Genetic and Environmental Contributions to Platelet Aggregation
The Framingham Heart Study

Christopher J. O'Donnell, MD, MPH; Martin G. Larson, ScD; DaLi Feng, MD; Patrice A. Sutherland, BA; Klaus Lindpaintner, MD; Richard H. Myers, PhD; Ralph A. D’Agostino, PhD; Daniel Levy, MD; Geoffrey H. Tofler, MD

Background—Platelet aggregation plays an important role in arterial thrombosis in coronary heart disease, stroke, and peripheral arterial disease. However, the contribution of genetic versus environmental influences on interindividual variation in platelet aggregability is poorly characterized.

Methods and Results—We studied the heritability of platelet aggregation responses in 2413 participants in the Framingham Heart Study. The threshold concentrations of epinephrine and ADP required to produce biphasic platelet aggregation and collagen lag time were determined. Mixed-model linear regression was used to calculate correlation coefficients within sibships and within spouse pairs. Variance and covariance component methods were used to estimate the proportion of platelet aggregation attributable to measured covariates versus additive genetic effects. After accounting for environmental covariates, the adjusted sibling correlations for epinephrine, ADP, and collagen lag time were 0.24, 0.22, and 0.31, respectively (P < 0.0001 for each). In contrast, adjusted correlations for spouse-pairs were −0.01, 0.05, and −0.02, respectively (all P > 0.30). The estimated heritabilities were 0.48, 0.44, and 0.62, respectively. Measured covariates accounted for only 4% to 7% of the overall variance in platelet aggregation, and heritable factors accounted for 20% to 30%. The platelet glycoprotein IIa Pl-A2 polymorphism and the fibrinogen Hind III β-148 polymorphism contributed <1% to the overall variance.

Conclusions—In our large, population-based sample, heritable factors play a major role in determining platelet aggregation, and measured covariates play a lesser role. Future studies are warranted to identify the key genetic variants that regulate platelet function and to lay the groundwork for rational pharmacogenetic approaches. (Circulation. 2001;103:3051-3056.)

Key Words: platelets • genetics • glycoproteins • fibrinogen
Data are sparse regarding the genetic epidemiology of abnormal platelet aggregability. Mutations in genes encoding platelet GPs, such as GP IIb/IIIa and GP Ib/IX, may be responsible for rare monogenic bleeding conditions such as Glanzmann thrombasthenia and Bernard-Soulier syndrome. However, there is not yet strong evidence for specific molecular variants that increase aggregability in complex cardiovascular diseases. Limited twin study data suggest that the platelet aggregation response to epinephrine and adrenergic receptor binding are influenced by genetic factors. Specific genetic defects have been implicated in platelet receptor number and function. In several studies, the 807T/C polymorphism in the gene encoding integrin α2 (GP Ia), the collagen receptor, is strongly associated with platelet receptor density. We found that the P(2) polymorphism of GP IIa is associated with platelet reactivity in vitro. Studying the genetic and environmental contributions to platelet aggregability provides an essential context for future studies to determine accurately both the cardiovascular risks of genetic variants for specific platelet GP receptors and the potential benefits conferred by genetic variants during treatment with platelet GP IIb/IIIa receptor antagonists and other antiplatelet therapies.

The Framingham Heart Study is a large, prospective, population-based study containing multiple sibships, so it is possible to test hypotheses regarding platelet aggregability in vitro among related and unrelated pairs of individuals. Therefore, in our subjects, we sought to assess the contribution of measured and unmeasured genetic and environmental influences on interindividual differences in platelet aggregability.

**Methods**

**Study Sample**

The selection criteria and study design of the Framingham Heart Study have been detailed previously. For this study, we collected blood samples for platelet aggregation analysis from 1792 men and 2007 women examined between April 1, 1991 and June 29, 1995 during the fifth Offspring cohort examination cycle. Each participant provided written informed consent. Subjects were excluded (n=1386) for the following reasons: presence of prevalent cardiovascular disease (n=176), platelet aggregation test not done (n=535), use of aspirin or anticoagulant medications (n=528), absence of a normal platelet aggregation response to arachidonic acid (n=15), incomplete platelet aggregation testing (n=99), or incomplete covariate data (n=33). After these exclusions, 2413 subjects (1050 men and 1363 women) were eligible for the present study.

**Measurements**

The methods for anthropomorphic measurements, physician history, physical examination, and blood assays for cardiovascular risk factor information have been described previously. Fibrinogen levels were measured by the Clauss method. The diagnosis of a prior cardiovascular disease event (coronary heart disease, cerebrovascular disease, peripheral vascular disease, or congestive heart failure) was established according to previously published criteria after review by a committee of 3 physicians.

**Determination of Platelet Aggregability**

Blood samples were collected between 8 and 9 AM from an antecubital vein with subjects in the supine position after an overnight fast to minimize circadian variation in platelet aggregability. To determine platelet aggregability, we employed a previously described method used in several prior studies. Brieﬂy, platelet aggregation was performed according to the method of Born. The reagents used were epinephrine (Sigma), ADP (Sigma), and collagen (Biodata). The percent extent of aggregation in duplicate to epinephrine and ADP was determined in varying concentrations (0.01 to 15 μmol/L) and with a fixed concentration of arachidonic acid (5 mg/mL). The median threshold concentrations for platelet aggregation to epinephrine and ADP were 1 and 3 μmol/L, respectively. The collagen lag time was measured in response to 1.9 μmol/L collagen. The median collagen lag time was 80 s (range, 45 to >160 s). We previously reported consistency in intraperson variability by testing done in the same persons on different days for platelet aggregation responses to ADP and epinephrine. In reproducibility testing, test–test threshold for aggregation to epinephrine was the same in 8 of 9 subjects (89%) for epinephrine and in 18 of 22 subjects (81%) for ADP.

**Genotyping the P(2) Polymorphism of the GP IIa Gene and the Hind III β-148 Polymorphism of the β-Fibrinogen Gene**

For genotyping, genomic DNA was isolated from whole blood. The method of genotyping the P(2) polymorphism of the GP IIa gene has been described previously. A modified polymerase chain reaction (PCR)–based restriction fragment length polymorphism analysis was used to detect the Hind III β-148 polymorphism, which results in a C for T substitution at position −148 in the promoter region of the fibrinogen β gene. The sequences of the sense primer and anti-sense primer were 5′attgtgcttgaagaaaagcaataagcatttatg3′ and 5′tgctgagtagctgaaagttatattgtcattgtcagaaaacataagcatttatg3′, respectively. DNA was amplified using PCR. In the presence of the Hind III restriction endonuclease recognition site that represents the more common allele variant (H1), the 400 base pair (bp) amplification product was cleaved into fragments of 114 bp and 286 bp. The H2 allele was not cleaved by Hind III. The Hind III-digested amplification product was size-fractionated on an agarose gel. PCR results were scored without knowledge of platelet aggregability results. A total of 98% of the subjects were successfully genotyped.

**Statistical Methods**

The untransformed distributions were skewed and non-normal for 50% threshold platelet aggregation to epinephrine and ADP and for collagen lag time; therefore, logarithmic transformation (base 10) was applied before further analyses.

To analyze genetic contributions to platelet aggregation, separate analyses were conducted on siblings (ie, full siblings only) and spouse pairs. A total of 1041 subjects were analyzed in sibships (425 sibships with sibship sizes ranging from 2 to 7). There were 464 spouse pairs and 881 sibling pairs (178 male-male, 435 male-female, and 268 female-female pairs). Spouse pairs, rather than randomly selected unrelated individuals, were included as a comparison group because, due to assortative mating, spouses may be similar in age, weight, and other variables, including household environment, that tend to be common among siblings. Mixed-model linear regression was used to calculate variance and covariance components, using the SAS procedure MIXED and to calculate interindividual correlation coefficients among siblings and between spouses. These methods accommodate different numbers of subjects in different sibships. Covariates in the adjusted model (continuous measures unless otherwise indicated) included age (linear spline on the age segments, with knots at 47 and 62 years), sex, body mass index, triglycerides, total cholesterol, HDL cholesterol, diabetes (yes or no), cigarette smoking status (yes or no), alcohol consumption (drinks per day), menopausal status (yes or no), estrogen replacement status (yes or no), systolic blood pressure, diastolic blood pressure, and use of antihypertensive drug therapy (yes or no). Secondary analyses were conducted by adding plasma fibrinogen to these covariates.

We also estimated the extent to which genes and measured environmental factors contributed to the variation in platelet aggregation in the fully adjusted model. Mixed models were used to estimate the proportion of the variance due to the additive effect of genes and the additive effect of measured covariates to the variation...
in platelet aggregation. The relative proportions of the variance explained by the measured environmental covariates and by genes were calculated as the variance attributable to that particular component divided by the total variance in platelet aggregability. The residual variance that was not accounted for by the 2 components is the proportion of the variance attributable to unmeasured environmental factors.

The SAS System (release 11) was used for all statistical analyses. All statistical tests were 2-sided, and a 0.05 was considered statistically significant.

## Results

### Participant Characteristics

The eligible study sample consisted of 1050 men and 1363 women. Characteristics of the sample are shown in Table 1. The age range was 26 to 82 years. Subjects free of prevalent cardiovascular disease who attended the examination but who were not included in the analysis were older and more likely to be male. After accounting for age and sex, these excluded subjects tended to have a lower mean total cholesterol and a higher prevalence of diabetes, antihypertensive therapy, and estrogen replacement therapy.

### Correlates of Platelet Aggregation to Epinephrine, ADP, and Collagen Lag Time

In men, the correlation between aggregation to epinephrine and aggregation to ADP was \( r = 0.55 \); between aggregation to epinephrine and collagen lag time, it was \( r = 0.31 \); and between aggregation to ADP and collagen lag time, it was \( r = 0.21 \). In women, these correlations were \( r = 0.55 \), \( r = 0.29 \), and \( r = 0.20 \), respectively.

Pearson correlation coefficients of platelet aggregation with age and other factors associated with coronary heart disease risk are listed in Table 2. A negative correlation with threshold concentration of epinephrine, ADP, or collagen lag time provides evidence for increasing platelet aggregability to these agonists (ie, greater aggregability). For example, the negative correlations of age with threshold aggregation to epinephrine (\(-0.10\) in men and \(-0.06\) in women) indicates that aggregability decreases with increasing age; conversely, the positive correlation of body mass index with threshold aggregation to epinephrine (0.07 in men and 0.04 in women) indicates that aggregability increases with increasing body mass index. In both sexes, there is increasing platelet aggregation to epinephrine with increasing age (\( r = -0.10 \) in men and \( r = -0.06 \) in women) and ADP (\( r = -0.12 \) in men and \( r = -0.09 \) in women). The results of correlations (Table 2) show statistically significant increasing aggregability to collagen with increasing triglyceride levels, HDL cholesterol, and presence of diabetes, as well as of increasing aggregability to epinephrine with increasing triglyceride levels, in both men and women. The magnitude of correlation was generally \( \leq 0.12 \) for all covariates.

### Familial and Spousal Correlations for Platelet Aggregation

The sibling correlations for platelet aggregation to epinephrine and ADP and for collagen lag time were 0.24, 0.22, and 0.31, respectively (\( P < 0.0001 \) for each) based on residuals from linear models. These did not differ materially from correlations based on raw data (Figure). In separate analyses of brother-sister pairs, the magnitude of fully adjusted correlations remained similar to the correlations among all sibling pairs and significantly different from zero (Figure). Similar correlations were also found among brother-only and sister-only pairs.

Unadjusted and fully adjusted spousal correlations for platelet aggregation to epinephrine, ADP, and collagen were not statistically different from zero (\( r = -0.01, 0.05, \) and \(-0.02, \) respectively; \( P > 0.30 \) for all correlations; Figure). Significant adjusted correlations were also seen for peak (\( >90\% \)) aggregation to epinephrine and ADP for sibling pairs but not spouse pairs (data not shown).

Simple estimates of heritability derived from the adjusted correlation coefficients were calculated to account for the

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**TABLE 1. Characteristics of Study Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n=468)</th>
<th>Women (n=573)</th>
<th>Men (n=464)</th>
<th>Women (n=464)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.5 (10.1)</td>
<td>52.5 (10.0)</td>
<td>55.8 (8.0)</td>
<td>54.0 (7.9)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.0 (4.2)</td>
<td>26.9 (6.0)</td>
<td>28.3 (4.4)</td>
<td>26.3 (5.4)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>153.0 (93.4)</td>
<td>129.7 (77.8)</td>
<td>151.7 (105.8)</td>
<td>129.3 (74.8)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>201.1 (34.9)</td>
<td>205.0 (38.1)</td>
<td>202.5 (32.9)</td>
<td>208.5 (35.9)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>43.4 (11.1)</td>
<td>56.0 (14.7)</td>
<td>44.1 (11.6)</td>
<td>57.5 (16.0)</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>6.0</td>
<td>4.5</td>
<td>6.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Cigarettes, %</td>
<td>19.9</td>
<td>19.4</td>
<td>17.9</td>
<td>15.7</td>
</tr>
<tr>
<td>Alcohol consumption, oz/wk</td>
<td>3.7 (4.7)</td>
<td>1.8 (2.5)</td>
<td>3.7 (4.7)</td>
<td>1.7 (2.3)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>126.8 (16.9)</td>
<td>122.3 (19.2)</td>
<td>128.7 (16.2)</td>
<td>123.4 (19.8)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>77.2 (9.4)</td>
<td>73.5 (9.9)</td>
<td>77.0 (9.9)</td>
<td>72.7 (9.7)</td>
</tr>
<tr>
<td>Estrogen replacement, %</td>
<td>NA</td>
<td>12.9</td>
<td>NA</td>
<td>18.5</td>
</tr>
<tr>
<td>Menopause, %</td>
<td>NA</td>
<td>59.5</td>
<td>NA</td>
<td>66.8</td>
</tr>
<tr>
<td>Hypertension treatment, %</td>
<td>12.4</td>
<td>14.0</td>
<td>13.6</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Values are mean (SD) or percent. BP indicates blood pressure; NA, not applicable.
average proportion of genes shared by pair members using the equation $h^2 = 2r$ ($h^2$ indicates heritability and $r$, sibling-pair correlations). Heritability is the proportion of variance in platelet aggregability phenotypes attributed to the additive effect of many genes. The estimated heritabilities were 0.48, 0.44, and 0.62 for the threshold platelet aggregability response to epinephrine, ADP, and collagen, respectively.

Components of Variance Analysis

The overall contribution of genetic factors and measured covariates to platelet aggregation to epinephrine and ADP and for collagen lag time are shown in Table 3. In the fully adjusted model, the contribution of genetic factors to overall variation in platelet aggregation responses was 21% for epinephrine, 22% for ADP, and 30% for collagen lag time. In the same models, the contribution of measured covariates to overall variation in responses to epinephrine, ADP, and collagen was 6%, 7%, and 4%, respectively. Addition of serum fibrinogen to the covariates in the fully adjusted model did not materially alter these estimates.

We examined the effect of the GP IIIa genotype on variation in aggregation responses to different agonists. When GP IIIa was added to the components of variance model, it contributed only 0.8% to the variation in platelet aggregation in response to epinephrine. The contribution of GP IIIa to overall variance in platelet aggregation to ADP and to collagen lag time was 0.3% and 0.2%, respectively. In a components of variance model with both the GP IIIa genotype and the fibrinogen Hind III b-148 genotype (Table 4), Hind III b-148 contributed 0.5% to the variance in platelet aggregation to epinephrine and ADP and to the variance in collagen lag time. Likewise, in a separate components of variance model without the GP IIIa genotype, the fibrinogen Hind III b-148 polymorphism made no significant contribution to overall variation in platelet aggregation responses to any of the 3 agonists (data not shown).

Discussion

In this study of sibships drawn from a population-based sample, we found evidence for significant heritability in platelet aggregation based on sibling correlations for platelet aggregability to epinephrine and ADP and for collagen lag time. Heritable factors contributed substantially more than all the measured covariates combined to the overall variation in platelet aggregation in response to these agonists; of the overall variance in platelet aggregation, 21% to 30% was due

### Table 2. Correlation of Platelet Aggregation With Age and Other Covariates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n=1050)</th>
<th>Women (n=1363)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epinephrine</td>
<td>ADP</td>
</tr>
<tr>
<td>Age (y)</td>
<td>-0.10†</td>
<td>-0.12‡</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.07*</td>
<td>-0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.08†</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Cigarettes (%)</td>
<td>-0.03</td>
<td>-0.02</td>
</tr>
<tr>
<td>Alcohol consumption (oz/wk)</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>0.08*</td>
<td>0.10†</td>
</tr>
<tr>
<td>Estrogen Replacement (%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hypertension treatment (%)</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Menopause (%)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

A negative correlation indicates that the variable is associated with increased platelet aggregability. BP indicates blood pressure.

*P<0.05, †P<0.01, ‡P<0.001.
to heritable factors compared with 4% to 7% due to measured covariates. Indeed, the magnitude of these adjusted sibling correlations exceed previously reported sibling correlations for interindividual differences in many heritable cardiovascular risk factors, including systolic blood pressure and HDL cholesterol. Our data are consistent with the limited available data from small twin studies regarding the contribution of additive genetic factors to platelet aggregability and to the presence and function of platelet surface GPs. However, the interpretation of twin data needs to be treated with caution, in part because unmeasured shared environmental effects in utero and in early life may lead to an overestimate in the degree of correlation among siblings. Further, estimates of environmental correlations in twins may not be comparable to estimates obtained in the general population.

It is possible that confounders such as hypertriglyceridemia, hypertension, or physical activity may explain some of the increase in familial clustering of platelet aggregability in twin studies. However, the magnitude and direction of correlation for many of these factors has not been consistent from study to study. In our data, increasing age was associated with decreasing platelet aggregability to epinephrine and ADP, and increasing levels of plasma triglycerides and decreasing HDL were significantly correlated with lower platelet aggregability to epinephrine and collagen lag time (Table 2). Although there are other significant and complex correlations of covariates to platelet aggregability, our estimates of sibling correlations remained significantly elevated even after multivariate adjustment. Further, the overall proportion of variance due to the combined contribution of measured environmental covariates was consistently <10%.

Platelet aggregation is regulated dynamically by extracellular excitatory and inhibitory signals leading to a transmembrane stimulus-response coupling that generates specific intracellular second messengers. We recently reported that the Pla2 allele of the GP IIIa receptor is associated with incrementally greater aggregability in response to epinephrine, as well as with a trend for greater aggregability in response to ADP. However, the Pla2 allele contributed only 0.8% to the overall variation in platelet aggregability to epinephrine and <0.5% to aggregation to the other agonists. There are conflicting data regarding the role of the Pla2 genotype with coronary heart disease and stroke. Possible explanations for the inconsistency of associations of Pla2 with clinically apparent disease include small sample sizes in available studies and inappropriate selection of control samples (genetic admixture), as well as a potential permissive role of environmental factors on recognition of genotype-phenotype interactions.

Given the substantial role that heritable factors play in overall variance in platelet aggregability, further studies are warranted to investigate the associations of platelet GP receptor candidate gene variants (besides GP IIIa) with platelet function. Given the modest effect to be anticipated from any individual genetic variants and given the highly polymorphic nature of the GP IIIa gene and those for other receptor GPs, large sample sizes may be required to enable reliable detection of individual genetic effects.

There are potential study limitations. The exclusion of subjects with prevalent cardiovascular disease may affect the generalizability of these findings. However, if a genetic predisposition to platelet hyperaggregability plays a substantial role in the onset of cardiovascular disease, exclusion of these subjects should only serve to lower the heritability estimates. Another potential limitation is that nearly all subjects were white, so our findings may only be generalizable to white populations. Future studies to test for a differential role of genetic influences on platelet function across racial groups will need to carefully examine the impact of gene-environment interactions. As a third limitation, we acknowledge that it may be difficult to extrapolate in vitro results to in vivo conditions. Finally, it is possible that intrasubject variability might adversely affect the reproducibility of these measures. To minimize the intrasubject and circadian variability, we used a standardized, previously described method of blood collection and platelet aggregometry. Any decreases in reproducibility, however, would only tend to decrease the estimates of sibling correlations and heritability toward the null.

In conclusion, in our population-based sample, heritable factors play a major role in determining platelet aggregation. Both the GP IIIa genotype and the fibrinogen Hind III β-148 genotype make only a small contribution to platelet aggregation. Thus, the majority of variation in platelet aggregation may be due to the cumulative effects of other genetic variants of these proteins or other proteins involved in the cascade of steps mediating platelet adhesion and aggregation. On the basis of our heritability estimates (ranging from 0.44 to 0.62), further genetic studies are warranted using both candidate gene and genome screen approaches to identify the effects of genetic variants of such proteins. Given the importance of platelet inhibition in the armamentarium of therapies for primary and secondary prevention of coronary heart disease, as well as stroke and peripheral arterial disease, these studies may form the basis for future pharmacogenetic approaches.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Fibrinogen β-148</th>
<th>GP IIIa</th>
<th>Other</th>
<th>Measured Covariates*</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>0.00</td>
<td>0.01</td>
<td>0.21</td>
<td>0.08</td>
<td>0.70</td>
</tr>
<tr>
<td>ADP</td>
<td>0.00</td>
<td>0.00</td>
<td>0.22</td>
<td>0.09</td>
<td>0.69</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.00</td>
<td>0.00</td>
<td>0.28</td>
<td>0.06</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Covariates in the adjusted model are listed in Statistical Methods.
Acknowledgment

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References

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