Genetic Variations Associated With Recurrent Venous Thrombosis

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Background—The prediction of recurrent venous thrombosis using individual genetic risk predictors has proven to be challenging. The aim of this study was to assess whether multiple genetic single nucleotide polymorphism (SNP) analysis would predict recurrent venous thrombosis.

Methods and Results—Patients with a first venous thrombosis were followed for a recurrent venous thrombosis up to 2009 (MEGA follow-up study), which occurred in 608 out of 4100 patients (2.7%/year). Thirty-one common thrombosis-associated single nucleotide polymorphisms (SNPs) were associated with the risk of recurrence. A genetic risk score (GRS) for each individual was calculated by summing the number of risk-increasing alleles for each of the 31 SNPs and for a simplified model consisting of 5 SNPs: rs6025, rs1799963, rs8176719, rs2066865, and rs2036914. The risk of recurrence associated with the GRS was calculated continuously and after stratification in a low and high score. All individual SNPs were at most mildly associated with recurrence risk. Regarding the 31-SNP GRS, recurrence risk was highest in patients with ≥31 and lowest in patients with <21 risk alleles. The discriminative power of the 5-SNP GRS was similar to that of the 31-SNP GRS. The 6-year cumulative incidence of recurrence was high for individuals with ≥5 (20.3%; 95% confidence interval, 16.5–24.1) and low for individuals with ≤1 (9.4%; 95% confidence interval, 6.7–12.1) risk alleles. Predictive power improved after stratification into provoked and unprovoked first events and sex.

Conclusions—Multiple genetic SNP analysis is useful in the prediction of recurrent thrombosis, even more so when combining this model with clinical risk factors. (Circ Cardiovasc Genet. 2014;7:806-813.)

Key Words: follow-up study ■ genetic testing ■ venous thrombosis
the SNPs included in that study had a relatively low allele frequency, and therefore, with the addition of each SNP, the number of carriers rapidly reduced. This indicated a limited clinical utility as it would apply only to a small proportion of patients.

The aim of this study was to assess whether the genetic risk score (GRS) based on SNPs consistently associated with the risk of a first venous thrombosis also has predictive value on the risk of recurrent venous thrombosis.

### Methods

#### Study Population

Patients were included from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, a large population-based case–control study into risk factors for a first venous thrombosis. Between March 1999 and September 2004, consecutive patients aged <70 years with a first episode of deep venous thrombosis (leg or arm) or pulmonary embolism were included from the files of 6 participating anticoagulation clinics in the Netherlands (Amersfoort, Amsterdam, The Hague, Leiden, Rotterdam, and Utrecht). Information on the diagnostic procedure was obtained from hospital records and general practitioners. A deep venous thrombosis was confirmed with Doppler ultrasonography. A pulmonary embolism was confirmed by a ventilation perfusion lung scan, spiral computed tomography, or angiogram. Exclusion criteria were severe psychiatric problems and the inability to speak Dutch. All patients filled in a detailed questionnaire on risk factors for venous thrombosis at the time of the first event. DNA was obtained from blood samples or buccal swabs. For the MEGA follow-up study, only patients with a short questionnaire and via the anticoagulation clinics, which monitored all outpatients’ anticoagulant treatment with vitamin K antagonists. For all potential recurrences, discharge letters were obtained from the clinician who diagnosed the recurrence.

Information on recurrent events was obtained from the patients via a short questionnaire and via the anticoagulation clinics, which monitored all outpatients’ anticoagulant treatment with vitamin K antagonists. For all potential recurrences, discharge letters were obtained from the clinician who diagnosed the recurrence.

The reported recurrences were classified into certain and uncertain recurrences according to a decision rule previously described. In this study, only certain recurrences were used as end point, and patients with an uncertain recurrence were censored at time of their uncertain recurrence (n=141).

The end of follow-up was defined as the date of the recurrence and, in the absence of a recurrence, the date of filling in the questionnaire. If a patient did not fill in a questionnaire, they were censored at the last date known to be recurrence free, that is, the last visit to the anticoagulation clinic, date of death or emigration, or the last time the patient was known to be recurrence-free from information of the MEGA case–control study. Start of follow-up was the date of the first event.

#### Genotype Determination

In total, 31 SNPs were tested that were repeatedly found to increase the risk of a first venous thrombosis in the Leiden Thrombophilia Study and MEGA study. Genotyping of individual DNA samples was performed with kPCR assays using 0.3 ng of DNA or using multiplexed oligo ligation assays. Genotyping accuracy of both systems have been assessed in previous studies, and the concordance of the genotype calls from these methods was >99.5%. For the current analyses, 628 (13%) patients were excluded because they did not provide a DNA sample (n=620) or the obtained material was of poor quality (n=8). Three additional patients were excluded (2 man–woman transitions and 1 Klinefelter syndrome), resulting in a maximum of 4100 patients in the analyses. The mean duration of anticoagulation in these patients was 10.0 months (range, 0.5–122.9 months). 3440 patients had a valid measurement for all 31 SNPs included.

#### Statistical Analysis

Cox regression models were used to calculate the hazard ratios (HR) with 95% confidence intervals (95% CI) for the association of each SNP with recurrent venous thrombosis adjusted for age and sex. The risk allele was defined as the allele found to increase the risk of a first venous thrombosis. Additionally, we calculated the risk allele HR from an additive model, which can be interpreted as the increase in risk per copy of risk allele.

We calculated the number of risk-increasing alleles carried and compared this between patients with and without a recurrence. Using the number of risk alleles as a continuous variable, we calculated the HR of recurrence per addition of risk allele adjusted for age and sex.

Because predictors of recurrence may be different from those of a first event, we also constructed a prediction model using the 5 genetic variants that were, in this study, most strongly associated with the risk of recurrence.

Analyses were performed in the overall patient group and after stratification into patients with a first unprovoked or provoked event and after stratification by sex. The risk of recurrent venous thrombosis associated with the 5-SNP GRS was also assessed after exclusion of patients with malignant disease at the time of the first venous thrombosis and in patients of white descent only (defined as both parents born in a North or West European country). Furthermore, we performed stratified analyses on the type of the first venous thrombosis (deep venous thrombosis of the leg and pulmonary embolism) and family history of venous thrombosis (father, mother, or sibling with venous thrombosis). Unprovoked thrombosis was defined as having none of the following provoking factors: malignant disease in the 5 years before the first thrombosis, surgery, trauma, hospitalization, immobilization, plaster cast, hormone use (oral contraceptives and hormone therapy), or pregnancy within 3 months before the first event, within 4 weeks postpartum, or long-haul flight (>4 hours) in the 2 months before the first thrombosis.

#### Results

The mean age of the 4100 patients at the time of the first event was 48 years (range, 18–70 years) and 2233 (54.5%) were women. The total volume of follow-up was 22,040 person-years with a mean follow-up of 5.4 years (range, 1.1
Table 1. The Risk of Recurrent Venous Thrombosis Associated With 31 Individual SNPs Previously Associated With the Risk of a First Venous Thrombotic Event (HR Adjusted for Age and Sex)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Chrom</th>
<th>Position</th>
<th>Risk Allele Freq, %</th>
<th>N</th>
<th>HR*</th>
<th>95% CI HR</th>
<th>HR†</th>
<th>95% CI HR</th>
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<td>1.34–1.89</td>
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<td>1.22</td>
<td>1.09–1.37</td>
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<td>6.153.534</td>
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<td>1206 indel</td>
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<td>0.73–1.10</td>
<td>0.99</td>
<td>0.88–1.10</td>
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(Continued)
months–10.7 years). Of these patients, 608 had a recurrent event resulting in an overall incidence of recurrence of 27.6 per 1000 person-years (95% CI, 25.4–29.8). 1133 (28.3%) patients had an unprovoked first event and 2870 (71.7%) had a provoked first event (information missing in 97 patients). The incidence of recurrent thrombosis was higher in patients with a first unprovoked event than in patients with a provoked first event (provoked: 21.8 per 1000 person-years, 95% CI, 19.5–24.1; unprovoked: 42.9 per 1000 person-years, 95% CI, 37.6–48.1). As expected, the risk of recurrence was higher in men than in women.

Table 1 shows the risk of recurrent venous thrombosis associated with the 31 SNPs. The SNPs were ordered according to the risk of recurrent thrombosis in an additive model. All individual SNPs were at most mildly associated with the risk of recurrence, with the strongest association for the factor V Leiden mutation (HR additive model, 1.60; 95% CI, 1.02–1.07).

Previously, the predictive value for a first venous thrombosis of a simplified model containing 5 genetic variants was equally strong as that of the extended model. We tested this 5-SNP model for recurrent venous thrombosis. With these 5 SNPs, the number of risk-increasing alleles ranged from 0 to 8. The overall discriminative power of the simplified 5-SNP GRS was similar to that of the 31-SNP GRS (P10 versus P90: risk difference at 6-year follow-up: 31-SNP model, 6%; 5-SNP model, 10.9%). However, this 5-SNP GRS showed better discriminative power in the period shortly after the first venous thrombosis (P10 versus P90: risk difference at 2 year follow-up: 31-SNP model, 1.1%; 5-SNP model, 6.9%). When analyzing the 5-SNP GRS continuously, the addition of 1 risk allele was associated with a 20% increased risk of recurrent thrombosis (HR, 1.2; 95% CI, 1.1–1.3). Individuals with many risk alleles (≥5 risk alleles, ie, ≥P90) had a substantially higher risk of recurrent thrombosis than individuals with few risk alleles (≤1 risk allele, ie, ≤P10; Figure 2, panel B) with a HR of 2.5, 95% CI of 1.7 to 3.6. In our analyses, start of follow-up was at the time of the first venous thrombosis; however, when performing the analyses using stop date of anticoagulation as the start of follow-up, results regarding the predictive power of the genetic models were essentially the same. The goodness-of-fit of our model was checked by adding a time-dependent covariate to the Cox regression analysis, thereby allowing the effect of the GRS to vary over time. This time-dependent covariate was not significant, and because this is a large study, the proportional hazard assumption was considered fulfilled.

The 6-year cumulative incidence of recurrence was high, that is, 20.3% (95% CI, 16.5–24.1) for individuals with ≥5 risk alleles (11.3% of the patients), and low, that is, 9.4% (95% CI 6.7–12.1) for individuals with <1 risk allele (11.9% of the patients; Table 2). When we analyzed the risk of recurrent
venous thrombosis associated with the 5-SNP GRS in patients without a cancer-related first venous thrombotic event, similar results were obtained as with the total study population (6-year cumulative incidence in patients with ≥5 risk alleles, 20.9%; in individuals with ≤1 risk allele, 9.6%; HR, 2.6 [95% CI, 1.8–3.9]). Of the total study population, 3569 (89.5%) were of white descent (information of birth country of parents available for 3986 patients). We performed a sensitivity analysis whereby restricting the analysis to the patients of white descent only. Again results were similar to the results obtained in the total study population (6-year cumulative incidence in patients with ≥5 risk alleles, 21.5%; in individuals with ≤1 risk allele, 10.0%; HR, 2.5 [95% CI, 1.7–3.6]).

The discriminative power of the 5 SNP risk score was also assessed in patients with a first deep venous thrombosis of the leg and patients with a first pulmonary embolism separately. In patients with a deep venous thrombosis of the leg, similar results were obtained as in the overall analysis with 6-year cumulative incidences of 11.3% in patients with a low GRS and 21.0% in patients with a high GRS (HR, 2.1; 95% CI, 1.3–3.4). Although the number of recurrences in patients with a pulmonary embolism with either a low or a high GRS was low, a similar difference between high and low GRS was found (HR, 1.9; 0.8–4.7). Furthermore, also stratification on family history of venous thrombosis did not affect the results, that is, both in patients with a positive as well as in patients with a negative family history of venous thrombosis, the risk of recurrent venous thrombosis was high in patients with ≥5 risk alleles and low in patients with ≤1 risk allele. In patients with a positive family history of venous thrombosis, the 6-year cumulative incidence of recurrence was 13.2% in patients with ≤1 risk allele and 25.6% in patients with ≥5 risk alleles (HR, 2.4; 95% CI, 1.3–4.7). In patients with a negative family history of venous thrombosis, the 6-year cumulative incidence of recurrence was 7.6% in patients with ≤1 risk allele and 16.6% in patients with ≥5 risk alleles (HR, 2.1; 95% CI, 1.2–3.7).

The discriminative power improved when combining the 5-SNP GRS with clinical provoking factors (Figure 2, panel C). The risk of recurrence was highest in individuals with an unprovoked first event who carried ≥5 risk alleles (6-year cumulative incidence, 29.5; 95% CI, 21.1–37.9) and lowest in individuals with a provoked first event carrying ≤1 risk allele (6-year cumulative incidence, 7.1; 95% CI, 4.3–9.9). Stratification into men and women showed that the highest risk of recurrence was found in men carrying ≥5 risk alleles: 6 years cumulative incidence, 29.4% (95% CI, 23.2–35.6), and the lowest risk of recurrence was found for women carrying ≤1 risk allele: 6 years cumulative incidence, 5.2% (95% CI, 2.5–7.9).

The 5 SNPs that were used in the 5-SNP GRS were among the 12 SNPs that were, in the present study, the most strongly associated with the risk of recurrence (Table 1). However, based on this study, the rank order of the risk of recurrence was different from that for a first venous thrombotic event. We tested a 5-SNP GRS using the 5 genetic variants that were most strongly associated (ie, by HR) with the risk of recurrent venous thrombosis in this study (rs6025, rs1799963, rs8176719, rs3136520, and rs3822057). This model appeared to discriminate less clearly between individuals with a high and individuals with a low risk of recurrence than the predefined 5-SNP GRS (Table 2).

To assess whether the factor V Leiden and the prothrombin 20210A mutation would be sufficient to stratify patients according to their risk of recurrence, we assessed the predictive value of a 2-SNP GRS (rs6025 and rs1799963; n=4098). Only 1 patient, who also had a recurrent event, carried 3 risk alleles. Fifty-three patients carried 2 risk alleles of whom 11 had a recurrent event and 790 patients carried 1 risk allele of whom 165 had a recurrent event. Although this 2-SNP GRS was able to stratify patients in high and low risk groups (ie, 6-year cumulative incidence: ≥2 risk alleles, 23.0%; 95% CI, 11.8–34.2; 0 risk alleles, 14.2%; 95% CI, 13.0–15.4), this model has little clinical relevance because very few patients were in the high risk group.

**Discussion**

The prediction of recurrent venous thrombosis using individual genetic risk predictors has proven to be
challenging. The present study shows that multiple genetic SNP analysis is useful in the prediction of recurrent thrombosis. A 5-SNP GRS, previously validated for first venous thrombosis, comprising factor V Leiden, prothrombin 20210 G>A, rs8176719 (ABO), rs2066865 (FGG 1034 C>T), and rs2036914 (F11), could stratify patients into high and low risk of recurrence, with an over 2-fold difference. The predictive power was even stronger after stratification into provoked and unprovoked first events. The measurement of 5 SNPs was sufficient.

The high frequency of recurrent venous thrombosis (12%–20% in 5 years) begs the question of long-term anticoagulant prophylaxis after a first thrombosis. However, because this therapy is associated with a risk of major hemorrhage of 2% per year, indiscriminate use in all patients is not warranted. Identification of individuals at low risk of recurrence in whom anticoagulant therapy can safely be withheld, and in those with high risk who should receive long-term treatment, will lead to a minimum of untoward events, that is, thrombotic and bleeding events. Groups with over 25% 6-year risk of recurrence were men with a high risk score and all individuals with a high risk score and an unprovoked first event. These constituted 6.0% of all patients, and in them, long-term anticoagulation could be considered. Vice versa, groups with ≤7% 6-year risk of recurrence were women with a low risk score and all individuals with a low risk score and a provoked first event. These constituted almost 10% of all patients, and in them anticoagulation may be safely discontinued.

Although the factor V Leiden and the prothrombin 20210A mutation were the strongest genetic markers among the 31 measured SNPs, neither were strongly associated with the risk of recurrence when considered individually. Furthermore, when the SNPs were ranked according to the risk of recurrence, the top 5 SNPs performed less well than the 5 SNPs previously identified as performing well in predicting first thrombosis, which have repeatedly proven their ability to predict the risk of a first thrombosis in multiple studies. Compared with studies on the risk of a first venous thrombosis, little information is available on the risk of recurrence regarding genetic variation. The 5-SNP GRS using the 5 genetic variants that were most strongly associated (ie, by univariate HR) with the risk of recurrent venous thrombosis was only based on information from the current study and so the risk estimates associated with the selected variants contain more uncertainty than those of the well-established 5 SNPs in the GRS. This most likely led to the somewhat worse predictive value of the former 5-SNP GRS.

Limitations of our study are that we excluded children and individuals aged >70 at the time of the first event. Therefore, our findings cannot be extrapolated to these patients. Per analysis, we also excluded patients with missing measurements. This resulted in the exclusion of 660 patients for the 31-SNP model and 223 for the 5-SNP model. Arguably, this exclusion may have led to a loss in power to detect potential effects on the risk of recurrence. Of the total study population, 3569 (89.5%) were of white descent. Therefore, we can apply only these findings to patients with a similar ethnic background. Further studies are needed to assess the predictive value of

Figure 2. Cumulative incidence of recurrence using the 31 single nucleotide polymorphism (SNP) genetic risk score (A) and the 5 SNP genetic risk score (B and C).
the genetic model in populations with different ethnic backgrounds. Strengths of our study are the large number of recurrent events observed in a homogeneous, well-characterized cohort of patients, which enabled us to assess the risk of venous thrombosis associated with different GRSs, including subgroup analyses and the prolonged follow-up.

In conclusion, we showed that, using genetic markers for venous thrombosis, it is possible to stratify patients who have had a first venous thrombosis into subgroups with a high and low risk of recurrence. Risk stratification became more pronounced using the GRSs with respect to the conservation of information regarding provoking factors at the time of the first event and with sex. We think that the results indicate that for some high-risk groups, long-term anticoagulant treatment is indicated. With the increasing use of exome and whole genome sequencing, novel genetic markers for venous thrombosis will be discovered. These will most likely be rare genetic variants with a high thrombosis risk. These genetic markers will be of great importance in further unravelling causes of venous thrombosis, but because of their low prevalence in the general population, these genetic markers may be of little clinical relevance when considered individually. However, the prediction of recurrence risk using multiple genetic and environmental markers may be further optimized.

Sources of Funding
The Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis was supported by The Netherlands Heart Foundation (grant NHS 98.113), the Dutch Cancer Foundation (RUL99/1992), and The Netherlands Organization for Scientific Research (grant 912-03-033l 2003). The MEGA follow-up study was supported by The Netherlands Heart Foundation (grant NHS 2008B86). The funding organizations played no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the article.

Disclosure
Lance A. Bare, James J. Devlin, Pieter H. Reitsma, and Frits R. Rosendaal hold or have applied for patents related to SNPs in this article (notably rs6025, rs2066865, and rs2036914). Andre R. Arellano, Carmen H. Tong, James J. Devlin, and Lance A. Bare are employees of Celera Corporation, a wholly owned subsidiary of Quest Diagnostics. The remaining authors declare no competing financial interests.

References
Our findings are replicated.


...low GRS and an unprovoked first event. In them, long-term anticoagulation could be considered.

...and most strongly associated with the risk of a first venous thrombosis in literature (rs6025 [factor V Leiden], rs1799963 [F2], rs8176719 [ABO], rs2066865 [FGG], and rs2036914 [F11]). Using this 5-single nucleotide polymorphism genetic risk score (GRS), we were able to stratify patients on their recurrence risk, that is, the 6-year cumulative incidence of recurrence was high for individuals with ≥5 (20.3%) and low for individuals with ≤1 risk alleles (9.4%). Predictive power improved after stratification into provoked/unprovoked first events and sex. Groups with >25% 6-year recurrence risk were men with a high GRS and all individuals with a low GRS and a provoked first event. These patients may be candidates in whom anticoagulation may be safely discontinued if our findings are replicated.

**Clinical Perspective**

The rate of recurrent venous thrombosis is high. Because anticoagulant therapy is associated with a risk of major hemorrhage, indiscriminate use in all patients is not warranted. Identification of individuals at low risk of recurrence in whom anticoagulant therapy can safely be withheld, and those with high risk who should receive long-term treatment, will lead to a minimum of untoward events, that is, thrombotic and bleeding events. However, the prediction of recurrent venous thrombosis with individual genetic risk variants has proven to be difficult. In this study, we assessed the predictive value of multiple single nucleotide polymorphism testing for the risk of recurrence using 5 genetic variants that were consistently and most strongly associated with the risk of a first venous thrombosis in literature (rs6025 [factor V Leiden], rs1799963 [F2], rs8176719 [ABO], rs2066865 [FGG], and rs2036914 [F11]). Using this 5-single nucleotide polymorphism genetic risk score (GRS), we were able to stratify patients on their recurrence risk, that is, the 6-year cumulative incidence of recurrence was high for individuals with ≥5 (20.3%) and low for individuals with ≤1 risk alleles (9.4%). Predictive power improved after stratification into provoked/unprovoked first events and sex. Groups with >25% 6-year recurrence risk were men with a high GRS and all individuals with a high GRS and an unprovoked first event. In them, long-term anticoagulation could be considered. Vice versa, groups with ≤7% 6-year recurrence risk were women with a low GRS and all individuals with a low GRS and a provoked first event. These patients may be candidates in whom anticoagulation may be safely discontinued if our findings are replicated.

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**References**


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Genetic Variations Associated With Recurrent Venous Thrombosis
Astrid van Hylckama Vlieg, Linda E. Flinterman, Lance A. Bare, Suzanne C. Cannegieter, Pieter H. Reitsma, Andre R. Arellano, Carmen H. Tong, James J. Devlin and Frits R. Rosendaal

Circ Cardiovasc Genet. 2014;7:806-813; originally published online September 10, 2014; doi: 10.1161/CIRCGENETICS.114.000682
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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