The Arg633His substitution responsible for the private platelet antigen Gro^a unravelled by SSCP analysis and direct sequencing

S. SIMSEK,¹ C. FOLMAN,¹ C. E. VAN DER SCHOOT¹ AND A. E. G. KR. VON DEM BORNE^{1,2} ¹Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam; ²Department of Haematology and Centre for Blood Cell Research, Academic Medical Centre, Amsterdam, The Netherlands

Received 16 August 1996; accepted for publication 24 January 1997

Summary. We have previously described the private or family platelet antigen, Gro^a, which was identified in a case of neonatal alloimmune thrombocytopenia. The Gro^a antigen was found to be located on the GP IIIa (β_3) subunit of the GP IIb/IIIa complex, the most prominent fibrinogen receptor of platelets. Initial experiments to characterize the Gro^a antigen at the molecular genetic level were unsuccessful. We therefore decided to use a different strategy to unravel the molecular basis of this antigen. Platelet GP IIIa mRNA of a Gro(a⁺) and a Gro(a⁻) donor was amplified with suitable primers in a reverse transcriptase–polymerase chain reaction (RT-PCR) and subjected to single-strand conformational polymorphism (SSCP) analysis. Three regions of the amplified GP IIIa cDNA derived from the Gro(a⁺) donor showed a different SSCP pattern when compared to that of the Gro(a⁻) donor. Direct nucleotide sequence analysis of these three segments revealed that two of them contained silent substitutions, A1163C,

A1553G and G1565A. The first and the latter changes were described previously. In the third segment a G1996A mutation was found, predicting an arginine → histidine substitution at position 633 of the mature glycoprotein. PCR-ASRA (allele-specific restriction enzyme analysis) performed on cDNA as well as on genomic DNA with the restriction enzyme *Mae*III showed that the His633 form of GPIIIa is restricted to the Gro(a⁺) phenotype. The observed mutation is three amino acids upstream of the mutation underlying the HPA-8/Sr system (Arg636Cys), suggesting this region of GP IIIa to be susceptible for mutations. Moreover, the presence of a silent mutation and two low-frequency forms of the silent polymorphisms strongly suggests that the G1996A mutation did not occur in a direct ancestral allele.

Keywords: NAITP, platelet GP IIIa, polymorphism, PCR-SSCP, PCR-ASRA.

Human platelet antigens (HPA) can be targets for alloantibodies in neonatal alloimmune thrombocytopenia (NAITP) and transfusion reactions. HPAs are antigenic determinants of platelet membrane glycoproteins (Simsek & von dem Bourne, 1994). The GP IIIa (β_3) subunit of the glycoprotein (GP) IIb/IIIa complex (Ginsberg *et al.*, 1988; Phillips *et al.*, 1991) (also known as the integrin $\alpha_{IIb}\beta_3$) (Hynes, 1987), which functions as a fibrinogen receptor on platelets, carries the majority of these alloantigen determinants, i.e. those belonging to the HPA-1, -4, -6W, -7W, -8W systems (van der Schoot *et al.*, 1986; Rosa & McEver, 1989; McFarland *et al.*, 1993; Kuijpers *et al.*, 1993; Kroll *et al.*, 1990). The recently described private platelet antigen Gro^a also resides on GP IIIa (Simsek *et al.*, 1994b).

Correspondence: Dr S. Simsek, Department of Haematology, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

The basis for all known HPA systems lies in single point mutations, leading to a single amino acid substitution (Kuijpers *et al.*, 1992, 1993; Newman *et al.*, 1989; Lyman *et al.*, 1990; Wang *et al.*, 1991, 1993; Simsek *et al.*, 1994a; Santos *et al.*, 1994; Noris *et al.*, 1995).

In a previous study the entire coding sequence of GP IIIa cDNA derived from a Gro(a⁺) donor was amplified in six overlapping fragments. Subsequent nucleotide sequence analysis of at least 16 randomly chosen subclones after subcloning the PCR products in the plasmid vector pUC18 did not show a consistent mutation that could be held responsible for the Gro^a antigen (Simsek *et al.*, 1994b), which suggested that the Gro^a antigen was the result of a post-translational modification of GP IIIa.

However, in the present study we found that, by using a combination of single-strand conformational polymorphism (SSCP) (Jin *et al.*, 1993; Orita *et al.*, 1989) analysis and direct

nucleotide sequence analysis, that also the Gro^a antigen is caused by a single point mutation. An Arg633His substitution in the DNA encoding platelet GP IIIa, near the polymorphism underlying the Sr^a/HPA-8 system (Arg636 Cys) (Santos *et al.*, 1994), was found to be associated with the Gro^a antigen.

MATERIAL AND METHODS

Blood. Blood from the family members and from unrelated healthy donors was drawn by venepuncture. The Gro(a) phenotype of the platelets was determined by the platelet immunofluorescence test (von dem Borne *et al.*, 1978) and monoclonal antibody-specific immobilization of platelet antigens (MAIPA)-assay (Kiefel *et al.*, 1987). In the MAIPA technique, a combination of a monoclonal antibody directed against the β_3 -chain of the GP IIb/IIIa (α IIb β_3) and anti-Gro^a antibodies was used.

Molecular characterization of the Gro^a polymorphism. Platelet RNA and genomic DNA was prepared from Gro(a⁺) and Gro(a⁻) donors, according to established techniques (Chomczynski & Sacchi, 1987; Ciulla *et al.*, 1988).

cDNA was synthesized with 200 U of MoMuLV reverse transcriptase (RT) (GIBCO BRL, Grand Island, N.Y., U.S.A.), in a volume of 25 μ l containing 7.5 μ l stretched RNA template, 30 OD pd(N)₆ (Pharmacia, Uppsala), 0.5 mM dNTP mix (dATP, dCTP, dGTP, dTTP), 10 mM DTT, 30 U RNasin (Promega, Madison, Wis.) and the 5 $\times$ reaction buffer at 42°C according to the manufacturer's instructions. 50 μ l of water were added to the cDNA mixture. Suitable oligonucleotide primers were used in the PCR to amplify the entire coding region of GP IIIa (Rosa *et al.*, 1988). PCR was performed with cDNA or genomic DNA as a template, 20 pmol of each primer and 2 U of Taq DNA polymerase in a buffer recommended by the manufacturer, in a total volume of 50 μ l. The first cycle of the PCR was performed at 95°C for 5 min, followed by 34 cycles of 1 min at 95°C, 1.5 min at 52°C, 2.5 min at 72°C, and finally one cycle of 1 min at 95°C, 1.5 min at 52°C and 9 min 72°C.

Single-strand conformational polymorphism (SSCP). 11 overlapping segments of the cDNA coding for GP IIIa derived from Gro(a⁺) and Gro(a⁻) individuals were amplified and subjected to SSCP analysis (Jin *et al.*, 1993). An aliquot of PCR product (2 μ l) was mixed with an equal volume (2 μ l) of a solution containing 95% formamide, 0.5% bromophenol blue and 0.5% xylene cyanol. After 3 min of denaturation at 100°C, the samples were placed immediately on ice for at least 2 min. The samples were loaded on a nondenaturing gel (7.5% homogenous PhastGel) and electrophoresed with a PhastSystem at 320 V for 15 min at 15°C (Peyruchaud *et al.*, 1995). The gels were silverstained according to the method described in the PhastSystem manual (silver staining method optimized for native PAGE). The PCR product (size 266 bp) in which an abnormal pattern was observed was amplified with oligonucleotide primers 21: 5'-tagaattC AAGTGTGAATGTGGCAGC-3' (nucleotides 1835-1853, extended at its 5' end with an EcoRI site) and 21A: 5'-GACGACACAGTCATCCTCATT-3' (nucleotides 2093-2073). The nucleotide sequence of the PCR products displaying

conformational polymorphisms were determined directly by the dideoxy-chain-termination method (Sanger *et al.*, 1977) using the Sequenase PCR Product Sequencing Kit (United States Biochemicals, Cleveland, Ohio, U.S.A.).

DNA typing for Gro^a by PCR-ASRA (allele-specific restriction enzyme analysis). Oligonucleotide primers corresponding to genomic DNA sequences that flank the Gro^a polymorphism, were designed in order to amplify this region (primers exon XIa: 5'-TTGCCTTAATCACTGTGTCCT-3' and exon XIb: 5'-CC TGGGTGTGTGCAACTCTAA-3') (Zimrin *et al.*, 1990). MaeIII restriction digestions of PCR products derived from genomic DNA were carried out under conditions recommended by the manufacturers (New England Biolabs, Beverly, Mass.). Restriction digests were analysed on 10% polyacrylamide gels.

RESULTS

RT-PCR and SSCP analysis

Eleven overlapping segments of the cDNA coding for GP IIIa derived from Gro(a⁺) and Gro(a⁻) individuals were amplified and subjected to SSCP analysis. Three segments from Gro(a⁺) subjects showed a different SSCP pattern when compared to these segments from Gro(a⁻) subjects. Fig 1 shows the abnormally migrating fragment that was generated from cDNA with oligonucleotide primers 21 and 21A, unique to the Gro(a⁺) sample (lane 2), compared to the Gro(a⁻) sample (lane 1). The nucleotide sequences of the three segments were determined in Gro(a⁺) and Gro(a⁻) individuals. They were compared with the previously reported sequence of GP IIIa (Rosa *et al.*, 1988). A single base change G $\rightarrow$ A at position 1996 (Fig 2) of the GP IIIa cDNA was identified, predicting an arginine (CGT) Gro(a⁻) allotype and histidine (CAT) in the Gro(a⁺) allotype at amino acid 633. Moreover, the Gro(a⁺) individual was found to be heterozygous for the reported silent nucleotide polymorphisms A $\leftrightarrow$ C at position 1163 (Val355) and G $\leftrightarrow$ A at position 1565. In addition, another silent nucleotide mutation G1553A (Glu485) was encountered in the Gro(a⁺) cDNA. No other mutations were found.

Fig 1. Single-strand conformational polymorphism (SSCP) analysis of amplified GP IIIa cDNA derived from a Gro(a⁻) and Gro(a⁺) donor. GP IIIa cDNA was PCR amplified from nucleotides 1835-2093 (primers 21 and 21A). After amplification, an aliquot of the PCR fragment was subjected to SSCP analysis using the PhastGel system (non-denaturing gel electrophoresis and silver staining method). The SSCP pattern of the Gro(a⁻) sample shows two bands (lane 1), whereas that of the Gro(a⁺) sample shows more than two bands (lane 2).

Fig 2. Direct sequence analysis of PCR-amplified cDNA. The region from GP IIIa 1989–2003 is shown. The sequence of the Gro(a⁻) individual is identical to the previously published sequence (Rosa *et al*, 1988), a G at position 1996. The sequence derived from the Gro(a⁺) individual has also the G at position 1996, but in addition an A at the same position can be seen. The G→A mutation results in an Arg (CGT) to a His (CAT) substitution at amino acid position 633 of GP IIIa.

Detection of the G→A polymorphism by MaeIII ASRA

Studies on cDNA. The G→A substitution at nucleotide 1996 results in the loss of a target site for the restriction enzyme MaeIII in the PCR product amplified from Gro(a⁺) cDNA. The digestion of the PCR products from Gro(a⁺) and

Gro(a⁻) (primers 21A and 21) yielded fragments of the expected size (data not shown).

Studies on genomic DNA. Oligonucleotide primers exon XIa and XIb were used to amplify the polymorphic region in the GP IIIa genomic DNA derived from Gro(a⁺) and Gro(a⁻)

Fig 3. (A) Schematic representation of the GP IIIa gene and the PCR-ASRA strategy. A 170 bp DNA segment of the GP IIIa gene, containing exon XI, was PCR amplified, with primers corresponding to sequences upstream and downstream of exon XI (horizontal arrows). The vertical arrows indicate the target sites for the restriction enzyme MaeIII in this 170 bp fragment. In the Gro(a⁻) allele, both target sites are present resulting in three restriction fragments, of 94 bp, 67 bp and 9 bp, after MaeIII digestion. Due to the G→A mutation, one of the target sites (left vertical arrow) is lost, resulting in two fragments of 103 bp and 67 bp, after MaeIII digestion. (B) Analysis of the MaeIII-digested PCR products on a 10% polyacrylamide gel. Lane M: 100 bp DNA size standards. Lane 1: the undigested 170 bp PCR product. Lane 2: the MaeIII-digested fragments from a Gro(a⁺) individual, of 103 bp, 94 bp and 67 bp. Lane 3: the MaeIII-digested fragments from a Gro(a⁻) individual, of 94 bp and 67 bp. The theoretically expected 9 bp fragment is not visible on this gel. All Gro(a⁺) individuals were found to be heterozygotes for the polymorphic allele.

individuals (Fig 3). The PCR product of 170 bp was subjected to *Mae*III digestion and subsequently electrophoresed on a 10% polyacrylamide gel and stained with ethidium bromide. Note the target site for *Mae*III present in both alleles, which can be regarded as an 'internal control' for the efficiency of the enzyme. Thus, in the *Gro*(a⁻) allele, the DNA comprising 170 bp is digested into three fragments of 94 bp, 67 bp and 9 bp, whereas in the *Gro*(a⁺) allele the 170 bp fragment is cut into two fragments of 103 bp and 67 bp because of the G → A change.

DISCUSSION

The platelet membrane GP IIb/IIIa complex (also called $\alpha_{IIb}\beta_3$) can bind fibrinogen, fibronectin, von Willebrand factor and vitronectin when platelets are activated (Phillips *et al.*, 1991). In addition to its pivotal function in haemostasis, the complex carries the majority of the human platelet alloantigens (Newman, 1994; von dem Borne *et al.*, 1995).

In previous studies the target of the private platelet alloantibody anti-Gro^a was found to reside on the platelet GP IIIa (Simsek *et al.*, 1994b), but we were not able to establish the molecular basis of the Gro^a antigen, i.e. none of the 16 subclones selected differed in Gro(a⁺) and Gro(a⁻) subjects. In the present study we applied the SSCP technique, which is

a PCR-based methodology for identifying single base changes in a fragment of DNA. Platelet GP IIIa mRNA was amplified by PCR. After amplification, the PCR fragments were subjected to SSCP analysis. The nucleotide sequences of the PCR fragments which displayed a different SSCP pattern compared to normal controls were determined. This revealed a heterozygosity for a G → A mutation at base pair 1996 of the coding region of the mature protein, predicting a substitution of arginine (AAG) by histidine (GAG) at amino acid position 633, in the Gro(a⁺) form of GP IIIa. In addition to the G → A substitution at base pair 1996, three silent changes in heterozygous form were identified: (1) A ↔ C polymorphism at position 1163 (silent at amino acid level: Val355Val); (2) G ↔ A mutation at position 1553 (Glu485Glu); (3) G ↔ A polymorphism at position 1565 (Arg489Arg). Of the four mutations detected in the cDNA of GP IIIa, G1996A was most likely to be responsible for the Gro^a alloantigen, because it represented the only mutation predicting an amino acid substitution, Arg633His. In Fig 4 a model for the generation of the Gro(a) haplotypes is shown. It is noteworthy that the nucleotide mutation, predicting to lead to the Gro(a) antigen, is contained within a GP IIIa isoform in which three other changes have occurred. This strongly suggests that the G1996A did not occur in an ancestral allele.

Fig 4. The Gro(a) haplotype. Schematic representation of glycoprotein IIIa on which targets (antigenic determinants) for anti-HPA-1a, -1b, -4a, -4b, -6b, -7b, -8b and Gro(a) reside. The DNA coding for the GPIIIaHis633 form carries three silent nucleotide changes (represented within boxes). The HPA-6a, -7a, -8a and Gro(b) are in parentheses because antibodies recognizing these targets have not yet been found.

The G1996A base change abolishes the target site for the restriction enzyme *Mae*III in the Gro(a⁺) form of the GP IIIa DNA. We demonstrated by allele-specific restriction enzyme analysis (ASRA) with *Mae*III of amplified cDNA, as well as of genomic DNA, that the G1996A mutation is indeed associated with the Gro^a antigen. PCR-ASRA typing on genomic DNA is a useful technique, because it eliminates the need for the isolation of platelet RNA. Interestingly, the Gro^a polymorphic site is located near the Sr^a polymorphic site (HPA-8wb). Moreover, the wild-type amino acid is arginine in both polymorphisms. These findings suggest that this region of the GP IIIa gene is susceptible to mutations. The amino acid substitution does not have an effect on the expression of the GP IIb/IIIa complex on the platelet membrane, as was demonstrated in previous immunochemical studies (Simsek et al, 1994b). The Gro(a⁺) individuals thus far studied have been found to be heterozygous and do not suffer from haemorrhagic diathesis or thrombosis.

In conclusion, we report that a G↔A polymorphism at base 1996 in the DNA encoding glycoprotein IIIa (β₃) is associated with the Gro^a alloantigen. This further illustrates the polymorphic nature of GP IIIa.

ACKNOWLEDGMENTS

We thank Dr L. Porcelijn, Mrs E. Huiskes and Mrs P. M. M. Bleeker for their technical support and Drs D. Roos and C. P. Engelfriet for critically reading the manuscript. We are grateful to the family concerned for their cooperation in this study.

REFERENCES

- Chomczynski, P. & Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*, **162**, 156–159.
- Ciulla, T.A., Sklar, R.M. & Hauser, S.L. (1988) A simple method for DNA purification from peripheral blood. *Analytical Biochemistry*, **174**, 458–488.
- Ginsberg, M.H., Loftus, J.C. & Plow, E.F. (1988) Cytoadhesins, integrins, and platelets. *Thrombosis and Haemostasis*, **59**, 1–6.
- Hynes, R.O. (1987) Integrins: a family of cell surface receptors. *Cell*, **48**, 549–554.
- Jin, Y., Dietz, H.C., Nurden, A. & Bray, P.F. (1993) Single-strand conformation polymorphism analysis is a rapid and effective method for the identification of mutations and polymorphisms in the gene for glycoprotein IIIa. *Blood*, **82**, 2281–2288.
- Kiefel, V., Santoso, S., Weisheit, M. & Mueller-Eckhardt, C. (1987) Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. *Blood*, **70**, 1722–1726.
- Kroll, H., Kiefel, V., Santoso, S. & Mueller-Eckhardt, C. (1990) Sr(a), a private platelet antigen on glycoprotein IIIa associated with neonatal alloimmune thrombocytopenia. *Blood*, **76**, 2296–2302.
- Kuijpers, R.W.A.M., Faber, N.M., Cuypers, H.T.M. & von dem Borne, A.E.G.K. (1992) The N-terminal globular domain of human platelet glycoprotein Ibα has a methionine145/threonine145 amino acid polymorphism which is associated with the HPA-2 (Ko) alloantigens. *Journal of Clinical Investigation*, **89**, 381–384.
- Kuijpers, R.W.A.M., Simsek, S., Faber, N.M., Goldschmeding, R., van Wermeskerken, R.V.K. & von dem Borne, A.E.G.Kr. (1993) Single point mutation in glycoprotein IIIa gives rise to a new platelet specific alloantigen (Mo) involved in neonatal alloimmune thrombocytopenia. *Blood*, **81**, 70–76.
- Lyman, S., Aster, R.H., Visentin, G.P. & Newman, P.J. (1990) Polymorphism of human platelet membrane glycoprotein IIb associated with the Baka/Bakb alloantigen system. *Blood*, **75**, 2343–2348.
- McFarland, J.G., Blanchette, V., Collins, J., Newman, P.J., Wang, R. & Aster, R.H. (1993) Neonatal alloimmune thrombocytopenia due to a new platelet-specific alloantibody. *Blood*, **81**, 3318–3323.
- Newman, P.J. (1994) Nomenclature of human platelet alloantigens: a problem with the HPA system. *Blood*, **83**, 1447–1451.
- Newman, P.J., Derbes, R.S. & Aster, R.H. (1989) The human platelet alloantigens, PlA1 and PlA2, are associated with a leucine33/proline33 amino acid polymorphism in membrane glycoprotein IIIa, and are distinguishable by DNA typing. *Journal of Clinical Investigation*, **83**, 1778–1781.
- Noris, P., Simsek, S., de Bruijne-Admiraal, L.G., Porcelijn, L., Huiskes, E., van der Hilst, G., van Leeuwen, E.F., van der Schoot, C.E. & von dem Borne, A.E.G.Kr. (1995) Neonatal alloimmune thrombocytopenia (NATP) caused by a new low-frequency platelet-specific alloantigen, Max(a) located on GP IIb. *Blood*, **86**, 1019–1026.
- Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 2766–2770.
- Peyruchaud, O., Nurden, A. & Bourre, F. (1995) Non-radioactive SSCP for genotyping human platelet alloantigens. *British Journal of Haematology*, **89**, 633–636.
- Phillips, D.R., Charo, I.F. & Scarborough, R.M. (1991) GP IIb–IIIa: the responsive integrin. *Cell*, **65**, 359–362.
- Rosa, J.P., Bray, P.F., Gayet, O., Johnston, G.I., Cook, R.G., Jackson, K.W., Shuman, M.A. & McEver, R.P. (1988) Cloning of glycoprotein IIIa cDNA from human erythroleukemia cells and localization of the gene to chromosome 17. *Blood*, **72**, 593–600.
- Rosa, J.-P. & McEver, R.P. (1989) Processing and assembly of the integrin, glycoprotein IIb–IIIa, in HEL cells. *Journal of Biological Chemistry*, **264**, 12596–12603.
- Sanger, F., Nicklen, S. & Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, **74**, 5463–5467.
- Santoso, S., Kalb, R., Kroll, H., Walka, M., Kiefel, V., Mueller-Eckhardt, C. & Newman, P.J. (1994) A point mutation leads to an unpaired cysteine residue and a molecular weight polymorphism of a functional platelet β₃ integrin subunit: the Sr(a) alloantigen system of GP IIIa. *Journal of Biological Chemistry*, **269**, 8439–8444.
- Simsek, S., Gallardo, D., Ribera, A. & von dem Borne, A.E.G.Kr. (1994a) The human platelet alloantigens, HPA-5(a⁺,b⁺) and HPA-5(a⁺,b⁺), are associated with a Glu505/Lys505 polymorphism of glycoprotein Ia (the α₂ subunit of VLA-2). *British Journal of Haematology*, **86**, 671–674.
- Simsek, S., Goldschmeding, R., Kuijpers, R.W.A.M., Vlekke, A.B.J. & von dem Borne, A.E.G.Kr. (1994b) A new private platelet alloantigen, Gro(a), localized on GP IIIa involved in neonatal alloimmune thrombocytopenia. *Vox Sanguinis*, **67**, 302–306.
- Simsek, S. & von dem Borne, A.E.G.Kr. (1994) Molecular genetics of human platelet antigens. *Infusionstherapie und Transfusionsmedizin*, **21**, (Suppl. 3), 29–33.
- van der Schoot, C.E., Wester, M., von dem Borne, A.E.G.Kr. & Huisman, J.G. (1986) Characterization of platelet-specific alloantigens by immunoblotting: localization of Zw and Bak antigens. *British Journal of Haematology*, **64**, 715–723.

- von dem Borne, A.E.G.Kr., Verheugt, F.W.A. & Oosterhof, F. (1978) A simple immunofluorescence test for the detection of platelet antibodies. *British Journal of Haematology*, **39**, 195–207.
- von dem Borne, A.E.G.Kr., Kaplan, C. & Minchinton, R. (1995) Nomenclature of human platelet alloantigens. *Blood*, **86**, 1409–1410.
- Wang, L., Juji, T., Shibata, Y., Kuwata, S. & Tokunaga, K. (1991) Sequence variation of human platelet membrane glycoprotein IIIa associated with the Yuka/Yukb alloantigen system. *Proceedings of the Japanese Academy*, **67**, 102–106.
- Wang, R., McFarland, J.G., Kekomäki, R. & Newman, P.J. (1993) Amino acid 489 is encoded by a mutational 'hot spot' on the $\beta 3$ integrin chain: the Ca/Tu human platelet alloantigen system. *Blood*, **82**, 3386–3391.
- Zimrin, A.B., Gidwitz, S., Lord, S., Schwartz, E., Bennett, J.S., White, G.C., II & Poncz, M. (1990) Genomic organization of platelet glycoprotein IIIa. *Journal of Biological Chemistry*, **265**, 8590–8595.