Supplementary Online Content

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SUPPLEMENTARY METHODS

Determination of bleeding phenotype in the discovery and replication cohort

The goal of this study was to identify genetic variation associated with bleeding risk; hence a phenotype that may have less influence from environmental causes of warfarin-related bleeding was chosen. At INRs of greater than 4, it is reasonable to assume that non-genomic factors (e.g. drug-drug interactions, organ dysfunction, accidental overdose) may have a greater impact on bleeding risk than genomic factors. Thus, the cases were limited to those that bled at INRs under 4 to provide a potential enrichment for people with a genetic predisposition to bleed. Additionally, the bleeding definition outlined below mirrors the adverse event definition in several warfarin pharmacogenomic clinical trials [1-3] which report INRs > 4 as an adverse drug reaction (ADR). Therefore, bleeding that occurred outside of this ADR definition was chosen, with the hypothesis that people who bleed at INR<4 may have a genetic predisposition to risk.

The Electronic Heath records (EHR) of participants from the International Warfarin Consortium recruited at the University of Chicago and the University of Illinois were manually curated to determine if warfarin-treated patients had a bleeding event at INR<4 (details related to the inclusion and exclusion criteria below). These subjects constituted the discovery cohort. To identify the replication cohort, the Clinical Research Data Warehouse at University of Chicago was first queried for African-American patients with warfarin listed within their current medications. Then, using the algorithm described in Cunningham *et al.*, which developed an automated case definition for warfarin-related bleeds,[4] a second query was conducted to identify potential warfarin-related bleeds. Briefly, bleeding-related hospitalizations were classified according to the probable site of the bleeding, based on the primary diagnosis code of the hospitalization. The algorithm did not consider hospitalizations in which there is no primary diagnosis associated with bleeding. This second query identified African-Americans on warfarin with the presence of the pre-specified ICD9 codes associated with warfarin-related bleeds. The algorithm included ICD9 codes associated with site of bleeding (which was confirmed via manual review) and excluded bleeding related to major trauma. After identification of a potential cohort, warfarin associated bleeding events were confirmed by manual chart review. In both cohorts, patients on warfarin with major bleeds occurring at an INR<4 were considered as cases while controls were patients with no documented bleeds while on warfarin therapy. Major bleeding was defined as bleeding requiring hospitalization and/or causing a decrease in hemoglobin level of >2 g/dL and/or requiring blood transfusion. Sixteen

subjects from the discovery cohort and 12 subjects from the replication cohort were excluded for bleeds that occurred at INRs > 4. Electronic medical records were reviewed retrospectively from 2014 to 2016 to obtain clinical variables and bleeding events data. If determined to have a bleed while on warfarin, all relevant clinical data at the time of the bleeding event were also collected. Since a subset of the discovery cohort and the complete replication cohort were recruited at University of Chicago, the medical record numbers of the patients were matched to prevent any sample overlap. All participants had at least 1 year of clinical data available from the warfarin initiation date. If patients died from a fatal warfarin-related bleed within one year of warfarin initiation, they were retained as cases in the analysis. INR at the time of bleed, data on potential bleeding risk factors and warfarin maintenance dose, defined as the same dose for at least three consecutive clinic visits that produced INRs within the therapeutic range, were collected. Patients with active malignancy, bleeding due to major trauma, or an INR≥4 at the time of bleed were excluded.

Quality control and SNP imputation

SNPs were excluded based on genotyping rate <95%, minor allele frequency <5%,[5] and failed Hardy-Weinberg equilibrium tests p <0.00001. SNPs were also excluded if they were: A/T or C/G SNPs to eliminate flip-strand issues, SNPs on the X and Y chromosomes or mitochondrial SNPs. Genome-wide genotype data was used to validate gender and identity-by-descent. No sample had a call rate of <95%, missingness >0.05, gender misspecification, or IBD >0.125. Additionally, principal components 1 and 2 were used to confirm ancestry of all individuals with any outliers removed. Genotypes were phased using SHAPEIT and imputed with IMPUTE2 using reference files from the 1,000 Genomes haplotypes -- Phase I integrated variant set release (v3) in NCBI build 37 (hg19) coordinates. [6-8] Post imputation quality control involved exclusion of SNPs if the minor allele frequency was <0.05, imputation quality <0.8, and failed Hardy-Weinberg equilibrium tests p <0.00001.

Bleeding risk prediction

Individual bleeding risk was evaluated by HAS-BLED (uncontrolled hypertension, abnormal renal and liver function, stroke, bleeding tendency or predisposition, labile INR, age >65 years, antiplatelet agents, NSAIDs or alcohol use) scoring at the time of warfarin initiation in the replication cohort. The criteria for HAS-BLED scoring involves assigning one point for each of the risk factors: hypertension (uncontrolled, >160 mmHg systolic), abnormal renal function (dialysis, transplant, Cr >2.6 mg/dL or >200 µmol/L), liver function (cirrhosis or bilirubin >2x normal or

AST/ALT/AP >3x normal), stroke, prior major bleeding, older age (>65 years), medication usage predisposing to bleeding (antiplatelet agents, NSAIDs), and alcohol use (≥ 8 drinks/week). The discovery cohort could not be scored as sufficient clinical details at warfarin initiation were not available. Since the aim of this analysis was to evaluate the predictive accuracy of HAS-BLED in determining bleeding risk prior to anticoagulation therapy, labile INR was scored zero, as done previously in studies predicting bleeding risk with anticoagulant initiation.[9,10]

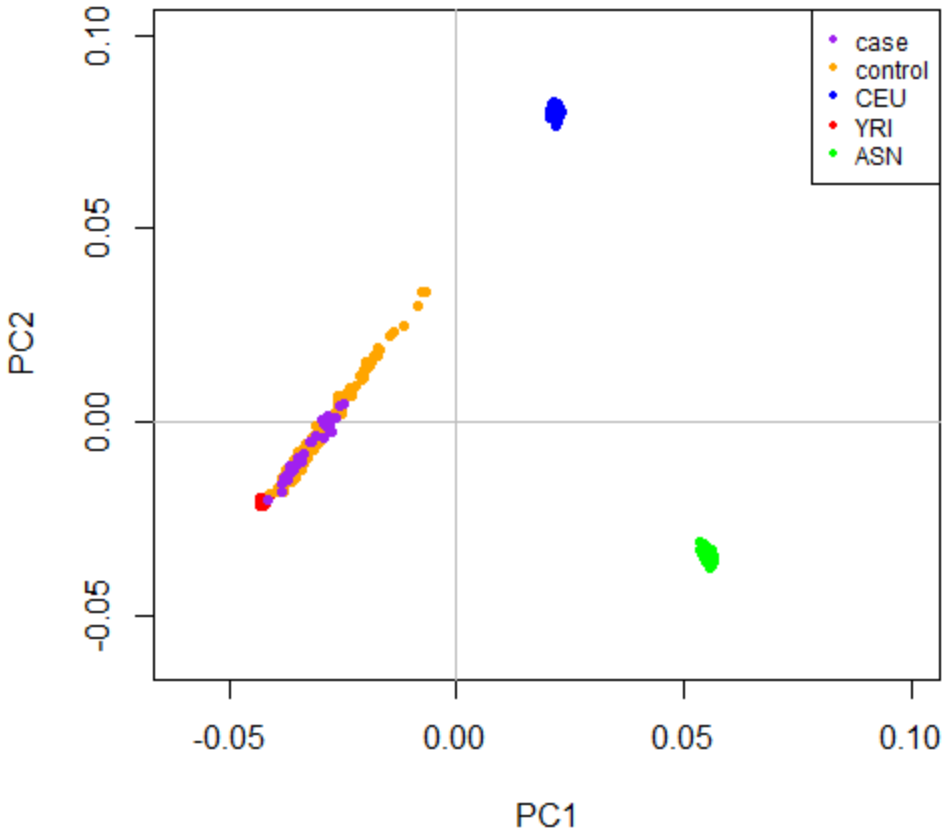
Patients were also scored for ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation). The ATRIA scoring involves assigning 3 points for anemia or severe renal disease, 2 points for age ≥ 75 years, and 1 point each for prior hemorrhage and diagnosed hypertension. The comparison of model performance between ATRIA and HAS-BLED is shown in eTable 6.

Statistical Analyses

Potential population stratification was examined in the discovery cohort by principal component (PC) analysis using PLINK 1.9[11] with an LD-pruned (r^2>0.2) set of 185,139 markers. A quantile-quantile (Q-Q) plot of expected and observed p-values revealed no evidence for systematic genotype calling error, and the genomic inflation factor (based on median chi-squared) was 1.014 indicating sufficient control for possible population stratification (eFigure 2). Covariates such as age, weight, gender, stable warfarin dose, INR at stable warfarin dose, abnormal renal function, and the first ten principal components were tested as single covariates for association with warfarin-related bleeding risk. GWAS analysis was adjusted for abnormal renal function ($p<0.0001$) principal component 1 ($p=0.016$) and principal component 2 ($p=0.015$). No other PCs showed association to the bleeding phenotype. Association analysis in the replication cohort was adjusted to HAS-BLED. HAS-BLED was assessed both as quantitative and dichotomized variable. The difference in the mean HAS-BLED score between cases and controls was determined and compared. As dichotomous variable, patients were stratified into low risk (score<3) and high risk (score≥3). Since gastrointestinal tract was the most common site of bleeding, the predictive performance of HAS-BLED was also tested for GI bleeding. Model calibration was assessed by Hosmer-Lemeshow goodness-of-fit statistics, which supported an appropriate model fit of HAS-BLED and HAS-BLED+SNP ($p>0.05$). A p value of less than 0.05 indicates a significant difference between the predicted and observed bleeding events and is considered lack of model fit. C-statistics of SNP+HAS-BLED was compared with that of only HAS-BLED using the DeLong non-parametric method.[12] Comparison of c-indices to evaluate the prediction increment of a new biomarker when added to an existing model has been the

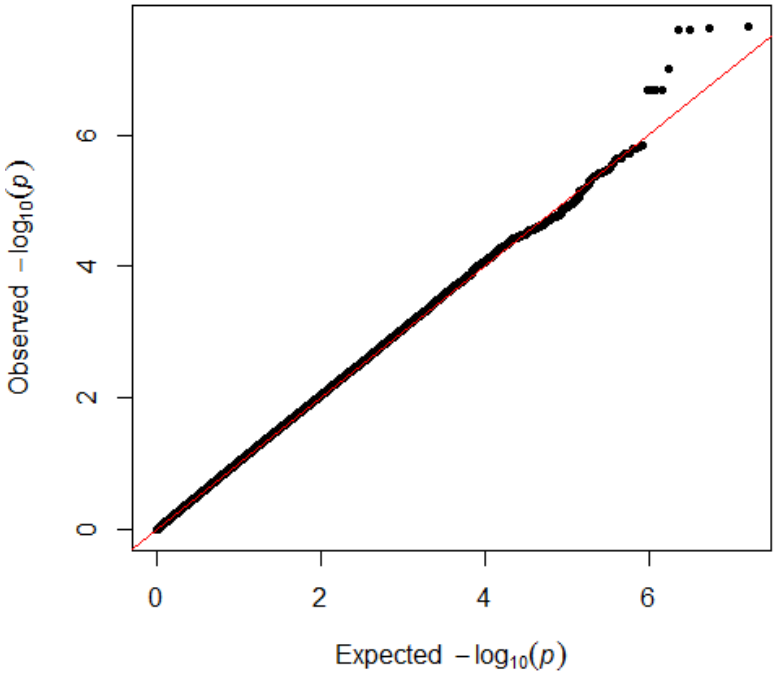
commonly used performance measure. However, several studies have suggested reclassification analysis to be of greater clinical importance.[40,41] Therefore, improvement in predictive accuracy was evaluated by calculating the net reclassification improvement (NRI) and integrated discrimination improvement (IDI), which is a measure of how well subjects with and without the outcome are discriminated by the new model compared to the old.[13]

eFigure 1. Principal component analysis of the Discovery Cohort and three HapMap polulations



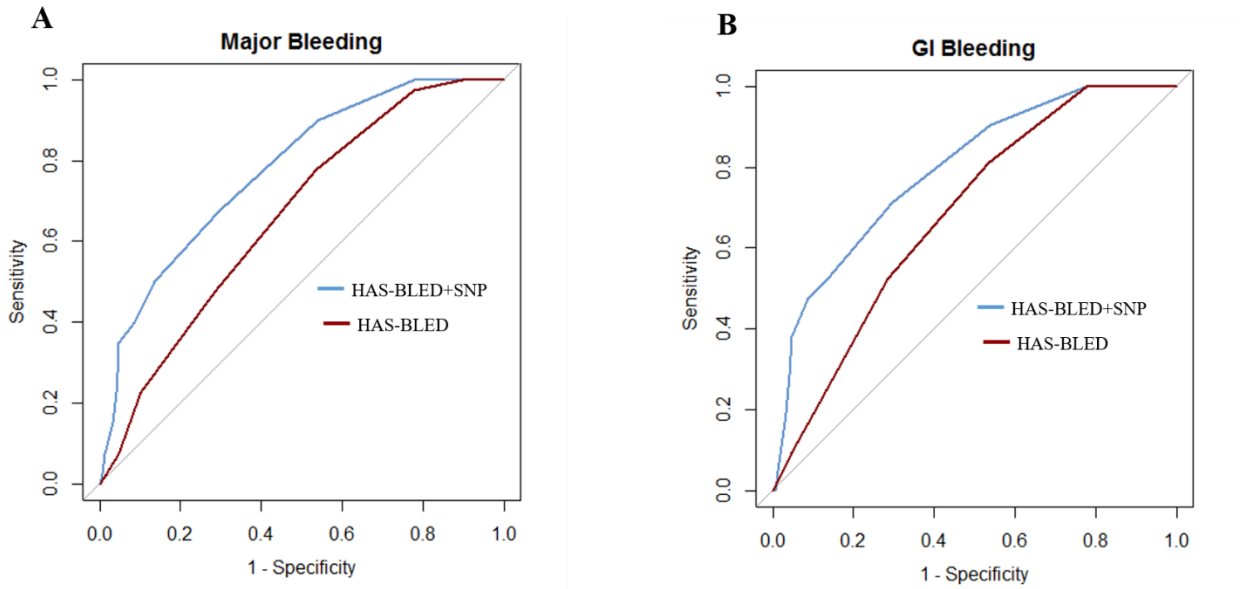
Principal components analysis was performed using the LD-pruned genome-wide SNP genotypes of discovery Cohort (cases shown in purple and controls shown in orange) and the three HapMap populations, CEU (Utah Residents with Northern and Western European Ancestry, shown in blue), YRI (Yoruba in Ibadan, Nigeria, shown in red) and ASN (Asian, shown in green) to infer continuous axes of genetic variation. The analysis accounts for population substructure, i.e. population-specific variations in allele distribution of the SNPs under investigation. The first two principal components of genetic ancestry (PC1 and PC2) shows that the HAPMAP populations formed three distinct clustures and the African-American discovery cohort aligned between the CEU and YRI as expected. Sample size: cases = 31, controls = 184, CEU= 112, YRI= 113, ASN= 170.

eFigure 2. Quantile-quantile (QQ) plot of the Discovery Cohort



Quantile-Quantile (QQ) plot showing observed (y-axis) vs expected (x-axis) p-values for bleeding risk from warfarin therapy. Genomic inflation factor (λ) = 1.014

eFigure 3. Receiver operating curve (ROC) for HAS-BLED+SNP vs. HAS-BLED for A) Major bleeding, B) Gastro-intestinal (GI) bleeding in the replication cohort

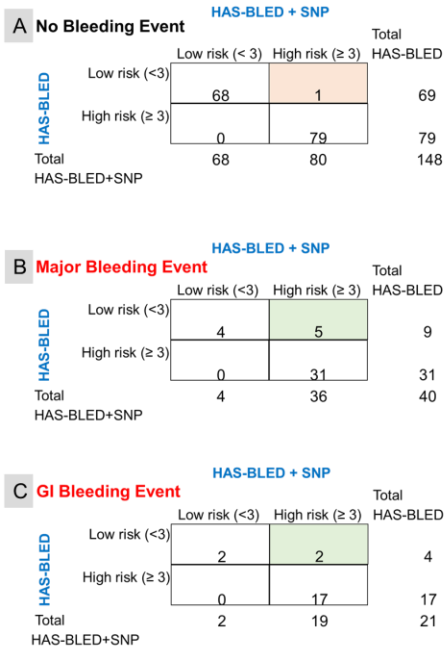


HAS-BLED: hypertension, abnormal renal/liver function, stroke, bleeding history, labile INR, elderly, antiplatelet agents, NSAIDs/alcohol use

SNP: single nucleotide polymorphism

Major bleeding was defined as bleeding requiring hospitalization and/or causing a decrease in hemoglobin level of >2 g/dL and/or requiring blood transfusion

eFigure 4: Predictive accuracy of HAS-BLED and HAS-BLED+SNP for detection of major bleeding and gastrointestinal (GI) bleeding in the replication cohort



D

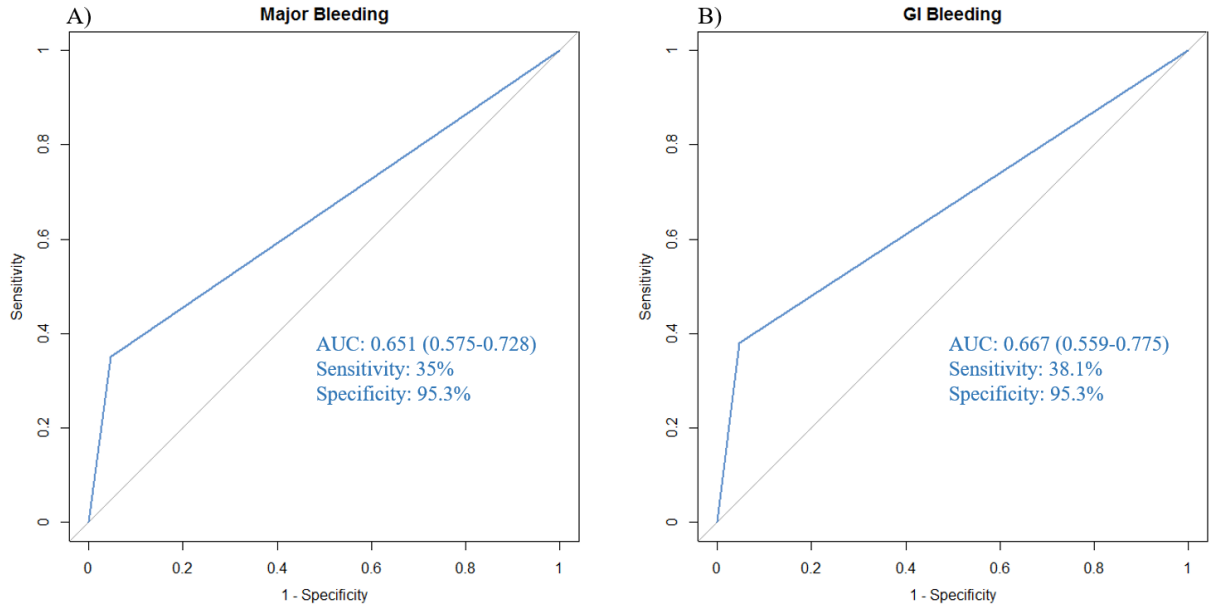
	Models	C-statistics (95%CI)	Difference in C-statistics (95%CI)	p value	Major bleeding Correctly Reclassified n(%)	No bleeding Correctly Reclassified n(%)	%NRI	p value	%IDI	p value
Major Bleeding	HAS-BLED	0.67 (0.59-0.74)	0.11 (0.10-0.12)	0.003	5 (12.5)	-1(-0.7)	11.8	0.024	13.6	0.00048
	HAS-BLED + SNP	0.78 (0.70-0.85)								
GI Bleeding	HAS-BLED	0.67 (0.58-0.76)	0.14 (0.13-0.15)	0.01	2 (9.5)	-1 (- 0.7)	8	0.16	12	0.0079
	HAS-BLED + SNP	0.79 (0.69-0.89)								

Incorrect reclassification
Correct reclassification
No reclassification

NRI = Net reclassification improvement
IDI = Integrated discrimination improvement
CI- Confidence interval
HAS-BLED: hypertension, abnormal renal/liver function, stroke, bleeding history, labile INR, elderly, drugs/alcohol
Major bleeding was defined as bleeding requiring hospitalization and/or causing a decrease in hemoglobin level of >2 g/dL and/or requiring blood transfusion

The figure shows the reclassification table for A) No bleeding event, B) Major bleeding event and C) Gastrointestional (GI) bleeding event in the replication cohort. Table D details the predictive performance of HAS-BLED+SNP versus HAS-BLED by C-statistics. Difference in the performance of the two m odels (HAS-BLED+SNP versus HAS-BLED) was evaulated by difference in C-statistics (95% CI), NRI and IDI, and the associated p values for each of these metrics.

eFigure 5. Receiver operating curves (ROC) for rs78132896 single nucleotide polymorphism (SNP) A) Major bleeding, B) GI bleeding in the replication cohort



AUC- Area under the curve

Major bleeding was defined as bleeding requiring hospitalization and/or causing a decrease in hemoglobin level of >2 g/dL and/or requiring blood transfusion

eTable1. Warfarin-related bleeding events at INR >4 by sites in the discovery and the replication cohort cases.

Bleeding events by site n (%)*	**Discovery cohort cases**	**Replication cohort cases**
Gastrointestinal	19 (61.3)	21 (52.5)
Genitourinary	7 (22.6)	4 (10)
Epistaxis	2 (6.4)	10 (25)
Esophageal	1 (3.2)	1 (2.5)
Cerebral/ICH	1 (3.2)	2 (5)
Hemoptysis	1 (3.2)	1 (2.5)
Hematoma	-	1 (2.5)
>1 major bleeding event n (%)	10 (32)	14 (35)

* Denotes first site of bleeding if multiple bleeds occurred.

eTable 2. Genome Wide Annotation of Variants (GWAVA) scores for the significant SNPs

ID	Classifiers*		
	Region score	Transcription start site score	Unmatched score
rs114504854	0.45	0.22	0.21
rs78132896[#]	0.44	0.69	0.9
rs16871327[#]	0.42	0.32	0.29
rs115112393	0.44	0.24	0.02

*Classifiers range from 0-1 with higher scores indicating variants predicted as more likely to be functional

[#]SNPs were prioritized for haplotype vector construction based on their high transcription start site score and unmatched score, as the region scores were nearly identical for all SNPs

Region score: The score is based on the SNP's disruption/ introduction of a DNAse hypersensitive site.

Transcription start site score: The score is based on the proximity of the SNP to the transcription start site

Unmatched score: The score is based on the matching of control SNPs that do not have regulatory function to the associated SNPs, with higher unmatched score predicting SNPs that are more likely functional.

eTable 3. Distribution of HAS-BLED scores for major bleeding outcome in the replication cohort

HAS-BLED Score	All Patients (N = 188) n (%)	Cases (N = 40) n (%)*	Controls (N = 148) n (%)
0	15 (7.5)	-[$]	15 (100)
1	19 (10.4)	1 (5.0)	18 (94.7)
2	44 (21.8)	8(18.2)	36 (81.8)
3	49 (25.2)	12 (23.5)	37 (75.5)
4	33 (19.8)	8 (20.0)	25 (75.7)
5	17 (9.4)	8 (42.1)	9 (52.9)
6	11 (5.9)	3 (25)	8 (72.7)

*the percentages are calculated as percentage of all patients

[$]No case had a HAS-BLED score of zero

HAS-BLED: hypertension, abnormal renal/liver function, stroke, bleeding history, labile INR, elderly, antiplatelet agents, NSAIDs/alcohol use

eTable 4 Allele Frequency Distribution of the haplotype (rs115112393, rs16871327, rs78132896, rs114504854) in populations from the 1000 Genomes Project Phase 3 (EUR- European, ASN- Asian, AFR- African, ASW- Americans of African Ancestry in SW USA) Linkage disequilibrium between SNPs, r^2 =1

Variants	Discovery Cohort allele frequency N (%)	1000 Genomes Population allele frequency N (%)			
		EUR (N= 1006)	ASN (N= 1986)	AFR (N=1260)	AMR(N=693)
rs115112393	T: 95% C: 5%	T:100%	T:100%	T:95% C:5% **ASW**- T:93% C:7%	T:100%
rs16871327	T: 95% C: 5%	T:100%	T:100%	T:95% C:5% **ASW**- T:93% C:7%	T:100%
Rs78132896	T: 95% C: 5%	T:100%	T:100%	T:95% C:5% **ASW**- T:93% C:7%	T:100%
rs114504854	T: 95% C: 5%	T:100%	T:100%	T:95% C:5% **ASW**- T:93% C:7%	T:100%

eTable 5. Risk alleles of bleeding disorders and their significance in the Discovery Cohort

Disorders	SNP (*Gene*)	Reference	Discovery Cohort			
			MA	MAF (%)	OR	p value
Polycythemia Vera, essential thrombocythaemia	rs10974944 (*JAK2*)	[14]	G	18	0.9	0.88
Von Willebrand	rs1221638 (*STXBP5*)	[15]	G	36	1.86	0.076
	rs216303 (*VWF*)	[15]	G	14	1.3	0.4
	rs4981022 (*STAB2*)	[15]	G	27	1.3	0.27
	rs8176704 *(ABO)*	[16]	A	5	0.95	0.8
Bernard Soulier Syndrome	rs6065 (*GP1BA*)	[17]	T	23	0.72	0.28

JAK2- Janus kinase 2

STXBP5 - syntaxin binding protein 5

VWF- von Willebrand factor

STAB2- stabilin 2

ABO- ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase

GP1BA- glycoprotein Ib platelet subunit alpha

eTable 6. Performance of ATRIA and HAS-BLED models for detection of major bleeding in the replication cohort

Model	C-statistics (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
ATRIA (threshold: 4)	0.66 (0.57-0.74)	67.5%(0.51-0.81)	59.5%(0.51-0.67)
ATRIA+SNP (threshold: 4)	0.76 (0.68-0.83)	82.5%(0.67-0.92)	59.5%(0.51-0.67)
HAS-BLED (threshold: 3)	0.67 (0.59-0.74)	77.5%(0.69-0.89)	46.6%(0.38-0.55)
HAS-BLED+SNP (threshold: 3)	0.78 (0.70-0.85)	90%(0.75-0.97)	45.9%(0.38-0.54)

HAS-BLED: hypertension, abnormal renal/liver function, stroke, bleeding history, labile INR, elderly, antiplatelet agents, NSAIDs/alcohol use

ATRIA: Anticoagulation and Risk Factors in Atrial Fibrillation

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