

Cover Page



Universiteit Leiden

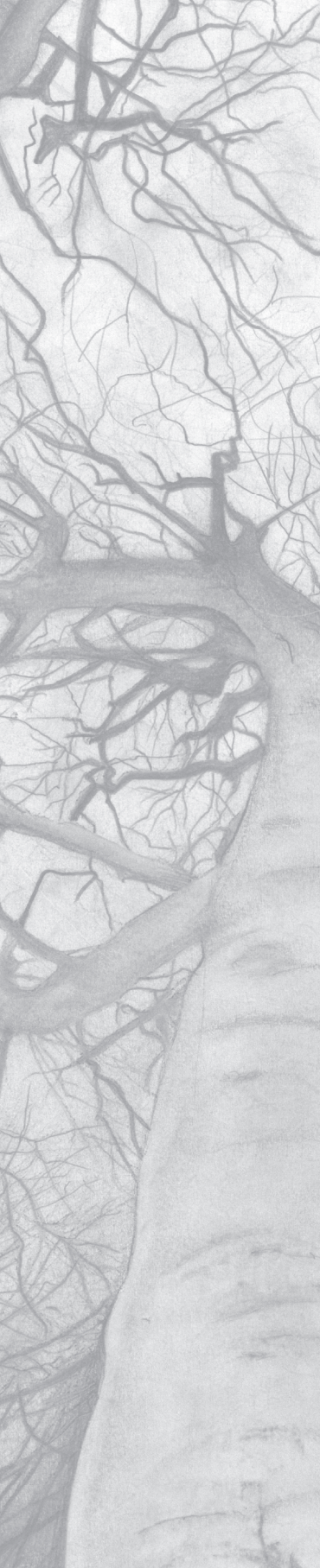


The handle <http://hdl.handle.net/1887/37409> holds various files of this Leiden University dissertation

Author: Engbers, Marissa

Title: Conventional and age-specific risk factors for venous thrombosis in older people : the AT-AGE study

Issue Date: 2016-01-28



CHAPTER 4

Genetic risk factors for venous thrombosis in older people

A. Karasu
M.J. Engbers
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

To be submitted

ABSTRACT

Introduction

As the incidence of venous thrombosis increases strongly with age and the number of older people is on the rise, the focus on the older people becomes more relevant. We aimed to assess whether common genetic risk factors, i.e., the factor V Leiden (FVL) and prothrombin G20210A variants (PT), and non-O blood group, as well as a positive family history of venous thrombosis are risk factors for a first venous thrombosis at an older age (≥ 70 years).

Methods

401 consecutive cases with a first-time thrombosis and 431 control subjects were included in the AT-AGE case-control study. All subjects were ≥ 70 years. Information on risk factors for thrombosis, including family history, was obtained from questionnaires. Unprovoked thrombosis was defined as thrombosis not related to surgery, fracture, plaster cast, minor injuries or immobility within three months prior to the venous thrombotic event. FVL and PT were determined in 394 cases and 426 control subjects. The risk of thrombosis was assessed by calculating odds ratios (OR) with 95% confidence intervals (CI95) after adjustment for age, sex, and study centre.

Results

The risk of venous thrombosis was 2.2-fold increased in FVL carriers (CI95 1.2-3.9). This risk was 1.4-fold increased in PT mutation carriers (CI95 0.5-3.9). A positive family history was associated with a 2.1-fold increased risk of thrombosis (CI95 1.5-3.1). The OR for non-O blood group was 1.3 (CI95 1.0-1.8). The highest risk of thrombosis was found in individuals who had both a positive family history and were carriers of one of the two prothrombotic variants.

Conclusion

Factor V Leiden, prothrombin G20210A mutation, non-O blood group and a positive family history for venous thrombosis were risk factors of venous thrombosis in older people.

INTRODUCTION

Venous thrombosis is a multicausal disease, associated with both environmental and genetic risk factors. [1] Factor V Leiden (rs6025) and the prothrombin G20210A mutation (rs1799963) are the most common prothrombotic variants (prevalence of 3-5%) in young and middle aged population and are associated with a 3-7-fold increase in the risk of venous thrombosis compared with non-carriers. [2,3] Another genetically determined risk factor, i.e., the non-O blood group, is an important determinant of venous and arterial disease. [4,5] In the young and middle aged population, blood group non-O is associated with a doubling in risk of thrombosis. [6] Aggregation of venous thrombosis cases in a family may reflect the presence of known and unknown genetic risk factors. However, conflicting results are published regarding a positive family history as a predictor for the presence of inherited thrombophilia. [7-10]

Most epidemiological studies include only young and middle-aged individuals. Older people are often excluded from clinical studies into aetiology and management because of co-morbidities, short life expectancies, and logistical difficulties. [11,12] Limited information is available regarding genetic risk factors for venous thrombosis in older people. Furthermore, it is unknown whether a positive family history of venous thrombosis is predictive of a venous thrombotic event at an older age. As the incidence of venous thrombosis increases strongly with age and the number of older people is on the rise, the focus on older people becomes more relevant. Venous thrombosis is rare in young individuals (<1 per 10 000 per year under the age of 18) but increases to nearly 1% per year at very old age. [13-14]

This study aimed to assess whether common genetic risk factors, i.e., the factor V Leiden and prothrombin G20210A variants, and non-O blood group, as well as a positive family history of venous thrombosis are risk factors for a first venous thrombosis at an older age (≥ 70 years).

METHODS

Study population and data collection

The Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study is a two-centre, population-based case-control study designed to study risk factors for venous thrombosis in older people. The design of the AT-AGE study was described in detail previously. [15] From June 2008 to August 2011 in Leiden, the Netherlands and December 2008 to July 2011 in Vermont, US, all consecutive cases 70 years and older with a first deep venous thrombosis of the leg (DVT) or pulmonary embolism (PE) were identified. Cases were identified from the anticoagulation clinics in Haarlem and Leiden

and from the Vascular Laboratory and the Radiology department of the University of Vermont Medical Centre (Burlington, Vermont, United States). Control subjects were randomly selected from five primary care practices in Leiden and four in Vermont. Subjects with an active malignancy or severe psychiatric or cognitive disorder were excluded. For all participants, a home visit took place, during which an extensive structured interview was completed by trained personnel and a blood sample or buccal swab was collected. The index date was defined as the date of diagnosis of the thrombosis for the cases and the date of the home visit for the control subjects. All participants provided written informed consent. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and by the Committee of Human Research of the University of Vermont.

Risk factor assessment

Self-reported information on the presence of first-degree relatives (parent, sibling, or offspring) who experienced venous thrombosis was obtained via the interview. Family history of venous thrombosis was considered positive if at least one first degree relative experienced thrombosis. Participants who indicated that they did not know whether a first degree relative has had venous thrombosis were classified as having a negative history.

Provoked venous thrombosis was defined as thrombosis after hospitalisation, fracture, plaster cast, splint, minor injuries of the lower extremities (such as a sprained ankle or contusion of the lower leg) or transient immobility at home ≥ 4 successive days in the three months before the index date.

During the home visits, blood samples were drawn into vacuum tubes containing 0.1-volume 0.106-mol/L trisodium citrate or when no blood sample could be drawn a buccal swab was collected (N=28). The blood sample was separated into plasma and cells through centrifugation. DNA analysis for the factor V Leiden mutation (rs6025) and the prothrombin G20210A mutation (rs1799963) was performed using a combined polymerase chain reaction method with the TaqMan assay. Blood group was determined by a 5' nuclease assay (Taqman; Applied Biosystems, Foster City, CA) using a standard PCR reaction mix (Eurogentec, Seraing, B) and an allele specific fluorescent probe equipped with a minor groove binding moiety (applied Biosystems, Foster City, CA).

Statistical analysis

As estimates of relative risks, we calculated odds ratios (ORs) with 95% confidence intervals (CI95) using logistic regression. We determined associations between venous thrombosis risk and factor V Leiden (FVL), the prothrombin G20210A mutation (PT), ABO blood group, and a positive family history. All reported ORs were adjusted for age (continuous), sex (categorical), and study centre (Leiden and Haarlem versus Vermont,

categorical) using multivariable logistic regression. Additional to the individual effect, the combined effect of the risk factors was studied. Furthermore, analyses were stratified for provoked and unprovoked thrombosis, and type of thrombosis (DVT only or PE with or without DVT).

Of all participants, 25 (6%) cases and 13 (3%) control subjects did not know whether one of their first degree family members had experienced a venous thrombotic event. In the overall analyses, these individuals were classified as having a negative family history, however, we performed a sensitivity analysis in which the risk of thrombosis associated with a positive family history was calculated after exclusion of these individuals. The risk of thrombosis associated with a positive family history of thrombosis was studied in more detail by calculating ORs for having any affected first-degree relative, having a first-degree relative affected before the age of 50 years, and for having multiple affected first-degree relatives. We calculated the positive predictive value of family history to identify FVL and PT. IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

RESULTS

In this study, 401 cases and 431 control subjects were included (Table 1). 166 Cases (41%) were diagnosed with an isolated DVT and 235 (59%) were diagnosed with PE with or without DVT. DNA analysis for factor V Leiden was available for 394 (98%) cases and 426 (99%) control subjects, for prothrombin G20210A mutation for 394 (98%) cases and 427 (99%) control subjects and for ABO blood group for 376 (94%) cases and 416 (97%) control subjects.

Table 1. Baseline characteristics of control subjects and cases

	Controls	Cases
N	431	401
Men, N (%)	209 (48.5)	166 (41.4)
Age, mean (range)	77.5 (70-96)	78.7 (70-101)
Type VT, N (%)		
Deep vein thrombosis (DVT)	N.A.	166 (41.4)
Pulmonary embolism (PE) (\pm DVT)	N.A.	235 (58.6)
Factor V Leiden, N (%)	18 (4.2)	34 (8.6)
Prothrombin G20210A mutation, N (%)	7 (1.6)	9 (2.3)
Non-O blood group, N (%)	232 (55.8)	231 (61.4)
Positive family history for VT, N (%)	54 (12.5)	97 (24.2)
Provoking factors, N (%)	67 (15.5)	170 (42.4)

N = number, VT = venous thrombosis

Prothrombotic variants, ABO blood group and risk of venous thrombosis

Out of 394 cases, 34 (8.6%) cases carried the FVL mutation (32 heterozygotes and 2 homozygotes). Of the control subjects, 18 (4.2%) were heterozygous for the FVL mutation and none of the control subjects were homozygous. Carriers of the FVL mutation (heterozygous and homozygous carriers combined) had a 2-fold increased risk of a first thrombosis (Table 2, OR 2.2, CI95 1.2-3.9), compared with participants who did not carry the FVL mutation. The FVL mutation was present in 19 of 162 cases with DVT (11.7%) and 15 of 232 cases with PE with or without DVT (6.5%), leading to odds ratios of 3.0 for DVT (CI95: 1.5-6.0), and 1.5 for PE (CI95 0.7-3.1). The odds ratios for provoked thrombosis and unprovoked thrombosis in the presence of FVL were similar (OR 2.2, CI95 1.0-4.4 for provoked thrombosis and OR 2.0, CI95 1.0-4.0 for unprovoked thrombosis). Nine cases (2.3%) and seven control subjects (1.6%) were heterozygous carriers of the prothrombin G20210A mutation, and none were homozygous. This led to an OR for prothrombin G20210A of 1.4 (OR 1.4, CI95 0.5-3.9). In presence of the PT mutation, the odds ratio for unprovoked thrombosis was 1.8 (CI95 0.6-5.4) while no association was observed for provoked thrombosis (OR 1.0, CI95 0.3-4.0). 231 (61.4%) cases had blood group non-O and 232 control subjects (55.8%), resulting in an OR of 1.3 (CI95 1.0-1.8) for blood group non-O. Blood group non-O was not associated with the risk of provoked thrombosis (OR 1.0, CI95 0.7-1.5), whereas the risk of unprovoked thrombosis was 1.6-fold increased (CI95 1.1-2.3).

We studied the combined effect of ABO blood group and the presence of either the two variants (i.e., FVL or PT mutation), with wildtype carriers of the FVL and PT variants with blood group O as the reference category. Individuals carrying either prothrombotic

Table 2. Genetic risk factors of venous thrombosis

Risk factor	Absent or present	Controls N	Cases (Nprovoked / Nunprovoked)	ORoverall* (CI95)	ORprovoked* (CI95)	ORunprovoked* (CI95)
Factor V Leiden	-	408	153/207	1†	1†	1†
	+	18	15/19	2.2 (1.2-3.9)	2.2 (1.0-4.4)	2.0 (1.0-4.0)
Prothrombin G20210A mutation	-	420	165/220	1†	1†	1†
	+	7	3/6	1.4 (0.5-3.9)	1.0 (0.3-4.0)	1.8 (0.6-5.4)
Blood group	O	184	71/74	1†	1†	1†
	Non-O	232	89/142	1.3 (1.0-1.8)	1.0 (0.7-1.5)	1.6 (1.1-2.3)
Family history VT	-	377	127/177	1†	1†	1†
	+	54	43/54	2.1 (1.5-3.1)	2.3 (1.4-3.6)	2.0 (1.3-3.1)

*OR adjusted for age, sex, study center

†reference category

N = number, VT = venous thrombosis

variant and blood group non-O had a 2.3-fold increased risk of venous thrombosis (CI95 0.9-5.9) and wildtype carriers of FVL and PT with blood group non-O had a 1.3-fold increased risk of venous thrombosis (CI95 1.0-1.8). Those with both blood group non-O and a prothrombotic variant had a similar risk as those with blood group O and a prothrombotic variant (Table 3).

Table 3. Prothrombotic variants, blood group non-O and risk of venous thrombosis

FVL/PT	Non-O blood group	Controls N	Cases (N provoked/N unprovoked)	OR _{Overall} * (CI95)	OR _{provoked} * (CI95)	OR _{unprovoked} * (CI95)
-	-	175	64/68	1†	1†	1†
+	-	8	7/6	2.3 (0.9-5.9)	2.5 (0.8-7.4)	2.1 (0.7-6.6)
-	+	216	78/126	1.3 (1.0-1.8)	1.0 (0.7-1.5)	1.6 (1.1-2.3)
+	+	16	11/16	2.2 (1.1-4.3)	1.8 (0.8-4.1)	2.5 (1.2-5.4)

*OR adjusted for age, sex, study center

†reference category

N = number, FVL = Factor V Leiden, PT= Prothrombin G20210A mutation

Family history and risk of venous thrombosis

Family history of thrombosis was positive for 97 cases (24.2%) and 54 control subjects (12.5%). Individuals with a positive family history of thrombosis had a more than two-fold increased risk of venous thrombosis compared with individuals without a positive family history of thrombosis (OR 2.1, CI95 1.5-3.1). The association was similar when subjects who did not know their family history were excluded from the analysis (OR 2.2, CI95 1.5-3.2). The risk of both provoked and unprovoked thrombosis was increased in the presence of a positive family history, i.e., OR provoked thrombosis: 2.3 (CI95 1.4-3.6); OR unprovoked thrombosis: 2.0 (CI95 1.3-3.1). The risk of venous thrombosis was not increased when only family members who had thrombosis before the age of 50 years were considered positive (OR 0.8, CI95 0.5-1.4), taking individuals without a positive family history as a reference. The number of affected relatives was also not associated with the risk of thrombosis, having more than 1 positive family member versus only 1 positive family member resulted in an OR of 0.8 (CI95 0.4-1.8). To assess whether a positive family history was mainly determined by the presence of the factor V Leiden or the prothrombin G20210A mutations, we studied the association between a family history of thrombosis and these prothrombotic variants. Out of 97 cases with a positive family history, 11 carried the FVL or PT variant. This results in a positive predictive value of positive family history for the FVL or PT variant of 11%.

In table 4 the associations of the combined risks of a positive family history of thrombosis and carrying the factor V Leiden or the prothrombin G20210A mutation are shown.

Table 4. Prothrombotic variants, family history of thrombosis and the risk of venous thrombosis

FVL/PT	Family history	Controls N	Cases (Nprovoked/N unprovoked)	ORoverall* (CI95)	ORprovoked* (CI95)	ORunprovoked* (CI95)
-	-	350	112/155	1†	1†	1†
-	+	51	38/48	2.1 (1.4-3.1)	2.2 (1.4-3.6)	2.0 (1.3-3.2)
+	-	23	13/17	1.7 (0.9-2.9)	1.7 (0.8-3.5)	1.6 (0.8-3.1)
+	+	2	5/6	7.6 (1.6-35.7)	7.1 (1.3-37.6)	7.7 (1.5-40.1)

*OR adjusted for age, sex, study center

†reference category

N = number, FVL = Factor V Leiden, PT= Prothrombin G20210A mutation

Individuals with a positive family history of venous thrombosis who also carried either a prothrombotic variant had a high risk of thrombosis (OR 7.6, CI95 1.6-35.7).

DISCUSSION

In this population-based case-control study of 832 individuals aged 70 years and older, we show that factor V Leiden, the prothrombin G20210A mutation, and non-O blood group are risk factors for venous thrombosis in older individuals (≥ 70 years) as they are in younger individuals, increasing the risk of venous thrombosis 2.2-, 1.4-, and 1.3-fold respectively. Furthermore, a positive family history of thrombosis increased the risk of thrombosis 2.1-fold, without an effect of the number of affected family members and their age of onset. The highest risk of thrombosis was found in individuals who had both a positive family history and were carriers of one of the two prothrombotic variants.

The FVL and PT variants are well established risk factors in young and middle-aged individuals. The risk is four- to sevenfold increased for FVL carriers and two- to threefold increased for the PT variant. [3,16] Our results indicate that the FVL and PT variants remain associated with the risk of thrombosis in older age, as previous small subgroup analyses showed. [17]

The risk of thrombosis was highest in the presence of multiple genetic risk factors. These results illustrate the multicausal character of thrombosis, which is still present in older people. [1] Furthermore, the results are in line with studies explaining that the heterozygous mutations of the FVL and PT mutations are relatively weak risk factors for venous thrombosis, unless another genetic or acquired risk factor is present. [18] The finding of lower relative risks in older people than in middle-aged individuals may be partly explained by the higher absolute risk of venous thrombosis in older people which leads to smaller relative effects of individual risk factors in older people than in middle-aged individuals. Or, in other words, whereas the relative risks are smaller than in

the young, absolute risk differences for carriers versus non-carriers are substantial, given the high baseline risk in older people. Furthermore, our results may be due to attrition of susceptibles, indicating that susceptible individuals with FVL or PT are more likely to develop venous thrombosis earlier in life, resulting in lower relative risks at an older age.

Compared with blood group O, non-O individuals had a 1.3-fold increased risk of thrombosis. The increased risk can be partly explained by higher levels of FVIII and VWF in ABO blood group. [6,19,20] High FVIII levels are associated with a lowered responsiveness to activated protein C (APC). In carriers of factor V Leiden, this association is strengthened, which explains the interaction between non-O blood group and factor V Leiden carriers. [6,21]

Family history and the two prothrombotic variants were poorly associated in this study, as was also indicated in previous studies. [7,10] The positive predictive value of family history as a test for genetic risk factors, i.e. FVL and PT, is low. This may indicate that unknown or unmeasured genetic risk factors are present in individuals with a positive family history. This hypothesis is supported by the finding of a 2.3-fold increased risk of thrombosis in persons with a positive family history and non-carriers of factor V Leiden and prothrombin G20210A variants.

The strength of our study is the specific focus on individuals aged 70 years and older. Home visits were performed in order to achieve a high participation rate (participation rate: cases 69%, control subjects 73%).

Our study has a number of limitations. Control subjects with a positive family history of venous thrombosis might be more willing to participate in a study of venous thrombosis than those who do not have a positive family history of thrombosis. However, this selection bias would only result in an underestimation of the true effect. Moreover, as in any case-control study, recall bias might have occurred when obtaining information on risk factors used for the classification into provoked and unprovoked thrombosis and family history. However, by using standardised interviews performed by trained personnel for both cases and control subjects, the risk of recall bias was minimised.

Our results may have clinical implications. A positive family history of venous thrombosis doubled the risk of venous thrombosis in older people. In clinical practice this information is easy to obtain, however it is not implemented in clinical decision rules of venous thrombosis. In older people these clinical decision rules show a high failure rate. [22] Potentially, obtaining information on family history of thrombosis in individuals aged 70 years or older could improve prediction of thrombosis in older people. [23]

In conclusion, factor V Leiden, prothrombin G20210A mutation, non-O blood group and a positive family history for thrombosis were risk factors of venous thrombosis in older people.

REFERENCES

1. Rosendaal FR. Venous thrombosis: a multicausal disease. *Lancet* 1999; 353: 1167-73.
2. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 1995; 85: 1504-8.
3. 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: 3698-703.
4. Jick H, Slone D, Westerholm B, Inman WH, Vessey MP, Shapiro S, Lewis GP, Worcester J. Venous thromboembolic disease and ABO blood type. A cooperative study. *Lancet* 1969; 1: 539-42.
5. Medalie JH, Levine C, Neufeld H, Riss E, Dreyfus F, Papier C, Goldbourt U, Kahn H, Oron D. Blood-groups, cholesterol, and myocardial infarction. *Lancet* 1970; 2: 723.
6. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet* 1995; 345: 152-5.
7. van Sluis GL, Sohne M, El Kheir DY, Tanck MW, Gerdes VE, Buller HR. Family history and inherited thrombophilia. *J Thromb Haemost* 2006; 4: 2182-7.
8. Amberger N, Masuhr F, Valdueza JM, Vetter B, Weih M. A negative personal and family history for venous thrombotic events is not sufficient to exclude thrombophilia in patients with cerebral venous thrombosis. *Eur J Neurol* 2004; 11: 555-8.
9. Ruud E, Holmstrom H, Brosstad F, Wesenberg F. Diagnostic value of family histories of thrombosis to identify children with thrombophilia. *Pediatr Hematol Oncol* 2005; 22: 453-62.
10. Bezemer ID, van der Meer FJ, Eikenboom JC, Rosendaal FR, Doggen CJ. The value of family history as a risk indicator for venous thrombosis. *Arch Intern Med* 2009; 169: 610-5.
11. McMurdo ME, Witham MD, Gillespie ND. Including older people in clinical research. *BMJ* 2005; 331: 1036-7.
12. Bugeja G, Kumar A, Banerjee AK. Exclusion of elderly people from clinical research: a descriptive study of published reports. *BMJ* 1997; 315: 1059.
13. Raleigh VS. World population and health in transition. *BMJ* 1999; 319: 981-4.
14. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost* 2007; 5: 692-9.
15. Engbers MJ, Blom JW, Cushman M, Rosendaal FR, van Hylckama Vlieg A. The contribution of immobility risk factors to the incidence of venous thrombosis in an older population. *J Thromb Haemost* 2014; 12: 290-6.
16. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De S, V, Cumming T, Arruda V, Hillarp A, Reny JL. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism--pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. *Thromb Haemost* 2001; 86: 809-16.
17. Rosendaal FR, van Hylckama Vlieg A, Doggen CJ. Venous thrombosis in the elderly. *J Thromb Haemost* 2007; 5 Suppl 1: 310-7.
18. Koeleman BP, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. *Blood* 1994; 84: 1031-5.
19. Pomp ER, le Cessie S, Rosendaal FR, Doggen CJ. Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. *Br J Haematol* 2007; 139: 289-96.

20. le Cessie S, Debeij J, Rosendaal FR, Cannegieter SC, Vandenbroucke JP. Quantification of bias in direct effects estimates due to different types of measurement error in the mediator. *Epidemiology* 2012; 23: 551-60.
21. Rodrigues CA, Rocha LK, Morelli VM, Franco RF, Lourenco DM. Prothrombin G20210A mutation, and not factor V Leiden mutation, is a risk factor for cerebral venous thrombosis in Brazilian patients. *J Thromb Haemost* 2004; 2: 1211-2.
22. Schouten HJ, Geersing GJ, Oudega R, van Delden JJ, Moons KG, Koek HL. Accuracy of the Wells clinical prediction rule for pulmonary embolism in older ambulatory adults. *J Am Geriatr Soc* 2014; 62: 2136-41.
23. Schouten HJ, Koek HL, Oudega R, van Delden JJ, Moons KG, Geersing GJ. Validation of the Oudega diagnostic decision rule for diagnosing deep vein thrombosis in frail older out-of-hospital patients. *Fam Pract* 2015; 32: 120-5.

