Unsolved issues in etiology and treatment of venous thrombosis

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Citation for published version (APA):
Wichers, I. M. (2010). Unsolved issues in etiology and treatment of venous thrombosis

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No linkage for venous thrombosis at a candidate region on chromosome 18 in Dutch thrombophilic families

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*Journal of Thrombosis and Haemostasis* 2009; 7:1239-40
Venous thrombosis (VTE) is a multicausal disease in which interaction of genetic (i.e. factor V Leiden, PT20210), acquired (i.e. elevated levels of factor VIII, lupus anticoagulans) and environmental (i.e. surgery, immobilization) factors determine the development of disease. A study in Danish twins showed that for VTE the proportion of the variance attributable to genetic effects was as high as 55% 1. Furthermore, in a family-based study, the Genetic project Analysis of Idiopathic Thrombophilia (GAIT) study, the heritability of thrombosis susceptibility was estimated by means of a novel variance component method using a multivariate threshold model 2. It was estimated that more than 60% of the variation in susceptibility to common thrombosis is attributable to genetic risk factors. Since only a limited number of genetic risk factors are known, it is expected that there are unidentified hereditary defects that contribute to the risk for VTE.

In a search for novel genetic risk factors a whole genome scan in large protein C deficient kindred identified candidate regions involved in the risk of VTE on chromosomes 10, 11 and 18 3. Supportive evidence for the region on chromosome 18 was obtained in the GAIT study. In this second study, linkage analysis pinpointed a region on chromosome 18 which simultaneously influenced the ‘activated protein C resistance’ (APCR) phenotype, factor VIII levels and VTE 4. The peak log of the odds (LOD) score for APCR occurred near microsatellite marker D18S53, i.e. very close to the peak LOD score in the first study. Since the fact that two independent studies point to the same region on chromosome 18 provides compelling evidence of a true finding, we were interested if we could confirm linkage of VTE to chromosome 18 in the GENES study, a study of families with unexplained thrombophilia in The Netherlands.

GENES is an ongoing study and at the time of the present analysis there were 22 families included 5. Inclusion criteria were a history of VTE and the absence of known thromophilic defects (factor V Leiden, deficiencies of protein C, of protein S and of antithrombin, the prothrombin G20210A mutation and (familial) elevated levels of factor VIII, IX and XI). A standardized history was taken using a validated questionnaire 6. Family members were defined as cases if they had had a history of symptomatic VTE.

A thrombotic event was classified as “definite” if objectively diagnosed (use of compression ultrasonography, plethysmography, venography, (spiral) CT scan or
ventilation/perfusion scan) and treated with anticoagulant therapy. A VTE event was defined as “probable” if not objectively diagnosed or documentation on diagnostic tests used was unavailable. In this study cases with probable and definite events were included for analysis. Family history was defined as ≥1 first degree and/or ≥2 second degree relatives with VTE.

The 22 pedigrees consisted of 889 individuals and 58 family members with VTE (6%). A heritability (h²) analysis for VTE was performed in the program Sequential Oligogenic Linkage Analysis Routines (SOLAR) ⁴⁷. The h² for VTE was 76% (SE 13%) and 46% (SE 27%) when only individuals with definite VTE (n=30) were included in the analysis. The median age of onset was 40 years (range 18-68 years). Family sizes ranged from six to 185 with a median size of 28.

To determine the power to detect linkage, a simulation study using SIMLINK was performed in five randomly selected GENES families ⁸. We assumed a dominant trait with a piecewise linear or cumulative normal penetrance function and an allele frequency of 0.01. Based on 1000 simulations, the maximum LOD score and the power to detect a LOD score > 3 or > 2 were 5.1, 85% and 95% at θ = 0 and 3.8, 62% and 82% at θ = 0.05. Results were similar for the two penetrance functions.

Genomic DNA was prepared from 10 ml whole blood using standard procedures. Two markers on chromosome 18 (D18S53 and D18S843) were chosen from the UniSTS human sequence database. Oligonucleotide primers were obtained from Bioreglo (Nijmegen, The Netherlands). The forward primers were labelled with a 5-FAM fluorescent dye, and reverse primers were modified by PIGtailing ⁷. Polymerase chain reactions (PCRs) were performed in a 10 μL volume containing 3 μL (D18S843) versus 1.2 μL (D18S53) DNA, 0.2 μL of each oligonucleotide primer, 1.5 μL of 2.5 mM dNTP’s, 0.2 μL Taq, 1.5 μL PCR buffer and 1.5 μL of 25 mM MgCl₂. DNA was initially denatured at 95°C for 3 min and was then subjected to 11 cycles of 94°C for 15 sec, 55°C for 15 sec and 72°C for 30 sec, followed by 31 cycles of 89°C of 15 sec, 55°C of 15 sec, 72°C of 30 sec, followed by a final step of 72°C of 10 min. PCR products were diluted four times with distilled water and 4 μL diluted product was mixed with 0.4 μL GS-500 size standard (Applied Biosystems) and 15.6 μL denaturated H₂O and separated on a 3100 Genetic Analyzer (Applied Biosystems).
Data were analyzed using Genemapper software (Applied Biosystems) for Windows NT. Two independent observers checked results for inconsistency. Genetic linkage analysis was performed in SOLAR \textsuperscript{4,8}.

Of the 889 individuals 421 individuals had genotype information of whom 51 with a history of VTE. We could not confirm the previous claims as we found no linkage between VTE and D18S53 or D18S843, located at 38 and 28 cM on chromosome 18 respectively, in the whole study population or in any of the individual families from GENES. In the analysis of all families the LOD score for marker D18S53 was 0.122 and for D18S843 0.147, both of which are far removed from a significance threshold of 3.0. Furthermore, a bivariate analysis of VTE and activated protein C-sensitivity ratio was performed but no linkage was found. Bivariate analysis for VTE and FVIII was performed in the largest family, but no linkage was found.

These negative results imply that if there is a genetic risk factor hidden at the locus on chromosome 18, its prevalence might be low in Dutch patients with thrombophilia. Also for other genetic risk factors like factor V Leiden and PT20210 a gradient has been observed in Europe, with factor V Leiden most common in northern parts of Europe, whereas the opposite seems the case for the prothrombin variant \textsuperscript{9}. Please note that the large PC family that was studied in Vermont is of French–Canadian descent, i.e. originated close to the geographic region from which the participants of the Spanish GAIT were recruited. Therefore our negative result should not be taken as final evidence that the linkage peak that was observed in the previous studies is not pointing at a true novel risk factor for VTE.

**Acknowledgements**

We would like to thank Prof. Dr. F. Baas (Neurogenetics Laboratory) and J. Benit-Deekman for technical support.

This study was supported by a grant from the Netherlands Heart Foundation (2005B248).
Chapter 5

References


