Supplementary material

Analytical methods

Analytical methods. For patients recruited in the Bichat and Angers university hospitals, INR was assayed on-site in the corresponding Laboratoire d'Hémostase, which both use a STARE coagulometer (Diagnostica Stago, 92600 Asnières, France) and thromboplastin STA®-Neoplastin CI with an international sensitivity index (ISI) of 1.63 (batch number 100675, Diagnostica Stago) during the whole study. The samples from the 4 patients in the Beaujon hospital were centralised at the Bichat hospital. INR was calculated from the prothrombin time (PT) as:

$$\mathrm{INR} = \left(\frac{\text{Patient PT}}{\text{mean normal PT}} \right)^{\mathrm{ISI}} \tag{8}$$

The two Laboratoires d'Hémostase both perform routine controls, which included for the study period successively C06, C07, C08 (Asqualab, Stago) that were daily checked on the coagulometer. PT coefficient of variation was between 1.7-2.4% during this period.

Determination of fluindione concentrations. The samples for the measurement of fluindione concentrations were frozen at -20°C, and shipped at regular intervals throughout the study to the Laboratoire de Pharmacologie-Toxicologie in the Angers university hospital. Plasma fluindione was assayed with use of an HPLC–UV system Surveyor (ThermoFinnigan), with ChromQuest software [48]. The UV spectrophotometer was set at a wavelength of 280 nm .The separation was achieved at 40°C temperature, with a reversed-phase 100X 4.6 mm internal diameter BetaBasic-8 column and 5 μm particle size packing (ThermoElectron). The mobile phase composition was optimized to a 0.067 mol.L^{-1} dibasic sodium phosphate buffer (adjusted to pH 6.3 with phosphoric acid) and acetonitrile (82:18, vol/vol) mixture. The flow rate was set at 1.5 mL.min^{-1}.The following extraction procedure was used: 100 μ1 of plasma from human heparinized blood (spiked plasma used for calibration and controls; patients' plasma samples) was added to a 1.5 ml tube that contained 50 μL of 20 mg.L^{-1} internal standard solution (warfarin) and 100 μL acetonitrile. The tube was vortexed for 30 seconds and centrifuged for 10 minutes at 3000g. A 150 μL volume of supernatant was transferred to another tube that contained 200 μL of phosphate buffer; 25 μL of the mixture was injected into the HPLC system. The calibration curve was linear over the range 0.05 to 6 mg.L^{-1}. The method was highly reproducible. The coefficient of variation was 6.1% for a fluindione concentration of 0.1 mg.L^{-1}, 2.8% for a concentration of 0.5 mg.L^{-1}, and 2.3% for a concentration of 4 mg.L^{-1} (10 measurements for each concentration). The estimated limit of quantification was 0.1 mg.L^{-1} under the conditions described above, with a signal-to-noise ratio of 3 and a coefficient of variation lower than 20%.

Determination of genetic polymorphisms. DNA was extracted from the blood samples of patients consenting to the genetic ancillary study. For patients from the Bichat university hospital, the sample was directly sent to the Centre de Ressources Biologiques (CRB, DNA bank) of the hospital, and the DNA was extracted. For patients from the other two hospitals, samples were frozen at -20°C and sent every 3 months to the CRB for extraction and storage.

CYP2C9*2 and CYP2C9*3 (rs number 1799853 and 1057910, respectively) as well as VKORC1 genetic polymorphism for the 1173 C>T (rs number 9934438) were determined using custom Taq Man allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) as in [40, 28]. They were performed all together at the end of study. The post-PCR-generated fluorescence intensity was quantified using an ABI 7000 Sequence detector System software version 1.2.3 (Applied Biosystems, Courtaboeuf, France). Each SNP genotyping procedure was performed in duplicate (separate experiments) for each patient. In cases of discordant results, samples were analyzed by DNA sequencing to confirm the genotype. Sequenced wild-type, homozygous and heterozygous patient samples were used as controls. All PCR reagents were purchased from Applied Biosystems.

We chose the VKORCI 1173 C>T SNP (rs9934438) to identify the major VKORCI haplotype groups A and B. The C allele of the 1173 C>T SNP corresponds to the group B VKORCI haplotype and the T allele to the group A VKORCI haplotype. This SNP is in complete linkage disequilibrium with at least four other SNPs, which all individually allow the identification of VKORCI haplotype groups [49, 50], which has been previously confirmed in a White French population [40].

Assessing model sensitivity

The PREPA study was an observational study, and to minimise the burden on patients, samples were taken only at the usual time for therapeutic monitoring. Figure S1 shows the dose-normalised fluindione concentrations versus time after the dose. Several patients skipped one or several doses, usually because the clinician was concerned about a quick rise in the INR, therefore for these patients the time after dose exceeds 24 hours, but most samples were taken 10 to 16 hours after the dose. The main plot displays the whole dataset, while the inset shows a zoom for time after dose lower than 48 hours, in order to better show the variability. Note that concentrations were normalised to the last dose taken before the measurement, so that for patients with changing doses the apparent variability may be larger since it does not take into account the whole dosing history. This figure shows that most of the samples were clustered between 10 and 15 hours.

Because there was concern regarding the ability of this design to properly estimate the parameters of the model, and also in light of the discrepancy with the results previously found in the ADAP study, we performed a small study to assess the robustness of the parameter

estimates. Due to time constraints this study was performed only for the PK model. We simulated 20 datasets with the population parameters estimated in the ADAP study and 20 datasets with the parameters estimated with the base model (without covariates) in the PREPA study. We then estimated the parameters of the PK model starting with initial estimates close to those of the ADAP study. The results are displayed in Supplementary Table S3, and show that despite the sparse design, the parameter estimates in both cases are close to the simulated values. There was more variability in the estimates from the datasets simulated with the parameters from the ADAP study (25% variability between runs versus 5%), partly because the IIV was larger in ADAP than in PREPA, but it was possible to clearly distinguish between the two sets of parameters.

Supplementary tables

Table S1: Drug classification used for the covariate analysis of the comedications in the PREPA study (from reference [42]).

Drugs increasing the thrombotic risk	
A1	enzymatic inducers (decreasing INR)
A2	other drugs inhibiting coagulation
Drugs increasing the haemorragic risk	
B11	anti-vitamin K agents
B12	aspirin, non-steroidal anti-inflammatory drugs
B13	heparins
B14	other drugs
B21	enzymatic inhibitors
B22	antibiotics with a negative effect on the intestinal flora
B23	drugs acting through another mechanism

Table S2: Range of parameter estimates for the covariate effects, obtained by multiple imputation (K=5 imputed datasets), compared to the estimate in the dataset where missing weight is imputed to the mean value.

Parameter	Estimate	Range MI
$\beta_{V,men}$	0.24 (41)	[0.17 – 0.25]
$\beta_{V,weight}$	0.57 (35)	[0.51 – 0.63]
$\beta_{CL,age}$	-3.26 (30)	[-3.38 – -2.96]
$\beta_{CL,cordarone}$	-0.18 (49)	[-0.19 – -0.17]
$\beta_{C_{50},surgery}$	-0.54 (25)	[-0.75 – -0.52]
$\beta_{\gamma,surgery}$	-0.46 (34)	[-0.68 – -0.50]
$\beta_{\gamma,deroxat}$	0.88 (31)	[0.81 – 2.03]

Table S3: Results from the simulation study: PK parameter estimates obtained on N=20 datasets simulated with the design of the original study, under two different set of parameters, ADAP=parameters estimated in the ADAP study [25]; PREPA=parameters estimated in the current study, with the base model (no covariates). We show the median and range of the parameters estimated in the 20 datasets for each simulation.

Parameter	ADAP			PREPA		
	Simulated	Estimated		Simulated	Estimated	
		Median	[range]		Median	[range]
V (L)	37.1	38.8	[23.0–65.3]	9.06	9.38	[8.73–10.20]
CL ($L.hr^{-1}$)	0.49	0.42	[0.26–0.74]	0.12	0.12	[0.10–0.14]

Supplementary figure

Figure S1: Dose-normalised fluindione concentrations versus time after dose. Main plot: whole dataset; Inset: zoom for time after dose lower than 48 hours. When dose changes occurred, the last non-null dose before the measurement was taken for normalising.