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Clopidogrel IBS Patients Have Higher Incidence of Gastrointestinal Symptoms Influenced by Age and Gender

Suren Soghomonyan¹, Mahmoud Abdel-Rasoul², Alix Zuleta-Alarcon¹, Iveta Grants¹, Victor Davila¹, Jeffrey Yu¹, Cheng Zhang³, Emmett E. Whitaker^{1,5}, Sergio D. Bergese¹, Nicoleta Stoicea¹, Razvan Arsenescu^{3,4}, and Fievos L. Christofi¹

¹Department of Anesthesiology, The Wexner Medical Center, The Ohio State University, 420 West 12th Ave, Room 226, Columbus, OH 43210, USA

²Department of Biomedical Informatics, Center for Biostatistics, The Ohio State University, 1800 Cannon Drive, Room 320, Columbus, OH 43210, USA

³Department of Internal Medicine, Wexner Medical Center, The Ohio State University, 395 West 12th Ave, Suite 200, Columbus, OH 43210, USA

⁴Atlantic Inflammatory Bowel Disease Center of Excellence, Atlantic Digestive Health Institute, 435 South Street, Suite 205, Morristown, NJ 07960, USA

⁵Department of Anesthesiology and Pain Medicine, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205, USA

Abstract

Background—Clopidogrel is an irreversible antagonist of P2Y₁₂ receptors (P2Y₁₂Rs) used as an antiplatelet drug to reduce risk of thrombosis. P2Y₁₂Rs are expressed in gastrointestinal (GI) tract where they might regulate GI function.

Aim—To evaluate if blockade of P2Y₁₂Rs by clopidogrel is associated with higher incidence of GI symptoms in patients with irritable bowel syndrome (IBS).

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Author's contribution Suren Soghomonyan Contributions to the design of the retrospective study, analysis and interpretation of data and preparation of the manuscript. Mahmoud Abdel-Rasoul Senior Biostatistician for the Wexner Medical Center and NIH CTSA programs involved in the statistical analysis, interpretation of data and contributed to the preparation and writing of the manuscript and discussion/interpretation of findings. Alix Zuleta-Alarcon Postdoctoral fellow who contributed to the literature search on clopidogrel, IBS and GI disorders, data interpretation, discussion and overall writing of the manuscript. Iveta Grants Contributed to all aspects of the study and significantly contributed to the immunochemical studies on P2Y₁₂R expression and distribution in the human intestinal tract. Victor Davila Contributed to the design of the study, writing of the manuscript and interpretation of the data. Jeffrey Yu Summer medical student supported by a Samuel J. Roessler Scholarship to analyze data from this study; contributed to preparation and writing of the manuscript. Cheng Zhang Gastroenterologist on the team who contributed to the design of the manuscript, interpretation of data and overall preparation and writing of the manuscript. Emmett E. Whitaker Contributed to the design and interpretation of data and overall completion of the manuscript, and in particular the discussion section. Sergio D. Bergese Contributed to the overall interpretation of data, preparation and writing of the manuscript. Nicoleta Stoicea Provided supervision of the summer medical student on a Samuel J. Roessler Scholarship to work on data analysis in the study. She contributed to study design, interpretation and writing of the manuscript. Razvan Arsenescu Senior gastroenterologist on the team who contributed to the design of the study, data analysis and interpretation and preparation of the manuscript. Fievos L. Christofi Principal Investigator of this retrospective study, who was responsible for the overall study design, conduct of the study, data analysis, interpretation and manuscript writing and submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Methods—A retrospective analysis of our institutional database was conducted for a 13-year period. IBS patients were identified, and their demographics, GI symptoms and clopidogrel therapy were collected. Logistic regression models were used to characterize symptoms in clopidogrel versus no-clopidogrel IBS-groups, adjusting for Age and Sex differences. An additional study characterized the P2Y₁₂R distribution in human gut.

Results—The search identified 7217 IBS patients (6761 no-clopidogrel/456 clopidogrel). There were a higher proportion of patients with GI symptoms on clopidogrel (68%) compared to controls (60%, $p = 0.0011$) that were Females (70 vs. 60%, $p = 0.0003$) not Males (61 vs. 60%; $p = 0.8312$). In Females, clopidogrel was associated with higher incidence of GI symptoms (Age adjusted; $p < 0.0001$) for pain, constipation, gastroparesis ($p = 0.0001$) and psychogenic pain ($p = 0.0006$). Age or Sex (adjusted models) influenced one or more GI symptoms (i.e., pain, $p < 0.0001$; constipation, $p < 0.0001$ / $p = 0.008$; diarrhea, flatulence, $p = 0.01$). P2Y₁₂R immunoreactivity was abundant in human ENS; glial-to-neuron ratio of P2Y₁₂Rs expressed in Females $\gg$ Males.

Conclusions—Irreversible blockade of P2Y₁₂R by clopidogrel is associated with higher incidence of GI symptoms in Female IBS patients, although Age or Sex alone contributes to symptomatology. Prospective studies can determine clinical implications of P2Y₁₂Rs in IBS.

Keywords

Clopidogrel; P2Y₁₂ receptors; Enteric nervous system; Enteric cells; Age; Gender; Irritable bowel syndrome; Abdominal pain; Gastroparesis; Constipation; Diarrhea; Flatulence

Introduction

Clopidogrel is a thienopyridine-class antiplatelet drug used to prevent intravascular clotting in high-risk patients. Its mechanism of action is based on irreversible binding and blockade of the purinergic P2Y₁₂ receptors (P2Y₁₂Rs) on platelet membranes. Adenosine diphosphate (ADP) is the natural ligand for P2Y₁₂Rs, which after binding triggers downstream reactions resulting in platelet adhesion, fibrin cross-linking and formation of the primary thrombus. These reactions are effectively blocked by treatment with clopidogrel [1–3]. Clopidogrel is a pro-drug converted into its active form in the liver by CYP2C19 and other enzymes (CYP1A2, CYP2B6, CYP2C9 and CYP3A4/5). Genetic variability of these enzymes is responsible for treatment failure in up to 14% of patients [1, 4, 5].

Clopidogrel is given as a prophylactic treatment to patients at high risk of vascular thrombosis, or who have symptomatic atherosclerosis, unstable angina, myocardial infarction, peripheral vascular or cerebrovascular disease [2]. The drug is either used as monotherapy or in combination with aspirin following the placement of cardiovascular stents. As a monotherapy, it is frequently prescribed as an alternative to aspirin in patients with aspirin intolerance [6]. In recent years, the number of patients receiving clopidogrel has consistently increased, and these numbers are expected to rise further because of the aging general population, more aggressive management of the chronic vascular diseases and because clopidogrel is increasingly being used in children [7]. About 1.6 million adults undergo coronary revascularization procedures each year, and, of these, percutaneous

intervention (PCI) is the technique used in 74% of cases. When a coronary arterial stent is placed at the time of the PCI in up to 90% of adults, clopidogrel is routinely used to reduce the risk of re-occlusion [8–10].

Purinergic signaling is involved in the regulation of most important physiological functions of the GI tract including mucosal reflexes, motility and sensory signaling in the enteric nervous system (ENS), synaptic transmission, as well as coordination of motility and secretion [11–13]. P2Y₁₂Rs are highly expressed in the gastrointestinal (GI) tract. Functional P2Y₁₂Rs are expressed in enterochromaffin cells lining the intestinal mucosa where they provide inhibitory modulation of 5-HT release, modulation of intestinal reflexes and transmission of visceral sensory signaling to the brain. They are also expressed on intrinsic primary afferent neurons of the ENS in animals, where they may potentially play a role in regulation of enteric neural reflexes, visceral sensation, GI motility and secretion. Overall, P2Y₁₂Rs are implicated in the sensory afferent limb of intrinsic and extrinsic gut reflexes [14, 15]. One would predict that clopidogrel could potentially disrupt gut physiology; resulting in GI symptoms.

Adverse effects of clopidogrel include bleeding, hyper-sensitivity reactions, thrombotic thrombocytopenic purpura, neutropenia, headache, dizziness, vertigo, numbness, neuralgia and paresthesias. Other adverse GI effects reported by patients taking clopidogrel include GI discomfort, diarrhea, constipation, dyspepsia, nausea and abdominal pain although the incidence is not known [3, 4, 7, 12, 16, 17]. It is also not clear to what extent the incidence of GI symptoms is higher in patients on clopidogrel than in the general population or whether clopidogrel may exacerbate similar symptoms in patients with functional GI disorders such as irritable bowel syndrome (IBS).

IBS is a relatively common functional bowel disorder characterized by visceral pain and hypersensitivity, abdominal cramps, bloating and episodes of either diarrhea and/or constipation. The disease is more prevalent in Females than Males and affects around 10–20% of adults in the USA [18–20]. IBS is associated with a significant economic burden, morbidity and worsened quality of life.

Overall, based on the distribution of P2Y₁₂ receptors in the GI tract, we hypothesized that irreversible blockade of P2Y₁₂ receptors in the gut in IBS patients could potentially disrupt gut physiology resulting in the increase in GI symptoms. Our prediction was that IBS patients taking clopidogrel would therefore be associated with a higher incidence of GI symptoms other than GI bleeding (i.e., abdominal pain, diarrhea, constipation, bloating, gas/flatulence, etc.). Our aim was two-fold: (1) to test whether IBS patients on clopidogrel had a higher incidence of GI symptoms in a retrospective study of IBS patients based on the information retrieved from the electronic medical database of our institution for over 7000 IBS patients. (2) Determine the expression and distribution of P2Y₁₂ receptors in the human ENS in small and large bowel to confirm findings in animals.

Materials and Methods

Human Subjects

This retrospective study was approved by the IRB Ethics Committee at the Ohio State University Wexner Medical Center (IRB # 2013H0249) and conducted on data obtained from the institutional electronic medical records database by our information warehouse (iWH) for the period between 01.01.2000 and 05.30.2013. A parallel study was approved to study purinergic receptor signaling including P2Y₁₂R distribution in human intestinal tissue (IRB # 2012H0231). Adult patients between the Age 18–75 years and diagnosed with IBS were included in the study. For all patients, the diagnosis of IBS was done by the healthcare providers at the time based on Rome II criteria used for patients treated before 2006 and Rome II or III criteria afterward [21]. Those patients who had a diagnosis of inflammatory bowel disease were excluded from the study. In collaboration with the iWH, we developed specific queries using SQL based on international classification of diseases (ICD) IX to screen the electronic medical records for patients diagnosed with IBS who received treatment in our hospital during the period 01.01.2000–05.30.2013. Additional queries were developed, and code information used to extract the demographic data (Age/Sex), all GI symptoms and evidence of clopidogrel therapy in this patient group. The patients' Age was determined for the time of data acquisition.

Statistical Analysis

SAS version 9.3 software (SAS Institute, Cary, NC) was used to analyze the data by a senior biostatistician on our team. Logistic regression models were used to characterize symptoms in treatment versus control (no-clopidogrel) groups and adjusted for Age and Sex differences. Models were developed to characterize the associations between the patients' demographic parameters, and treatment with clopidogrel on presence of GI symptoms. Logistic regression models for each symptom or for all symptomatic patients as a group were developed and analyzed. The odds ratios, 95% confidence intervals and *p* values of the parameters were calculated. *p* values less than 0.05 were considered to be statistically significant.

1. Data were analyzed for differences in the incidence of GI symptoms between clopidogrel and no-clopidogrel and expressed as a percentage of patients with GI symptoms (Table 1).
2. Multivariate logistic regression models were used to compare clopidogrel, Sex and Age differences in all IBS patients (Table 2). The first model was the “naïve model,” comparing clopidogrel versus no-clopidogrel groups (not adjusted for Age or Sex). The second model was clopidogrel versus no-clopidogrel (adjusted for Age and Sex). The third model tested for differences in Females only (clopidogrel vs. no-clopidogrel) or for the influence of Age in Females. The fourth model tested for differences in Males only (clopidogrel vs. no-clopidogrel) or for the influence of Age in Males. Age was analyzed as a continuous variable, and the difference was tested for a “1 year change in Age.”

3. Multivariate logistic regression models were used to test differences for each outcome (symptom) in IBS patients (Table 3). To test for effect of clopidogrel, we analyzed the data for differences between clopidogrel and no-clopidogrel groups (adjusting for Sex and Age, confounding variables) in all symptomatic patients. In the same model(s), to test for Sex differences, we analyzed differences between Female and Male groups in GI symptoms in all symptomatic patients (adjusted for clopidogrel and Age). To test if Age is significantly associated with a specific GI symptom in the same model(s), Age was analyzed as a continuous variable after adjusting for clopidogrel and Sex.
4. Multivariate logistic regression models adjusted for Age for each outcome variable (symptom) was used to analyze differences in each symptom in Females only or Males only (Table 4).

Immunochemical Labeling of P2Y₁₂ Receptors in the Human ENS

P2Y₁₂ receptor immunoreactivity (P2Y₁₂ receptor-ir) with a single antibody or co-labeling studies with 2 antibodies were used to determine the expression and distribution of P2Y₁₂Rs in the human ENS. The details of the methods were previously described from our team [22]. Whole-mount preparations of freshly microdissected human submucous plexus obtained from jejunum of patients who underwent resection and Roux-en-Y anastomosis were used in immunofluorescent labeling studies for P2Y₁₂Rs and HuC/HuD protein expression in enteric neurons. Separate experiments were done for immunohistochemical identification of P2Y₁₂Rs in paraffin sections of jejunum or immunofluorescent labeling of normal colon from surgical colectomy cases (i.e., for polyps, neoplasms, cancer; tissue was from a region away from the involved region); 8 surgical specimens included 7 descending colon cases and 1 transverse colon. Semiquantitative analysis was done in the colon for P2Y₁₂ receptor expression. Supplemental Table 1 summarizes the antisera characteristics used for staining. Omission of primary antibodies was used as control for the secondary antibody. Pre-absorption of the primary antibody with its immunogenic peptide was used to test specificity of the antibody for P2Y₁₂Rs. Isotype IgG antibodies were used as additional controls for primary antibodies.

Results

Incidence of GI Symptoms in IBS Patients on Clopidogrel

The database search identified 7217 IBS patients who underwent treatment in our medical center during the study period. Among those patients, 6761 did not have any associated records of treatment with clopidogrel. Within this group, 4058 (60.02%) had GI symptoms on record. We found 456 IBS patients who had a documented history of taking clopidogrel, and, among them, 309 of 456 patients (67.76%) had active GI symptoms, which was significantly higher compared to the no-clopidogrel group ($p = 0.0011$). The rate of occurrence for the most characteristic GI symptoms among the selected patient population is shown in Fig. 1, for both patients taking and those not taking clopidogrel. Among 7217 IBS patients identified in the electronic medical records database, there were 5728 Females and 1489 Males. The data on recorded GI symptoms among Male and Female patients are shown

in Table 1. The incidence of GI symptoms was significantly higher among Female patients treated with clopidogrel compared to those not treated with the medication (69.9 vs. 59.8%, $p < 0.0003$). Treatment with clopidogrel was not associated with a higher rate of GI symptoms in Males (60.2 vs. 61.3%, $p = 0.8312$).

Overall, the incidence of GI symptoms in IBS patients was significantly higher among patients treated with clopidogrel compared to those not treated with the medication.

The mean Age of patients not taking clopidogrel was 54.92 ± 16.35 years, while patients taking clopidogrel had a higher mean Age of 69.18 ± 13.32 years. The difference between the groups was significant ($p < 0.0001$).

Among 6761 IBS patients not treated with clopidogrel, 1378 were Males and 5383 Females. In the treatment group (456 patients), there were 111 Males and 345 Females. The Female to Male ratio was 80:20 in IBS patients with no-clopidogrel compared to the ratio of 76:24 in the clopidogrel group ($p = 0.043$).

Multivariate logistic regression models comparing clopidogrel, Sex and Age in IBS patients for all patients (Table 2).

1. In the naïve clopidogrel model (not adjusted for Age or Sex), patients on clopidogrel had a higher incidence of GI symptoms than control IBS patients ($p < 0.0011$).
2. In the multivariate model adjusted for Age and Sex, patients on clopidogrel were more likely to have GI symptoms than patients not on clopidogrel (OR 1.75; 95% CI 1.42–2.15; $p < 0.0001$).
3. Younger Age (adjusted for clopidogrel and Sex) is associated with greater odds of having GI symptoms ($p < 0.0001$). In other words, older patients are less likely (have lower odds) than younger patients to have GI symptoms.
4. The odds of having GI symptoms did not differ significantly between Males and Females (OR 1.01; 95% CI 0.90–1.14) ($p = 0.8356$).
5. In Females only, patients on clopidogrel had a higher incidence of GI symptoms than controls after adjusting for Age ($p < 0.0001$). Age was also significant indicating that Age does matter and that older Female patients are less likely to be symptomatic.
6. In contrast to Females, in Males, there was not a significant difference between clopidogrel and control ($p = 0.4137$). Age, however, was significant. Older Males had lower odds of being symptomatic ($p = 0.0072$).

Association Between Clopidogrel and Specific GI Symptoms in IBS Patients

Multivariate logistic regression models were used to analyze differences in the presence of each specific GI symptom (outcome variable) in symptomatic IBS patients. The data are summarized in Table 3 for the influence of clopidogrel, Sex and Age.

1. *Influence of Clopidogrel on Specific GI Symptoms* IBS patients on clopidogrel (adjusted for Age and Sex) were more likely to display certain GI symptoms compared to control IBS patients: These included epigastric pain ($p < 0.0001$), generalized abdominal pain ($p = 0.0001$), abdominal pain of other specific site ($p < 0.0001$), acute pain not elsewhere classified (NEC) ($p < 0.0001$), constipation NEC ($p < 0.0001$), constipation not otherwise specified (NOS) ($p < 0.0001$), gastroparesis ($p < 0.0001$) and gastro-duodenal disease NEC ($p = 0.0007$).
2. *Influence of Sex on Specific GI Symptoms* Significant Sex differences occurred in certain outcomes in Female versus Male patients (adjusted for clopidogrel and Age): these included epigastric pain ($p = 0.0001$), generalized abdominal pain ($p = 0.0015$), abdominal pain in right upper quadrant ($p = 0.0034$), acute pain NEC ($p = 0.0013$), constipation ($p = 0.0079$), constipation NEC ($p = 0.0057$) and constipation NOS ($p < 0.0001$).
3. *Influence of Age on Specific GI Symptoms* Age as a continuous variable (adjusted for clopidogrel and Sex) was strongly associated with epigastric pain ($p < 0.0001$), generalized abdominal pain ($p < 0.0001$), abdominal pain in left upper quadrant ($p < 0.0001$), left lower quadrant ($p < 0.0001$), right upper quadrant ($p < 0.0001$), right lower quadrant ($p < 0.0001$), abdominal pain of other specific site ($p < 0.0001$), abdominal pain ($p = 0.0454$), periumbilical pain ($p < 0.0001$), acute pain NEC ($p < 0.0001$), epigastric abdominal tenderness ($p < 0.0087$), constipation ($p < 0.0001$) and gastroparesis ($p = 0.0042$), diarrhea ($p = 0.0102$) and flatulence/eructation/gas pain ($p = 0.015$). In interpreting the odds ratios, an odds ratio < 1.0 means older individuals are less likely to have the same symptom; > 1.0 means older patients are more likely to have the symptom. Note: odds ratios are highly significant but marginally different with Age, but this is reflective of the influence of 1-year difference in Age, and the greater the Age differences the more the influence. Also, for Female to Male comparisons, the odds ratios are much higher than 1.0, indicating that Females are more likely to be associated with certain GI symptoms. This is not the case for gastroduodenal disease NEC (OR 0.77; $p = 0.0305$) and noninfectious gastroenteritis NEC (OR 0.81, $p = 0.0344$).
4. *Influence of Clopidogrel in Males or Females Adjusted for Age* Data are summarized in Table 4 for Females or Males with clopidogrel versus no-clopidogrel (adjusted for Age) for each outcome (symptom). In general, the effect of clopidogrel on being symptomatic differed between Females and Males. In separate models stratified by Sex, a significant difference between clopidogrel and no-clopidogrel was only evident for Females ($p < 0.0001$). In addition, clopidogrel was also associated with a greater effect in Females than Males in specific GI symptoms. Specific symptoms that were strongly associated with clopidogrel in IBS Female patients included epigastric pain ($p = 0.0005$), generalized abdominal pain ($p < 0.0001$), abdominal pain of other specific site ($p < 0.0001$), acute gastritis without hemorrhage ($p = 0.0013$), acute pain NEC ($p < 0.0001$), constipation NEC ($p = 0.0001$), constipation NOS ($p < 0.0001$), gastroparesis ($p < 0.0001$), gastroduodenal disease NEC (0.0007), noninfectious

gastroenteritis NEC ($p = 0.0039$) and psychogenic pain ($p = 0.0006$). In contrast, in Males (adjusted for Age) clopidogrel had little influence on GI symptoms in IBS patients. Only epigastric pain ($p = 0.0328$) and gastroparesis ($p = 0.0494$) were significant $p < 0.05$.

P2Y₁₂ Receptor Immunoreactivity in the Human Enteric Nervous System

Immunochemical identification of P2Y₁₂ receptor expression was determined in 4 surgical specimens of small bowel and 8 surgical specimens of colon. P2Y₁₂ immunoreactivity (ir) was highly expressed in the human enteric nervous system in both cell bodies of enteric neurons and neurites or long tapering processes running through the ganglia and some varicose fibers (Fig. 2). P2Y₁₂ immunoreactivity was co-localized with a majority of HuC/HuD immunopositive neurons in the human jejunum. Findings from both IHC (or immunofluorescent/IF) staining in paraffin sections and whole-mount surgical tissues confirmed expression of P2Y₁₂ immunoreactivity in enteric neurons. Expression of P2Y₁₂ receptor immunoreactivity occurred in >50% of submucous neurons but varied from 40 to 60% in different preparations, and included ganglia without any staining.

In the colon, P2Y₁₂ receptor immunoreactivity occurred in both HuC/HuD⁺ neurons and s100b⁺ glial cells in both jejunum and colon (Figs. 2, 3, 4). Only Female IBS patients who received clopidogrel were associated with higher incidence of GI symptoms, and therefore, in a small number of surgical patients (4 Males and 4 Females), in a proof of concept study, we targeted neurons and glial cells among the many different cell types existing in the gut wall, to evaluate potential Sex differences in expression and distribution of P2Y₁₂ receptors. A semiquantitative analysis in whole-thick sections of human colon showed that P2Y₁₂ receptors are expressed in the ENS of colon surgical tissues from both Male and Female patients. However, P2Y₁₂ receptors are expressed more in myenteric neurons in Males than in Females (Fig. 4a–c). In addition, neurons are usually lightly stained for P2Y₁₂ receptor immunoreactivity (Fig. 4d, e), with occasional neurons with strong expression (Fig. 4f). On the other hand, analysis in glial cells showed that P2Y₁₂ receptors are expressed much more in Females than Males (Fig. 4d, e); quantitative analysis in glial cells is most difficult for paraffin sections, and our analysis remains descriptive. Other cells in the gut wall that may also express P2Y₁₂ receptors were not analyzed.

Discussion

Clopidogrel is increasingly used worldwide to reduce the risk of vascular thrombotic events and improve outcome in patients with cardiac stents as well as patients with cerebrovascular or peripheral vascular disease. Its therapeutic action is based on specific blockade of the purinergic P2Y₁₂Rs on the platelets, which prevents intravascular clot formation [2].

Our retrospective study intended to find associations between the irreversible blockade of P2Y₁₂Rs within the GI tract and manifestation of GI symptoms among patients diagnosed with IBS. Our study revealed that IBS patients on clopidogrel have a higher incidence of GI symptoms, and Age and gender are significant factors influencing the manifestation of GI symptoms.

A total of 6.32% of the patient population in our study had received clopidogrel therapy. This number is close to the reported 7% prevalence of coronary arterial disease in adults [9] meaning that the vast majority of patients might have received clopidogrel to treat coronary insufficiency or following coronary stenting, although this was not determined in our analysis.

The retrospective nature of the study and variability in records during the long study period (around 13 years) did not allow for associating the time periods of clopidogrel therapy and the course of IBS, which was a limitation of the study. However, we took into account the facts that IBS is a chronic disorder, and that patients requiring therapy with clopidogrel received their treatment for prolonged time periods, sometimes for life. Based on these considerations, we assumed that chronic treatment with clopidogrel could have influenced the course of IBS GI symptoms in study patients, and such an association can be found by analyzing our electronic medical records database, which contains a large number of records. Another limitation of our retrospective data search was related with the incomplete records of all GI symptoms for some patients, and lack of recorded information on severity of GI symptoms. Nevertheless, our study revealed important associations between P2Y₁₂R blockade, possibly in the GI tract and development of various GI symptoms.

All patients included in the study were diagnosed in a high-performing academic gastroenterology practice at The Ohio State University. Although no validated questionnaires were used, IBS diagnosis was based on Rome criteria. Moreover, all patients evaluated for IBS routinely underwent: imaging CT/MR, upper, lower endoscopy; celiac markers; stool studies; stool calprotectin/lactoferrin. Thus, organic gastrointestinal conditions were thoroughly excluded. Furthermore, potential NSAID enteropathy as possibly relating to use of aspirin was addressed. Significant differences with clopidogrel were noted in relation to IBS recognized symptoms predominantly in Female IBS patients. On the other hand, IBS patients are not expected to have only symptoms that satisfy the Rome criteria. The majority of the symptoms recorded in our analysis fall under: abdominal pain, gas production, stool pattern changes—constipation/diarrhea that are essentially hallmark symptoms of IBS.

As expected, there was a significant Age difference among patients taking or not taking clopidogrel. IBS is known to be prevalent mostly in Female patients. In western countries, Females are two to three times more likely to have IBS than Males [19, 20]. To rule out any selection bias related to patient Age and Sex, we developed logistic regression models for GI symptoms in groups normalized for both Age and Sex. The analysis showed a strong correlation exists between the most common symptoms and clopidogrel therapy, especially for Females (Tables 3, 4). The associations between development of various types of diarrhea and treatment with clopidogrel were less obvious.

Cardiovascular problems in patients on clopidogrel (not associated with IBS in any way) rather than clopidogrel itself blocking the P2Y₁₂R could potentially explain differences between the groups (clopidogrel vs. no-clopidogrel), since we could not control for this potential confounder effect of the disease. Overall, comorbid disorders of IBS, especially in patients who received clopidogrel therapy (i.e., atherosclerosis with some metabolic

syndrome related disease), may also influence GI symptoms. However, the gender-specific effect and the Age-dependent effect of clopidogrel argues against this possibility—suggesting that the effect is not a generalized one in the clopidogrel population due to other existing comorbidities in these patients such as cardiovascular or other complications, etc. Rather, it is more likely a result of clopidogrel acting to cause irreversible blockade of P2Y₁₂Rs in the GI tract. A point worth mentioning is that not everyone with coronary artery disease is on clopidogrel and frequently clopidogrel patients are on dual antiplatelet therapy with aspirin (ASA). We have not analyzed the usage of ASA in these patients. On the other hand, most patients on clopidogrel used low-dose 81 mg ASA. This low-dose ASA is not expected to cause any significant symptoms [23]. Mucosal abnormalities, IBD, Celiac, NSAID's were systematically evaluated as part of the symptom work up as noted earlier.

Sixty-eight percent of clopidogrel IBS patients had active GI symptoms compared to 60% in control IBS patients. In general, differences existed between Females and Males for any symptoms for the influence of clopidogrel (if data were analyzed for all symptomatic patients). The incidence of GI symptoms was significantly higher among Female patients treated with clopidogrel compared with those not treated with the medication, whereas clopidogrel did not affect the rate of GI symptoms in Males.

Our study revealed a significant influence of clopidogrel on specific GI symptoms in IBS patients. Thus, after adjusting for any confounding influence of Sex and Age, IBS patients receiving clopidogrel compared to control IBS patients were more likely to have certain GI symptoms such as abdominal pain, epigastric pain, constipation and gastroparesis.

However, Age (or Sex) alone had an additional influence on the incidence of GI symptoms independent of clopidogrel effects. These symptoms included diarrhea, gas-related symptoms (i.e., distension, eructation and flatulence) as well as abdominal pain. Overall, either clopidogrel, Age or Sex alone could have an independent effect on GI symptoms in IBS patients. One possible explanation of this phenomenon may be the possibility of Age-dependent changes in P2Y₁₂R in the GI tract and ENS that can influence the action of clopidogrel at P2Y₁₂R and, therefore, induce GI effects (see later discussion).

In addition, clopidogrel was also associated with a greater effect in Females than Males in specific GI symptoms. Specific symptoms that were strongly associated with clopidogrel in IBS patients included epigastric pain, generalized abdominal pain, abdominal pain of other specific site, acute gastritis without hemorrhage, acute pain NEC, constipation NEC, constipation NOS, gastroparesis, non-infectious gastroenteritis NEC and psychogenic pain. In contrast, in Males (adjusted for Age) clopidogrel had little influence on GI symptoms in IBS patients.

Clearly, there was a sexual dimorphism in the influence of clopidogrel in Females and Males (adjusted for Age) for the GI symptoms in general, or for specific symptoms. The underlying mechanisms are unknown, although sexual dimorphism in P2Y₁₂R expression in Males and Females may in part, explain the higher incidence in GI symptoms associated with Clopidogrel. We found that the P2Y₁₂R is expressed in the ENS of colon surgical tissues from both Male and Female patients. However, the P2Y₁₂R expression is expressed more in

myenteric neurons in Males than in Females. On the other hand, in glial cells, P2Y₁₂Rs are more highly expressed in Females than Males. Glial cells are important regulators of motility and secretion [24, 25] and may be involved in chronic morphine-induced constipation [26] and visceral pain [27]. P2Y₁₂Rs regulate microglial activation and surveillance during neuropathic pain [28]. Expression of P2 receptors varies with Age and Sex [29] and Male and Female hormones influence expression of P2Y₁₂R [30]. Sex-specific differences in P2Y₁₂Rs in glia (and other cells) clearly deserve further consideration. A detailed in vitro investigation of human gut physiology of P2Y₁₂Rs in Male and Female is needed to better understand how clopidogrel irreversible blockade of P2Y₁₂Rs could potentially influence GI symptoms that are significant only in Female IBS patients. The more important question that has not yet been answered is whether P2Y₁₂ receptor expression and distribution in the human colon is different between Male and Female IBS patients (compared to healthy controls) to test whether a sex-dependent change in P2Y₁₂ receptor expression occurs in IBS that could potentially explain the effect of clopidogrel on GI symptoms in Female IBS patients suggested from our study.

The agonist bound structure of the human P2Y₁₂ ADP receptor has been recently identified by X-ray crystallography [31]. The G protein-coupled receptor is linked to a G_i subunit of adenylyl cyclase and is a chemoreceptor for ADP. Effect of clopidogrel on GI symptoms in IBS patients could potentially be explained by off target effects at P2Y₁₂R in the GI tract (see Fig. 5 for proposed working model). The P2Y₁₂Rs are abundant in the GI tract and are highly expressed on neurons, EC cells and variably expressed in enteric glia, suggesting that P2Y₁₂Rs may potentially serve as important regulators of GI motility, secretomotor functions, visceral sensation and perhaps visceral pain. In animal studies, P2Y₁₂R immunoreactivity is expressed in 42–46% of enteric neurons in both myenteric and submucous plexus. P2Y₁₂R-ir neurons were immunopositive for calbindin, representing Dogiel II/AH, intrinsic primary afferent neurons—these neurons represent the afferent sensory limb of enteric neural reflexes controlling all motor functions of the GI tract, including motility, secretion and coordination of these motor functions [14]. The functional role of these receptors and whether they are excitatory or inhibitory receptors in the ENS is unknown, but blockade of these receptors by clopidogrel is expected to have a profound influence on gut neural reflexes and motor behaviors of the human gut. In the current study we were able to show P2Y₁₂R is highly expressed in the human ENS in both small bowel and colon.

Our previous study also provided proof for functional inhibitory P2Y₁₂R in sensory enterochromaffin cells (EC) that provide inhibitory modulation of human EC (5-HT) signaling in the gut [15]. Activation of EC cells during peristaltic reflexes triggers release of 5-HT that is involved in enteric neural reflexes by activating the intrinsic primary afferent neurons of the ENS [32]. Therefore, irreversible blockade of inhibitory P2Y₁₂R on EC cells by clopidogrel would block an ongoing inhibition of 5-HT release, thereby enhancing the release of 5-HT and facilitating enteric neural reflexes. This could potentially explain a higher incidence of GI symptoms in IBS patients with clopidogrel, although influence of cardiovascular disease in patients treated with clopidogrel cannot be ruled out as a contributing factor. It is also not known whether the number, activity or sensitivity of P2Y₁₂Rs is altered or increased in IBS patients or animal models of IBS; but gut cells

expressing P2Y₁₂ receptors (i.e., EC, neurons, glia) are all implicated in IBS. What is also known is that activation of P2Y₂ and P2Y₄ receptors (not P2Y₁₂Rs) sensitize mouse and human colonic nociceptors [33], but effects of P2Y₁₂Rs in the gut remain unknown. Future studies are required to determine the expression, distribution and function of the P2Y₁₂R throughout the gastrointestinal tract that may be relevant to IBS.

Altogether, clinical data also seem to suggest a potential role of P2Y₁₂R blockage in influencing GI motility and secretomotor function, and association with changes in bowel habits (constipation or diarrhea), pain generation and propagation, GI discomfort/pain and dysmotility. A potential complication in the interpretation of pain in patients with IBS on clopidogrel is that blockade of P2Y₁₂R in microglia of the CNS (i.e., by generating P2Y^{-/-} 12 KO mice) ameliorates pain hypersensitivity in a mouse model of neuropathic pain [28]. It is not clear if clopidogrel treatment would influence pain of IBS as well by a central mechanism in the current population that would serve to offset the observed increase in pain sensation in IBS patients.

Our analysis had several other limitations: (1) it did not discriminate between IBS phenotypes, i.e., diarrhea-IBS, constipation-IBS, alternators between diarrhea and constipations and post-infectious IBS. The etiologies for these disorders may be different, and they cannot be assumed to behave similarly in the clopidogrel population. (2) Our study excluded IBD in our queries, but did not have any other exclusion criteria. In our IBS population, the ratio of Female to Male was 80:20, which was similar for the clopidogrel group (76:24). The ratio is similar to what is reported in the literature for IBS patients, keeping in mind, previously reported gender ratio differences associated with geography, clinical-based versus community-based studies and IBS subtype [19, 20]. So, we are confident that the study group was IBS. Still, our study could not exclude the possibility that patients had other comorbidities, in addition to their cardiovascular disease, that could contribute to their GI symptom profile irrespective of IBS. It is evident from our analysis that some of the GI symptoms are not linked to IBS. (3) Information on severity of IBS, duration of clopidogrel and type/severity of cardiovascular disease was not reliably accessible from the iWH. (4) The Age of each patient was taken at the time of data acquisition, and although Age, differences can be tested the exact Age of each patient may not be accurate. (5) The kind of treatment/drugs that patients received for IBS symptoms would also influence the symptoms; the frequency and type of symptoms could be different between treated and not treated patients. (6) Reliance on Rome II and III rather than Rome IV criteria for diagnosis of IBS would have identified more patients meeting the criteria for IBS as a result of tightening of the criteria with Rome IV among other differences.

Further basic research into P2Y₁₂Rs in the GI tract, and P2Y₁₂^{-/-} knockout models [33] or their irreversible blockade in the GI tract by clopidogrel administration could provide deeper insight into the role of P2Y₁₂R. For example, the antithrombotic phenotype produced by clopidogrel in vivo could be mimic by knockout of P2Y₁₂R (in P2Y₁₂^{-/-} KO mice). It would be justified and important for clinical practice to design a prospective clinical trial to clarify the temporal relationships between P2Y₁₂R block-age and development of GI symptoms in patients diagnosed with IBS and other GI disorders. One problem with designing a clinical trial is lack of a control population (to evaluate effects of clopidogrel of GI function in the

absence of disease). As an alternative, a surrogate in vivo study can be conducted in P2Y₁₂^{-/-} KO mice in an IBS model or test the responses to administration of clopidogrel in the IBS model to clarify if blocking of P2Y₁₂R can exacerbate motor abnormalities, diarrhea/or constipation, dyspepsia, gastroparesis, visceral pain or other GI symptoms.

In summary (1) a higher incidence of GI symptoms or GI illness occurs in IBS patients on clopidogrel than in IBS controls. (2) The influence of clopidogrel occurs in a sex-specific manner, and is evident mainly in Females. (3) Age or Sex alone contributes to symptomatology in IBS patients. (4) Not all GI symptoms associated with clopidogrel are necessarily those typically linked to IBS, i.e., abdominal pain, diarrhea and/or constipation. Our study did not discriminate between post-infectious IBS and other sub-types of IBS, and the post-infectious IBS phenotype could be a contributing factor. (5) P2Y₁₂Rs are expressed in the human enteric nervous system, and functional P2Y₁₂Rs exist in EC cells [15]. The glial-to-neuron ratio of P2Y₁₂ receptors expressed in the human enteric nervous system of the colon in Females >> Males. This raises the possibility that irreversible blockade of intestinal P2Y₁₂R by clopidogrel is associated with higher incidence of GI symptoms in Female IBS patients. We speculate that the mechanism of this gender difference may be associated with a greater influence exerted by glial P2Y₁₂ receptors in Females versus Males on motor functions and visceral pain sensation in the human gut of IBS patients. Clopidogrel, therefore, would block the influence of glia on gut functions that are mediated through P2Y₁₂ receptors.

Overall, findings have potential implications for IBS, other functional GI disorders or GI diseases, incidence of GI symptoms, types of GI symptoms and treatment strategies for patients on clopidogrel. Whether clopidogrel can induce GI symptoms or is associated with higher prevalence of GI symptoms in subjects without IBS or diseases affecting the GI tract was not answered. To further evaluate the impact of clopidogrel on GI symptoms, a prospective study is warranted in a standardized cohort of IBS patients (Male and Female) in a single IBS phenotype versus non-IBS patients (excluding anyone with any known GI pathology, i.e., IBD, neurological disorders, metabolic disorders, autism, etc.) with or without clopidogrel. Prospective studies can determine clinical implications of P2Y₁₂Rs in IBS and GI disorders [29–31] with a focus on a single IBS phenotype using the Rome IV criteria for diagnosis (IBS-D, IBS-C, IBS-C/D or IBS-PI). Functional studies in a chronic “Plavix” mouse, P2Y₁₂R global knockout mouse or cell-specific knockouts of the EC cells or primary afferent neurons of the ENS, can pinpoint the role of P2Y₁₂R in the GI tract and potentially link it to visceral pain, abnormal GI motility and bowel habits in IBS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Differences in incidence of GI symptoms between IBS patients and IBS patients treated with clopidogrel. The *bars* show rates of typical gastrointestinal symptoms among patients treated and not treated with clopidogrel. Abbreviations: *NEC* not elsewhere classified, *NOS* not otherwise specified, *Acute gastritis w/o hmrhg* acute gastritis without hemorrhage, *Diarrhea of infect orig* diarrhea of infectious origin, *Flatul/eructat/gas pain* flatulence/eructation/gas pain, *oth spf gastrit w/hmrhg* other specific gastritis with hemorrhage

Fig. 2. Strong P2Y₁₂ receptor – immunoreactivity is expressed in the human enteric nervous system. **a–f** Black and white laser confocal images of immunoreactivity (P2Y₁₂ receptor-ir) in submucosal neurons of the human jejunum. **g–l** Co-labeling for P2Y₁₂ receptor– ir and HuC/D (a human enteric neuronal marker). *Green* the primary antibody for P2Y₁₂ receptors was conjugated with an FITC donkey anti-rabbit IgG secondary antibody, *red* the primary antibody to HuC/D was conjugated with Texas-Red donkey anti-mouse IgG, *yellow* staining indicates co-labeled neurons with P2Y₁₂-ir. Labeling for P2Y₁₂-ir occurs in both the cell

bodies and axonal processes of neurons. **m–o** Immunohistochemical staining shows strong immunoreactivity in a majority of submucous neurons and their processes; color, biotin-SP donkey anti-rabbit IgG and VIP substrate

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Fig. 3.
P2Y₁₂ receptor immunoreactivity in myenteric ganglia of the human colon in a Male surgical subject. **a–d** P2Y₁₂R-ir occurs primarily in neurons (*red colored* cells surrounded by *green* s100β⁺ glial cells); P2Y₁₂R-ir is occasionally expressed in glia (*yellow color*); a No labeling occurs in s100β⁺ glia or other cells (neurons) with isotype IgG control substituted for anti-P2Y₁₂ antiserum. *Green* the primary antibody for s100β was conjugated with FITC donkey anti-mouse IgG. The isotype IgG control or anti-P2Y₁₂ antibody was conjugated with Texas-Red donkey anti-rabbit IgG secondary antibody. **e, f** Pre-absorption of the P2Y₁₂ antiserum with its control peptide (CP, immunogenic peptide) blocks all P2Y₁₂R-ir in the ganglia. *Sold arrows* indicate positive P2Y₁₂ neurons; *c open white arrows* indicate possible glial co-localization of P2Y₁₂R-ir; *f open yellow arrows* indicate co-localization of P2Y₁₂R-ir with HuC/D⁺ neurons

Fig. 4.

Sex-dependent differences of P2Y₁₂—receptor expression in myenteric neurons and glial cells of the human colon. **a, b** Expression of P2Y₁₂ receptor immunoreactivity in myenteric neurons is greater in Males than Females. Significant differences were calculated using a Mann–Whitney test. *Box-plots* are shown in **a** and **b** with each point representing numbers of P2Y₁₂—positive neurons in a single ganglion. **c** Neuronal expression of P2Y₁₂ receptors occurs in 95% of the ganglia in surgical tissue from Males, whereas it is only expressed in 32% of the ganglia in Females. **d, e** Abundant P2Y₁₂ receptor immunoreactivity (*red*) is co-localized with s100β⁺ glial cells (*green*) in Females; this is in contrast to Males (see Fig. 3); *yellow* co-expression of P2Y₁₂ and s100β; **f** An example of several P2Y₁₂⁺ neurons in one

ganglion; glial cells were identified by their s100 β immunoreactivity (*green*); co-labeled cells are yellow. Examples are representative of glial expression of P2Y₁₂ receptor immunoreactivity in Females from 4 surgical cases; *open arrows* glial cells expressing P2Y₁₂ immunoreactivity; *solid arrows* neurons expressing P2Y₁₂ immunoreactivity

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Fig. 5.

Clodipogrel hypothesis for GI symptoms in IBS patients. We previously showed that functional P2Y₁₂R on sensory enterochromaffin cells (EC) lining the gut mucosa is involved in inhibitory modulation of serotonin (5-HT) release. Release of 5-HT from EC cells triggers enteric neural reflexes (motility and secretion) and transmits information to the brain about visceral sensation. Therefore, irreversible blockade of P2Y₁₂R on EC cells would cause disinhibition resulting in over secretion of 5-HT and more intense stimulation of such intrinsic and extrinsic gut reflexes. P2Y₁₂R in the ENS is expressed primarily in calbindin-positive, Dogiel Type II/intrinsic primary afferent neurons of the guinea pig small intestine (and GI tract). Our current study confirmed that P2Y₁₂R immunoreactivity is abundant in human ENS, although sex-specific differences in expression of P2Y₁₂ receptors suggest preferential expression of glial P2Y₁₂ receptors relative to neurons in Females unlike Males, and Sex differences in P2Y₁₂ receptors may be relevant for GI symptoms in IBS patients with clodipogrel. The glial-to-neuron ratio of P2Y₁₂ receptor expressed in Females $\gg$ Males in the colon. Glial cells modulate motility by influencing the behavior of the enteric nervous system. P2Y₁₂Rs are distributed in the afferent limb of gut reflexes, and while our understanding of the physiology of P2Y₁₂ receptors in the gut remains poor, it is likely that irreversible blockade of P2Y₁₂ receptors could potentially lead to exacerbation of GI symptoms associated with IBS predominantly in Females

Table 1

Incidence of GI symptoms is higher in IBS patients with clopidogrel (and specifically in Female IBS patients) compared to IBS patients with no-clopidogrel

Patient groups	Number of patients	% of patients with GI symptoms	Significance of difference
All patients	7217		
No-clopidogrel	6761 (93.68%)	4058 (60.02%)	0.0011 *
Clopidogrel	456 (6.32%)	309 (67.76%)	
Females	5728 (79.37%)		
No-clopidogrel	5383 (93.98%)	3217 (59.77%)	0.0003 *
Clopidogrel	345 (6.02%)	241 (69.86%)	
Males	1489 (20.63%)		
No-clopidogrel	1378 (92.55%)	830 (60.23%)	0.8312
Clopidogrel	111 (7.45%)	68 (61.26%)	

*
The difference is significant

Table 2

Logistic regression models comparing incidence of symptoms for clopidogrel/Sex/Age in IBS patients

	Odds ratio	95% CI	<i>p</i> value	
Naïve clopidogrel versus no-clopidogrel group	1.4	1.14	1.72	<0.0011
Clopidogrel versus no-clopidogrel (adjusted for Age and Sex)	1.75 *	1.42	2.15	<0.0001
Sex differences (Female vs. Male) after adjusting for clopidogrel and Age	1.01	0.90	1.14	0.8356
Age as a continuous variable (adjusted for clopidogrel and Sex)	0.99 *	0.98	0.99	<0.0001
Females only				
Clopidogrel versus no-clopidogrel	1.98 *	1.55	2.53	<0.0001
Age	0.98 *	0.98	0.99	<0.0001
Males only				
Clopidogrel versus no-clopidogrel	1.18	0.79	1.78	0.4137
Age	0.99 *	0.99	1.00	0.0072

CI confidence interval*
The difference is significant

Table 3

Presence of specific symptoms in patient groups (clopidogrel vs. no-clopidogrel)

Outcome variables (GI symptoms)	Clopidogrel versus no-clopidogrel All symptomatic patients (adjusted for Age and Sex)		Female versus Male differences (adjusted for clopidogrel and Age)		Age as a continuous variable (adjusted for clopidogrel and Sex)	
	Odds ratio	p value	Odds ratio	p value	Odds ratio	p value
Epigastric pain	1.76	<0.0001	1.46	0.0001	0.98	<0.0001
Generalized abdominal pain	1.72	0.0001	1.37	0.0015	0.99	<0.0001
Abdominal pain in left upper quadrant	1.30	0.3183	1.20	0.2657	0.98	<0.0001
Abdominal pain in left lower quadrant	1.26	0.2012	1.15	0.2141	0.98	<0.0001
Abdominal pain of other specific site	1.77	<0.0001	1.05	0.5489	0.98	<0.0001
Periumbilical pain	1.62	0.1662	1.34	0.2118	0.97	<0.0001
Abdominal pain in right lower quadrant	1.22	0.3124	1.30	0.0306	0.98	<0.0001
Abdominal pain in right upper quadrant	1.32	0.0722	1.36	0.0034	0.99	<0.0001
Abdominal pain in unspecified site	1.28	0.0459*	1.20	0.015	1.00	0.0123
Epigastric abdominal tenderness	4.35	0.0771	1.75	0.4597	0.95	0.0087
Abdominal pain	0.31	0.2534	1.48	0.4208	1.02	0.0454
Acute gastritis without hemorrhage	2.44	0.0949	0.99	0.0644	0.99	0.0715
Acute pain NEC	2.56	0.0001	1.92	0.0013	0.96	<0.0001
Anal or rectal pain	1.00	0.999	0.78	0.2623	0.99	0.0819
Constipation	1.29	0.2605	1.72	0.0079	1.03	<0.0001
Constipation NEC	2.70	<0.0001	1.92	0.0057	0.99	0.2325
Constipation NOS	1.75	<0.0001	1.51	<0.0001	1.00	0.2061
Diarrhea	1.29	0.0392	1.01	0.9492	1.00	0.0102
Flatulence/eructation/gas pain	0.96	0.8627	1.23	0.2329	1.01	0.015
Gastritis/duodenitis NOS	N/A	N/A	N/A	N/A	N/A	N/A
Gastroduodenal disease NEC	1.82	0.0007	0.77	0.0305	1.00	0.9526
Gastroduodenal disease NOS	2.29	0.0032	1.20	0.4486	1.00	0.5455
Gastroparesis	2.79	<0.0001	1.37	0.0289	0.99	0.0042
Infectious diarrhea NOS	2.36	0.282	0.89	0.836	0.98	0.1475
Noninfectious gastroenteritis NEC	1.31	0.0848	0.81	0.0344	1.00	0.0544
Psychogenic pain NEC	4.83	0.1457	0.75	0.1865	0.94	0.2088

Bold values are statistically significant ($p < 0.05$)

Table 4

Sex-stratified logistic regression models adjusted for Age for each outcome variable in symptomatic patients with IBS (clopidogrel vs. no-clopidogrel groups)

Outcome variables (GI symptoms)	Females only adjusted for Age (clopidogrel vs. no-clopidogrel)		Males only adjusted for Age (clopidogrel vs. no-clopidogrel)	
	Odds ratio	<i>p</i> value	Odds ratio	<i>p</i> value
Any symptoms	1.98	<0.0001	1.18	0.4137
Epigastric pain	1.71	0.0005	2.03	0.0328
Generalized abdominal pain	1.88	<0.0001	1.07	0.868
Abdominal pain in left upper quadrant	1.34	0.3033	1.11	0.871
Abdominal pain in left lower quadrant	1.46	0.0466	0.47	0.2115
Abdominal pain of other specific site	1.81	<0.0001	1.64	0.0862
Periumbilical pain	1.93	0.0647	N/A	N/A
Abdominal pain in right lower quadrant	1.30	0.2012	0.81	0.702
Abdominal pain in right upper quadrant	1.39	0.999	1.01	0.9802
Abdominal pain in unspecified site	1.34	0.0426	1.13	0.6497
Epigastric abdominal tenderness	5.46	0.0464	N/A	N/A
Abdominal pain	0.37	0.3328	N/A	N/A
Acute gastritis without hemorrhage	3.19	0.0013	N/A	N/A
Acute pain NEC	2.90	<0.0001	N/A	N/A
Anal or rectal pain	1.15	0.7578	0.57	0.5861
Constipation	1.32	0.2615	1.15	0.8053
Constipation NEC	2.65	0.0001	2.97	0.0723
Constipation NOS	1.88	<0.0001	1.27	0.4244
Diarrhea	1.35	0.0277	1.07	0.7946
Flatulence/eructation/gas pain	0.89	0.6934	1.20	0.712
Gastritis/duodenitis NOS	1.90	0.5771	N/A	N/A
Gastroduodenal disease NEC	1.99	0.0007	1.39	0.3759
Gastroduodenal disease NOS	2.16	0.0157	2.84	0.0827
Gastroparesis	2.92	<0.0001	2.20	0.0494
Infectious diarrhea NOS	1.54	0.6912	5.18	0.1991
Noninfectious gastroenteritis NEC	1.64	0.0039	0.54	0.1184
Psychogenic pain NEC	5.85	0.1485	N/A	N/A
Psychogenic pain NOS	29.41	0.0006	N/A	N/A

Bold values are statistically significant ($p < 0.05$)

N/A, insufficient data to calculate significance. Some outcomes were not included in the table because of insufficient data. These include slow transit constipation, gastric anomaly, NEC, gastritis with hemorrhage, functional diarrhea, diarrhea of infectious origin, adverse effects—antidiarrheal agent