



Original Article

Sex Differences in Clopidogrel Effects Among Young Patients With Acute Coronary Syndrome: A Role for Genetics?

Amanpreet Kaur, MSc,^a Rachel P. Dreyer, PhD,^{b,c} Thomas W. Marsh, MSc,^{d,e}
George Thanassoulis, MD, MSc,^{a,d,f} Valeria Raparelli, MD, PhD,^{g,h} Gail D'Onofrio, MD, MS,^c
James C. Engert, PhD,^{d,e,f} and Louise Pilote, MD, MPH, PhD^{a,f}

^a Centre for Outcomes Research and Evaluation, Research Institute, McGill University Health Centre, Montreal, Quebec, Canada

^b Centre for Outcomes Research and Evaluation (CORE), Yale-New Haven Hospital, New Haven, Connecticut, USA

^c Department of Emergency Medicine, Yale School of Medicine, New Haven, Connecticut, USA

^d Preventive and Genomic Cardiology, McGill University Health Centre, Montreal, Quebec, Canada

^e Department of Human Genetics, McGill University, Montreal, Quebec, Canada

^f Department of Medicine, McGill University, Montreal, Quebec, Canada

^g Department of Translational Medicine, University of Ferrara, Ferrara, Italy

^h Faculty of Nursing, University of Alberta, Edmonton, Alberta, Canada

ABSTRACT

Background: Poorer health outcomes experienced by young women with acute coronary syndrome may be related to sex differences in the safety and efficacy of antiplatelet agents, such as clopidogrel. Polymorphisms in drug metabolism enzyme (cytochrome P450 [CYP] family) genes are independent factors for the variability in response to clopidogrel. However, a sex-specific impact of genetics to explain worse clinical outcomes in women has not been explored extensively. Therefore, our objective was to determine whether an interaction of sex with CYP variants occurs among users of clopidogrel, and if so, its

RÉSUMÉ

Contexte : Les piètres résultats cliniques observés chez les jeunes femmes atteintes d'un syndrome coronarien aigu pourraient être liés à des différences entre les sexes en ce qui concerne l'innocuité et l'efficacité des antiplaquetaires, comme le clopidogrel. Le polymorphisme génétique des enzymes intervenant dans le métabolisme des médicaments (famille des cytochromes P450 [CYP]) est un facteur indépendant de la variabilité de la réponse au clopidogrel. Cependant, jamais la possibilité d'un effet génétique propre au sexe qui expliquerait les résultats cliniques défavorables chez les femmes n'a été

Dual antiplatelet therapy with aspirin and clopidogrel has been the standard regimen for treatment of acute coronary syndrome (ACS) for many years, specifically after

percutaneous coronary intervention to prevent stent thrombosis and ACS recurrence.¹ Clopidogrel has been the most common P2Y₁₂ inhibitor prescribed, with a safe profile, despite a wide variability in the pharmacodynamic response linked to several factors, including genetic polymorphisms.^{2,3} The recent availability of new P2Y₁₂ inhibitors (ie, prasugrel and ticagrelor) has changed the pattern of clopidogrel use, which is now recommended for patients with ACS who cannot receive newer thienopyridines, such as those who have previously experienced intracranial bleeding or who have an indication for anticoagulation.^{4,5} Of note, in patients who do not respond to clopidogrel due to the presence of a genetic polymorphism, the risk of ischemic events via high platelet reactivity is increased.^{2,6}

Female sex has been shown to be an independent risk factor for ACS recurrence, especially in young patients.⁷

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Ethics Statement: Institutional review board approval was obtained at Yale University and the McGill University Health Centre to create a merged database and perform secondary analyses of the merged data. In both studies, we included participants with an available blood sample and who provided informed written consent for study participation and data sharing. The research reported has adhered to the relevant ethical guidelines.

Corresponding author: Dr Louise Pilote, Centre for Outcomes Research and Evaluation, McGill University Health Centre, 5252 de Maisonneuve West, 2B.39, Montréal, Québec H4A 3S5, Canada. Tel.: +1-514 934-1934 x44722; fax: +1-514 843-1676.

E-mail: louise.pilote@mcgill.ca

See page 976 for disclosure information.

impact on 1-year adverse clinical outcomes.

Methods: We used data from a combined cohort of 2272 patients (median age 49 years; 56% female) hospitalized for acute coronary syndrome. We examined interactions between sex and CYP variants among clopidogrel users at admission and discharge to assess associations with 1-year readmission due to cardiac events.

Results: The case-only analysis of 177 participants on clopidogrel at the time of presentation showed that the risk of an atherothrombotic event was greater in female carriers of the CYP2C9*3 loss-of-function allele (odds ratio = 3.77, 95% confidence interval = 1.54-9.24). The results of the multivariable logistic regression model for users of clopidogrel at discharge (n = 1733) indicated that women had significantly higher risk of atherothrombotic readmissions at 1 year (odds ratio = 1.55, 95% confidence interval = 1.16-2.07), compared to the risk for men, but the loss-of-function alleles, either individually or through a genetic risk score, were not associated with 1-year readmissions.

Conclusion: This study highlights the need for an improved understanding of the role of sex-by-gene interactions in causing sex differences in drug metabolism.

Other than traditional risk factors, such as diabetes and dyslipidemia that lead to poorer health outcomes and higher mortality rates in young female patients with ACS, differences in the efficacy and safety of many commonly used antiplatelet drugs, such as clopidogrel, can explain the poorer outcomes in female patients.^{8,9} Clopidogrel is a prodrug that requires metabolic activation, and it is metabolized differently in female patients than in male patients.¹⁰ Critical enzymes involved in clopidogrel metabolism and activation in the liver are members of the cytochrome P450 (CYP) family. Reduced efficacy of clopidogrel has been shown to be modulated by polymorphisms in cytochrome P450 (CYP) genes.^{3,11} Depending upon the presence of gain-of-function (GOF) or loss-of-function (LOF) alleles in CYP loci, on-treatment platelet reactivity has been observed to be lower or higher, respectively.¹¹⁻¹³ Many CYP enzymes (eg, CYP3A5) are also known to have sex-dependent expression patterns.^{12,13} Furthermore, platelet response to clopidogrel has been shown to be heterogeneous in female patients, who exhibit a higher baseline of on-treatment platelet reactivity, compared with that of male patients.^{14,15}

A reduced efficacy of clopidogrel due to genetic polymorphisms could also be heterogeneous, explaining, in part, the observed worse clinical outcomes in young female patients as compared to male patients with ACS. However, the interaction between sex and genes for clopidogrel-related outcomes has not been examined. Hence, we sought to address the need to explore these differences.

Our objective was to determine whether young female patients who are carriers of CYP variants are at higher risk of cardiac events than young male patients, among users of clopidogrel who present with an ACS, and among users of

examined in profondeur. Notre objectif était donc de déterminer s'il se produit une interaction entre le sexe et les variants de CYP chez les personnes prenant du clopidogrel et, si tel est le cas, quels sont ses effets sur les résultats cliniques indésirables après un an.

Méthodologie : Nous avons utilisé les données d'une cohorte combinée de 2 272 patients (âge médian : 49 ans; 56 % de femmes) hospitalisés en raison d'un syndrome coronarien aigu. Nous avons examiné les interactions entre le sexe et les variants du CYP chez les utilisateurs de clopidogrel au moment de leur admission à l'hôpital et de leur congé pour évaluer les liens entre ces variables et les réhospitalisations après un an en raison d'événements cardiaques.

Résultats : L'analyse de cas de 177 participants prenant du clopidogrel au moment de leur admission à l'hôpital a montré que le risque d'événement athérothrombotique était plus élevé chez les femmes porteuses de l'allèle non fonctionnel CYP2C9*3 (rapport de cotes = 3,77; intervalle de confiance [IC] à 95 % = 1,54 à 9,24). Les résultats obtenus avec le modèle de régression logistique multivariée pour les utilisateurs de clopidogrel au moment du congé (n = 1 733) indiquent que les femmes présentaient un risque significativement plus élevé de réhospitalisation en raison d'un événement athérothrombotique après un an (rapport de cotes = 1,55; [IC] à 95 % = 1,16 à 2,07), comparativement aux hommes. Toutefois, les allèles non fonctionnels, considérés individuellement ou sur la base d'un score de risque génétique, n'étaient pas liés à la réhospitalisation après un an.

Conclusion : Cette étude fait ressortir la nécessité de mieux comprendre le rôle de l'interaction sexe-gène dans les différences entre les sexes relativement au métabolisme des médicaments.

clopidogrel at discharge after ACS. Given the role of the CYP genes in clopidogrel metabolism, our hypotheses were as follows: (i) individuals on clopidogrel who develop ACS are more likely to carry CYP LOF alleles (single-nucleotide polymorphisms [SNPs]); and (ii) these effects are modified by sex.

Methods

Study population

We combined 2 large cohort studies of young adults who sustained an ACS at ages between 18 and 55 years: GENESIS-PRAXY (Gender and Sex Determinants of Cardiovascular Disease: from Bench to Beyond Premature Acute Coronary Syndrome)⁸ and VIRGO (Variation in Recovery: Role of Gender on Outcomes of Young AMI Patients),¹⁶ which resulted in a total sample size of 4782 participants (58% female). The methodology and design of both studies have been described previously.^{8,16} Institutional review board approval was obtained at Yale University and the McGill University Health Centre to create a merged database and perform secondary analyses of the merged data. In both studies, we included only participants with an available blood sample and who provided informed written consent for study participation and data sharing. Moreover, we included only European ancestry participants in our study to avoid population stratification.

GENESIS-PRAXY and VIRGO cohorts

GENESIS-PRAXY is a prospective observational cohort study of 1210 young adults (30% female patients), aged 18-55 years, hospitalized with ACS. Participants were recruited across 24 sites in Canada, 1 site in the US, and 1

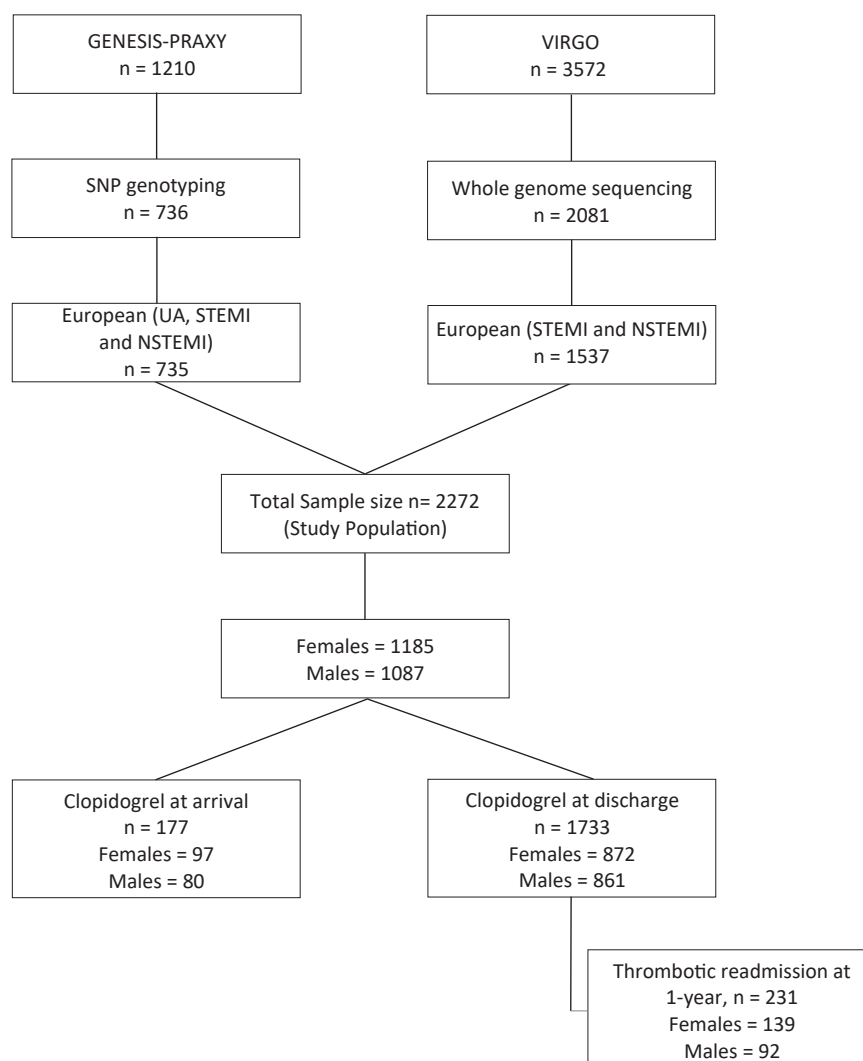


Figure 1. Study flow: flowchart of study participants in the cohort. GENESIS-PRAXY, **G**ender and **S**ex Determinants of Cardiovascular Disease: from Bench to Beyond **P**remature **A**cute Coronary **S**ndrome; NSTEMI, non-ST-elevation myocardial infarction; SNP, single-nucleotide polymorphism; STEMI, ST-elevation myocardial infarction; VIRGO, **V**ariation in **R**ecovery: Role of **G**ender on **O**utcomes of Young AMI Patients; UA, unstable angina.

site in Switzerland, between January 2009 and April 2013.⁸ Clinical and demographic information was collected via baseline questionnaires completed by participants during hospitalization, and medical chart reviews.^{17,18} The diagnosis of ACS was based on the standardized criteria^{19,20} for symptoms, signs, electrocardiogram findings, and elevations in cardiac enzyme levels. Blood samples were collected at time of event.

The VIRGO cohort consists of 3572 participants with acute myocardial infarction (AMI; 67% female patients) aged 18-55 years who were enrolled from 103 US sites, 24 Spanish sites, and 3 Australian sites, between August 2008 and May 2012.^{16,21} Data on demographics, clinical presentation, and treatment were collected via medical chart review and in-person interviews conducted by trained personnel during the AMI admission.²² The diagnosis of AMI was confirmed by the presence of elevated levels of cardiac enzymes and supporting evidence of myocardial ischemia, including at least one of the following: symptoms of ischemia;

electrocardiogram changes suggestive of new ischemia; other evidence of myocardial necrosis on imaging.²² Blood samples were collected 1 month after discharge.

Common variables in both cohorts, including participant demographic information, clinical risk factors and characteristics, were merged for this study population. Both cohorts have follow-up information on readmission for up to 12 months postdischarge.

Study sample

From a total sample size of 4782 patients, we included a combined sample of 2272 European ancestry participants with any ACS (N = 1185; 52% female) who had genotype data available (Fig. 1). From the 1210 participants in the GENESIS-PRAXY cohort, 736 participants were genotyped based on availability of blood samples at the time of genotyping. Of these, 735 European ancestry participants were included. From the 3572 participants in the VIRGO cohort, 2081 had sequencing data available, of which 1537 European

Table 1. Participant characteristics at baseline

Characteristic	Female participants	Male participants	P
	(n = 1185)	(n = 1087)	
Demographics			
Age, y	48.19 ± 5.45	47.85 ± 5.79	0.145
Clinical risk factors			
Hypertension	697 (58.82)	576 (52.99)	0.005
Dyslipidemia	931 (78.57)	812 (74.70)	0.032
Diabetes	401 (33.84)	204 (18.77)	< 0.0001
Obesity (BMI > 30 kg/m ²)	587 (49.75)	495 (45.83)	0.063
Smoking currently	372 (31.39)	384 (35.33)	0.049
Clinical characteristics			
Type of ACS			
STEMI	606 (51.14)	661 (60.81)	< 0.0001
NSTEMI	559 (47.17)	395 (36.34)	
Unstable angina	20 (1.69)	31 (2.85)	
Reperfusion therapy			
Primary PCI, STEMI patients	446 (75.68)	453 (70.89)	0.061
PCI, NSTEMI patients	398 (76.54)	279 (79.49)	0.32

Values are mean ± standard deviation, or n (%), unless otherwise indicated.

ACS, acute coronary syndrome; BMI, body mass index; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction.

participants were included in our analysis. The ancestry of the participants was based on self-reported questionnaire responses and then confirmed using their genetic data. Of these, 177 patients were on clopidogrel prescription at the time of admission. At the time of discharge, 1733 patients were prescribed clopidogrel.

Genotyping and/or sequencing of the study samples

DNA extraction, plating, quantification, and quality-control tests of the GENESIS-PRAXY samples were carried out by McGill University and the Genome Quebec Innovation Centre. SNP genotyping was performed using the Sequenom iPLEX platform (Sequenom Inc, San Diego, CA).²³ Genotypes for the VIRGO cohort were obtained from whole-genome sequencing data provided by the Broad Institute of Harvard and MIT (Cambridge, MA).²⁴ Deep-coverage sequencing was performed using the Illumina HiSeqX platform (Illumina Inc, San Diego, CA).

Selection of SNPs

The *CYP* polymorphisms were selected based on the available literature on clopidogrel metabolism and absorption. Minor allele frequencies (MAFs) for each cohort were calculated and were compared to European MAFs reported in the large international open-source databases called 1000 Genomes and gnomAD.^{25,26} The 1000 Genomes database includes genome data from 2504 individuals from different locations around the globe, whereas gnomAD is a collection of exome and genome sequencing data of 141,456 individuals, predominantly Europeans, from a wide variety of large-scale sequencing projects. *CYP* allele SNPs with MAFs greater than 0.001 were considered for genotyping and were then selected based on successful primer design during sequencing (Supplemental Table S1).

Calculation of the genetic risk score

As multiple SNPs are involved in clopidogrel metabolism, we calculated a genetic risk score (GRS) to assess their cumulative effect.²⁷ We calculated an unweighted GRS from 7 *CYP* alleles involved in clopidogrel metabolism (*CYP2C9*3*, *CYP2C19*2*, *CYP2C19*4*, *CYP2C19*17*, *CYP3A5*2*, *CYP3A5*3*, and *CYP3A5*6*) for 2272 participants. The mean unweighted GRS of LOF *CYP* alleles in 2272 European participants was 2.07 ± 1.05 (Supplemental Fig. S1).

GRS was calculated by summation of the number of the 7 *CYP* risk alleles, using the following formula:

$$GRS = \sum_{i=1}^k Ni$$

with *k* = number of genetic variants,
and *N_i* = number of risk alleles (0,1,2).

Cardiac readmission for VIRGO and GENESIS-PRAXY

Recurrent cardiac events were defined as a readmission for an ACS within 1-year postdischarge.

Statistical analysis

Sociodemographic and clinical characteristics of participants were summarized using means (standard deviation [SD]) for continuous variables, and percentages for categorical variables. We used the t-test and the Pearson χ^2 test to compare baseline characteristics between sexes. Statistical significance was set at *P* < 0.05. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

For all genetic variants, among clopidogrel users at ACS admission, we tested sex-by-gene interactions via a case-only design. The case-only design can be used to analyze interactions without the use of controls and requires a smaller sample size than case-control studies because of the increased efficiency.²⁸ In case-only studies, because controls are not used, the issue of control selection bias is also eliminated,²⁹ but the main effect cannot be estimated. This approach is cost effective and has greater precision for detecting interactions, compared with the case-control design.^{29,30} With the case-only design, we tested sex-by-gene interactions among patients who were on clopidogrel at the time of an admission for ACS. The odds ratio (OR) obtained from a case-only logistic regression measures the multiplicative joint effect of genotype and sex; and it is interpreted as the multiplicative interaction between gene and sex.²⁹

Among clopidogrel users at discharge from hospital after an ACS, we investigated the possibility of a sex-by-gene interaction for the risk of readmission for a cardiac event using a cohort design. We used multivariable logistic regression to investigate associations among sex, GRS, and cardiac readmission risk. Variables included in the model (hypertension, dyslipidemia, diabetes, smoking, and previous myocardial infarction) were selected based on their statistically significant relationship with cardiac readmission in univariate analyses, and their clinical relevance (Supplemental Table S2).

Table 2. Sex-by-gene interactions (CYP genes) for acute coronary syndrome risk (case-only analysis)

Allele	rsID	Odds ratio	95% confidence limits	
<i>CYP2C9*3</i>	rs1057910	3.767	1.536	9.243
<i>CYP2C19*2</i>	rs4244285	0.63	0.295	1.348
<i>CYP2C19*4</i>	rs28399504	0.822	0.051	13.352
<i>CYP3A5*2</i>	rs28365083	1.663	0.148	18.682
<i>CYP3A5*3</i>	rs776746	0.531	0.144	1.95
<i>CYP3A5*6</i>	rs10264272	0.822	0.051	13.352
<i>CYP2C19*17</i>	rs12248560	1.131	0.608	2.103

Odds ratios provided are for female patients.

rsID, reference single-nucleotide polymorphism cluster identification number.

Results

Baseline characteristics

The median age of participants from both cohorts was 49 years (the mean age was 48.19 ± 5.45 years for female patients and 47.85 ± 5.79 years for male patients [Table 1]). Female patients had significantly higher rates of hypertension, diabetes, and dyslipidemia, as compared to those of male patients, whereas male patients had higher rates of current smoker status. However, female and male participants did not differ significantly in the prevalence of obesity. Male patients had significantly higher rates of ST-elevation myocardial infarction (STEMI), whereas female patients had higher rates of non-ST-elevation myocardial infarction (NSTEMI).

At the time of admission, 177 participants (55% female) were on clopidogrel treatment, whereas 1733 participants (50% female) were prescribed clopidogrel at time of discharge.

SNP minor allele frequencies

We calculated MAFs for all selected SNPs in our study population (Supplemental Table S3), and they were similar in both cohorts and were similar to frequencies reported in public databases (Supplemental Table S1).^{25,26}

Sex-by-gene interaction for recurrent ACS risk

For users of clopidogrel at admission, the risk of ACS was greater in female carriers of *CYP2C9*3* (total n for 1,2 risk alleles = 388; clopidogrel users at arrival = 72; clopidogrel users at discharge = 205), an LOF allele, as compared to that in male carriers (OR = 3.77, 95% confidence interval = 1.54-9.24; Table 2). The other 6 *CYP* LOF alleles, including the GOF *CYP2C19*17* allele, did not show any statistically significant interactions.

Cardiac readmissions at 1-year postdischarge

Among the users of clopidogrel at discharge, 231 cardiac readmissions occurred. We compared the readmissions for cardiac events at 1-year postdischarge (60% female patients) for LOF *CYP* variants (*CYP2C9*3*, *CYP2C19*2*, *CYP3A5*2*, *3) for female vs male patients (Table 3). Female patients had significantly higher risk of cardiac readmission (OR = 1.55, 95% confidence interval = 1.16-2.07) at 1 year, compared to that of male patients (Table 4), but the interaction analysis between the sex of participants and *CYP* LOF variants was not statistically significant. No significant

Table 3. Sex-by-gene (CYP gene) interactions for clopidogrel users at the time of discharge

Allele	rsID	Odds ratio	95% confidence limits	
<i>CYP2C9*3</i>	rs1057910	0.637	0.286	1.421
<i>CYP2C19*2</i>	rs4244285	0.786	0.351	1.757
<i>CYP3A5*2</i>	rs28365083	1.355	0.099	18.65
<i>CYP3A5*3</i>	rs776746	0.7	0.123	3.981
<i>CYP2C19*17</i>	rs12248560	0.873	0.477	1.599

Odds ratio for gene \times sex (female) interaction term for the model: thrombotic readmission outcome (yes/no) = female (sex) + gene + gene \times female (sex).

rsID, reference single-nucleotide polymorphism cluster identification number.

association was seen between GRS and the risk of readmission at 1 year for a cardiac event.

Discussion

Young female patients with ACS have been shown to have poorer outcomes than male patients.^{31,32} Indeed, in this analysis of 2 large cohorts of young patients with ACS, in patients who were on clopidogrel at discharge, female patients had a significantly higher risk of readmission for a cardiac event at 1 year postdischarge, compared to that of male patients. Our hypothesis that individuals on clopidogrel who develop recurrent ACS are more likely to carry *CYP* LOF alleles was supported by our case-only analysis, which revealed that, among clopidogrel users at ACS onset, a *CYP2C9* LOF allele (*3) was associated with a higher risk of cardiac events in young female patients, as compared to the risk in male patients. However, such an association was not found in our analysis of risk of a recurrent cardiac event.

These findings suggest a possible genetic mechanism underlying sex differences in the efficacy of clopidogrel. The presence of LOF *CYP* alleles may be associated with increased risk of recurrent thrombosis in young female patients with ACS who are treated with clopidogrel, and thus could partly explain the difference in efficacy of clopidogrel between female and male patients.

LOF *CYP* alleles confer higher platelet reactivity, which has been associated with a higher risk of cardiac events.^{2,33} Therefore, patients with a poor response to clopidogrel are likely to have an increased risk of recurrence of ischemic complications.³ Also, *CYP* group enzymes are known to have sex-specific expression patterns and appear to contribute to varying cardiac risks, as well as cardiovascular event outcomes, between female and male patients.^{12,13} For example, the *CYP3A* class of enzymes has been shown to exhibit expression levels in female patients that can be double that in male patients.¹⁵ Other factors that have been reported to be responsible for sex differences in health outcomes after cardiovascular events and drug metabolism are hormonal mechanisms, differences in platelet biology, variability in age, comorbidities, and body size.³⁴⁻³⁶

The association between the LOF *CYP* alleles, specifically *CYP2C19* and *CYP2C9*, and increased risk of poor outcomes in individuals on clopidogrel, due to decreased exposure to the active metabolite of clopidogrel, has been reported previously.^{37,38} For example, Visser and colleagues reported that drugs that are metabolized by *CYP2C9* increase the risk of

Table 4. Multivariate analysis of atherothrombotic readmission at 1 year postdischarge

Cardiac readmission		Frequency	Percentage
No		1474	86.45
Yes		231	13.55
Odds ratio			
Variable	Parameter estimate	95% confidence limits	
Model 1			
Female (sex)	1.64	1.24	2.18
Model 2			
GRS	1.08	0.95	1.23
Model 3			
GRS	1.08	0.95	1.23
Female (sex)	1.63	1.23	2.17
Model 4			
Female_GRS (sex × GRS)	1.01	0.77	1.31
GRS	1.07	0.88	1.31
Female (sex)	1.61	0.86	3.02
Model 5			
Female (sex)	1.55	1.16	2.07
GRS	1.04	0.91	1.19
Age	0.97	0.95	0.99
Hypertension	1.10	0.81	1.5
Dyslipidemia	1.29	0.88	1.89
Diabetes	1.30	0.94	1.79
Smoking	0.71	0.52	0.98
History of renal disease	1.06	0.64	1.76
Previous MI	1.43	1.01	2.05
PCI	0.78	0.57	1.08

GRS, genetic risk score; MI, myocardial infarction; PCI, percutaneous coronary intervention.

AMI in female patients.^{39,40} In another study by Harmsze and colleagues, the *CYP2C9*3* allele was shown to play an important role in the clopidogrel response in individuals on dual-antiplatelet therapy undergoing coronary stenting.⁴¹ In a case-control study, Harmsze et al. showed that the LOF alleles *CYP2C19*2* and *CYP2C9*3* increased the risk of stent thrombosis after percutaneous coronary intervention.⁴² In addition, Li et al. showed that carriers of the GOF *CYP2C19*17* variant have an increased therapeutic responsiveness to clopidogrel.⁴³ A recent study by Gronich et al. has shown that Europeans who are homozygous for the *CYP2C19* LOF allele and are on clopidogrel may be at a higher risk of an ischemic event.⁴⁴ The detrimental outcomes in response to clopidogrel could vary because of a sex-by-gene interaction, with female carriers of LOF alleles having a higher risk of event recurrence than male carriers. As shown in this study, among clopidogrel users at ACS admission, cardiac event risk was greater in female, compared with male, carriers of the *CYP2C9*3* LOF allele.

Commercial pharmacogenetic tests available to identify the likely response of patients to the standard clopidogrel regimen are available.^{6,45} However, these tests are for the genotype variants of the *CYP2C19* LOF allele only, which is the most extensively studied variant for gene-dose response for clopidogrel.⁴⁰ Alternative therapies, such as prasugrel, are recommended for patients who are carriers of *CYP2C19*2* and *3** LOF alleles. Nevertheless, these commercial tests do not capture all of the genetic variability in clinical response to clopidogrel.⁴⁶ This limitation could preclude the assessment of clopidogrel resistance. Currently, no standardized clinical guidelines are available for patients on clopidogrel to reliably

identify dosing regimens specifically in relation to the sex of patient. Moreover, female patients are underrepresented in cardiovascular studies, and therefore, the information on drug safety and efficacy in female patients suffering from cardiovascular diseases remains limited.^{14,47,48} Our results indicate that the interaction of the *CYP2C9*3* allele with sex could, at least partly, explain the increased risk of recurrent ACS in young female patients. Given the important role of *CYP2C9* genes in drug metabolism, pharmacogenetic tests for anti-thrombotic drugs like warfarin include *CYP2C9* alleles for determining dosage regimen.⁴⁹ Hence, this study underscores the importance of inclusion of *CYP* loci in addition to the commonly used *CYP2C19* locus, specifically the *CYP2C9* allele, in clopidogrel pharmacogenetic tests.

Study limitations

Although our merging of 2 cohorts of individuals with premature ACS is unique, a notable limitation of this study remains the relatively limited sample size, especially for the number of participants who are on clopidogrel or are readmitted for cardiac events. Therefore, findings should be replicated in a larger study sample.

Also, this study is limited to European ancestry participants. Differences in the distribution of pharmacogenetically important allelic variants involved in drug metabolism between major populations may indicate ethnic differences in drug metabolism.⁵⁰ For instance, Asian individuals have been reported to have a high prevalence of *CYP2C19* LOF alleles: almost twice that of European populations.⁵¹ This finding warrants replication of the present study in different ethnicities.

One possible reason that an association was found in the case-only analysis but not in the cohort study is that participants who present with an event at admission, and are on clopidogrel, represent a group with a possible clopidogrel failure,⁵² making them more likely to be a carrier of the *CYP* LOF mutation.² In contrast, in the cohort study, all clinically eligible patients were prescribed clopidogrel at discharge. Hence, no selection process was at play. Moreover, the power to detect an association in the cohort analysis was much lower compared to that in the case-only approach, which may also explain this discrepancy.

Moreover, we did not find any interaction of sex with *CYP2C19* alleles, although these alleles have been shown to be associated with the most resistance, suggesting that the magnitude of the effect is the same in both sexes. Given that only one interaction with a *CYP* allele (*2C9*3*) was statistically significant in our study, this finding likely represents a chance bias. However, a point to note is that *CYP2C9* is the most abundant *CYP2C* subfamily enzyme in the human liver (accounting for approximately 20% of total hepatic P450 protein) and is known to be an important contributor to drug metabolism.⁵³ The 2 polymorphisms, *CYP2C9*2* and **3* alleles, are the most common variants found at the highest *CYP2C* allele frequencies in white Europeans/Caucasians, and functional effects on drug metabolism are well established. These factors might, therefore, explain the presence of (female) sex interaction with the *CYP2C9*3* allele in our European study population.

Although chances are high that our study population, which was on a clopidogrel prescription, is likely

heterogeneous, with a proportion of patients suffering from spontaneous coronary artery dissection, we had very few patients who had it (8%) available in our cohort for analysis. Nevertheless, given that we employed a clopidogrel user design, our results are unlikely to have been affected by this small group. Furthermore, including death as an endpoint would have accounted for the possibility of fatal myocardial infarction in patients who did not arrive at the hospital in time to be admitted. However, the number of cases was insufficient to account for death as an endpoint. Hence, the likelihood of the competing risk of death is low.

We have shown that the LOF allele *CYP2C9*3* might confer a higher risk of ACS in young women, which likely could be explained by higher on-clopidogrel platelet reactivity. Given that a measure of platelet reactivity is not available for this dataset, additional research is required to confirm the platelet reactivity data in order to strengthen our understanding of the sex–*CYP* association, with increased likelihood of cardiac events in female patients.

Our use of 2 large, highly characterized cohorts of young adults with premature ACS allowed us to investigate whether a sex-by-gene interaction contributes to the differences in clinical outcomes between female and male patients on clopidogrel. Factors underlying the finding that worse outcomes occur in female patients who sustain an ACS, compared with those in males, are incompletely understood. Sex-by-gene interaction in *CYP* genes responsible for clopidogrel metabolism could in part explain differences in risk among female and male patients on clopidogrel. Genotyping for drug metabolism polymorphisms may be more helpful in stratifying the benefits and risks associated with clopidogrel, in female patients.

Conclusion

This study highlights the need for an improved understanding of the role of sex-by-gene interactions in causing sex differences in drug metabolism and the need to focus on sex-based analyses. Our results also indicate the need for standardized clinical guidelines to address the different adverse effects in female vs male patients, and the gene-dose response for clopidogrel. Use of such a pharmacogenetics approach could consequently lead to the development of sex-specific therapies.⁵⁴

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

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