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Aspirin Resistance in Healthy Drug-Naïve Men versus Women (From the Heredity and Phenotype Intervention [HAPI] Heart Study)

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Abstract

This study was designed to determine the factors that contribute to inter-individual variation in the antiplatelet effects of aspirin. We measured platelet response to aspirin in 745 (400 men and 345 women) drug-naïve asymptomatic subjects of the Heredity and Phenotype Intervention (HAPI) Heart Study. Whole blood platelet aggregometry [WBPA] was performed to assess response to arachidonic acid, adenosine diphosphate (ADP) and collagen at baseline and after 14 days of 81 mg/day of aspirin. There was wide inter-individual variation in platelet aggregation in response to aspirin with no clear biological threshold to define aspirin resistance. Variation in platelet function before and after aspirin was heritable. Women exhibited greater platelet aggregability in response to ADP and collagen, at baseline and after aspirin administration. The degree to which aspirin inhibited collagen-induced platelet aggregation was also significantly less in women compared to men [mean % $\pm$ SD inhibition of collagen-induced (1 μ g/ml) platelet aggregation: 49.9 $\pm$ 30.9 vs. 57.5 $\pm$ 42.5 in women and men, respectively; $p = 0.005$). Using a cutoff of < 70% inhibition of collagen-induced platelet aggregation, 21% of the total population demonstrated aspirin resistance which occurred in 30% of women and 16% of men ($p = 0.0002$). Aspirin resistant subjects were older, had significantly higher total cholesterol and LDL-C levels, lower hematocrit, and higher platelet count compared to aspirin sensitive subjects. In conclusion, in this study group, platelet function is heritable. There is wide inter-individual variation in platelet response to aspirin as defined by WBPA with women having lower mean percent inhibition of platelet aggregation and greater prevalence of aspirin resistance than men.

Introduction

The mechanisms underlying aspirin resistance or failure of aspirin therapy are largely unknown. Several studies have reported less effective inhibition of platelet function in

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response to aspirin in women than in men¹⁻³. In a recent study, women were found to have greater baseline platelet reactivity than men and also women retained modestly more platelet reactivity than did men after aspirin therapy⁴. Response to aspirin therapy may additionally be mediated by genetic variation, although specific polymorphisms definitively linked to aspirin responsiveness have yet to be identified. A better understanding of factors influencing aspirin responsiveness may help identify patients who may not benefit from aspirin therapy and in whom substitution of an alternative therapy might prove more beneficial in decreasing cardiovascular disease (CVD) morbidity and mortality. In the current study, we have characterized the platelet response to low dose aspirin in a large number of drug-naïve individuals asymptomatic for CVD from a population homogeneous with respect to genetics and lifestyle. We measured platelet aggregation in whole blood in response to several agonists both before and after administration with aspirin. We additionally measured urinary 11-dehydrothromboxane as a marker of aspirin-induced inhibition of thromboxane generation. Our specific goals were to characterize the distribution of aspirin responsiveness across this population, estimate the heritability of platelet reactivity and aspirin responsiveness, and to assess gender differences in aspirin responsiveness and its clinical correlates.

Methods

The Heredity and Phenotype Intervention (HAPI) Heart Study is part of the NHLBI PROGENI Network and was designed to identify genes that interact with environmental exposures to modify risk factors for CVD⁵. From 2003 to 2006, Old Order Amish subjects from Lancaster, PA, aged 20 years and considered to be relatively healthy were recruited. Exclusion criteria included severe hypertension (blood pressure > 180/105 mm Hg), malignancy, kidney, liver or untreated thyroid disease, and inability to safely discontinue all vitamins, nutritional supplements, and prescription and non-prescription medications. For the aspirin response substudy, subjects with platelet count <100,000/ μ l or >500,000/ μ l and a white blood cell count > 20,000/ μ l were excluded. Of the 868 total participants in the HAPI Heart Study, 756 completed the aspirin intervention of whom 745 had evaluable whole blood platelet aggregometry [WBPA] measurements before and after aspirin. By virtue of the fact that the Amish are a closed founder population⁶, virtually all of these individuals can be connected into a single 14-generation pedigree. The 745 examined individuals included a large number of relative pairs suitable for estimating heritability; among these were 241 parent-offspring pairs, 493 sibling pairs, 11 grandparent-grandchild pairs, 368 avuncular pairs, and 159 first cousin pairs.

All participants underwent a medical history interview including assessment of CVD risk factors, vitamin, nutritional supplements, and prescription and nonprescription medication usage, and questions about prior history of CVD. Subjects taking aspirin (2.3%) were withdrawn from aspirin 14 days prior to initiation of the study. All other prescription or non-prescription medications and herbal or nutritional supplements or vitamins were discontinued 7 days prior to examination for the duration of the study. Physical examinations were conducted at the Amish Research Clinic in Strasburg, PA, and blood samples were obtained following an overnight fast. Height and weight were measured using a stadiometer and calibrated scale with shoes removed and in light clothing, and body mass index (BMI) (kg/m^2) was calculated. Systolic blood pressure (BP) (1st phase) and diastolic BP (5th phase) were obtained in triplicate using a standard sphygmomanometer with the subject sitting for at least 5 minutes. Hypertension was defined as systolic BP $\geq$ 140 mm Hg, and/or diastolic BP $\geq$ 90 mm Hg, and/or reported current use of BP lowering medications. Diabetes was defined as a fasting glucose $\geq$ 126 mg/dl or reported current use of prescription diabetes medications. Current smokers included use of a pipe, cigar or cigarettes. Fasting

serum lipid concentrations were assayed by Quest Diagnostics (Horsham, PA). All subjects had triglyceride < 400 mg/dl and low density lipoprotein cholesterol levels were calculated.

At the initial clinic visit clinic visit 1, baseline WBPA studies were performed in the fasting state and a first morning urine sample was collected. The aspirin intervention began the day after clinic visit 1; the subject took 81 mg aspirin every day for 14 consecutive days. One to three days prior to the second clinic visit a nurse and liaison performed a home visit, to ensure compliance. On the 14th day the subject took his/her aspirin shortly before arriving at the Amish Research Clinic for the second clinic visit 2 and collected his/her first morning urine sample. At clinic visit 2, fasting blood was drawn and WBPA studies were repeated in identical fashion to clinic visit 1. Compliance was assessed by a pill count and review of the subject's study diary. Subjects were permitted to miss up to four aspirin doses over the two week period and still be included in the final analysis, provided that he/she took the aspirin for at least three consecutive days prior to clinic visit 2. The aspirin intervention could be extended for up to three days (17 days total) to meet this criterion.

Venous blood (9 ml) for platelet aggregation studies was collected from the antecubital vein by gentle aspiration using a 21 gauge butterfly cannula and a 10 ml sterile syringe charged with 1 ml of 0.105 M sodium citrate anti-coagulant. In addition, one ethylenediaminetetraacetic acid (EDTA) Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ) was drawn and sent to Quest Diagnostics (Horsham, PA) where a complete blood cell count with differential was performed. Whole blood platelet impedance aggregometry was performed by the same technician at baseline and after aspirin therapy using a Chrono-Log four channel aggregometer (Havertown, PA) within three hours after the blood was drawn. Instrument incubation wells were set to 37°C, and stirring speed set at 1000 rpm. Pre-warmed cuvettes were filled with 0.5 ml Hank's Balanced Salt Solution (Sigma-Aldrich, St. Louis MO), 0.5 ml citrate anti-coagulated whole blood and a stir bar. After a five minute incubation period, a pre-warmed probe was inserted into each cuvette and the cuvettes were moved to the reaction wells. The aggregation baseline was set to zero and the impedance circuit was calibrated to 50%. Aggregation was initiated with the addition of one of three agonists, collagen, adenosine diphosphate (ADP) or arachidonic acid, purchased from Chronolog, Horsham, PA. Each channel of the 4 channel aggregometer was dedicated to a particular agonist. A dose response was performed with collagen 0.5, 1, 2, and 5 µg/ml final concentrations in channels 1, 2, 3, and 4, respectively. After completion of collagen reactions, new reactions were performed containing ADP at a final concentration of 10 µM in channels 1 and 2, and arachidonic acid at a final concentration of 0.5 mM in channels 3 and 4. Reactions were allowed to run for 10 minutes, but all calculations were based on a 5 minute test time.

First morning void urine samples, stored at -80° C were thawed and assayed for urine 11-dehydro thromboxane B2 by an enzyme-linked immunoassay kit (Cayman Chemical Co., Ann Arbor, Michigan) according to the manufacturer's recommendation. Thromboxane levels were normalized to urinary creatinine.

Descriptive characteristics of the study participants were compared between men and women adjusting for age. Variables having non-normal distributions were logarithm (triglycerides) or inverse normal (urine 11-dehydro thromboxane B2) transformed prior to analysis. Categorical variables were compared between groups using the Chi-square statistic. Pearsonian correlations were estimated among the blood platelet aggregation measures using different agonists, and the partial correlations presented following adjustment for age and sex. We defined aspirin resistance as <70% inhibition of platelet aggregation to 1 µg/ml collagen, similar to the threshold reported by others⁷. P-values (double sided) 0.05 is considered significant.

Comparisons between men and women in mean levels of baseline and post-aspirin platelet aggregation and urine 11-dehydro thromboxane B2 levels were adjusted for age, BMI, blood pressure, total cholesterol, triglyceride, hematocrit, WBC, platelet count, and smoking. The post-aspirin administration values were further adjusted for baseline value in addition to the above covariates. To account for the correlations in trait values owing to study subjects being related, we accounted for the nonindependence of study subjects using the variance components approach as implemented in the SOLAR software package (http://www.sfbr.org/Departments/genetics_detail.aspx?p=37)⁸. This approach allowed us to estimate the effect of variable of interest (e.g., sex) on the quantitative trait while simultaneously adjusting for the effects of covariates as well as a polygenic component, computed as a function of the kinship matrix.

Heritability analyses were performed using the variance components methodology as implemented in the SOLAR software program. Variation in baseline and post-aspirin platelet aggregation and urine 11-dehydro thromboxane B2 levels was modeled as a function of measured environmental covariates, additive genetic effects, and a residual error component. The heritability of those phenotypes was estimated as the proportion of the total phenotypic variation that could be attributable to additive genetic effects. Additive genetic effects were parameterized as a function of the kinship matrix.

Results

Characteristics of the study population by sex are shown in Table 1. The distributions of the platelet aggregatory response to collagen in different concentrations are shown in Figure 1. With the exception of collagen 0.5 µg/ml, all the distributions before and after aspirin administration were approximately normally distributed. The correlations of platelet aggregatory response among different concentrations of collagen (both before and after aspirin administration) were relatively high (Table 2). The correlation was less when comparing the response among different agonists, e.g., collagen vs. ADP or arachidonic acid.

At baseline, there was a significant heritable component to variation in each platelet aggregation phenotype to different agonists and in urinary thromboxane B2 excretion, with the estimated heritabilities ranging from 0.18 - 0.42 (Table 3). Age and sex explained only small proportions of the phenotypic variation and had little impact on the heritability estimate. After aspirin administration, the heritability decreased for all phenotypes ($h^2 = 0.10 - 0.22$), except for aggregation to ADP whose heritability remained 0.42. Baseline aggregation levels were significantly associated with aggregation levels post aspirin administration. After controlling for baseline aggregation levels, the heritability of aggregation to ADP decreased from 0.42 to 0.24, while heritability of aggregation to collagen at 1 µg/ml remained at 0.22 and heritability of aggregation to collagen at other concentrations decreased to the point where they were no longer significantly greater than zero.

At baseline (pre-aspirin), women exhibited consistently greater platelet aggregatory response to different agonists (Table 4). These included all four concentrations of collagen as well as ADP. Consistent with these observations, women had greater urinary thromboxane B2 excretion than men. After multivariate adjustment for age, BMI, systolic BP, total cholesterol, triglyceride, hematocrit, platelet count, WBC, and smoking status, women still exhibited significantly greater platelet aggregatory response to collagen at 1 µg/ml and greater urinary thromboxane B2 excretion than men. After two weeks of daily low dose aspirin ingestion, arachidonic acid-induced platelet aggregation was markedly depressed in both women and men, with complete inhibition reached in the majority of

subjects. Furthermore, there was no difference in urinary 11-dehydro thromboxane B2 levels after aspirin administration between men and women. However, despite this similar degree of cyclo-oxygenase-1 (COX-1)-dependent pathway inhibition, women continued to show increased platelet aggregatory response to collagen and ADP. The degree of inhibition was significantly less in women as compared to men for collagen at both the 1 $\mu\text{g/ml}$ and 2 $\mu\text{g/ml}$ doses and for the ADP 10 μM dose, even after adjusting for age, BMI, systolic BP, total cholesterol, hematocrit, platelet count, WBC count, baseline aggregation value, and family structure. Mean percent inhibition of collagen-induced (1 $\mu\text{g/ml}$) platelet aggregation in response to aspirin in women was significantly lower than in men (mean $\pm$ SD: 49.9 ± 30.9 vs. 57.5 ± 42.5 ; $p = 0.005$).

We also used a cutoff of $\geq 70\%$ inhibition of platelet aggregation with collagen 1 $\mu\text{g/ml}$ as agonist to define aspirin resistance. Table 5 shows clinical characteristics of the 156 aspirin resistant and 588 aspirin sensitive subjects. By these criteria, 21% (156/744) of study subjects were aspirin resistant, including 30% of women and 16% of men ($p = 0.0002$ for sex difference). Using other threshold values to define aspirin resistance similarly resulted in a female excess (data not shown). Aspirin resistant subjects were older and had significantly higher total cholesterol levels, higher platelet number, and higher urinary 11-dehydro thromboxane B2 level after aspirin administration than aspirin sensitive subjects.

Discussion

Large scale epidemiologic studies of prevention of thromboembolic manifestations of CVD have generally shown benefit from chronic aspirin use. However, all subgroups appear not to enjoy the same benefits of aspirin therapy. The main finding in this study of a large number of asymptomatic drug-naïve subjects is that women have greater platelet aggregation at baseline and are less responsive to the platelet inhibitory activity of aspirin. Other factors that were associated with decreased aspirin response in our study include age, diastolic BP, cholesterol (total), and increased platelet number. Our results are consistent with large scale trials of aspirin for primary prevention in which benefit occurs predominantly in men⁹.

Developing reliable bioassays of aspirin's effectiveness has the potential to identify a subpopulation at risk for aspirin failure or a subpopulation in whom the use of aspirin is more likely to harm than to benefit. However, this has been difficult since there is a multiplicity of non-standardized methodologies to measure platelet function and there is no consensus with regard to how to define aspirin resistance. We chose to use whole blood platelet aggregometry and urinary thromboxane, methods that have been used previously to identify individuals less responsive to aspirin^{2, 10, 11}. We found that these platelet aggregation traits measured before and after aspirin therapy, as well as calculated changes in response to aspirin were approximately normally distributed in our sample of relatively healthy drug-naïve subjects, suggesting that aspirin response is a complex trait in which there is no clear cut-off to define aspirin resistance. Like others¹², we found a moderate correlation in platelet aggregatory response to collagen, ADP and arachidonic acid, and to a lesser extent, urinary thromboxane B2 levels (data not shown). These relationships held both before and after aspirin therapy. This moderate degree of correlation among these platelet function traits are likely due to the fact that stimulators of platelet aggregation act through different, albeit converging, pathways and thus query common, as well as different, aspects of the platelet aggregation cascade. Measurement error is also a factor since technical and other variables can affect the strength of correlations.

Our study also confirmed the contribution of heritable factors to the variability in platelet function both before and after aspirin therapy and the heritability estimates we observed

were consistent with those previously reported from Caucasian subjects from the GeneSTAR Study¹³. The baseline platelet function contributed substantially to the heritability of the platelet function after aspirin therapy. Further studies are needed to identify the genetic determinants of those platelet functions.

The best understood effect of aspirin is its direct inhibition of COX-1. Urinary thromboxane levels and whole blood platelet aggregation in response to arachidonic acid may reflect variation that is directly attributable to inhibition of COX. In contrast, aspirin-related effects on whole blood aggregation in response to collagen and ADP, while also important mediators of arterial thrombosis, may involve biochemical pathways that are not directly related to COX inhibition. It is likely that aspirin does not have a single mechanism of action with regard to its anti-platelet effect and, more broadly, with regard to its clinical effect. Additionally, one mechanism of action may be more important in some subgroups than others or may be more important in some disease states than others. For example, one mechanism may be more important in women than in men and this could explain why aspirin used for primary prevention in women may be more effective in preventing stroke than in preventing myocardial infarction. In our study, the sex difference in platelet function in response to aspirin was most pronounced with respect to collagen-induced aggregation, an indirect COX pathway, and less so (if at all) with respect to arachidonic acid-induced platelet aggregation, a direct COX pathway. These findings suggest that the mechanism of aspirin resistance in women may be independent of its direct effect on COX inhibition. An example of a gender specific mechanistic effect was found in recent work by Chiang, et al¹⁴. This study demonstrated that low dose aspirin has a sex dependent impact on anti-inflammatory 15-epi-lipoxin A4 production, which may contribute in part to the sex dependent clinical benefits of aspirin.

Prior studies of sex differences in platelet aggregation related phenotypes have yielded mixed results. One study, using whole blood aggregometry, reported a relative higher prevalence of aspirin non-responders in women as compared to men²; another study, using optical platelet aggregometry, similarly reported a higher incidence of aspirin resistance among women¹. By contrast, in a large study by Becker et al⁴, women experienced increased baseline (pre-aspirin) platelet reactivity with similar or greater absolute changes in response to aspirin compared to men. Our study demonstrates both increased baseline platelet activity and decreased response to aspirin in women. However, both our study and the one reported by Becker et al showed that women retained more platelet reactivity compared with men. Possibly, these contrasting results are due to different demographics among the studied populations. For example, the Becker study comprised subjects at higher risk of CVD who were first-degree relatives of CVD patients, slightly older than our subjects, and with higher BMI. All of these factors increase the likelihood for the presence of atherosclerotic vascular disease, and the presence of atherosclerotic vascular disease may heighten platelet reactivity.

Our study has several strengths, including the large number of subjects, all recruited from a homogeneous population. We minimized confounding influences by performing these studies in asymptomatic drug-naïve subjects using a well-controlled prospective study design. However, there are also a number of limitations. Subjects were relatively healthy and the intervention was performed for only two weeks. Furthermore, we studied only Caucasian subjects. Longer term studies in more mixed populations examining CVD events and mortality will be required to understand further the clinical relevance of aspirin resistance relevant to disease.

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Figure 1.
Distribution of whole blood platelet aggregation to collagen before and after aspirin administration.

Table 1
Clinical characteristics of the study population

Variable	Men (n=400)	Women (n=345)	P value
Age (years)	41.4 (13.0)	45.3 (14.0)	<0.0001
Body mass index (kg/m ²)	25.5 (3.2)	27.7 (5.1)	<0.0001
Systolic blood pressure (mmHg)	120.5 (11.6)	119.9 (15.7)	0.045
Diastolic blood pressure (mmHg)	77.3 (8.7)	75.2 (8.0)	<0.0001
Total cholesterol (mg/dL)	204.4 (44.4)	214.6 (46.2)	0.063
High density lipoprotein cholesterol (mg/dL)	53.1 (12.9)	59.5 (15.7)	<0.0001
Low density lipoprotein cholesterol (mg/dL)	138.7 (40.9)	140.3 (42.8)	0.534
Triglyceride (mg/dL)	62.5 (36.0)	73.9 (45.9)	0.034
White blood cell count (10 ³ /μL)	5.34 (1.20)	5.23 (1.08)	0.168
Hematocrit (%)	43.1 (2.4)	38.4 (2.5)	<0.0001
Platelet count (10 ³ /μL)	228.8 (47.2)	241.3 (48.7)	0.002
Hypertension medication	0	0.3%	0.28
Diabetes medication	0	0	na
Current smoker	17.3%	0	<0.0001
Menopausal status	na	38.3%	na
Hormone use in postmenopausal women	na	2.0%	na

Mean (SD) or frequency.

Adjusted for age (except for age).

Current smokers include pipe, cigar, and cigarette users.

P value for triglycerides is based on logarithm-transformed value.

Table 2
Age and sex- adjusted correlation between platelet aggregation using different agonists before and after aspirin administration

	Collagen (0.5 µg/ml)	Collagen (1µg/ml)	Collagen(2 µg/ml)	Collagen(5 µg/ml)	Adenosine diphosphate(10 µM)	Arachidonic acid (0.5 mM)
Collagen (0.5 µg/ml)			0.585	0.505	0.487	0.351
Collagen (1µg/ml)	0.742		0.705	0.704	0.504	0.412
Collagen (2 µg/ml)	0.539	0.786		0.669	0.421	0.386
Collagen (5 µg/ml)	0.348	0.517	0.668		0.345	0.372
Adenosine diphosphate (10 µM)	0.280	0.408	0.455	0.389		0.654
Arachidonic acid (0.5 mM)	0.337	0.308	0.242	0.142	0.274	

Pearson correlation coefficients (above diagonal were before aspirin administration and below diagonal were after aspirin administration).

All p value <0.0001.

Table 3
Heritability of platelet aggregation and urinary 11-dehydro thromboxane B2 before and after aspirin administration

Variable	Before Aspirin Administration **				Post Aspirin Administration **				***			
	H ²	SE	P value	%variation explained by covariates	H ²	SE	P value	%variation explained by covariates	H ²	SE	P value	%variation explained by covariates and baseline
Collagen 0.5 µg/mL	0.28	0.10	0.0005	0.07	0.10	0.09	0.12	0.05	0.06	0.09	0.24	0.08
Collagen 1 µg/mL	0.25	0.08	0.0006	0.05	0.22	0.10	0.01	0.09	0.22	0.10	0.01	0.12
Collagen 2 µg/mL	0.31	0.10	0.0005	0.04	0.22	0.10	0.01	0.10	0.14	0.10	0.08	0.16
Collagen 5 µg/mL	0.18	0.08	0.007	0.03	0.12	0.09	0.07	0.03	0.11	0.09	0.11	0.09
Adenosine diphosphate 10 µM	0.42	0.10	0.000004	0.05	0.42	0.10	0.000003	0.11	0.24	0.10	0.004	0.32
Arachidonic acid 0.5mM*	0.28	0.09	0.0004	0.07								
Urinary 11-dehydro thromboxane B2, ng/ mg creatinine	0.23	0.12	0.02	0.03	0.15	0.09	0.03	0.03	0.10	0.08	0.09	0.14

* The majority of post-aspirin administration values for arachidonic acid were zero, hence no heritability was calculated.

** Adjusted for age, age², and sex.

*** Adjusted for age, age², sex, and before aspirin administration value.

Urinary thromboxane B2 values were inverse normal transformed.

Table 4
Platelet aggregation and urinary thromboxane B2 before and after aspirin administration

Variable	Before Aspirin Administration				Post Aspirin Administration			
	Men (n = 400)	Women (n = 345)	Unadjusted p value	Covariates adjusted p value	Men (n = 400)	Women (n = 345)	Unadjusted p value	Covariates adjusted p value
Whole blood indirect COX-1 pathways								
Collagen 0.5 µg/mL (Q)	10.6 (0.3)	11.9 (0.2)	<0.0001	0.13	1.9 (0.1)	2.6 (0.2)	0.0003	0.15
Collagen 1 µg/mL (Q)	11.7 (0.3)	12.7 (0.2)	<0.0001	0.01	4.7 (0.4)	6.2 (0.2)	<0.0001	0.04
Collagen 2 µg/mL (Q)	13.1 (0.3)	14.0 (0.2)	<0.0001	0.42	9.2 (0.4)	10.9 (0.2)	<0.0001	0.0003
Collagen 5 µg/mL (Q)	16.2 (0.4)	17.1 (0.2)	0.0003	0.12	14.9 (0.2)	15.9 (0.2)	<0.0001	0.09
Adenosine diphosphate 10 uM (Q)	9.2 (0.4)	10.4 (0.2)	<0.0001	0.09	9.0 (0.4)	10.9 (0.2)	<0.0001	0.0006
Whole blood direct COX-1 pathway								
Arachidonic acid 0.5 mM* (Q)	100.0	100.0	1	1	23.5	16.8	0.02	0.60
Urine direct COX-1 pathway								
Urinary thromboxane B2 (ng/mg creatinine)	0.98 (0.12)	1.15 (0.06)	0.002	0.001	0.28 (0.04)	0.32 (0.02)	0.19	1.00
Mean (SE)								0.25

* The majority of post-aspirin therapy values for arachidonic acid were zero; values are thus expressed as the percentage who failed to be inhibited completely.

Covariate-adjusted p value were adjusted for age, body mass index, systolic blood pressure, total cholesterol, triglyceride, hematocrit, platelet count, white blood cell count, smoking status and family structure.

Covariate and baseline-adjusted p value were further adjusted for covariates above and baseline (before aspirin therapy) aggregation value (or thromboxane level).

Urinary thromboxane B2 values were inverse normal transformed.

Table 5
Patient characteristics stratified by aggregation test (<70% inhibition of platelet aggregation using collagen 1 µg/ml)

Variable	Aspirin resistant (n = 156)	Aspirin sensitive (n = 588)	P value
Age (years)	47.9 (1.2)	41.9 (1.4)	<0.0001
Women (n, %)	93, 59.6%	252, 42.9%	0.003
Body mass index (kg/m ²)	26.5 (0.4)	26.5 (0.7)	0.06
Systolic blood pressure (mmHg)	121.8 (1.2)	119.8 (1.6)	0.89
Diastolic blood pressure (mmHg)	75.7 (0.7)	76.5 (1.1)	0.14
Total cholesterol (mg/dL)	222 (4)	205 (8)	0.03
High density lipoprotein cholesterol (mg/dL)	58 (1)	56 (3)	0.17
Low density lipoprotein cholesterol (mg/dL)	149 (8)	137 (4)	0.07
Triglyceride (mg/dL)	76 (4)	66 (7)	0.99
White blood cell count (10 ³ /µL)	5.32 (0.1)	5.28 (0.2)	0.84
Hematocrit	40.0 (0.2)	41.2 (0.4)	0.49
Platelet count (10 ³ /µL)	246.2 (4.0)	231.3 (10.1)	0.005
Urinary thromboxane B2 (ng/mg creatinine) (after aspirin administration)	0.33 (0.02)	0.27 (0.04)	0.02
Hypertension medication	0	0.2%	0.40
Current smoker	7.1%	9.9%	0.81
Menopausal status	47.3%	34.9%	0.89
Hormone use among postmenopausal women	1.6%	3.2%	0.17

Mean (SE) or frequency.

Continuous variables were adjusted for age (other than age), sex and family structure; Chi-square test for categorical variables.

P values for triglycerides and thromboxane B2 were based on logarithm or inverse normal transformed values.

Current smokers include pipe, cigar, and cigarette users.