Association of VKORC1 polymorphisms and major bleedings in patients who are treated with vitamin K antagonists

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Dear Editor,

Research results are conflicting on whether the single nucleotide polymorphism rs9923231 (−1639G>A) of vitamin K epoxide reductase complex subunit-1 (VKORC1) is associated with increased major bleeding during treatment with vitamin K antagonists (VKAs) [1–6]. Furthermore, it is unclear whether additional risk factors, such as a high international normalized ratio (INR), further increase the risk for major bleeding. Our aim was to determine the association of VKORC1 1639 G>A with major bleeding and to determine whether a high INR further increased the risk of major bleeding.

In short, patients were eligible for the BLEED cohort study if they were at least 18 years old and started VKAs for a planned duration of at least 6 weeks at one of the three participating anticoagulation clinics [7]. Consent was given by means of an opt-out procedure which led to 16,570 (99%) included patients out of 16,706 eligible patients. The BLEEDS was approved by the medical ethical committee of the Leiden University Medical Center [7].

Baseline characteristics and follow-up data were collected from Dutch anticoagulation clinics. Follow-up started from initiation of VKA until the outcome major bleeding, death or the end of the study. Information about major bleeding was acquired through standardized interviews during every anticoagulation clinic visit and were classified by physicians not involved in the study. Bleeding events were classified as major if these were fatal, intracranial, an objectively diagnosed joint bleed, a bleeding event in a critical organ, or if they led to a blood transfusion or to a hospital admission [8]. Furthermore, DNA was collected from 13,790 patients (83%) 3 weeks after initiating VKAs.

For this study, we assembled a case–cohort study, containing all 326 cases with major bleeding and a random sample of 978 patients at baseline (subcohort). We genotyped these patients by spotting commercially available primers, made by Thermo Fisher, for the VKORC1 genotype rs9923231 on an open array system.

DNA results were available from 239 cases and 789 subcohort members. We calculated incidence rates (IR) of major bleedings per 100 patient years (PYs). The distribution of each genotype in the whole BLEEDS was extrapolated from the distribution of the subcohort. Hazard ratios and 95% confidence intervals (CIs) were estimated by means of weighted Cox regression. We adjusted the analyses for VKA dosage and INR to study the effect of both on major bleeding. Analyses were restricted to the first 3 and 6 months of follow-up and stratified time-dependently by high INR (>4).

The mean age of the patients was 71 years (standard deviation 13), with 7342 (53%) male participants. The subcohort is representative of the whole cohort. The allele distribution of G1639A in the subcohort did not deviate from the Hardy–Weinberg equilibrium (p-value = 0.31).

AA carriers had a higher major bleeding rate (2.23/100 PYs [95% CI 1.66–2.93]) when compared with GA (1.71/100 PYs [95% CI 1.41–2.05]) and GG carriers (1.50/100 PYs [95% CI 1.20–1.85]) (Table 1). AA carriership was associated with a 1.5-fold (95% CI 0.98–2.30) increased major bleeding risk when compared with GG carriers. This increased risk for AA carriers was similar to the results of several other studies [1, 2, 9]. However, differing results were also found [3, 5, 6]. These findings may be explained by differing racial makeup of study populations and statistical variation due to a lower number of patients [3, 5, 6].

After adjustment for INR or VKA dosage, relative risk estimates decreased. After adjustment for both INR and VKA dosage, relative risk estimates

Table 1. *Incidence of major bleedings, stratisfied by international normalized ratio (INR), and association between genotype and major bleeding in vitamin K antagonist (VKA)-treated patients*

SNP	Cases	Person years in subcohort[a]	Person years[b]	Incidence rate (per 100 PY; 95% CI)	HR (95% CI)	aHR (95% CI)[c]	aHR (95% CI)[d]	aHR (95% CI)
Complete follow-up								
GG	**81**	**342**	**5408**	**1.50 (1.20 – 1.85)**	**1.00**	**1.00**	**1.00**	**1.00**
INR < 4	70	325	5167	1.36 (1.06 – 1.70)	1			
INR ≥ 4	11	15	243	4.53 (2.38 – 7.87)	3.13 (1.61 – 6.08)			
GA	**110**	**407**	**6436**	**1.71 (1.41 – 2.05)**	**1.14 (0.82 – 1.59)**	**1.08 (0.78 – 1.51)**	**0.91 (0.63 – 1.31)**	**0.88 (0.60 – 1.27)**
INR < 4	88	381	6053	1.45 (1.17 – 1.78)	1.08 (0.75 – 1.54)			
INR ≥ 4	22	24	382	5.76 (3.70 – 8.58)	3.80 (2.24 – 6.44)			
AA	**48**	**136**	**2151**	**2.23 (1.66 – 2.93)**	**1.50 (0.98 – 2.30)**	**1.33 (0.87 – 2.04)**	**0.95 (0.56 – 1.59)**	**0.87 (0.52 – 1.46)**
INR < 4	26	126	1997	1.30 (0.87 – 1.88)	0.98 (0.59 – 1.64)			
INR ≥ 4	22	10	153	14.4 (9.24 – 21.4)	8.39 (4.68 – 15.0)			
First 3 months of follow-up								
GG	**30**	**74**	**1126**	**2.66 (1.83 – 3.76)**	**1.00**	**1.00**	**1.00**	**1.00**
INR < 4	29	68	1046	2.77 (1.89 – 3.93)	1			
INR ≥ 4	1	5	77	1.30 (0.06 – 6.41)	0.51 (0.07 – 3.83)			
GA	**37**	**86**	**1309**	**2.83 (2.02 – 3.86)**	**1.06 (0.64 – 1.75)**	**1.00 (0.61 – 1.66)**	**0.98 (0.55 – 1.75)**	**0.94 (0.53 – 1.68)**
INR < 4	30	77	1184	2.53 (1.74 – 3.57)	0.92 (0.54 – 1.56)			
INR ≥ 4	7	8	123	5.69 (2.49 – 11.3)	2.03 (0.87 – 4.76)			
AA	**21**	**29**	**441**	**4.76 (3.03 – 7.16)**	**1.79 (0.99 – 3.25)**	**1.57 (0.88 – 2.80)**	**1.52 (0.71 – 3.28)**	**1.38 (0.65 – 2.93)**
INR < 4	9	25	384	2.34 (1.14 – 4.30)	0.88 (0.40 – 1.91)			
INR ≥ 4	12	4	62	19.4 (10.5 – 32.9)	6.64 (3.09 – 14.3)			
First 6 months of follow-up								
GG	**40**	**140**	**2176**	**1.84 (1.33 – 2.48)**	**1.00**	**1.00**	**1.00**	**1.00**
INR < 4	38	131	2053	1.85 (1.33 – 2.51)	1			
INR ≥ 4	2	8	125	1.60 (0.27 – 5.29)	0.88 (0.21 – 3.69)			
GA	**56**	**164**	**2548**	**2.20 (1.68 – 2.83)**	**1.20 (0.78 – 1.85)**	**1.15 (0.72 – 1.72)**	**0.98 (0.61 – 1.58)**	**0.93 (0.58 – 1.51)**
INR < 4	41	150	2351	1.74 (1.27 – 2.34)	0.95 (0.60 – 1.51)			
INR ≥ 4	15	12	188	7.98 (4.64 – 12.9)	3.93 (2.06 – 7.49)			
AA	**30**	**53**	**824**	**3.64 (2.50 – 5.13)**	**1.95 (1.16 – 3.27)**	**1.62 (0.97 – 2.70)**	**1.29 (0.68 – 2.44)**	**1.12 (0.59 – 2.12)**
INR < 4	11	47	737	1.49 (0.78 – 2.59)	0.82 (0.40 – 1.65)			
INR ≥ 4	19	6	94	20.2 (12.5 – 31.0)	9.83 (5.12 – 18.9)			

Note: The GG carriers (unstratified) were used as reference for calculation of HRs for GA and AA carriers (unstratified). The GG carriers with low INR were used as reference for calculation of HRs for GG (high INR), GA and AA (low and high INR) carriers.

Abbreviations: aHR, adjusted Hazard Ratio; CI, confidence interval; HR, hazard ratio; PY, patient year; SNP, single nucleotide polymorphism.

[a]Calculated from patient time of genotyped patients.

[b]Calculated from patients with available DNA material and extrapolated from distribution in subcohort.

[c]Adjusted for INR.

[d]Adjusted for VKA dosage; adjusted for INR and VKA dosage.

decreased toward unity (Table 1). This indicates that INR and VKA dosage may cause the increased risk for major bleeding.

Stratification for INR revealed high major bleeding rates among high INR AA, GA and GG carriers (14.4/100 PYs, 95% CI 9.24–21.1, 5.76/100 PYs, 95% CI 3.70–8.58 and 4.53/100 PYs, 95% CI 2.38–7.87, respectively). The major bleeding rates of AA carriers with high INR were even higher in the first 6 months of therapy and reached as high as 20.2/100 PY. Major bleeding rates were similar for all genotypes with low INRs (1.30–1.45/100 PYs). The relative risk estimate and incidence rates of major bleeding in the AA genotype may be even higher than presented in this letter. Because DNA material was collected in the third week of therapy, patients who experienced a major bleeding in the first 2 weeks of therapy and ceased VKA treatment could not be genotyped due to the absence of DNA. As the prevalence of VKORC1 1639 AA carriers is probably higher in those patients who stopped in the first 2 weeks, our results could have been diluted.

The very high major bleeding rates among AA carriers may be explained because individuals with the A allele require low VKA maintenance dosages [10], potentially making them more susceptible to changes in INR influencing factors which may have a greater relative impact than the relative impact in patients with other genotypes and higher dosages.

The AA genotype of VKORC1 G1639A is associated with an increased risk of major bleeding. Whether the AA genotype is consequently associated with a decreased thrombosis risk is unknown due to a lack of data on thrombosis. The high major bleeding rates among AA carriers with a high INR warrant extra monitoring in these patients.

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Conflict of interest

The authors declare no conflict of interest.

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