Association of CYP2C19 Polymorphism With Proton Pump Inhibitors Effectiveness and With Fractures in Real-Life: Retrospective Cohort Study

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Symptom refractoriness of patients treated with proton pump inhibitors (PPIs) might be explained by polymorphism in CYP2C19. This is a retrospective cohort study in which we used the computerized database of Clalit Health Services to compose a cohort from cancer case-control studies' participants that had been genotyped, and that have been dispensed PPI (January 1, 2002 to November 10, 2020). We retrieved demographic and clinical variables on date of PPI initiation (cohort entry), and studies' questionnaires-reported consumption of foods/beverages known to increase peptic-related symptoms. Primary outcome was an abdominal pain diagnosis; secondary outcome was a composite of abdominal pain, visit to a gastroenterology clinic, change to another PPI, PPI dose increase, or metoclopramide prescription, reflecting symptoms persistence/recurrence; in a 2-year follow-up. We also evaluated the association between genetic groups and hip/wrist/spine fractures, in a long-term follow-up. Of 3,326 PPI initiators, there were 66 (2.0%), 739 (22.2%), 1394 (41.9%), 947 (28.5%), and 180 (5.4%) CYP2C19 poor, intermediate, normal, rapid, and ultra-rapid metabolizers, respectively. Being a poor metabolizer was associated with lower risk for the primary outcome, hazard ratio (HR) = 0.50 (95% confidence interval (CI) 0.27–0.91), HR = 0.52 (95% CI 0.28–0.94); and for the secondary outcome, HR = 0.57 (95% CI 0.38–0.86), HR = 0.58 (95% CI 0.39–0.87), in univariate and multivariable cox regression analyses, respectively. In long-term follow-up with 20,142 person-years of follow-up: 7.6% (5 cases) within the poor metabolizers group, and 11.6%, 12.9%, 12.8%, and 11.1% in the normal, intermediate, rapid, and ultra-rapid metabolizers groups, respectively, had a new fracture (nonsignificant). We conclude that CYP2C19 poor metabolizer status is associated with higher effectiveness of PPIs, and is not associated with higher risk for fractures.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Only 58% of proton-pump inhibitor (PPI) recipients report being satisfied with their treatment. PPIs are metabolized through the polymorphic CYP2C19 enzyme. Most CYP2C19 studies evaluating PPIs were conducted in Asian populations.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Is there association in White patients, between CYP2C19 polymorphism and measures of PPI effectiveness, and fractures?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ CYP2C19 poor metabolizer status is associated, in real-life, with significantly lower risk for peptic-related symptoms' persistence/recurrence, but not with risk for fractures.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ We found that in most patients treated with PPIs effectiveness is not optimal, probably due to decreased exposure to PPIs in serum. Future studies should evaluate ways to increase effectiveness in non-CYP2C19 poor-metabolizers treated with PPIs.

Proton pump inhibitors (PPIs) are one of the most commonly prescribed medication types, used by an estimated 7%–9% of adults worldwide, and by more than 20% of adults aged 65 years or older.[1] They are the most potent inhibitors of gastric acid secretion by irreversibly inhibiting the hydrogen-potassium ATPase pump. PPIs are recommended as first-line treatment for patients

with gastroesophageal reflux disease (GERD), peptic ulcer, Zollinger-Ellison syndrome, non-steroidal anti-inflammatory drug-associated ulcers, the eradication of *Helicobacter pylori* infection, and for severe or frequent peptic-related symptoms even without confirmed disease.[2,3] PPIs are frequently administered for other upper gastrointestinal symptoms, such as dyspepsia.

Patients who failed to obtain satisfactory symptomatic response or complete esophageal healing after a full course of standard dose PPI are defined as having PPI failure; a definition that allows inclusion of patients who perceived their remaining symptoms on PPI therapy as bothersome.[4–6] A study in 11,064 patients with chronic heartburn found that, although PPIs were the preferred class of medications for preventing heartburn, only 58% of PPI recipients reported being totally satisfied with their anti-reflux treatment.[7] A systematic review that included 9 studies of 14,774 patients showed improved health-related quality of life in patients who responded well to PPI treatment, but not in nonresponders.[8,9]

In addition to reduced quality of life, non-resolution of symptoms is likely an expensive clinical problem[1] as patients tend to repeatedly utilize health care resources, such as clinic visits, diagnostic studies, and prescription medications.[4,10–12] Reduced work productivity has been reported.[13]

A range of mechanisms can result in insufficient suppression of gastric acid and refractory symptoms. Among them are physiological mechanisms, like delayed gastric emptying, or visceral hypersensitivity, or drug-related mechanisms, such as poor compliance with PPI timing or adherence[4] and rapid PPI metabolism. PPIs are metabolized through the hepatic cytochrome system, with CYP2C19 having the dominant role. CYP2C19 gene is highly polymorphic.[14] Approximately 2% of White, 3% of sub-Saharan Africans, 4% of African-American/Afro-Caribbean, 8–13% of Asians, and 57% of Oceanian patients are homozygous for a CYP2C19 polymorphism that renders them to low activity of the enzyme (i.e., poor metabolizers). Intermediate, normal, and rapid metabolizers constitute together the vast majority in most populations, whereas percentages of ultrarapid metabolizers, carrying two alleles with higher-than-normal activity, are about 5% in Europeans and lower in other populations (Clinical Pharmacogenetics Implementation Consortium (CPIC) CYP2C19 polymorphism frequency table).[15]

Because the metabolites of PPIs are pharmacologically inactive, it was thought that rapid metabolizers might demonstrate lower efficacy of PPIs and contribute to PPI failure. In contrast, poor metabolizers may show increased efficacy due to increase in exposure to the drug. The highest impact of CYP2C19 pathway on PPIs metabolism has been reported in omeprazole.[16]

Side effect frequency might vary by metabolizers' status as well,[14] but there is paucity of data regarding the clinical and the genetic associations.[17] Bone fractures were previously associated with high exposure or with long-term PPI use, but the associations remained uncertain.[1,18,19]

A few studies demonstrated increased PPI treatment success in CYP2C19 poor and intermediate metabolizers compared with normal metabolizers for the first-generation PPIs (omeprazole, lansoprazole, and pantoprazole).[14] However, in the recently published CPIC guidelines[14] it was noted that most CYP2C19 studies evaluating PPIs were conducted in Asian populations, where

rapid and ultra-rapid metabolizer phenotypes are rare and thus no clinical data existed for dose recommendations for rapid and ultra-rapid metabolizers. As for the second-generation PPIs (esomeprazole, rabeprazole, and dexlansoprazole) there was less evidence linking CYP2C19 genotype with effectiveness and recommendations could not be made.[14]

Our goal was to evaluate in real life association of CYP2C19 polymorphism with measures of effectiveness and with fractures. Because symptoms guide management of patients treated with PPIs, we exploited the electronic medical records to ascertain indices of symptoms' non-resolution and of utilization of health care resources for gastrointestinal symptoms, to reflect persistence/recurrence of symptoms, as well as for diagnoses of fractures in the long-term follow-up.

METHODS

Study population

The source population is an unselected sample from breast, colorectal, and lung cancer case-control studies in Northern Israel, active for more than 20 years at the Clalit National Cancer Control Center, which collected biological samples including DNA from every consenting participant to study cancer etiology. The controls had been randomly sampled from the population register and matched to their corresponding cancer cases by age, sex, ethnicity, and socio-economic status.[20] All participants were interviewed in person, and completed a food-frequency questionnaire during the interview. There were 11,950 subjects (cases and controls), consecutively chosen from the above studies, who underwent genomewide association study. Carmel Medical Center ethics committee approved the study.

Genotyping

Genomewide association study analyses were performed under GAME-ON initiative using the OncoArray by Illumina that included a backbone and a customized panel for dense mapping of known susceptibility regions, including pharmacogenetic markers. Details on the genotyping calling were described in more detail elsewhere.[21] In brief, standard quality control was performed on all scans. All individuals with low call rate ($< 1 \times 10^{-5}$), single-nucleotide polymorphisms (SNPs) with minor allele frequency < 1%; call rate < 95%; or call rate < 99%, and minor allele frequency < 5% and all SNPs with genotype frequencies that departed from Hardy-Weinberg equilibrium at $P < 1 \times 10^{-6}$ were excluded. For highly significant SNPs, genotype intensity cluster plots were examined manually to judge reliability.[22] CYP2C19 metabolizer status was assessed by alleles previously associated with CYP2C19 activity, that were included in the OncoArray (CYP2C19 *1, *2, *3, *5, *7, *10, and *17). As rare *5 and *7 CYP2C19 variants were not found in the cohort, definitions of metabolizer status were as follows: poor metabolizer = homozygous decreased activity (*2/*2, *2/*3, *3/*3, and *2/*10); intermediate metabolizer = heterozygote or intermediate activity (*1/*2, *1/*3,*1/*10, *2/*17, and *10/*17); normal metabolizer = homozygous wild-type or normal activity (*1/*1); rapid metabolizer (*1/*17); and ultra-rapid metabolizer = increased activity (*17/*17).[14] Cohort participants and the medical teams were unaware of CYP2C19 results.

Study cohort

This is a retrospective cohort study in which we followed the genotyped studies participants that have been also using PPIs. The clinical measurements are based on data from the computerized database of Clalit Health Services, the largest of 4 integrated health care organizations in Israel, which insures 4.7 million patients (53% of the population). Health care coverage in Israel is mandatory according to the National Health

Insurance Law (1995), and is provided by four groups akin to not-for-profit health maintenance organizations. All members of the different organizations have a similar health insurance plan and similar access to health services, including low medications copayment. The electronic medical records of Clalit include data from multiple sources: records of primary care physicians, community specialty clinics, hospitalizations, laboratories, and pharmacies. Diagnoses are captured in the registry by diagnosis-specific algorithms, using International Classification of Diseases Ninth revision ninth revision (ICD-9) code reading, text reading, laboratory test results, and disease-specific drug usage. This database has been formerly described and validated.[23,24]

Deriving from genotyped case-control studies' participants described above, we assembled the cohort of PPI initiators for the current study. We used the Clalit pharmacy database to assemble the cohort of patients that have been newly dispensed at least one PPI prescription (omeprazole, pantoprazole, lansoprazole, or esomeprazole) any time between January 1, 2002, and November 10, 2020. In this new cohort, we disregarded the original cases-controls matching. The day of the first PPI prescription defined the cohort entry date. As PPIs are dispensed in Israel only as prescription drugs, we had the full information on PPIs acquired by the cohort participants.

We retrieved, from the electronic medical records, demographic and clinical variables on cohort entry date. These inlcuded date of birth, sex, ethnicity, socio-economic status, alcohol abuse, smoking, and body mass index as well as chronic diseases diagnoses of hypertension, diabetes mellitus, hyperlipidemia, ischemic heart disease, congestive heart failure, cerebrovascular accident, asthma, chronic obstructive pulmonary disease, dementia, chronic renal failure, malignancy, osteoporosis, anxiety, and depression. We also retrieved, at an assessment window of 4 months before cohort entry date, prescriptions dispensed of histamine type 2 receptor blocker; prescriptions dispensed of potential CYP2C19 inhibitors including cimetidine, fluoxetine, fluvoxamine, isoniazid, ketoconazole, systemic hormonal contraceptives, ticlopidine, and voriconazole; and prescriptions dispensed for potential CYP2C19 inducers, including efavirenz, rifampicin, and ritonavir.[25] In addition, we ascertained from a food-frequency questionnaire filled out during an in-person interview upon entering the case-control study, doses/week consumed, as reported by the participant, of high-fat foods, and coffee/tea/carbonated beverages, due to their association with peptic-related symptoms.

Study outcome and follow-up

The primary outcome was a new diagnosis of abdominal pain from a primary physician visit or an emergency department visit (for ICD-9 codes see **Table S1**). We made a record any time a diagnosis of abdominal pain was given in these medical encounters. The first time happened during follow-up, at least 1 day following the cohort entry date, was regarded as the primary outcome.

A secondary effectiveness outcome was occurrence of any of 5 measures reflecting non-resolution/recurrence of symptoms, in a 2-year follow-up, if it happened at least 1 day following cohort entry date, including: (1) ICD-9 code of abdominal pain (from a primary physician visit or an emergency department visit) as described above; (2) PPI daily dose increase from initial dose, ascertained by records of the PPI dose dispensed each date on Clalit pharmacies, and the number of pills/day in the prescription; (3) a change to another PPI, ascertained by pharmacy records of the level 5 Anatomic Therapeutic Chemical (ATC) code of dispensed PPI; (4) dispensing of metoclopramide prescription in Clalit pharmacies; and (5) a visit to a gastroenterology clinic as recorded in Clalit database. We chose these outcome measures to follow management of patients treated with PPI who still suffer peptic symptoms: the first steps are usually dose increase or change to another PPI.[4] Next, it is the common practice to refer the patient to a gastroenterology clinic. Sometimes, the prokinetic metoclopramide or baclofen are being added to treatment (the latter is not in use in Israel). Anti-reflux surgeries are only very rarely used and thus were not included as outcomes.

For effectiveness analysis, patients were followed for up to 2 years starting on each participant's cohort entry date, until an outcome event, death, end of registration in Clalit, or end of study date (November 10, 2020), whichever came first.

We also retrieved new diagnoses of hip/spine/wrist fractures from hospitalizations, or diagnoses written in the patients' medical records in outpatients medical encounters, using ICD-9 codes (**Table S1**). For this analysis, we performed a long-term follow-up until outcome date, death, end of study (November 10, 2020), or end of registration in Clalit, whichever came first.

Statistical analysis

We compared baseline characteristics of patients by genotypes with the χ^2 test for categorical variables and Fisher exact test for small numbers, as needed. Student's t-test /ANOVA was applied for continuous variables.

To describe the frequency of non-resolution/recurrence outcome events according to time since PPI initiation, we constructed a Kaplan-Meier curve for cumulative event-free survival for the primary outcome, stratified according to CYP2C19 genotype.

We undertook Cox regression analyses to calculate the hazard ratio (HR) to achieve a first non-resolution/recurrence event according to genotype. In a multivariable Cox regression analysis, we adjusted for confounders, following the stepwise process to identify true confounders (associated with both exposure and outcome). Comparisons are expressed as univariate and multivariate HRs and 95% confidence intervals (CIs). In a sensitivity analysis, we stratified the cohort by the different PPI types. Cox regression analysis was used also to analyze association between CYP2C19 genetic group and fractures in a long-term follow-up.

All analyses were performed using SPSS software (version 24) (IBM, New York, NY, USA). All P values are two-sided, and $P < 0.05$ was considered significant.

RESULTS

There were 3,326 of 11,950 genotyped participants who had been dispensed at least one PPI prescription between January 1, 2002, and November 10, 2020, and entered this study cohort. Mean follow-up time was 426 days (median 508 days) for effectiveness analysis, with 3,887 person-years of follow-up.

Mean (SD) duration of treatment in 2-years follow-up, was 336 (310) days. There were 1,362 (41.0%) patients who had received a diagnosis of abdominal pain before entering the cohort; others were diagnosed with acid reflux (502, 15.1%), gastritis (293, 8.8%), peptic ulcer (71, 2.1%), Helicobacter pylori infection (43, 1.3%), and esophagitis (13, 0.4%).

Of the 3,326 patients in the cohort, there were 66 (2.0%), 739 (22.2%), 1,394 (41.9%), 947 (28.5%), and 180 (5.4%) poor, intermediate, normal, rapid, and ultra-rapid metabolizers, respectively, similar to frequencies reported in Europeans.[15] There was no deviation from Hardy-Weinberg within the cohort (**Table 1**).

Demographic and clinical characteristics at PPI initiation by genotype groups are presented in **Table 2**. The primary outcome occurred in 1,098 (33.0%) in a 2-year follow-up. Only 16.7% of poor metabolizers had new abdominal pain diagnosis during the 2-year follow-up, whereas 30–34.4% of patients from other genetic groups had the primary outcome (**Figure 1**). Risk for the primary outcome in poor metabolizers compared with normal metabolizers was 0.50 (95% CI 0.27–0.91, P = 0.02; **Table 3**). Additional outcomes that composed the secondary outcome occurred as follows: PPI dose increase from initial

Table 1 CYP2C19 metabolizer phenotype by diplotype, Israel (n = 3,326)

	Normal metabolizers n = 1,394 (41.9%)	Intermediate metabolizers n = 739 (22.2%)	Poor metabolizers n = 66 (2.0%)	Rapid metabolizers n = 947 (28.5%)	Ultra-rapid metabolizers n = 180 (5.4%)
*1/*1	1394				
*1/*2		547			
*1/*3		1			
*1/*10		1			
*2/*17		188			
*10/*17		2			
*2/*2			65		
*2/*10			1		
*1/*17				947	
*17*17					180

Unselected sample from breast, colorectal and lung cancer case-control studies in Northern Israel.

dose in 381 patients (11.5%); change to another PPI in 399 patients (12.0%); dispensing metoclopramide prescription in 535 patients (16.1%); and visit to a gastroenterology clinic in 342 patients (10.3%).

Within poor metabolizers, 36.4% had at least one composite outcome event in 2 years, whereas within all other groups there were 51.1–56.2% with at least one event (log rank test P = 0.009). Risk for a composite outcome event was 0.57 (0.38–0.86) in poor metabolizers compared with normal metabolizers (P = 0.007); and no difference in risk between the other groups compared with normal metabolizers: HR = 0.91 (95% CI 0.81–1.03), HR = 1.03 (95% CI 0.93–1.15), and HR = 0.94 (95% CI 0.76–1.17) in intermediate, rapid, and ultra-rapid metabolizers, respectively (**Table 3**). Interestingly, reduction in PPI dose have been identified in 18.2% of poor metabolizers, 14.6% of intermediate metabolizers, and in 13.4%, 13.3%, and 13.3% in normal, rapid, and ultra-rapid metabolizers, respectively.

For a multivariable analysis, we performed stepwise process to include confounders from all demographic/clinical characteristics. Age and ethnicity were independently associated with both exposure (**Table 2**) and the outcome (P < 0.001) and were introduced into the multivariable model. Poor metabolizer status was associated with reduced risk in the multivariable analysis, HR = 0.52 (95% CI 0.28–0.94, P = 0.03), HR = 0.58 (95% CI 0.39–0.87, P = 0.009) for the primary and secondary outcomes, respectively (**Table 3**).

In stratification by PPI, low risk associated with CYP2C19 poor metabolizer status was mostly apparent in omeprazole users HR = 0.50 (95% CI 0.26–0.98, P = 0.04); HR = 0.53 (95% CI 0.27–1.02, P = 0.057), for the primary and secondary outcomes, respectively (**Table 3**). There was nonstatistically significant lower risk in poor metabolizers using lansoprazole, HR = 0.26 (95% CI 0.04–1.85, P = 0.18); HR = 0.25 (95% CI 0.04–1.82, P = 0.17), for the primary and secondary outcomes, respectively (**Table 3**). Pantoprazole and the second-generation PPI, esomeprazole, groups had low numbers of users, which did not permit separate analyses.

For long-term adverse effect analysis, mean follow-up was 2.6 years (maximum 18.5 years), with 20,142 person-years of follow-up. Mean (SD) time (months) from first to last prescription of PPI was 96.8 (73.3), 92.8 (74.3), 98.1 (78.5), 95.9 (73.0), and 98.2 (74.2), in normal, intermediate, poor, rapid, and ultra-rapid metabolizers, respectively, with no significant difference between groups. There were 403 cases of fractures. Within these fractures there were 156 hip fractures (4.7% of the cohort), 152 spine fractures (4.6% of the cohort), and 95 wrist fractures (2.9% of the cohort). Fractures occurred in 7.6% within the poor metabolizers group, and in 11.6%, 12.9%, 12.8%, and 11.1% in normal, intermediate, rapid, and ultra-rapid metabolizers groups, respectively. The lower fracture risk associated with being poor metabolizer was not significant (HR=0.6, 95% CI 0.25–1.47; **Table 3**), possibly due to low power (0.67) for this analysis.

DISCUSSION

We describe, in a real-life cohort of White patients, higher PPI effectiveness associated with being CYP2C19 poor metabolizer, and lower effectiveness associated with the other genetic groups. Rapid and ultra-rapid metabolizers have the same PPI effectiveness as normal metabolizers. There was no association between CYP2C19 poor metabolizing status and fractures.

It has been acknowledged that patients determine the success of PPI therapy. This may vary from one individual to another, based on patient's expectations from therapy, which is likely to be influenced by sex, age, ethnic background, and other demographic factors.[4] We have ascertained demographic and clinical characteristics of the cohort participants, as well as diet components known to increase peptic-related symptoms, to allow multivariable analysis encompassing potential confounders. We used variable measures (ICD-9 codes, visits in outpatient clinics, and pharmacy data) to reflect symptoms persistence/recurrence, and found that symptoms relief was associated with being CYP2C19 poor metabolizer, when compared with normal metabolizers (HR = 0.52 (95% CI 0.28–0.94); HR = 0.58 (95% CI 0.39–0.87), primary and secondary outcomes, respectively).

PPI failure is increasing in proportion with the expanding indications for their use.[26] Although in erosive esophagitis trials, PPIs at standard doses for 8 weeks relieved symptoms and healed esophagitis in up to 86% of patients,[27] about 30% of patients with

Table 2 Demographic and clinical characteristic of proton-pump inhibitors initiators cohort from case-control studies in Northern Israel, 2002–2020

	Normal metabolizers n = 1,394 (41.9%)	Intermediate metabolizers n = 739 (22.2%)	Poor metabolizers n = 66 (2.0%)	Rapid metabolizers n = 947 (28.5%)	Ultra-rapid metabolizers n = 180 (5.4%)	Total n = 3,326	P value
Sex, female	1,197 (85.9)	603 (81.6)	59 (89.4)	789 (83.3)	151 (83.9)	2,799 (84.2)	0.073
Age, mean (SD), (min, max)	72.06 (12.52) (28,105)	72.24 (12.07) (38,100)	74.82 (11.07) (47,99)	70.5 (12.53) (31,104)	72.68 (12.05) (31,96)	71.75 (12.39) (28,105)	0.002
Ethnicity, Jewish	909 (65.2)	527 (71.3)	45 (68.2)	620 (65.5)	118 (65.6)	2,219 (66.7)	0.054
Socioeconomic status, low, medium, high	712,495,171 (51.7, 35.9, 12.4)	354,279,93 (48.8, 38.4, 12.8)	34,22,9 (52.3,33.8,13.8)	496,314,116 (53.6,33.9,12.5)	94,67,18 (52.5,37.4,10.1)	1,690,1177,407 (51.6,35.9,12.4)	0.717
BMI, mean (SD)	28.85 (9.48)	28.53 (5.74)	28.82 (5.82)	29.27 (6.50)	29.11 (5.35)	28.84 (8.33)	0.30
Hypertension	697 (50.0)	381 (51.6)	38 (57.6)	442 (46.7)	85 (47.2)	1,643 (49.4)	0.165
Diabetes mellitus	340 (24.4)	165 (22.3)	21 (31.8)	212 (22.4)	37 (20.6)	775 (23.3)	0.268
Hyperlipidemia	728 (52.2)	407 (55.1)	36 (54.5)	467 (49.3)	90 (50.0)	1,728 (52.0)	0.198
Ischemic heart disease	231 (16.6)	133 (18.0)	12 (18.2)	159 (16.8)	35 (19.4)	570 (17.1)	0.826
Congestive heart failure	41 (2.9)	27 (3.7)	3 (4.5)	34 (3.6)	8 (4.4)	113 (3.4)	0.735
s/p CVA	68 (4.9)	36 (4.9)	4 (6.1)	48 (5.1)	10 (5.6)	166 (5.0)	0.99
Asthma	79 (5.7)	46 (6.2)	3 (4.5)	51 (5.4)	9 (5)	188 (5.7)	0.928
COPD	80 (5.7)	30 (4.1)	2 (3.0)	48 (5.1)	6 (3.3)	166 (5.0)	0.332
Dementia	24 (1.7)	17 (2.3)	0 (0)	12 (1.3)	7 (3.9)	60 (1.8)	0.083
Renal failure	46 (3.3)	31 (4.2)	4 (6.1)	36 (3.8)	8 (4.4)	125 (3.8)	0.662
Malignancy	512 (36.7)	265 (35.9)	23 (34.8)	340 (35.9)	57 (31.7)	1,197 (36.0)	0.766
Osteoporosis	208 (14.9)	91 (12.3)	8 (12.1)	106 (11.2)	24 (13.3)	437 (13.1)	0.111
Anxiety	74 (5.3)	36 (4.9)	3 (4.5)	45 (4.8)	8 (4.4)	166 (5.0)	0.966
Depression	116 (8.3)	62 (8.4)	5 (7.6)	79 (8.3)	11 (6.1)	273 (8.2)	0.884
Alcohol abuse	5 (0.4)	3 (0.4)	1 (1.5)	10 (1.1)	0 (NA)	19 (0.6)	0.114
Smoking	338 (24.2)	211 (28.6)	13 (19.7)	219 (23.1)	41 (22.8)	822 (24.7)	0.073
Fluoxetine[a]	11 (0.8)	9 (1.2)	1 (1.5)	8 (0.8)	1 (0.6)	30 (0.9)	0.826
High-fat dishes, servings/week, mean (SD)	23.21 (13.40)	22.33 (12.04)	22.57 (12.14)	22.21 (11.82)	21.72 (12.58)	22.86 (12.90)	0.05
Coffee/tea/carbonated beverages, serving/week, mean (SD)	23.52 (15.53)	23.19 (15.30)	24.66 (15.43)	25.55 (16.39)	24.19 (15.99)	23.73 (15.61)	0.003
PPI initiation							
Omeprazole	1,106 (79.3)	615 (83.2)	55 (83.3)	760 (80.3)	138 (76.7)	2,674 (80.4)	
Daily dose of omeprazole (mg) median, mean (SD)	20, 23.9 (9.2)	20, 23.2 (8.7)	20, 24.0 (9.3)	20, 23.6 (8.6)	20, 23.6 (8.9)	20, 23.7 (8.9)	0.64
Lansoprazole	236 (16.9)	100 (13.5)	9 (13.6)	156 (16.5)	32 (17.8)	533 (16.0)	

(Continued)

Table 2 (Continued)

	Normal metabolizers n = 1,394 (41.9%)	Intermediate metabolizers n = 739 (22.2%)	Poor metabolizers n = 66 (2.0%)	Rapid metabolizers n = 947 (28.5%)	Ultra-rapid metabolizers n = 180 (5.4%)	Total n = 3,326	P value
Daily dose of lansoprazole (mg) median, mean (SD)	30, 32.6 (12.9)	30, 34.2 (12.8)	30, 26.7 (6.6)	30, 33.8 (14.5)	30, 33.8 (15.2)	30, 33.2 (13.4)	0.48
Esomeprazole	38 (2.7)	13 (1.8)	1 (1.5)	23 (2.4)	9 (5.0)	84 (2.5)	
Daily dose of esomeprazole (mg) median, mean (SD)	40, 33.7 (16.8)	40, 33.8 (17.1)	20, 20 (NA)	40, 40 (20.9)	20, 28.9 (10.5)	40, 34.8 (17.6)	0.48
Pantoprazole	14 (1.0)	11 (1.5)	1 (1.5)	8 (0.8)	1 (0.6)	35 (1.1)	
Daily dose of pantoprazole (mg) median, mean (SD)	40, 35.7 (16.0)	40, 38.2 (16.6)	20, 20.0 (NA)	40, 35.0 (9.3)	20, 20.0 (NA)	40, 35.4 (14.6)	0.43

n(%), unless otherwise noted.
BMI, body mass index; CVA, cerebrovascular accident; COPD, chronic obstructive pulmonary disease; PPI, proton-pump inhibitor.
[a]Number of users of other potential CYP2C19 inhibitors/inducers at 4-months assessment window before cohort entry was low and did not permit analysis (including: ketoconazole, voriconazole, oral hormonal contraceptives, cimetidine, ticlopidine, fluvoxamine, isoniazid, rifampicin, and ritonavir).

GERD remain symptomatic on standard dose of PPI, and the vast majority of them will continue to experience GERD symptoms on even higher doses of PPI.[4] Moreover, the proportion of patients with non-erosive reflux disease responding to a standard dose of PPI is 20–30% lower than what has been documented in patients with erosive esophagitis and, among them, patients with functional heartburn demonstrate the lowest symptom response rate.[4]

A large body of literature from studies in Asian populations reported an association between CYP2C19 normal metabolizers and decreased therapeutic effectiveness with PPIs compared with CYP2C19 intermediate and poor metabolizers.[14] For example, a study by Furuta et al.[28] demonstrated 45.8% healing rates in normal metabolizers with erosive esophagitis treated with lansoprazole 30 mg daily for 8 weeks when compared with 84.6% in poor metabolizers with erosive esophagitis. Accordingly, poor metabolizers of lansoprazole had significantly higher blood levels of lansoprazole, and patients that were successfully treated had higher levels of lansoprazole than patients who failed therapy.[28] Esophageal healing rates of patients with erosive esophagitis treated with lansoprazole 30 mg daily for normal, intermediate, and poor metabolizers were 57%, 69%, and 73% in 4 weeks and 77%, 95%, and 100% at 8 weeks, respectively.[29] There were no data related to symptom relief among patients based on CYP2C19 genotype.

In the current cohort, intermediate metabolizers were suffering persistence/recurrent symptoms, similar to normal metabolizers. In line with our findings, evidence of high/moderate degree (for omeprazole, lansoprazole, and pantoprazole) have shown that CYP2C19 poor metabolizers, when compared with intermediate metabolizers, had decreased metabolism, better acid secretion indices, and increased efficacy (table S1 of CPIC guidelines).[14] It should be noted, that the diplotype *2/*17, comprising most of the intermediate metabolizers group in our cohort, is rare in Asians,[15] and it might be that the function of *2/*17 is higher, at least for PPI metabolism, than other diplotypes used to define intermediate metabolizers in Asian populations.

In the sensitivity analysis we performed by PPI type, better symptom relief associated with being CYP2C19 poor metabolizer was apparent in omeprazole users (the majority of the current cohort). Although no differences in effectiveness of acid suppression were described between the different types of PPIs,[1] the PPIs differ in the role CYP2C19 plays in their metabolism. There is a substantial body of evidence that goes in line with our data linking CYP2C19 genotype with variability in plasma concentrations and efficacy of first-generation PPIs (omeprazole, lansoprazole, and pantoprazole),[14] mostly with omeprazole.[16] Age and ethnicity were identified as being associated with both exposure (genetic group) and outcome in this cohort. We did not find reports of survival advantages of CYP2C19 poor metabolizers. However, age had been reported as being associated with omeprazole metabolism.[30]

This study might suggest that in most White patients treated with PPIs, effectiveness is not optimal, specifically when using omeprazole. High dose PPI therapy has been suggested to increase cure rate in helicobacter pylori infection.[31] Studies might be needed to evaluate ways to increase effectiveness of PPIs, in which CYP2C19 seems to play a major role in metabolism, when prescribed for various indications.
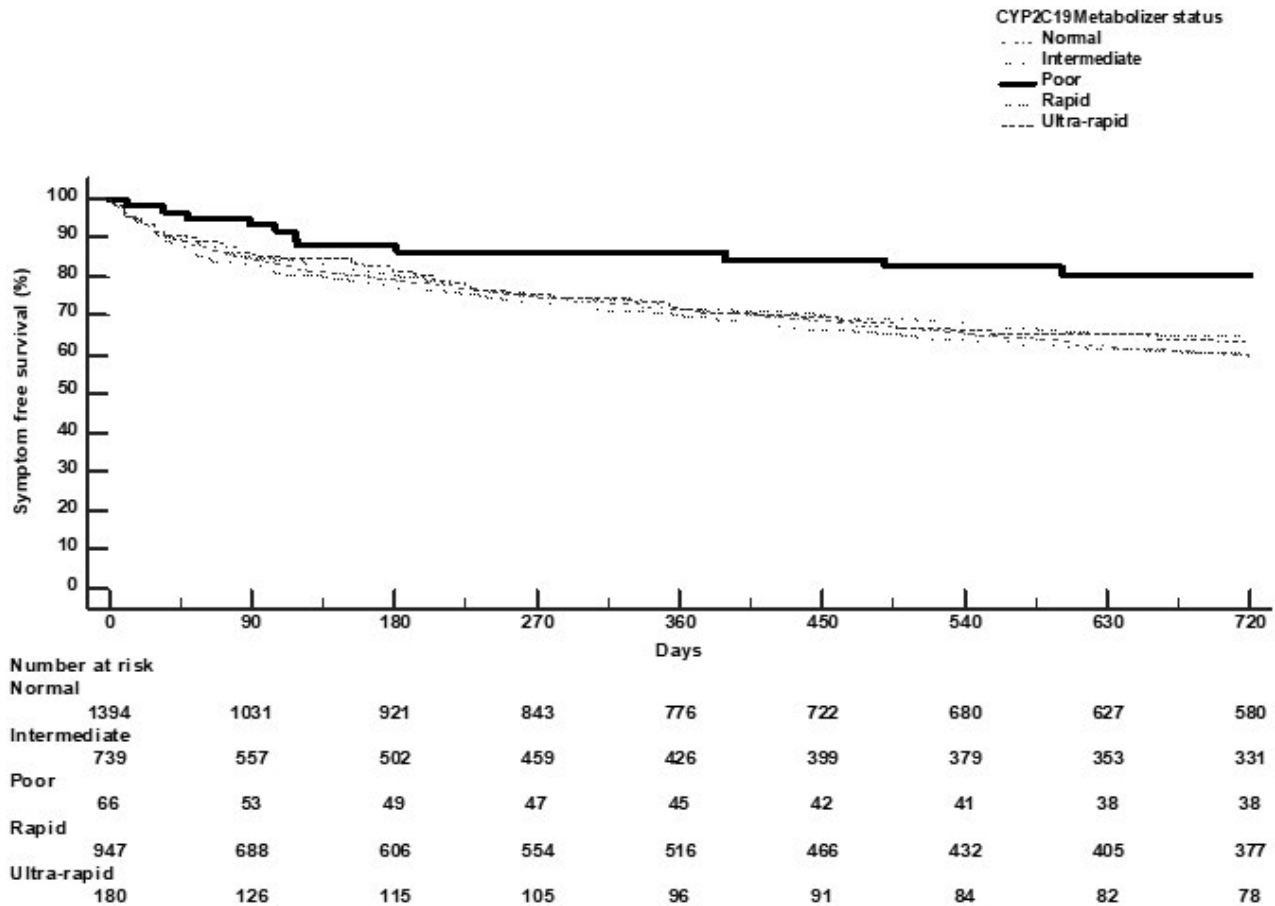
Figure 1 Kaplan-Meier curve of cumulative event-free survival for a new diagnosis of abdominal pain, in proton-pump inhibitors initiators, reflecting non-resolution/recurrence of symptoms, in 2-years follow-up; stratified according to CYP2C19 genotype.

Higher risk for bone fractures[1,18,19] had been previously associated with higher exposure of PPIs,[17,18] or with long-term use.[18,32] We have not found evidence of an association between higher exposure (in CYP2C19 poor metabolizers) and fractures. Further, there was a nonsignificant decreased risk of fractures associated with being a poor metabolizer.

This study had several limitations. First, due to the retrospective nature of the study cause and effect could not be ascertained. A second limitation was the heterogeneity of cohort participants that included both patients with various forms of cancer and controls. The third limitation was lack of data on lifestyle modifications, such as elevation of the head end of the bed to reduce GERD symptom. However, these maneuvers were expected to be non-differential, and were likely to bias the results toward the null. A fourth limitation was our assumption of the usual step-up therapy approach in treating peptic-related symptoms/disease; such that increasing daily dose was a proxy to non-resolution of symptoms. This, however, might not reflect a different attitude in which high PPI dose is administered as the first step and the dose is then gradually decreased until symptoms recur (step-down approach).[33] Nonetheless, in a step-down approach, dose increase if existed, reflected symptoms recurrence. In addition, because pharmacogenetic data were not known to patients and prescribers, the step-down clinical approach

was expected to be non-differential, and thus was likely to bias the results toward the null. A fifth limitation was the absence of endoscopic diagnoses, before and following PPI treatment. However, a large portion of patients treated with PPIs do not have endoscopic evaluation[1,34] or do not have erosive peptic disease on endoscopic evaluation. A sixth limitation was inclusion of a few clinical and pharmacy indices as well as data on visits to clinics as our secondary outcome measure, which had not been used before as an outcome measure in real-life studies for effectiveness of PPI, and should be validated in additional studies.

A notable strength of this study is the population-based nature of the study with a relatively large number of PPI users, encompassing genetic, clinical, and dietary data that allowed us evaluation of PPI effectiveness, and of a probable adverse effect, in real life, where participants and prescribers were unaware of genetic results.

The management of peptic-related symptoms and diseases influences quality of lives, and has implications for healthcare utilization and costs.[1] We demonstrate in this real-life cohort of White paients higher effectiveness of PPIs that is associated with being a CYP2C19 poor metabolizer, in particular with omeprazole use, whereas being a non-poor metabolizer (which means belonging to the large majority of population in White patients) is associated with decreased PPI effectiveness. Higher exposure to PPIs as in poor metabolizers was not associated with increased risk of

Table 3 Number of events and risk, for effectiveness and safety outcomes, associated with CYP2C19 polymorphism, in patients initiating proton-pump inhibitors, Clalit, Israel

Analysis	Normal metabolizers n = 1,394 (41.9%) N(%)	Intermediate metabolizers n = 739 (22.2%) Univariate N (%), HR (95% CI), P value	Intermediate metabolizers Multivariate HR (95% CI), P value	Poor metabolizers n = 66 (2.0%) Univariate N (%), HR (95% CI), P	Poor metabolizers Multivariate HR (95% CI), P value	Rapid metabolizers n = 947 (28.5%) Univariate N (%), HR (95% CI), P value	Rapid metabolizers Multivariate HR (95% CI), P value	Ultra-rapid metabolizers n = 180 (5.4%) Univariate N (%), HR (95% CI), P value	Ultra-rapid metabolizers Multivariate HR (95% CI), P value
Primary outcome: abdominal pain, all PPIs	479 (34.4), Ref.	229 (31.0), 0.91 (0.78–1.07), 0.25	0.93 (0.80–1.09), 0.39	**11 (16.7), 0.50 (0.27–0.91), 0.02**	**0.52 (0.28–0.94), 0.03**	325 (34.3), 1.02 (0.89–1.18), 0.78	1.01 (0.88–1.16), 0.92	54 (30.0), 0.89 (0.67–1.17), 0.40	0.89 (0.67–1.18), 0.42
Primary outcome: abdominal pain, stratification by PPI[a]									
Omeprazole	372 (33.6), Ref.	189 (30.7), 0.93 (0.78–1.11), 0.41	0.95 (0.80–1.13), 0.57	**9 (16.4), 0.50 (0.26–0.98), 0.04**	**0.53 (0.27–1.02), 0.057**	255 (33.6), 1.03 (0.88–1.21), 0.71	1.02 (0.87–1.20), 0.79	38 (27.5), 0.84 (0.60–1.17), 0.29	0.85 (0.61–1.18), 0.33
Lansoprazole	91 (38.6), Ref.	33 (33.0), 0.83 (0.56–1.24), 0.36	0.85 (0.57–1.26), 0.42	1 (11.1), 0.26 (0.04–1.85), 0.18	0.25 (0.04–1.82), 0.17	63 (40.4), 1.02 (0.74–1.41), 0.91	1.00 (0.73–1.38), 0.99	13 (40.6), 1.04 (0.58–1.85), 0.91	1.02 (0.57–1.83), 094
Composite outcome	Ref.	0.91 (0.81–1.03), 0.13	0.92 (0.81–1.04), 0.917	**0.57 (0.38–0.86), 0.007**	**0.58 (0.39–0.87), 0.009**	1.03 (0.93–1.15), 0.57	1.02 (0.91–1.13), 0.80	0.94 (0.76–1.17), 0.60	0.95 (0.76–1.18), 0.63
Composite outcome, stratification by PPI[a]									
Omeprazole	Ref.	0.91 (0.79–1.04), 0.17	0.92 (0.80–1.05), 0.23	**0.56 (0.36–0.89), 0.01**	**0.57 (0.36–0.90), 0.016**	1.06 (0.93–1.20), 0.37	1.05 (0.93–1.19), 0.46	0.92 (0.72–1.18), 0.51	0.93 (0.72–1.19), 0.54
Lansoprazole	Ref.	0.89 (0.66–1.21), 0.46	0.88 (0.64–1.19), 0.39	0.43 (0.14–1.35), 0.15	0.41 (0.13–1.27), 0.12	0.96 (0.74–1.25), 0.76	0.94 (0.72–1.21), 0.61	1.07 (0.66–1.73), 0.78	1.07 (0.67–1.73), 0.78
New Fracture, all PPIs	162 (11.6), Ref.	95 (12.9), 1.13 (0.88–1.45), 0.35		5 (7.6), 0.60 (0.25–1.47), 0.26		121 (12.8), 1.12 (0.88–1.41), 0.36		20 (11.1). 0.93 (0.58–1.48), 0.75	
New fracture, stratification by PPI[a]									
Omeprazole	Ref.	1.16 (0.88–1.53), 0.28		0.57 (0.21–1.54), 0.27		1.16 (0.90–1.50), 0.27		1.03 (0.63–1.69), 0.89	
Lansoprazole	Ref.	1.06 (0.50–2.23), 0.88		0.97 (1.31–7.22), 0.98		1.17 (0.63–2.16), 0.63		0.63 (0.15–2.65), 0.52	

Cox regression analyses to calculate HRs (95% CI) to achieve a first event according to genotype.

Primary effectiveness outcome was diagnosis of abdominal pain in primary physician visit or an emergency department visit in a 2-years follow-up. Secondary effectiveness outcome was the composite outcome of: diagnosis of abdominal pain (from a primary physician visit or an emergency department visit), gastroenterology clinic visit, a change to another PPI, PPI dose increase from initial dose, or dispensing metoclopramide prescription, in a 2-years follow-up.

In the new fracture analysis, outcome was a new diagnosis of hip/spine/wrist fractures, from hospitalizations, or from diagnoses written in the patients' medical records in outpatient medical encounters, in long-term follow-up until outcome date, death, end of study (November 10, 2020), or end of registration in Clalit, whichever came first.

CI, confidence interval; HR, hazard ratio; PPI, proton-pump inhibitor.

[a]Pantoprazole and esomeprazole smaller groups did not permit separate analyses by genetic groups.

fractures. Future studies might be needed to evaluate ways to increase effectiveness in non-poor-metabolizers, treated with PPIs.

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