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Influence of coronary artery disease-associated genetic variants on risk of venous thromboembolism

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ABSTRACT

Introduction: We investigated whether genetic variations robustly associated with coronary artery disease are also associated with risk of venous thromboembolism in a well-defined, female case–control study (n = 2753) from Sweden.
Materials and Methods: 39 single nucleotide polymorphisms in 32 loci associated with coronary artery disease in genome-wide association studies were identified in a literature search and genotyped in the ThromboEmbolism Hormone Study (TEHS). Association with venous thromboembolism was assessed by logistic regression.
Results: Only rs579459 in the *ABO* locus demonstrated a significant association with VTE. A tentative association between *ANRIL* and VTE in the discovery analysis failed to replicate in a meta-analysis of 4 independent cohorts (total n = 7181).
Conclusions: It appears that only the *ABO* locus is a shared risk factor for coronary artery disease and VTE.

Introduction

A plausible causal link between arterial and venous thrombosis has been advocated [1,2], and a large meta-analysis indicates an impact of established risk factors for atherosclerosis on both disease entities [3]. Several studies have reported increased risks of cardiovascular disease amongst patients previously diagnosed with venous thromboembolism (VTE) suggesting that VTE is a predictor of subsequent arterial thrombosis [4,5]. Similarly, patients with atherosclerosis and manifest cardiovascular disease have been reported to be at an increased risk of developing VTE [6,7]. In addition, arterial and venous thrombosis is influenced by common mechanisms, i.e. ABO blood group [8,9] activation of blood coagulation [10–12], hypofibrinolysis [13,14] and inflammation [15,16]. Recent studies have highlighted that risk of VTE is reduced in subjects

Abbreviations: BMI, body mass index; CAD, Coronary artery disease; DVT, deep vein thrombosis; PE, pulmonary embolism; SNP, single nucleotide polymorphism; VTE, venous thromboembolism; TEHS, The ThromboEmbolism Hormone Study; FARIVE, The Facteurs de risque et de récidive de la maladie thromboembolique veineuse study; HVH, The Heart and Vascular Health Study; MARTHA, The Marseille Thrombosis Association study; EOVT, The Early Onset of Venous Thrombosis study.

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treated with statins or antiplatelet drugs, which today are established regimens for primary and secondary prevention of cardiovascular complications in atherosclerotic disease [17,18].

Despite these epidemiological findings, arterial and venous thromboses have been considered as two separate entities, with different pathophysiology and treatment. Indeed, two large prospective population studies have refuted an impact of atherosclerosis on risk of VTE [19,20]. However, controversy remains whether this conclusion is valid [1]. Increasing age and body mass index (BMI) remain the only cardiovascular risk factors with robust influences on VTE risk in prospective population studies, whereas for smoking, hyperlipidemia, hypertension and diabetes mellitus the observed associations are inconsistent [21–23].

In terms of genetic predisposition, it is known that common variants in the *ABO* [24], factor V *(F5)* [25], prothrombin (*F2)* [26], fibrinogen γ-chain (*FGG)* [27] and factor XI (*F11)* [28] genes influence risk of VTE. However, no study has systematically investigated whether known coronary artery disease (CAD)-associated SNPs that have emerged through genome-wide association (GWA) studies of CAD also increase the risk of VTE. Here, we investigated the genetic variants which have been robustly associated with CAD, in a well-defined, Swedish female case–control study of VTE, with the aim of clarifying whether there is a common genetic predisposition for CAD and VTE.

Materials and Methods

Discovery Cohort

The ThromboEmbolism Hormone Study (TEHS) is a case–control study of VTE in women initiated by the Medical Product Agency (MPA) in Uppsala and conducted through the Centre for Pharmacoepidemiology at Karolinska Institutet in Stockholm. TEHS was designed to investigate how environmental and genetic factors affect the risk of VTE, with a particular focus on women taking different combined hormonal contraceptives or hormone replacement therapy [9]. In brief, a total of 1433 cases and 1402 controls, aged between 18 and 65, who contributed DNA samples, were recruited between 2003 and 2009. Cases were out- and in-patients, recruited from 43 hospitals across Sweden, with first time, incident deep vein thrombosis (DVT) in lower limbs and/or pulmonary embolism (PE). All cases of VTE were objectively confirmed with established diagnostic imaging methods, and treatment with anticoagulants was initiated. Exclusion criteria were previous VTE, current malignancy or pregnancy. Women who had previously been diagnosed with cancer were included only if treatment had been completed at least five years before the VTE diagnosis. Female controls were selected randomly from the population register held by the National Board of Taxation and matched to birth year of the cases. Extensive information was obtained through telephone interviews, precluding participation of non-Swedish-speaking women. Table 1 reports the characteristics of the population studied. Informed written consent was obtained in accordance with the Declaration of Helsinki. The study was approved by the regional research ethics committees.

SNP Selection and Genotyping

After a literature survey (May 2011), 32 loci were selected (Supplementary Table 1). In a number of loci, 2 signals appear to be independent so both signals were included; thus, a total of 39 SNPs robustly associated with CAD and/or MI (having attained conventional genome-wide significance ($P < 5 \times 10^{-8}$) in large genome-wide or candidate gene meta-analyses) were included (Supplementary Table 1). Established genetic variants predisposing to VTE were also genotyped, including those tagging ABO phenotypes (rs514659 tagging non-O blood group; rs8176704, rs512770 and rs8176749) [29], *F5* (rs6025), *F2* (rs1799963), *FGG* (rs2066285) and *F11* (rs2036914). It has been reported that the following SNPs are able to discriminate between; rs514659, O versus non-O; rs8176704, A1 versus A2; rs512770, O1 versus O1v/O2; rs8176749, A1 versus B [29].

Forty-four SNPs were assayed using the Illumina Goldengate platform, and genotypes were read and analysed using the Illumina BeadXpress and Illumina GenomeStudio 2011.1 software, respectively, at the SNP&SEQ Technology Platform, Uppsala, Sweden. Rs6025 and rs1799963 were genotyped by Pyrosequencing™ technology (ISO standard 2004) at the Royal Institute of Technology, Stockholm, Sweden. Rs512770 was genotyped with a Taqman SNP genotyping assay (C__997884_20, Applied Biosystems) and analyzed using StepOne software v2.1. Standard quality control procedures were performed, with SNP exclusion due to call rate (<95%) and/or deviation from Hardy-Weinberg equilibrium ($p < 0.005$). Subjects with a low call rate (<90%) were excluded. Genotyping was performed on plates containing

Table 1
Characteristics of TEHS.

	Cases			Controls			
	mean	N	freq.	mean	N	freq.	p-value
		1426			1395		
Pulmonary embolism		457	0.32				
Deep venous thrombosis		997	0.70				
PE + DVT		28	0.02				
Women		1426	1.00		1395	1.00	
Age (years)	46			47			
18-49		746	0.52		692	0.50	
50-64		680	0.48		703	0.50	
BMI (kg/m^2)	27			25			<0.001
BMI 30 or more		351	0.25		176	0.13	<0.001
Current smoking		377	0.26		303	0.22	0.005
Hypertension		303	0.21		285	0.20	NA
Hyperlipidaemia		167	0.12		174	0.13	NA
Diabetes Mellitus		61	0.04		38	0.03	0.026
Cardiovascular disease		74	0.05		43	0.03	0.005
Contraceptives							
Combined oestrogen and progesterone		326	0.44*		109	0.16*	<0.001
Progesterone only		143	0.21*		180	0.26*	NA
Menopausal replacement therapy		195	0.29†		120	0.17†	<0.001
Positive family history of VTE		376	0.27		205	0.15	<0.001

Where: PE, pulmonary embolism; DVT, deep vein thrombosis; BMI, body mass index; Cardiovascular disease, including previous myocardial infarction, angina pectoris, peripheral artery disease, transient ischemic attack and stroke; Hornome replacement therapy defined as per oral or patch application; * frequency of women age 18–49 and † frequency of women age 50; p-value calculated by T-test. NA, not associated.