Genetic Determinants of Dabigatran Plasma Levels and Their Relation to Bleeding

Running title: Paré et al.; Genetic determinants of dabigatran activity

Guillaume Paré, MD1; Niclas Eriksson, PhD2; Thorsten Lehr, PhD3,4; Stuart Connolly, MD1; John Eikelboom, MD1; Michael D. Ezekowitz, MD, PhD5; Tomas Axelsson, PhD6; Sebastian Haertter, PhD3; Jonas Oldgren, MD, PhD2; Paul Reilly, PhD7; Agneta Siegbahn, MD, PhD6; Ann-Christine Syvanen, PhD6; Claes Wadelius, MD, PhD8; Mia Wadelius, MD, PhD6; Heike Zimdahl-Gelling, PhD3; Salim Yusuf, MD1; Lars Wallentin MD PhD2

1Population Health Research Institute, Hamilton Health Sciences and McMaster University, Hamilton, ON, Canada; 2Uppsala Clinical Research Center and Dept of Medical Sciences; 6Dept of Medical Sciences; 5Dept of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden; 3Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany; 4Clinical Pharmacy, Saarland University, Saarbrücken, Germany; 5Thomas Jefferson Medical College, Cardiovascular Research Foundation, Philadelphia, PA; 7Boehringer Ingelheim Pharma Inc., Ridgefield, CT

Address for Correspondence:
Guillaume Paré MD, MSc, FRCPC
Population Health Research Institute
Hamilton Health Sciences and McMaster University
David Braley Cardiac Vascular and Stroke Research Institute
237 Barton St. East Rm. C3-103
Hamilton, Ontario, L8L 2X2 Canada
Tel: 905-527-4322 #40356
Fax: 905-528-2814
E-mail: parreg@McMaster.ca

Abstract:

**Background**—Fixed-dose unmonitored treatment with dabigatran etexilate is effective and has a favorable safety profile in prevention of stroke in atrial fibrillation patients compared to warfarin. We hypothesized that genetic variants could contribute to inter-individual variability in blood concentrations of the active metabolite of dabigatran etexilate, and influence the safety and efficacy of dabigatran.

**Methods and Results**—We successfully conducted a genome-wide association study in 2,944 RE-LY participants. The *CES1* SNP rs2244613 was associated with trough concentrations, and the *ABCB1* SNP rs4148738 and *CES1* SNP rs8192935 were associated with peak concentrations at genome-wide significance (P<9 x 10⁻⁸) with a gene-dose effect. Each minor allele of the *CES1* SNP rs2244613 was associated with lower trough concentrations (15% decrease per allele, 95%CI 10-19%; P=1.2 x 10⁻⁸) and a lower risk of any bleeding (OR=0.67, 95%CI 0.55-0.82; P=7 x 10⁻⁵) in dabigatran-treated participants, with a consistent but non-significant lower risk of major bleeding (OR=0.66, 95%CI 0.43-1.01). The interaction between treatment (warfarin versus all dabigatran) and carrier status was statistically significant (P=0.002) with carriers having less bleeding with dabigatran than warfarin (HR=0.59, 95%CI 0.46-0.76; P=5.2 x 10⁻⁵) in contrast to no difference in noncarriers (HR=0.96, 95%CI 0.81-1.14; P=0.65). There was no association with ischemic events, and neither rs4148738 nor rs8192935 was associated with bleeding or ischemic events.

**Conclusions**—Genome-wide association analysis identified that carriage of *CES1* rs2244613 minor allele occurred in 32.8% of patients in RELY and was associated with lower exposure to active dabigatran metabolite. The presence of the polymorphism was associated with a lower risk of bleeding.

**Clinical Trial Registration Information**—ClinicalTrials.gov; Identifier: NCT00262600

**Key words**: stroke prevention, genetics, human, cardiovascular disease, genomics, anticoagulant, pharmacogenetics, atrial fibrillation
Introduction

The Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial demonstrated that dabigatran etexilate 110 mg bid was as effective and 150 mg bid was superior to warfarin for stroke prevention in patients with non-valvular atrial fibrillation. Both dabigatran etexilate doses were associated with less major and minor bleeding, including lower rates of intracranial hemorrhage. Unlike vitamin K antagonists, dabigatran etexilate was given in fixed doses without coagulation monitoring, a major advantage for patients and physicians. However, considerable inter-individual variability exists in blood concentrations of the active metabolite of dabigatran, with coefficient of variation estimated at 30% for systemic exposure. There is therefore an interest to investigate the reasons for this variability.

Dabigatran etexilate is an oral prodrug that is rapidly converted by esterases – notably liver esterase CES1 – to dabigatran, a reversible direct thrombin inhibitor. Dabigatran etexilate has a mean absolute bioavailability of 6.5%, which is independent of dose and not meaningfully influenced by coadministration with food. Dabigatran etexilate, but not dabigatran, is a substrate of the P-glycoprotein intestinal efflux transporter (ABCB1) and strong P-glycoprotein inhibitors increase dabigatran bioavailability by 12-23%. Conversion of dabigatran etexilate to dabigatran is assumed to be effectively complete such that the prodrug and its intermediates are hardly detectable in plasma. Maximum plasma concentrations of dabigatran occur approximately 1-3 h after oral dosing. Renal excretion is the predominant (80%) elimination pathway, and cytochrome P450 enzymes or other oxidoreductases are not involved in metabolism of dabigatran.

We hypothesized that genetic factors are responsible for some of the inter-individual variability in blood concentrations of the active metabolite of dabigatran etexilate, and that these
factors are associated with dabigatran safety and efficacy. In this report, we first present a genome-wide analysis in 1,490 RE-LY participants of European Caucasian ancestry in whom we had both genetic information and data on dabigatran concentration in the blood. In a second phase, we tested newly identified genetic determinants of dabigatran concentration for association with efficacy and safety outcomes in 1,694 dabigatran etexilate-treated patients with genetic information. Finally, we confirmed genetic associations were specific to dabigatran by testing in 807 warfarin-treated RE-LY patients.

Methods

RE-LY Trial

The RE-LY study design\(^1\) and results\(^2\) have been described previously. Briefly, RE-LY was a randomized trial designed to compare two fixed doses of dabigatran etexilate — 110 mg or 150 mg twice daily — each administered in a blinded manner, with open-label use of warfarin in patients who had atrial fibrillation and at least one additional risk factor for stroke. Patients were eligible for inclusion if they had atrial fibrillation documented on electrocardiography performed at screening or within 6 months beforehand and at least one of the following additional risk factors for stroke: previous stroke or transient ischemic attack, a left ventricular ejection fraction of less than 40\%, New York Heart Association class II or higher heart-failure symptoms within 6 months before screening, and an age of at least 75 years or an age of at least 65 years plus diabetes mellitus, hypertension, or coronary artery disease. Patients with severe valvular heart disease, stroke within 14 days or severe stroke within 6 months before screening, and a creatinine clearance of less than 30 ml per minute were not eligible for inclusion. A total of 18,113 patients were enrolled and the median duration of follow-up was 2.0 years.
In this genetic substudy, the primary safety outcome was any bleeding, a composite of major and minor bleeding. Major bleeding was defined as a reduction in the hemoglobin level of at least 20 g per liter, transfusion of at least 2 units of blood, or symptomatic bleeding in a critical area or organ. All other bleeding was considered minor. The efficacy outcome was ischemic stroke or systemic embolism. The secondary outcome was any ischemic event, defined as a composite of ischemic stroke, systemic embolism, myocardial infarction and pulmonary embolism. All events were adjudicated as part of the RE-LY trial by two independent investigators who were unaware of the treatment assignments.

The Institutional Review Board at each center approved each study, and all patients provided written informed consent. Only those patients who also consented to participate in the genetic study were eligible for these analyses.

**Genotyping**

Genotyping at 620,901 markers was attempted in 3,076 RE-LY participants using the Illumina Human610-quad DNA analysis beadchip. Standard quality control procedures were applied. Briefly, participants were kept for further analysis if total call rate was >98%, self-reported sex matched genetic sex, and the maximum estimated genetic relatedness with any other participant was <5%, leaving 2,944 individuals for analysis. Only participants of European Caucasian ancestry were included in the present report since >85% of individuals were European Caucasians, a limited number of individuals were available for analysis in remaining ancestries, and population stratification can lead to type I error inflation. Consistent results were obtained when analyzing all samples (Supplementary Table 1). Self-reported European Caucasian ancestry was confirmed by visual inspection of the first two genetic principal components. 1,694 dabigatran etexilate-treated RE-LY participants were included in the main analyses (1,490
of which also had data on dabigatran concentrations), with an additional 807 warfarin-treated participants included to test for treatment interaction. Single nucleotide polymorphisms (SNPs) were retained for association analysis when call rate was >98%, minor allele frequency (MAF) >1%, and Hardy-Weinberg equilibrium P-value > 10^-6. 551,203 SNPs were tested for association with dabigatran concentration. Data management and quality control was done using PLINK. All genetic coordinates are based on NCBI human genome build 36 and dbSNP build 130. Imputation of untyped SNPs was performed with MACH on HapMap phase 3 data.

Pharmacokinetic Measurements

Peak and trough samples were collected for determination of dabigatran concentration at 1, 3, 6 and 12 months post randomization. Only samples collected within 10 to 16 hours after the previous dabigatran etexilate dose were considered for trough concentration, and only samples collected within 1 to 3 hours after dose were considered for peak concentration. For analyses reported here, the first trough and post-dose samples fulfilling the time-window rule were used from subjects with multiple blood samples. Plasma concentrations of total dabigatran were determined after alkaline cleavage of conjugates by a validated high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method at AAIPharma Deutschland GmbH & Co. KG, Neu-Ulm, Germany. 1,490 dabigatran-treated participants with both genetic and pharmacokinetic data were available for analysis.

Statistical Analysis

Genome-wide analysis of peak and trough dabigatran concentration was performed by linear regression in PLINK, assuming an additive contribution of each minor allele. Dabigatran concentration was log-transformed to approximate a normal distribution, but results are presented in original units throughout for ease of interpretation. Log-transformed dabigatran
concentration was further adjusted for dabigatran dose, age, sex, log(BMI), log(Cockcroft-Gault creatinine clearance), use of proton pump inhibitors, use of moderate or strong P-gp inhibitors (amiodarone, verapamil, diltiazem and quinidine), and the first ten genetic principal components to correct for residual population stratification. Clinical variables were chosen because they were associated with dabigatran concentration in univariate analyses (except for the principal components). The genome-wide p-value significance threshold was conservatively set at 0.05/551,203 = 9 x 10^{-8} using a Bonferroni correction to adjust for multiple hypothesis testing.

Association of SNPs with events was performed using logistic regression in PLINK, assuming an additive genetic model and using a nominal level of significance (P<0.05) since only SNPs previously identified as genetic determinants of dabigatran concentrations were tested. Logistic regression models included age, sex, dabigatran dose, CHADS2 score, use of aspirin, log(Cockcroft-Gault creatinine clearance), and the first ten genetic principal components as independent variables. Power estimates were derived through simulations (N=1,000) of genetic effects according to the specified models using logistic regression. Associations of identified SNPs with time to event outcomes were examined using Cox Proportional Hazards regression, as implemented in R. The assumption of proportionality of hazards was tested by using scaled Schoenfeld residuals (P>0.05 in all cases). Regional plots were generated using LocusZoom \textsuperscript{14} and all other figures were created with R. Unless otherwise specified, a two-sided P<0.05 was considered significant.

Results

Patient characteristics

Patients’ characteristics are presented in Table 1. Treatment groups were well balanced with
respect to baseline characteristics, with the exception of diastolic blood pressure. Mean baseline
diastolic blood pressure was higher in the dabigatran 150 mg group as compared to the 110 mg
group (78.7 mmHg versus 77.4 mmHg; P=0.02), but the differences were modest and this was
one of multiple comparisons. 1,490 patients had pharmacokinetic data, representing 88.0% of
dabigatran-treated participants in our genetic substudy. Mean peak and trough dabigatran
concentrations were higher in the dabigatran 150 mg group as compared to the 110 mg group, as
expected.

Genetic association with dabigatran concentrations

We performed a genome-wide association analysis of peak and trough dabigatran concentrations
in 1,490 patients. All SNPs with association p-value <5 x 10⁻⁷ are presented in Table 2.
Manhattan plots of peak and trough concentrations genome-wide association are shown in
Figure 1. There was no apparent inflation of type I error upon inspection of quantile-quantile
plots (Supplementary Figure 1). Two SNPs exceeded our significance threshold for
association with peak concentrations. Each minor allele of the CES1 SNP rs8192935 was
associated with a 12% decrease in adjusted peak concentrations (95% CI 8-16%; P=3.2 x 10⁻⁸).
Conversely, each minor allele of the ABCB1 SNP rs4148738 was associated with a 12% increase
in adjusted peak concentrations (95%CI 8-17%; P=8.2 x 10⁻⁸). Two SNPs were significantly
associated with trough concentrations, both at the CES1 locus. The SNP with the strongest
effect, rs2244613, was associated with a 15% decrease in adjusted trough concentrations per
minor allele (95%CI 10-19%; P=1.2 x 10⁻⁸). Based on these results, as compared to the 67.2% of
individuals without any rs2244613 minor allele, the 29.4% of patients carrying 1 minor allele are
expected to have 15% lower trough concentrations, and the 3.4% of the population carrying 2
minor alleles are expected to have 28% lower concentrations.
Association analysis at each genome-wide significant locus was repeated after conditioning on the respective lead SNP to test whether multiple SNPs were independently associated at each locus. No SNP within 500 Kb of each lead SNP was convincingly associated (P>0.001) and therefore only lead SNPs were considered for further analysis (Figure 2). The two CES1 SNPs - rs8192935 and rs2244613 - are in linkage disequilibrium (r²=0.45 and D’=1.00), but neither haplotype analysis nor association with untyped imputed SNPs yielded more significant results (data not shown). Genetic associations were consistent across both doses of dabigatran, as evidenced by non-significant heterogeneity p-values (Supplementary Table 2).

We used population pharmacokinetic modeling technique\(^4, 15\) to estimate the effect of the three lead SNPs on bioavailability, volume of distribution and clearance. All three SNPs were identified as having a statistically significant impact on the bioavailability and to a lesser extent clearance of dabigatran (Supplementary Table 3). Compared to individuals without any minor allele for each of the three SNPs, combinations of genetic effects can result in modulation of exposure (area under the curve) from a 29.6% decrease up to 20.2% increase (Supplementary Figure 2).

**Genetic association with clinical outcomes**

Association between each lead SNP and clinical outcome was tested in 1,694 dabigatran-treated patients (Table 3). The CES1 SNP rs2244613 was significantly associated with any bleeding (N=587), with OR=0.67 per minor allele (95%CI 0.55-0.82; P=7 x 10\(^{-5}\)). The results for major bleeding (N=101) were consistent but not significant (OR=0.66, 95%CI 0.43-1.01; P=0.06). Minor bleeding (N=545), the more frequent component of all bleeding, was consistent and significant (OR=0.70, 95%CI 0.57-0.85; P=4 x 10\(^{-4}\)). Genetic associations were consistent across both doses of dabigatran (interaction P=0.81; Supplementary Table 4). Upon multivariate
analysis, CES1 SNP rs2244613 remained significantly associated with bleed along with age and proton pump inhibitor use (Supplementary Table 5). Gastrointestinal bleeding was increased with the new oral anticoagulants, dabigatran\textsuperscript{16} and rivaroxaban\textsuperscript{17}, compared to warfarin. In our analyses, genetic associations were consistent whether the bleeding site was intra or extra gastrointestinal (heterogeneity p-value > 0.05), although these analyses were limited by the small numbers of gastrointestinal minor bleeds (N=36) and extra-gastrointestinal major bleeds (N=38). No association (OR=0.95, 95%CI 0.59-1.51; P=0.82) was noted with ischemic events (Supplementary Figure 3). Power to identify a similar increase (OR=1.33 per minor allele) in risk of secondary efficacy outcome (i.e. composite of ischemic stroke, systemic embolism, myocardial infarction and pulmonary embolism) was estimated at 24\% assuming an alpha of 0.05.

Since only a small fraction (3.4\%) of participants were homozygous for the minor allele of rs2244613, we tested for association with bleeding using Cox Proportional Hazard regression under a dominant genetic model (i.e. pooling individuals with 1 or 2 minor alleles; Figure 3). Minor allele carriage was associated with an unadjusted HR of 0.70 (95%CI 0.58-0.84; P=2 x 10\textsuperscript{-4}). In other words, 27.8\% (154/553) of rs2244614 minor allele carriers suffered from a bleeding event as compared to 37.9\% (432/1139) of noncarriers, corresponding to a relative risk of 0.73 (95%CI 0.63-0.86). Importantly, no such association was observed in 807 warfarin-treated patients with genetic information (HR=1.13, 95%CI 0.90-1.42; P=0.29) and the interaction between treatment (warfarin versus all dabigatran) and carrier status was statistically significant (P=0.002). Dabigatran-treated carriers had significantly less bleeding than warfarin-treated carriers (HR=0.59, 95%CI 0.46-0.76; P=5.2 x 10\textsuperscript{-5}). However, no statistically significant difference was observed between the two treatment groups in noncarriers (HR=0.96, 95% CI
We further tested for association with bleeding after adjustment for trough and peak concentrations, and even after adjustment minor allele carriage was associated with decreased risk of bleeding (HR=0.72, 95%CI 0.58-0.90; P=0.0032).

Neither the CES1 SNP rs8192935 nor the ABCB1 SNP rs4148738 SNPs associated with peak concentrations were associated with bleeding or ischemic events under additive genetic models. It should be noted that trough concentrations had a stronger association with bleed (OR=1.25, 95% CI 1.11-1.40; p=0.0002) per standard deviation than peak concentrations (OR=1.13, 1.01-1.26; p=0.03). Nevertheless, the CES1 SNP rs8192935 was nominally associated with bleeding (OR=0.80, 95%CI 0.65-0.99; p=0.041) when using a dominant genetic model.

Discussion

We performed a genome-wide pharmacogenetic analysis of dabigatran in 1,694 RE-LY participants. The rs2244613 SNP intronic to the esterase gene CES1 was associated with decreased trough concentrations as well as decreased risk of bleeding. The CES1 SNP rs8192935 and ABCB1 SNP rs4148738 were associated with peak concentrations, but not with clinical outcome. These results reflect the important role of esterases and the P-glycoprotein transporter in determining dabigatran drug concentrations and suggest a role of CES1 rs2244613 as a determinant of clinical outcome.

CES1 encodes for the liver carboxylesterase 1 enzyme, an esterase responsible for the biotransformation of dabigatran etexilate into the active metabolite, dabigatran, as well as hydrolysis of multiple other ester- and amide-bond-containing drugs such as quinapril, methylphenidate, cocaine and heroin. To the best of our knowledge, this is the first report of
common genetic variants at the CES1 locus with genome-wide significant associations\textsuperscript{25, 26}, a finding with implications for the pharmacogenetics of other drugs metabolized by this enzymatic system. While itself a compelling biological candidate, CES1 lies within a cluster of esterase genes (e.g. CES4, CES7, CES5A, etc.) and we cannot exclude an effect of identified SNPs on other esterase genes. The clustering of esterase genes could potentially explain the apparent association of two partially correlated CES1 SNPs - rs2244613 and rs8192935 - with predominantly trough and peak concentrations, respectively.

The ABCB1 gene encodes for P-glycoprotein, an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity\textsuperscript{27, 28}. Dabigatran etexilate, but not dabigatran, is an ABCB1 substrate and ABCB1 inhibitors increase dabigatran bioavailability by 10-20\%\textsuperscript{5}. The ABCB1 SNP rs4148738 associated with peak concentration is in linkage disequilibrium (R\textsuperscript{2}=0.51) with the C3435T SNP (rs1045642) widely reported to be associated with drug metabolism\textsuperscript{29}.

Association of CES1 SNP rs2244613 with bleeding is consistent with its effect on trough dabigatran concentration. Importantly, no association was observed in warfarin-treated patients. There was a significant treatment-genotype interaction, ruling out an effect of rs2244613 through dabigatran-independent pathways. Relative risk of bleeding was 0.73 (95\%CI 0.63-0.86) for rs2244613 minor allele carriers versus noncarriers, consistent across both dabigatran doses. In comparison, the reported relative risk of bleeding was 0.86 (95\% CI 0.81-0.93) for the lower (110 mg bid) versus higher dose (150 mg bid) of dabigatran in the overall study\textsuperscript{16}. Observed genetic effect was thus larger than the effect of drug dosage in the parent study. There were no or only small differences in bleeding rates between dabigatran doses in our substudy, but this could be attributed to the smaller sample size as compared to the overall study (N=1,694 versus
N=12,091). No association was observed with ischemic events, but again statistical power to identify such association was estimated at 24%. In addition the relationship between dabigatran concentrations and ischemic events may be weaker than for bleeding. Larger studies will be needed to confirm the absence of association.

The lack of association of peak concentrations SNP rs4148738 (ABCB1) with bleeding - and modest association of rs8192935 (CES1) - could be the result of insufficient statistical power or lower relevance of peaks for bleeding, as also observed for the factor Xa inhibitor edoxaban. Indeed, trough concentrations showed a stronger association with bleeding than peak concentrations, suggesting they are a better biological correlate of bleeding events. Lower trough concentrations may facilitate hemostatic plug formation at the site of vascular injury, thereby reducing the risk of bleeding. Further, the variability due sampling time differences is much smaller for trough compared with peak concentrations. While the CES1 SNP rs2244613 was associated at genome-wide significance with trough concentrations, the association of rs8192935 and rs4148738 with trough concentrations was modest. Finally, genetic variants might influence the response to dabigatran independently of pharmacokinetics, or influence the concentrations of active metabolite over time in ways not properly captured by peak and trough concentrations. The latter hypothesis is supported by the observation that the association of rs2244613 carrier status with bleeding was only slightly attenuated after adjustment for trough and peak concentrations. Stronger genetic associations with outcomes than predicted by intermediate markers are not uncommon. For example, a recent large-scale genetic analysis has shown that a 1 SD increase in low-density lipoprotein cholesterol (LDLc) from genetic variants is associated with an OR of 2.13 (95%CI 1.69-2.69) for myocardial infarction whereas the corresponding OR from an equivalent increase in LDLc is 1.54 (95%CI 1.45-1.63) 32.
Our study has a few potential limitations. First, our study was underpowered to detect the effect of modest exposure changes on the frequency of ischemic events. Second, reported effect sizes might be inflated by the “winner’s curse” effect. However, the stringent p-value threshold used should minimize this potential bias. Finally, only participants of European Caucasian ancestry could be adequately analyzed. Even though there is no reason to suspect different results in other populations \textit{a priori}, further studies in diverse populations will be needed. According to 1000 Genomes data (accessed July 2012), frequency of the C allele of rs2244613 varies from 15% in Europeans to 60% in Asians.

In conclusion, our study shows that the \textit{CES1} and \textit{ABCB1} loci are associated with dabigatran pharmacokinetics. The SNP with the largest effect on trough concentrations, rs2244613, was present in 32.8% of participants and was associated with bleeding. These results confirm the importance of these two loci in dabigatran metabolism and suggest that rs2244613 is a clinical determinant of systemic exposure in dabigatran-treated patients. Further studies will be needed to determine the association of rs2244613 with ischemic events. The magnitude of excess bleeding risk associated with rs2244613 is even greater than that seen when comparing the two doses of dabigatran tested in the RE-LY trial, raising the possibility that routine genotyping may enable clinicians to tailor the dose of dabigatran for individual patients and thereby optimize the balance between efficacy and safety.

\textbf{Acknowledgments:} Guillaume Pare, Niclas Eriksson, Thorsten Lehr, Claes Wadelius, and Mia Wadelius contributed to study design. Guillaume Pare, Thorsten Lehr, and Niclas Eriksson performed statistical analyses. Guillaume Pare wrote the manuscript. Guillaume Pare, John Eikelboom, Stuart Connolly, Salim Yusuf, Paul Reilly, Thorsten Lehr, Niclas Eriksson and Lars Wallentin contributed to interpretation of results. All contributed to data collection and critical review of the final report.
Funding Sources: The RELY study was funded by Boehringer Ingelheim Pharma Inc.

Conflict of Interest Disclosures: Dr. Pare reports receiving lecture fees from Boehringer Ingelheim; Dr. Connolly reports receiving consulting fees, lecture fees, and grant support from Boehringer Ingelheim; Dr. Eikelboom, consulting fees, lecture fees, and grant support from Boehringer Ingelheim, AstraZeneca, Sanofi-Aventis, and GlaxoSmithKline, consulting fees and lecture fees from Eisai Pharmaceuticals, Eli Lilly, and McNeil, and consulting fees from Bristol-Myers Squibb, Corgenix Medical Corporation, and Daiichi-Sankyo; Dr. Ezekowitz, consulting fees, lecture fees, and grant support from Boehringer Ingelheim and Aryx Therapeutics, consulting fees from Sanofi-Aventis, and lecture fees and grant support from Portola Pharmaceuticals; Dr. Yusuf, consulting fees, lecture fees and grant support from Boehringer Ingelheim and consulting fees from AstraZeneca, Bristol-Myers Squibb, and Sanofi-Aventis; Dr. Oldgren, consulting fees, lecture fees, and grant support from Boehringer Ingelheim and lecture fees from AstraZeneca; Drs. Lehr, Haertter, Reilly, and Zimdahl-Gelling report being employees of Boehringer Ingelheim; and Dr. Wallentin, consulting fees, lecture fees, and grant support from Boehringer Ingelheim, consulting fees from Regado and Athera, lecture fees from AstraZeneca, and Eli Lilly, and grant support from AstraZeneca, Bristol-Myers Squibb, GlaxoSmithKline, and Schering Plough.

References:


4. Liesenfeld KH, Lehr T, Dansirikul C, Reilly PA, Connolly SJ, Ezekowitz MD, Yusuf S,


10. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559-575.


### Table 1. Baseline Characteristics of Dabigatran-Treated Patients Enrolled in RELY-Genetics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Warfarin</th>
<th>Dabigatran 110 mg</th>
<th>Dabigatran 150 mg</th>
<th>Warfarin versus all dabigatran (p-value)</th>
<th>Dabigatran 110 mg versus dabigatran 150 mg (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>807</td>
<td>849</td>
<td>845</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td>72.2 (7)</td>
<td>71.7 (7.5)</td>
<td>71.9 (7.6)</td>
<td>0.23</td>
<td>0.58</td>
</tr>
<tr>
<td>Female (%)</td>
<td>273 (33.8%)</td>
<td>259 (30.5%)</td>
<td>272 (32.2%)</td>
<td>0.21</td>
<td>0.46</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.4 (5.6)</td>
<td>28.9 (5.7)</td>
<td>29.2 (5.3)</td>
<td>0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>CHADS2</td>
<td>2.0 (1.1)</td>
<td>2.0 (1.2)</td>
<td>2.0 (1.1)</td>
<td>0.85</td>
<td>0.53</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>134.9 (19)</td>
<td>132.6 (20)</td>
<td>134.2 (18)</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>78.1 (11)</td>
<td>77.4 (12)</td>
<td>78.7 (11)</td>
<td>0.89</td>
<td>0.02</td>
</tr>
<tr>
<td>History of Stroke (%)</td>
<td>83 (10.3%)</td>
<td>91 (10.7%)</td>
<td>77 (9.1%)</td>
<td>0.77</td>
<td>0.27</td>
</tr>
<tr>
<td>History of Diabetes (%)</td>
<td>158 (19.6%)</td>
<td>178 (21.0%)</td>
<td>159 (18.8%)</td>
<td>0.85</td>
<td>0.27</td>
</tr>
<tr>
<td>Aspirin Use (%)</td>
<td>228 (28.3%)</td>
<td>265 (31.2%)</td>
<td>234 (27.7%)</td>
<td>0.54</td>
<td>0.11</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min)</td>
<td>76.6 (26)</td>
<td>76.8 (28)</td>
<td>76.4 (27)</td>
<td>0.96</td>
<td>0.79</td>
</tr>
</tbody>
</table>

#### Dabigatran concentration

<table>
<thead>
<tr>
<th></th>
<th>Warfarin</th>
<th>Dabigatran 110 mg</th>
<th>Dabigatran 150 mg</th>
<th>Warfarin versus all dabigatran (p-value)</th>
<th>Dabigatran 110 mg versus dabigatran 150 mg (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number with measurements (%)</td>
<td>-</td>
<td>752 (88.6%)</td>
<td>738 (87.3%)</td>
<td>-</td>
<td>0.43</td>
</tr>
<tr>
<td>Trough Concentration (ng/mL)</td>
<td>-</td>
<td>73 (48)</td>
<td>105 (72)</td>
<td>-</td>
<td>1 × 10⁻²¹</td>
</tr>
<tr>
<td>Peak Concentration (ng/mL)</td>
<td>-</td>
<td>156 (94)</td>
<td>220 (133)</td>
<td>-</td>
<td>9 × 10⁻²⁵</td>
</tr>
</tbody>
</table>

#### Events

<table>
<thead>
<tr>
<th>Event</th>
<th>Warfarin</th>
<th>Dabigatran 110 mg</th>
<th>Dabigatran 150 mg</th>
<th>Warfarin versus all dabigatran (p-value)</th>
<th>Dabigatran 110 mg versus dabigatran 150 mg (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic Stroke or Systemic Emboli (%)</td>
<td>12 (1.5%)</td>
<td>15 (1.8%)</td>
<td>17 (2.0%)</td>
<td>0.48</td>
<td>0.71</td>
</tr>
<tr>
<td>Any Ischemic Event (%)</td>
<td>25 (3.1%)</td>
<td>34 (4.0%)</td>
<td>32 (3.8%)</td>
<td>0.32</td>
<td>0.82</td>
</tr>
<tr>
<td>Any Bleed (%)</td>
<td>325 (40.3%)</td>
<td>289 (34.0%)</td>
<td>298 (35.3%)</td>
<td>0.0064</td>
<td>0.60</td>
</tr>
<tr>
<td>Major Bleed (%)</td>
<td>45 (5.6%)</td>
<td>49 (5.8%)</td>
<td>52 (6.2%)</td>
<td>0.70</td>
<td>0.74</td>
</tr>
<tr>
<td>Minor Bleed (%)</td>
<td>306 (37.9%)</td>
<td>270 (31.8%)</td>
<td>275 (32.5%)</td>
<td>0.0046</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Quantitative traits are presented as mean (SD).
**Table 2.** Suggestive (P<5 x 10^{-7}) and Significant (P<9 x 10^{-8}) Genetic Associations with Dabigatran Concentrations

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chrom.</th>
<th>Position (bp)</th>
<th>Locus</th>
<th>Function</th>
<th>MAF (Allele)</th>
<th>H-W P</th>
<th>Fold Change per Minor Allele (95%CI)*</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak Concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4148738</td>
<td>7</td>
<td>87000985</td>
<td>ABCB1</td>
<td>Intron</td>
<td>0.45 (G)</td>
<td>0.06</td>
<td>1.12 (1.08-1.17)</td>
<td>8.2 x 10^{-8}</td>
</tr>
<tr>
<td>rs2235046</td>
<td>7</td>
<td>87012002</td>
<td>ABCB1</td>
<td>Intron</td>
<td>0.45 (A)</td>
<td>0.08</td>
<td>1.12 (1.07-1.17)</td>
<td>1.9 x 10^{-7}</td>
</tr>
<tr>
<td>rs1128503</td>
<td>7</td>
<td>87017537</td>
<td>ABCB1</td>
<td>Synonymous</td>
<td>0.44 (A)</td>
<td>0.09</td>
<td>1.12 (1.07-1.17)</td>
<td>2.3 x 10^{-7}</td>
</tr>
<tr>
<td>rs10276036</td>
<td>7</td>
<td>87018134</td>
<td>ABCB1</td>
<td>Intron</td>
<td>0.44 (G)</td>
<td>0.09</td>
<td>1.12 (1.07-1.17)</td>
<td>2.3 x 10^{-7}</td>
</tr>
<tr>
<td>rs1202169</td>
<td>7</td>
<td>87033786</td>
<td>ABCB1</td>
<td>Intron</td>
<td>0.44 (G)</td>
<td>0.11</td>
<td>1.12 (1.07-1.17)</td>
<td>3.2 x 10^{-7}</td>
</tr>
<tr>
<td>rs1202168</td>
<td>7</td>
<td>87033898</td>
<td>ABCB1</td>
<td>Intron</td>
<td>0.44 (A)</td>
<td>0.11</td>
<td>1.12 (1.07-1.17)</td>
<td>3.2 x 10^{-7}</td>
</tr>
<tr>
<td>rs1202167</td>
<td>7</td>
<td>87034995</td>
<td>ABCB1</td>
<td>Intron</td>
<td>0.44 (A)</td>
<td>0.12</td>
<td>1.12 (1.07-1.17)</td>
<td>3.2 x 10^{-7}</td>
</tr>
<tr>
<td>rs8192935</td>
<td>16</td>
<td>54419295</td>
<td>CES1</td>
<td>Intron</td>
<td>0.33 (A)</td>
<td>0.86</td>
<td>0.88 (0.84-0.92)</td>
<td>3.2 x 10^{-8}</td>
</tr>
<tr>
<td><strong>Trough Concentration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4580160</td>
<td>16</td>
<td>54326141</td>
<td>CES1P2</td>
<td>Intron</td>
<td>0.30 (A)</td>
<td>0.20</td>
<td>0.88 (0.84-0.92)</td>
<td>1.7 x 10^{-8}</td>
</tr>
<tr>
<td>rs4784563</td>
<td>16</td>
<td>54333986</td>
<td>CES1P2</td>
<td>Intron</td>
<td>0.27 (C)</td>
<td>0.24</td>
<td>0.88 (0.84-0.92)</td>
<td>2.3 x 10^{-7}</td>
</tr>
<tr>
<td>rs2244613</td>
<td>16</td>
<td>54402110</td>
<td>CES1</td>
<td>Intron</td>
<td>0.18 (C)</td>
<td>0.07</td>
<td>0.85 (0.81-0.90)</td>
<td>1.2 x 10^{-8}</td>
</tr>
<tr>
<td>rs4122238</td>
<td>16</td>
<td>54414218</td>
<td>CES1</td>
<td>Intron</td>
<td>0.15 (A)</td>
<td>0.02</td>
<td>0.86 (0.81-0.91)</td>
<td>3.5 x 10^{-7}</td>
</tr>
<tr>
<td>rs8192935</td>
<td>16</td>
<td>54412925</td>
<td>CES1</td>
<td>Intron</td>
<td>0.33 (A)</td>
<td>0.86</td>
<td>0.89 (0.85-0.93)</td>
<td>1.2 x 10^{-7}</td>
</tr>
</tbody>
</table>

Chrom.: Chromosome; MAF: Minor allele frequency; H-W P: Hardy-Weinberg P-Value; Synonymous: Coding synonymous SNP.

Bolded SNPs represent lead SNPs at each locus.

*Fold change in residualized dabigatran concentration.
Table 3. Association of Lead SNPs with Bleeding and Ischemic events in Dabigatran-treated Participants

<table>
<thead>
<tr>
<th>Event</th>
<th>rs4148738* (ABCBI; Peak concentration)</th>
<th>rs8192935* (CESI; Peak concentration)</th>
<th>rs2244613* (CESI; Trough concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)†</td>
<td>P</td>
<td>OR (95%CI)†</td>
</tr>
<tr>
<td>Ischemic Stroke or Systemic embolism</td>
<td>0.88(0.53-1.46)</td>
<td>0.62</td>
<td>0.76(0.43-1.34)</td>
</tr>
<tr>
<td>Any Ischemic Event</td>
<td>0.98(0.69-1.40)</td>
<td>0.92</td>
<td>1.04(0.72-1.51)</td>
</tr>
<tr>
<td>Any Bleeding</td>
<td>0.94(0.82-1.09)</td>
<td>0.44</td>
<td>0.89(0.76-1.03)</td>
</tr>
<tr>
<td>Major Bleeding</td>
<td>1.14(0.85-1.52)</td>
<td>0.40</td>
<td>0.88(0.64-1.21)</td>
</tr>
<tr>
<td>Minor Bleeding</td>
<td>0.94(0.81-1.09)</td>
<td>0.38</td>
<td>0.89(0.76-1.05)</td>
</tr>
</tbody>
</table>

*SNP (candidate locus; original association with dabigatran concentration)

†Odds ratio per minor allele

Significant (P<0.05) results are bolded. Association of SNPs with events was performed using logistic regression assuming an additive genetic model. Logistic regression models included age, sex, dabigatran dose, CHADS2 score, use of aspirin, log(Cockcroft-Gault creatinine clearance), and the first ten genetic principal components as independent variables.
Figure Legends:

**Figure 1.** A. Manhattan plot of association with peak dabigatran concentrations. B. Manhattan plot of association with trough dabigatran concentrations. SNPs are shown according to their physical location and $-\log_{10} p$-values for association. The black line represents the genome-wide significance threshold of $9 \times 10^{-8}$.

**Figure 2.** Regional association plots. A. Genetic associations with trough concentrations at the $CESI$ locus. B. Genetic associations with peak concentrations at the $ABCB1$ locus. C. Genetic associations with peak concentrations at the $CES1$ locus. SNPs are shown according to their physical location and $-\log_{10} p$-values for association. Also shown is the recombination rate in cM/Mb (blue line) and the linkage disequilibrium ($r^2$) of each SNP with the SNP having the lowest P-value.

**Figure 3.** Kaplan-Meier survival curves of bleeding according to rs2244613 ($CES1$) carrier status.
Figure 1A
Figure 1B
Figure 2A
Figure 2B
Figure 2C
Figure 3

Freedom from Bleed
According to rs2244613 Carrier Status

Dabigatran only: HR=0.70, 95% CI 0.58–0.84, P=0.00016
Warfarin only: HR=1.13, 95% CI 0.90–1.42, P=0.29
Carrier Status X Treatment Interaction P=0.0015

<table>
<thead>
<tr>
<th></th>
<th>Carriers/Dabigatran (N events=154)</th>
<th>Noncarriers/Dabigatran (N events=432)</th>
<th>Carriers/Warfarin (N events=110)</th>
<th>Noncarriers/Warfarin (N events=215)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. at Risk</td>
<td>553</td>
<td>1139</td>
<td>257</td>
<td>549</td>
</tr>
<tr>
<td>Days After Randomization</td>
<td>465</td>
<td>911</td>
<td>204</td>
<td>451</td>
</tr>
<tr>
<td></td>
<td>434</td>
<td>821</td>
<td>175</td>
<td>387</td>
</tr>
<tr>
<td></td>
<td>314</td>
<td>587</td>
<td>124</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td>178</td>
<td>335</td>
<td>71</td>
<td>179</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>72</td>
<td>15</td>
<td>36</td>
</tr>
</tbody>
</table>
Genetic Determinants of Dabigatran Plasma Levels and Their Relation to Bleeding
Guillaume Paré, Niclas Eriksson, Thorsten Lehr, Stuart Connolly, John Eikelboom, Michael D. Ezekowitz, Tomas Axelsson, Sebastian Haertter, Jonas Oldgren, Paul Reilly, Agneta Siegbahn, Ann-Christine Syvänen, Claes Wadelius, Mia Wadelius, Heike Zimdahl-Gelling, Salim Yusuf and Lars Wallentin

Circulation. published online March 6, 2013;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2013/03/05/CIRCULATIONAHA.112.001233

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2013/03/05/CIRCULATIONAHA.112.001233.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at: http://circ.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL
**Supplementary Table 1.** Association results of lead SNPs in all RE-LY genetic participants.

<table>
<thead>
<tr>
<th>SNP</th>
<th>European Caucasians (N=1,694)</th>
<th>Latin American (N=66)</th>
<th>Other/Undetermined (N=190)</th>
<th>All (N=1,950)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rs2244613 (CES1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentrations⁠</td>
<td>0.89 (0.84-0.95), P=0.00018</td>
<td>1.06 (0.81-1.38), P=0.70</td>
<td>0.86 (0.73-1.01), P=0.063</td>
<td>0.89 (0.84-0.94), P=4e-05</td>
</tr>
<tr>
<td>Trough concentrations⁠</td>
<td>0.86 (0.81-0.92), P=1.7e-06</td>
<td>0.95 (0.69-1.30), P=0.76</td>
<td>0.87 (0.72-1.05), P=0.14</td>
<td>0.86 (0.82-0.91), P=4.9e-07</td>
</tr>
<tr>
<td>Any bleeding⁠</td>
<td>0.67 (0.55-0.81), P=4.2e-05</td>
<td>0.69 (0.20-2.34), P=0.55</td>
<td>0.96 (0.59-1.56), P=0.87</td>
<td>0.70 (0.58-0.83), P=6.2e-05</td>
</tr>
<tr>
<td>Major bleeding⁠</td>
<td>0.66 (0.43-1.01), P=0.054</td>
<td>0.25 (0.01-6.31), P=0.40</td>
<td>2.40 (0.96-6.00), P=0.062</td>
<td>0.79 (0.55-1.14), P=0.21</td>
</tr>
<tr>
<td><strong>rs4148738 (ABCB1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentrations⁠</td>
<td>1.11 (1.06-1.17), P=6.6e-06</td>
<td>0.82 (0.65-1.04), P=0.10</td>
<td>1.14 (0.98-1.33), P=0.10</td>
<td>1.10 (1.06-1.15), P=1e-05</td>
</tr>
<tr>
<td>Trough concentrations⁠</td>
<td>1.07 (1.02-1.12), P=0.006</td>
<td>0.88 (0.66-1.17), P=0.37</td>
<td>1.04 (0.87-1.24), P=0.69</td>
<td>1.06 (1.01-1.11), P=0.014</td>
</tr>
<tr>
<td>Any bleeding⁠</td>
<td>0.94 (0.82-1.09), P=0.43</td>
<td>1.45 (0.51-4.16), P=0.49</td>
<td>0.62 (0.38-0.99), P=0.045</td>
<td>0.92 (0.80-1.05), P=0.21</td>
</tr>
<tr>
<td>Major bleeding⁠</td>
<td>1.17 (0.88-1.55), P=0.29</td>
<td>0.40 (0.045-3.65), P=0.42</td>
<td>1.58 (0.65-3.84), P=0.31</td>
<td>1.17 (0.89-1.53), P=0.25</td>
</tr>
<tr>
<td><strong>rs8192935 (CES1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentrations⁠</td>
<td>0.88 (0.84-0.93), P=1.0e-06</td>
<td>0.98 (0.78-1.23), P=0.87</td>
<td>0.95 (0.81-1.11), P=0.49</td>
<td>0.89 (0.85-0.93), P=9.5e-07</td>
</tr>
<tr>
<td>Trough concentrations⁠</td>
<td>0.88 (0.84-0.93), P=1.7e-06</td>
<td>0.92 (0.70-1.20), P=0.52</td>
<td>0.97 (0.81-1.15), P=0.70</td>
<td>0.89 (0.85-0.93), P=2.3e-06</td>
</tr>
<tr>
<td>Any bleeding⁠</td>
<td>0.87 (0.75-1.02), P=0.08</td>
<td>0.70 (0.28-1.77), P=0.45</td>
<td>0.74 (0.46-1.18), P=0.20</td>
<td>0.85 (0.74-0.98), P=0.03</td>
</tr>
<tr>
<td>Major bleeding⁠</td>
<td>0.88 (0.65-1.20), P=0.42</td>
<td>1.17 (0.20-6.66), P=0.86</td>
<td>1.26 (0.50-3.18), P=0.62</td>
<td>0.92 (0.69-1.22), P=0.56</td>
</tr>
</tbody>
</table>

All results are for dabigatran-treated patients only.

¹Fold-change (95%CI) per minor allele, adjusted for age, sex and reported ancestry (when appropriate) only.
²Odds ratio (95%CI) per minor allele, adjusted for age, sex and reported ancestry (when appropriate) only.
³Based on the following self-reported ancestries: Arab (N=1), Black African (N=5), Chinese (N=21), Colored African (N=1), Japanese (N=4), Other Asian (N=15), South Asian (N=9), and undetermined (N=134).
**Supplementary Table 2.** Associations of lead SNPs with peak and trough concentrations in participants on dabigatran 110 mg BID and 150 mg BID.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Locus</th>
<th>Phenotype</th>
<th>Dabigatran 110 mg bid</th>
<th>Dabigatran 150 mg bid</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fold Change(^\d) (95% CI)</td>
<td>P-value</td>
<td>Fold-Change(^\d) (95% CI)</td>
</tr>
<tr>
<td>rs2244613</td>
<td>CES1</td>
<td>Trough Concentration</td>
<td>0.82(0.76-0.89)</td>
<td>2x10(^{-6})</td>
<td>0.89(0.83-0.95)</td>
</tr>
<tr>
<td>rs4148738</td>
<td>ABCB1</td>
<td>Peak Concentration</td>
<td>1.12(1.05-1.19)</td>
<td>3x10(^{-4})</td>
<td>1.13(1.07-1.20)</td>
</tr>
<tr>
<td>rs8192935</td>
<td>CES1</td>
<td>Peak Concentration</td>
<td>0.86(0.81-0.91)</td>
<td>2x10(^{-6})</td>
<td>0.90(0.85-0.96)</td>
</tr>
</tbody>
</table>

\(^\d\)Fold change per minor allele in adjusted dabigatran concentration.
**Supplementary Table 3.** Influence of genetic polymorphisms on pharmacokinetic parameters estimated from mixed-effect models.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Univariate Analysis</th>
<th></th>
<th>Univariate Analysis</th>
<th></th>
<th>Univariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bioavailability</td>
<td>Clearance</td>
<td>Volume of distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Effect $^{5}$</td>
<td>P-Value</td>
<td>Effect $^{5}$</td>
<td>P-Value</td>
<td>Effect $^{5}$</td>
</tr>
<tr>
<td>rs4148738</td>
<td>+10.2%</td>
<td>5.8*10^{-8}</td>
<td>-4.4%</td>
<td>3.1*10^{-3}</td>
<td>+0.9%</td>
</tr>
<tr>
<td>(ABCB1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs8192935</td>
<td>-12.0%</td>
<td>2.5*10^{-13}</td>
<td>+6.2%</td>
<td>8.7*10^{-7}</td>
<td>+0.0%</td>
</tr>
<tr>
<td>(CES1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2244613</td>
<td>-12.6%</td>
<td>2.3*10^{-10}</td>
<td>+6.7%</td>
<td>7.4*10^{-6}</td>
<td>-0.8%</td>
</tr>
<tr>
<td>(CES1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{5}$ percent change per minor allele
**Supplementary Table 4.** Association of rs2244613 with Bleeding and Ischemic events in each treatment group.

<table>
<thead>
<tr>
<th>Event</th>
<th>Dabigatran 110 mg</th>
<th>Dabigatran 150 mg</th>
<th>All dabigatran</th>
<th>Warfarin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR(95% C)</td>
<td>P-value</td>
<td>OR(95% C)</td>
<td>p-value</td>
</tr>
<tr>
<td>Any bleed</td>
<td>0.69(0.53-0.91)</td>
<td>0.009</td>
<td>0.64(0.49-0.85)</td>
<td>0.0018</td>
</tr>
<tr>
<td>Major bleed</td>
<td>0.54(0.28-1.02)</td>
<td>0.059</td>
<td>0.79(0.45-1.38)</td>
<td>0.40</td>
</tr>
<tr>
<td>Ischemic Stroke or Systemic Emboli</td>
<td>0.68(0.24-1.97)</td>
<td>0.48</td>
<td>0.63(0.22-1.79)</td>
<td>0.39</td>
</tr>
<tr>
<td>Any Ischemic Event</td>
<td>0.76(0.38-1.51)</td>
<td>0.43</td>
<td>1.21(0.65-2.23)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Association of rs2244613 with events was performed using logistic regression assuming an additive genetic model. Logistic regression models included age and sex (only) as independent variables.
### Supplementary Table 5. Multivariate analysis of trough dabigatran concentration and risk of bleed.

<table>
<thead>
<tr>
<th></th>
<th>Variance in trough concentration explained (%)</th>
<th>Fold-change in trough concentration (per SD or exposure yes/no)</th>
<th>OR for bleed (per SD or exposure yes/no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.1%</td>
<td>1.03 (0.99-1.07)</td>
<td>1.35 (1.17-1.55)*</td>
</tr>
<tr>
<td>Female (yes/no)</td>
<td>0.3%</td>
<td>1.07 (1.00-1.14)</td>
<td>0.86 (0.68-1.10)</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min)</td>
<td>13.1%</td>
<td>0.71 (0.68-0.75)*</td>
<td>0.90 (0.76-1.07)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>2.8%</td>
<td>1.12 (1.08-1.16)</td>
<td>1.04 (0.92-1.19)</td>
</tr>
<tr>
<td>P-glycoprotein Inhibitor (yes/no)</td>
<td>0.3%</td>
<td>1.08 (1.00-1.16)*</td>
<td>1.10 (0.85-1.42)</td>
</tr>
<tr>
<td>Proton Pump Inhibitor (yes/no)</td>
<td>0.9%</td>
<td>0.85 (0.77-0.93)*</td>
<td>1.43 (1.05-1.94)*</td>
</tr>
<tr>
<td>rs2244613 Carrier (yes/no)</td>
<td>1.8%</td>
<td>0.85 (0.80-0.91)*</td>
<td>0.62 (0.49-0.79)*</td>
</tr>
<tr>
<td>Dabigatran 150 mg versus 110 mg (yes/no)</td>
<td>9.5%</td>
<td>1.44 (1.35-1.53)*</td>
<td>0.99 (0.80-1.23)</td>
</tr>
</tbody>
</table>

*: P<0.05. 1 SD for age = 7.5 years, 1 SD for creatinine clearance = 27.5 mL/min, and 1 SD for BMI = 5.5 kg/m². Frequency of P-glycoprotein inhibitor use was 21.3%, frequency of proton pump inhibitor use was 13.0%, and carrier frequency of rs2244613 was 32.7%.
Supplementary Figure 1.

A) Quantile-Quantile plot of peak concentrations association.
B) Quantile-Quantile plot of trough concentrations association.
Supplementary Figure 2. Simulated median plasma concentration-time profiles at steady-state for 150 mg dabigatran etexilate twice daily according to genotype combination.

Simulated plasma concentration-time profiles at steady-state for 150 mg dabigatran etexilate twice daily. In a multivariate analysis considering all three SNPs simultaneously, only an effect on bioavailability was significant. The effect per minor allele was +10.1%, -9.3% and -5.5% for rs4148738, rs8192935 and rs2244613, respectively. Solid line and shaded area represents the predicted median time profile and the 10th and 90th percentile of a typical RE-LY participant with no minor allele. Broken lines illustrate median time profiles of patients with 0 or 2 minor allele of each SNPs.
Supplementary Figure 3. Kaplan-Meier survival curves of primary efficacy endpoint according to rs2244613 (CES1) carrier status.

**Freedom from Primary Efficacy Endpoint According to rs2244613 Carrier Status**

Dabigatran only: HR=0.48, 95%CI 0.20–1.17, P=0.11
Warfarin only: HR=0.44, 95%CI 0.097–2.02, P=0.29
Carrier Status X Treatment Interaction P=0.93

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>Days After Randomization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers Dabigatran</td>
<td>553</td>
</tr>
<tr>
<td>Noncarriers Dabigatran</td>
<td>1139</td>
</tr>
<tr>
<td>Carriers Warfarin</td>
<td>257</td>
</tr>
<tr>
<td>Noncarriers Warfarin</td>
<td>549</td>
</tr>
</tbody>
</table>