LETTER TO THE EDITOR

CYP3A4*22 and bleeding risk in ticagrelor users

Dear Editor,

We read with great interest the article by Liedes et al., who investigated the association between *CYP3A4*22* (rs35599367), *CYP3A5*3* (rs776746), and *CYP4F2* rs3093135 and bleeding risk associated with ticagrelor use. They analyzed 368 ticagrelor-treated adult (≥ 18 years) patients from three Finnish biobanks with ischemic heart disease.[1] Crude and age/sex-adjusted hazards ratio (HR) for incident bleeding were, respectively, 3.74 (95% CI 1.26–11.05) and 3.67 (95% CI 1.21–11.08) in *CYP3A4*22* carriers. Conversely, *CYP3A5*3* and *CYP4F2* rs3093135 were not associated with bleeding risk. We have conducted a similar analysis using the UK Biobank[2] (approved application number: 56653) to replicate these findings.

We used the ICD-10 codes I20-I25 reported by Liedes et al. and additional codes (HDRUK Phenotype Library, https://phenotypes.healthdatagateway.org/phenotypes/PH1027/version/2263/detail/; another study[3]) to include 560 ischemic heart disease patients (72.0% male, median 61 [interquartile range/IQR 55–65] years) who a) had British, Irish, or any other white background (to be similar to the Finnish population which is almost 98% White)[4]; b) had linked primary care data; c) had genotype data for the above three SNPs; d) were not prescribed ticagrelor or other antiplatelets (clopidogrel and prasugrel) within two years after the date of registration into the primary care database; e) had at least one ticagrelor prescription two years after primary care database registration and before October 2017; and f) had at least one day of follow-up. Similar to Liedes et al., patients were continuously followed up (median follow-up 290 days, IQR 118 to 362 days, range 6 to 1367 days) from the date of the first ticagrelor prescription to the earliest of consumption of all prescribed ticagrelor with no prescription within 30 days, prescription of either clopidogrel/prasugrel, the patient had a bleed or end of prescription records (last prescription date: 13th July 2017). Using the ICD-10 bleeding codes provided by Liedes et al., 79 (14.1%) participants had at least one bleed (12 [2.1%] major bleeds) corresponding to 19.9 bleeding (3.0 serious bleeding) events per 100 patient-years. Analysis using the cox proportional hazards model revealed that none of the SNPs were associated with incident bleeding (Table 1), results which are consistent for *CYP3A5*3*, and *CYP4F2* rs3093135, but not *CYP3A4*22*.

We did not replicate Liedes et al.'s finding that *CYP3A4*22*, an intronic SNP that alters RNA splicing[5] and that reduces the elimination of ticagrelor,[6] increases bleeding risk. Because our study was larger, with relatively precise findings, there is a possibility that previous findings were false positives. However, the discrepant results could also be due to differences in the studied populations (e.g. the Finnish biobank had a median age of 68 [interquartile range 60–73] years while the UK Biobank had a median age of 61 [interquartile range 55–65] years), study design (register-based Finnish biobank vs prospective UK Biobank study), and exposure definition (dispensation vs prescription records). Despite similar follow-up (290 [IQR 118 to 362] versus 366 [IQR 204 to 394] days), our study (14% participants with at least one bleed) had more bleeding events than Liedes et al.'s (6% bleeds); nevertheless, such high bleeding rates have been previously reported in ticagrelor treated patients.[7] We did not identify any aspirin users, which is likely because aspirin is usually taken as an over-the-counter drug and may not be recorded in the prescription records.[1] Liedes et al. could also not identify aspirin usage using the drug purchase registry but were able to identify 61% aspirin users

using patient records. It is important to note that although drug dispensation data may be a more accurate indicator of drug use than drug prescription data, none of the two sources tell us whether the patients actually took the drugs and/or were compliant to the drug use instructions, which could lead to exposure misclassification.

In conclusion, we have used the UK biobank to try replicating Liedes et al.'s work. Consistent with their findings, *CYP3A5*3* and *CYP4F2* rs3093135 were not associated with bleeding risk. However, we did not replicate their finding that *CYP3A4*22* increases bleeding risk.

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CONFLICT OF INTEREST

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KEYWORDS

Bleeding, CYP3A4, genome-wide association study, pharmacogenetics, Ticagrelor

Innocent G. Asiimwe[1] and Munir Pirmohamed[1]

[1]*The Wolfson Centre for Personalized Medicine, MRC Centre for Drug Safety Science, Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom.*

Correspondence

Innocent G. Asiimwe/Munir Pirmohamed. The Wolfson Centre for Personalized Medicine, and Centre for Drug Safety Science, Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool. Block A, Waterhouse Buildings, 1-5 Brownlow Street, Liverpool, United Kingdom. L69 3GL.

Email: i.asiimwe@liverpool.ac.uk, munirp@liverpool.ac.uk

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Table 1. Associations between single-nucleotide variants and bleeding events in ticagrelor users

Variant[a]	All subjects (N = 560)	No bleeding (N = 481)	Bleeding (N = 79)	Bleeding events per 100 patient-years (total = 19.9)	Model 1: unadjusted		Model 2: age and sex adjusted[b]	
					HR (95% CL)	P-value	HR (95% CL)	P-value
Additive mode of inheritance								
*CYP3A4*22*					1.09 (0.52–2.31)	0.817	1.12 (0.53–2.37)	0.763
Non-carrier, n (%)	502 (89.6%)	430 (89.4%)	72 (91.1%)	20.1				
Heterozygote, n (%)	57 (10.2%)	50 (10.4%)	7 (8.9%)	19.4				
Homozygote, n (%)	1 (0.2%)	1 (0.2%)	0 (0%)	0.0				
MAF	0.053	0.054	0.044					
*CYP3A5*3*					0.93 (0.50–1.72)	0.813	0.94 (0.50–1.77)	0.855
Non-carrier, n (%)	482 (86.1%)	414 (86.1%)	68 (86.1%)	20.0				
Heterozygote, n (%)	75 (13.4%)	64 (13.3%)	11 (13.9%)	20.5				
Homozygote, n (%)	3 (0.5%)	3 (0.6%)	0 (0%)	0.0				
MAF	0.072	0.073	0.070					
CYP4F2 rs3093135					1.19 (0.80–1.75)	0.389	1.15 (0.77–1.71)	0.490
Non-carrier, n (%)	398 (71.1%)	346 (71.9%)	52 (65.8%)	18.2				
Heterozygote, n (%)	143 (25.5%)	118 (24.5%)	25 (31.6%)	25.9				
Homozygote, n (%)	19 (3.4%	17 (3.5%)	2 (2.5%)	15.0				
MAF	0.162	0.158	0.184					
Dominant mode of inheritance								
*CYP3A4*22*					1.14 (0.52–2.49)	0.736	1.17 (0.53–2.58)	0.691
Non-carrier, n (%)	502 (89.6%)	430 (89.4%)	72 (91.1%)	20.1				
Carrier, n (%)	58 (10.4%)	51 (10.6%)	7 (8.9%)	18.8				
*CYP3A5*3*					0.96 (0.51–1.82)	0.905	0.97 (0.51–1.86)	0.932
Non-carrier, n (%)	482 (86.1%)	414 (86.1%)	68 (86.1%)	20.0				
Carrier, n (%)	78 (13.9%)	67 (13.9%)	11 (13.9%)	19.6				
CYP4F2 rs3093135					1.34 (0.84–2.14)	0.218	1.28 (0.80–2.06)	0.302
Non-carrier, n (%)	398 (71.1%)	346 (71.9%)	52 (65.8%)	18.2				
Carrier, n (%)	162 (28.9%)	135 (28.1%)	27 (34.2%)	24.5				

[a] The chromosome 7 SNPs (*CYP3A4*22* and *CYP3A5*3*) were genotyped while the chromosome 19 SNP *CYP4F2* rs3093135 was imputed. Hardy-Weinberg Equilibrium Chi-squared test P-values were 0.639, 0.964, and 0.172 for *CYP3A4*22*, *CYP3A5*3* and *CYP4F2* rs3093135 respectively. [b] Adding the first 10 principal components of genetic ancestry did not change the conclusions. Abbreviations: HR, hazard ratio; CI, confidence interval; CYP, cytochrome P450; MAF = minor allele frequency, N/n = number of participants.