been linked to components of the coagulation cascade, including greater plasma levels of PC10 and coagulation factor VII, according to GWAS of cardiovascular intermediate characteristics. The causal relevance of these intermediate features to cardiovascular illnesses, on the other hand, is unknown. The thrombomodulin-protein C pathway is an important modulator of the coagulation-inflammatory process cross-talk. It is made up of molecular components that can react to a variety of pathophysiological conditions in various vascular beds. Thrombomodulin binds to thrombin at the vascular endothelium, limiting its clotting and cell activation potential and converting PC to Activated PC (APC) [2]. When PC is provided by EPCR17, a type I transmembrane protein that is primarily expressed on the endothelium of major blood arteries, PC activation by the Thrombin-thrombomodulin (TM) complex is significantly improved. After dissociating from EPCR, APC attaches to protein S, inactivating the coagulation factors Va and VIIIa and preventing further thrombin production. APC also enhances fibrinolysis by lowering plasminogen activator inhibitor type 1 (PAI-1) levels and lowers inflammation by reducing the generation of Tumour Necrosis Factor (TNF) and interleukin-1 (IL-1).

Plasma contains a soluble version of EPCR (sEPCR), which is produced by ectodomain shedding of EPCR from the endothelium. Plasma sEPCR

levels in healthy people have a bimodal distribution, with greater levels being related with one of the four common PROCR haplotypes. The minor allele of the p.S219G mutation is associated with this haplotype (designated A3 or H3). The variation increases EPCR shedding from the endothelium surface by making the receptor more susceptible to metalloprotease cleavage and producing an alternatively spliced, shortened transcript, according to functional studies. TNF- and IL-125 are potent regulators of shedding. The ability of sEPCR to bind both PC and APC is preserved, but it does not improve PC activation. The specific molecular mechanism behind the PROCR-p.S219G functional variation and its impact on cardiovascular intermediate phenotypes that may affect the risk of CAD and VTE is unknown [3].

METHODS

Phenome-scan PROCR-rs867186

We used PhenoScanner v2, a database of human genotype-phenotype associations, to compile data from the most recent available genome-wide association studies to investigate the effects of PROCR-rs867186 genotype on cardiovascular intermediate characteristics and outcomes. Wherever available, we used data from people of European heritage to allow for comparisons. Because we concentrated our research on cardiometabolic characteristics and outcomes, not all significant genome-wide associations were reported [4].

The equilibrium binding constants must be determined

Filter binding experiment was used to evaluate the equilibrium binding constants (Kd values) of modified aptamers. In SB18T buffer (40 mM Hepes pH 7.5, 102 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 0.01 percent Tween-20), the Kd values of modified aptamers were determined. T4 polynucleotide kinase (New England Biolabs) and [³²P] ATP were used to 5 end-labeled modified aptamers (Perkin-Elmer). Protein C, APC, sEPCR, thrombin, factor V, factor VIIa, protein S, and thrombomodulin were biotinylated in the filter binding experiment using EZ-Link NHS-PEG4-Biotin (Thermo Scientific) according to the manufacturer's procedure [5].

RESULTS

PROCR-p.S219G is linked to cardiovascular illnesses and risk factors

SAIGE, a generalised mixed model association test that accounts for case-control imbalance and sample relatedness, was used to examine the

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association of PROCRA-p.S219G with each of these codes (Methods). The findings pointed to circulatory system disorders such as phlebitis/thrombophlebitis (PheWAS code 451; $P=4.2 \times 10^{-8}$) and coronary artery disease (PheWAS code 411.4; $P=2.9 \times 10^{-5}$). We got the greatest available genetic association dataset for each trait. In the UK Biobank; and INVENT analyses, the minor (G) allele of rs867186 (219Gly) was consistently linked with a greater risk of VTE. In the UK Biobank, we also found a greater risk of deep vein thrombosis and pulmonary embolism, all of which are symptoms of VTE. In a comprehensive GWAS meta-analysis of the UK Biobank and CARDIoGRAMplusC4D consortium, however, rs867186-G was linked to a decreased risk of CAD [6]. We linked the rs867186-G allele to a variety of intermediate phenotypes relevant to the cardiovascular system to investigate the genetic basis for this connection pattern (Methods). We concentrated on features that have a direct impact on the protein C pathway. In the ARIC investigation, we discovered that rs867186-G is highly associated with increased PC levels in plasma, as determined by an Enzyme-linked Immunosorbent Test (ELISA). Using modified aptamers ('SOMamer reagents,' this assay measures the relative quantities of plasma proteins or protein complexes. Furthermore, the genotype was linked to higher APC plasma levels and coagulation factor VII activity. We found no correlations ($P>0.05$) between plasma levels of other measured proteins in the coagulation cascade and protein C pathway, including protein S (the cofactor of APC), or risk factors for thrombosis, such as fibrinogen, von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1) and the thrombolytic agent tissue plasminogen activator (tPA) [7].

The PROCRA locus has been linked to a common genetic aetiology

Despite evidence linking the rs867186 mutation at the PROCRA locus to CAD and VTE, it's unclear whether the two diseases share a causative variant and mechanism. We used statistical colocalization analysis to address this. Hypothesis Prioritization in Multi-trait Colocalization (HyPrColoc), a Bayesian technique, was used to assess colocalization across numerous complicated traits at the same time.

Protein C in arterial and venous diseases: a causal analysis

The findings imply that PC and APC abundance, FVII activity, and susceptibility to CAD and DVT are all influenced by genetic variations at the PROCRA locus. These findings, however, do not necessarily imply that these molecular features are linked to disease manifestations. We used Mendelian Randomization (MR) techniques to better clarify this association, employing genetic variants as instrumental factors to reduce confounding and reverse causation. To quantify the causal relationships between potential risk variables and cardiovascular outcomes, we created a multi-allelic genetic score (Methods). At the PROCRA region, the score was made up of roughly independent ($r^2 \leq 0.1$) SNPs with a P value of 5×10^{-8} . These analyses were likewise carried out using APC, yielding similar effect sizes and associated P values. The results were consistent across a variety of MR methods, including the Inverse-variance Weighting (IVW) method, median-based methods (simple and weighted), and MR-Egger regression (Methods). Further sensitivity analyses were performed to test the validity of our findings in the face of probable violations of the MR assumptions. By modelling disease phenotypes as the exposure and PC or APC level as the outcome using genome-wide significant predictors of disease, we used reverse MR to evaluate evidence for causal effects in the reverse direction. These studies found no evidence of CAD or DVT/VTE having a negative impact on PC or APC levels [8].

EPCR expression and shedding in endothelial cells were measured

We discovered that PROCRA is strongly expressed in human umbilical vein endothelial cells (HUVECs) and mildly expressed in macrophages, but not in any other cell type investigated, using transcriptome data from the BLUEPRINT Blood Atlas spanning 27 mature hematopoietic cell types. We found substantial expression of membrane-bound EPCR in HUVECs in flow cytometric analyses, which is consistent with these findings [9]. When homozygotes of the rs867186-G allele were compared to homozygotes of the A allele, we found 1.9-fold lower levels of EPCR in untreated HUVECs ($P=0.0051$). We discovered decreased levels of EPCR in HUVECs treated with phorbol 12-myristate 13-acetate (PMA), a strong drug for enhancing ectodomain shedding, compared to HUVECs treated with vehicle control

in both homozygote groups.

DISCUSSION

The discovery of the molecular basis of cross-disease correlations represents a significant step forward in our understanding of disease genesis. We demonstrate an integrative, multi-modal approach to addressing this dilemma by using recent breakthroughs in population biobanks, statistical genomics, and translational epidemiology. We used this method to study two vascular illnesses that are oppositely linked to the PROCRA missense mutation p.S219G (rs867186). Through a series of molecular processes, we discovered that PROCRA-219Gly protects against CAD but increases vulnerability to VTE. PROCRA-219Gly was shown to be related with higher circulating plasma sEPCR and lower EPCR levels on endothelial cells, which is consistent with an increase in EPCR membrane shedding and matches prior findings. Because only the membrane-bound version of EPCR can activate PC26, we expected that lowering EPCR would result in higher PC but lower APC levels. As a result, we and others have found that PROCRA-219Gly carriers have greater plasma PC levels. However, using the SomaScan technology, we discovered an unexpected spike in APC levels during our phenome-scan. Because the TM complex, not EPCR, is the major driver of PC activation in vivo, the increased levels of APC seen in PROCRA-219Gly carriers could be attributed to an elevation of TM activity in these people. In this scenario, an increase in APC would be a homeostatic mechanism seeking to compensate for PROCRA-219Gly carriers' enhanced thrombotic potential, and it could be suggestive of acquired APC resistance. Given that both PC and APC bind to sEPCR with the same affinity as EPCR26, it's possible that the increased sEPCR levels found in PROCRA-219Gly carriers help to stabilise and prolong PC/stay APC's in the circulation [10].

Given APC's short half-life of 15 minutes, this would be very important. APC is also unable to inactivate FV or FVIII when linked to sEPCR. As a result, APC sequestration by sEPCR in PROCRA-219Gly carriers may limit APC's anticoagulant function, leading to APC resistance and increased thrombotic risk in these people. In our phenome scan, however, we found no statistically significant relationships between PROCRA-219Gly and FV or FVIII levels.

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