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ORIGINAL ARTICLE

Diversity of Glanzmann thrombasthenia in southern India: 10 novel mutations identified among 15 unrelated patients

E. J. R. NELSON,* S. C. NAIR,† H. PERETZ,‡ B. S. COLLER,§ U. SELIGSOHN,¶ M. CHANDY* and A. SRIVASTAVA*

*Departments of *Hematology and †Clinical Pathology, Christian Medical College, Vellore, India; ‡Clinical Biochemistry Laboratory, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel; §Laboratory of Blood and Vascular Biology, The Rockefeller University, New York, NY, USA; and ¶Institute of Thrombosis and Hemostasis, The Chaim Sheba Medical Center, Tel-Hashomer, Israel*

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Summary. *Background:* Glanzmann thrombasthenia (GT) is a congenital bleeding disorder caused by either a lack or dysfunction of the platelet integrin $\alpha_{IIb}\beta_3$. *Objectives:* To determine the molecular basis of GT in patients from southern India. *Patients:* Fifteen unrelated patients whose diagnosis was consistent with GT were evaluated. *Results:* Platelet surface expression of $\alpha_{IIb}\beta_3$ was < 10%, 10%–50%, and > 50% of controls in five, nine, and one patient(s), respectively. Immunoblotting of the platelet lysates showed no α_{IIb} in 14 patients, and no β_3 in 10 patients, although severely reduced in four patients. Platelet fibrinogen was undetectable in 13 patients, and severely reduced in one patient. One patient showed normal surface $\alpha_{IIb}\beta_3$ expression, and normal α_{IIb}, β_3 and fibrinogen levels in the lysate. Ten novel candidate disease-causing mutations were identified in 11 patients. The missense mutations included Gly128Ser, Ser287Leu, Gly357Ser, Arg520Trp, Leu799Arg in α_{IIb}, and Cys575Gly in β_3. We have already shown that Gly128Ser, Ser287Leu, and Gly357Ser mutations variably affect $\alpha_{IIb}\beta_3$ surface expression. The Cys575Gly mutation may disrupt the disulphide link with Cys586 to cause the GT phenotype. The molecular pathology of the other missense mutations is not clear. Two nonsense mutations, Trp-16Stop and Glu715Stop in α_{IIb}, and a 7-bp deletion (330-336TCCCCAG) in β_3 are predicted to result in truncated proteins. An IVS15(−1)G → A mutation in α_{IIb} induced a cryptic splice site as confirmed by reverse transcription-polymerase chain reaction (RT-PCR) analysis. Thirteen polymorphisms were also identified (five in α_{IIb} and eight in β_3), among which five were novel. *Conclusions:* While identifying a significant number of novel mutations causing GT, this study confirms the genetic heterogeneity of the disorder in southern India.

Correspondence: Alok Srivastava, Department of Hematology, Christian Medical College, Vellore – 632 004, Tamil Nadu, India. Tel.: +91 416 2222102; fax: +91 416 2232035; e-mail: aloks@cmcvellore.ac.in

Present address: E. J. R. Nelson, Hematology and Medical Oncology, The Samuel Bronfman Department of Medicine, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, USA.

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Introduction

Glanzmann thrombasthenia (GT) is a rare, autosomal recessive bleeding disorder characterized by a life-long mucocutaneous bleeding tendency, absent or severely reduced platelet aggregation in response to the physiological agonists adenosine 5′-diphosphate (ADP), epinephrine, and collagen, and a relatively normal initial phase of aggregation in response to ristocetin [1–3]. The disease is caused either by a lack or dysfunction of the platelet integrin $\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa), which serves as a receptor for fibrinogen, von Willebrand factor, and perhaps other adhesive glycoproteins [4]. More than 100 candidate disease-causing mutations in either α_{IIb} and/or β_3 genes have been identified in patients with GT worldwide, including deletions, insertions, inversions, single nucleotide substitutions that lead to missense or nonsense mutations, and splicing defects [5–8]. Certain mutations predominate in a given population, such as Iraqi Jews and French Gypsies [9,10]. Although rare in the general population (1:200 000 among a population of 60 million in Iran) [11], it occurs in high frequency in certain ethnic populations with an increased incidence of consanguinity such as in south Indians, Iranians, Iraqi Jews, Palestinian and Jordanian Arabs, and French Gypsies [9–12].

In this paper, clinical, hematological, biochemical and molecular genetic data on 15 unrelated GT patients from southern India are presented, bringing to light 10 novel mutations and five novel polymorphisms in the α_{IIb} or β_3 genes.

Materials and methods

Sample processing

The study was approved by the ethics committee at the Christian Medical College, Vellore, India. Platelet-rich plasma (PRP) was prepared from blood that was anticoagulated with citrate or ethylenediaminetetraacetic acid (EDTA). Peripheral blood was collected after obtaining informed consent from patients diagnosed as having GT based on clinical and hematological evaluation. A portion of the citrated PRP was used for flow cytometry analysis. The EDTA-anticoagulated PRP and the remaining citrated PRP were washed separately in Tris–saline–EDTA buffer (TSE, pH 7.4). The citrated platelet pellet was resuspended in 70% ethanol and stored at −80 °C. The EDTA pellet was resuspended in the wash buffer to a final platelet count of 2×10^9 cells mL^{-1}, lysed with an equal volume of lysis solution [6 mM *N*-ethyl maleimide in 3.3% sodium dodecylsulfate (SDS)], heated immediately to 100 °C for 3 min and then cooled and frozen at −80 °C. Genomic DNA was extracted from the buffy coat using a standard phenol–chloroform method.

Analysis of platelet surface expression of $\alpha_{IIb}\beta_3$

Mouse monoclonal antibodies (mAbs) 10E5 (anti-$\alpha_{IIb}\beta_3$, CD41/61), 7E3 (anti-$\alpha_{IIb}\beta_3$ + anti-$\alpha V\beta_3$, CD41/61 and CD51/61, respectively) and 6D1 (anti-GPIb, CD42b) were added to the citrated PRP, followed by incubation with FITC-conjugated goat-antimouse antibody (Cappel; ICN Pharmaceuticals, Columbus, OH, USA). Appropriate IgG1 and IgG2a isotype control antibodies were also analyzed [gifts from Dr Kenneth Bradstock (Westmead Hospital, Sydney, Australia)]. Surface expression was analyzed in a flow cytometer (Bryte-HS, Bio-Rad, Hercules, CA, USA) using WinBryte software.

Total platelet content of $\alpha_{IIb}\beta_3$

Lysates of washed platelets solubilized in SDS [13] were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using precast gels (PhastGel Homogenous 7.5; Pharmacia Biotech, Uppsala, Sweden). Equivalent amounts of protein were added to each lane. Samples for α_{IIb} were tested reduced to display both pro-α_{IIb} and the heavy chain of mature (cleaved) α_{IIb}, and samples for β_3 were tested non-reduced. The separated proteins were blotted onto polyvinylidene fluoride (PVDF) membranes and incubated separately with murine mAbs specific for α_{IIb} (CD41, SZ22) and β_3 (CD61, SZ21) (Immunotech, Marseille, Cedex, France). Secondary labeling was done with a gold-conjugated goat antimouse IgG (Auroprobe One GAM; Amersham International PLC, Buckinghamshire, UK), followed by enhancement of the gold stain using a silver reagent (IntenSE BL; Amersham).

Mutation analysis of α_{IIb} and β_3 genes

A total of 38 PCR reactions were performed on genomic DNA using intragenic primers as previously described [14,15] or additional primers and conditions that can be obtained by contacting one of us (A.S.). Briefly, PCR amplification was carried out using ~100 ng genomic DNA, 0.2–0.4 μM primers, 125 μM dNTPs, 1.5–2.0 mM $MgCl_2$, 1 U of DNA polymerase (DyNAzyme II; Finnzymes, Espoo, Finland), and reaction buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.3) in a total volume of 25 μL. Single-strand conformation polymorphism (SSCP) analysis was performed by either an automated (PhastGel System; Pharmacia Biotech) or manual method (Protean II Cell; Bio-Rad). The gels were silver-stained as per the manufacturer's protocol (Pharmacia Biotech).

Amplicons that showed mobility shifts by SSCP analysis were purified from 2.5% agarose gel (GeneClean II Kit; Bio 101, Carlsbad, CA, USA) and their DNA sequence was determined (Big Dye terminator kit; Applied Biosystems, Foster City, CA, USA) using an automated sequencer (ABI 310 Genetic Analyzer; Applied Biosystems).

mRNA isolation was performed from platelets stored in 70% ethanol at −80 °C according to the manufacturer's instructions (QuickPrep Micro mRNA purification kit; Amersham Biosciences, Piscataway, NJ, USA). The cDNA was synthesized using a random hexamer according to the manufacturer's protocol (Superscript First-strand Synthesis System; Invitrogen Corporation, Carlsbad, CA, USA). PCR for β-actin was done to check the efficiency of platelet mRNA isolation. For the α_{IIb} IVS15(−1) G → A mutation, PCR was done using the primers, 5′ 1252: GGCCAAGTGCTGGTGTTC and CTCCATGCAGCACGACAG: 1858 3′, spanning α_{IIb} exons 13–18. For the β_3 TCCCCAG 330–336 deletion in exon 3, primers spanning β_3 exons 1–6 were used, namely 5′: 28 CTCTGGGTGACTGTGCTGG and CCGTCATTAGGCTGGACAAT: 890 3′.

Restriction fragment length polymorphism (RFLP) analysis

RFLP was performed for the previously unpublished polymorphisms, using *Nla*III, *Ban*II, *Bsa*JI, *Bsl*I, and *Hae*III restriction enzymes (MBI Fermentas GmbH, St Leon-Rot, Germany).

Results

Patient characteristics

The clinical, hematological, immunophenotypic, surface membrane protein, and molecular genetic data on the 15 patients evaluated at our center are summarized in Tables 1 and 2. All the patients were from southern India, predominantly from the states of Tamil Nadu and Andhra Pradesh. The age of onset of symptoms ranged from 0 to 7 years (mean 2 years), but the age at definitive diagnosis ranged from 4 to 41 years (median 14 years). Eleven patients were born to known consanguineous

Table 1 Clinical, hematological, biochemical, and molecular genetic data of Glanzmann thrombasthenia (GT) patients with a mutation in either α_{IIb} or β_3 genes

							Hematological evaluation						Platelet proteins					
														Western blot				
UPN	Sex /age	Ori	CG	FH	Bleeding symptoms	Tx	BT min	CR	ADP	EPI	COL	RIS	FCM, % ($\alpha_{IIb}\beta_3$)	α_{IIb}	β_3	Fibgn	Mutation (predicted consequence)	Polymorphisms
1	M/13	AP	Y	N	Th, Ep, Pe, GI, Gu, Tr	25	> 15	Nil	Nil	Nil	Nil	Nor	5.6	0	0	0	**α_{IIb} 48G > A (−16Trp > Stop)** (PTC within SP)	**α_{IIb} IVS16(+11)T > C**
2	M/10	AP	Y	N	Ep, Ec, GI, Gu	1	> 15	Nil	Nil	Nil	Nil	ND	16.4	0	0	0	**α_{IIb} 475G > A (128Gly > Ser)**	**β_3 5′-UTR (−114)G > C** **β_3 5′-UTR (−35)insG**
3	M/6	AP	Y	N	Ec, Gu, Tr	1	ND	Poor	Nil	Nil	Nil	Nor	20.1	0	+	0	**α_{IIb} 953C > T (287Ser > Leu)**	Nil
4	M/22	TN	N	Y	Th, Ep, GI, Gu	0	> 15	Poor	Nil	Nil	Nil	Nor	31.4	0	+	+	**α_{IIb} 1162G > A (357Gly > Ser)**	**β_3 5′-UTR (−114)G > C** **β_3 5′-UTR (−35)insG** **β_3 5′-UTR (−24)G > C**
5	F/28	GA	N	N	Th, Pe, Ec, GI, Gu, Tr, Mr	50	12.5	Nil	Nil	Nil	ND	Nor	14.9	0	0	0	**α_{IIb} IVS15(−1)G > A** (alternative splicing)	α_{IIb} IVS21(−7)C > G α_{IIb} 2622T > G (843Ile > Ser) α_{IIb} 3063C > T (990Val > Val) **β_3 5′-UTR (−35)insG**
6	M/14	TN	Y	N	Th, Ep, Pe, Gu, Tr	4	> 15	Nil	Nil	Nil	Nil	Nor	1	0	0	0	**α_{IIb} 1651C > T (520Arg > Trp)**	**β_3 5′-UTR (−114)G > C** **β_3 5′-UTR (−35)insG** **β_3 5′-UTR (−24)G > C**
7	M/11	WB	N	Y	Th, Ep, Pe, Ec, Gu	2	> 15	Nil	Nil	Nil	Nil	Nor	8.3	0	0	0	**α_{IIb} 1651C > T (520Arg > Trp)**	β_3 176T > C (33Leu > Pro) (HT) β_3 1533G > A (485Glu > Glu) (HT) β_3 1545A > G (489Arg > Arg) (HT) β_3 IVS10(+23)C > G (HT)
8	M/31	TN	Y	N	Ep, Gu	18	> 15	Nil	Nil	Nil	Nil	Nor	11.9	0	0	0	**α_{IIb} 2236G > T (715Glu > Stop)**	α_{IIb} IVS21(−7)C > G α_{IIb} 2622T > G (843Ile > Ser) α_{IIb} 3063C > T (990Val > Val)
9	M/20	TN	Y	Y	Th, Ep, Ec, Gu	1	> 15	Nil	Nil	Nil	Nil	Poor	10	0	0	0	**α_{IIb} 2489T > G (799Leu > Arg)**	α_{IIb} IVS21(−7)C > G α_{IIb} 2622T > G (843Ile > Ser) **α_{IIb} IVS26(+22)G > T** α_{IIb} 3063C > T (990Val > Val) **β_3 5′-UTR (−114)G > C (HT)** **β_3 5′-UTR (−35)insG** β_3 1641C > T (HT)
10	F/12	TN	Y	N	Th, Gu	1	> 15	Nil	Nil	Nil	Nil	Nor	3	0	0	0	**β_3 330–336 TCCCCAGdel** (FS PTC141)	α_{IIb} IVS21(−7)C > G α_{IIb} 2622T > G (843Ile > Ser) **α_{IIb} IVS26(+22)G > T** α_{IIb} 3063C > T (990Val > Val) β_3 176T > C (33Leu > Pro)

Table 1 Continued

UPN	Sex /age	Ori	CG	FH	Bleeding symptoms	Hematological evaluation						Platelet proteins				Mutation (predicted consequence)	Polymorphisms
						Tx	BT min	CR	ADP	EPI	COL	RIS	FCM, % ($\alpha_{IIb}\beta_3$)	Western blot			
														α_{IIb}	β_3	Fibgn	
11	F/16	TN	Y	Y	Th, Ec, Gu	10	> 15	Nil	Nil	Nil	Nil	Nor	15.7	0	0	0	**β_3 1801T > G (575Cys > Gly)**	α_{IIb} IVS21(−7)C > G α_{IIb} 2622T > G (843Ile > Ser) **α_{IIb} IVS26(+22)G > T** α_{IIb} 3063C > T (990Val > Val)

For nucleotide numbering the A nucleotide of the ATG start codon was designated +1. For amino acid numbering, the first amino acid of the mature protein (not including signal peptide) was designated +1. Novel mutations and polymorphisms are indicated in bold.

UPN, unique patient number; Ori, State of origin of parents; CG, parental consanguinity; FH, family history; Tx, number of times blood or platelets were transfused; BT, bleeding time; CR, clot retraction; ADP, adenosine diphosphate; EPI, epinephrine; COL, collagen; RIS, ristocetin; FCM, flow cytometry; Fibgn, fibrinogen; AP, Andhra Pradesh; TN, Tamil Nadu; GA, Goa; WB, West Bengal; Y, Yes; N, No; Th, bleeding during tooth eruption; Ep, epistaxis; Pe, petech,iae; GI, gastrointestinal bleeding; Gu, gum bleeding; Tr, bleeding during tooth extraction; Mr, menorrhagia; Ec, ecchymoses; FS, frame shift; HT, heterozygous; Ins, insertion; PTC, premature termination codon; SP, signal peptide; Nor, normal response; ND, not done; Del, deletion.

parents, and six of them had a family history of bleeding. The predominant clinical manifestations included skin and mucosal bleeding both affecting nearly all patients. Five patients (33.3%) had received red cell or platelet transfusions on more than five occasions and 11 of them had been transfused at least once. Though there were no platelet aggregation responses to physiological agonists in any of the patients, some patients had residual, albeit reduced, clot retraction (3/15, 20%), and some had poor initial phase responses to ristocetin (3/15, 20%).

Platelet surface expression of GPIb and $\alpha_{IIb}\beta_3$ receptors

The mean platelet surface expression level of GPIb was 79.4% of controls (range 44.8%–96.4%) as shown by the binding of anti-GPIb antibody, 6D1 (CD42b). Platelet $\alpha_{IIb}\beta_3$ expression was < 10% of controls in five patients, 10–50% in nine patients, and 95% in one patient.

α_{IIb}, β_3 and fibrinogen in the platelet lysates

Fibrinogen was undetectable in the platelet lysates in 13 patients, and severely reduced in one, as revealed by Coomassie blue-stained SDS-PAGE gels. Immunoblot analysis of the platelet lysates revealed no traces of α_{IIb} in 14 patients. β_3 was undetectable in the platelet lysates in 10 patients, and severely reduced in four. However, in one patient, α_{IIb}, β_3 and fibrinogen were present at normal levels.

Mutations in the α_{IIb} and β_3 genes

Probable disease-causing homozygous mutations were found in 11 out of 15 patients (73%), nine in α_{IIb} and two in β_3, of which 10 were novel (eight in α_{IIb} and two in β_3). Of the 10 candidate disease-causing mutations identified, six were missense, two were nonsense, one was a small deletion (7 bp) and one was a splice site mutation (Tables 1 and 2).

Missense mutations (n = 6) Six novel missense mutations were identified in α_{IIb} exons 4, 11, 12, 17, and 25, namely Gly128Ser (GGT > AGT), Ser287Leu (TCG > TTG), Gly357Ser (GGC > AGC), Arg520Trp (CGG > TGG), and Leu799Arg (CTC > CGC). Arg520Trp was identified in patients 6 and 7, and Cys575Gly was found in β_3 exon 11, TGC > GGC in patient 11.

Nonsense mutations (n = 2) Two novel nonsense mutations were identified in α_{IIb} exons 1 and 22 in patients 1 and 8, respectively. The mutation in exon 1 occurred within the signal peptide sequence (Trp-16Stop; TGG > TGA) and hence it is predicted to completely prevent synthesis of α_{IIb} protein. The other mutation, Glu715Stop (GAG > TAG) identified in patient 8 is predicted to result in a truncated α_{IIb} protein.

Splice mutation (n = 1) A novel point mutation at the splice acceptor site of α_{IIb} intron 15, IVS15(−1)G → A [3′ splice site, A**G** → A**A**] was seen in patient 5, and this

Table 2 Clinical, hematological, biochemical, and molecular genetic data of the Glanzmann thrombasthenia (GT) patients with no mutation in either α_{IIb} or β_3 genes

							Hematological evaluation						Platelet proteins					
														Western blot				
UPN	Sex /age	Ori	CG	FH	Bleeding symptoms	Tx	BT (min)	CR	ADP	EPI	COL	RIS	FCM, % ($\alpha_{IIb}\beta_3$)	α_{IIb}	β_3	Fibgn	Mutation	Polymorphisms
12	F/15	KA	Y	Y	Th, Ep, Pe, Ec, Gu, Mr	0	> 15	Nil	Nil	Nil	Nil	Poor	32.7	0	+	0	No mutation	Nil
13	F/41	AP	N	Y	Ep, Pe, Gu, Mr	15	> 15	Nil	Nil	ND	ND	Nor	0.5	0	0	0	No mutation	β_3 1533G > A (HT) β_3 1545A > G (HT) β_3 176T > C (33Leu > Pro) (HT)
14	M/4	AP	Y	N	Ep, Pe, GI, Gu	0	> 15	Poor	Nil	Nil	Nil	Nor	95.5	4+	4+	4+	No mutation	α_{IIb} IVS21(−7)C > G α_{IIb} 2622T > G (843Ile > Ser) α_{IIb} 3063C > T (990Val > Val)
15	F/13	TN	Y	N	Th, Ep, Ec, Gu	0	> 15	Nil	Nil	Nil	Nil	Poor	14.4	0	+	0	No mutation	β_3 176T > C (33Leu > Pro) (HT) β_3 IVS10(+23)C > G (HT) β_3 1533G > A (HT) β_3 1545A > G (HT)

For nucleotide numbering the A nucleotide of the ATG start codon was designated +1. For amino acid numbering, the first amino acid of the mature protein (not including signal peptide) was designated +1.

UPN, unique patient number; Ori, State of origin of parents; CG, parental consanguinity; FH, family history; Tx, number of times blood or platelets were transfused; BT, bleeding time; CR, clot retraction; ADP, adenosine diphosphate; EPI, epinephrine; COL, collagen; RIS, ristocetin; FCM, flow cytometry; Fibgn, fibrinogen; AP, Andhra Pradesh; TN, Tamil Nadu; KA, Karnatak,a; FH, family history; Y, Yes; N, No; Nor, normal response;; Th, bleeding during tooth eruption; Ep, epistaxis; Ec, ecchymoses; Pe, petechiae; GI, gastrointestinal bleeding; Gu, gum bleeding; ND, not done; FCM, flow cytometry; Mr, menorrhagia; HT, heterozygous.

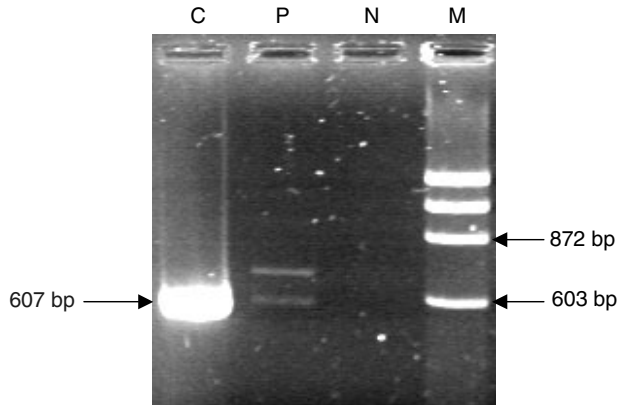

Fig. 1. Reverse transcription-polymerase chain reaction (RT-PCR) analysis for the α_{IIb} IVS15(−1)G → A splice site mutation showing diminished amounts of the two splice variants (607 bp and ~750 bp). C, control cDNA; P, patient cDNA; N, PCR negative; M, ϕX 174 *Hae*III marker.

mutation was confirmed by digestion with *Alu*I restriction enzyme. RT-PCR analysis using primers encompassing α_{IIb} exons 13–18 revealed two cDNA products of equal amounts, but grossly diminished when compared with the amount of cDNA in the normal control. One of these matched the control amplicon size of 607 bp while the other amplicon was about 750 bp in length (Fig. 1).

Deletion (n = 1) A novel 7-bp deletion, namely TCCCCAG 330–336, in β_3 exon 3 was identified in patient 10. This mutation is predicted to result in a shift in the reading frame at Ser110 and eventually lead to premature termination at the new codon position 141. RT-PCR analysis using primers encompassing β_3 exons 1–6, revealed a cDNA product moving very closely with the control amplicon of size 863 bp (Fig. 2), sequencing of which confirmed the 7-bp deletion.

Polymorphisms in the α_{IIb} and β_3 genes

Apart from the 10 candidate disease-causing mutations, 13 polymorphisms were also identified, five in α_{IIb} and eight in β_3, of which five have not been previously reported, namely α_{IIb} IVS16(+11)T → C, and IVS26(+22)G → T, and β_3 5′-UTR-114G → C, 5′-UTR-35insG, and 5′-UTR(−24)G → C. One private polymorphism, α_{IIb} IVS16(+11)T > C, was not observed in any of the 53 unrelated healthy controls tested. The polymorphism α_{IIb} IVS26(+22)G > T was present in all those tested, indicating that this could be a very common polymorphism in the south Indian population, or an error in the published sequence (GenBank accession no. J02764). Three α_{IIb} polymorphisms previously reported to be in linkage disequilibrium [16] were also found in six patients (homozygous), IVS21(−7)C → G, 2622T → G in exon 26, and 3063C → T in exon 30. The 2622T → G (843Ile → Ser) substitution is part of the human platelet alloantigen HPA-3 system. The HPA-1 system, β_3 176T → C (33Leu → Pro) substitution in exon 3 was identified in four patients, three of whom were heterozygous. Three patients were heterozygous for two previously reported polymorphisms in β_3 exon 10, 1533G → A (485Glu → Glu) and 1545A → G (489Arg → Arg) [15]. One patient was heterozygous for another polymorphism in β_3 exon 10, 1641C → T (495Cys → Cys), whereas another β_3 IVS10 (+23)C → G polymorphism was identified in the heterozygous form in two patients.

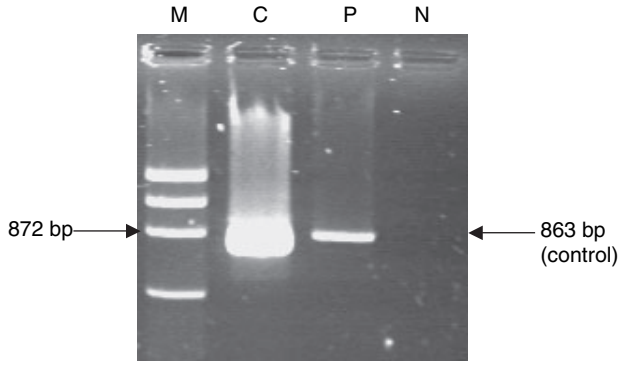

Fig. 2. Reverse transcription-polymerase chain reaction (RT-PCR) analysis for the β_3 330–336 TCCCCAG deletion showing a transcript moving very closely with the normal control transcript of 863 bp, consistent with the 7-bp deletion. C, control cDNA; P, patient cDNA; N, PCR negative; M, ϕX 174 *Hae*III marker.

Discussion

GT has been reported among Indian patients by several groups [17–22], including a series of 42 patients from southern India which was described from our center in 1981 [12]. GT is the fourth most common inherited bleeding disorder in southern India, after hemophilia A and B and von Willebrand disease (S. C. Nair, S. Baidya, M. Chandy, A. Srivastava, unpublished observation). We have recently reported 23 mutations, of which 20 were novel, among 40 patients with GT, mostly from southern India [23] seen at our center between 1996 and 1998 during a collaborative study involving our center and those in Tel-Hashomer, Israel and New York, USA. The current paper describes the results of evaluation of 15 unrelated patients with GT who presented subsequently at our center in 1999 and 2000. Ten more novel mutations were found among them. Fourteen novel mutations have also been described in the β_3 gene among patients from western India [24–26].

There is significant heterogeneity in clinical presentations among the patients included in the current study, with the age of onset of symptoms varying from birth to 7 years, though most were symptomatic by their second year. Diagnosis was delayed by almost a decade in most cases, reflecting the lack of awareness and facilities available for detecting this rare disorder. It is also significant that the median age of the patients was about 14 years and no patient was older than 41 years. It is likely therefore that GT affects survival in our population where the current life expectancy is about 65 years. Parental consanguinity (73% vs. 63%), family history of bleeding (40% vs. 38%) and absence of clot retraction (80% vs. 88%) were found to be consistent with our previous study [23].

The clinical presentation was similar to previous reports with respect to consanguinity, sites of bleeding, and transfusion frequencies [1]. Gingival bleeding alone was the most frequent symptom, being reported in 93.3% of our patients. This symptom has been particularly difficult to manage in one family with two affected siblings with almost continuous bleeding requiring multiple platelet transfusions. The cause for this remains unclear. Menorrhagia was present in all female patients above the age of puberty.

Different types of mutations were identified among 11 patients in this study, such as missense, nonsense, deletion, and splice site mutations, thus conforming to the genetic diversity of this disorder in southern India [23]. Recently, we described in detail, the α_{IIb} mutations, namely G128S, S287L, and G357S, that are located in the highly conserved 'cage' motif within the β-propeller domain [27]. Including the above, 11 candidate mutations have been reported within the β-propeller domain of α_{IIb} affecting receptor biogenesis and/or function [28–34].

The 575Cys → Gly mutation is located in the region encoding the fourth cysteine-rich repeat of β_3 (I-EGF-4 domain). There are a total of 56 cysteine residues in β_3 that are involved in intra-chain disulphide bond formations. Mutation of Cys575 is predicted to disrupt the disulphide bond with Cys586, and hence could affect β_3 folding [24]. Evidence points to the development of a GT phenotype if the short-range disulphide bonds in the protease-sensitive region of β_3 are interfered with, resulting in a misfolded protein [35]. A mutation affecting the same Cys575 residue namely, Cys575Arg was previously reported in a homozygous form in three affected siblings (two of them dizygotic twins) and in a heterozygous form in three other patients in Italy [7].

The exact mechanism by which the other α_{IIb} missense mutations identified in this study (Arg520Trp and Leu799Arg) cause GT has not been determined. The two α_{IIb} nonsense mutations which were also identified in this study, namely Trp16Stop and Glu715Stop are predicted to lead to premature termination of translation.

The point mutation at the splice acceptor site (A**G** → A**A**) of intron 15 of α_{IIb} resulted in diminished expression of an alternatively spliced mRNA transcript, in addition to a transcript resulting from normal splicing. However, no corresponding protein products were detectable in the platelet lysate, suggesting that the mutant α_{IIb} mRNA was unstable.

The 7-bp deletion within exon 3 of β_3, 330–336 TCCCCAG is predicted to produce an out-of-frame deletion. RT-PCR analysis revealed a product of about the size of the normal control cDNA product, consistent with normal splicing, sequencing of which confirmed the 7-bp deletion. This deletion was followed by premature termination of translation at the new codon position 141 of the mature β_3 protein.

In conclusion, these data confirm the genetic diversity of mutations and polymorphisms in α_{IIb} and β_3 genes in patients with GT in southern India and provides insights into the structure/function relationships in these genes. As we could not identify any clear genotype–phenotype correlations and because the range of bleeding symptoms was quite broad, it is clear that other genetic and/or environmental factors, still to be defined, affect the clinical expression of the disorder.

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Disclosure of Conflict of Interests

B. S. Coller is an inventor of abciximab and in accord with Federal Law and the policies of the Research Foundation of the State University of New York, shares in royalties paid to the Foundation for sales of abciximab. No other authors have a conflict of interest.

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