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Prothrombin and risk of venous thromboembolism, ischemic heart disease and ischemic cerebrovascular disease in the general population

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ABSTRACT

Objective: We tested the hypotheses that *Prothrombin G20210A* heterozygosity associate with increased risk of venous thromboembolism (VTE), ischemic heart disease (IHD), and ischemic cerebrovascular disease (ICVD) in the general population and re-tested risk of IHD and ICVD in two case-control studies.
Methods: 9231 individuals from the Danish general population were followed for VTE (VTE = DVT + PE), deep venous thrombosis (DVT), pulmonary embolism (PE), IHD, myocardial infarction (MI), ICVD, and ischemic stroke (IS) for a median of 24 years. Case-control studies included 2461 IHD cases and 867 ICVD cases.
Results: In the general population, *Prothrombin G20210A* heterozygotes had 1.3 (95% CI: 0.6–2.9) fold risk for VTE, 0.6 (0.2–2.0) for DVT, 1.7 (0.6–4.8) for PE, 1.5 (1.1–2.1) for IHD, 1.7 (1.1–2.7) for MI, 1.1 (0.6–1.9) for ICVD, and 1.1 (0.5–2.1) for IS compared to non-carriers. Double heterozygotes for *Prothrombin G20210A* and *Factor V Leiden* versus double non-carriers had a multifactorially adjusted hazard ratio for IHD of 6.0 (2.0–19). In case-control studies, multifactorially adjusted odds ratios for *Prothrombin G20210A* heterozygotes versus non-carriers were 2.0 (1.1–3.4) for IHD, 2.0 (1.0–3.8) for MI, 1.4 (0.7–3.1) for ICVD, and 2.1 (0.8–5.4) for IS.
Conclusion: Prothrombin G20210A heterozygosity alone and in combination with *Factor V Leiden* R506Q heterozygosity predicts 1.5 and 6.0 fold risk of IHD compared to non-carriers.

1. Introduction

The *Prothrombin G20210A* variant is the second most common hereditable thromophilic defect found in patients with VTE [1]. Indeed, testing for *Prothrombin G20210A* together with *Factor V R506Q* is part of routine laboratory workup of patients with unexplained VTE in most thrombophilia centers. Additionally, some experts advocate testing of patients with unexplained arterial events, most notably premature MI (<40 years), premature IS and arterial events in women receiving oral contraceptives or postmenopausal hormonal replacement therapy [2–4].

The *Prothrombin G20210A* polymorphism involves a single base-pair substitution in the 3′-untranslated region of the prothrombin gene. Heterozygosity for *G20210A* results in ∼25% higher levels of prothrombin in plasma, a change presumably responsible for the propensity to develop VTE [5,6].

Numerous mainly case-control studies have tested the association between *Prothrombin G20210A* and venous thrombosis and reported odds ratios between 1.7–5.4 [6–12]. Meta-analyses of mainly case-control studies on *Prothrombin G20210A* and arterial thrombosis suggest a 30–40% increased risk of IHD and IS. The effects of *Prothrombin G20210A* on venous and arterial thrombosis have never previously been studied in a large sample from the general population.

We first tested the hypothesis that *Prothrombin G20210A* heterozygosity increases the risk of VTE, DVT, PE, IHD, MI, ICVD, and IS in individuals in the general population. We retested the hypothesis on risk of IHD, MI, ICVD, and IS in two independent case-control studies of IHD and ICVD patients with matched controls. These case-control studies, in contrast to the general population study, do not address novel questions, as numerous case-control studies have been published previously [9,13]. Nevertheless, we included the two case-control studies to confirm previous findings and to compare the results from

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our general population study with those in case-control studies.

2. Methods

2.1. Study design

2.1.1. General population study

9231 individuals were sampled at random from the Danish general population in Copenhagen. All individuals participated in the 1991–1994 examination of the Copenhagen City Heart Study, and the majority of these also participated in the 1976–1978 (n = 6450) and 1981–1983 (n = 6643) examinations. The protocol included a detailed questionnaire, a physical examination, and blood samples for DNA extraction [14,15]. Endpoints were ascertained from the national Danish Patient Registry and the national Danish Causes of Death Registry, public registers to which all hospitalisations and deaths in Denmark are reported. Endpoints were: VTE (n = 290) including DVT and PE; DVT (n = 192; ICD-8 codes 451.0, 451.9, 671.1, and 671.9 and ICD-10 codes I80.1- I80.3, O22.3, O87.1), and PE (n = 127; ICD-8 codes 450 and 673.9 and ICD-10 codes I26.0, O88.2). IHD including MI and other conditions (n = 1453; ICD-8 codes 410–414 and ICD-10 codes I20-I25), MI (n = 720; ICD-8 codes 410 and ICD-10 codes I21-I22), ICVD including IS and other conditions (n = 614; ICD-8 codes 431–438 and ICD-10 codes I61-I66+G45), and IS (n = 436; ICD-8 codes 433-434 and ICD-10 codes I63). MI, ICVD and IS events in The Copenhagen City Heart Study were verified by experienced cardiologists and neurologists; 72% of VTE events recorded in the registry mentioned above met standard diagnostic criteria, which include ultrasonography for DVT and ventilation/perfusion scintigraphy for PE [15]. All VTEs were included in our analysis. IHD was angina pectoris or MI. A diagnosis of MI required the presence of at least 2 of the following criteria: (1) characteristic chest pain, (2) elevated cardiac enzymes, and (3) electrocardiographic changes indicative of MI. ICVD was amarousis fugax (monocular blindness), transient ischemic attack (focal neurological symptoms lasting <24 h) or IS (focal neurological symptoms lasting >24 h). To distinguish between cerebral infarction, intracerebral haemorrhages and subarachnoid haemorrhages, either CT or MR scan, autopsy, spinal fluid examination or surgical description was necessary. Endpoints were gathered until December 31st 2000 (ICVD and IS), December 31st 2001 (DVT and PE), and December 31st 2003 (IHD and MI). Study entry was defined as first examination day. Median follow up was 24 years. Follow-up was 100% complete.

2.1.2. Case-control studies

2,461 IHD patients (of whom 1,123 had MI) and 867 ICVD patients (of whom 348 had IS) were compared with controls from the Copenhagen City Heart Study who were free from IHD and ICVD. Cases and controls were matched on age within 1 year strata using all available controls. The number of controls accordingly varies by disease group.

Patients with IHD were identified among patients from the greater Copenhagen area referred for coronary angiography from 1991 through 2003. IHD was diagnosed by experienced cardiologists based on characteristic symptoms of stable angina pectoris, plus at least one of the following: stenosis/atherosclerosis on coronary angiography, a previous MI, or a positive exercise electrocardiography test.

Patients with ICVD were identified among patients from the greater Copenhagen area referred from 1994 through 2002 for outpatient ultrasonography of the carotid artery. Experienced neurologists and vascular surgeons diagnosed ICVD on the basis of amarousis fugax, transient ischemic attack or IS, together with at least 50% stenosis of the carotid artery. Haemorrhage was excluded on computed tomography.

2.2. Ethics

The Danish ethics committees for Copenhagen, Frederiksberg, and Copenhagen County, as well as Herlev Hospital, Copenhagen University Hospital approved this study. Informed consent was obtained from participants.

2.3. Analyses

Prothrombin G20210A genotype was determined by RFLP using the *BanII* enzyme after PCR amplification of a 134 bp fragment with two primers (sense 5′-TTTGGAGAGTAGGGGGCCACTCA-3′; antisense 5′-GAATAGCACTGGGAGCATTGGGG-3′). Non-carriers were identified as individuals with bands of 23, 32 and 79 bps length on an agarosis gel. Heterozygotes displayed bands of 23, 32, 79 and 102 bps and homozygotes displayed bands of 32 and 102 bp. *Factor V R506Q* were genotyped as described previously [14,16].

2.4. Statistical analyses

Data was analyzed using Stata. A 2-sided P-value below 0.05 was statistically significant. Hazard ratios for VTE, DVT, PE, IHD, MI, ICVD and IS were calculated using Cox proportional hazard models with age as time scale, which automatically adjust for age. Multifactorially adjusted models for VTE, DVT and PE included age, gender, body mass index (<25, 25–30, and >30 kg/m^2), smoking (current smoker/non smoker), leisure time physical activity (<2 h/week, 2–4 h of light exercise/week, 2–4 h of demanding exercise/week, or >4 h of demanding exercise/week), fibrinogen, alcohol consumption (0, 12–72, 84–156,168–252, 264–336, >336 g/week), use of oral contraceptives, menopausal status, use of hormone replacement therapy, *Factor V R506Q* genotype (non-carriers, heterozygotes, homozygotes), and year of study entry. Multifactorially adjusted models on IHD, MI, ICVD and IS included age, gender, body mass index, smoking, leisure time physical activity, cholesterol, high density lipoprotein cholesterol, lipoprotein(a), fibrinogen, triglycerides, use of lipid lowering therapy, diabetes mellitus, hypertension, alcohol consumption, *Factor V Leiden* R506 genotype, and year of study entry. The proportional hazard assumption was tested by plotting -ln(-ln(survival)) versus ln(analysis time). No violations of the proportional hazard assumption were observed. Interaction between *Prothrombin G20210A* and each of the covariates adjusted for in the models were investigated by including two-factor interaction terms one at a time, and testing them for significance using a Wald-test.

In case-control studies we calculated odds ratios for IHD, MI, ICVD and IS using conditional logistic regression models. Cases and controls were matched within 1year age groups. Multifactorially adjusted models included age, gender, body mass index, smoking, diabetes mellitus, cholesterol, high density lipoprotein cholesterol, triglycerides, use of lipid lowering therapy, hypertension, and *Factor V R506Q* genotype.

All hazard and odds ratios were calculated for *Prothrombin G20210A* heterozygotes versus non-carriers only, due to the low number of, or absence of homozygotes.

The authors had full access to the data in the study and take responsibility for its integrity.

3. Results

3.1. General population

Characteristics of participants are shown in Suppl. Table 1. In the general population, 2.1% (n = 196) were *Prothrombin G20210A*