Patients With Glanzmann Thrombasthenia Lacking Platelet Glycoprotein \( \alpha_{\text{IIb}}\beta_3 \) (GPIIb/IIIa) and \( \alpha,\beta_3 \) Receptors Are Not Protected From Atherosclerosis

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Background—Platelets have been suggested to play a role in the early development of atherosclerosis. As one test of this hypothesis, we assessed whether patients with Glanzmann thrombasthenia who lack platelet glycoprotein \( \alpha_{\text{IIb}}\beta_3 \) (GPIIb/IIIa) complexes or both \( \alpha_{\text{IIb}}\beta_3 \) and the more ubiquitous \( \alpha,\beta_3 \) cell membrane complexes are protected from development of atherosclerosis.

Methods and Results—Seven patients with Glanzmann thrombasthenia, 45 to 66 years of age, underwent bilateral carotid artery ultrasonography and screening for risk factors of atherosclerosis. Findings consistent with early atherosclerosis evaluated by measurement of intima-media thickness and presence of atherosclerotic plaques were observed in 6 of the 7 patients. Intima-media thickness values higher than the 75th and 90th percentiles of age- and sex-matched white control subjects of the Atherosclerosis Risk in Communities (ARIC) study were observed in 30 and 8 of 56 carotid artery measurements, respectively. Five of the 6 patients with signs consistent with early atherosclerosis lacked both \( \alpha_{\text{IIb}}\beta_3 \) and \( \alpha,\beta_3 \) complexes and 1 only lacked \( \alpha_{\text{IIb}}\beta_3 \).

Conclusions—Glanzmann thrombasthenia does not protect affected individuals from development of atherosclerosis.

Key Words: atherosclerosis ■ carotid arteries ■ imaging ■ platelets

Platelets have been implicated in the pathophysiology of the acute ischemic events that result in unstable angina and myocardial infarction because a wide range of platelet inhibitors that target thromboxane \( \mathrm{A_2} \) formation, the \( \mathrm{P2Y_12} \) ADP receptor, and the glycoprotein (GP) IIb/IIIa (\( \alpha_{\text{IIb}}\beta_3 \)) receptor have demonstrated efficacy in preventing and treating such events in large clinical trials.\(^{1,2} \) Less certain, however, is whether platelets contribute significantly to the development of the atherosclerosis that underlies the development of acute ischemic events.

A number of different theories have been proposed to explain the development of atherosclerosis, with each suggesting variable contributions from the oxidation and intimal trapping of cholesterol, monocyte/macrophage recruitment, medial smooth muscle cell proliferation and migration, platelet deposition, tissue factor–induced fibrin formation, and inflammation.

Platelets have been suggested to contribute to the development of atherosclerosis by at least two separate mechanisms. In the “response to injury” model proposed by Ross,\(^{3,4} \) platelet deposition at sites of endothelial denudation caused by a number of different insults is followed by the release of platelet-derived growth factor and other agents that elicit smooth muscle cell proliferation and/or migration. In the “incorporation of mural thrombus” model of Rokitansky, atherosclerosis progresses in a stepwise fashion as episodes of mural thrombus formation that are followed by incorporation of the thrombus into the atherosclerotic lesion.\(^{5} \) In addition to these two models, platelets may participate in the development of atherosclerosis in other ways. For example, platelets have been shown to affect both monocyte adhesion to endothelial cells and macrophage accumulation of cholesterol.\(^{6,7} \) Moreover, with activation, platelets can express and/or release a number of agents that can contribute to the inflammatory response, including chemokines and CD40 ligand (CD154).\(^{8} \)

The platelet GPIIb/IIIa receptor plays a vital role in platelet thrombus formation because it is required for platelet-platelet interactions. Patients with Glanzmann thrombasthenia (GT) who lack this receptor have a moderate to severe mucocutaneous bleeding disorder. The disorder can result from mutations in the genes encoding either \( \alpha_{\text{IIb}} \) (GPIIb) or \( \beta_3 \) (GPIIIa).
Despite the profound defect in platelet aggregation in GT, the ability of platelets to adhere and form a single layer of platelets on subendothelium remains intact because adhesion is mediated by interactions of other receptors with adhesion molecules in the subendothelium. Platelets from patients with GT can undergo the release of their internal granule contents when stimulated appropriately, but their spreading is altered by the lack of \( \alpha_{IIb} \beta_{3} \) receptors.

We have previously identified and characterized patients with GT in 2 major ethnic populations in Israel: the Iraqi Jews and Arabs. Most Iraqi-Jewish patients harbor an 11-base pair deletion in exon 12 of the \( \beta_{3} \) gene, whereas most Arab patients have a 13-base deletion in exon 4 of the \( \alpha_{IIb} \) gene. \( \beta_{3} \) is also a component of the \( \alpha_{IIb} \beta_{3} \) receptor, Iraqi-Jewish patients lack both \( \alpha_{IIb} \beta_{3} \) and \( \alpha_{IIb} \beta_{1} \), whereas Arab patients lack \( \alpha_{IIb} \beta_{3} \) but their \( \alpha_{IIb} \beta_{1} \) is preserved. Patients from both populations have a moderate to severe bleeding tendency, very prolonged bleeding times, absent platelet aggregation in response to ADP, absent clot retraction, and virtually no immunodetectable \( \alpha_{IIb} \beta_{3} \).

Noninvasive methods can detect early stages of atherosclerosis and preclinical cardiovascular disease. High-resolution ultrasound imaging can directly identify atherosclerotic plaques and their encroachment on the lumen in the carotid artery. This method was shown to be highly reproducible, and the finding of plaques in carotid arteries was shown to be associated with coronary artery disease and acute myocardial infarction. In addition, ultrasound measurements of the intima-media thickness (IMT) are highly reproducible and directly correlate with known risk factors for atherosclerosis (age, blood pressure, history of coronary heart disease, serum LDL cholesterol level, and smoking). Because atherosclerotic plaques develop in the carotid arteries earlier than in the coronary arteries and because a linear relation exists between the extent of carotid and coronary atherosclerosis, carotid ultrasonography is a valuable method for detection of early atherosclerosis.

In the present study, we assessed patients with GT for signs of carotid artery atherosclerosis.

**Methods**

**Patients**

Seven Iraqi-Jewish patients with GT (age >45 years) participated in the study. Five were homozygous for the 11-bp deletion in the \( \beta_{3} \) gene \(^1^1 \) and 2 were homozygous for a splice site mutation of the \( \alpha_{IIb} \) gene. \(^2^6 \) The study was approved by the Human Subject Research committee of the Sheba Medical Center, and all subjects gave informed consent.

**Four-Stage Evaluation**

**Interview**

The subjects were asked for demographic data and about their personal medical history, with particular attention to venous or arterial thrombotic events, atherosclerosis-related chronic disorders, and smoking history and status.

**Blood Sampling and Laboratory Tests**

Blood was drawn between 8 and 9:30 AM after fasting for at least 10 hours. Tests included glucose, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, apolipoprotein (Apo) A\(_{1}\), ApoB, lipoprotein [Lp](a), and homocysteine (the last measured by high-performance liquid chromatography). Levels of fibrinogen and factor VII activity were determined by standard methods. DNA analyses of factor V G1691A, factor II G20210A, methylenetetrahydrofolate reductase C677T, and ApoE isoforms were performed as previously described. \(^2^8 \) **Anthropometric Measurements**

The parameters measured were weight, height, blood pressure, and waist-to-hip diameter. The body mass index was expressed as kilograms (body weight)/meters squared (m=height in meters).

**Assessment of Signs Consistent With Early Atherosclerosis**

Bilateral carotid ultrasonography was performed by duplex scanner of the carotid arteries. A Diagnostics Ultramark 9 duplex scanner with a 4 to 7 imaging probe and Doppler was used. All patients were examined in the supine position, and measurements were performed by the same sonographer. The sonographer scanned the right and left common carotid arteries, the carotid bulbs, and the first 1.5 cm of the internal and external carotid arteries. For each location, the sonographer imaged the vessel in multiple planes and then focused on the interfaces required to measure IMT and any areas of focal plaques. The best images were taped and later digitized for scoring of focal plaques. Trained readers measured the average IMT across 1-cm segments of the near and far walls of the distal common carotid artery, the far wall of the carotid bulb, and the internal carotid artery on both right and left sides. Measures from each location were then averaged to produce an overall measure of carotid IMT. A computerized reading program was used. Readers also scored the ultrasound images for plaques in the common carotid, carotid bulb, internal carotid, and external carotid. A plaque was defined as a distinct area protruding into the vessel lumen with \( \geq 50\% \) greater thickness than the surrounding area.

**Data Analysis**

**End Point Definitions**

The study was designed to independently assess current and past evidence of clinical atherosclerosis, present evidence of anatomic

| TABLE 1. Cardiovascular Risk Factors in Patients With Glanzmann Thrombasthenia |
|-------------------------|--------|-----------------|----------|----------|-----------------|
| Patient | Sex | Age, y | Smoking Status | Pack-Years | BMI, kg/m\(^2\) | History of Hypertension | Dyslipidemia |
| DM | F | 65 | – | – | 30.4 | – | – |
| RY | M | 61 | – | – | 26.5 | + | + |
| YH | F | 66 | – | – | 21.8 | – | – |
| AF | F | 54 | + | 15.0 | 24.3 | – | – |
| SE | F | 51 | + | 11.5 | 26.8 | – | – |
| SS | F | 46 | – | – | 29.3 | – | – |
| IS | M | 62 | – | – | 25.8 | – | – |

+ indicates positive; –, negative.
atherosclerosis, and past evidence of acute thrombosis. Clinical atherosclerosis was defined as a history of effort angina pectoris, intermittent claudication, aortic aneurysm, coronary angioplasty, coronary artery bypass operation, carotid endarterectomy, or angioplasty of lower extremity arteries. Anatomic atherosclerosis was defined as the presence of one or more atherosclerotic plaques or an IMT greater than the 75th percentile of age- and sex-matched white control subjects in the Atherosclerosis Risk in Communities (ARIC) study. 30 Cardiovascular thrombosis was defined as a history of myocardial infarction, unstable angina, transient ischemic attack, thrombotic stroke, or arterial thromboembolism.

Statistical Analysis
Continuous variables are expressed as mean±SD. A probability value <0.05 was considered statistically significant.

Results
Five of the patients with GT were female and two were male. The age of the patients ranged between 46 and 66 years. Several cardiovascular risk factors were identified, as depicted in Table 1. None of the patients, who have been followed for more than 25 years, has manifested or given a history of acute arterial or venous thrombosis.

The lipid profile of the 7 patients is presented in Table 2. One patient had increased levels of VLDL cholesterol, ApoB, LP(a), and triglycerides, which were consistent with type IV/V dyslipidemia. Two additional patients had increased levels of Lp(a), and one of them also had an elevated level of ApoB. Six patients were homozygous for the ApoE3 isoform, and one was heterozygous for ApoE2 and ApoE3 isoforms.

Levels of fibrinogen, factor VII, and homocysteine were normal in all patients (Table 2). Molecular analyses revealed that none of the patients carried the factor V G1691A or factor II G20210A mutations. One patient was homozygous for the MTHFR C677T gene alteration.

The IMT values for the 7 patients were higher than the 75th percentile of the ARIC reference population 30 in 30 of 56 carotid artery measurements and higher than the 90th percentile in 8 measurements (Table 3). In 4 patients, between 1 and 7 plaques were observed, and in 3 of them calcifications were present as well. The median IMT values in 5 women with GT was higher than the median IMT values in age-matched white women of the ARIC study for all sites of the right and left carotid arteries (Table 4). Because of the small number of patients, statistical analysis of the difference did not seems appropriate. Of the 2 male patients, the one also affected by type IV/V dyslipidemia had IMT values higher than the 75th percentile in 7 of 8 carotid artery sites as well as 7 plaques and calcifications, whereas the other patient had no signs of atherosclerosis.

### Table 2. Laboratory Results

<table>
<thead>
<tr>
<th>Patient</th>
<th>HDL, mmol/L</th>
<th>LDL, mmol/L</th>
<th>VLDL, mmol/L</th>
<th>ApoA1, g/L</th>
<th>ApoB, g/L</th>
<th>Lp(a), mg/L</th>
<th>Total Triglycerides, mmol/L</th>
<th>Fibrinogen, μmol/L</th>
<th>Factor VII, U/dL</th>
<th>Homocysteine, μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>1.68</td>
<td>2.95</td>
<td>0.36</td>
<td>1.64</td>
<td>1.10</td>
<td>100</td>
<td>0.81</td>
<td>3.77</td>
<td>105</td>
<td>8.6</td>
</tr>
<tr>
<td>RY</td>
<td>0.91</td>
<td>2.66</td>
<td>1.68</td>
<td>1.15</td>
<td>1.49</td>
<td>410</td>
<td>5.44</td>
<td>2.96</td>
<td>150</td>
<td>7.8</td>
</tr>
<tr>
<td>YH</td>
<td>1.91</td>
<td>2.33</td>
<td>0.41</td>
<td>1.73</td>
<td>0.91</td>
<td>100</td>
<td>0.93</td>
<td>2.85</td>
<td>140</td>
<td>10.9</td>
</tr>
<tr>
<td>AF</td>
<td>1.97</td>
<td>1.78</td>
<td>0.23</td>
<td>1.59</td>
<td>0.76</td>
<td>100</td>
<td>0.52</td>
<td>2.94</td>
<td>...</td>
<td>7.5</td>
</tr>
<tr>
<td>SE</td>
<td>1.60</td>
<td>3.08</td>
<td>0.28</td>
<td>1.53</td>
<td>1.08</td>
<td>310</td>
<td>0.61</td>
<td>3.81</td>
<td>146</td>
<td>8.1</td>
</tr>
<tr>
<td>SS</td>
<td>1.09</td>
<td>3.34</td>
<td>0.57</td>
<td>1.27</td>
<td>1.40</td>
<td>420</td>
<td>1.25</td>
<td>2.92</td>
<td>121</td>
<td>7.6</td>
</tr>
<tr>
<td>IS</td>
<td>1.09</td>
<td>3.21</td>
<td>0.54</td>
<td>1.19</td>
<td>1.12</td>
<td>100</td>
<td>1.19</td>
<td>2.71</td>
<td>130</td>
<td>12.6</td>
</tr>
<tr>
<td>Normal Range</td>
<td>0.91–2.09</td>
<td>0.78–4.14</td>
<td>0.10–1.55</td>
<td>1.00–2.00</td>
<td>0.40–1.25</td>
<td>0–100</td>
<td>0.34–2.26</td>
<td>2.00–4.00</td>
<td>55–145</td>
<td>5–15</td>
</tr>
</tbody>
</table>

### Table 3. Ultrasonography Findings in Carotid Arteries of Patients With Glanzmann Thrombasthenia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, y</th>
<th>No. of Plaques</th>
<th>IMT CCA—Far</th>
<th>IMT CCA—Near</th>
<th>IMT Bulb</th>
<th>IMT ICA</th>
<th>IMT CCA—Far Calcifications</th>
<th>IMT CCA—Near Calcifications</th>
<th>IMT Bulb Calcifications</th>
<th>IMT ICA Calcifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM†</td>
<td>F</td>
<td>65</td>
<td>5</td>
<td>0.79</td>
<td>0.75</td>
<td>0.76</td>
<td>0.85‡</td>
<td>1.62§</td>
<td>2.54§</td>
<td>1.32§</td>
<td>0.80</td>
</tr>
<tr>
<td>RY†</td>
<td>M</td>
<td>61</td>
<td>7</td>
<td>0.94‡</td>
<td>0.99‡</td>
<td>0.98‡</td>
<td>1.05‡</td>
<td>1.00</td>
<td>1.29†</td>
<td>1.32‡</td>
<td>1.41†</td>
</tr>
<tr>
<td>YH∥</td>
<td>F</td>
<td>66</td>
<td>4</td>
<td>0.55</td>
<td>0.94§</td>
<td>0.64</td>
<td>1.05‡</td>
<td>1.03</td>
<td>1.32‡</td>
<td>0.64</td>
<td>0.85‡</td>
</tr>
<tr>
<td>AF†</td>
<td>F</td>
<td>54</td>
<td>1</td>
<td>0.74‡</td>
<td>0.77‡</td>
<td>0.74‡</td>
<td>0.66</td>
<td>0.82</td>
<td>1.15§</td>
<td>0.90‡</td>
<td>0.86‡</td>
</tr>
<tr>
<td>SE†</td>
<td>F</td>
<td>51</td>
<td>0</td>
<td>0.72§</td>
<td>0.77‡</td>
<td>0.73‡</td>
<td>0.80‡</td>
<td>0.83</td>
<td>0.63</td>
<td>0.67</td>
<td>0.60</td>
</tr>
<tr>
<td>SS†</td>
<td>F</td>
<td>46</td>
<td>0</td>
<td>0.72§</td>
<td>0.69§</td>
<td>0.67‡</td>
<td>0.63‡</td>
<td>0.50</td>
<td>0.84‡</td>
<td>0.53</td>
<td>0.60</td>
</tr>
<tr>
<td>IS‡</td>
<td>M</td>
<td>62</td>
<td>0</td>
<td>0.75</td>
<td>0.56</td>
<td>0.71</td>
<td>0.75</td>
<td>0.69</td>
<td>0.74</td>
<td>0.76</td>
<td>0.55</td>
</tr>
</tbody>
</table>

CCA indicates common carotid artery; ICA, internal carotid artery; +, positive; and −, negative.
†Homozygotes for the 11– base pair deletion of the β3 (GPIIIa) gene.
∥Homozygotes for a splice junction mutation in the GPIIb gene.
‡Above the 75th percentile of age- and sex-matched white women of the ARIC study. 30
§Above the 90th percentile of age- and gender-matched values in ARIC study. 30
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Of the 6 patients in whom the imaging sites were consistent with early atherosclerosis, 5 had total lack of both \( \alpha_{\text{m}} \beta_3 \) and \( \alpha_c \beta_3 \) and 1 had only lack of \( \alpha_{\text{m}} \beta_3 \).

**Discussion**

The presented data indicate that early signs of atherosclerosis can be observed in patients with GT. In 6 of 7 patients examined, including 5 who lacked \( \alpha_c \beta_3 \) as well as \( \alpha_{\text{m}} \beta_3 \), ultrasonography of the carotid arteries demonstrated signs consistent with early atherosclerosis (Table 3). Each of the 3 parameters for defining early atherosclerosis, that is, increased IMT values, presence of plaques, and calcifications, have repeatedly been shown to correlate with atherosclerotic cardiovascular diseases and atherosclerosis risk factors.\(^{13-17,19}\)

Five of the 6 patients with affected carotid arteries had atherosclerotic risk factors, that is, increased body mass, hypertension, hyperlipidemia, smoking, or increased Lp(a) level. In contrast to our findings with regard to signs consistent with early atherosclerosis, none of our patients had a history of a thrombotic cardiovascular event. Our sample size was too small, however, to conclude that GT offers protection against such events.

The lack of protection against atherosclerosis in GT may suggest that platelet–vessel wall interaction plays only a minor role or no role in the pathogenesis of atherosclerosis. Alternatively, it can be argued that those platelet functions that remain intact in patients with GT such as adhesion and release of granule contents in response to some stimuli are sufficient to support the development of atherosclerosis. Thus, our data are insufficient to exclude any role for platelets in the process.

Our finding that atherosclerosis can develop in patients lacking the \( \alpha_c \beta_3 \) receptor is also of note because \( \alpha_c \beta_3 \) is upregulated on smooth muscle cells in response to experimental vascular injury,\(^{31,32}\) and it has been implicated in smooth muscle cell migration and proliferation and in atherosclerosis progression.\(^{33,34}\) Our data indicate either that this receptor is not required for the atherosclerotic process or that there is sufficient redundancy from related receptors such as \( \alpha_c \beta_3 \) to serve the equivalent function.

Interestingly, a recent study also demonstrated lack of protection against atherosclerosis in patients with hemophilia A and von Willebrand disease.\(^{35}\) The authors of this study found indistinguishable carotid artery IMT values in patients and healthy control subjects whose mean ages and prevalences of atherosclerotic risk factors were similar. The femoral artery IMT values, however, were slightly lower in the patients with these coagulopathies. Collectively, these observations suggest that atherosclerosis can develop despite reduced thrombin generation as in patients with hemophilia A, absent platelet aggregation as in patients with GT, and reduced platelet adhesion as in patients with von Willebrand disease.

**Acknowledgments**

The authors are grateful to Ivonne Telner for her skilled technical assistance in the ultrasonographic examinations.

**References**


**TABLE 4. Median IMT Values (nm) in 5 Women With GT Patients and Age-Matched White Women From the ARIC Study**

<table>
<thead>
<tr>
<th>Carotid Artery</th>
<th>Location</th>
<th>GT Patients*</th>
<th>ARIC Study*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>CCA</td>
<td>0.73†</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>BULB</td>
<td>0.83</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>ICA</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>Left</td>
<td>CCA</td>
<td>0.77†</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>BULB</td>
<td>1.15</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>ICA</td>
<td>0.80</td>
<td>0.58</td>
</tr>
</tbody>
</table>

CCA indicates common carotid artery; ICA, internal carotid artery.

*Each value represents the median of the mean measurements.

†Average values of near and far sites.


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