CORRESPONDENCE

Genotype-Guided Dosing of Vitamin K Antagonists

TO THE EDITOR: Three articles in the December 12 issue[1-3] address pharmacogenetics and dosing of coumarin anticoagulants. Because it is more usual for elderly patients to have an international normalized ratio (INR) outside the targeted therapeutic range and to have frank bleeding, it would be of clinical interest to know whether the authors stratified patients by age and whether coexisting conditions or the use of concomitant medications contributed to any effects related to age.[4-6] In addition, because the frequency of polymorphisms varies according to ethnic background, it would be of clinical interest to know the differential effects of coumarin anticoagulants in different populations.[7] Last, because drug exposure may be a better determinant of drug metabolism than dosage, it would be of clinical value to know whether the authors had pharmacokinetic data.[8]

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No potential conflict of interest relevant to this letter was reported.

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TO THE EDITOR: Kimmel and colleagues report that patients in the group receiving genotype-guided warfarin dosing did not show an improvement in the mean percentage of time in the therapeutic range. Using pharmacokinetic–pharmacodynamic data from our genotype-guided dose-initiation algorithm for warfarin, we found genotype-based dosing to be superior in patients who had atrial fibrillation as compared with patients who had venous thromboembolism, with the benefit increasing with increasing numbers of variant alleles.[1-3] We applied this model to simulate the data provided by Kimmel and colleagues and found no significant difference in INR response between dosing groups overall, as Kimmel and colleagues reported, but we did find that the genetics-based algorithm had clear benefits when the participants harbored 2 or more variant alleles (see Fig. 1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). We believe that in the large proportion of patients with venous thromboembolism,

the dilution of effects in a majority of participants with a low allele burden who were not expected to benefit and the inclusion of patients of African descent, for whom novel genetic predictors were not considered,[4] contributed to the negative findings. We suggest that the authors provide outcomes data for increasing numbers of variants. Pharmacogenetic interventions largely benefit the outliers, an important consideration when designing randomized, controlled trials that are intended to show the effectiveness of genotype-guided dosing.

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No potential conflict of interest relevant to this letter was reported.

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4. Perera MA, Cavallari LH, Limdi NA, et al. Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. Lancet 2013;382:790-6.

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TO THE EDITOR: Two pharmacogenetic trials of warfarin therapy, European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) and Clarification of Optimal Anticoagulation through Genetics (COAG), reported by Pirmohamed and colleagues and Kimmel and colleagues, respectively, had contradictory messages. Could the results of a genotyping intervention differ according to the prevalence of a genotype that has the greatest effect on warfarin dosing? The prevalence of homozygotes, who required the most significant dosing changes, was 17% in EU-PACT versus 11% in the COAG trial for the *VKORC1* variant and 3.4% in EU-PACT versus 1% in the COAG trial for the *CYP2C9*2* and *CYP2C9*3* variants. The possibility that genotype prevalence can attenuate outcomes was highlighted in the black cohort in the COAG trial, in which 75% of black patients and 25% of nonblack patients had no genotyped variants of *CYP2C9* and *VKORC1*, and the prevalence of homozygotes for these genes in black and nonblack patients was less than 1% and 17%, respectively. Sample size in genotyping trials (e.g., Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response after Percutaneous Coronary Intervention [TAILOR-PCI] trial, ClinicalTrials.gov number, NCT01742117) should be calculated on the basis of the prevalence of reduced-function or loss-of-function alleles that affect the phenotype, since we do not anticipate a difference in outcomes in patients without such mutations.

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TO THE EDITOR: Kimmel and colleagues note that a genotype-guided dosing algorithm using *CYP2C9*2*, *CYP2C9*3*, and *VKORC1* (–1639G→A) is statistically inferior to a clinical-dosing algorithm in patients of African descent. However, the single-nucleotide polymorphisms (SNPs) used in the study's pharmacogenetic dosing algorithm are known to occur at significantly lower frequencies in persons of African descent than in persons of European descent (Table 1).[1-4] The authors' ability to draw appropriate conclusions about the usefulness of genetics when determining dosages of warfarin for patients of African descent is thus very limited, and the benefits for this population have not been adequately tested. Physicians should not assume that self-reported race is an accurate proxy for the influence of genetic ancestry.[5] Rather, studies testing the usefulness of pharmacogenetics in a specific population should test variants with high frequency and measurable effect in that population.

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Dr. Altman reports being the founder of and receiving consulting fees from Personalis; and Dr. Klein, receiving consulting fees from Personalis. No other potential conflict of interest relevant to this letter was reported.

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Table 1. Frequencies of Tested Warfarin Variants in Persons of European and African Descent.*

Variant	Genotype Frequencies		P Value*
	Persons of European Descent	Persons of African Descent	
*CYP2C9*2*†	TT = 0.0175 TC = 0.227 CC = 0.756	TT = 0.00182 TC = 0.0499 CC = 0.948	$P<2.2\times10^{-16}$
*CYP2C9*3*†	CC = 0.00349 CA = 0.125 AA = 0.872	CC = 0.000454 CA = 0.0277 AA = 0.972	$P<2.2\times10^{-16}$
VKORC1 (3673G→A)‡	AA = 0.195 GA = 0.407 GG = 0.398	AA = 0.061 GA = 0.082 GG = 0.857	$P=1.564\times10^{-7}$

* P values were calculated for the comparison of genotype distributions with the use of Fisher's exact test and the R Statistical Package, version 2.15.3.
† Data are from the GO Exome Sequencing Project and are based on 4300 persons of European descent and 2203 persons of African descent. See the project home page.[3]
‡ Data are from the HapMap Project as reported by the dbSNP and are based on 113 persons of European descent and 49 persons of African descent. See the International HapMap 3 Consortium.[4]

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TO THE EDITOR: Results from the EU-PACT and COAG trials conflict with regard to the clinical usefulness of genotype-guided warfarin dosing. Whereas the EU-PACT trial showed a benefit with this approach in a homogeneous population in whom important variants are well defined, the COAG study failed to show a benefit in a racially diverse population. Genotyping for both trials was limited to *VKORC1* and *CYP2C9*2* and *CYP2C9*3*, which is an appropriate selection for a population of European descent but not necessarily for non-European populations. We and others have shown distinct associations with genetic warfarin dosing in patients of African descent, who comprised nearly 30% of the COAG population.[1-3] Approximately 20% of persons of African descent carry the allele *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, or *CYP2C9*11*, and 44% carry an *A* allele for SNP rs12777823, all of which portend lower dose requirements.[2,3] The COAG trial would have misclassified these genotypes as variant, which probably explains why more African patients received an overdose when their regimen was determined with the genotype-guided strategy. We contend that until race-specific variants are accounted for in pharmacogenetic algorithms applied to minorities, the benefits of genotype-guided dosing in these patients will not be sufficiently addressed.

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Dr. Cavallari reports being listed as a coinventor on U.S. utility patent application number 12/572,908, entitled *CYP2C9*8* Alleles Correlate with Decreased Warfarin Metabolism and Increased Warfarin Sensitivity. No other potential conflict of interest relevant to this letter was reported.

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DR. KIMMEL AND COLLEAGUES REPLY: We appreciate the comments of Koller and colleagues and Schwarz and colleagues and have performed the suggested analyses. There were no significant differences between the dosing strategies in the primary outcome when stratified by age (<65 years vs. ≥65 years; P=0.24 for the interaction) or by primary indication (deep-vein thrombosis or pulmonary embolism vs. other; P=0.16 for the interaction). Similar results were obtained when the data were stratified by number of variants (≥2 vs.

0 or 1) (P=0.92 for the interaction in the entire study population; P=0.51 for the interaction in the population of African descent; and P=0.28 for the interaction in the population not of African descent). We do not have pharmacokinetic data.

Pereira and colleagues suggest that the results of a genotyping intervention could differ according to the prevalence of the genotype that has the greatest effect on warfarin dosing. We tested this hypothesis; it is not supported by the data (see Table 2 in our article). Furthermore, it should be noted that patients with no variants or with more than one variant in either *CYP2C9* or *VKORC1* (for whom pharmacogenetic dosing algorithms predict a higher and lower dose, respectively, than the clinical algorithms) would be expected to benefit the most.[1] In its sample size calculations, the COAG trial considered the presence of genetic variants most likely to have an effect on dosing and powered the trial to examine the group most likely to benefit from genotyping (those with predicted dose difference of ≥1 mg or more per day between the pharmacogenetic and clinical algorithms).[2]

We agree with Daneshjou and colleagues and with Cavallari and colleagues that patients of African descent might benefit from algorithms that use additional genetic variants. However, we note that there were 701 patients in the trial who were not of African descent, a sample size larger than that of either of the EU-PACT studies, and there was no significant benefit of pharmacogenetics in this group, despite the excellent dose prediction when the dosing algorithm variants were used. Thus, whether better prediction for initial dosing in patients of African descent will lead to better anticoagulation control remains an untested hypothesis. For now, the best available evidence is that the use of existing genetic variants approved by the Food and Drug Administration for the prediction of dose may worsen 30-day anticoagulation control in this population. We also agree that self-reported race is not necessarily an accurate proxy for the influence of genetic ancestry. However, clinical use of warfarin pharmacogenetics relies on self-reported race. In comparing trials, we believe that the most important factor is the hypothesis that each trial tested. The COAG trial of warfarin and the EU-PACT trial of acenocoumarol and phenprocoumon tested the hypothesis that genetic information would improve anticoagulation control when added to clinical information. Neither trial showed a benefit in the primary outcome. In contrast, the EU-PACT trial of warfarin tested a different hypothesis: that an algorithm using both genetic and clinical information would improve anticoagulation control as compared with a strategy that was not tailored to clinical factors other than age.

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Since publication of their article, the authors report no further potential conflict of interest.

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DR. PIRMOHAMED AND COLLEAGUES REPLY: We agree with Koller and colleagues that exposure is more important than dosage. We therefore incorporated *CYP2C9* genetic status into our algorithms from the first day of dosing, since this is the main determinant of warfarin exposure. In the trial by Kimmel and colleagues, the first dose of warfarin was not informed by genotyping in the majority of the patients, which may be one reason for the differences in outcomes. As evidenced by the older age of the patients in our study, there was a higher proportion with atrial fibrillation than in the COAG trial, and the effects of age and interacting medications were taken into account by the algorithms. Given the pragmatic nature of our trial, we did not obtain blood samples for pharmacokinetic analysis.

Our further analyses, to be published separately, have indicated that the greatest effect of the genotype-guided dosing algorithm on the percentage of time in the therapeutic range was in patients with variant alleles. Thus, we would agree with Pereira and colleagues that the differences between the two trials in variant genotypes may have contributed to the divergent results. The differences in algorithmic strategies may also have been a factor, since a loading dose of warfarin accelerated the attainment of a steady state, and therefore a change in the INR, on day 4,

when the same dose revision algorithm was used in the two trials.

Another reason for the difference between the two trials relates to the differences in the homogeneity of the populations. Thus, we would agree with Cavallari and colleagues that patients in the COAG trial who were of African descent may have benefited from algorithms that used additional genetic variants. It has also been suggested that in the EU-PACT trial, genotyping-guided dosing was associated with a greater time in the therapeutic range because genotype-guided dosing was compared with a standard fixed-dose regimen, whereas in the COAG trial a clinical-dosing algorithm was used as a comparator. However, the time in the therapeutic range in our groups was either equivalent to or superior to that reported for the COAG trial at 4 weeks, even when the patients in the COAG trial who were not of African descent were used for comparison. The difference in blinding (single vs. double) is unlikely to have accounted for the differences in results, since in the EU-PACT trial we used an objective biomarker (i.e., INR) as the end point, and dose changes were handled in both groups after day 5 by entering the latest INR into computerized dosing software programs routinely used in our anticoagulant clinics.

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DR. MAITLAND-VAN DER ZEE AND COLLEAGUES REPLY: Koller and colleagues asked whether patients in the EU-PACT trial were stratified by age (Table 1). Among patients who were younger than 75 years of age (334 patients), the difference in the percentage of time in the INR range between the groups that did or did not undergo genotyping was 5.1 percentage points during the 12 weeks after the initiation of therapy (P=0.05) and 7.5 percentage points during the first 4 weeks (P=0.01). Among the patients who were 75 years of age or older (150 patients), the genotyped group spent less time in the INR range during the 12-week period (difference, 7.1 percentage points; P=0.06), and there was no significant difference in the time spent during the first 4 weeks (difference, 0.4 percentage points; P=0.93). Therefore, it seems that the genetic information is more important for younger patients.

In our trial, it was not possible to study the effect of race because almost all patients

Table 1. Percentage of Time in the Therapeutic INR Range in the EU-PACT Trial in the Genotype-Guided Group and the Control Group, According to Age (with Data for Acenocoumarol and Phenprocoumon Combined).*

Age Group and Study Week	No. of Persons in Age Group	Time in INR Range		Difference between Groups	P Value
		Genotype-Guided Group (N=239)	Control Group (N=245)		
		percent		*percentage points (95% CI)*†	
<75 yr	334				
Wk 1–12		64.4	59.3	5.1 (–0.03 to 10.1)	0.05
Wk 1–4		54.1	46.6	7.5 (2.1 to 13.0)	0.01
Wk 5–8		68.3	61.7	6.5 (–0.7 to 13.7)	0.08
Wk 9–12		70.3	69.5	0.8 (–6.7 to 8.3)	0.83
≥75 yr	150				
Wk 1–12		54.9	62.0	–7.1 (–14.4 to 0.2)	0.06
Wk 1–4		49.6	49.3	0.4 (–8.1 to 8.9)	0.93
Wk 5–8		56.0	68.3	–7.3 (–18.4 to 3.8)	0.20
Wk 9–12		59.5	72.8	–13.2 (–24.6 to –1.8)	0.02

* The therapeutic international normalized ratio (INR) was 2.0 to 3.0. EU-PACT denotes European Pharmacogenetics of Anticoagulant Therapy.
† The between-group difference was calculated as the value for the genotype-guided group minus the value for the control group.

were white. Finally, because we had INR measurements for the dosing of coumarin, we did not consider it useful to gather pharmacokinetic data.

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Intussusception Risk after Rotavirus Vaccination in U.S. Infants

TO THE EDITOR: Yih et al. (Feb. 6 issue)[1] report that a rotavirus vaccine was associated with approximately 1.5 excess cases of intussusception per 100,000 recipients of the first dose among infants in the United States. Changes in temporal trends in the incidence of intussusception have been described in a few studies based on hospitalization data.[2-5] This surveillance allows a historical baseline view of the incidence of intussusception. Five-year data from the network of regional emergency departments in southern France included more than 1 million visits by patients younger than 18 years of age. These data showed an increase in the incidence of intussusception, defined according to the diagnosis code,[2] from 31.9 cases per 100,000 visits in 2009 to 74.1 cases per 100,000 visits in 2013 (odds ratio, 2.32; 95% confidence interval, 1.74 to 3.11) (Fig. 1). The vaccine coverage among less than 10% of the population in France could not explain this variation. During the same 2009–2013 period, the global incidence of intussusception was highest among children between 1 and 2 years of age (average incidence in this age group, 97.7 cases per 100,000 visits) and the increase in the incidence among children in this age group was similar to the increase among all children. Emergency department databases permit real-time surveillance of the incidence of intussusception, but because of natural changes in incidence, information obtained from these databases should be interpreted with caution. These data alone are not sufficient in pharmacovigilance for adverse effects of rotavirus vaccine.

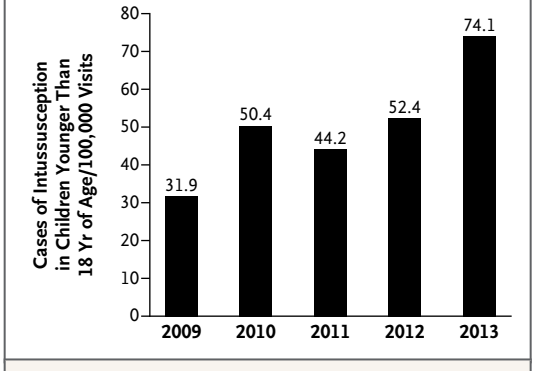

Figure 1. Incidence of Intussusception in Children Younger Than 18 Years of Age in Emergency Departments in Southern France.

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No potential conflict of interest relevant to this letter was reported.

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