Mean Platelet Volume and Integrin Alleles Correlate With Levels of Integrins $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$ in Acute Coronary Syndrome Patients and Normal Subjects

Thomas J. Kunicki, Shirley A. Williams, Diane J. Nugent, Mark Yeager

Objective—The interindividual variation in platelet $\alpha_2\beta_1$ exceeds a 2-fold variance in platelet $\alpha_{IIb}\beta_3$ level. Our objective was to parse the contribution of mean platelet volume (MPV) and integrin gene alleles to this variation in large cohorts of patients with acute coronary syndrome (ACS) and normal subjects.

Methods and Results—Platelet $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$ levels were measured by flow cytometry in whole blood from 320 ACS patients and 128 normal subjects and compared with MPV, platelet count, ITGA2 rs28095, and ITGB3 rs5918 alleles. In all subjects, a strong direct correlation was found between MPV and $\alpha_{IIb}\beta_3$ level ($P<0.001$). Neither MPV nor $\alpha_{IIb}\beta_3$ level correlated with ITGB3 rs5918 alleles. In the case of $\alpha_2\beta_1$ level, MPV contributed modestly, whereas ITGA2 rs28095 exerted a greater effect. An inverse correlation was found between MPV and the rs28095 minor allele.

Conclusion—MPV is the major effector of platelet $\alpha_{IIb}\beta_3$ level, whereas the ITGA2 rs28095 alleles influence $\alpha_2\beta_1$ level more than MPV does. The rs28095 minor allele, associated with lower MPV, likely exerts this effect via the influence of $\alpha_2\beta_1$ on megakaryocyte maturation. Because of the hyperactivity of larger platelets, MPV is an accurate metric of risk for adverse outcome in ACS. (Arterioscler Thromb Vasc Biol. 2012;32:147-152.)

Key Words: arterial thrombosis ■ platelets ■ thrombosis ■ integrins ■ mean platelet volume
ACS patients included in this study were referred to the Division of Cardiovascular Diseases of Scripps Clinic for coronary angiography, with or without a preexisting history of ACS, as defined by American Heart Association/American College of Cardiology guidelines.12

Quantitation of \( \alpha_{ib} \beta_3 \) and \( \alpha_2 \beta_1 \) byFlow Cytometry

12F1 and 8C12, murine monoclonal antibodies specific for \( \alpha_2 \beta_1 \), and AP2, a murine monoclonal antibody specific for \( \alpha_{ib} \beta_3 \), were used to measure platelet plasma membrane levels of these integrins by flow cytometry of whole blood samples, as previously described.13,14

Using a 19-G butterfly needle and Vacutainer tubes, an initial volume of blood was discarded, and a subsequent blood sample was anticoagulated by mixing with one-sixth volume acid citrate dextrose-formula A. Not later than 4 hours after phlebotomy, 5 μL of blood was mixed with 40 μL of 2 mmol/L MgCl₂, 138 mmol/L NaCl, 12 mmol/L NaHCO₃, 2.6 mmol/L KCl, pH 7.4 (Tyrode buffer), containing 1% (wt/vol) bovine serum albumin. Five microliters of primary antibody (100 μg/mL AP2, 12F1, 8C12, or nonimmune mouse IgG1) was then added to the mixture, which was gently inverted and incubated for 30 minutes at room temperature. Then, 950 μL of FITC–goat anti-mouse IgG (heavy + light chains, Zymed, Inc, 62-6312) was then added, and the mixture was incubated for an additional 30 minutes at room temperature. Then, 950 μL of ice-cold 1% (v/v) parafomaldehyde in phosphate-buffered saline pH 7.4 was added, and the sample was stored at 4°C in the dark until it was assayed by flow cytometry, which was performed within the subsequent 3 days. Neither the time delay between phlebotomy and antibody assay (1–4 hours) nor the delay between antibody assay and flow cytometry (1–3 days) had an effect on the outcome of receptor quantitation (data not shown).

Levels of bound monoclonal antibody were expressed as geometric mean fluorescence intensity after subtraction of geometric mean fluorescence intensity obtained from addition of control nonimmune murine IgG1.

Measurement of Platelet Count and MPV

Whole blood platelet count and MPV were measured using a Coulter 9000 apparatus (Mallinckrodt Baker, Phillipsburg, NJ).

Genotyping

Genotypes were determined using primer sequences previously reported in a primer extension–based assay15 or a customized Nanogen-based single-nucleotide polymorphism analysis (Nanogen Inc., San Diego, CA, USA).16 When necessary, single-nucleotide polymorphisms were confirmed by direct Sanger sequencing. The single-nucleotide polymorphisms analyzed in this study were ITGB3 rs5918 (minor allele frequency=0.17)17 and ITGA2 rs28095 (minor allele frequency=0.38).6

Statistics

Statistical calculations were performed using SigmaStat, version 3.01 (SPSS Inc, Chicago, IL). For continuous variables (eg, platelet count, MPV, age, \( \alpha_2 \beta_1 \) level, \( \alpha_{ib} \beta_3 \) level), descriptive statistics were calculated and reported as mean and standard deviation. Linear and multilinear regression, using the adjusted \( r^2 \) statistic, was used to determine the contribution of independent variables that predict the dependent variable. Associations between discontinuous variables (eg, single-nucleotide polymorphisms) and continuous variables were described using the Pearson product moment correlation or Spearman rank order association, and probability values were corrected for multiple testing. Pairwise associations between variables were analyzed by 1-way ANOVA, Kruskal-Wallis 1-way ANOVA on ranks, or \( t \) test. All tests are 2-sided and considered significant at \( P<0.05 \) after correction for multiple testing. The coefficient of variation is defined as the ratio of the standard deviation to the mean (SD/mean).

Results

Subject Parameters

The ACS patient cohort was deidentified, including sex and age. In the control subject cohort, the percentage of males was 51%, and the mean age was 39.3±9.2 years. The MPV of ACS patients (7.46±1.43 fl; n=109) was not significantly different (\( P=0.079 \)) from that of control subjects (7.11±1.64 fl; n=128), and the same was true for the platelet count (214±58 and 225±56×10^3/μL, respectively; \( P=0.219 \)) (Supplemental Table I, available online at http://atvb.ahajournals.org).

Quantitation of Integrin Levels

Intradonor variation in \( \alpha_2 \beta_1 \) or \( \alpha_{ib} \beta_3 \) levels was analyzed using 11 control and 6 ACS subjects by flow cytometric measurements performed on 2 separate occasions at least 30 days apart (Supplemental Figure I). The coefficient of variation in geometric mean fluorescence intensity for each of the three monoclonal antibodies was as follows: AP2, 0.11; 12F1, 0.21; and 8C12, 0.10. On this basis, bound 8C12 is a more precise metric than bound 12F1 for the quantitation of \( \alpha_2 \beta_1 \). The intersubject variation was similar in either ACS patients or control subjects with regard to platelet levels of \( \alpha_{ib} \beta_3 \) (Figure 1) or \( \alpha_2 \beta_1 \) (Figure 2), as summarized in Table 1. The metric of \( \alpha_2 \beta_1 \) depicted in Figure 2 was bound 8C12, but...
similar findings were made for bound 12F1 (Supplemental Figure II), and very high correlation was observed between bound 8C12 and 12F1 (adjusted $r^2 = 0.583; P < 0.001$) (Supplemental Figure III).

Multivariate analysis in ACS patients (Supplemental Table II) indicated a statistically significant direct association between MPV and the level of AP2 ($P < 0.001$), 12F1 ($P < 0.001$), or 8C12 ($P = 0.001$) bound per platelet (geometric mean fluorescence intensity). Not unexpectedly, there was also a very strong direct correlation between platelet-bound 12F1 and 8C12 (data not shown). Likewise, among control subjects (Figure 4), MPV accounted for 49% (bound AP2) of the variation in levels of $\alpha_{IIb}\beta_3$, whereas identical findings were observed for bound 12F1 as for bound 8C12 (data not shown). As shown in Table 2, the $ITGA2$ rs28095 alleles exert a significant effect on the levels of platelet $\alpha_{IIb}\beta_3$. In addition, there was a significant negative correlation between the $rs28095$ minor allele T and MPV. This likely accounts for the weak but significant correlation between the presence of the minor allele and decreased bound AP2 (that is, a decreased level of $\alpha_{III}\beta_1$).

**Table 1. Platelet Integrin Levels**

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<th>SD</th>
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<th>Maximum</th>
<th>CV</th>
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**Control Subjects**

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ACS indicates acute coronary syndrome; CV, coefficient of variation.

*Geometric mean fluorescence intensity.

**Table 2. Control Subjects: Influence of ITGA2 rs28095**

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**Figure 3.** Acute coronary syndrome (ACS) patients: relationship between mean platelet volume (MPV) and level of bound AP2 (left) or 8C12 (right). Corresponding regression lines are plotted. For AP2, adjusted $r^2 = 0.456, P < 0.001$; for 8C12, adjusted $r^2 = 0.885, P = 0.001$. The powers of the performed tests with $\alpha = 0.05$ were 1.0 for MPV vs AP2 and 0.9 for MPV vs 8C12.

**Discussion**

MPV is the most common metric of platelet size and correlates very well with platelet reactivity. Larger platelets have greater prothrombotic potential, and elevated MPV is associated with increased platelet aggregation, thromboxane synthesis, $\beta$-thromboglobulin release, and expression of adhesion molecules. A relationship between MPV and platelet count has not been established, and most studies do not find a significant correlation between these 2 variables. On the
other hand, several studies have demonstrated a statistically significant association between MPV and acute myocardial infarction or other cardiovascular events, even after adjusting for platelet count,21–22 whereas the majority of studies have not found a statistically significant association between increased platelet count and incidence of acute myocardial infarction, restenosis, or long-term mortality in cardiovascular disease.21,22,24,26

Previous studies have found that the levels of αthβ3 and glycoprotein (GP)Ibα vary over a 2-fold range among normal subjects or patients with ACS.5,11,27–29 As expected, these increased levels of αthβ3 are associated with increased platelet reactivity and decreased sensitivity to αthβ3 antagonists.11,28 Very recently, it has also been shown that the ability of platelets to support thrombin generation may be defined by a subpopulation of larger, perhaps reticulated, platelets bearing nondissociable platelet-derived Factor Va and exhibiting increased adhesion receptor density (P-selectin, GPIbα, and integrin αthβ3).30 However, this procoagulant subpopulation can represent as little a 8% to as much as 54%, depending on the donor, and an association with MPV has not been established. As in most studies of platelet physiology, another confounding factor is platelet activation, because this has been shown to increase the level of surface receptor expression, including αthβ3, by as much as 40% when the activation is conducted in vitro.31,32 However, the percentage of platelet activation (percentage of maximum expression of total αthβ3) in whole blood from patients is not likely to be as high as the maximum inducible in vitro. On the other hand, there are no accurate data concerning the effect of platelet activation on platelet volume, but any increase would certainly be affected by the nature of the agonist and the conditions of the medium. As a relevant observation, it has been reported that during storage of platelet concentrates, the surface activation markers CD62P and PAC-1 increase up to 600% and 150%, respectively, but there is absolutely no significant change in MPV.33

The best way to minimize this confounding factor of platelet activation during platelet processing is to fix platelets in whole blood before flow cytometric analysis, as we have done in this study.

Our study examines larger cohorts of both ACS patients and control subjects than hitherto reported and confirms that levels of αthβ3 vary at least 2-fold. In addition, our results establish that MPV accounts for 49% of the observed variation. Logically, the number of glycoprotein receptors per platelet increases proportionately with MPV, and because receptor density remains unchanged,11 the increase is likely due to the increased surface area of platelet plasma membrane. Although we found no influence of the common ITGB3 alleles on expression level, a genetic component cannot be ruled out, perhaps because of as yet unidentified extragenic regulatory elements. At the same time, it is not likely that ITGB3 rs5918 is in linkage disequilibrium with genetic variants that contribute to MPV, because our results and the results of a previous study34 found that neither MPV nor platelet count correlates with the ITGB3 rs5918 minor allele.

In the case of α2β1, the genetic component of variation is more profound and exceeds the contribution of MPV. We previously described a genetically determined variation in the level of the collagen receptor α2β1 on platelets and argued that this variation is largely independent of MPV. The fact that genetically determined α2β1 levels are found on cells other than platelets is consistent with this argument. Nonetheless, differences in MPV must contribute to the overall variability, and our objective in this study was to parse the contributions of MPV and the genetic component. Our findings indicate that allelic differences in ITGA2 rs28095, previously documented to profoundly influence transcription of this gene, are the most important factor that regulates α2β1 levels (46% based on bound 8C12; 57% based on bound 12F1), with MPV exerting an independent but smaller contribution (13% based on bound 8C12; 8% based on bound 12F1).

Perhaps the most intriguing finding of our study is the inverse association of the ITGA2 rs28095 minor allele T with MPV. This suggests that expression levels of integrin α2β1 may be involved in the regulation of platelet size, which is certainly consistent with previous observations that α2β1 modulation of proplatelet formation is an important factor in the production and size of platelets.55,56 In the bone marrow, the interaction of hematopoietic stem cells with osteoblasts inhibits megakaryocyte maturation and proplatelet formation through a mechanism that is dependent on the engagement of collagen I by megakaryocyte α2β1.36 Enhanced binding of megakaryocyte α2β1 to collagen I attenuates or delays proplatelet formation. Conversely, in Wiscott-Aldrich syndrome and X-linked thrombocytopenia, a deficiency of the protein WASp is associated with loss of α2β1-mediated inhibition of proplatelet formation, resulting in ectopic shedding of platelets into the bone marrow space and microthrombocytopenia.55 Likewise, familial thrombocytopenia 2 is characterized by mutations of the gene ANKRD26, decreased MPV, and decreased expression of integrin α2β1.37 Thus, it is reasonable that megakaryocyte expression of the rs28095 minor allele T, which is known to attenuate ITGA2 transcription, thereby leading to diminished α2β1 expression and reduced platelet adhesion to collagen I, may also result in a mild form of accelerated platelet formation and consequently a decrease in MPV.

The degree of αthβ3 variation that is observed likely influences platelet reactivity and the risk of thrombosis but can also complicate the detection of carriers of the hereditary
deficiency of αIIbβ3, Glanzmann thrombasthenia (GT). In one of the earliest studies, McEver et al.1 used radiolabeled monoclonal antibody Tab to measure αIIbβ3 on platelets of 4 GT patients and their immediate family members. They found 30,000 to 50,000 molecules bound to platelets of normal subjects and normal members of the GT families, but 20,000 to 35,000 molecules bound per platelet by obligate GT heterozygotes (n=5). Four other possible GT heterozygotes expressed 15,000 to 30,000 molecules per platelet. This was the first indication that the range of platelet αIIbβ3 expression might overlap the range of expression in normal subjects. Coller et al.3 produced similar results using radiolabeled monoclonal antibody 10E5, finding a range of αIIbβ3 levels in 37 obligate GT carriers of 18,000 to 47,000 per platelet, compared with a normal range of 27,000 to 50,000. It is possible that normalization of αIIbβ3 levels based on MPV may serve to increase the accuracy of detection of obligate GT carriers using monoclonal antibody-based methods.

Two other platelet-specific receptors have been found to vary to a degree similar to that seen with αIIbβ3 (2-fold). These are the von Willebrand factor receptor GPIba11 and the collagen receptor GPVI.1,4,38 Logically, the observed variation in these receptors is most probably attributable to differences in MPV, although a direct correlation has yet to be reported. Indeed, several reports from one research group have noted that platelet GPVI levels are significantly elevated in ACS patients and strongly associated with myocardial ischemia.39–41 None of these reports, however, included a measurement of MPV.

In summary, our results provide compelling evidence that MPV is the greatest contributor to variation in levels of platelet αIIbβ3 among either normal individuals or patients with ACS and may be the major contributor to the variation seen in several other platelet receptors, such as GPIbα or GPVI. An exception is the collagen receptor αcβ1, whose level is influenced in part by MPV but to a larger extent by allelic variation in ITGA2. In addition, our results suggest that allelic variation in ITGA2 may itself contribute to the modulation of platelet size and MPV. Our findings also contribute to the argument that increased levels of platelet receptors, particularly integrins αIIbβ3 and αcβ1, resulting from increased MPV, are responsible for the resultant increase in platelet reactivity. Larger platelets are hyperactive, owing in large part to the increased surface expression of all receptors, but most notably the integrins, which play a key role in initiation of platelet activation and perpetuation of the nascent thrombus. Additional studies of the clinical implications of our findings are warranted.

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Disclosures

None.

References


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A. Bound AP2 ($\alpha_{\text{IIb}\beta_3}$)  B. Bound 8C12 ($\alpha_{2\beta_1}$)

Supplemental Figure I. Intra-donor variation in A) bound AP2, as a measure of integrin $\alpha_{\text{IIb}\beta_3}$, and B) bound 8C12, as a measure of integrin $\alpha_{2\beta_1}$. GMFI was determined on two occasions (1st and 2nd) for each of 17 subjects (11 normal controls; 6 ACS patients). Comparable findings were obtained with measurements of bound 12F1 (not shown).

Supplemental Figure II. Inter-donor variation in platelet-bound 12F1, as a metric of the level of integrin $\alpha_{2\beta_1}$. GMFI is plotted on the ordinate. Measurements for each individual (320 ACS patients; 128 controls) are plotted in chronological order (left to right) on the abscissa.
Supplemental Figure III. Linear correlation between bound 12F1 (abscissa) and bound 8C12 (ordinate) for each donor (combined control subjects and ACS patients; n = 448). Adjusted $r^2 = 0.583$, $p < 0.001$.

Supplemental Table I. Subject Parameters

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*fL; §platelets x 10^-3/µl; SD, standard deviation; CV, coefficient of variation

Supplemental Table II. ACS Patients: Pearson Product Moment Correlation

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*Correlation Coefficient; §p-value; Highly significant correlations are in **bold.**
**Supplemental Table III. Control Subjects: Pearson Product Moment Correlation**

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*Correlation Coefficient; \(p\)-value; Highly significant correlations are in **bold**.
For all comparisons, \(n = 128\). At \(n = 128\), the power of the performed test with \(\alpha = 0.05\) will be \(\geq 0.08\) given a correlation coefficient \(\geq 0.246\).
### LABORATORY RESULTS

<table>
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<th>AP2</th>
<th>12F1</th>
<th>8C12</th>
<th>MPV</th>
<th>AGE</th>
<th>PLT</th>
<th>Hb</th>
<th>Hct</th>
<th>WBC</th>
<th>CHOL</th>
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<th>TG</th>
<th>PT</th>
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<th>PTT</th>
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**Supplementary Table IV:** Correlation between Laboratory Variables in ACS Patients determined by Pearson product moment correlation (SigmaStat 3.01).

- Correlation Coefficient (top)
- p-value (center)
- number of pair-wise observations (bottom)

For each variable entry, the following parameters are depicted:

- CHOL: Correlation Coefficient (top)
- p-value (center)
- number of pair-wise observations (bottom)

p-values are corrected for multiple testing, as described by Bonferroni et al.

Entries highlighted in yellow are statistically significant (p < 0.05) after correction for multiple testing. In each case, the power of the performed test with \( \alpha = 0.05 \) is > 0.8.

A positive correlation coefficient indicates that there is a direct correlation between the two variables; a negative coefficient indicates that there is an inverse correlation.

### ABBREVIATIONS

- AP2: Flow Cytometry GMFI anti-\( \alpha \text{IIb}\beta3\)
- 12F1: Flow Cytometry GMFI anti-\( \alpha \text{IIb}\beta1\)
- 8C12: Flow Cytometry GMFI anti-\( \alpha \text{IIb}\beta1\)
- MPV: Mean Platelet Volume (Coulter Counter)
- AGE: PLT, platelet count (Coulter Counter)
- Hb, Hemoglobin
- Hct, Hematocrit
- WBC, white blood cell count
- CHOL, blood cholesterol level
- LDL, blood low density lipoprotein level
- HDL, blood high density lipoprotein level
- TG, blood triglyceride level
- PT, prothrombin time
- INR, international normalized ratio
- PTT, partial thromboplastin time
### CORONARY ARTERY DISEASE

#### Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Description</th>
<th>Categories</th>
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</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Hypercholesterolemia</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Angina</td>
<td>Angina-like symptoms</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Diabetes mellitus</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Blood pressure &gt; 130/80 mmHg</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Smoking</td>
<td>Current or former smoker</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Family History</td>
<td>Family history of CAD</td>
<td>Yes/No</td>
</tr>
</tbody>
</table>

#### Medications

<table>
<thead>
<tr>
<th>Medication</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Antiplatelet agent</td>
</tr>
<tr>
<td>Statins</td>
<td>Lipid-lowering agent</td>
</tr>
<tr>
<td>ACE Inhibitors</td>
<td>Blood pressure-lowering agent</td>
</tr>
</tbody>
</table>

### Cardiovascular Risk Evaluation

#### Coronary Artery Disease Detection

**Patients with known CAD:**

1. **Calculation of Risk Score**
   - Add 1 point for each of the following:
     - Male sex
     - Age > 60 years
     - Smoker
     - Hypertension
     - Hypercholesterolemia
   - Add 0.5 points for:
     - Family history of CAD
     - Diabetes
   - Subtract 1 point for:
     - No smoking

#### 10-Year Cardiovascular Risk

- **Low Risk:** 0-1 points
- **Moderate Risk:** 2-3 points
- **High Risk:** ≥4 points

### References

- ACS Council on Clinical Cardiology/American Heart Association: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
- ACC/AHA: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
- NHLBI: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
- AHA: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
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- AHA: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)

### Analysis

- **Supplementary Table:**
  - Correlation Matrix for Demographic and Clinical Variables in ACS Patients (data source: Framingham Heart Study).
- **Logistic Regression Analysis:**
  - Predictors: Age, Sex, Smoking Status, Hypertension, Hypercholesterolemia, Diabetes, Family History.
  - Outcome: Presence or Absence of Coronary Artery Disease.

### Notes

- **Abbreviations:**
  - CAD: Coronary Artery Disease
  - MI: Myocardial Infarction
  - ACS: Acute Coronary Syndrome
  - DM: Diabetes Mellitus
- **Clinical Notes:**
  - Hypertension: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
  - Hypercholesterolemia: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
  - Diabetes: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
  - Smoking Status: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)

### References

- ACC/AHA: \( < 0.01 \) (Correlation Coefficient: 0.078 < 0.05, 0.05 ≤ Correlation Coefficient < 0.1, 0.15 ≤ Correlation Coefficient < 0.2, Correlation Coefficient ≥ 0.25)
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