Research Article

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Prothrombotic genetic risk factors are associated with an increased risk of liver fibrosis in the general population The Rotterdam Study

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See Editorial, pages 1311–1313

Background & Aims: The coagulation system is known to be involved in fibrogenesis in patients with liver disease. We investigated whether common genetic prothrombotic risk factors are associated with an increased risk of fibrosis in the general population.

Methods: This investigation was part of the Rotterdam Study, an ongoing, population-based cohort study. Liver stiffness (LS) was measured using transient elastography (Fibroscan®) and associated with single nucleotide polymorphisms determining blood group type and presence of the Factor V Leiden (FVL) mutation or prothrombin G20210A gene variant.

Results: Reliable LS measurements and genetic data were obtained from 1055 Caucasian participants. LS ⩾8.0 kPa, suggestive of clinically relevant fibrosis, was observed in 101 subjects (9.6%). Presence of FVL or prothrombin G20210A was independently associated with an increased risk of LS ⩾8.0 kPa (OR 2.09, 95%CI 1.07–4.07, $p = 0.03$). Combination of blood group type non-O and the FVL mutation or prothrombin G20210A variant resulted in an even higher risk of LS ⩾8.0 kPa (OR 3.36, 95%CI 1.50–7.56, $p = 0.003$). Presence of the FVL mutation or prothrombin G20210A variant in participants with blood group non-O was associated with a predicted probability of 14.3% (7.7–23.8) of LS ⩾8.0 kPa.

Conclusions: Participants carrying the FVL mutation or prothrombin G20210A variant have an increased risk of clinically relevant liver fibrosis, which is even higher in blood group type non-O carriers. The fact that genetic prothrombotic risk factors are associated with an increased risk of liver fibrosis suggests that coagulation plays an important role in fibrogenesis in the general population.

Introduction

Liver fibrosis develops as the result of a complex interplay between chronic liver injury, genetic susceptibility and environmental risk factors. Hypercoagulability is considered to be one of the factors influencing liver fibrosis development and progression in liver disease [1]. The exact mechanism of this influence has not yet been fully elucidated, but thrombin seems to play a key role. Thrombin could cause liver fibrosis by directly activating hepatic stellate cells by binding the protease-activated receptor 1 [2–5]. An alternative hypothesis describes that thrombin production causes the formation of occlusive thrombi which eventually results in tissue ischemia, parenchymal extinction and ultimately liver fibrosis and cirrhosis [6,7].

The Factor V Leiden (FVL) mutation, prothrombin G20210A gene variant and ABO blood group type non-O are well-known genetic prothrombotic risk factors. Presence of these risk factors is associated with a two- to fivefold increased risk of venous thrombosis [8–11]. In addition, the combined presence of FVL

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Abbreviations: FVL, factor V Leiden; LS, liver stiffness; TE, transient elastography; SNP, single nucleotide polymorphism; VTE, venous thromboembolism; BMI, body mass index; ALT, alanine aminotransferase; DM, diabetes mellitus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; APC, activated protein C.

or prothrombin G20210A with blood group type non-O is known to result in an additionally increased risk of venous thrombosis. In previous studies, patients with both FVL and blood group type non-O were reported to have a 4- to 23-fold increased risk of venous thrombosis compared to patients with blood group O and without FVL [12–16]. Combined presence of prothrombin G20210A and blood group type non-O was associated with a ninefold increased risk of venous thrombosis compared to controls and a twofold increased risk compared to patients with prothrombin G20210A and blood group type O [15,17].

Several studies have reported an association between presence of FVL, prothrombin G20210A, blood group type non-O and liver fibrogenesis in liver disease. Two studies in patients with chronic hepatitis C showed that the rate of fibrosis progression and the risk of cirrhosis was increased in patients carrying the FVL mutation [18,19]. In addition, presence of FVL caused a significant acceleration of liver fibrogenesis when exposing mice to chronic liver injury [20]. An increased rate of liver fibrosis development was also observed in patients with hepatitis C carrying the prothrombin G20210A gene variant [21]. Finally, increased severity of liver fibrosis was observed in chronic hepatitis C patients with blood group type non-O, the most common prothrombotic genetic risk factor [12,22]. It is unknown whether these prothrombotic risk factors also play a role in liver fibrogenesis in the general population. Therefore, the aim of the current study was to investigate whether presence of the FVL mutation, prothrombin G20210A gene variant and/or blood group type non-O is associated with an increased risk of liver fibrosis, assessed by using liver stiffness (LS) as proxy, in a population-based study.

Materials and methods

Study population and design

This study was part of the Rotterdam Study, a large ongoing prospective population-based cohort study in the Netherlands. The rationale and study design of the Rotterdam Study have been described elsewhere [23]. At the start of the Rotterdam Study in 1990, all inhabitants of Ommoord, a suburb in the city of Rotterdam, aged 55 years and over were asked to participate in this study. In 2000, a new cohort was added to the initial study population. This second cohort consisted of participants who had moved to Ommoord or who had turned 55 years of age since the start of the study. Participants are evaluated at the designated research center every 3–4 years. Each assessment cycle consists of an extensive home interview and a range of physical examinations, including fasting blood collection, at the research center. As of 2009, each examination cycle additionally includes an abdominal ultrasound and transient elastography (TE) measurement. Abdominal ultrasound and TE were performed after obtaining fasting blood samples at the research center. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC University Medical Center and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study and permission to obtain information from their treating physicians.

Liver stiffness measurement and abdominal ultrasonography

Liver fibrosis was assessed non-invasively by measuring LS using TE (Fibroscan®, Echosens™). A single, experienced ultrasonographer measured LS. Measurements were performed on the right lobe of the liver, through the intercostal spaces, with the participant lying flat on his/her back with the right arm laying in maximal abduction and in between breathing movements. The failure rate of TE in our study was 4.3%. LS measurements were considered reliable if 10 valid measurements were obtained with a success rate of at least 60% and an interquartile range of less than 30% of the median LS. Based on these criteria, 21.6% of LS measurements was considered unreliable, which is comparable to other studies [24,25]. Either an M or XL-probe was used to obtain LS measurements, according to the manufacturer's recommendations. XL-probe was used to measure LS in participants with a skin capsule distance larger than 2.5 cm, as assessed by abdominal ultrasound. In the remaining patients LS was measured using the M-probe. Participants with an intracardiac device were excluded from the current study, since TE is contraindicated in these participants. Consistent with others, an LS cut-off value of 8.0 kPa was used to identify clinically relevant liver fibrosis [26,27]. In a subgroup analysis, LS $\geqslant$9.5 kPa was used as cut-off to determine presence of severe fibrosis or cirrhosis [28].

Abdominal ultrasonography (Hitachi HI VISION 900) was used to assess presence of steatosis, to measure spleen size, and to examine the hepatic parenchyma among others. Images were stored digitally and re-evaluated by an expert hepatologist with large experience in abdominal ultrasound. The diagnosis of steatosis was determined by the ultrasound technician according to the protocol by Hamaguchi *et al.* [29]. Presence of steatosis was reassessed by the expert hepatologist in all cases.

Evaluation of the Factor V Leiden mutation, prothrombin G20210A gene variant and ABO blood group type

Presence of FVL and prothrombin G20210A was determined by assessing the genotype present at rs6025 (G→T) and rs1799963 (G→A) respectively. Participants were classified as having either ABO blood group type O or ABO blood group type non-O based on the genotype present at rs505922, used as proxy for rs687289 (R^2 = 1.0, D' = 1.0) (presence of genotype GG at rs687289 corresponds with presence of blood group type O). To determine presence of these single nucleotide polymorphisms (SNPs), DNA was isolated from whole blood samples and extracted according to standard automated procedures [30]. Genotyping in the Rotterdam study cohort was performed in batches in the Erasmus MC University Medical Center, Rotterdam, The Netherlands using the Infinium II HumanHap 550K Genotyping Bead-Chip® version 3 (Illumina Inc., San Diego, CA, USA). Sample-specific quality control included filters for low call rate, heterozygosity and sex mismatch. SNP-specific quality control measures comprised filters for call rate, minor allele frequency and Hardy-Weinberg equilibrium. The Markov Chain Haplotyping package [31] was applied for imputation using the cohort of the 1000 Genomes Project as reference population [32]. A detailed description of these methods has been published elsewhere [33–36].

Interview, anthropometry and biochemistry

During the home interview, extensive data was obtained on demographics, medical history, comorbidity, alcohol consumption, smoking behavior, and drug use. Excessive alcohol consumption was defined as an intake of 14 or more international units (IU) of alcohol per week for women. For men, an intake of >21 IU per week was considered to be excessive. History of venous thromboembolism (VTE) was determined by using medical charts. VTE was defined as presence of pulmonary embolism or deep venous thrombosis. Trained research nurses performed anthropometric measurements at the research center, from which body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

During the assessment cycle, fasting venous blood samples were collected and stored at −80 °C. Alanine aminotransferase (ALT) and glucose levels were determined using automated procedures (Roche Diagnostics GmbH, Mannheim, Germany). Diabetes mellitus (DM) was defined as fasting plasma glucose $\geqslant$7.0 mmol/L or drug treatment for elevated blood glucose. To determine presence of viral hepatitis, hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (HCV) antibodies were measured using immunoassays (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Baseline characteristics are expressed as numbers with proportions for categorical variables and as median with interquartile range or mean with standard deviation for continuous variables. Significance of differences in baseline characteristics was assessed by using Chi-squared tests (counts), Student *t* tests (means) or Mann-Whitney *U* tests (medians) respectively. Logistic regression analysis was used to examine associations between presence of FVL, prothrombin G20210A and/or blood group type and LS $\geqslant$8.0 kPa. In multivariable logistic regression analysis, we adjusted for age, sex, and logarithmically transformed ALT. A second multivariable model was adjusted for age, sex, ALT, and steatosis. We adjusted for additional possible confounders in a multivariable model includ-