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Vitamin K epoxide reductase complex subunit 1 (*VKORC1*) polymorphism influences the anticoagulation response subsequent to vitamin K intake: a pilot study

E. A. SCONCE,*† P. J. AVERY,‡ H. A. WYNNE† and F. KAMALI*†
**School of Clinical & Laboratory Sciences; †Institute of Ageing and Health; and ‡Schools of Mathematics and Statistics, Newcastle University, Newcastle upon Tyne, UK*

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We recently demonstrated that daily supplementation with oral vitamin K can improve the stability of anticoagulation control in previously unstable patients receiving chronic therapy with warfarin [1]. Vitamin K supplementation also antagonized the pharmacologic activity of warfarin, but to varying degrees in different patients. Vitamin K epoxide reductase (VKOR) is the pharmacologic target for warfarin and influences the warfarin dose requirement [2,3]. Because of its influence upon VKOR activity, we explored, in this pilot study, whether the *VKORC1* genotype has any impact upon the extent to which vitamin K affects the anticoagulation response and warfarin dose requirements.

Correspondence: Farhad Kamali, Wolfson Unit of Clinical Pharmacology, School of Clinical and Laboratory Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK.
Tel.: +44 191 222 8043; fax: +44 191 222 5827.
E-mail: farhad.kamali@ncl.ac.uk

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Blood samples were obtained from the 70 Caucasian patients taking part in the earlier published vitamin K supplementation study [1] for *post hoc* analysis. All were receiving chronic therapy with warfarin for stroke prophylaxis and had unstable control of anticoagulation. Unstable control was defined as the patient having a standard deviation of International Normalized Ratio (INR) of > 0.5 (i.e. from among the most unstable 20% of our anticoagulated population) and having had at least three dose changes during the previous 6 months [1]. In this study, patients had been randomly allocated to two groups in a double-blinded fashion. One group received a once-daily oral supplement of 150 μg of vitamin K, and the other matching placebo, with their warfarin evening dose for 6 months. The criteria for patient selection and monitoring are described elsewhere [1]. The study had prior approval from the Joint Newcastle University and Health Authority Ethics Committee, and written informed consent was acquired from all participants. An overnight-fasted blood sample was taken at study entry (day 0) and 1 week after supplementation with vitamin K for determination of plasma vitamin K and vitamin K 2,3-epoxide concentrations, as vitamin K concentrations show large temporal variations and are influenced by vitamin K consumed in a meal [4]. The same blood sample was also used

for the measurement of venous INR and for *VKORC1* genotyping. Plasma vitamin K and vitamin K 2,3-epoxide concentrations were determined by high-performance liquid chromatography using post-column reduction and fluorimetric detection [5]. The limits of detection of the extracted samples were 100 pg mL^{-1} and 200 pg mL^{-1} for vitamin K and vitamin K 2,3-epoxide respectively (signal/noise ratio = 3). The interday coefficients of variation for vitamin K at 1430 pg mL^{-1} and vitamin K 2,3-epoxide at 4150 pg mL^{-1} were 8.6% and 9.7% respectively. After study completion, only patients who were identified as having received vitamin K supplementation were genotyped for the *VKORC1* 1639G > A polymorphism, using polymerase chain reaction and restriction fragment length polymorphism [2].

Minitab version 14 (Minitab, Coventry, UK) was used to carry out statistical analysis. The change in warfarin dose requirement after vitamin K supplementation was calculated for each patient by calculating the difference between the dose at study entry and the dose that remained unchanged for at least two clinic visits, indicating that the patient had reached a new treatment equilibrium after vitamin K supplementation. The plasma vitamin K and vitamin K 2,3-epoxide concentrations were found not to be normally distributed. The corresponding data were therefore log-transformed for comparisons between *VKORC1* genotypes. One-way ANOVA was used to demonstrate the effect of the *VKORC1* genotype on the differences in INR and log plasma vitamin K and vitamin K 2,3-epoxide concentration between day 0 (study entry) and after 1 week of vitamin K supplementation and on the difference in stable warfarin dose requirement, which was achieved on average 2–3 weeks after the initiation of vitamin K supplementation.

In 17 males and 18 females with a median (range) age of 76 years (58–82 years) and 73 years (58–83 years), daily warfarin dose requirements increased from 3.8 on day 0 to 4.4 mg at new treatment equilibrium as a result of vitamin K supplementation [mean difference 0.55; 95% confidence interval (CI) 0.37–0.72; $P < 0.001$]. Whereas plasma vitamin K concentrations increased after 1 week of vitamin K supplementation as compared to baseline (mean difference of log concentration 0.76; 95% CI 0.65–0.87; $P < 0.001$), plasma vitamin K 2,3-epoxide concentrations decreased (mean difference of log concentration − 0.19; 95% CI − 0.26 to − 0.11; $P < 0.001$). There were no significant differences in plasma vitamin K concentrations at entry or after 1 week of supplementation with vitamin K between the three different genotypes (Table 1). There were no significant differences in plasma

Table 1 Median (range) of patient age, mean (95% confidence interval) of International Normalized Ratio (INR), plasma vitamin K and vitamin K 2,3-epoxide concentration, plasma vitamin K/vitamin K 2,3-epoxide ratio and warfarin dose at study entry and after 7 days of vitamin K supplementation, and difference in INR, log plasma vitamin K and vitamin K 2,3-epoxide concentration and warfarin dose on day 0 and after 7 days of supplementation with vitamin K according to *VKORC1* genotype; plasma vitamin K and vitamin K 2,3-epoxide concentrations were log-transformed and then back-transformed to produce the estimated mean and 95% confidence intervals

	GG (n = 11)	GA (n = 18)	AA (n = 6)	P-value
Age, years (range)	74 (58–81)	74 (60–81)	75 (60–83)	
Plasma vitamin K concentration before supplementation (pg mL^{-1})	597 (448–795)	607 (486–760)	778 (528–1147)	> 0.1
Plasma vitamin K concentration after supplementation (pg mL^{-1})	1375 (1090–1736)	1240 (1034–1488)	1682 (1227–2306)	> 0.1
Difference in log plasma vitamin K concentration	0.83 (0.56–1.11)	0.71 (0.50–0.93)	0.77 (0.40–1.15)	> 0.1
Plasma vitamin K 2,3-epoxide concentration before supplementation (pg mL^{-1})	1189 (988–1430)	1316 (1139–1520)	1456 (1135–1871)	> 0.1
Plasma vitamin K 2,3-epoxide concentrations after supplementation (pg mL^{-1})	849 (680–1061)	1147 (964–1365)	1365 (1010–1846)	0.030
Difference in log plasma vitamin K 2,3-epoxide concentration	− 0.34 (− 0.46 to − 0.21)	− 0.14 (− 0.23 to − 0.04)	− 0.06 (− 0.23 to 0.10)	0.016
Plasma vitamin K/vitamin K 2,3-epoxide ratio before supplementation	0.53 (0.41–0.65)	0.49 (0.39–0.58)	0.63 (0.47–0.80)	> 0.1
Plasma vitamin K/vitamin K 2,3-epoxide ratio after supplementation	1.77 (1.42–2.11)	1.13 (0.87–1.40)	1.24 (0.77–1.71)	0.019
Difference in plasma vitamin K/vitamin K 2,3-epoxide ratio	1.23 (0.88–1.58)	0.65 (0.38–0.92)	0.61 (0.14–1.08)	0.025
INR before supplementation	2.47 (1.95–3.00)	2.86 (2.45–3.27)	2.58 (1.87–3.29)	> 0.1
INR after supplementation†	1.52 (1.14–1.90)	1.98 (1.69–2.28)	2.40 (1.88–2.92)	0.024
Change in INR after supplementation	− 0.95 (− 1.34 to − 0.57)	− 0.88 (− 1.18 to − 0.58)	− 0.18 (− 0.70–0.33)	0.045
Warfarin dose requirement (mg) before supplementation	3.23 (2.23–4.23)	4.52 (3.74–5.30)	3.50 (2.14–4.86)	> 0.1
Warfarin dose requirement (mg) after supplementation*	4.05 (2.96–5.14)	4.86 (4.01–5.71)	3.50 (2.03–4.97)	> 0.1
Increase in warfarin dose after supplementation	0.82 (0.45–1.19)	0.34 (0.05–0.63)	0.00 (− 0.50–0.50)	0.028

P-values refer to the overall test of differences between the three genotypes.

*The warfarin dose was determined when it remained unchanged for at least two clinic visits after vitamin K supplementation.

†Mean INR values at stable warfarin dose following vitamin K supplementation were not significantly different between the three *VKORC1* genotypes.

vitamin K 2,3-epoxide concentrations between the three genotypes at entry, but the differences were of borderline significance after 7 days of vitamin K supplementation (Table 1). Plasma vitamin K/vitamin K 2,3-epoxide concentration ratios between the three genotypes were not significantly different from each other at entry. However, the ratio in the *VKORC1* GG genotype patients was significantly higher than in the GA and AA genotype patients after 1 week of vitamin K supplementation (Table 1). Those patients carrying the GG genotype demonstrated a significantly larger decrease in INR and required a significantly greater increase in stable warfarin dose (mean: 95% CI) than those carrying the GA and AA genotypes following vitamin K supplementation (0.82; 0.45–1.19 vs. 0.34; 0.05–0.63 vs. 0.00; − 0.50–0.50; $P = 0.028$) (Table 1). The frequency of the *VKORC1* G allele (0.57) was similar to that in the general anticoagulated population [2].

Vitamin K, in its hydroquinone form, is an essential cofactor for the post-translational carboxylation of clotting proteins II, VII, IX and X. Coumarins, including warfarin, reduce the regeneration of vitamin K hydroquinone from vitamin K 2,3-epoxide via vitamin K quinone by reversibly inhibiting the action of VKOR in the vitamin K cycle, resulting in a dose-dependent increase in plasma vitamin K 2,3-epoxide concentration, as its conversion back to vitamin K is inhibited [6]. Warfarin inhibits VKOR because of its structural similarity to the normal substrates of VKOR, vitamin K quinone and vitamin K 2,3-epoxide. In this reversible competitive inhibition process, warfarin competes with either vitamin K or vitamin K 2,3-epoxide for binding to the active site of VKOR. The *VKORC1* 1639G > A polymorphism has been demonstrated to contribute to interindividual differences in warfarin dose requirements, with the GG genotype patients requiring a significantly higher daily dose of warfarin than the GA and AA genotype patients to achieve the same target INR [2]. However, warfarin dose requirements were not significantly different between the three genotype groups of patients in the current study, either prior to or after vitamin K supplementation. This is possibly because of a small sample size and/or other genetic confounders, notably CYP2C9 polymorphism, which influence warfarin dose requirement [7]. Increased availability of vitamin K (through daily vitamin K supplementation), is expected to diminish the inhibitory activity of warfarin. The decrease in warfarin anticoagulant activity, made evident by reductions in INR and the subsequent increase in warfarin dose following vitamin K supplementation, differed between individuals and appears to be related to the *VKORC1* 1639G > A polymorphism. Vitamin K 2,3-epoxide is converted by VKOR to vitamin K more efficiently in *VKORC1* 1639GG genotype patients than in GA or AA genotype patients; hence the observation that vitamin K 2,3-epoxide levels were lower in patients with this genotype than in those with the GA or AA genotype following vitamin K supplementation. It is therefore plausible to suggest that supplementation with vitamin K leads to a greater regeneration of vitamin K hydroquinone and subsequently a larger increase in the carboxylation of the vitamin K-dependent clotting factors in patients with the GG genotype than in patients with the AA genotype. The extent of the impact of vitamin K supplementation on anticoagulation status was ultimately reflected in the warfarin dose; in patients with the GG genotype, this increased by an average of 25%, as compared to 8% for those with the GA genotype and 0% for those with the AA genotype. Although in all patient with the GG genotype the warfarin dose requirement was increased, in GA genotype patients it increased in only nine, was unaltered in seven, and marginally decreased in two, and in AA genotype patients it remained unchanged in four, marginally increased in one, and marginally decreased in one.

At this stage, it is difficult to extend the current findings of the impact of the *VKORC1* genotype on vitamin K-induced alterations in the anticoagulation response to the whole anticoagulated population, as the results obtained according to genotype are potentially influenced by lack of power or unidentified genetic and environmental confounders underpinning the present findings. Further larger studies comparing stable with unstable patients will be required in order to assess the precise relationships between dietary vitamin K intake, *VKORC1* genotype and the stability of anticoagulation control, and indicate whether patients with the *VKORC1* 1639GG genotype are more sensitive to fluctuations in INR in response to dietary intake of vitamin K than are other patients. This could then indicate what role genotyping of patients and tailoring advice about dietary intake of vitamin K should have, if any, in clinical practice.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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