Platelets From a Patient Heterozygous for the Defect of P2\textsubscript{CYC} Receptors for ADP Have a Secretion Defect Despite Normal Thromboxane A\textsubscript{2} Production and Normal Granule Stores

Further Evidence That Some Cases of Platelet ‘Primary Secretion Defect’ Are Heterozygous for a Defect of P2\textsubscript{CYC} Receptors

Marco Cattaneo, Anna Lecchi, Rossana Lombardi, Christian Gachet, Maddalena L. Zighetti

Abstract—Two unrelated patients with a congenital bleeding diathesis associated with a severe defect of the platelet ADP receptor coupled to adenylate cyclase (P2\textsubscript{CYC}) have been described so far. In one of them, platelet secretion was shown to be abnormal. We recently showed that platelets with the primary secretion defect (PSD; characterized by abnormal secretion but normal granule stores, thromboxane A\textsubscript{2} production, and ADP-induced primary wave of aggregation) have a moderate defect of P2\textsubscript{CYC}. Therefore, the interaction of ADP with the full complement of its receptors seems to be essential for normal platelet secretion, and PSD patients may be heterozygotes for the congenital severe defect of P2\textsubscript{CYC}. In this study, we describe 2 new related patients with a severe defect of P2\textsubscript{CYC} and the son of one of them, who is to be considered an obligate heterozygote for the defect. The 2 patients with the severe defect had lifelong histories of abnormal bleeding, prolonged bleeding times, abnormalities of platelet aggregation and secretion, lack of inhibition of adenylate cyclase by ADP, and a deficiency of platelet-binding sites for [\textsuperscript{33}P]2 MeS-ADP (240 and 225 sites per platelet; normal range, 530 to 1102). The son of one of them had a mildly prolonged bleeding time and abnormalities of platelet aggregation and secretion similar to those found in patients with PSD. In addition, his platelets showed a moderate defect of binding sites for [\textsuperscript{33}P]2 MeS-ADP (430 sites per platelet) and of adenylate cyclase inhibition by ADP. This study of a family with the platelet disorder characterized by a defect of the platelet P2\textsubscript{CYC} receptor supports our hypothesis that the full complement of the platelet ADP receptors is essential for normal platelet secretion and that some patients with the common, ill-defined diagnosis of PSD are actually heterozygous for the defect. (Arterioscler Thromb Vasc Biol. 2000;20:e101-e106.)

Key Words: ADP ■ purinoceptors ■ platelet secretion ■ congenital disorders of platelet function ■ thromboxane A\textsubscript{2}

Platelet secretion defects are the most common congenital abnormalities of platelet function.\textsuperscript{1,2} They include storage pool deficiency (characterized by a diminished content of platelet granule constituents), defective arachidonate metabolism (aspirin-like defects), and an ill-defined and heterogeneous group of abnormalities characterized by abnormal secretion but a normal content of platelet granule constituents and arachidonate metabolism (sometimes referred to as primary secretion defects, or PSD).\textsuperscript{1-3}

We recently showed that released ADP potentiates platelet secretion induced by the thromboxane mimetic U46619 independently of the formation of large aggregates and thromboxane A\textsubscript{2} production, and that at least some of the PSD patients have a partial reduction of platelet-binding sites for [\textsuperscript{33}P]2 MeS-ADP.\textsuperscript{3} This finding suggested to us that some PSD patients have a partial defect of platelet ADP receptors, which is responsible for their secretion defect. Because the inhibition of adenylate cyclase in PSD platelets was partially impaired, we hypothesized that these individuals may be heterozygotes for the severe defect of platelet ADP receptors coupled to adenylate cyclase and termed P2TAC or P2\textsubscript{CYC}.\textsuperscript{4-11} Two unrelated patients with a congenital, severe defect of P2\textsubscript{CYC} have been described so far.\textsuperscript{12,13} Their platelets, when exposed to ADP, change shape normally but undergo no or only very slight and rapidly reversible aggregation and do not exhibit the normal inhibition of prostaglandin (PG) E\textsubscript{1}-stimulated adenylcyclase. In addition, they have a severely reduced number of binding sites for the ADP analogue 2 MeS-ADP.\textsuperscript{13,14} Platelet secretion induced by several agonists was studied in 1 of the 2 patients and found to be severely impaired.\textsuperscript{12}

Received March 14, 2000; revision accepted June 14, 2000.


Correspondence to M. Cattaneo, MD. Hemophilia and Thrombosis Center, Via Pace 9, 20122 Milano, Italy. E-mail marco.cattaneo@unimi.it

© 2000 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
In the present study, we describe 2 sisters with a severe defect of platelet-binding sites for 2 MeS-ADP and abnormalities of platelet function identical to those of the 2 previously described patients, suggesting that they also have a severe defect of P2Y12. The son of one of them had a moderate deficiency of platelet-binding sites for 2 MeS-ADP, and his platelets had a secretion defect and partial impairment of inhibition of adenylyl cyclase by ADP, similar to that found in platelets with PSD. The results of this study, therefore, support our initial hypothesis that some patients with PSD are heterozygous for the congenital, severe defect of platelet-binding sites for 2 MeS-ADP, and abnor-

Methods

Patients

The proband (II-4 in Figure I), a 57-year-old woman born from first cousins and living in southern Italy, was referred to our center for a lifelong history of easy bruising, menorrhagia, and severe bleeding complications after dental extractions or major surgery. Of her family members, only her 48-year-old sister (II-6), who was living in Milan, could be studied. She had a lifelong history of easy bruising, epistaxis, menorrhagia, and moderate bleeding complications after dental extractions. Her 13-year-old son (III-1) and 45-year-old daughter (III-2) were also accepted to be investigated. All studied subjects had normal coagulation tests (prothrombin time and activated partial thrombo-

Materials

2 MeS-ADP was from Boehringer. [33P]2 MeS-ADP was from New England Nuclear. ADP, adenosine 2-phosphate 5-phosphate (A2P5P), epinephrine, collagen, the thromboxane/prostaglandin endoperoxide analogue 9,11-dideoxy-11-epoxymethano-prostaglan-
din F1 (U66619), platelet-activating factor (PAF-acether), o-phthalaldehyde, prostaglandin I2 (PGI2), prostaglandin E1 (PGE1), and acetylsalicylic acid (ASA) were from Sigma. AR-C69931 MX, a P2Y12 antagonist, was a kind gift of AstraZeneca R&D (Charnwood, UK). Fura 2 AM was from Calbiochem. Apyrase was a kind gift of Dr R.L. Kinlough-Rathbone (McMaster University, Hamilton, Ontario, Canada). All other products were of reagent grade or better. Commercial preparations of luciferin/luciferase reagent were used to measure the platelet ATP and ADP contents (ATP assay kit, BioOrbit Oy) and platelet secretion concurrently with platelet aggregation (ChronoLume). The radioimmunoassay kit for measurement of TxB2 was from New England Nuclear. The bleeding time was measured with the Sympate II disposable device (Organon Teknika Corp).

Preparation of Platelet-Rich Plasma (PRP) and Washed Platelet Suspensions

Nine volumes of blood was drawn into 1 volume of 129 mmol/L trisodium citrate for preparation of PRP, and 6 volumes was drawn into 1 volume of acid-citrate-dextrose anticoagulant for preparation of washed platelet suspensions. Twice, washed platelet suspensions were prepared according to the method described by Mustard et al,15 with the exception that 500 mmol/L PGI2 was added during the first and second wash.14 Platelet counts were adjusted to 3×10^10/L in PRP and to 400×10^9/L in washed platelet suspensions. For measurement of cytoplasmic concentrations of ionized calcium ([Ca^2+]i), platelets were loaded with 2 μmol/L fura 2 AM for 45 minutes at 37°C as previously described.12

Platelet Aggregation and Secretion Studies

Samples of PRP (0.45 mL) were incubated with 50 μL of luciferin/ luciferase reagent at 37°C for 30 seconds and stirred at 1000 rpm in a lumiggregometer (Lumig-grometer, Chrono-log Corp). After incubation, 10 μL of an aggregating agent was added, and the aggregation and ATP secretion tracings were recorded for 3 minutes.

Measurement of Platelet Shape Change

Platelet shape change induced by 0.1 or 1 μmol/L ADP was measured in the aggregometer by using citrated PRP to which 2 mmol/L EDTA had been added to prevent platelet aggregation. Decreases in oscillations of the basal tracings and increases in optical density were interpreted as being caused by platelet shape change.

Measurement of [Ca^2+]i

Aliquots of fura 2 AM–loaded platelets were transferred to quartz cuvettes maintained at 37°C. Fluorescence was monitored continuously before and after stimulation with ADP by using a spectrophluo-

Measurement of Platelet Granule Content

Total platelet ADP and ATP content was measured by the firefly luciferin/luciferase method16 in a luminometer (luminometer 1250, LKB, BioOrbit Oy). Platelet serotonin (5-HT) content was measured with the o-phthalaldialdehyde method.17 Fibrinogen was measured in washed platelet lysates by an ELISA with a polyclonal antibody (Atlantic Antibodies) conjugated with peroxi-

Measurement of Platelet Thromboxane B2 Production

Thromboxane B2 (TxB2) was measured in supernatant serum of blood samples collected in glass tubes containing no anticoagulant and clotted at 37°C for 2 hours.

Binding of [33P]2 MeS-ADP to Washed Platelets

Binding of [33P]2 MeS-ADP (872 to 1044 Ci/mm) to washed platelets was measured as described.14 [33P]2 MeS-ADP (0.1 mmol/L) with increasing concentrations of unlabeled ligand (0 to 49.9 mmol/L) was incubated with washed platelet suspensions (1 mL final volume) at 37°C for 5 minutes. The ligand bound to platelets was separated from free ligand by filtration through Whatman GF/C glass filters under vacuum. Radioactivity bound to the platelets on the filters was measured by scintillation counting (Minibetta 1211, LKB). Nonspecific binding, determined by incubation in the presence of 1 μmol/L 2 MeS-ADP, amounted to 1% to 3% of total binding. Data were analyzed by a computer program (LIGAND).19
Measurement of Platelet cAMP
Platelet cAMP was measured by a radioisotopic assay with a commercially available kit (Amersham International). Duplicate samples of 1 mL of citrated PRP containing 1 mmol/L theophylline were incubated with Tyrode’s solution, PGE2 (1 μmol/L) plus Tyrode’s solution, or PGE2 plus ADP (0.1, 1, and 10 μmol/L). After incubation at 37°C for 2 minutes, 1 mL of 5% trichloroacetic acid was added, and the samples were snap-frozen in dry ice and methanol, thawed at room temperature, and then shaken at 4°C for 45 minutes. After centrifugation at 4°C for 30 minutes, the supernatant was extracted 3 times with 5 mL of water-saturated ether, dried under a stream of N2 at 60°C, and stored at −20°C. Before assay, the samples were reconstituted with 0.05 mol/L Tris buffer containing 4 mmol/L EDTA.

Results
The bleeding time was prolonged in the proposita (II-4), her sister (II-6), and her nephew (III-1) and normal in II-7, her brother-in-law (II-6 in Table I). The platelet count and contents of ADP, ATP, 5-HT, and fibrinogen were normal in all subjects except in II-7, whose platelet content of 5-HT and ADP was slightly lower than normal (Table I), suggesting that he had a mild δ-storage-pool deficiency.

Platelet Aggregation and ATP Secretion
ADP at concentrations ranging from 2 to 20 μmol/L induced a very small and rapidly reversible wave of platelet aggregation in PRP of II-4 and II-6 (Figure II). Platelet aggregation induced by 2 or 4 μmol/L ADP was reversible in III-1 (son of II-6 and II-7) and comparable to that of normal platelets that had been incubated in vitro with 0.5 mmol/L ASA for 30 minutes (Figure II). Higher concentrations of ADP (up to 20 μmol/L) induced normal platelet aggregation in PRP of III-1 (Figure II). The P2Y1 antagonist A2P5P (1 mmol/L), which was tested in II-6 (Figure III) and III-1 (not shown), completely abolished platelet aggregation induced by 2 μmol/L ADP. Platelet aggregation induced by 0.2 μmol/L PAF-acether or 0.5 μmol/L U46619 was reversible in II-4, II-6, and III-1, whereas it was irreversible in normal controls (not shown). The extent of platelet aggregation induced by collagen (2 μg/mL) was normal in PRP of III-1 and ≈50% of normal in that of II-4 and II-6 (not shown). Platelet aggregation induced by any agonist tested was normal in II-7 (husband of II-6), despite his mild δ-storage-pool deficiency, confirming that platelet aggregation may be normal in patients with this abnormality.20

The platelet ATP secretion induced by any of the tested agonists was absent or severely impaired in the proposita (II-4) and her sister (II-6) (Table II). In III-1 (son of II-6 and II-7), it was absent or severely reduced when induced by ADP or U46619 and borderline-low when induced by PAF-acether or collagen (Table II). It was borderline-low with any tested agonist in II-7 (Table II), the husband of II-6 and father of III-1, whose platelet concentration of ADP and 5-HT was slightly reduced.

Platelet Shape Change
ADP (0.1 and 1 μmol/L) caused normal shape change of platelets from II-6 (Figure III) and III-1 (not shown), which was completely inhibited by 1 mmol/L A2P5P. Platelet shape change was not studied in II-4 and II-7.

ADP-Induced Increase in [Ca2+]i
In the presence of 2 mmol/L external CaCl2, stimulation with 5 μmol/L ADP caused a rapid increase in [Ca2+]i, which was

---

**Table I. Routine Parameters of Platelet (plt) Function in the 4 Patients Studied**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>II-4</th>
<th>II-6</th>
<th>III-1</th>
<th>II-7</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time, min</td>
<td>&gt;30</td>
<td>20</td>
<td>13</td>
<td>3.5</td>
<td>2–8</td>
</tr>
<tr>
<td>Platelet count, ×10⁹/μL</td>
<td>335</td>
<td>147</td>
<td>174</td>
<td>263</td>
<td>150–347</td>
</tr>
<tr>
<td>Platelet 5-HT, nmol/10⁹ plt</td>
<td>2.6</td>
<td>3.5</td>
<td>4.5</td>
<td>1.6</td>
<td>2.1–6.7</td>
</tr>
<tr>
<td>Platelet ADP, nmol/10⁹ plt</td>
<td>18.6</td>
<td>28.1</td>
<td>50.4</td>
<td>12.0</td>
<td>13.5–33.9</td>
</tr>
<tr>
<td>Platelet ATP, nmol/10⁹ plt</td>
<td>38.9</td>
<td>64.2</td>
<td>67.3</td>
<td>49.5</td>
<td>35.7–80.5</td>
</tr>
<tr>
<td>Platelet fibrinogen, nmol/10⁹ plt</td>
<td>0.35</td>
<td>0.23</td>
<td>0.18</td>
<td>0.17</td>
<td>0.1–0.6</td>
</tr>
<tr>
<td>Serum TxB₂, nmol/10⁹ plt</td>
<td>ND</td>
<td>13.5</td>
<td>14.1</td>
<td>3.4</td>
<td>1.8–26</td>
</tr>
</tbody>
</table>

Patient values are the mean of at least 2 different determinations. Normal ranges are set to the 2.5th and 97.5th percentiles of the distribution of values obtained in 55 healthy subjects.

---

Figure II. Tracings of platelet aggregation (upper) and ATP secretion (lower) recorded simultaneously with a lumiaggregometer (see Methods for details). ADP at indicated concentrations (2, 4, and 20 μmol/L) was added to samples of citrated PRP from a normal subject before and 30 minutes after incubation with 0.5 mmol/L ASA and from patients II-4, II-6, II-7, and III-1 (see Figure I). The recordings were stopped 3 minutes after addition of ADP.
similar in platelets from II-6 and a normal control; in the absence of external Ca\(^{2+}\) (ie, with 0.2 mmol/L EGTA), the mobilization of \([\text{Ca}^{2+}]_{i}\) was lower, but again it was similar in II-6 and a normal control; the ADP-induced increases in \([\text{Ca}^{2+}]_{i}\) were completely abolished by 0.5 mmol/L A2P5P in both II-6 and normal platelets (data not shown). The mobilization of \([\text{Ca}^{2+}]_{i}\) was not studied in the other patients.

### Inhibition of PGE\(_{1}\)-Induced Increase in Platelet cAMP

The basal levels of platelet cAMP in the 4 patients studied were normal (5.9 pmol/10\(^9\) in II-4, 12.5 in II-6, 9.1 in III-1, and 12.5 in II-7; normal range, 6 to 14.2 pmol/10\(^9\), \(n=18\)) and increased normally after stimulation with PGE\(_{1}\) (32.3 in II-4, 54.8 in II-6, 35.6 in III-1, and 44.3 in II-7; normal range, 22.5 to 68.6). ADP (0.1 to 10 \(\mu\)mol/L) did not affect the PGE\(_{1}\)-induced increase in cAMP levels in platelets from patients II-4 and II-6, whereas it inhibited the cAMP increase in a concentration-dependent manner in platelets from healthy subjects and patients II-7 and III-1 (Figure IV). However, although the degree of inhibition of the PGE\(_{1}\)-induced increase in cAMP levels in platelets from patients II-4 and II-6, whereas it inhibited the cAMP increase in a concentration-dependent manner in platelets from healthy subjects and patients II-7 and III-1 (Figure IV). However, although the degree of inhibition of the PGE\(_{1}\)-induced increase in platelet cAMP was very similar to that seen in healthy subjects for patient II-7, it was much less for patient III-1 at all ADP concentrations tested (Figure III).

### [\(^{33}\text{P}\)]2 MeS-ADP Binding to Washed Platelets

Binding experiments were performed in 29 healthy subjects and in at least 2 different sessions in the 4 patients studied. Specific binding of [\(^{33}\text{P}\)]2 MeS-ADP to washed normal and patient platelets was saturable and the Scatchard plot\(^{21}\) was linear. For all patients, the \(K_d\) values were comparable to those calculated for normal volunteers (Table III). In contrast, the number of binding sites was severely decreased in the proposita II-4 and her sister II-6, moderately decreased in III-1, and normal in II-7 (Table III).

### Discussion

Three purinergic receptors are currently believed to contribute separately to the complex platelet responses to ADP: the P2X\(_1\) ionotropic receptor, responsible for rapid influx of ionized calcium into the cytosol; the P2Y\(_1\) metabotropic receptor, responsible for mobilization of ionized calcium from internal stores, platelet shape change, and initial aggregation; and an as-yet-unidentified receptor coupled to G\(_{\alpha}\), which is essential for the full aggregation response to ADP and is termed P2\(_{\text{cyc}}\), or P2T\(_{\text{AC}}\).\(^{4–11}\) The elusive P2\(_{\text{cyc}}\) receptor is probably the molecular target of thienopyridine compounds and other selective inhibitors of ADP-induced platelet aggregation.\(^{10,22–25}\) In addition, it is probably defective in patients

### Table II. Platelet (plt) Secretion Induced by Different Agonists From Platelets of the 4 Patients Studied

<table>
<thead>
<tr>
<th>Agonist</th>
<th>ATP Secreted, pmol/10(^9) plt</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP, 2 (\mu)mol/L</td>
<td>0</td>
<td>0.2–1.1</td>
</tr>
<tr>
<td>ADP, 4 (\mu)mol/L</td>
<td>0</td>
<td>0.3–6.4</td>
</tr>
<tr>
<td>ADP, 20 (\mu)mol/L</td>
<td>0</td>
<td>0.7–6.2</td>
</tr>
<tr>
<td>PAF-acether, 0.2 (\mu)mol/L</td>
<td>0</td>
<td>0.5–9.6</td>
</tr>
<tr>
<td>U46619, 0.5 (\mu)mol/L</td>
<td>0</td>
<td>1.2–9.2</td>
</tr>
<tr>
<td>Collagen, 2 (\mu)g/mL</td>
<td>0.7</td>
<td>2.1–14.5</td>
</tr>
</tbody>
</table>

Patient values are the mean of at least 2 different determinations. Normal ranges are set to the 2.5th and 97.5th percentiles of the distribution of values obtained in 18 to 50 healthy subjects.

### Table III. Binding Parameters of [\(^{33}\text{P}\)]2 MeS-ADP to Patient and Control Platelets

<table>
<thead>
<tr>
<th>Patients</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-4</td>
<td>240</td>
</tr>
<tr>
<td>II-6</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Patient values are the mean of at least 2 different determinations. Normal ranges are set to the 2.5th and 97.5th percentiles of the distribution of values obtained in 29 healthy subjects.
with a congenital bleeding diathesis characterized by a severe
defect of platelet responses to ADP. Two such patients have
been described so far by our group and by Nurden et al: the
many similarities between the 2 patients suggest that they
are affected by the same type of abnormality of platelet ADP
receptors.2,6

In this study, we describe 2 sisters with a congenital
bleeding tendency characterized by a platelet function defect
that is similar to that of the previously described patients:
(1) severe impairment of aggregation but normal shape
change induced by ADP; (2) lack of adenylyl cyclase
inhibition by ADP; (3) normal mobilization of Ca2+ from
internal stores induced by ADP, which was completely
inhibited by the P2Y1 receptor antagonist A2P5P; and (4)
decreased number of binding sites for 13P]2 MeS-ADP. In
addition, similar to those of the first patient described by our
group,12 the patients’ platelet secretion of δ-granule consti-
ituents induced by several agonists was severely impaired,
confirming our previous observation that ADP plays an
important role in the potentiation of platelet secretion stimu-
lated by release-inducing agonists.3

Another similarity between the 2 patients described in this
study and those previously described12,13 is that all were born
from consanguineous parents, indicating that their platelet
defect is most likely inherited as a recessive trait. As
expected, the platelets of the son of 1 of them, who is an
obligate heterozygote, had a number of binding sites for 2
MeS-ADP that was intermediate between normal and that of
his mother and aunt. ADP induced a normal primary wave of
aggregation of his platelets and partially inhibited adenylate
cyclase. In addition, a variety of agonists, including the TxA2
mimetic U46619, PAF-acether, and collagen, induced im-
paired or borderline-low secretion of ATP from his platelets.
This secretion defect was not caused by impaired production
of TxA2 or low concentrations of platelet granule contents
and is therefore, very similar to that described in patients with
an ill-defined and probably heterogeneous group of congen-
ital defects of platelet secretion, sometimes referred to by the
general term “primary secretion defect” (PSD).3 This defect,
which is the most common congenital abnormality of platelet
secretion, is characterized by abnormal/borderline-low plate-
let secretion induced by different agonists, a normal primary
wave of aggregation induced by ADP, normal granule stores,
and normal arachidonate metabolism.1 The results of this
study therefore confirm our previous hypothesis that some
patients with PSD are heterozygotes for the severe defect of
P2CYC, the platelet ADP receptor that is coupled to adenylyl
cyclase.3 The important role of ADP interaction with this
receptor in primary hemostasis is emphasized by the finding
that patient III-1, like others with PSD, has, despite the mild
defect of P2CYC, a mild prolongation of the bleeding time. The
bleeding history, which is usually mild in PSD, was negative
in patient III-1, probably because he had never been exposed
to situations at risk for excessive bleeding owing to his young
age.

ADP is a weak agonist because by itself it does not trigger
platelet secretion directly.27 When exogenous ADP is added
to a normal platelet suspension, it causes shape change and
aggregation. It is the close platelet-to-platelet contact brought
about by the aggregation process that, in a minority of
individuals, triggers the formation of trace amounts of TxA2,
which stimulates the secretion of small amounts of δ-granule
constituents and reinforces aggregation.10,28–30 This effect is
greatly enhanced and can be observed in most individuals
when the concentration of ionized calcium in the extracellular
medium is artificially decreased to the micromolar level, such as
in citrated PRP.28,30 On the other hand, the mechanism that
is responsible for the potentiation of platelet secretion by
ADP released from the platelet δ-granules, which was demon-
strated for the first time by our group in 1997,3 is quite
different from that triggered by exogenous ADP because it is
independent of TxA2 synthesis and the formation of large
platelet aggregates.3 It is likely that although ADP by itself
cannot directly stimulate the secretion of δ-granule constitu-
ents, it does potentiate platelet secretion induced by other
agonists. The results of the present study and of our previous
one3 indicate that the full complement of platelet ADP receptors
is necessary for the potentiation of platelet secretion by ADP, whereas it is not essential for normal aggregation.

Based on current knowledge, it is unknown whether the
potentiation of platelet secretion by ADP is mediated solely
by its interaction with P2CYC or also by the other platelet
purinoceptors. In 2 preliminary reports of congenital defects of
platelet P2Y1,31 and P2X132 receptors, both associated with
abnormal platelet aggregation and a mild bleeding diathesis,
platelet secretion was not studied. However, recent unpublished data in P2Y1 knockout mice34 (Gachet et al, 2000) and
in human platelets incubated with selective P2Y1 and P2CYC
agonists (Cattaneo et al, 2000) indicate that the P2CYC
receptor is the main receptor responsible for the potentiation
of platelet secretion, suggesting that the Gi pathway cross-
talks with other pathways of platelet activation. The molec-
ular mechanisms for this are still unknown but could involve
phosphoinositide 3-kinase activation,35 tyrosine kinases spe-
cifically linked to this receptor and/or the vasodilator-
stimulated phosphoprotein.36,37 Thus, the patients described
here should help in better defining the selective role of the
P2CYC receptor in primary hemostasis.

In conclusion, our study describes 2 new patients with a
congenital bleeding diathesis associated with a severe defect
of platelet P2CYC receptors. In addition, the possibility of
studying the son of 1 of the 2 patients, who is an obligate
heterozygote for the disease, allowed us to confirm our
previous hypothesis that some patients with the common PSD
are heterozygotes for the disease and that the interaction of
released ADP with the full complement of its platelet recep-
tors is essential for normal platelet secretion.

References

1. Rao AK. Congenital disorders of platelet function: disorders of signal
2. Bennett JS. Hereditary disorders of platelet function. In: Hoffman R,
Benz EJ Jr, Shattil SS, Furie B, Cohen HJ, Silberstein LE, McGlave P.
Livingstone; 2000;2154–2172.
3. Cattaneo M, Lombardi R, Zighetti ML, Gachet C, Ohlman P, Cazenev-
J-P, Mannucci PM. Deficiency of 13P]2 MeS-ADP binding sites on
platelets with secretion defect, normal granule stores and normal
thromboxaneA2 production: evidence that ADP potentiates platelet
secretion independently of the formation of large platelet aggregates
Molecular basis for ADP-induced platelet activation, I: evidence for three
distinct ADP receptors on human platelets. J Biol Chem. 1998;273:
2024–2029.


33. Deleted in proof.


Platelets From a Patient Heterozygous for the Defect of P2\textsubscript{CYC} Receptors for ADP Have a Secretion Defect Despite Normal Thromboxane A\textsubscript{2} Production and Normal Granule Stores: Further Evidence That Some Cases of Platelet 'Primary Secretion Defect' Are Heterozygous for a Defect of P2CYC Receptors

Marco Cattaneo, Anna Lecchi, Rossana Lombardi, Christian Gachet and Maddalena L. Zighetti

Arterioscler Thromb Vasc Biol. 2000;20:e101-e106
doi: 10.1161/01.ATV.20.11.101

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/11/e101

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/