A trial of type 12 purinergic ($P2Y_{12}$) receptor inhibition with prasugrel identifies a potentially distinct endotype of patients with aspirin-exacerbated respiratory disease

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Background: Aspirin-exacerbated respiratory disease (AERD) is characterized by asthma, recurrent nasal polyposis, and respiratory reactions on ingestion of COX-1 inhibitors. Increased numbers of platelet-leukocyte aggregates are present in the sinus tissue and blood of patients with AERD compared with that of aspirin-tolerant patients, and platelet activation can contribute to aspirin-induced reactions.

Objective: We sought to determine whether treatment with prasugrel, which inhibits platelet activation by blocking the type 12 purinergic ($P2Y_{12}$) receptor, would attenuate the severity of sinonasal and respiratory symptoms induced during aspirin challenge in patients with AERD.

Methods: Forty patients with AERD completed a 10-week, double-blind, placebo-controlled crossover trial of prasugrel. All patients underwent oral aspirin challenges after 4 weeks of prasugrel and after 4 weeks of placebo. The primary outcome was a change in the provocative dose of aspirin that would elicit an increase in Total Nasal Symptom Score (TNSS) of 2 points. Changes in lung function, urinary eicosanoids, plasma tryptase, platelet-leukocyte aggregates, and platelet activation were also recorded.

Results: Prasugrel did not significantly change the mean increase in TNSS of 2 points (79 ± 15 for patients receiving placebo and 139 ± 32 for patients receiving prasugrel, $P = .10$), platelet-leukocyte aggregates, or increases in urinary leukotriene E_4 and prostaglandin D_2 metabolite levels during aspirin-induced reactions in the study population as a whole. Five subjects (responders) reacted to aspirin while receiving placebo but did not have any reaction to aspirin challenge after the prasugrel arm. In contrast to prasugrel nonresponders (35 subjects), the prasugrel responders had smaller reaction-induced increases in TNSS; did not have significant aspirin-induced increases in urinary leukotriene E_4, prostaglandin D_2 metabolite, or thromboxane B_2 levels; and did not display increases in serum tryptase levels during aspirin reactions on the placebo arm, all of which were observed in the nonresponders.

Conclusion: In the overall study population, prasugrel did not attenuate aspirin-induced symptoms, possibly because it failed to decrease the frequencies of platelet-adherent leukocytes or to diminish aspirin-induced mast cell activation. In a small subset of patients with AERD who had greater baseline platelet activation and milder upper respiratory symptoms during aspirin-induced reactions, $P2Y_{12}$ receptor antagonism with prasugrel completely inhibited all aspirin-induced reaction symptoms, suggesting a contribution from $P2Y_{12}$ receptor signaling in this subset. (J Allergy Clin Immunol 2019;143:316-24.)

Key words: *Aspirin-exacerbated respiratory disease, double-blind, randomized, placebo-controlled crossover trial, prasugrel, Samter triad, NSAID-exacerbated respiratory disease, leukotrienes, $P2Y_{12}$*

Aspirin-exacerbated respiratory disease (AERD) is a distinctive clinical syndrome affecting approximately 7% of adults with asthma and approximately 14% of those with severe asthma.[1] AERD is characterized clinically by asthma, rhinosinusitis with nasal polyposis, respiratory tissue eosinophilia, and respiratory reactions to ingestion of aspirin and other COX-1 inhibitors. Biochemically, AERD is marked by overactivity of the 5-lipoxygenase (5-LO)/leukotriene C_4 synthase (LTC_4S) pathway, resulting in generation of high levels of leukotriene C_4 (LTC_4) and therefore high urinary levels of leukotriene E_4 (LTE_4), the stable end-product of cysteinyl leukotriene (CysLT) metabolism. CysLTs are powerful bronchoconstrictors and facilitate vascular leak and tissue eosinophilia. They play a major pathogenic role in patients with AERD, particularly in clinical reactions to aspirin challenge/desensitization.[2]

Although eosinophils[3] and mast cells,[4] which express both 5-LO and LTC_4S, likely account for the majority of CysLTs released in patients with AERD during clinical reactions, platelets can also play a role in CysLT overproduction. Platelets lack 5-LO

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Abbreviations used
AERD: Aspirin-exacerbated respiratory disease
Cr: Creatinine
CysLT: Cysteinyl leukotriene
$CysLT_1R$: Type 1 CysLT receptor
FENO: Fraction of exhaled nitric oxide
5-LO: 5-Lipoxygenase
LTC_4: Leukotriene C_4
LTC_4S: Leukotriene C_4 synthase
LTE_4: Leukotriene E_4
PD2: Increase in TNSS of 2 points
PG: Prostaglandin
PGD-M: Prostaglandin D_2 metabolite
PRP: Platelet-rich plasma
$P2Y_{12}$: Type 12 purinergic
TNSS: Total Nasal Symptom Score
TXB_2: Thromboxane B_2

but can convert leukocyte-derived leukotriene A_4 to LTC_4 because of their expression of LTC_4S.[5] This conversion requires platelet activation and CD62P expression, resulting in CD62P-dependent adherence of platelets to 5-LO–expressing leukocytes (platelet-leukocyte aggregates). We have shown previously that both peripheral blood and sinonasal tissue of patients with AERD contain greater numbers of platelet-leukocyte aggregates than do aspirin-tolerant patients and that their numbers correlate with basal urinary LTE_4 levels.[6] Moreover, mouse studies implicate platelet-leukocyte aggregates in CysLT generation during reactions to aspirin.[7] These observations suggest that agents capable of interfering with the formation of platelet-leukocyte aggregates could have therapeutic efficacy in patients with AERD. Based on these findings, we hypothesized that antiplatelet therapy might be efficacious in patients with AERD, particularly if it interferes with transcellular generation of LTC_4.

Many stimuli that activate platelets elicit their release of adenosine diphosphate, which signals through the type 12 purinergic ($P2Y_{12}$) receptor to amplify CD62P expression and the interaction of platelets with leukocytes. Thienopyridine drugs (prasugrel and clopidogrel) that block $P2Y_{12}$ receptors inhibit platelet activation in patients with cardiovascular disease.[8] This double-blind, placebo-controlled trial aimed to test the hypothesis that blockade of $P2Y_{12}$ receptors with prasugrel would attenuate the severity of sinonasal and respiratory symptoms that occur during aspirin challenges in patients with AERD by blocking platelet activation and reducing CysLT generation by decreasing the frequency of platelet-leukocyte aggregates.

METHODS

Study design

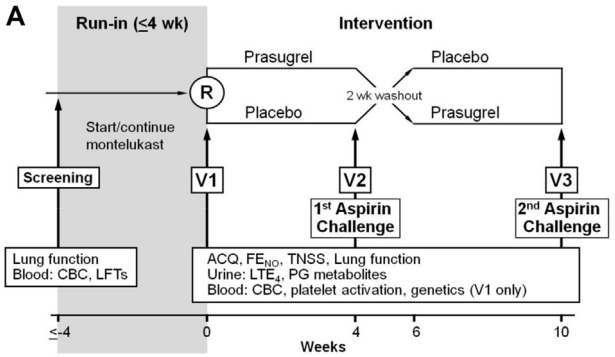
Participants were enrolled in a 10-week, double-blind, placebo-controlled crossover trial of prasugrel. Each treatment arm lasted 4 weeks, with a 2-week washout phase in between. An oral aspirin challenge was conducted at the end of each treatment arm (Fig 1). In a separate continuation study patients continued onto treatment with high-dose aspirin (650 mg twice daily).

Participants

Patients with AERD had a history of physician-diagnosed asthma, nasal polyposis, and at least 1 clinical reaction to aspirin or another nonselective COX inhibitor, with features of lower and/or upper airway involvement. They had refractory AERD with respiratory symptoms, which were not adequately responsive to other standard therapies, including corticosteroids and leukotriene modifiers, thus qualifying for aspirin desensitization and treatment with high-dose aspirin. Subjects had stable asthma, which was defined as a postbronchodilator FEV_1 of 70% of predicted value or greater; no increase in baseline dose of oral glucocorticoids for at least 3 months; and no history of hospitalization or emergency department visits because of asthma for at least 6 months before enrolling in the study to ensure the successful and safe completion of the protocol.

Subjects were excluded if they had current severe gastroesophageal reflux disease or a history of peptic ulcer disease or gastrointestinal bleed. All subjects were between the ages of 18 and 65 years and were not pregnant, breast-feeding, or smoking while participating in the trial.

This was a single-site study conducted at the Asthma Research Center, Brigham and Women's Hospital, Boston, Massachusetts. The Institutional Review Board of Brigham and Women's Hospital/Partners Healthcare approved this study, and all subjects provided written informed consent. Patients were recruited from August 2012 through April 2016, with the last patient's final visit in December 2016. The trial ended when recruitment goals were met. Compliance with montelukast and daily study drug administration was confirmed with both diary cards and pill counts.



B

	Baseline	Minutes after reaction onset: 0	30	60	90	120	150	180
Urine	✓	✓			✓			✓
TNSS	✓	✓	✓	✓	✓	✓	✓	✓
Spirometry	✓	✓		✓		✓		✓
CBC/differential	✓			✓				

FIG 1. Detailed schema of the trial. **A,** Study schema showing timeline and schedule of assessments. *CBC,* Complete blood count; *LFTs,* liver function tests. **B,** Table of time points when assessments were collected on the day of aspirin challenge.

Clinical procedures

After a 4-week run-in period on stable asthma treatment and 10 mg/d montelukast, subjects were randomized to begin taking either prasugrel (5 mg/d for patients weighing <60 kg or 10 mg/d for patients weighing ≥60 kg after a 60-mg loading dose) or equivalent placebo for 4 weeks, followed by a 4-dose aspirin challenge (40.5, 81, 162, and 325 mg) with 90-minute intervals,[9] which was terminated when patients experienced either an increase in Total Nasal Symptom Score (TNSS) of at least 2 greter than baseline values or a decrease in FEV_1 of at least 15% from baseline or reached a dose of 325 mg of aspirin without any reaction symptoms. The TNSS questionnaire is a 9-symptom patient-recorded outcome measurement on a total scale of 0 to 40, which includes assessment of nasal congestion, runny nose, sneezing, nasal itching, and eye and throat symptoms (see Fig E1 in this article's Online Repository at www.jacionline.org for the full questionnaire). Baseline assessments were collected the morning of the aspirin challenge.

Patients were observed for a 3-hour period after onset of their reaction and challenge termination, with serial measurements of lung function and TNSSs and collection of blood and urine samples. Subjects were discharged to home to continue daily montelukast and wash out the study drug from the first treatment phase. At the end of the 2-week washout period, subjects crossed over to alternate treatment for 4 weeks and returned for the second aspirin challenge, which proceeded as above.

Laboratory procedures

Blood was drawn into heparinized tubes, kept at room temperature, and assayed within 1 hour of collection. For aspirin challenge visits, blood was drawn in the morning before administration of aspirin and 1 hour after onset of the aspirin-induced reaction. If no reaction occurred, blood was drawn again 3 hours after the 325-mg dose of aspirin. Platelet-rich plasma (PRP) was obtained from the top layer of blood samples after a 20-minute centrifugation at 200*g*.

To monitor platelet activation and quantify platelet-leukocyte aggregates, we incubated 10 μL of PRP with directly conjugated antibodies specific for CD61 and CD62P and 20 μL of whole blood with antibodies specific for CD61, CD45, CD14, and CCR3 or appropriate isotype controls for 20 minutes; CCR3 antibody was from BioLegend (San Diego, Calif), and all others were from BD Biosciences (San Jose, Calif). We lysed red blood cells with FACS Lysing Solution (BD Biosciences) and then fixed the cells in 1% paraformaldehyde. At least 20,000 $CD45^+$ cells for whole blood analyses or 30,000 platelets for PRP analyses were recorded for each sample in a BD FACSCanto flow cytometer. Analyses were completed with FlowJo software (Version 10; TreeStar, Ashland, Ore). $CD45^+$ leukocytes were classified as eosinophils ($CCR3^+$ granulocytes), neutrophils ($CCR3^-$ granulocytes), monocytes ($CD14^+$), and lymphocytes. The presence of adherent platelets was determined based on relative expression of CD61 on each cell type. An aliquot of plasma from each blood draw was also analyzed at Virginia Commonwealth University for total tryptase levels.

Levels of urinary eicosanoids were measured by using gas chromatography−mass spectrometry at Vanderbilt University, as previously described.[6] For aspirin challenge visits, urine samples were collected in the morning before aspirin administration and again at the onset of and 90 and 180 minutes after the aspirin-induced reaction. For those visits, during which no reaction was elicited, urine was collected 180 minutes after the 325-mg dose of aspirin.

DNA from peripheral blood leukocytes was used to sequence the *P2RY12* locus (gene encoding the $P2Y_{12}$ receptor) in 28 subjects. DNA was extracted with an AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, Calif). Five overlapping PCR amplicons covering the entire gene were sequenced by using the Illumina MiSeq platform (Illumina, San Diego, Calif) to generate 300-bp end reads, which were aligned to the reference human genome (GRCh37/hg19) using Bowtie2 (version 2.2.6). SAM tools (version 1.4) were used to call genetic variants from sorted and indexed BAM files. Association testing of genotype with response to prasugrel was performed by means of logistic regression on selected single nucleotide polymorphisms.

Outcomes

Because all subjects received montelukast throughout the study (which substantially attenuates aspirin-induced decreases in lung function), we used changes in nasal symptoms as the main indicator of reaction. The primary outcome compared the effect of 4 weeks of prasugrel versus placebo on the provocative dose that caused an increase in TNSS of 2 points (PD_2) during an aspirin challenge. PD_2 is modeled after the aspirin PD_{20} value, which was established in the 2007 European Academy of Allergy and Clinical Immunology (EAACI) guidelines,[10] and was calculated as follows (see detailed description of each variable in Fig E2 in this article's Online Repository at www.jacionline.org):

$$inverselog_{10}\left(\frac{(2-(PrevTNSS-BaselineTNSS))\times(log_{10}ProvocDose-log_{10}PrevDose)}{(MaxTNSS-BaselineTNSS)-(PrevTNSS-BaselineTNSS)}+(log_{10}PrevDose)\right).$$

Participants who did not have any clinically apparent reaction to aspirin during an aspirin challenge (a decrease in FEV_1 of <15% and no significant development of any naso-ocular or nonrespiratory symptoms, as quantified by the lack of increase in TNSS) were designated as having a negative challenge and were assigned a PD_2 of 650, which is double the maximum dose of aspirin given during either challenge.

Secondary outcomes included differences in provocative dose of aspirin, maximum increase in TNSS, change in urinary eicosanoid level, and platelet activation during aspirin challenge while receiving prasugrel versus placebo. Additional predefined outcomes included the effect of prasugrel on chronic asthma control, urinary eicosanoid metabolite levels, platelet activation, and fraction of exhaled nitric oxide (FENO) values in patients with AERD.

Sample size

The study was powered to detect an effect of prasugrel on PD_2 during aspirin desensitization. The sample size of 40 using a 2-sided paired *t* test comparing the mean PD_2 value between treatment with prasugrel and placebo provided 80% power to detect a 1.74-fold (0.8 after log_2 transformation) change in PD_2 at a .05 level of significance.

Randomization

Randomization consisted of 3 randomly selected blocks for each stratum of dose (5 or 10 mg) with a block size of 5 patients randomly assigned to treatment or control groups for each randomly selected block. We used permuted block randomization with SAS software (Version 9.2; SAS Institute, Cary, NC) proc plan, ensuring equal numbers of patients with AERD were randomized to each group. The Brigham and Women's Hospital Investigational Drug Services pharmacy conducted the randomization and kept the records to maintain the blind of the study. The Investigational Drug Services pharmacy also prepared the placebo and prasugrel through encapsulation.

Blinding

All participants, clinical care providers, and research staff were kept blind to intervention, and all analyses of mechanistic data (eg, flow cytometric measurements of platelet activation and urinary eicosanoid measurements) were completed before unblinding of research investigators.

Statistical methods

For the crossover study, we compared the difference in PD_2 values between the 2 (prasugrel vs placebo) aspirin challenges by using a 2-sided paired *t* test as the main comparison and used the same analysis for the change in maximum reduction in FEV_1 during aspirin reaction. We used mixed-model diagnostics, such as residual plots for goodness of fit, and before undertaking the primary analysis, we checked for a period effect. We compared levels of urinary LTE_4 and prostaglandin (PG) metabolites measured during the reaction to aspirin for subjects in both treatment arms, each separately by using a paired *t* test. We compared both the preaspirin baseline and the aspirin reaction-induced percentages of activated platelets and platelet-leukocyte aggregates for subjects pretreated with prasugrel and placebo, each by using a 2-sided paired *t* test. For the additional *post hoc* analyses done on the responder versus nonresponder subgroups, the false discovery rate method was used to correct for multiple comparison testing, with a rate greater than .05 considered significant.[11] Raw *P* values are presented, and significant values using the false discovery rate. Data are presented as ± SEs, unless

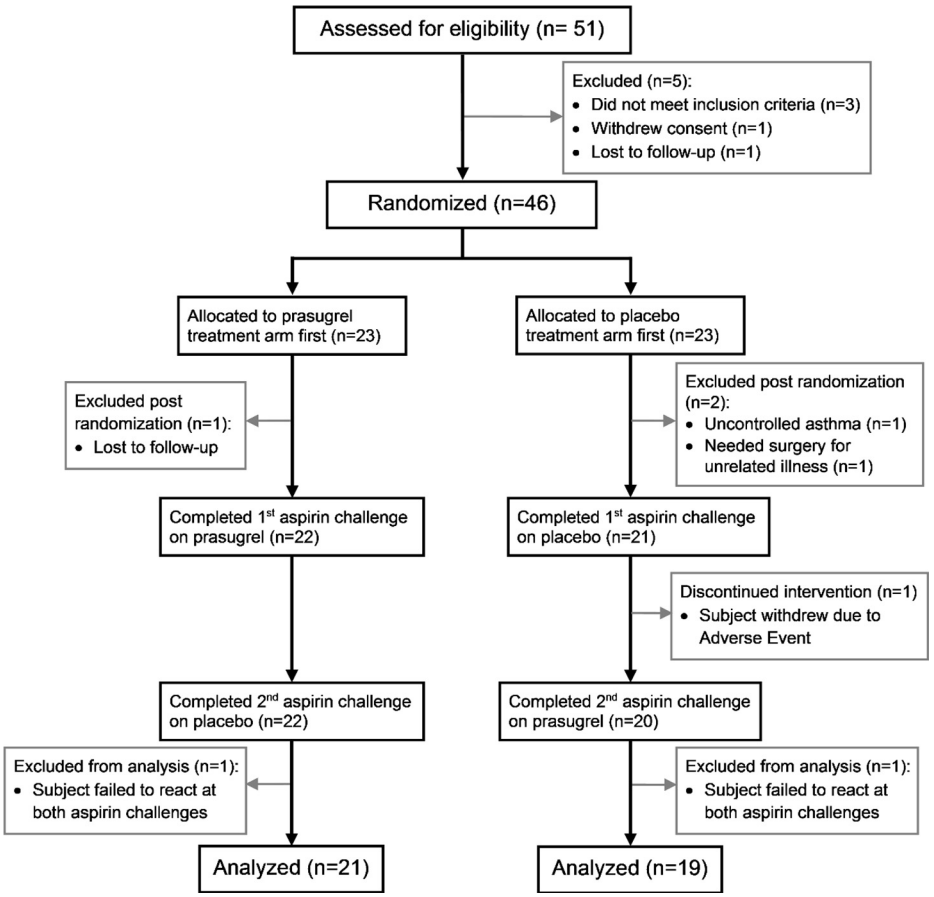


FIG 2. Patient flow through the study.

otherwise specified. This study was registered at clinicaltrials.gov as NCT01597375.

RESULTS

Patients

A total of 51 potential participants were screened, 46 of whom underwent randomization per protocol, and 40 of whom completed the trial and were analyzed (Fig 2). The baseline characteristics of the patients who underwent randomization are summarized in Table I. Thirty-nine of 40 patients had confirmed (diary cards and pill counts) compliance with both daily montelukast and daily study drug of greater than 90%.

Primary outcome

The mean PD_2 value in the prasugrel arm was 79 ± 15, and the mean PD_2 value in the placebo arm was 139 ± 32 ($P = .10$; Fig 3, *A*). Of the 40 patients with AERD who completed the crossover trial, 5 did not have any clinically apparent reaction to aspirin during the arm when they had been treated with prasugrel (prasugrel responders) and were assigned a PD_2 value of 650. The clinical reactions of the 5 responders during the placebo arm challenge showed significantly smaller increases in TNSS compared with those of the 35 nonresponders (maximum TNSS increase, 3.4 ± 0.8 for 5 responders vs 7.5 ± 0.9 for 35 nonresponders; $P = .003$), although the average decrease in FEV_1 was similar (9.9% ± 3.5% vs 12.8% ± 2.0%, $P = .50$). There was also 1 patient who did not have any clinically apparent reaction to aspirin during the placebo arm but did have a reaction during the prasugrel arm.

For the entire study population, the mean difference in maximum increase in TNSS for patients in the prasugrel arm compared with the placebo arm was 0.5 ± 1.0 ($P = .32$), with a mean increase in TNSS on the prasugrel arm of 6.5 ± 0.8 and a mean increase in TNSS on the placebo arm of 7.0 ± 0.8 (Fig 3, *B*). The administered aspirin dose that provoked a reaction for patients in the prasugrel arm was 0.2 ± 0.2−fold greater ($P = .38$) than the administered provocative aspirin dose for patients on the placebo arm.

Secondary outcomes

Treatment with prasugrel did not alter baseline percentages of $CD62P^+$ platelets compared with percentages after placebo in all 40 patients with AERD who completed the trial and did not change the percentages of any leukocyte subset with adherent platelets (Fig 4, *A-E*). In the 40 patients as a group, neither the percentages of $CD62P^+$ platelets nor the percentages of leukocytes with adherent platelets changed 1 hour into aspirin-induced reactions, and prasugrel did not alter these percentages when compared with placebo (Fig 4, *A-E*). Plasma tryptase levels increased significantly during aspirin-induced reactions (increase of 44% ± 12% for the placebo arm [$P = .02$] and 60% ± 21% for the prasugrel arm [$P = .004$]), although treatment with prasugrel did not alter either the preaspirin levels of tryptase or the aspirin-induced increases (Fig 4, *F*).

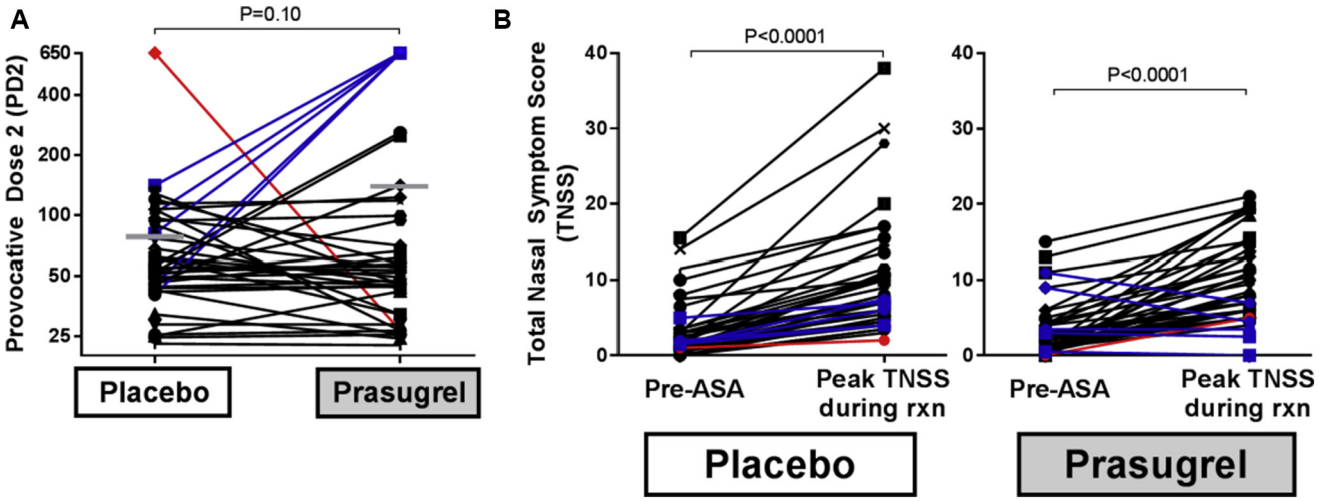


FIG 3. Effect of prasugrel on PD_2 values and TNSSs. **A,** The primary outcome, change in the provocative dose of aspirin that would elicit PD_2 is shown, with *horizontal bars* representing the mean PD_2 value for each treatment arm. **B,** The change in TNSS from the preaspirin baseline to the maximum TNSS recorded during each aspirin challenge is shown. Thirty-four subjects reacted to aspirin at both challenges *(black lines)*, 1 subject reacted to aspirin only on the prasugrel arm *(red line)*, and 5 subjects reacted only on the placebo arm *(blue lines). ASA*, Aspirin.

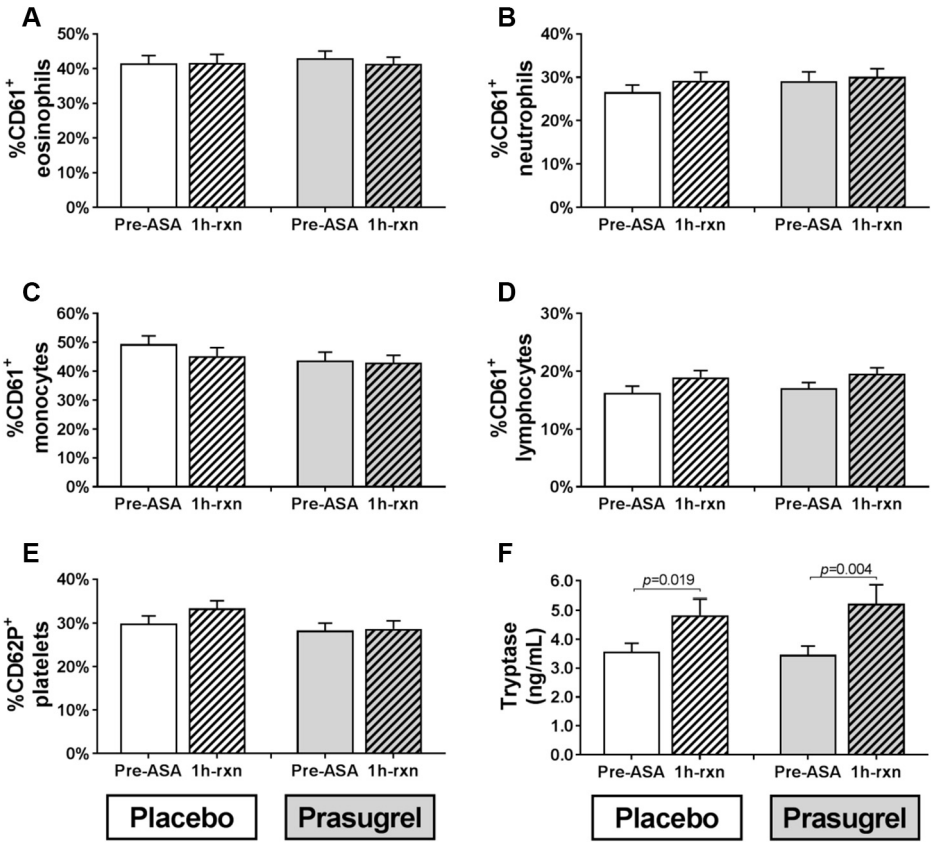


FIG 4. Platelet-leukocyte aggregation, platelet activation, and plasma tryptase level measurement. **A-D,** Percentages of leukocytes with adherent platelets (as determined by staining with CD61) before aspirin *(ASA)* administration *(solid columns)* and 1 hour after reaction onset *(striped columns)* are shown for each treatment arm for eosinophils (Fig 4, *A*), neutrophils (Fig 4, *B*), monocytes (Fig 4, *C*), and lymphocytes (Fig 4, *D*). **E** and **F,** Percentages of platelets that expressed surface CD62P (Fig 4, *E*) and total plasma tryptase levels (Fig 4, *F*) are shown at the same time points. Data are expressed as means + SEs.

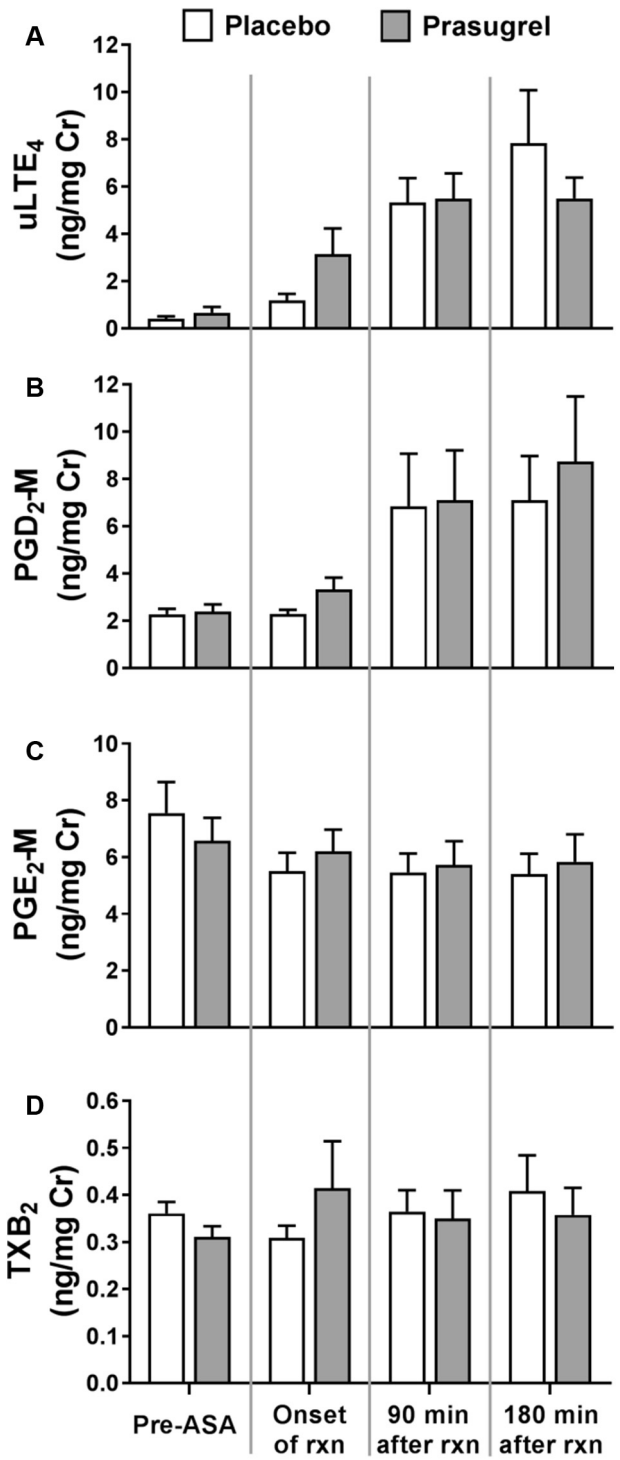


FIG 5. Urinary eicosanoid levels. Baseline preaspirin and aspirin-induced reaction levels of urinary eicosanoid levels analyzed by using gas chromatography-mass spectrometry are shown for both treatment arms: **A,** LTE_4; **B,** PGD2 mediator *(PGD_2-M)*; **C,** PGE_2 mediator *(PGE_2-M)*; and **D,** TXB_2. Data are expressed as means + SEs. *ASA*, Aspirin; *rxn*, reaction.

Prasugrel did not alter baseline levels of urinary eicosanoids measured before aspirin administration (Fig 5). Urinary eicosanoid levels for patients receiving prasugrel treatment increased during aspirin-induced reactions to the same extent as those in patients receiving placebo treatment.

Characteristics of responder/nonresponder subgroups

The 5 prasugrel responders did not differ from the 35 nonresponders in terms of baseline demographics, Asthma Control Questionnaire scores, FEV_1, TNSSs, FENO values, or preaspirin differential cell counts during either the prasugrel or placebo treatment arms, and their PD_2 values during the placebo arm challenge also did not differ (see Table E1 in this article's Online Repository at www.jacionline.org). The 5 responders had lower preaspirin baseline urinary levels of LTE_4 (0.14 ± 0.03 vs 0.44 ± 0.14 ng/mg creatinine [Cr], $P = .042$) and thromboxane B_2 (TXB_2; 0.26 ± 0.03 vs 0.38 ± 0.03 ng/mg Cr, $P = .022$) while receiving placebo compared with the 35 nonresponders and tended to show reduced prostaglandin D_2 metabolite (PGD-M) levels (1.73 ± 0.20 vs 2.35 ± 0.26 ng/mg Cr, $P = .067$). Responders had significantly lower peak levels of urinary LTE_4 (0.46 ± 0.20 vs 9.91 ± 2.50 ng/mg Cr, $P = .0009$), PGD-M (2.11 ± 0.50 vs 10.37 ± 2.97 ng/mg Cr, $P = .011$), and TXB_2 (0.19 ± 0.04 vs 0.53 ± 0.08 ng/mg Cr, $P = .001$) in response to aspirin challenge (Fig 6, *A-C*). Compared with baseline prechallenge levels, responders showed no significant aspirin-induced increases in urinary LTE_4 (Fig 6, *A*) or PGD-M (Fig 6, *B*) levels. Furthermore, although there was a clear aspirin-induced increase in plasma tryptase levels 1 hour into the aspirin-induced reactions in the 35 nonresponders (increase of 52% ± 14% for the placebo arm, $P = .004$), the 5 responders trended toward a slight decrease in plasma tryptase levels (change of –9% ± 4%, $P = .112$) during their placebo arm aspirin challenge, with a significant difference in aspirin-induced change in tryptase levels between the 2 groups ($P = .001$; Fig 6, *D*). The maximum increase in TNSS during the placebo arm challenges correlated with the fold increase in urinary LTE_4 levels ($r = 0.58$, $P = .001$), with a trend toward an increase in TNSS correlations with the increase in urinary PGD-M and serum tryptase levels.

Peripheral blood absolute eosinophil count decreased in the 35 nonresponders during the aspirin-induced reaction in the placebo arm (−155 ± 41 cells/μL) but did not change in the responders (+40 ± 68 cells/μL, $P = .043$). Although their baseline eosinophil numbers did not differ, the 5 responders had significantly higher percentages of eosinophils with platelets attached than did the nonresponders at the preaspirin baselines while in the prasugrel arm (62% ± 5% vs 40% ± 5%, $P = .001$; Fig 7, *A*). The 5 responders also had a trend toward prasugrel-induced decrease in preaspirin baseline activated platelet ($CD62P^+$ platelet) counts in the prasugrel treatment arm compared with the placebo arm (reduction from 38% ± 5% on placebo to 25% ± 3% on prasugrel, $P = .046$), although this difference was not significant when corrected for multiple comparison testing. In contrast, prasugrel treatment did not lead to a decrease in platelet activation for the nonresponders (Fig 7, *B*).

Urine collected 3 hours after the 325-mg dose of aspirin from the patients who did not manifest any reaction is not biologically equivalent in time point or dose to that collected during aspirin-induced reactions. However, we analyzed these samples for eicosanoid levels from the 4 patients from whom they were collected. The change in urinary LTE_4 and urinary PGD-M levels (preaspirin baseline to 3 hours after 325 mg) during the prasugrel arm did not differ from the change (preaspirin baseline to 3 hours after reaction) measured during the placebo arm nor did the

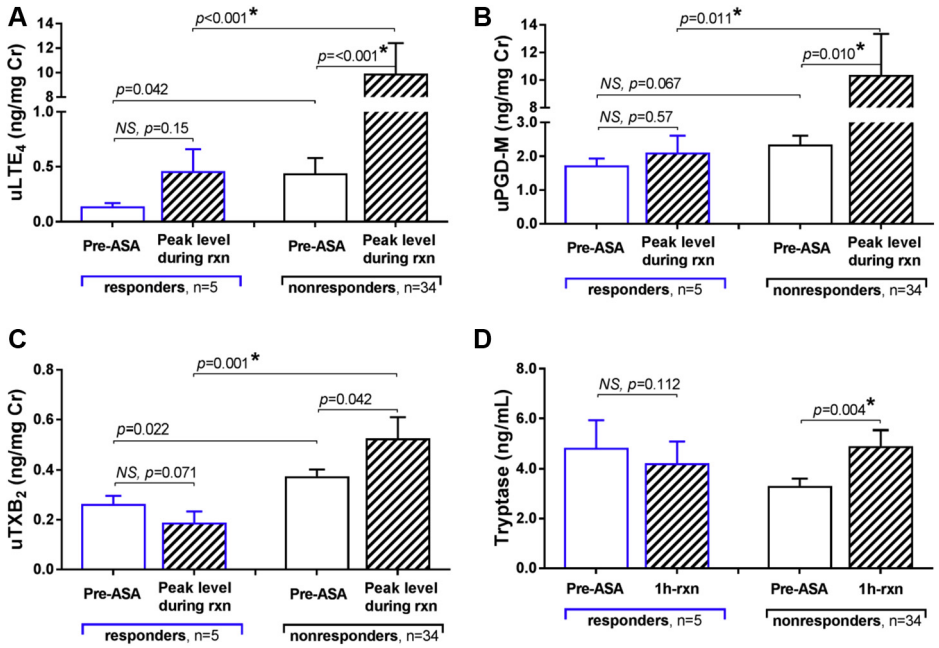


FIG 6. Responder versus nonresponder differences in aspirin *(ASA)*–induced urinary eicosanoid and plasma tryptase levels. **A-C,** Placebo arm urinary levels of LTE_4 (Fig 6, *A*), PGD-M (Fig 6, *B*), and TXB_2 (Fig 6, *C*) before aspirin administration *(white columns)* and at the aspirin reaction–induced peak *(striped columns)* are shown for the responders *(blue lines)* and nonresponders. **D,** Placebo arm plasma tryptase levels before aspirin administration and 1 hour after the reaction onset are shown for the same patient subgroups. Data are expressed as means + SEs. *P* values marked with *asterisks* are significant by using a false discovery rate of .05 to correct for multiple comparisons.

magnitude of the mediator production (see Fig E3 in this article's Online Repository at www.jacionline.org).

We conducted a *post hoc* analysis of *P2RY12* sequencing of the 5 responders and 23 nonresponders for whom we had sufficient genomic DNA. Of the 26 selected single nucleotide polymorphisms, 19 of which were previously associated with *P2RY12* gene expression, 2 were exonic, 23 were intronic, and 1 was upstream of the gene. No variant was predicted to disrupt amino acid composition, and none were associated with drug response (see Table E2 in this article's Online Repository at www.jacionline.org).

Safety

The number of total and severe adverse events was similar between the placebo and prasugrel arms (see Table E3 in this article's Online Repository at www.jacionline.org). Prasugrel was associated with a higher likelihood of bruising ($P = .006$). No patients withdrew participation because of any prasugrel-related adverse events. One patient withdrew participation after the first aspirin challenge, which had been during the placebo arm, because of a systemic reaction to the aspirin challenge that included vomiting.

DISCUSSION

Aberrantly increased CysLT production, eosinophilic respiratory tract inflammation, and persistent mast cell activation are all steady-state features of AERD that are further amplified during reactions to drugs that block COX-1. These reactions are thought to involve a prominent contribution from CysLTs and mast cell–derived mediators. We[6] and others[12] have demonstrated increased numbers of platelet-leukocyte complexes in the blood of patients with AERD and also observed such complexes in sinonasal tissues.[6] The CD62P-dependent adherence of platelets to leukocytes has several physiologic consequences, including transcellular conversion of leukotriene A_4 to LTC_4 by platelets.[5,13] Adherent platelets accounted for the majority of LTC_4S activity of blood granulocyte fractions from patients with AERD.[6] Platelets were also necessary for the surge of CysLT generation induced by aspirin challenge in AERD-like mice.[6,7]

We aimed to determine whether a similar platelet-dependent mechanism contributed to the aspirin-induced symptoms in patients with AERD. A previous study suggested that 6 days of treatment with clopidogrel, a thienopyridine antagonist of the $P2Y_{12}$ receptor, reduced numbers of platelet-monocyte aggregates in the blood of healthy patients.[14] Therefore we conducted this crossover study to determine whether prasugrel could block CysLT-dependent reactions to aspirin challenge and inhibit the formation of platelet-leukocyte aggregates in well-characterized patients with AERD.

Along with sinonasal congestion and ocular symptoms, reactions to aspirin in patients with AERD frequently result in sharp reductions in FEV_1. These reductions in part reflect the actions of CysLTs at the type 1 CysLT receptor ($CysLT_1R$). To ensure the safety of the challenges in this study, all subjects were treated with montelukast, an inhibitor of $CysLT_1R$, during challenge. $CysLT_1R$ blockade attenuates the decrease in lung function induced by aspirin but does not tend to eliminate the upper respiratory tract symptoms indicative of a reaction.[15] Therefore we used TNSS, rather than a change in FEV_1, as the basis for assessing reactions, and calculated the PD_2 value for aspirin on prasugrel versus placebo as the primary outcome based on TNSS.

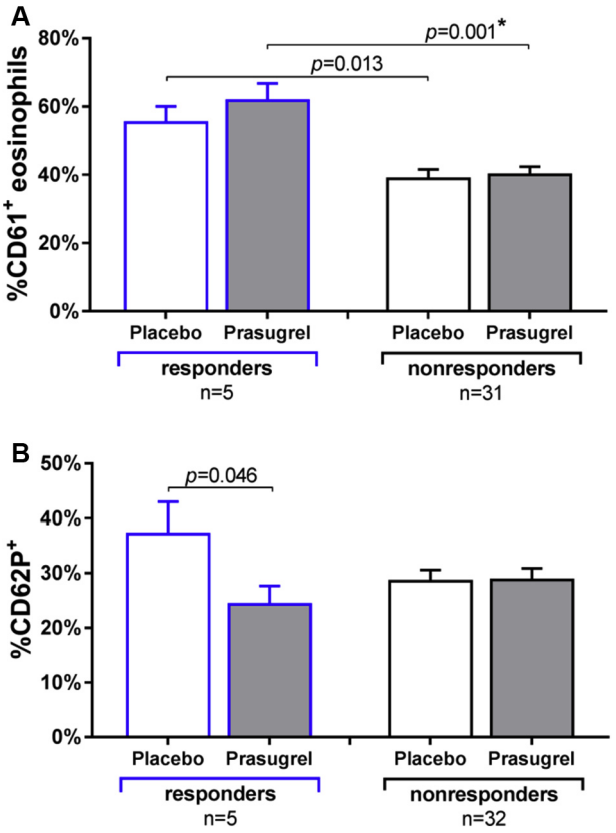


FIG 7. Responder versus nonresponder platelet activation and platelet-leukocyte aggregates. **A,** Percentages of eosinophils with adherent platelets before aspirin administration are shown for each treatment arm for responders (n = 5, *blue lines*) and nonresponders. **B,** Percentages of platelets that expressed surface CD62P are shown for the 2 patient subgroups at the same time points. Data are expressed as means + SEs. *P* values marked with *asterisks* are significant by using a false discovery rate of .05 to correct for multiple comparisons.

TABLE I. Patients' baseline demographics and characteristics

	Patients with AERD (n = 44)
Age (y)	47 ± 10
Sex (female)	24 (55%)
Race	
White	40 (91%)
Black	1 (2%)
Asian	0
Other	3 (7%)
Ethnicity	
Hispanic	3 (7%)
FEV_1 (L)	3.15 ± 0.11
FEV_1 (% predicted)	93.7 ± 2.0
FVC (L)	4.22 ± 0.16
F_{ENO} (ppb)	45 ± 4
Peripheral eosinophil count (/μL)	423 ± 53
Peripheral basophil count (/μL)	47 ± 8
ACQ-7 score	0.65 ± 0.09
TNSS	4.5 ± 0.7
Low-dose ICS (≤200 μg)*	15 (34%)
Medium-dose ICS (201-500 μg)*	18 (41%)
High-dose ICS (>500 μg)*	4 (9%)
Oral glucocorticoid use	2 (5%)
Long-acting β-agonist use	28 (64%)
Long-acting muscarinic antagonist use	0

Data are presented as means ± SEs.
ACQ, Asthma Control Questionnaire; *FVC*, forced vital capacity.
*Fluticasone propionate dry powder equivalent.

All 40 subjects experienced characteristic reactions on at least 1 of the 2 challenges, and most reacted to both challenges, confirming their diagnosis of AERD. Although treatment with prasugrel increased PD_2 values, this did not reach significance ($P = .10$, Fig 3). Moreover, in the population of patients as whole, prasugrel did not alter the baseline TNSS or Asthma Control Questionnaire score, baseline urinary eicosanoid level, F_{ENO} value, or change in eicosanoid level with aspirin challenge. In general, the maximum decrease in FEV_1 during aspirin-induced reactions correlated with the maximum increase in TNSS ($r = 0.50$, $P = .001$; see Fig E4 in this article's Online Repository at www.jacionline.org). Of the 74 aspirin-induced reactions in the study, 28 of those involved a decrease in FEV_1 of 15% or greater (38% of the reactions), which is consistent with previous reports of the rates of lower respiratory tract symptoms.[15] As previously reported, $CD62P^+$-free platelets (Fig 4, *A*) and platelet-leukocyte aggregates (Fig 4, *B-D*) were readily detectable in the blood of all subjects and did not change with aspirin challenge.[6,12] As expected, mean urinary LTE_4 levels increased sharply during reactions for the group as a whole (Fig 5) along with metabolite levels of PGD_2, the dominant COX product of mast cells, and plasma tryptase levels, which are all potential or verified indices of mast cell activation.

Five of the 40 subjects reacted while receiving placebo but did not exhibit any signs or symptoms of an aspirin-induced reaction (neither TNSS increase of ≥2 points nor FEV_1 decrease of ≥15%) while receiving prasugrel. Although the PD_2 value and the change in FEV_1 in responders were similar to the remainder of the cohort, the severity of upper respiratory symptoms, as measured by maximum increase in TNSS, was significantly milder in responders than in nonresponders (see Table E1).

Several features suggest that responders could represent an endotype of AERD with mild upper respiratory reactions. First, the baseline fraction of blood eosinophils with adherent platelets in the responder subgroup significantly exceeded those in nonresponders. This did not change as a result of prasugrel treatment (Fig 7, *A*). Second, in marked contrast to the nonresponders, responders did not display significant aspirin-induced increases in urinary LTE_4, PGD-M, and TXB_2 levels or aspirin-induced increases in plasma tryptase levels (all of which reflect mast cell activation; Fig 6)[16,17] during reactions on the placebo arm. Finally, blood eosinophil numbers decreased significantly during reactions on the placebo arm in the 35 nonresponders (presumably reflecting their recruitment to the respiratory tissue)[18,19] but not in the responders. Both LTE_4[20] and PGD_2[21] recruit eosinophils to the respiratory tissue. Thus the lack of change in blood eosinophil counts during reactions in the responder group could reflect low release of these lipid mediators by tissue mast cells. Collectively, our findings strongly suggest a small group of patients with AERD whose relatively low levels of mast cell mediator release reveal a $P2Y_{12}$-mediated component of their aspirin-induced reactions. This work, in conjunction with previous reports of a highly PGD_2-driven subgroup, confirms that multiple endotypes exist within the AERD phenotype of asthma.[17]

Our previous studies and those of others demonstrated that baseline blood platelet activation in patients with AERD exceeds that found in aspirin-tolerant control subjects by several fold.[6,12] Given that $P2Y_{12}$ receptors are required for autocrine activation of platelets in response to several classical agonists, it is surprising that prasugrel did not alter platelet CD62P levels in the entire cohort and did not change the percentages of platelet-leukocyte aggregates. Thus aberrant levels of steady-state platelet activation in patients with AERD might be driven by mechanisms that do not require $P2Y_{12}$-dependent amplification. Because $P2Y_{12}$ receptors are also expressed by M2-type macrophages,[22] dendritic cells,[23] and eosinophils,[24] it is possible that cell types other than platelets contribute to the reactions in the responder group. Polymorphic variants of the *P2RY12* gene have been associated with the risk of airway obstruction in house dust mite–sensitized children[25] and with indices of platelet and eosinophil activation in patients with AERD.[26] Although our identification of a responder subgroup of patients with AERD suggests a potential genetic mechanism, we did not identify any *P2RY12* variants that distinguished the 2 groups.

Because the mechanism by which platelet activation occurs in patients with AERD does not appear to require the $P2Y_{12}$ receptor amplification loop (except for the responder subgroup), our study can neither verify nor refute the potential role of platelets in facilitating leukocyte adhesion and recruitment or transcellular synthesis of LTC_4 in patients with AERD. Determining the mechanism responsible for AERD-associated platelet activation will be necessary to address this role by using a different target for intervention. Although prasugrel treatment is not effective for most patients with AERD, this study uncovered a potential subset of patients with AERD whose aspirin-induced reactions might involve $P2Y_{12}$ receptors, potentially because of reduced mast cell mediator release. Future larger studies will be necessary to validate this observation and reveal mechanisms.

Clinical implications: $P2Y_{12}$ antagonism with prasugrel prevents aspirin-induced reactions in only a subset of patients with AERD with a component of $P2Y_{12}$-dependent mechanisms of reaction to aspirin.

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Aspirin

Total Nasal Symptoms Score (TNSS) Worksheet

Participant ID:
Participant Initials:
Visit Date:
Visit:
Coordinator ID:

Test each nostril for stuffiness. Place finger to block end of right nostril. Try to breathe through the left nostril and determine severity of blockage/congestion. Then Repeat process to evaluate right nostril.

	None	A Little	Moderate	Quite a bit	Severe	Very Severe
1. Nasal Congestion (Left nostril)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2. Nasal Congestion (Right nostril)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3. Runny Nose	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
4. Itchy Nose	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5. Sneezing	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
6. Itchy Eyes	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
7. Teary Eyes	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
8. Itchy Ears or Throat	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
9. Eye Redness	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. Total Score _____

Participant Initials: __ __ __

Date: __ __/__ __/20 __ __

Time __ __:__ __

FIG E1. TNSS questionnaire. Scores for items 1 and 2 are averaged, so that the final range is between 0 and 40 points.

$$inverselog_{10}\left(\frac{\left(2-(PrevTNSS-BaselineTNSS)\right)\times(log_{10}ProvocDose-log_{10}PrevDose)}{(MaxTNSS-BaselineTNSS)-(PrevTNSS-BaselineTNSS)}+(log_{10}PrevDose)\right)$$

→ *BaselineTNSS:* The patient's first morning TNSS score, prior to the administration of aspirin.

→ *ProvocDose:* The administered dose of aspirin that provoked the reaction (40.5, 81, 162, or 325)

→ *PrevDose:* The administered dose of aspirin that was given just previously to the dose of aspirin that provoked the reaction. In all cases, the *PrevDose* is ½ of the *ProvocDose*.

→ *PrevTNSS*: The patient's previous TNSS score that was taken after the *PrevDose* was given. For patients whose reaction was induced by the first dose of aspirin (40.5mg), the *PrevTNSS* was the *BaselineTNSS*, as there were no intervening TNSS questionnaires given.

→ *MaxTNSS*: The patient's maximum TNSS recorded during the 3-hour observation of reaction. All patients recorded a TNSS at 30-minute intervals during that 3-hour period, and so the *MaxTNSS* is the highest TNSS recorded at one of the following timepoints: Onset of reaction, 30 minutes post onset of reaction, 60 minutes post onset of reaction, 90 minutes post onset of reaction, 120 minutes post onset of reaction, 150 minutes post onset of reaction, 180 minutes post onset of reaction.

FIG E2. PD_2 value calculation.

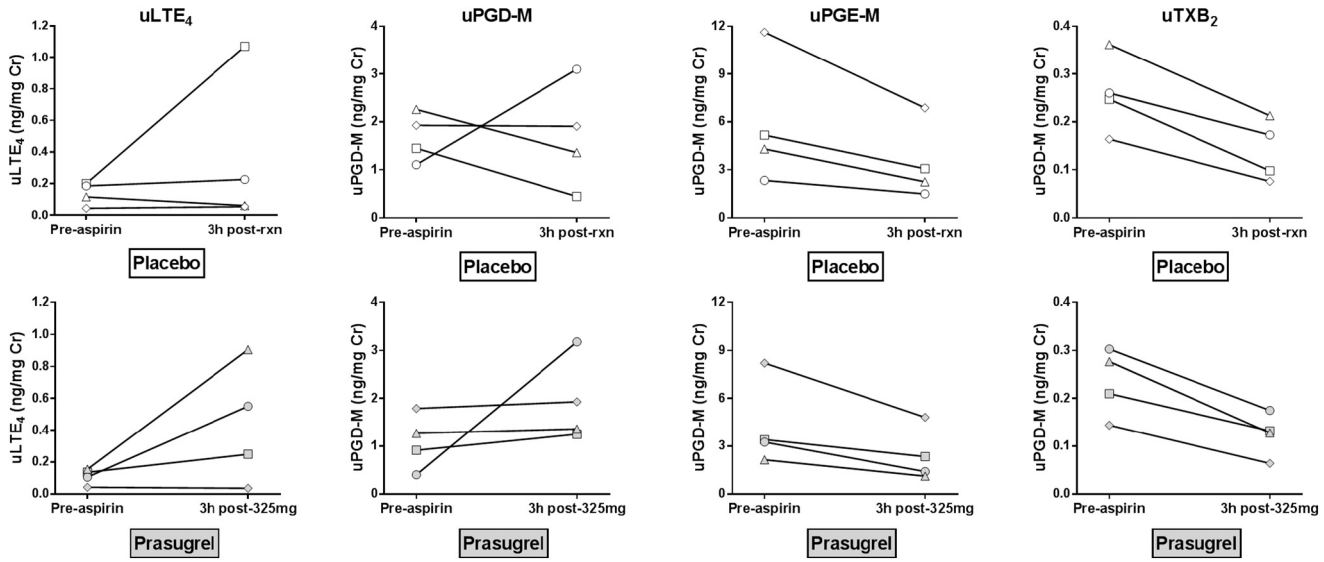

FIG E3. Urinary eicosanoid levels are shown for the 4 prasugrel responders before and 3 hours after the aspirin-induced reaction on the placebo arm *(top row)* and before and 3 hours after the 325-mg dose of aspirin on the prasugrel arm *(bottom row)*.

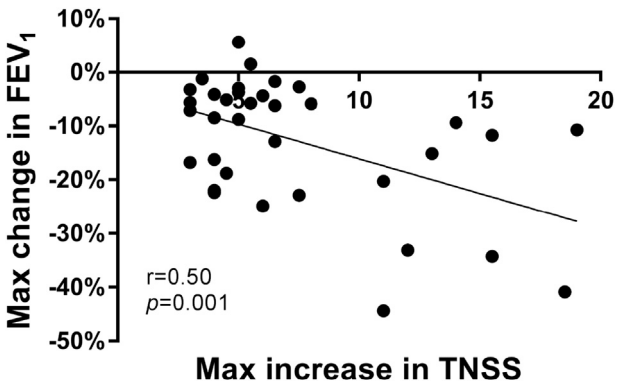

FIG E4. Correlation between maximum change in FEV_1 and maximum increase in TNSS during aspirin-induced reactions in the prasugrel treatment arm.

TABLE E1. Demographic/clinical and laboratory characteristics of the 5 responders and 35 nonresponders

	Prasugrel responders (n = 5)		Prasugrel nonresponders (n = 35)		*P* value (responder vs nonresponder)	
	Placebo	Prasugrel	Placebo	Prasugrel	Placebo	Prasugrel
Age (y)	44 ± 4		47 ± 2		.946	
Sex (female)	3/5		19/35		.834	
FEV_1 (L)	2.74 ± 0.26	2.74 ± 0.30	3.19 ± 0.14	3.16 ± 0.15	.178	.258
FEV_1 (% predicted)	84.5 ± 5.4	84.0 ± 6.6	93.1 ± 2.3	92.2 ± 2.2	.206	.294
F_{ENO} (ppb)	39.6 ± 8.9	39.2 ± 9.1	54.1 ± 8.4	45.9 ± 4.7	.258	.533
Blood AEC (/μL)	640 ± 166	500 ± 148	500 ± 62	435 ± 50	.464	.696
Blood eosinophils (%)	8.4 ± 1.7	7.2 ± 1.5	7.1 ± 0.7	6.4 ± 0.7	.499	.619
ACQ-7 score	0.93 ± 0.24	0.74 ± 0.11	0.77 ± 0.38	0.70 ± 0.11	.495	.865
TNSS, baseline	2.3 ± 0.7	5.4 ± 2.0	3.2 ± 0.7	3.2 ± 0.6	.342	.343
TNSS, maximum during rxn	5.7 ± 0.7	3.5 ± 1.2	10.8 ± 1.4	10.6 ± 0.9	.002	.0007
PD_2	82 ± 18	650	78 ± 18	66 ± 10	.881	
$uLTE_4$ (ng/mg Cr)	0.14 ± 0.03	0.10 ± 0.14	0.44 ± 0.13	0.75 ± 0.28	.041	.026
uPGD-M (ng/mg Cr)	1.73 ± 0.20	1.48 ± 0.45	2.37 ± 0.26	2.54 ± 0.33	.067	.234
uPGE-M (ng/mg Cr)	5.36 ± 1.64	4.31 ± 1.05	7.97 ± 1.25	6.94 ± 0.87	.443	.264
$uTXB_2$ (ng/mg Cr)	0.26 ± 0.03	0.33 ± 0.10	0.38 ± 0.03	0.31 ± 0.02	.022	.811

Clinical and laboratory values are at the preaspirin baseline after each of the treatment arms. Data are presented as means ± SEs.
ACQ, Asthma Control Questionnaire; *rxn*, reaction; *$uLTE_4$*, urinary LTE_4; *uPGD-M*, urinary PGD-M; *uPGE-M*, urinary PGE mediator; *$uTXB_2$*, urinary TXB_2.

TABLE E2. Results of logistic regression for association between genotype and response to prasugrel for selected SNPs

SNP	Position	Location	Alleles	MAF	Estimate	SE	*z* Value	Pr(>|z|)
rs6809699	151056598	Exon	A/C	0.143	18.467	3802.118	0.005	0.996
rs6785930	151056616	Exon	G/A	0.429	0.523	0.867	0.603	0.547
rs2046934	151057642	Intron	G/A	0.130	17.467	2465.326	0.007	0.994
rs9848789	151058963	Intron	C/T	0.089	0.114	1.012	0.113	0.910
rs7615865	151073033	Intron	T/C	0.214	1.105	1.144	0.966	0.334
rs1491978	151080070	Intron	C/T	0.196	0.939	1.132	0.830	0.407
rs7634096	151087637	Intron	C/T	0.143	−0.432	1.082	−0.399	0.690
rs7637803	151089226	Intron	C/T	0.125	−0.256	1.060	−0.242	0.809
rs3732765	151090424	Intron	G/A	0.286	0.089	0.784	0.113	0.910
rs11708287	151090863	Intron	A/C	0.286	0.089	0.784	0.113	0.910
rs9859538	151090963	Intron	G/A	0.339	0.285	0.679	0.420	0.674
rs12497065	151096112	Intron	T/C	0.278	0.136	0.776	0.175	0.861
rs12497089	151096164	Intron	T/A	0.259	0.243	0.761	0.319	0.750
rs17283010	151097391	Intron	G/A	0.278	0.136	0.776	0.175	0.861
rs12489121	151098519	Intron	A/G	0.321	−0.119	0.752	−0.159	0.874
rs6787801	151099741	Intron	A/G	0.446	0.343	0.804	0.427	0.670
rs9289836	151100121	Intron	C/T	0.339	0.285	0.679	0.420	0.674
rs3821663	151100677	Intron	T/G	0.286	0.089	0.784	0.113	0.910
rs11713504	151100956	Intron	A/G	0.278	0.136	0.776	0.175	0.861
rs10935840	151101083	Intron	A/G	0.278	0.136	0.776	0.175	0.861
rs7429509	151101167	Intron	C/T	0.321	0.416	0.721	0.577	0.564
rs12488803	151101358	Intron	T/G	0.250	0.296	0.756	0.392	0.695
rs10935841	151101691	Intron	C/T	0.286	0.089	0.784	0.113	0.910
rs12485508	151101746	Intron	C/T	0.286	0.089	0.784	0.113	0.910
rs1491974	151102452	Intron	A/G	0.464	0.205	0.759	0.270	0.787
rs4603933	151103070	Upstream	T/G	0.125	17.403	2465.326	0.007	0.994

Genomic position is given relative to the reference human genome (GRCh37/hg19).
MAF, Minor allele frequency; *Pr(>|z|)*, probability that value is greater than the normal distribution; *SNP*, single nucleotide polymorphism.

TABLE E3. Adverse events

	Placebo arm	Prasugrel arm	*P* value
Total adverse events reported	25	36	
Bruising	3	12	.006
Upper respiratory tract infection/sinusitis	5	7	.75
Worsening of asthma	5	4	1
Muscle injury/myalgia	2	0	.49
Headache	2	2	.49
Throat infection	1	1	1
Accidental ingestion of NSAID	1	0	1
Systemic reaction to aspirin challenge	1	0	1
Skin rash	1	1	1
Conjunctivitis	0	1	1
Ear infection	0	1	1
Finger tingling/numbness	0	1	1
Joint stiffness	0	1	1
Light-headedness	1	0	1
Lower leg edema	0	1	1
Mood change	1	0	1
Spider bite	1	0	1
Stomach pain	1	0	1
Urinary tract infection	0	1	1
Viral gastroenteritis	0	1	1

All events that occurred after randomization through 2 weeks after the second aspirin challenge were included. Data listed are the number of participants reporting each type of adverse event.

NSAID, Nonsteroidal anti-inflammatory drug.