

# Accepted Manuscript

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PII: S0091-6749(18)30843-1

DOI: [10.1016/j.jaci.2018.06.001](https://doi.org/10.1016/j.jaci.2018.06.001)

Reference: YMAI 13463

To appear in: *Journal of Allergy and Clinical Immunology*

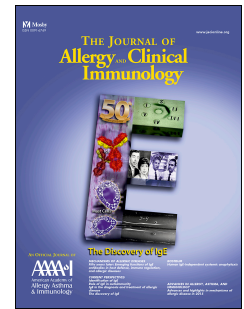
Received Date: 30 October 2017

Revised Date: 15 May 2018

Accepted Date: 3 June 2018

Please cite this article as: Laidlaw TM, Cahill KN, Cardet JC, Murphy K, Cui J, Dioneda B, Kothari P, Raby BA, Israel E, Boyce J, A trial of P2Y<sub>12</sub> receptor inhibition with prasugrel identifies a potentially distinct endotype of patients with aspirin-exacerbated respiratory disease, *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.06.001.

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**A trial of P2Y<sub>12</sub> receptor inhibition with prasugrel identifies a potentially distinct endotype of patients with aspirin-exacerbated respiratory disease.**

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Funding: This work was supported by the National Institutes of Health (NIH grant nos U19AI095219, K23HL111113, K23AI118804, K23AI125785, R01HL128241, T32AI00730, AI078908, HL117945, and AI52353), and by generous contributions from the Vinik and Kaye Families.

Conflict of Interest: T Laidlaw has served on scientific advisory boards for GlaxoSmithKline and Allakos and has received consultancy fees from Knopp Biosciences and Sanofi-Genzyme. K Cahill has served on scientific advisory boards for Teva. J Cardet, K Murphy, J Cui, B Dioneda, P Kothari, B Raby, and J Boyce have no conflicts of interest to disclose. E Israel has received consultancy fees from AstraZeneca, Philips Respironics, Regeneron Pharmaceuticals, Birk Rock Bio, Nuvelution Pharmaceuticals, Vitaeris Inc, Sanofi, and Merck.

## Abstract

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

**Objective:** We sought to determine whether treatment with prasugrel, which inhibits platelet activation by blocking the P2Y<sub>12</sub> receptor, would attenuate the severity of sinonasal and respiratory symptoms induced during aspirin challenge in subjects with AERD.

**Methods:** 40 subjects with AERD completed a 10-week, double-blinded, placebo-controlled crossover trial of prasugrel. All subjects 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 of 2 points (PD<sub>2</sub>). 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 PD<sub>2</sub> (79 ±15 on placebo and 139 ±32 on prasugrel, P = 0.10), platelet-leukocyte aggregates, or increases in urinary leukotriene E<sub>4</sub> (LTE<sub>4</sub>) and prostaglandin D<sub>2</sub> metabolite (PGD-M) levels during aspirin-induced reactions in the study population as a whole. Five subjects ("responders") reacted to aspirin while on placebo but did not develop 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 show significant aspirin-induced increases in urinary LTE<sub>4</sub>, PGD-M, or thromboxane B<sub>2</sub> levels, and failed to display increases in serum tryptase levels during the 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 higher baseline platelet activation and milder upper respiratory symptoms during aspirin reactions, P2Y<sub>12</sub> antagonism with prasugrel completely inhibited all aspirin-induced reaction symptoms, suggesting a contribution from P2Y<sub>12</sub> signaling in this subset.

**Clinical Implications:** P2Y<sub>12</sub> antagonism with prasugrel prevents aspirin-induced reactions in only a subset of AERD patients with a component of P2Y<sub>12</sub>-dependent mechanisms of reaction to aspirin.

**Capsule summary:** Platelet activation increased in AERD. In a randomized placebo-controlled cross-over trial, platelet inhibition through P2Y<sub>12</sub> antagonism with prasugrel neither prevented nor lessened aspirin-induced reactions in most patients with AERD, though did identify a small subset of responders who may have a distinct P2Y<sub>12</sub>-dependent mechanism of reaction to aspirin.

**Key words:** Aspirin-exacerbated respiratory disease, double-blinded randomized placebo controlled crossover trial, prasugrel, Samter's triad, N-ERD, leukotrienes, P2Y<sub>12</sub>

**Abbreviations:**

|                            |                                         |
|----------------------------|-----------------------------------------|
| AERD                       | Aspirin Exacerbated Respiratory Disease |
| TNSS                       | Total Nasal Symptom Score               |
| BWH                        | Brigham and Women's Hospital            |
| PD2                        | Increase in TNSS of 2 points            |
| LTE <sub>4</sub>           | Leukotriene E <sub>4</sub>              |
| PG                         | Prostaglandin                           |
| COX                        | Cyclooxygenase                          |
| ACQ                        | Asthma Control Questionnaire            |
| FeNO                       | Fractional Exhaled Nitric Oxide         |
| EP <sub>2</sub>            | E prostanoid 2                          |
| LTA <sub>4</sub>           | Leukotriene A <sub>4</sub>              |
| CysLT                      | Cysteinyl leukotriene                   |
| LTC <sub>4</sub>           | Leukotriene C <sub>4</sub>              |
| FEV <sub>1</sub>           | Forced expiratory volume in 1 second    |
| 5-LO                       | 5-lipoxygenase                          |
| P2Y <sub>12</sub> receptor | Type 12 purinergic receptor             |

## Introduction

Aspirin-exacerbated respiratory disease (AERD) is a distinctive clinical syndrome affecting ~7% of adults with asthma and ~14% of those with severe asthma.<sup>1</sup> AERD is characterized clinically by asthma, rhinosinusitis with nasal polyposis, respiratory tissue eosinophilia, and respiratory reactions to ingestion of aspirin and other inhibitors of cyclooxygenase (COX)-1. Biochemically, AERD is marked by over-activity of the 5-lipoxygenase (5-LO)/leukotriene C<sub>4</sub> synthase (LTC<sub>4</sub>S) pathway, resulting in generation of high levels of leukotriene (LT)C<sub>4</sub> and, therefore, high urinary levels of LTE<sub>4</sub>, 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 AERD, particularly in clinical reactions to aspirin challenge/desensitization.<sup>2</sup>

Although eosinophils<sup>3</sup> and mast cells,<sup>4</sup> which express both 5-LO and LTC<sub>4</sub>S, likely account for the majority of cysLTs released in AERD during clinical reactions, platelets may also play a role in cysLT overproduction. Platelets lack 5-LO, but can convert leukocyte-derived LTA<sub>4</sub> to LTC<sub>4</sub> due to their expression of LTC<sub>4</sub>S.<sup>5</sup> 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 previously shown that both peripheral blood and sinonasal tissue of individuals with AERD contain higher numbers of platelet-leukocyte aggregates than do aspirin-tolerant patients, and their numbers correlate with basal urinary LTE<sub>4</sub> levels.<sup>6</sup> Moreover, mouse studies implicate platelet-leukocyte aggregates in cysLT generation during reactions to aspirin.<sup>7</sup> These observations suggest that agents capable of interfering with the formation of platelet-leukocyte aggregates could have therapeutic efficacy in AERD. Based on these findings, we hypothesized that antiplatelet therapy may be efficacious in AERD, particularly if it interferes with the transcellular generation of LTC<sub>4</sub>.

Many stimuli that activate platelets elicit their release of adenosine diphosphate (ADP), which signals through the type 12 purinergic (P2Y<sub>12</sub>) receptor to amplify CD62P expression and the interaction of platelets with leukocytes. Thienopyridine drugs (prasugrel, clopidogrel) that block P2Y<sub>12</sub> receptors inhibit platelet activation in cardiovascular disease.<sup>8</sup> This double-blind placebo-controlled trial aimed to test the hypothesis that blockade of P2Y<sub>12</sub> receptors with prasugrel would attenuate the severity of sinonasal and respiratory symptoms that occur during aspirin challenges in subjects with AERD by blocking platelet activation and reducing cysLT generation by decreasing the frequency of platelet-leukocyte aggregates.

## Methods

### Study design

Participants enrolled in a 10-week, double-blinded, 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 (**Figure 1**). In a separate continuation study, patients continued onto high-dose aspirin (650 mg twice daily).

### Participants

Subjects with AERD had a history of physician-diagnosed asthma, nasal polyposis, and at least one clinical reaction to aspirin, or another non-selective 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. To ensure the successful and safe completion of the protocol, subjects had

stable asthma, defined as a post-bronchodilator FEV<sub>1</sub> of 70% of predicted or better, no increase in baseline dose of oral glucocorticoids for at least 3 months, and no history of hospitalization or emergency room visits due to asthma for at least 6 months prior to enrolling in the study.

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

This was a single-site study, conducted at the Asthma Research Center, Brigham and Women's Hospital. The Institutional Review Board of Brigham and Women's Hospital/Partners Healthcare approved this study, and all subjects gave 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.

#### Clinical Procedures

After a 4-week run-in period on stable asthma treatment and montelukast 10 mg daily, subjects were randomized to begin taking either prasugrel (5 mg (for patients <60kg) or 10mg (≥ 60kg) daily, following a 60mg loading dose) or equivalent placebo for 4-weeks, followed by a 4-dose aspirin challenge (40.5mg, 81mg, 162mg, and 325mg) with 90-minute intervals,<sup>9</sup> which was terminated when patients experienced either an increase in the total nasal symptom score (TNSS) of at least two points above baseline, or a decline in FEV<sub>1</sub> of at least 15% from baseline, or reached a dose of 325mg of aspirin without any reaction symptoms. The TNSS questionnaire is a 9-symptom patient recorded outcome measurement on a total scale of 0-40, which includes

assessment of nasal congestion, runny nose, sneezing, nasal itching, and eye and throat symptoms (see **Supplemental Figure E1** for the full questionnaire). Baseline assessments were collected the morning of the aspirin challenge.

Patients were observed for a 3-hour period following the onset of their reaction and challenge termination, with serial measurements of lung function, TNSS, and collection of blood and urine samples. Subjects were discharged to home to continue daily montelukast and washout the study drug from the first treatment phase. At the end of the 2-week washout period, subjects crossed over to the 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 prior to administration of aspirin, and 1 hour after the onset of the aspirin-induced reaction. If no reaction occurred, blood was drawn again 3 hours after the 325mg dose of aspirin. Platelet-rich plasma (PRP) was obtained from the top layer of blood samples after a 20-minute centrifuge at 200g.

To monitor platelet activation and quantify platelet-leukocyte aggregates, we incubated 10  $\mu$ L of PRP with directly-conjugated antibodies specific for CD61 and CD62P and 20  $\mu$ L of whole blood with antibodies specific for CD61, CD45, CD14 and CCR3, or appropriate isotype controls for 20 minutes (CCR3 antibody from BioLegend, San Diego CA, all others from BD Biosciences, San Jose CA. We lysed the red blood cells with FACS™ Lysing Solution (BD Biosciences, San Jose CA) then fixed the cells in 1% paraformaldehyde. At least 20,000 CD45<sup>+</sup> cells for the whole blood analyses or 30,000 platelets for the PRP analyses were recorded for

each sample in a BD FACSCanto flow cytometer. Analyses completed with FlowJo Version 10 (TreeStar, Ashland OR). CD45<sup>+</sup> leukocytes were classified as eosinophils (CCR3<sup>+</sup> granulocytes), neutrophils (CCR3<sup>-</sup> granulocytes), monocytes (CD14<sup>+</sup>), and lymphocytes. The presence of adherent platelets was determined by the 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.

Urinary eicosanoids were measured by gas chromatography-mass spectrometry (GC-MS) at Vanderbilt University as previously described.<sup>6</sup> For the aspirin-challenge visits, urine samples were collected in the morning before the administration of aspirin, and again at the onset of, 90, and 180 minutes after the aspirin-induced reaction. For those visits where no reaction was elicited, urine was collected 180 minutes after the 325mg dose of aspirin.

DNA from peripheral blood leukocytes was used to sequence the *P2RY12* locus (gene encoding the P2Y<sub>12</sub> receptor) in 28 subjects. DNA was extracted with an AllPrep DNA/RNA Mini Kit (Qiagen, Valencia CA). Five overlapping PCR amplicons, covering the entire gene, were sequenced using the Illumina MiSeq platform to generate 300 base paired-end reads which were aligned to the reference human genome (GRCh37/hg19) using Bowtie2 (v2.2.6). SAMtools (v1.4) was used to call genetic variants from sorted and indexed BAM files. Association testing of genotype with response to prasugrel was performed by logistic regression on selected SNPs.

## 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 vs. placebo on the provocative dose that caused an increase in TNSS of 2 (PD<sub>2</sub>) points during an aspirin challenge. PD<sub>2</sub> is modeled after the aspirin PD<sub>20</sub> (the provocative dose causing a 20% fall in baseline FEV<sub>1</sub>) established in the 2007 EAACI guidelines,<sup>10</sup> and was calculated as follows (see detailed description of each variable in **Supplemental Figure E2**):

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

Participants who did not develop any clinically apparent reaction to aspirin during an aspirin challenge (a fall in FEV<sub>1</sub> of less than 15% and no significant development of any nasocular or non-respiratory symptoms, as quantified by the lack of increase in TNSS) were designated as having a “negative challenge” and were assigned a PD<sub>2</sub> 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 eicosanoids, and platelet activation during aspirin challenge while on prasugrel versus placebo. Additional pre-defined outcomes included the effect of prasugrel on chronic asthma control, urinary eicosanoid metabolites, platelet activation, and fractional exhaled nitric oxide (FeNO) in patients with AERD.

### Sample size

The study was powered to detect an effect of prasugrel on the PD<sub>2</sub> during aspirin desensitization. The sample size of 40, using a 2-sided paired t-test comparing the mean PD<sub>2</sub> between treatment with prasugrel and placebo, provided 80% power to detect a 1.74 (0.8 after log<sub>2</sub> transformation) fold change in the PD<sub>2</sub>, at an 0.05 level of significance.

## Randomization

Randomization consisted of 3 randomly-selected blocks for each stratum of dose (5 mg 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 proc plan, ensuring equal numbers of AERD subjects were randomized to each group. The BWH Investigational Drug Services (IDS) pharmacy conducted the randomization and kept the records to maintain the blind of the study. IDS pharmacy also prepared the placebo and prasugrel via encapsulation.

## Blinding

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

## Statistical methods

For the crossover study, we compared the difference in  $PD_2$  between the two (prasugrel vs. placebo) aspirin challenges using a two-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 the levels of urinary  $LTE_4$  and 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 pre-aspirin 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 two-sided paired t-test. For the additional post hoc analyses done on the “responder” vs

“nonresponder” subgroups, the False Discovery Rate method was used to correct for the multiple comparison testing, with a rate greater than 0.05 considered significant.<sup>11</sup> The raw p-values are presented, and the significant values using the False Discovery Rate are identified in Figures 6 and 7. Data are presented  $\pm$  standard error (SE) unless otherwise specified.

## 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 (**Figure 2**). The baseline characteristics of the patients who underwent randomization are summarized in **Table 1**. Thirty-nine out of forty patients had confirmed (diary cards and pill counts) compliance with both daily montelukast and daily study drug of over 90%.

### Primary Outcome

The mean PD<sub>2</sub> on the prasugrel arm of  $79 \pm 15$  and the mean PD<sub>2</sub> on the placebo arm of  $139 \pm 32$  ( $P=0.10$ ) (**Figure 3A**). Of the 40 AERD patients who completed the cross-over trial, 5 did not develop any clinically apparent reaction to aspirin during the arm when they had been treated with prasugrel (prasugrel “responders”), and were assigned a PD<sub>2</sub> of 650. The clinical reactions of the 5 responders during the placebo-arm challenge showed significantly smaller increases in TNSS, compared to that of the 35 nonresponders (maximum TNSS increase of  $3.4 \pm 0.8$  for 5 responders, vs  $7.5 \pm 0.9$  for 35 nonresponders,  $P=0.003$ ), though the average fall in FEV<sub>1</sub> was similar ( $9.9 \pm 3.5\%$  vs  $12.8 \pm 2.0$ ,  $P=0.50$ ). There was also 1 patient who did not develop any clinically apparent reaction to aspirin during the arm when they had been treated with placebo, but did develop a reaction during the prasugrel arm. For the entire study population, the mean difference in maximum increase in TNSS for patients on the prasugrel arm compared to the placebo arm was  $0.5 \pm 1.0$  ( $P=0.32$ ), with mean increase in TNSS on the

prasugrel arm of  $6.5 \pm 0.8$  and the mean increase in TNSS on the placebo arm of  $7.0 \pm 0.8$  (**Figure 3B**). The administered aspirin dose that provoked a reaction for patients on the prasugrel arm was  $0.2 \pm 0.2$ -fold higher ( $P=0.38$ ) than the administered provocative aspirin dose for patients on the placebo arm.

### Secondary Outcomes

Treatment with prasugrel did not alter baseline percentages of CD62P<sup>+</sup> platelets compared with placebo in all 40 AERD patients who completed the trial, and did not change the percentages of any leukocyte subset with adherent platelets (**Figure 4, A-E**). In the 40 patients as a group, neither the percentage of CD62P<sup>+</sup> platelets nor the percentages of leukocytes with adherent platelets changed one hour into the aspirin-induced reactions, and prasugrel did not alter these percentages when compared with placebo (**Figure 4, A-E**). Plasma tryptase levels rose significantly during the aspirin-induced reactions (increase of  $44 \pm 12\%$  for placebo arm,  $P=0.02$ , and  $60 \pm 21\%$  for prasugrel arm,  $P=0.004$ ) though treatment with prasugrel did not alter either the pre-aspirin levels of tryptase, nor the aspirin-induced increases (**Figure 4, F**).

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

### Characteristics of Responder/Nonresponder Subgroups

The 5 prasugrel “responders” did not differ from the 35 nonresponders in terms of baseline demographics, ACQ, FEV<sub>1</sub>, TNSS, FeNO, or pre-aspirin differential cell counts during either the prasugrel or placebo treatment arms, and their PD<sub>2</sub> during the placebo-arm challenge also did not differ (**Supplemental Table E1**). The 5 responders had lower pre-aspirin baseline

urinary levels of  $\text{LTE}_4$  ( $0.14 \pm 0.03$  vs  $0.44 \pm 0.14$  ng/mg Cr,  $P=0.042$ ) and  $\text{TXB}_2$  ( $0.26 \pm 0.03$  vs  $0.38 \pm 0.03$  ng/mg Cr,  $P=0.022$ ) while on placebo compared to the 35 nonresponders, and tended to show reduced PGD-M levels ( $1.73 \pm 0.20$  vs  $2.35 \pm 0.26$  ng/mg Cr,  $P=0.067$ ). The responders showed significantly lower peak levels of urinary  $\text{LTE}_4$  ( $0.46 \pm 0.20$  vs  $9.91 \pm 2.50$  ng/mg Cr,  $P=0.0009$ ), PGD-M ( $2.11 \pm 0.50$  vs  $10.37 \pm 2.97$  ng/mg Cr,  $P=0.011$ ), and  $\text{TXB}_2$  ( $0.19 \pm 0.04$  vs  $0.53 \pm 0.08$  ng/mg Cr,  $P=0.001$ ) in response to aspirin challenge (**Figure 6, A-C**). Compared to baseline pre-challenge levels, the responders showed no significant aspirin-induced increases in urinary  $\text{LTE}_4$  (**Figure 6, A**) or PGD-M (**Figure 6, B**). Furthermore, although there was a clear aspirin-induced rise in plasma tryptase one hour into the aspirin-induced reactions in the 35 nonresponders (increase of  $52 \pm 14\%$  for placebo arm,  $P=0.004$ ), the 5 responders trended toward a slight decrease in plasma tryptase levels (change of  $-9 \pm 4\%$ ,  $P=0.112$ ) during their placebo arm aspirin challenge with a significance difference in aspirin-induced change in tryptase between the two groups ( $P=0.001$ ) (**Figure 6, D**). The maximum rise in TNSS during the placebo-arm challenges correlated with the fold increase in urinary  $\text{LTE}_4$  ( $r=0.58$ ,  $P=0.001$ ), with a trend toward rise in TNSS correlations with the increase in urinary PGD-M and serum tryptase.

Peripheral blood absolute eosinophil count decreased in the 35 nonresponders during the aspirin-induced reaction while on the placebo arm ( $-155 \pm 41$  cell/ $\mu\text{L}$ ), but did not change in the responders ( $+40 \pm 68$  cell/ $\mu\text{L}$ ,  $P=0.043$ ). While 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 pre-aspirin baselines while on the prasugrel ( $62 \pm 5\%$  vs  $40 \pm 5\%$ ,  $P=0.001$ ) arm (**Figure 7A**). The 5 responders also had a trend toward prasugrel-induced decrease in pre-aspirin baseline activated platelets ( $\text{CD62P}^+$  platelets) while on the prasugrel treatment arm compared to the placebo arm (reduction from  $38 \pm 5\%$  on placebo to  $25 \pm 3\%$  on prasugrel,  $P=0.046$ ), though 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 (**Figure 7B**).

The 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 timepoint or dose to that collected during the aspirin-induced reactions. However, we did analyze these samples for eicosanoid levels from the 4 patients for whom they were collected. The change in urinary  $\text{LTE}_4$  and urinary PGD-M (pre-aspirin baseline to 3-hour post-325mg) during their prasugrel arm did not differ from the change (pre-aspirin baseline to 3-hour post-reaction) measured during their placebo arm, nor did the magnitude of the mediator production (**Supplemental Fig. E3**).

We conducted a post hoc analysis of the *P2RY12* sequencing of the 5 responders and 23 nonresponders for whom we had sufficient genomic DNA. Of the 26 selected SNPs, 19 of which were previously associated with *P2RY12* gene expression, 2 were exonic, 23 intronic, and 1 up-stream of the gene. No variant was predicted to disrupt amino acid composition and none were associated with drug response (**Supplemental Table E2**).

### Safety

The number of total and severe adverse events was similar between the placebo arm and the prasugrel arm (**Supplemental Table E3**). Prasugrel was associated with a higher likelihood of bruising ( $P=0.006$ ). No patients withdrew participation due to any prasugrel-related adverse events. One patient withdrew participation following the first aspirin challenge, which had been during the placebo arm, due to a systemic reaction to the aspirin challenge that included vomiting.

## Discussion

Aberrantly increased production of cysLTs, 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<sup>6</sup> and others<sup>12</sup> have demonstrated increased numbers of platelet-leukocyte complexes in the blood of patients with AERD, and also observed such complexes in sinonasal tissues.<sup>6</sup> The CD62P-dependent adherence of platelets to leukocytes has several physiologic consequences, including transcellular conversion of LTA<sub>4</sub> to LTC<sub>4</sub> by platelets.<sup>5,13</sup> Adherent platelets accounted for the majority of LTC<sub>4</sub>S activity of blood granulocyte fractions from subjects with AERD.<sup>6</sup> Platelets were also necessary for the surge of cysLT generation induced by aspirin challenges of AERD-like mice.<sup>6,7</sup> 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<sub>12</sub> receptor, reduced the numbers of platelet-monocyte aggregates in the blood of healthy patients.<sup>14</sup> We therefore 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 subjects with AERD.

Reactions to aspirin in AERD frequently result in sharp reductions in FEV<sub>1</sub>, along with sinonasal congestion and ocular symptoms. These reductions in part reflect the actions of cysLTs at the type 1 cysLT receptor (CysLT<sub>1</sub>R). To ensure safety of the challenges in this study, all subjects were treated with montelukast, an inhibitor of CysLT<sub>1</sub>R, during the challenges. CysLT<sub>1</sub>R 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.<sup>15</sup> We therefore

used TNSS, rather than a change in FEV<sub>1</sub>, as the basis for assessing reactions, and calculated the PD<sub>2</sub> for aspirin on prasugrel vs. placebo as the primary outcome based on TNSS.

All 40 subjects experienced characteristic reactions on at least one of the two challenges, and most reacted to both challenges, confirming their diagnosis of AERD. Although treatment with prasugrel increased PD<sub>2</sub>, this did not reach significance (**Fig. 3**, P=0.10). Moreover, in the population of patients as whole, prasugrel did not alter the baseline TNSS or ACQ scores, baseline urinary eicosanoids, FeNO, or changes in eicosanoids with aspirin challenges. In general, the maximum fall in FEV<sub>1</sub> during aspirin-induced reactions correlated with the maximum rise in TNSS (**Supplemental Fig. E4**, r=0.50, P=0.001). Of the 74 aspirin-induced reactions in the study, 28 of those involved a fall in FEV<sub>1</sub> of 15% or more (38% of the reactions), which is consistent with previous reports of the rates of lower-respiratory symptoms.<sup>15</sup> As previously reported, CD62P<sup>+</sup>-free platelets (**Fig. 4A**) and platelet-leukocyte aggregates (**Fig. 4B-D**) were readily detectable in the blood of all subjects and did not change with aspirin challenges.<sup>6,12</sup> As expected, the mean urinary levels of LTE<sub>4</sub> rose sharply during reactions for the group as a whole (**Figure 5**), along with the metabolite levels of PGD<sub>2</sub>, 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 on placebo but failed to exhibit any signs or symptoms of aspirin-induced reaction (neither TNSS increase of  $\geq 2$  points nor FEV<sub>1</sub> decrease of  $\geq 15\%$ ) while receiving prasugrel. Although the PD<sub>2</sub> and the change in FEV<sub>1</sub> 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 the non-responders (**Supplemental Table E1**). Several features suggest that responders could represent an endotype of AERD patients with mild upper respiratory reactions. First, the

baseline fraction of blood eosinophils with adherent platelets in the responder subgroup significantly exceeded those in the nonresponders. This did not change as a result of prasugrel treatment (**Figure 7A**). Second, in marked contrast to the nonresponders, responders failed to display significant aspirin-induced increases in urinary  $\text{LTE}_4$ ,  $\text{PGD}_2$ -M, and  $\text{TXB}_2$  levels, or aspirin-induced increases in plasma tryptase levels (all of which reflect mast cell activation) (**Figure 6**)<sup>16,17</sup> during reactions on the placebo arm. Finally, the numbers of blood eosinophils significantly declined during reactions on the placebo arm in the 35 nonresponders (presumably reflecting their recruitment to the respiratory tissue)<sup>18,19</sup> but not in the responders. Both  $\text{LTE}_4$ <sup>20</sup> and  $\text{PGD}_2$ <sup>21</sup> recruit eosinophils to the respiratory tissue. Thus, the lack of change in blood eosinophils during the 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 subjects with AERD whose relatively low levels of mast cell mediator release reveal a  $\text{P2Y}_{12}$ -mediated component of their aspirin-induced reactions. This work, in conjunction with previous reports of a highly  $\text{PGD}_2$ -driven subgroup, confirms that multiple endotypes exist within the AERD phenotype of asthma.<sup>17</sup>

Our previous studies, and those of others, demonstrated that baseline blood platelet activation in AERD exceeds that found in aspirin-tolerant controls by several fold.<sup>6,22</sup> Given that  $\text{P2Y}_{12}$  receptors are required for autocrine activation of platelets in response to several classical agonists, it is surprising that prasugrel failed to alter platelet  $\text{CD62P}$  levels in the entire cohort, and did not change the percentages of platelet-leukocyte aggregates. Thus, the aberrant levels of steady-state platelet activation in AERD may be driven by mechanisms that do not require  $\text{P2Y}_{12}$ -dependent amplification. Since  $\text{P2Y}_{12}$  receptors are also expressed by M2-type macrophages,<sup>23</sup> dendritic cells,<sup>24</sup> and eosinophils,<sup>25</sup> 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,<sup>26</sup> and with indices of platelet and eosinophil activation in subjects with AERD.<sup>27</sup>

Although our identification of a “responder” subgroup of AERD suggests a potential genetic mechanism, we did not identify any *P2RY12* variants that distinguished the two groups.

Because the mechanism by which platelet activation occurs in AERD does not appear to require the P2Y<sub>12</sub> 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<sub>4</sub> in AERD. Determining the mechanism responsible for AERD-associated platelet activation will be necessary to address this role 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 may involve P2Y<sub>12</sub> receptors, potentially due to reduced mast cell-mediator release. Future larger studies will be necessary to validate this observation and reveal mechanism.

#### Registration

Clinicaltrials.gov NCT01597375

#### **Acknowledgements**

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546 **TABLE I.** Patients' baseline demographics and characteristics. Data are mean  $\pm$ SE.

|                                         | <b>AERD<br/>(n = 44)</b> |
|-----------------------------------------|--------------------------|
| Age (y)                                 | 47 $\pm$ 10              |
| Sex (female)                            | 24 (55%)                 |
| Race                                    |                          |
| White                                   | 40 (91%)                 |
| Black                                   | 1 (2%)                   |
| Asian                                   | 0                        |
| Other                                   | 3 (7%)                   |
| Ethnicity                               |                          |
| Hispanic                                | 3 (7%)                   |
| FEV1 (L)                                | 3.15 $\pm$ 0.11          |
| FEV1 predicted (%)                      | 93.7 $\pm$ 2.0           |
| FVC (L)                                 | 4.22 $\pm$ 0.16          |
| FeNO (ppb)                              | 45 $\pm$ 4               |
| Peripheral Eosinophil Count (/ $\mu$ L) | 423 $\pm$ 53             |
| Peripheral Basophil Count (/ $\mu$ L)   | 47 $\pm$ 8               |
| ACQ-7                                   | 0.65 $\pm$ 0.09          |
| TNSS                                    | 4.5 $\pm$ 0.7            |
| Low-dose ICS ( $\leq$ 200 mcg*)         | 15 (34%)                 |
| Medium-dose ICS (201-500 mcg *)         | 18 (41%)                 |
| High-dose ICS ( $>$ 500 mcg*)           | 4 (9%)                   |
| Oral Glucocorticoid Use                 | 2 (5%)                   |
| Long-acting Beta Agonist Use            | 28 (64%)                 |
| Long-acting Muscarinic Antagonist Use   | 0                        |

\* = Fluticasone Propionate dry powder equivalent

547

548

## FIGURE LEGENDS

**FIG 1. Detailed Schema of the trial.** **A**, Study schema showing timeline and schedule of assessments. **B**, Table of time-points when assessments were collected on the day of the aspirin challenge.

**FIG 2.** Patient flow through the study.

**FIG 3. Effect of prasugrel on PD<sub>2</sub> and TNSS.** **(A)** The primary outcome, change in the provocative dose of aspirin that would elicit an increase on the total nasal symptom score of 2 points (PD<sub>2</sub>) is shown, with horizontal bars representing the mean PD<sub>2</sub> for each treatment arm. **(B)** The change in TNSS from the pre-aspirin baseline to the maximum TNSS recorded during each aspirin challenge is shown. 34 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).

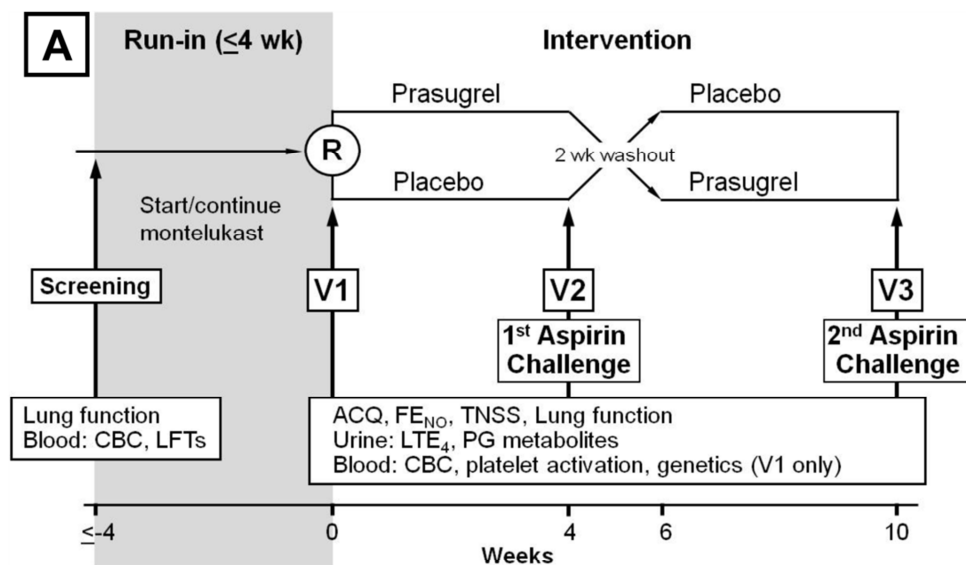
**FIG 4. Platelet-leukocyte aggregation, platelet activation, and plasma tryptase.**

Percentages of leukocytes with adherent platelets (as determined by staining with CD61) before aspirin administration (solid columns) and 1 hour after the onset of reaction (striped columns) are shown for each treatment arm for eosinophils **(A)**, neutrophils **(B)**, monocytes **(C)**, and lymphocytes **(D)**. Percentages of platelets that expressed surface CD62P **(E)** and total plasma tryptase levels **(F)** are shown at the same timepoints. Data are expressed as mean +SE.

**FIG 5. Urinary eicosanoid levels.** Baseline pre-aspirin and aspirin-induced reaction levels of urinary eicosanoid levels analyzed by gas chromatography-mass spectrometry are shown for both treatment arms. **(A)** LTE<sub>4</sub>, **(B)** PGD<sub>2</sub>-M, **(C)** PGE-M, and **(D)** TXB<sub>2</sub>. Data are expressed as mean +SE.

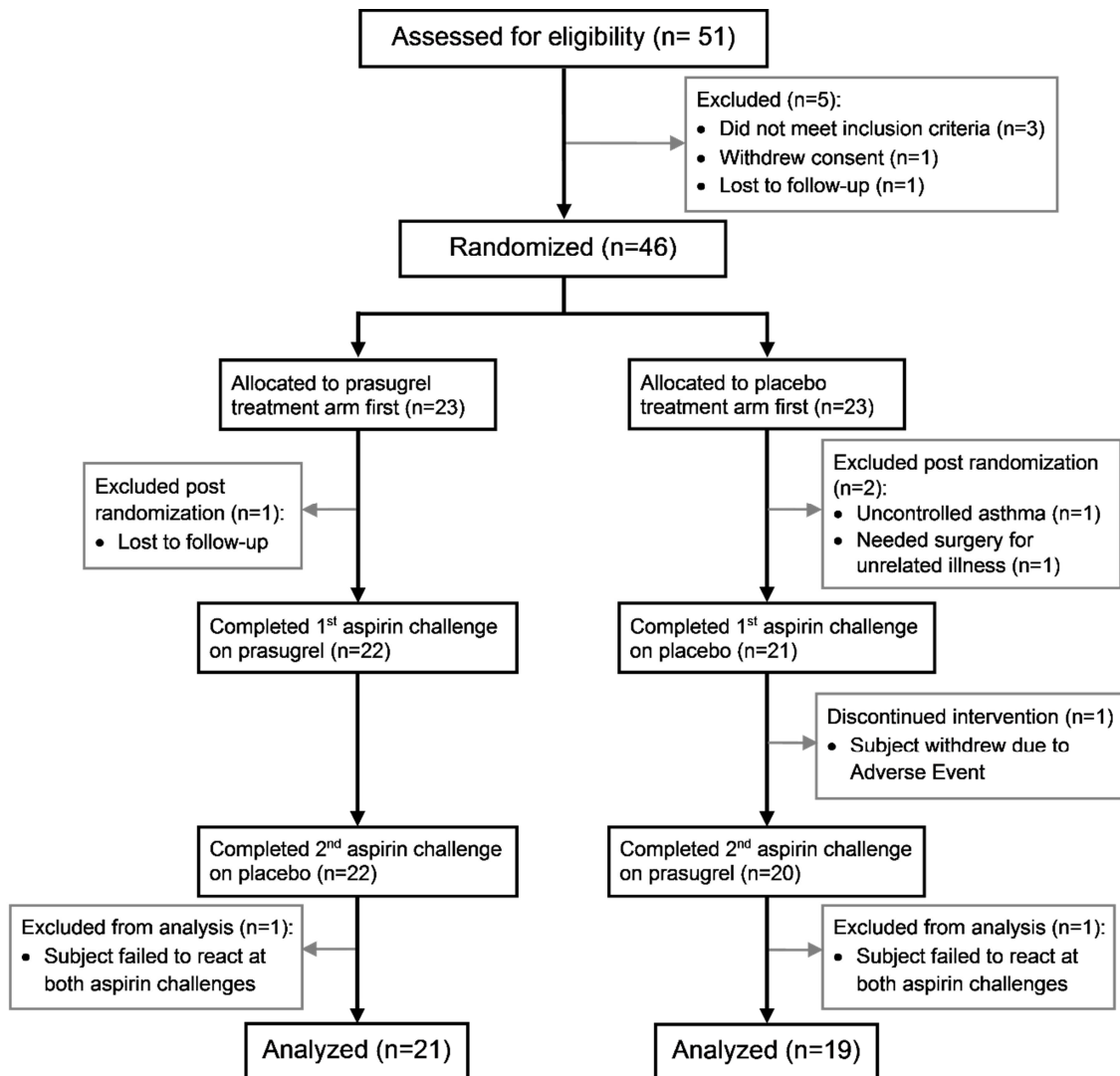
**FIG 6. Responder vs non-responder differences in aspirin-induced urinary eicosanoid and plasma tryptase levels.** Placebo arm urinary levels of  $\text{LTE}_4$  **(A)**,  $\text{PGD-M}$  **(B)**, and  $\text{TXB}_2$  **(C)** before aspirin administration (white columns) and at the aspirin reaction-induced peak (striped columns) are shown for the responders (blue lines) and the nonresponders. Placebo arm plasma tryptase levels **(D)** before aspirin administration and 1 hour after the onset of reaction are shown for the same patient subgroups. Data are expressed as mean +SE. P-values with a \* are significant using a False Discovery Rate of 0.05 to correct for multiple comparisons.

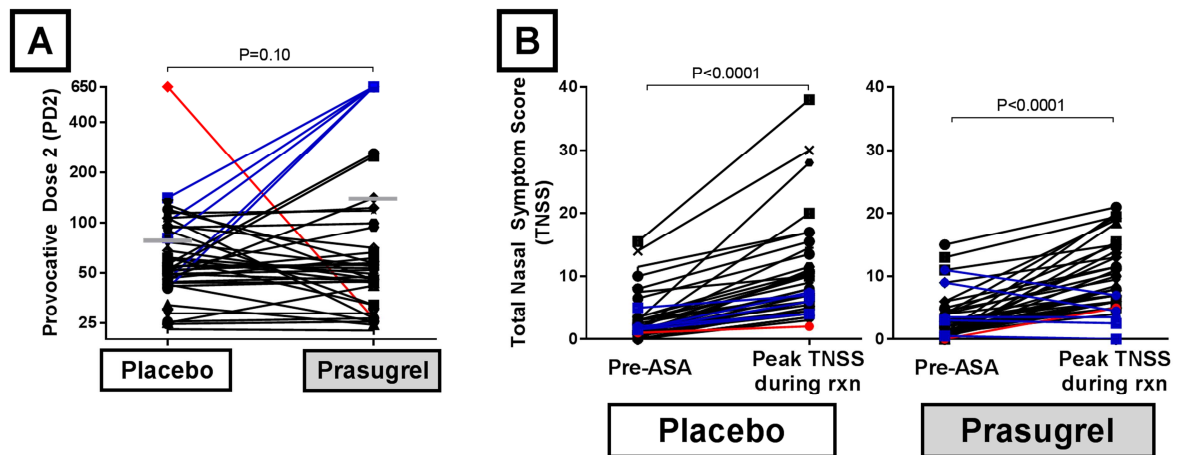
**FIG 7. Responder vs non-responder platelet activation and platelet-leukocyte aggregates.** **(A)** Percentages of eosinophils with adherent platelets before aspirin administration are shown for each treatment arm for the responders (n=5, blue lines) and the non-responders. **(B)** Percentages of platelets that expressed surface  $\text{CD62P}$  are shown for the two patient subgroups, at the same timepoints. Data are expressed as mean + SE. P-values with a \* are significant using a False Discovery Rate of 0.05 to correct for multiple comparisons.

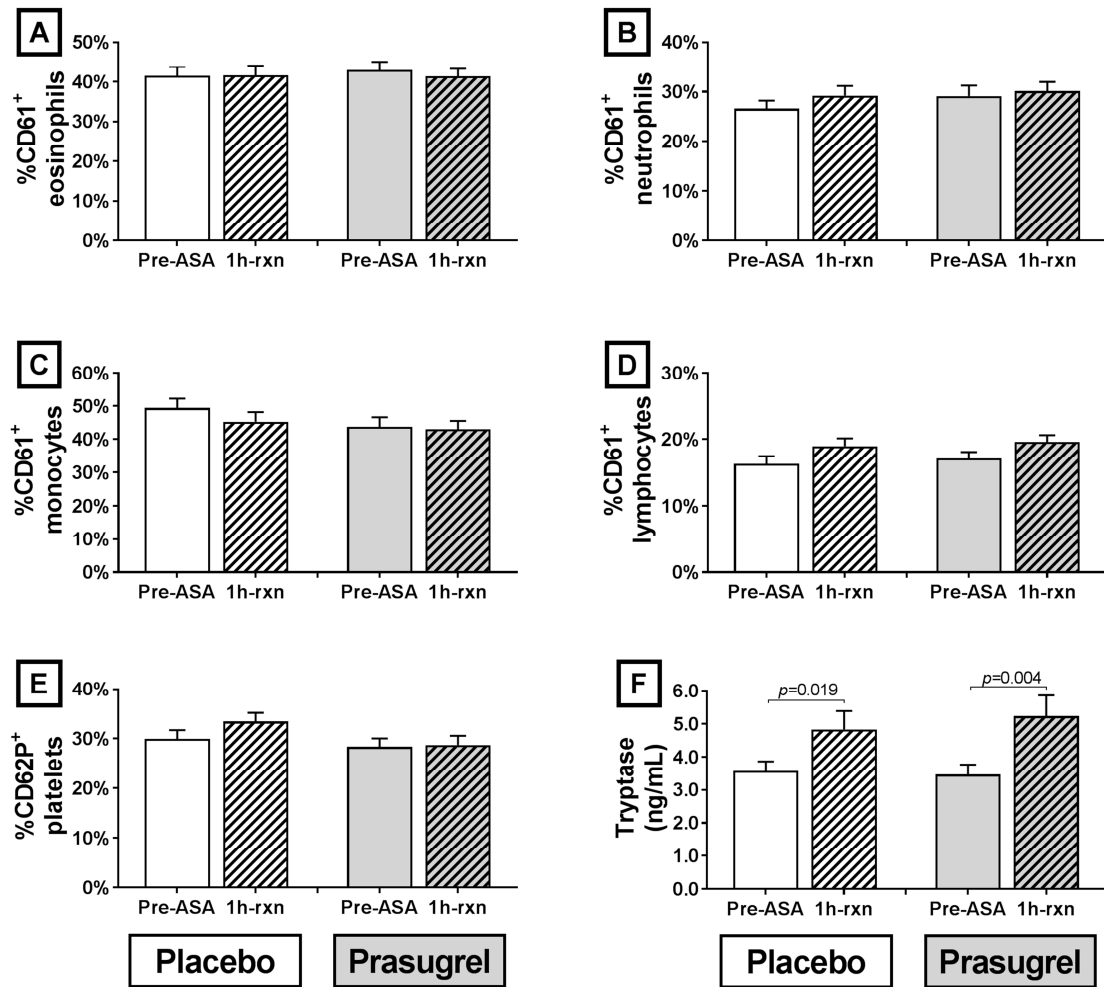


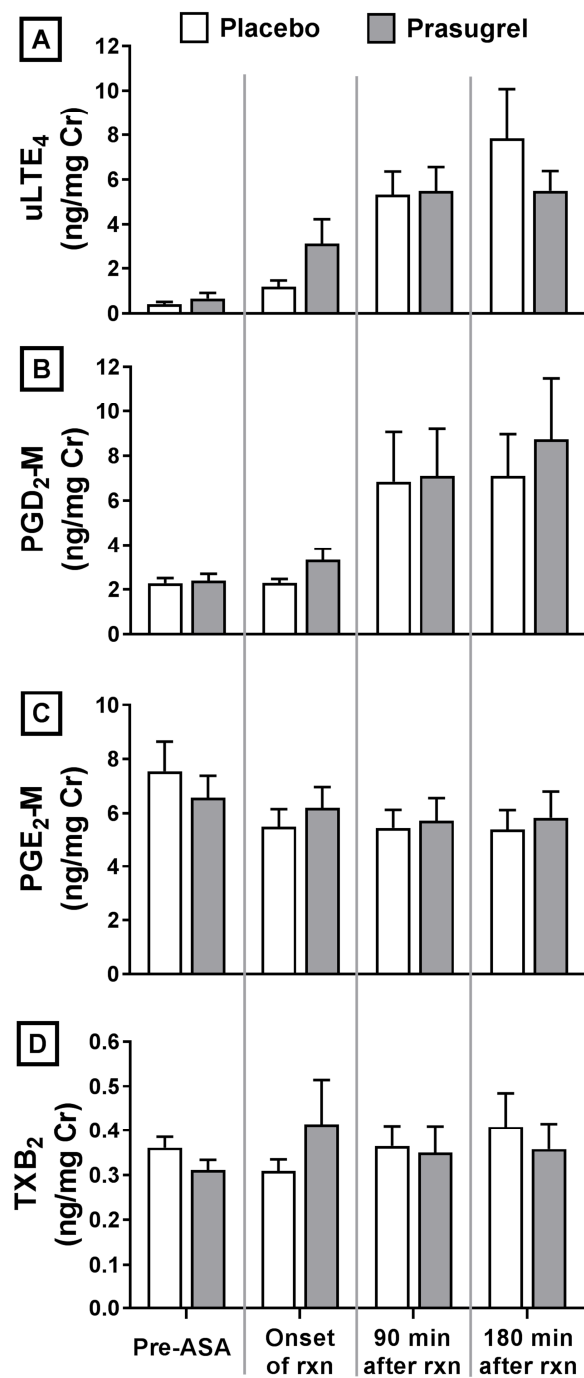
**B**

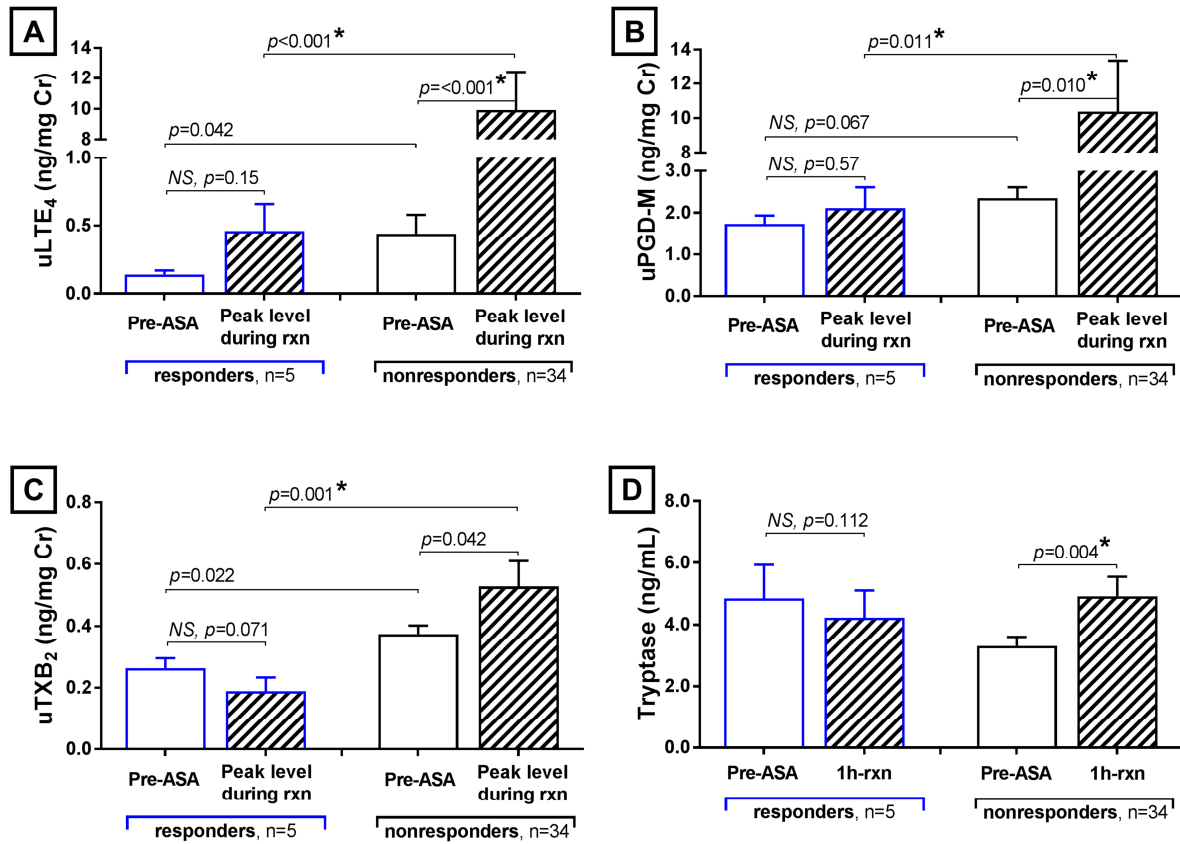
|                  | Baseline | Minutes after reaction onset: |    |    |    |     |     |     |
|------------------|----------|-------------------------------|----|----|----|-----|-----|-----|
|                  |          | 0                             | 30 | 60 | 90 | 120 | 150 | 180 |
| Urine  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

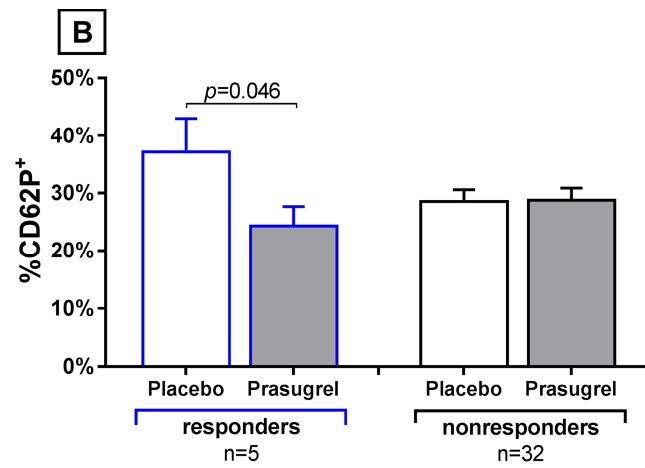
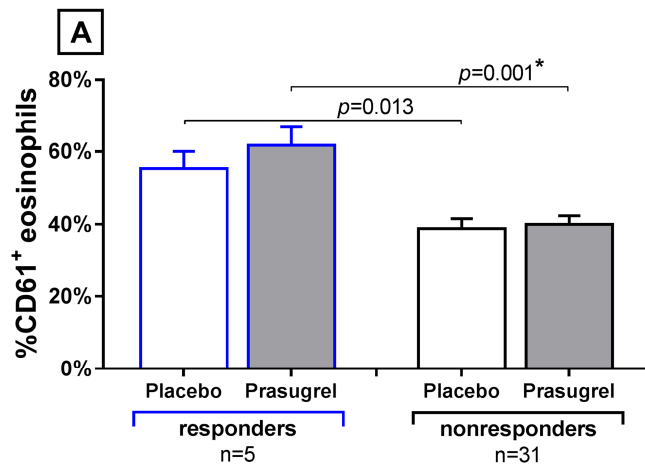












**Aspirin****Total Nasal Symptoms  
Score (TNSS) Worksheet**

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)  | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 2. Nasal Congestion (Right nostril) | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 3. Runny Nose                       | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 4. Itchy Nose                       | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 5. Sneezing                         | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 6. Itchy Eyes                       | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 7. Teary Eyes                       | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 8. Itchy Ears or Throat             | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 9. Eye Redness                      | <input type="checkbox"/> 0 | <input type="checkbox"/> 1 | <input type="checkbox"/> 2 | <input type="checkbox"/> 3 | <input type="checkbox"/> 4 | <input type="checkbox"/> 5 |
| 10. Total Score _____               |                            |                            |                            |                            |                            |                            |

Participant Initials: \_\_\_\_\_

Date: \_\_\_\_/\_\_\_\_/20\_\_\_\_

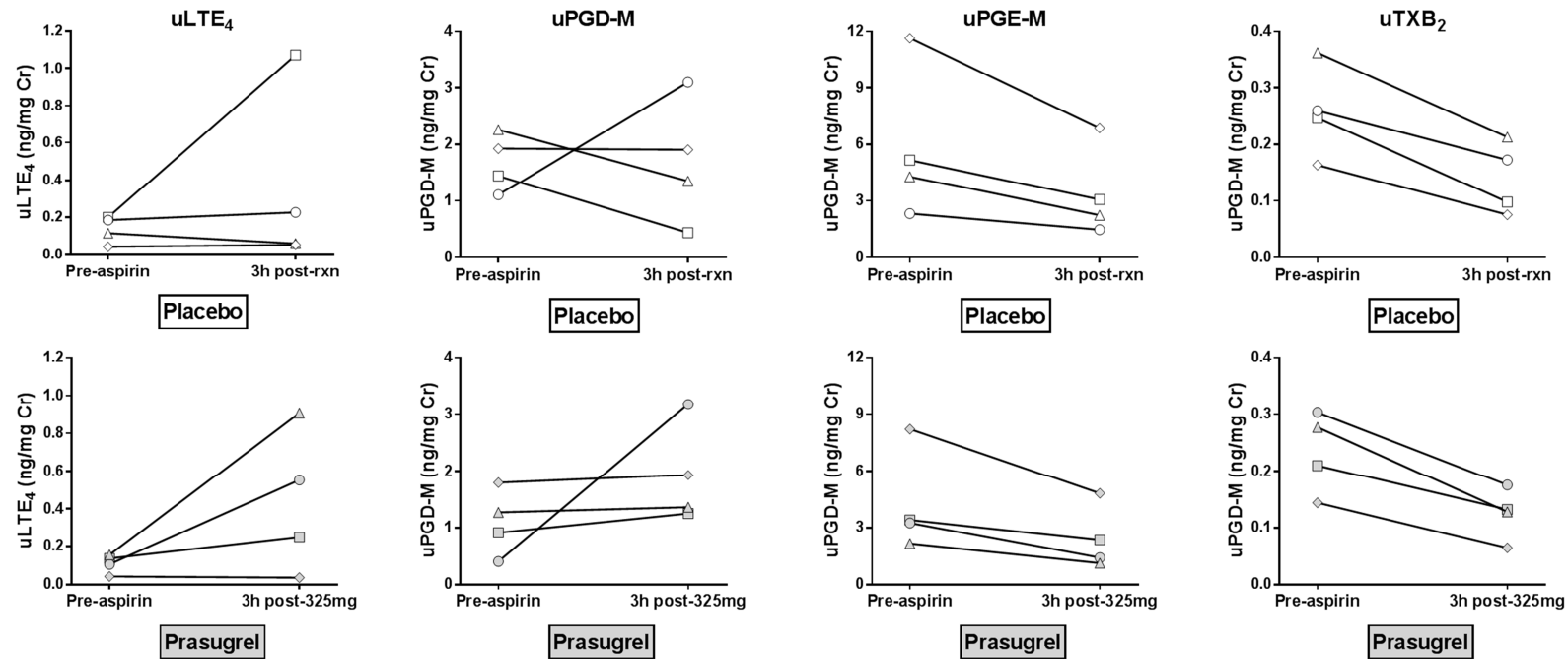
Time \_\_\_\_:\_\_\_\_

**Supplemental FIG. E1.** Total Nasal Symptom Score (TNSS) questionnaire. The scores for items 1 and 2 are averaged, so that the final range is between 0-40 points.

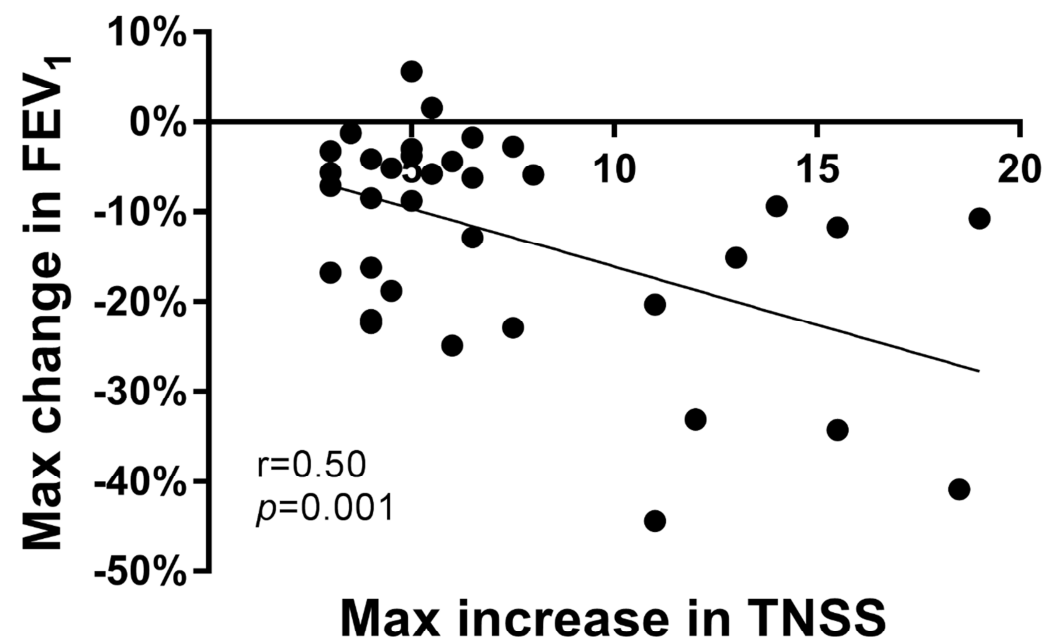
$$\text{inverselog}_{10} \left( \frac{(2 - (PrevTNSS - BaselineTNSS)) \times (\log_{10} ProvocDose - \log_{10} PrevDose)}{(MaxTNSS - BaselineTNSS) - (PrevTNSS - BaselineTNSS)} + (\log_{10} PrevDose) \right)$$

- *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  $\frac{1}{2}$  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.

**Supplemental FIG. E2.** PD<sub>2</sub> Calculation (provocative dose of aspirin that would cause an increase in TNSS of 2 points during an aspirin challenge).



**Supplemental 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 325mg dose of aspirin on the prasugrel arm (bottom row).



**Supplemental FIG. E4.** Correlation between maximum change in FEV<sub>1</sub> and maximum increase in total nasal symptom score (TNSS) during aspirin-induced reactions on the prasugrel treatment arm is shown.

**Supplemental Table E2.** Results of logistical regression for association between genotype and response to prasugrel for selected SNPs. Genomic position is given relative to the reference human genome (GRCh37/hg19).

| SNP        | Position  | Location | Alleles | MAF   | Estimate | Std. Error | 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    |

MAF - minor allele frequency

Std. Error - standard error

**Supplemental Table E3.** Adverse events.

|                                        | Placebo arm | Prasugrel arm | P-value |
|----------------------------------------|-------------|---------------|---------|
| Total adverse events reported          | 25          | 36            |         |
| Bruising                               | 3           | 12            | 0.006   |
| Upper respiratory infection/sinusitis  | 5           | 7             | 0.75    |
| Worsening of asthma                    | 5           | 4             | 1       |
| Muscle injury/myalgia                  | 2           | 0             | 0.49    |
| Headache                               | 2           | 2             | 0.49    |
| 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 two weeks after the second aspirin challenge were included. Data listed is number of participants reporting each type of adverse event.

**Supplemental Table E1.** Demographic/clinical and laboratory characteristics of the 5 responders and the 35 non-responders. Clinical and laboratory values are at the pre-aspirin baseline following each of the treatment arms. Data are mean  $\pm$  SE.

|                              | Prasugrel “responders”<br>(n = 5) |                 | Prasugrel<br>“nonresponders”<br>(n = 35) |                 | p-value (responder vs<br>nonresponder) |           |
|------------------------------|-----------------------------------|-----------------|------------------------------------------|-----------------|----------------------------------------|-----------|
|                              | Placebo                           | Prasugrel       | Placebo                                  | Prasugrel       | Placebo                                | Prasugrel |
| Age (y)                      | 44 $\pm$ 4                        |                 | 47 $\pm$ 2                               |                 | 0.946                                  |           |
| Sex (female)                 | 3 of 5                            |                 | 19 of 35                                 |                 | 0.834                                  |           |
| FEV1 (L)                     | 2.74 $\pm$ 0.26                   | 2.74 $\pm$ 0.30 | 3.19 $\pm$ 0.14                          | 3.16 $\pm$ 0.15 | 0.178                                  | 0.258     |
| FEV1 predicted (%)           | 84.5 $\pm$ 5.4                    | 84.0 $\pm$ 6.6  | 93.1 $\pm$ 2.3                           | 92.2 $\pm$ 2.2  | 0.206                                  | 0.294     |
| FeNO (ppb)                   | 39.6 $\pm$ 8.9                    | 39.2 $\pm$ 9.1  | 54.1 $\pm$ 8.4                           | 45.9 $\pm$ 4.7  | 0.258                                  | 0.533     |
| Blood AEC ( $\mu$ L)         | 640 $\pm$ 166                     | 500 $\pm$ 148   | 500 $\pm$ 62                             | 435 $\pm$ 50    | 0.464                                  | 0.696     |
| Blood eosinophils (%)        | 8.4 $\pm$ 1.7                     | 7.2 $\pm$ 1.5   | 7.1 $\pm$ 0.7                            | 6.4 $\pm$ 0.7   | 0.499                                  | 0.619     |
| ACQ-7                        | 0.93 $\pm$ 0.24                   | 0.74 $\pm$ 0.11 | 0.77 $\pm$ 0.38                          | 0.70 $\pm$ 0.11 | 0.495                                  | 0.865     |
| TNSS, baseline               | 2.3 $\pm$ 0.7                     | 5.4 $\pm$ 2.0   | 3.2 $\pm$ 0.7                            | 3.2 $\pm$ 0.6   | 0.342                                  | 0.343     |
| TNSS, max during rxn         | 5.7 $\pm$ 0.7                     | 3.5 $\pm$ 1.2   | 10.8 $\pm$ 1.4                           | 10.6 $\pm$ 0.9  | 0.002                                  | 0.0007    |
| PD <sub>2</sub>              | 82 $\pm$ 18                       | 650             | 78 $\pm$ 18                              | 66 $\pm$ 10     | 0.881                                  |           |
| uLTE <sub>4</sub> (ng/mg Cr) | 0.14 $\pm$ 0.03                   | 0.10 $\pm$ 0.14 | 0.44 $\pm$ 0.13                          | 0.75 $\pm$ 0.28 | 0.041                                  | 0.026     |
| uPGD-M (ng/mg Cr)            | 1.73 $\pm$ 0.20                   | 1.48 $\pm$ 0.45 | 2.37 $\pm$ 0.26                          | 2.54 $\pm$ 0.33 | 0.067                                  | 0.234     |
| uPGE-M (ng/mg Cr)            | 5.36 $\pm$ 1.64                   | 4.31 $\pm$ 1.05 | 7.97 $\pm$ 1.25                          | 6.94 $\pm$ 0.87 | 0.443                                  | 0.264     |
| uTXB <sub>2</sub> (ng/mg Cr) | 0.26 $\pm$ 0.03                   | 0.33 $\pm$ 0.10 | 0.38 $\pm$ 0.03                          | 0.31 $\pm$ 0.02 | 0.022                                  | 0.811     |