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ORIGINAL ARTICLE

Genetic Polymorphisms in Venous Thrombosis and Pulmonary Embolism After Total Hip Arthroplasty

A Pilot Study

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Abstract Deep venous thrombosis (DVT) after major orthopaedic surgery is a substantial concern. We asked whether the single or combined presence of thrombophilic genetic polymorphisms might further increase the already high risk for venous thrombosis and pulmonary embolism (PE) after THA. We therefore compared the prevalence of factor V Leiden, prothrombin G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, and plasminogen activator inhibitor 4G/5G polymorphisms between 50 patients with symptomatic DVT within 3 weeks after elective THA and an asymptomatic control group of 85 patients. We found no major difference for the presence of a single mutation between the groups. Factor V Leiden and homozygous MTHFR C667T mutations were of borderline significance with odds ratios (95% confidence intervals) of 3.73 (0.89–15.63) and 2.93 (0.92–9.29), respectively. Patients with homozygous or combined heterozygous status of MTHFR C677T and A1298C mutation had a higher frequency of DVT after elective THA (odds ratio, 2.86; 95% confidence interval, 1.32–6.35) than those with wild-type. The presence of a single mutation may not further increase the already high risk for symptomatic DVT after THA, whereas combinations of mutations of distinct polymorphisms might be important. However, prospective studies with a larger number of patients are needed before we would recommend preoperative screening.
Level of Evidence: Level III, prognostic study. See the Guidelines for Authors for a complete description of levels of evidence.

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Each author certifies that his or her institution has approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

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Introduction

Major orthopaedic surgery is associated with a high risk of postoperative DVT with or without PE. Despite routinely performed prophylactic chemical and mechanical measures during the perioperative period, a considerable number of patients still experience DVT after major orthopaedic surgery [18, 21, 29]. The residual risk for venographically diagnosed DVT after THA remains within a range of 9% to 36% [17, 18, 21, 29]. This risk is even higher after

TKA, with risks of as much as 52% [18]. Less than 10% of all DVT, however, may become symptomatic [18]. Furthermore, the type of DVT varies after these different orthopaedic procedures with a higher rate of distal DVT (89% versus 60%) and a lower rate of proximal DVT (9% versus 37%) after TKA compared with THA [18]. This probably is related to local tissue trauma at the site of surgery, manipulation of the knee during surgery, and the use of a tourniquet with subsequent distal venous stasis [18].

Therefore, optimization of perioperative antithrombotic management is still required. However, intensifying perioperative anticoagulation might be associated with a higher risk of bleeding. Therefore, more aggressive antithrombotic measures should be performed only for patients having an additionally increased thrombophilic risk. Preoperative identification of such patients would enable the orthopaedic surgeon to target intensified antithrombotic measures individually accepting a higher bleeding risk in the perioperative setting.

Several exogenous thrombophilic risk factors are known and may be analyzed easily without the need for additional laboratory tests, such as higher age, obesity, intake of estrogens, cancer, infection, smoking, former thromboembolic events, or a positive family history. For other and especially endogenous thrombophilic risks, additional laboratory tests are necessary. Several genetic polymorphisms such as factor V Leiden or prothrombin G20210A mutation have been associated with a substantially increased risk for DVT in general [9, 24, 27, 28]. For other genetic markers, such an association is suspected and discussed controversially, eg, methylenetetrahydrofolate reductase (MTHFR) C677T and/or A1298C and plasminogen activator inhibitor 1 (PAI-1) 4G/5G polymorphisms [1–3, 10, 11, 16, 22, 30, 32, 34, 37, 44]. Results of former studies are conflicting regarding whether the single or combined presence of such genetic mutations may further increase the already very high risk for DVT after major orthopaedic surgery, justifying costly preoperative screening and additional antithrombotic prophylactic measures in these patients [5, 6, 8, 18, 20, 21, 23, 29, 31, 36, 40–43]. One reason for the conflicting results may be found in the different clinical end points set in the studies varying among venographically diagnosed DVT, clinically symptomatic DVT with or without PE, or symptomatic PE only. Most of these authors found no association between one of the previously named genetic polymorphisms and the incidence of symptomatic or asymptomatic DVT after major orthopaedic surgery [18, 21, 23, 29, 31, 36, 40, 42]. When concentrating their analysis on symptomatic DVT, however, results are conflicting [5, 6, 8, 20, 31, 40, 41]. Asymptomatic DVT diagnosed by venogram may be associated with an increased risk for asymptomatic and symptomatic PE or have an increased mortality rate compared with those without thrombosis [15, 38]. Nonetheless, as long as no routine screening for postoperative asymptomatic DVT is implemented, only symptomatic events have an impact on daily practice leading directly to additional therapeutic measures.

We therefore asked whether the presence of one of the previously named genetic polymorphisms may further increase the already high postoperative risk for symptomatic DVT in patients undergoing elective THA. Additionally, we asked whether a combined mutation status may even further increase this risk.

Materials and Methods

We retrospectively studied patients who underwent elective THA between 1996 and 2003. The observation group consisted of 34 patients from the Waldkrankenhaus Erlangen, Germany, and 16 patients from the Clinic for Rehabilitation, Herzogenaurach, Germany. All patients had acute ascending thrombosis up to the popliteal or more proximal veins with or without PE (four and 46 patients, respectively) developed within 3 weeks postoperatively. No patient had isolated PE. Recruitment of the patients participating in this study was different in the two hospitals as described subsequently.

We recruited patients from Waldkrankenhaus Erlangen by retrospectively screening the patient files of 1966 patients who underwent elective THA between 1996 and 2003. All patients were included who had acute symptomatic ascending DVT at least up to the popliteal vein confirmed by complete compression ultrasound within 3 weeks after elective THA. Thirty-six patients met these inclusion criteria and were contacted by mail or phone and informed about the aim of the study. One patient had developed cancer and one patient had died. Therefore, 34 patients were able to participate in the study and provided informed consent.

The patients of the Clinic for Rehabilitation Herzogenaurach were recruited by screening all patient files between 1996 and 2003 for diagnosis of proximal DVT, PE, and THA according to the International Classification of Diseases, Tenth Revision, developed by the World Health Organization and translated into German by the German Institute for Medical Documentation and Information [14]. Twenty-three patients fulfilled the inclusion criteria and were contacted by mail or phone and informed about the aim of the study. One patient did not provide informed consent, one patient had died, and five patients were lost to followup. The remaining 16

patients provided informed consent and participated in the study.

The control group consisted of 85 randomly selected age-matched patients who underwent elective THA at the Waldkrankenhaus Erlangen with no symptomatic or clinically suspected DVT up to 6 months after the operation. We did not routinely screen asymptomatic patients of the control group for DVT or PE. All patients in both institutions and both groups received perioperatively the same standardized antithrombotic prophylactic treatment with compression stockings and subcutaneous application of nadroparin (Fraxiparin™; GlaxoSmithKline, Munich, Germany) in high prophylactic dosage starting approximately 12 hours preoperatively as recommended by the manufacturer. The second dose of nadroparin was given within 6 to 12 hours postoperatively. No intermittent pneumatic calf compression was used for any patient in this study. All patients in the observation and control groups were of Caucasian origin.

All patients in the observation group presented with symptomatic proximal DVT within 3 weeks after elective THA. The diagnosis of proximal DVT was confirmed by complete compression ultrasound. Four patients had additional clinical symptoms of PE. The diagnosis of PE was confirmed by conventional pulmonary angiography. DVT occurred within 8.96 ± 4.85 days (mean ± standard deviation) postoperatively (maximum, 21 days; minimum, 2 days). Because the patients were not fully mobilized within 3 weeks postoperatively, all DVTs occurred while being on treatment with low-molecular-weight heparin in high prophylactic dosage and wearing compression stockings. No patient in the control group had any clinical symptoms of DVT within 6 months after elective THA. None of the patients participating in the study had a malignant disease or any other chronic illness, especially no chronic cardiovascular disease except for varicose veins.

The patients in the observation group and the control group were similar regarding gender, body mass index, smoking habits, varicose veins, hormone intake (women), medical history, family medical history for DVT, and age (Table 1).

All patients answered a standardized questionnaire (Appendix 1); the following data were collected: age, height, weight, smoker or nonsmoker, personal and family medical history with a special focus on former DVT events, and the intake of hormones (women only).

For the genetic testing, we took a sample of 10 mL 0.05 M EDTA-anticoagulated blood. DNA was extracted according to a standard salting-out procedure. For DNA analysis, we used commercially available kits and the procedure was performed in accordance with the manufacturer's instructions. Mutation status (normal, heterozygous, homozygous) was determined for all mutations. We performed the analysis of factor V Leiden and prothrombin G20210A mutations, and PAI-1 variants, using Genespector Kit™ (Variom Biotechnology, Berlin, Germany). For determination of the mutation status of the MTHFR C677T and A1298C mutations, we used PCR amplification and restriction fragment length polymorphism analysis was performed according to Frosst et al. [13] and van der Put et al. [39], respectively. The 677C>T mutation introduces a new *Hin*fI restriction site that results in digestion of a 198-bp PCR amplicon into 175-bp and 23-bp fragments. The 1298A>C mutation abolishes a *Mbo*II restriction site resulting in a 163-bp amplicon that is digested into four fragments of 84, 31, 30, and 18 bp. The wild-type 1298AA genotype yields an amplicon digest of five fragments of 56, 31, 30, 28, and 18 bp. Digested PCR fragments were separated electrophoretically using a 10% polyacrylamide gel.

To be able to exclude influence of risk factors, we tested the observation and control groups for differences in gender, smoking habit, varicose veins, personal and family history for thrombosis, intake of hormones, and diabetes using the chi square test. Differences in age and body mass index between these groups were determined with the Mann-Whitney U test. The differences in prevalences of MTHFR, factor V Leiden, prothrombin G20210A, and PAI-1 mutations between patients and control subjects were tested using Fisher's exact test. Furthermore, we calculated sample odds ratios (ORs), their 95% confidence intervals (CIs), and p values. Fisher's exact test also was used to determine differences in the prevalence of combined mutation status between both groups. Because our sample size was not very large, we performed a power

Table 1. Demographic and clinical data of patients

Parameter	Observation group	Control group	p Value
Number of patients	50	85	
Gender: female (%)	72	73	> 0.9
Age* (years; mean ± SD)	68.1 ± 6.4	66.3 ± 7.3	0.15
Body mass index (kg/m^2; mean ± SD)	28.7 ± 5.9	27.2 ± 4.3	0.35
Smoker (%)	12	18	0.53
Varicosis (%)	38	42	0.75
Former venous thrombosis (%)	20	14	0.51
Positive family history for venous thrombosis (%)	12	5	0.22
Type 2 diabetes mellitus (%)	0	6	0.20
Intake of hormones† (%)	14	18	0.34

* Age at the time of the THA; †women only; SD = standard deviation.

analysis regarding the Fisher tests. We calculated the power of our study to detect ORs as high as 3.0 for a Bonferroni corrected alpha of 0.006 for single genetic polymorphisms and 0.01 for combined genetic polymorphisms. Additionally, the sample sizes required to detect differences in the prevalence of factor V Leiden and homozygous MTHFR C677T mutations between observation and control groups based on observed prevalences were calculated. The computations were performed using the statistical programming language R 2.7.1 [25] and SPSS® for Windows® (Release 11.5.0; SPSS Inc, Chicago, IL).

Table 3. Power for detecting odds ratios

Genetic polymorphism	Power
Factor V Leiden	10%
Prothrombin G20210A	10%
MTHFR C677T (C/T)	52%
MTHFR C677T (T/T)	20%
MTHFR A1298C (A/C)	52%
MTHFR A1298C (C/C)	22%
PAI-1 4G/5G	32%
PAI-1 4G/4G	22%

MTHFR = methylenetetrahydrofolate reductase; PAI-1 = plasminogen activator inhibitor 1.

Results

The presence of only one of the studied genetic polymorphisms did not increase the risk of symptomatic DVT after elective THA. Compared with the control group, the prevalence of each studied genetic polymorphism was not considerably higher in the observational group (Table 2). For factor V Leiden and MTHFR 667TT mutations, however, the higher number of carriers in the patient cohort was of borderline significance with ORs of 3.73 (95% CI, 0.89–15.63, $p = 0.08$) and 2.93 (95% CI, 0.92–9.29, $p = 0.08$, Bonferroni corrected alpha = 0.006), respectively. Our post hoc power analysis showed a sample size of 205 would be needed to detect a major difference in the proportions of factor V Leiden mutations between the observation and the control groups calculated with a power of 80% for a Bonferroni corrected alpha of 0.006. The required size of the groups to determine differences in homozygous MTHFR C677T mutations was 125. The power for detecting an OR of 3.0 comparing the prevalence of mutations of factor V Leiden, prothrombin G20210A, MTHFR C677T, MTHFR A1298C, and PAI-1 was calculated for a Bonferroni corrected alpha of 0.006 for the single genetic polymorphisms (Table 3).

Except for the two MTHFR mutations, a combined positive status of the studied genetic polymorphisms does not further increase the risk for symptomatic DVT after elective THA (Table 4). Compared with patients negative or no more than simple heterozygous status for both MTHFR mutations, patients with at least one homozygous or two heterozygous mutations of MTHFR C677T and A1298C polymorphism, hereafter referred to as MTHFR*, had increased risk for symptomatic DVT after elective THA with an OR of 2.89 (95% CI, 1.40–5.96; $p = 0.006$, Bonferroni corrected alpha = 0.01). The sample OR calculated for a combination of MTHFR* and factor V Leiden was the highest of all combinations, albeit not significant (OR, 5.36; 95% CI, 0.54–53.01; $p = 0.143$, Bonferroni corrected alpha = 0.01). The power for detecting ORs as high as 3.0 with combinations of these genetic polymorphisms was calculated for a Bonferroni-corrected alpha of 0.01 (Table 5).

Table 2. Comparison of the prevalence of the single tested genetic polymorphisms

Genetic polymorphism	Observation group	Control group	Odds ratio	95% confidence interval	p Value (Fisher's exact test)
Factor V Leiden (%)*	12	4	3.73	0.89–15.63	0.08
Prothrombin G20210A (%)	6	4	1.74	0.34–8.99	0.67
MTHFR C677T (C/T) (%)	38	42	1.03	0.48–2.20	> 0.9
MTHFR C677T (T/T) (%)	18	7	2.93	0.92–9.29	0.08
MTHFR A1298C (A/C) (%)	42	44	1.06	0.50–2.23	> 0.9
MTHFR A1298C (C/C) (%)	14	8	1.86	0.58–6.00	0.36
PAI–1 4G/5G	58	49	1.32	0.55–3.14	0.66
PAI-1 4G/4G	20	26	0.87	0.31–2.47	> 0.9

* One patient of the observation group was homozygous for factor V Leiden mutation; MTHFR = methylenetetrahydrofolate reductase; PAI-1 = plasminogen activator inhibitor 1; Bonferroni-corrected alpha = 0.006.

Table 4. Comparison of the combined prevalence of tested genetic polymorphisms

Genetic polymorphism	Observation group	Control group	Odds ratio	95% confidence interval	p Value (Fisher's exact test)
MTHFR* (%)	56	31	2.89	1.40–5.96	0.006†
MTHFR* and factor V Leiden (%)	6	1	5.36	0.54–53.01	0.143
MTHFR* and PAI-1 4G/4G (%)	42	25	2.21	1.05–4.66	0.053
Factor V Leiden and PAI-1 4G/4G (%)	8	2	3.61	0.64–20.46	0.194
Prothrombin G20210A and PAI-1 4G/4G (%)	4	2	1.73	0.24–12.67	0.627

* Both heterozygous or at least one homozygous; †significant; MTHFR = methylenetetrahydrofolate reductase; PAI-1 = plasminogen activator inhibitor 1.

Table 5. Power for detecting odds ratios

Genetic polymorphism	Power
MTHFR*	67%
MTHFR* and factor V Leiden	4%
MTHFR* and PAI 4G/4G	64%
Factor V Leiden and PAI 4G/4G	9%
Prothrombin G20210A and PAI 4G/4G	9%
MTHFR*	67%
MTHFR* and factor V Leiden	4%
MTHFR* and PAI 4G/4G	64%

* Both heterozygous or at least one homozygous; MTHFR = methylenetetrahydrofolate reductase; PAI = plasminogen activator inhibitor 1.

Only one patient who presented with DVT already on Day 2 postoperatively was positive for the factor V Leiden (homozygous) and prothrombin G20210A mutation (heterozygous).

Discussion

Carriers of several genetic polymorphisms such as factor V Leiden or prothrombin G20210A mutation are known to have an increased thrombophilic risk in general [9, 24, 27, 28]. For other genetic markers, such as MTHFR C677T and/or A1298C and PAI-1 4G/5G polymorphisms, a controversial discussion regarding the relevance as thrombophilic risk factors is ongoing [1–3, 10, 11, 16, 22, 30, 32, 34, 37, 44]. It is unclear, however, whether the presence of such genetic mutations could further and independently increase the already high thrombotic risk in major orthopaedic surgery. We therefore asked whether the presence of one of the previously named genetic polymorphisms might increase the postoperative risk for symptomatic DVT in patients undergoing elective THA. We also asked whether a combined mutation status might even further increase this risk.

Our study has several limitations. First, the number of patients is low resulting in relatively wide 95% CIs when calculating the ORs. The result of our power calculation confirms this drawback. Second, we missed determining homocysteine levels in our patients in parallel to testing for the MTHFR mutations. The presence of a homozygous, combined heterozygous, and to a lesser extent, single heterozygous state of the MTHFR polymorphisms might predispose to elevated homocysteine levels, especially when levels of folate, vitamin B6, and/or vitamin B12 are low [12, 33]. The resulting hyperhomocysteine is supposed to cause endothelial damage and is associated with an increased risk for arterial and venous thromboembolic events [7, 12, 19, 26]. It also is unclear whether the presence of a heterozygous and especially a homozygous state of MTHFR C677T and/or A1298C polymorphism might result in an increased risk for DVT independently from homocysteine levels. Only the latter would imply the need for screening for the MTHFR mutations before THA. Otherwise, determination of fasting homocysteine levels might be sufficient. Third, we did not test our patients and control subjects for further thrombophilic conditions, such as deficiency of antithrombin, proteins C and S, or the presence of antiphospholipid antibodies or elevated factor VIII activity. However, none of our patients was known to have inheritable deficiency of antithrombin or protein C or S. Additionally, because the average age of our study population was well above 60 years, it is unlikely these patients would sustain such a severe inheritable risk. Those risk factors are rare in an unselected population and frequently result in venous thromboembolic events at a young age [4, 35]. Finally, we concentrated our analysis on symptomatic DVT as the clinical end point. However, focusing on one distinct clinical setting, such as the incidence of symptomatic DVT after elective THA despite a definite antithrombotic prophylaxis, might increase the relevance of our data.

We found no major association between the presence of one single mutation and an increased risk for

symptomatic DVT after elective THA. However, we found borderline differences for hetero-homozygous factor V Leiden and the homozygous MTHFR C677T mutation, which might have become significant with an increased sample size of 205 and 125 patients in both groups, respectively. Overall, our results are in agreement with most of the currently available published data which are rare, except for those for factor V Leiden mutation. When comparing our results with published results, however, it is important to be aware of the different clinical end points set in the different studies. Whereas some authors concentrated their analysis on all DVTs with or without PE, others used symptomatic DVT as the clinical end point, which might lead to different results as discussed subsequently for the factor V Leiden mutation. Except for Svensson et al. [36] who reported an association with an OR of 4.2 for a subgroup of patients on short-term prophylaxis, others found no association between the incidence of all DVTs after major orthopaedic surgery and the presence of factor V Leiden mutation or resistance to activated protein C [18, 21, 23, 29, 36, 40]. Using symptomatic DVT and/or PE as the clinical end point, however, data are more conflicting. Three groups did not find an association between the presence of factor V Leiden mutation and symptomatic DVT with and without PE or isolated PE after THA or TKA [8, 31, 41]. In contrast to these authors, Lindahl et al. [20] investigated 645 patients after THA or TKA and estimated an OR of 5.0 (95% CI, 1.9–12.9) for the association between activated protein C and symptomatic postoperative DVT. Recently, Baba-Ahmed et al. [5] reported an association (OR, 10.5; 95% CI, 1.3–86) between factor V Leiden mutation and symptomatic DVT and/or PE in patients who underwent major orthopaedic surgery, including THA. As indicated by the large 95% CI, the number of cases in this specific subgroup was low. Furthermore, the authors did not analyze their data separately for the incidence of symptomatic DVT in the subgroup of patients undergoing THA. Similar to factor V Leiden, some authors have reported no increased risk for all DVT and PE after elective THA or TKA for carriers of prothrombin G20210A mutation [18, 40]. For symptomatic DVT and PE, some authors have reported an association [31, 40, 41], but others, including us, found none [8]. No association between homozygous MTHFR C677T mutation and an increased incidence of DVT with or without PE after major orthopaedic surgery has been reported by authors who have addressed this issue [18, 31, 41].

We found patients with a double heterozygous status for the two MTHFR mutations or at least one homozygous mutation of either MTHFR mutation had a higher risk for symptomatic DVT after elective THA compared with patients with the wild-type for these two MTHFR mutations (OR, 2.89). Facing the conflicting published data on a possible and independent association of the presence of the homozygous state of these polymorphisms and DVT with or without PE in general [1–3, 10, 11, 16, 22, 30, 32, 34, 37, 44], our data may be not that surprising. We are aware of only one group of authors who compared the prevalence of both MTHFR polymorphisms and elevated homocysteine levels (15 μmol/L or greater) between patients without or with symptomatic DVT and PE after THA [31]. Those authors reported no difference for either parameter in their small retrospective study.

Based on our current and limited data, we do not recommend testing for genetic polymorphisms before elective THA because the presence of a single mutation seems not to increase risk of postoperative risk symptomatic DVT. The borderline differences for hetero-homozygous factor V Leiden and homozygous MHTFR C677T mutations, and the results of the combined analysis for these two polymorphisms, including also the MTHFR A1293C mutation, however, may justify focusing on these polymorphisms in future prospective studies with a larger number of patients. In these studies, the determination of the homocysteine level is important to further clarify whether the presence of hetero-homozygous MTHFR C677T and/or A1298C polymorphism might result in an increased risk for DVT independent of homocysteine levels.

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Appendix 1

Questionnaire*

Name of patient: _____ Phone number: _____

Date of blood sampling: __.__.____

Date of birth: __.__.____. Gender [female] [male]

Family doctor:_____

Height: _____ cm Weight: ____ kg Body mass index: ____ kg/m^2

Age at the time of operation: _____ years

Side of operation: [right] [left]

Time interval between operation and thrombosis: ____ days

Previous thrombosis? [yes] [no] Side [right] [left]

Thrombosis in the family? [yes] [no] Who? _____

Varicose veins? [yes] [no] Side [right] [left]

Menopause since: _____

Hormone therapy? [yes] [no] Name of medication: _____

Smoking? [yes] [no] For how long? ____ years How many cigarettes? ___ per day

Other known diseases:

_____

_____

_____

*Translated from German into English

References

1. Akar N, Akar E, Akcay R, Avcu F, Yalcin A, Cin S. Effect of methylenetetrahydrofolate reductase 677 C-T, 1298 A-C, and 1317 T-C on factor V 1691 mutation in Turkish deep vein thrombosis patients. *Thromb Res.* 2000;97:163–167.
2. Akar N, Yilmaz E, Akar E, Avcu F, Yalcin A, Cin S. Effect of plasminogen activator inhibitor–1 4G/5G polymorphism in Turkish deep vein thrombotic patients with and without FV1691 G-A. *Thromb Res.* 2000;97:227–230.
3. Alamawi WY, Tamim H, Kreidy R, Timson G, Rahal E, Nabulsi M, Finan RR, Irani-Hakime N. A case control study on the contribution of factor V-Leiden, prothrombin G20210A, and MTHFR C677T mutations to the genetic susceptibility of deep venous thrombosis. *J Thromb Thrombolysis.* 2005;19:189–196.
4. Allaart CF, Poort SR, Rosendaal FR, Reitsma PH, Bertina RM, Briet E. Increased risk of venous thrombosis in carriers of hereditary protein C deficiency defect. *Lancet.* 1993;341:134–138.
5. Baba-Ahmed M, Le Gal G, Couturaud F, Lacut K, Oger E, Leroyer C. High frequency of factor V Leiden in surgical patients with symptomatic venous thromboembolism despite prophylaxis. *Thromb Haemost.* 2007;97:171–175.
6. Beksac B, Gonezález Della Valle A, Salvati EA. Thromboembolic disease after total hip arthroplasty: who is at risk? *Clin Orthop Relat Res.* 2006;453:211–224.
7. Böhm G, Al-Khaffa H. Thrombophilia and arterial diseases: an up-to-date review of the literature for the vascular surgeon. *Int Angiol.* 2003;22:116–124.
8. Campos M, Morais S, Cabeda JM, Justica B. Absence of venous thromboembolism during orthopaedic surgery in patients with FV

Leiden mutation or 20210 A allele of prothrombin gene. *Haemostasis.* 2000;30(suppl 1):35.
9. Dahlback B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. *Haemostasis.* 1994;24:139.
10. Domagala TB, Adamek L, Nizankowska E, Sanak M, Szczeklik A. Mutations C677T and A1298C of the 5,10-methylenetetrahydrofolate reductase gene and fasting plasma homocysteine levels are not associated with the increased risk of venous thromboembolic disease. *Blood Coagul Fibrinolysis.* 2002;13:423–431.
11. Franco RF, Morelli V, Lourenco D, Maffei FH, Tavella MH, Piccinato CE, Thomazini IA, Zago MA. A second mutation in the methylenetetrahydrofolate reductase gene and the risk of venous thrombotic disease. *Br J Haematol.* 1999;105:556–559.
12. Franco RF, Reitsma PH. Genetic risk factors of venous thrombosis. *Hum Genet.* 2001;109:369–384.
13. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995;10:111–113.
14. German Institute for Medical Documentation and Information. The International Classification of Diseases, Tenth Revision (ICD–10), German modification, 2008. Available at: www.dimdi.de/static/de/klassi/diagnosen/icd10/htmlgm2008/fr-icd.htm. Accessed January 2008.
15. Haas SB, Tribus CB, Insall JN, Becker MW, Windsor RE. The significance of calf thrombi after total knee arthroplasty. *J Bone Joint Surg Br.* 1992;74:799–802.
16. Hanson NQ, Aras O, Yang F, Tsai MY. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. *Clin Chem.* 2001;47:661–666.
17. Imperiale TF, Speroff T. A meta-analysis of methods to prevent venous thromboembolism following total hip replacement. *JAMA.* 1994;271:1780–1785.
18. Joseph JE, Low J, Courtenay B, Neil MJ, McGrath M, Ma D. A single-centre prospective study of clinical and haemostatic risk factors for venous thromboembolism following lower limb arthroplasty. *Br J Haematol.* 2005;129:87–92.
19. Kelly PJ, Rosand J, Kistler J, Shih VE, Silveira S, Plomaritoglou A, Furie KL. Homocysteine, MTHFR 677C->T polymorphism, and risk of ischemic stroke: results of a meta-analysis. *Neurology.* 2002;59:529–536.
20. Lindahl TL, Lundahl TH, Nilsson L, Andersson CA. APC-resistance is a risk factor for postoperative thromboembolism in elective replacement of the hip or knee: a prospective study. *Thromb Haemost.* 1999;81:18–21.
21. Lowe GD, Haverkate F, Thompson G, Turner RM, Bertina RM, Turpie AG, Mannucci PM, The ECAT DVT Study Group. Prediction of deep vein thrombosis after elective hip replacement surgery by preoperative clinical and haemostatic variables: The ECAT DVT Study. *Thromb Haemost.* 1999;81:879–886.
22. Naushad S, Jamal NJ, Angalena R, Prasad CK, Devi AR. Hyperhomocysteinemia and the compound heterozygous state for methylene tetrahydrofolate reductase are independent risk factors for deep vein thrombosis among South Indians. *Blood Coagul Fibrinolysis.* 2007;18:113–117.
23. Philipp CS, Dilley A, Saidi P, Evatt B, Austin H, Zawadsky J, Harwood D, Ellingsen D, Barnhart E, Philips DJ, Hooper WC. Deletion polymorphism in the angiotensin-converting enzyme gene as a thrombophilic risk factor after hip arthroplasty. *Thromb Haemost.* 1998;80:869–873.
24. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood.* 1996;88:3698–3703.
25. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2008. Available at: www.R-project.org. Accessed January 2008.
26. Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. *Arch Intern Med.* 1998;158:2101–2106.
27. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *Lancet.* 1995;346:1133–1134.
28. Rosendaal FR, Doggen CJM, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210G to A prothrombin variant. *Thromb Haemost.* 1998;79:706–708.
29. Ryan DH, Crowther MA, Ginsberg JS, Francis CW. Relation of factor V Leiden genotype to risk for acute deep venous thrombosis after joint replacement surgery. *Ann Intern Med.* 1998;128:270–276.
30. Salomon O, Rosenberg N, Zivelin A, Steinberg DM, Kornbrot N, Dardik R, Inbal A, Seligsohn U. Methionine synthase A2756G and methylenetetrahydrofolate reductase A1298C polymorphisms are not risk factors for idiopathic venous thromboembolism. *Hematol J.* 2001;2:38–41.
31. Salvati EA, Della Valle AG, Westrich GH, Rana AJ, Specht L, Weksler BB, Wang P, Glueck CJ. The John Charnley Award: heritable thrombophilia and development of thromboembolic disease after total hip arthroplasty. *Clin Orthop Relat Res.* 2005;441:40–55.
32. Sartori MT, Danesin C, Saggiorato G, Tormene D, Simioni P, Spiezia L. The PAI–1 gene 4G/5G polymorphism and deep vein thrombosis in patients with inherited thrombophilia. *Clin Appl Thromb Hemost.* 2003;9:299–307.
33. Sass AE, Neufeld EJ. Risk factors for thromboembolism in teens: when should I test? *Curr Opin Pediatr.* 2002;14:370–378.
34. Segui R, Estelles A, Miar Y, Espana F, Villa P, Falco C, Vaya A, Grancha S, Ferrando F, Aznar J. PAI-1 promoter 4G/5G genotype as an additional risk factor for venous thrombosis in subjects with genetic thrombophilic defects. *Br J Haematol.* 2000;111:122–128.
35. Simmonds RE, Ireland H, Lande DA, Zöller B, Garcia de Frutos P, Dahlbäck B. Clarification of the risk for venous thrombosis associated with hereditary protein S deficiency by investigation of a large kindred with a characterized gene defect. *Ann Intern Med.* 1998;128:8–14.
36. Svensson PJ, Benoni G, Fredin H, Björgell O, Nilsson P, Hedlund U, Nylander G, Bergqvist D, Dahlbaeck B. Female gender and resistance to activated protein C (FV:Q506) as potential risk factors for thrombosis after elective hip arthroplasty. *Thromb Haemost.* 1997;78:993–996.
37. Tsantes AE, Nikolopoulos GK, Bagos PG, Rapti E, Mantzios G, Kapsimali V, Travlou A. Association between the plasminogen activator inhibitor-1 4G/5G polymorphism and venous thrombosis: a meta-analysis. *Thromb Haemost.* 2007;97:907–913.
38. Vaitkus PT, Leizorovicz A, Cohen AT, Turpie AG, Olsson CG, Goldhaber SZ; PREVENT Medical Thromboprophylaxis Study Group. Mortality rates and risk factors for asymptomatic deep vein thrombosis in medical patients. *Thromb Haemost.* 2005;93:76–79.
39. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet.* 1998;62:1044–1051.
40. Wahlander K, Larson G, Lindahl TL, Andersson C, Frison L, Gustaffsson D, Bylock A, Eriksson BI. Factor V Leiden

(G1691A) and prothrombin gene G20210A mutations as potential risk factors for venous thromboembolism after total hip or total knee replacement surgery. *Thromb Haemost.* 2002; 87:580–585.
41. Westrich GH, Weksler BB, Glueck CJ, Blumenthal BF, Salvati EA. Correlation of thrombophilia and hypofibrinolysis with pulmonary embolism following total hip arthroplasty. *J Bone Joint Surg Am.* 2002;84:2161–2167.
42. Woolson ST, Zehnder JL, Maloney WJ. Factor V Leiden and the risk of proximal venous thrombosis after total hip arthroplasty. *J Arthroplasty.* 1998;13:207–210.
43. Wu O, Robertson L, Twaddle S, Lowe GD, Clark P, Greaves M, Walker ID, Langhorne P, Brenkel I, Regan L, Greer I. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. *Health Technol Assess.* 2006;10:1–110.
44. Zetterberg H, Coppola A, D'Angelo A, Palmér M, Rymo L, Blennow K. No association between the MTHFR A1298C and transcobalamin C776G genetic polymorphisms and hyperhomocysteinemia in thrombotic disease. *Thromb Res.* 2002;108:127–131