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Association of impaired renal function with venous thrombosis: A genetic risk score approach

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ABSTRACT

Objective: The association between impaired kidney function and venous thrombosis has been previously reported but supportive data are still sparse. We here wish to strengthen this association by investigating, by use of a genetic risk score approach, whether single nucleotide polymorphisms (SNPs) known to decrease the estimated glomerular filtration rate (eGFR), a surrogate marker for renal dysfunction, are associated with increased risk of venous thrombosis.

Approach and results: Fifty-one polymorphisms selected from the literature to robustly associate with eGFR were first tested for association with venous thrombosis in a French case-control collection of 1953 patients and 2338 healthy individuals. This led to the identification of a genetic risk score based on 9 polymorphisms that strongly associated with increased risk (odds ratio (OR) = 1.09 [1.06–1.15], $p = 1.44 \cdot 10^{-7}$). This genetic score association replicated (OR = 1.18 [1.11–1.26], $p = 8.86 \cdot 10^{-8}$) in an independent sample of 1289 patients and 1049 healthy controls part of the Dutch MEGA study. We then categorized the genetic score distribution observed in the combined samples into quintiles. Compared with the lowest quintile, the OR for increased risk of disease associated with the second, third, fourth and fifth quintiles were 1.13 [0.94–1.16], 1.47 [1.22–1.77], 1.52 [1.26–1.82] and 1.70 [1.41–2.05], respectively.

Conclusions: Using a genetic risk score analysis, our study provides new elements supporting the association between impaired renal function and the risk of venous thrombosis.

1. Introduction

Venous thrombosis (VT), that encompasses both deep vein thrombosis and pulmonary embolism, is the second most common cardiovascular disease and the third in terms of mortality. Its incidence is estimated to be ~200 per 100,000 person-years in Europe [1] and its

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GRS, genetic risk score; GWAS, genome wide association study; SNP, single nucleotide polymorphisms; VT, venous thrombosis

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recurrence rate within 10 years is close to 30%. The disease also represents a significant spending in health care [2], it is for example associated with a total annual cost ranging from €1.5 to 13.2€ billion for the EU [3]. The nature of the complex pathophysiological mechanisms that lead to VT is not fully characterized. Disturbances of the coagulation and fibrinolysis cascade leading to hypercoagulable states are clearly the most important contributor to VT etiology. Obesity-related mechanisms, inflammation [4], platelets [5] and complement cascade [6] are additional biological systems that contribute to the development of the disease. However, despite these results of intensive research efforts, there are so far limited prognosis tools that help clinicians to predict which individuals are at risk for a VT event. More investigations are thus needed to better disentangle the etiological architecture underlying VT.

A meta-analysis of 5 community-based cohorts has shown that decreased estimated glomerular filtration rate (eGFR) was associated with increased VT risk [7]. It was further hypothesized that this association was partially mediated by elevated levels of coagulation Factor VIII and von Willebrand factor [8]. Inflammation and coagulation biomarkers are associated with decreasing kidney function in ambulatory adults without established cardiovascular disease or chronic kidney disease. Investigations in the ARIC study [9] demonstrated that increased levels of factor VIIIc, fibrinogen, and von Willebrand Factor were significantly associated with increased risk of incident chronic kidney disease, of which decreased eGFR is a surrogate marker, in European Americans. The pathophysiology underlying the association of hemostatic factors to kidney function decline is unclear, although triggers of hemostatic activation, including vascular injury, endothelial dysfunction, and inflammation, have been proposed as potential mechanisms.

To provide additional support to this hypothesis, we here propose the first genetic risk score approach addressing where genetic polymorphisms that have been robustly associated with eGFR, a marker for renal function, associate with VT risk.

Main outlines of the workflow are summarized in Fig. 1. In a first step, we sought the literature for single nucleotide polymorphisms (SNPs) robustly associated with eGFR. These SNPs were then tested for association with VT risk in 1953 VT patients and 2338 healthy individuals from a French case control study (MARTHA/EOVT) [10]. We derived a genetic risk score (GRS) based on the VT-associated SNPs and tested it for replication in an independent sample of 1289 VT patients and 1049 controls from a Dutch case control study (MEGA study) [11]. We finally confirmed the association of the derived GRS with eGFR in a third independent study.

2. Material & method

2.1. Studied populations

Informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and the study met all institutional ethics requirements.

2.1.1. Discovery VT case-control samples

The discovery cohort was composed of 1953 VT patients and 2338 healthy individuals from two French VTE case-control studies, MARTHA and EOVT that have been extensively described before [10,12]. Patients were individuals with documented personal VTE history and lacking strong genetic risk factors (antithrombin, protein C or protein S deficiencies, FV Leiden homozygosity, FII G20210A homozygosity). Controls were apparently healthy individuals free of any chronic conditions and of a personal VT history.

2.1.2. Replication VT case-control samples

The replication stage was based on the same data from the Multiple Environmental and Genetic Assessment of risk factor for venous thrombosis (MEGA) study [11] as those that were used in a recent meta-analysis of Genome-Wide Association Study (GWAS) data for VT [12].1289 Dutch VT patients with no prior event of VT and no cancer were compared with 1049 controls genetically matched for geographical ancestry [[12].].

2.1.3. Additional validation cohorts

The Genesis/Genediab study group was composed of 1370 individuals with Type 1 diabetes from the French population [13].

Clinical characteristics of this population are provided in Supplementary Table A.

2.2. Genotyping

All individuals used in the work have previously been typed for genome-wide genotype SNPs using dedicated DNA microarrays and imputed with 1000 Genomes reference. Genotyping, quality controls and imputation analyses of the genotype data have been previously described in [12] for the MARTHA, EOVT and MEGA studies and in [14] for Genesis/Genediab.

2.3. SNP selection

Sixty-nine SNPs were identified to robustly associate with eGFR in different GWAS studies [15–18]. These were selected as they have demonstrated genome-wide significance ($p < 5 \cdot 10^{-8}$) association with eGFR and replicated in independent studies. From this list of candidate SNPs, we discarded SNPs from any pair of SNPs in linkage disequilibrium and excluded SNPs with bad imputation quality ($r^2 < 0.3$) in both French GWAS cohorts. This resulted in 51 candidate SNPs (Supplementary Table B).

2.4. Statistical methods

2.4.1. Derivation of the genetic risk score (GRS)

Each of the 51 selected candidate SNPs was tested for association with VT in the French case-control samples using logistic regression analysis where the expected number of alleles (often referred to as imputed dose) at each SNP was used as a covariate in a model adjusting for age, sex and principal components derived from genotype data. Of note all SNPs had imputation quality $r^2 > 0.30$. From these analyses, we selected only SNPs for which the risk-allele was the one that was reported in the literature to associate with eGFR decrease, as compatible with the hypothesized relationship between renal dysfunction and VT risk. Only SNPs (Supplementary Table B) satisfying this condition were kept for the subsequent GRS analysis. In order to identify the most parsimonious and information SNP combination with respect to VT, an Akaike Information Criterion (AIC) [19] strategy was adopted using a backward logistic regression procedure, while adjusting for sex, principal components and French sub-study group. SNPs selected by this AIC procedure were then entered into a GRS defined, for each individual, as the sum of the imputed risk-allele dose at each selected SNP. We confirmed that selected SNPs were also well imputed in the replication MEGA study.

2.4.2. Association of the GRS with VT risk

Association of the derived GRS with VT was then tested using logistic regression analysis using the same covariates as for the AIC strategy, both in the discovery (MARTHA/EOVT) and replication (MEGA) studies. Results observed in the discovery and replication studies were then pooled using fixed effect model (as implemented in the rmeta R package).

2.4.3. Association analysis with eGFR

The association of the proposed GRS was investigated in relation to eGFR in the Genesis/Genediab study where eGFR was estimated using plasma creatinin and the CKD-EPI eq. [20] Association was tested using