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Congenital Macrothrombocytopenia is a heterogeneous disorder in India

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Abstract

Introduction: Inherited macrothrombocytopenia represents a heterogeneous group of disorders which are characterised by the presence of a reduced number of abnormally large platelets in the circulation, which may or may not be associated with a bleeding tendency. In spite of several causative genes having been identified, the underlying genetic defects remain to be identified in approximately half of the cases.

Aims: To understand the molecular pathology of isolated giant platelet disorder from India.

Materials and Methods: We studied 112 cases who were referred for investigation of macrothrombocytopenia. Agonist induced platelet aggregation and platelet GP1b/IX/V receptor expression were investigated to assess GP1b/IX/V receptor expression and the *GP1BA*, *GP1BB*, *GP9*, *ABCG5, ABCG8, TUBB1* and *MYH9* genes were analysed to identify candidate gene defects.

Results: Twenty three candidate gene defects were identified in 48 of 112 cases, 20 of which were novel. Of the candidate defects identified, 91% were missense and 9% were nonsense variations. The missense variations were in *GP9* (9), *ABCG5* (4), *GP1BB* (3), *GP1BA* (3), and *MYH9* (2), while the nonsense defects occurred in *MYH9* (1) and *GP1BA* (1).

Conclusions: This study increases the understanding of the molecular basis of an isolated giant platelet disorder, a common heterogeneous condition prevalent in North and Eastern India.

Introduction

Defined as a reduction in the number of platelets in the circulation, thrombocytopenia is usually an acquired disorder. However, defects in the genes regulating megakaryocyte differentiation and platelet production, which result in autosomal dominant or recessive, and X-linked forms of inherited thrombocytopenia, are increasingly being recognised [1-6]. Thus, while previously considered to be a rare disorder, it is now thought that the frequency of inherited thrombocytopenia may be underestimated. The variable clinical expression of inherited thrombocytopenia may contribute to its under diagnosis since some patients are asymptomatic and the relatively mild bleeding symptoms in others can frequently be overlooked until a low platelet count is detected often as part of a routine blood test [7]. In contrast, patients with more severe forms of inherited thrombocytopenia are usually identified early in the perinatal period, or at times of haemostatic challenge.

Inherited macrothrombocytopenia, the most frequent form of inherited thrombocytopenia, represents a heterogeneous group of disorders characterised by a reduction in the number and an increase in the size of platelets. The molecular defects have been elucidated in a number of cases. A significant proportion of patients have autosomal dominantly inherited MYH9-related disorders which are due to defects in *MYH9* that disrupt the assembly or stability of the myosin complex and cause profound abnormalities in the platelet cytoskeleton that can also be associated nephritis, deafness and cataracts [2].

In 2002, *Naina et al.* described a form of inherited thrombocytopenia which appeared to be highly prevalent among healthy blood donors in the North-Eastern states of the Indian subcontinent, affecting up to one third of healthy blood donors in West Bengal [5]. Termed Harris Platelet Syndrome (HPS), it was characterized by mild to severe macrothrombocytopenia, normal platelet function and an absence of inclusion bodies in the neutrophils. None of the donors had a history of excessive bleeding. Preliminary family studies suggested an autosomal dominant mode of inheritance and also that defects in *MYH9* were not involved. In contrast, none of the randomly selected donors from Tamil Nadu in Southern India were found to have macrothrombocytopenia. The apparent absence of MYH9-RD in HPS suggests that the spectrum of molecular defects underlying macrothrombocytopenia in the West Bengal population differs from that observed in other well characterised cohorts of patients with inherited thrombocytopenia which, to date, have mainly been of European and Eastern origins. These findings also suggest that founder effects may contribute to the high prevalence of macrothrombocytopenia in the North East, compared to the South of India.

In this study we have investigated the clinical expression and molecular basis of inherited macrothrombocytopenia in 112 index cases, the majority of whom originated from West Bengal and the North-Eastern states of India and Nepal. Analysis of genes that have previously been associated with inherited macrothrombocytopenia identified candidate defects in 48 index cases

leading us to conclude that inherited macrothrombocytopenia is a heterogeneous disorder in this population.

Materials and Methods

Study subjects and Methods

One hundred and twelve unrelated cases, who were referred to the Department of Haemostasis, National Institute of Immunohaematology, Mumbai or the Department of Haematology, NRS Medical College, Kolkata for investigation of macrothrombocytopenia, were studied. Cases were enrolled in the study if they had a reduced platelet count (<150 X 10^9/L) and a mean platelet volume (MPV) greater than 10fl which was associated with the presence of giant platelets as revealed by light microscopic examination of a peripheral blood smear. In some cases, the MPV was greater than 18.5fl and could not be determined by the cell counter, but the presence of giant platelets was confirmed by examination of the peripheral blood smear.

All cases had normal anti-platelet antibody [8], and ferritin levels, and were grouped according to whether they had mild (100-150 X 10^9/L), moderate (50-100 X 10^9/L) or severe (<50 X 10^9/L) thrombocytopenia. A detailed clinical history, including age of onset and type of any bleeding symptoms was recorded for each case, using the World Health Organisation (WHO) bleeding scale (grade 0, no bleeding; grade 1, petechiae; grade 2, mild blood loss; grade 3, gross blood loss; grade 4, debilitating blood loss) to score the severity of bleeding symptoms. A family history of bleeding symptoms and information on consanguinity, were also obtained.

Candidate gene defects identified in the index cases were sought in genomic DNA from 100 healthy control subjects (Age Range: 10-65 years; Male/Female: 52/48) all of whom had normal platelet counts (>200 X 10^9/L), and MPV less than 10 fl and were also enrolled in the study.

The study was approved by the ethics committee and undertaken in accordance with the ethical guidance of the institution involved (No. IIH/IEC/11-2007). Written, informed consent was obtained from all study subjects (cases and controls) prior to the collection of citrated and EDTA blood samples for phenotypic and genotypic analyses.

Laboratory Investigations

Coagulation tests to exclude a plasma factor deficiency included a prothrombin time (PT) with neoplastin CI plus (Diagnostica Stago, Paris, France), activated partial thromboplastin time (APTT) with actin activated cephaloplastin reagent (Dade Behring FSL, Marburg, Germany), and thrombin time (TT) with commercial bovine thrombin (Baxter Diagnostic Inc. Dade Dives-USA). All assays were carried out in a semi-automated coagulometer (Start 4, Diagnostica Stago, France) as described previously [9]. Full blood counts were determined on samples taken into EDTA using the XT-2000i cell counter (Transasia Bio-Medical Ltd, Mumbai, India) within 2 hours of collection. Peripheral blood smears were stained using modified Leishman's stain and evaluated by light microscopy.

Platelet rich plasma (PRP) was prepared by centrifugation at 100g for 10 mins and *in vitro* platelet aggregation in response to ristocetin (0.5 and 1.25 mg/ml), adenosine diphosphate (ADP) (5μM), arachidonic acid (AA) (0.75mM) and collagen (2 μg/ml) was assessed using a lumi-aggregometer (Chronolog, Haverton, PA, USA). Expression of platelet membrane glycoproteins was assessed by flow cytometry in samples of PRP which were diluted with phosphate buffered saline, before being incubated with fluorescein isothiocyanate (FITC) conjugated monoclonal anti-human GPIb (CD42b), GPIX (CD42a) or phycoerythrin (PE) conjugated anti-GPIIb/IIIa (CD41a) antibodies, or the appropriate IgG1 and IgG2a isotype control antibodies (BD Biosciences, Pharmingen, San Jose, CA, USA). Following incubation, platelets were analysed for cell bound fluorescence in a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Genotyping Studies

Genomic DNA was extracted from peripheral blood samples using a standard phenol-chloroform method and quantified using a NanodropTM1000 spectrophotometer (Thermo Scientific). Sequencing of coding and non-coding regions of *GP1BA, GP1BB, GP9, TUBB1, MYH9, ABCG5 and ABCG8* was then undertaken to identify candidate gene defects.

PCRs contained DFS10X complete reaction buffer and DFS-Taq polymerase (5U/μl) (BIORON GmbH, Germany), 10pmoles of each primer, 25mM dNTPs, 25mM $MgCl_2$ and 100ng of genomic DNA in a final volume of 25μl. PCRs were

subjected to an initial denaturation step at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing of the primers at different temperatures for 45 seconds and elongation at 72°C for 1 minute. The final elongation step was carried out at 72°C for 10 minutes before incubating the PCR products at 4°C. The sequences of all primers and annealing temperatures of PCRs are shown in supplementary Table 1. PCR products were purified either by electrophoresis followed by gel extraction (QIAGEN Ltd, Manchester, UK) or by treatment with ExoSAP-IT (GE Healthcare, Little Chalfont, UK), before being sequenced in both directions using BigDye® Terminator v3.1 Cycle Sequencing (Applied Biosystems, Paisley, UK) and sequence analysis was performed on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

In Silico Analysis

Sequencher v4.9 (http://www.genecodes.com) was used for DNA sequence assembly. The functional importance of candidate missense variations was investigated using Align GVGD (http://agvgd.iarc.fr), Sorting Intolerant from Tolerant (SIFT; http://sift.bii.a-star.edu.sg/) and Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph/) [10]. The positions of amino acids and nucleotides were denoted using Human Genome Variation Society (HGVS) nomenclature (www.hgvs.org) and checked using Mutalyzer (https://mutalyzer.nl/check?name). The effects of amino acid substitutions caused by candidate single nucleotide variations on protein stability were predicted using I-Mutant2.0 (http://folding.biofold.org/i-mutant/i-

mutant2.0.html) [11] and MUpro (http://mupro.proteomics.ics.uci.edu/) [12]. All the above online tools were accessed in December 2014.

Statistical Analysis

Data were analysed using INSTAT Graphpad analyser (graphpad.com/scientific-software/instat/) using Spearman's rank correlation method. *P* values <0.05 were considered significant [13].

Results

Clinical and Phenotypic Features of the Index cases

The clinical features of the index cases are summarised in Table 2. The cases comprised 55 females (median age 25 years; range 8-70 years) and 57 males (median age 30 years; range 8- 70 years) (Table 3). The geographic distribution of cases across India is shown in (Fig. 1). Of the cases, 68 (61%) reported no history of bleeding symptoms (grade O), and 44 (39%) of the patients had bleeding manifestations; 6(5%) had grade 1 bleeding (petechiae), 18 (16%) had grade 2 bleeding (mild blood loss), 16 (14%) had grade 3 bleeding (gross blood loss) and only 4 (4%) patients reported grade 4 bleeding symptoms (debilitating blood loss). Predominant clinical manifestations were easy bruising 4 (4%), ecchymosis 8 (7%), epistaxis 18 (16%), frequent gum bleeding 6 (5%), menorrhagia 11 (10%) and a prolonged history of bleeding after trauma 7 (6%). One male, BM35.01, who had a history of frequent gum bleeds and two females who reported menorrhagia, BM78.01 & BM105.01, had previously required transfusions for excessive bleeding. Of the 68 asymptomatic cases, the majority of whom were

identified incidentally during routine analysis of blood samples, 12 had a family history of bleeding and 3 had a personal history of transfusion in the past. There was no known consanguinity amongst the cases studied. The study of inheritance pattern in two index cases (BM15.01 & BM27.01) and their family members showed dominant mode of inheritance (Fig. 2).

Examination of peripheral blood smears, which were available for all but one index case, BM46.01, confirmed the diagnosis of macrothrombocytopenia, and revealed the presence of stomatocytes in the red blood cells from 10 cases (Fig. 3), and the absence of leukocyte inclusion bodies, in all cases. There was a wide variation in platelet count among the cases; 45(40%) had mild thrombocytopenia, 54 (48%) had moderate thrombocytopenia, and 12 (11%) had severe thrombocytopenia which has resulted in their referral for investigation of bleeding problems. One patient had a normal platelet count but the peripheral blood smear revealed the presence of abnormally large platelets. The median MPV among the cases was 13.25 fl (range 12 - 16.5fl). The WHO bleeding score showed a significant inverse correlation with platelet count (P=0.016) and direct significant correlation with MPV (P=0.010) among the cases (Fig 3).

The results of screening coagulation tests were within the normal ranges in all the cases. Platelet aggregation and receptor study showed reduced expression for cases with low platelet count to normal expression for cases with mild to moderate platelet count.

Circulating platelet counts were within the normal range (mean platelet count 197.3, SEM 3.275), and peripheral blood smears confirmed the presence of normal sized platelets (mean MPV 11.09, SEM 0.074) in samples from all control subjects.

Genetic Investigations

A total of 23 heterozygous candidate single nucleotide variations (SNVs) affecting *GP9* (18 cases), *ABCG5* (12 cases), *MYH9* (11cases), *GP1BA* (4 cases) and *GP1BB* (3 cases) were identified in 48 of the 112 index cases (Table 2). Of these, 21 were non-synonymous variations that predicted amino acid substitutions in the encoded protein. The remaining two were nonsense variations, one in *GP1BA* and another in *MYH9*. The majority of the SNVs identified (20/23) were novel. Three SNVs had been previously reported; c.5797C>T (p.Arg1933*) in *MYH9,* and c.148C>T (p.Arg50Cys) and c.293C>G (p.Ala98Gly) in *ABCG5* (Table 2). Ten recurrent SNVs were identified, three in *MYH9* (p.Asp1948Asn, n=7; p.Glu1946Lys, n=2; p.Arg1933*, n=2), four in *ABCG5* (p.Arg50Cys, n=6; p.Asn551Lys, n=2; p.Ala98Gly, n=2; p.Asp71Asn, n=2) and three in GP9 (p.Ser62Thr, n=4; p.Arg97Pro, n=4; p.Arg39Gly, n=4). No sequence alterations were detected in either *TUBB1* or *ABCG8* in DNA from the first 50 index cases investigated. These genes were therefore not analysed in the remainder of the cases. None of the alterations identified among the cases, were detected among the control subjects.

Comparison of the allelic distributions of several common polymorphisms of *GP9*, *GPIBA*, *ABCG5* and *TUBB1* revealed a significant association between

two *GP9* polymorphisms and macrothrombocytopenia. SNP rs6069 [c.132 G>A (p.Thr44=), a silent change (OR= 0.16 95% CI=0.07-0.37, P< 0.0001)] and rs3796130 [c.466G>A (p.Ala156Thr) (OR= 0.13 95% CI=0.03 to 0.51], P=0.0034)], the risk/rare allele A for both SNP was found to be significantly associated with macrothrombocytopenia (Table 4).

***In silico* predictions of effects of candidate SNVs**

Seventeen of the 20 novel missense changes identified in the study were predicted to be deleterious to protein function and stability (Table 5). Three SNVs, two predicting p.Leu176Arg and p.Gln76His substitutions in GPIX, and a third predicting a p.Glu1946Lys substitution in non-muscle myosin heavy chain 9 were predicted to have benign or borderline effects on protein function using two bioinformatic tools (PolyPhen and SIFT). However, all three of these alterations were also predicted to decrease the stability of the corresponding proteins using the MUPRO/I-Mutant 2.0 predictive tool (Table 5).

Genotype-Phenotype Correlation

We examined the association between candidate gene defects and bleeding severity (as indicated by the WHO bleeding score) among those index cases who were heterozygous for recurrent candidate gene defects. The c.115 A>G (p.Arg39Gly) SNV in *GP9* was detected in 4 cases; 1 with severe thrombocytopenia, menorrhagia and with a bleeding score of 3, and three with moderate thrombocytopenia, two of whom had no bleeding diathesis (bleeding score 0) and a third who had frequent gum bleeds (bleeding score 3). The c.293 C>G (p.Ala98Gly) SNV in *ABCG5* was identified

in 2 index cases with moderate thrombocytopenia, one having a bleeding of score of 0 and the other having a score of 4. A The c.148C>T (p.Arg50Cys) SNV in *ABCG5* was associated with mild to moderate thrombocytopenia among 6 index cases, four of whom were asymptomatic, while the other two index cases reported histories of either ecchymosis and epistaxis (bleeding score 2), or of ecchymosis alone (bleeding score 1). Similarly, the c.211G>A (p.Asp71Asn) *ABCG5* SNV was present in one asymptomatic index case and another with a history of epistaxis. The c.5797C>T (p.Arg1933*) nonsense alteration in *MYH9* was detected in two cases, one of whom was asymptomatic, while the second had a bleeding score of 2. Similarly, the c.5842 G>A (p.Asp1948Asn) SNV in *MYH9* was associated with variable symptoms among the 7 index cases who inherited this alteration (Table 2).

No correlation could be established between mutations and phenotype in Bengal Macrothrombocytopenia cases (BM) (Table 2).

Discussion

Previous studies which have observed a high prevalence of a mild to severe form of non-*MYH9* related macrothrombocytopenia among healthy blood donors in the north Eastern states of India, suggest that founder effects may contribute to the prevalence of macrothrombocytopenia in this geographic region. In this study, we have described the clinical expression of inherited macrothrombocytopenia in 112 index cases originating primarily from West Bengal and the North-Eastern states of India and Nepal. The majority (61%) of the index cases were asymptomatic and were identified incidentally as a

result of routine blood tests, while the remainder of the index cases reported symptoms consistent with the presence of a platelet bleeding disorder.

Analysis of a panel of genes which have been associated with inherited macrothrombocytopenia in other populations revealed candidate gene defects in 48 of the 112 (43%) index cases confirming the underlying heterogeneity of the macrothrombocytopenia in this population, with candidate gene defects being identified in *GP1BA* (n=4), *GP1BB* (n=3), *GP9* (n=18), *ABCG5* (n=12) and *MYH9* (n=11).

Examination of peripheral blood smears was possible for all but one index case. Interestingly, none of the MYH9 gene defects identified in this study was associated with the presence of neutrophil inclusion bodies, and further work using immunofluorescent staining of non-muscle myosin would be required to determine whether the novel *MYH9* defects identified in these patients are associated with abnormal distribution of myosin in peripheral blood neutrophils. Unfortunately, it was not possible to examine a peripheral blood smear from the index case who was heterozygous for the *MYH9* nonsense variation, p.Arg1933* which we would expect to be associated with the presence of neutrophil inclusion bodies [14]. Of note, the *MYH9* defects predicting the p.Asp1948Asn, p.Glu1946Lys alterations affected residues in the non-helical tail region of the myosin heavy chain defects in which have previously been reported to be associated with a milder bleeding diathesis [15-16].

Examination of peripheral blood smears revealed the presence of stomatocytes in the red blood cells from 10 index cases, which, in addition to

the presence of giant platelets, is a recognised haematological feature of sitosterolaemia [3, 17]. Subsequent analysis of *ABCG5* and *ABCG8*, the genes which have previously been shown to harbour defects in patients with sitosterolaemia and macrothrombocytopenia [18], revealed candidate defects in *ABCG5* in all ten cases; p.Asn551Lys (BM7.01); p.Ala98Gly (BM38.01); p.Asp71Asn (BM109.01 and BM110.01) and p.Arg50Cys (BM14.01, BM68.01, BM83.01, BM89.01, BM90.01 & BM96.01).Interestingly, the *ABCG5* defect predicting the p.Arg50Cys substitution was present in 6 index cases suggestive of a possible founder effect for this alteration in the population studied.

Several SNVs were identified in the genes encoding the platelet GPIb-IX-V receptor complex. Thus, heterozygous SNVs affecting *GP1BA* were identified in four cases, three non-synonymous SNVs predicting a p.Leu10Val substitution in the signal peptide of GPIBA, a p.Pro454Ser substitution in the Proline/Threonine rich region, and a p.Leu213Arg substitution in the Leucine rich repeat region, and a nonsense variant, introducing a premature stop codon (p.Leu488*). Three non-synonymous SNVs were identified in *GP1BB,* all predicting substitutions of amino acids in the conserved leucine rich repeats of the cytoplasmic tail of GPIBB (c.285C>G, p.Cys95Trp; c.338A>T, p.Tyr113Phe; c.320G>C, p.Arg107Pro).

Bernard Soulier Syndrome (BSS) and Sitosterolaemia (Mediterranean stomatocytosis/macrothrombocytopenia) are classically described as recessive disorders and heterozygous carriers of these disorders are usually asymptomatic or have mild bleeding symptoms [19-22]. Supporting this, the

cases studied here who were heterozygous for candidate defects in *GPIBA*, *GPIBB* and *ABCG5* were either asymptomatic or had mild to moderate bleeding symptoms. Three *GP1BA* defects which predict p.Ala172Val, p.Tyr70Asp, and p.Leu73Phe substitutions in *GPIBA*, have been reported to give rise to BSS with a dominant mode of inheritance along with the classical form of BSS when the defects are homozygously inherited [23-26]. One of these, p.Ala172Val, which is also known as the Bolzano mutation is frequently found in the Italian population [19]. In *GP1BB*, heterozygous defects predicting the amino acid substitutions, p.Arg42Cys and p.Ala133Pro give rise to isolated giant platelet disorder [27-28] while the p.Tyr113Cys substitution reported in several Japanese families suppresses expression of the GP1b/IX/V complex, giving rise to a BSS-like bleeding disorder when homozygously inherited and to isolated giant platelet disorder when heterozygously inherited [29].

The frequency of 2 SNVs in *GP*9; rs6069 (p.Thr44=), a synonymous change with minor allele frequency of 0.0583 and rs3796130 (p.Ala156Thr) with a minor allele frequency of 0.0675, were observed to be more prevalent in cases than in control subjects and were significantly associated with the disorder (Table 4). p.Ala156Thr was reported to be associated with low platelet count and also alters the protein function [30] and has a damaging effect on the protein stability as predicated by different tools (Table 5).

The identification of candidate defects in genes which have previously been associated with macrothrombocytopenia in 48 of the 112 cases studied here, lead us to conclude that inherited macrothrombocytopenia is a

heterogeneous disorder in India. Further study will be required to identify the gene defects underlying macrothrombocytopenia in the remaining 64 (57%) cases without mutations in the genes investigated here.

Greater awareness of this condition will reduce the risk of misdiagnosis and the potential for inappropriate treatment of affected individuals which is important given that congenital macrothrombocytopenia is a common and under diagnosed condition in India [5, 31].

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Table 1: Oligonucleotide Primer Sequences

Gene Name	Location	Primer Name	Orientation	Sequence 5'-3'	Annealing Temperature	Product Size (bp)
GP1BA	Exon2	1b1_F	Forward	AGGTCTTTCTGCCTGCCTGT	60^{0}C	774
		1b1_R	Reverse	TAGCCAGACTGAGCTTCTCC	60^{0}C	
	Exon2	1b2_F	Forward	AAGGCAATGAGCTGAAGACC	59^{0}C	597
		1b2_R	Reverse	CTTGTGTTGGATGCAAGGAG	59^{0}C	
	Exon2	1b3_F	Forward	TCCACTGCTTCTCTAGACAG	65^{0}C	515
		1b3_R	Reverse	GGCTGATCAAGTTCAGGGAT	65^{0}C	
	Exon2	1b4_F	Forward	CACAAGCCTGATCACTCCAA	59^{0}C	585
		1b4_R	Reverse	TTCTCTCAAGGTCCCCAAAC	59^{0}C	
GP1BB	5'UTR	GP1bb1_F	Forward	AGGATCCTGGGTCTGTTCCC	71^{0}C	775
		GP1bb1_R	Reverse	CGACCGGACTCCAGACTCAC	71^{0}C	
	Exon 2	GP1bb2_F	Forward	TTACTGCGGCGCTTCCCTTG	65^{0}C	492
		GP1bb2_R	Reverse	AAGGCCAGCAGCGCAAGCT	65^{0}C	
	3'UTR	GP1bb3_F	Forward	AGCTTGCGCTGCTGGGCCTT	65^{0}C	545
		GP1bb3_R	Reverse	TGTCCCCTTGAACCGCCTCC	65^{0}C	
GP9	981-1560	GPIX_F	Forward	TGTTCCTGCTCTGGGCCACA	60^{0}C	586
		GPIX_R	Reverse	TTGGTGGAGTCTGGGGACCT	60^{0}C	
MYH9	Exon 2	MYH9_2F	Forward	GAGGTGTGAGCATGAGTGATCTTG	68^{0}C	512
		MYH9_2R	Reverse	CGTGAGGGTGATGGGAAGACC	68^{0}C	
	Exon 3	MYH9_3F	Forward	CTCACGATGACAAAGACATCTCTC	68^{0}C	598
		MYH9_3R	Reverse	CTTAGCACCTGCAAAGGTGTCAAT	68^{0}C	
	Exon 4	MYH9_4F	Forward	GGGCAGCTCTTTGGGAGCAAGGTGGG	68^{0}C	205
		MYH9_4R	Reverse	TGGGGGACTCTGCAAGCCCCAGTTGTG	68^{0}C	
	Exon 5	MYH9_5F	Forward	GTTGGGTCCTTCACGGGCACC	68^{0}C	319
		MYH9_5R	Reverse	CAAAGCATCCTCTTGTAAAGCTGAAGCC	68^{0}C	
	Exon 6	MYH9_6F	Forward	CGGCTCTGCCATCGTCCCCCTT	68^{0}C	255
		MYH9_6R	Reverse	AAAGGCAGCATGAGCCAAAGCTCCG	68^{0}C	
	Exon 7	MYH9_7F	Forward	CCGTCTCTGGGTTTTCTCCCTCCAA	68^{0}C	324
		MYH9_7R	Reverse	CTCCACAGAGAAGGTGTCAGGATGG	68^{0}C	

	Exon 8	MYH9_8F	Forward	AGAATCGCTTGAATCCAGGAGGTG	68^{0}C	354
		MYH9_8R	Reverse	TTCATTTCCCAAATGATGTCTACGG	68^{0}C	
	Exon 9	MYH9_9F	Forward	TCTGTCCCAGTCTCTCCAACCTTT	68^{0}C	389
		MYH9_9R	Reverse	AGGAATCATTTTCCCATACACTGAAGG	68^{0}C	
	Exon 10	MYH9_10F	Forward	CTTGTCTGGCTTGAGGATCCCTAGAT	68^{0}C	277
		MYH9_10R	Reverse	AATTTCCGCAAGACCTTCCCTCCTGA	68^{0}C	
	Exon 11	MYH9_11F	Forward	GGGTCTAATTAGAACTTTCTCTCTTGGG	68^{0}C	314
		MYH9_11R	Reverse	GGAATCATGTGAAAGTGCCTGACAC	68^{0}C	
	Exon 12	MYH9_12F	Forward	AAAGTGAAATTACTGGGGCATAGGG	68^{0}C	316
		MYH9_12R	Reverse	GAAGCAGGGTTCTTAACCAAGGATAA	68^{0}C	
	Exon 13	MYH9_13F	Forward	TTCCTGTATCCCTGCCCCACCCTCCTT	68^{0}C	359
		MYH9_13R	Reverse	CAACCAACACAGAGCTGAGGTGAGGAG	68^{0}C	
	Exon 14	MYH9_14F	Forward	GATTCAGGGGATTCTGATGTCCGGG	68^{0}C	346
		MYH9_14R	Reverse	TCCTGGTCCTAGAGAGCCTCGAC	68^{0}C	
	Exon 15	MYH9_15F	Forward	TCGCTCCCCTTATCCTCACCACTTCCT	68^{0}C	393
		MYH9_15R	Reverse	TCAGGGGGCACATGTGTACCCCTTGT	68^{0}C	
	Exon16	MYH9_16F	Forward	TCCGACGTGTGCCTGTCTCTCTCT	68^{0}C	364
		MYH9_16R	Reverse	TTTGCTGGGGAGACAGACAAGGGC	68^{0}C	
	Exon 17	MYH9_17F	Forward	CCCTGTCAGGTTCATAGGGGTTC	68^{0}C	347
		MYH9_17R	Reverse	GGCCAGACTCAGTTCTACATGGATG	68^{0}C	
	Exon 18	MYH9_18F	Forward	GGTGGGATTTGCCTGTGTCTTCTTTCC	68^{0}C	332
		MYH9_18R	Reverse	GGCATCCACCGACCACTGATATAGCAA	68^{0}C	
	Exon 19	MYH9_19F	Forward	TCAGCCAGTGAGAAGAAGGGTGAA	68^{0}C	435
		MYH9_19R	Reverse	CCTCAAAGGTAGAAATCCAGGAACAG	68^{0}C	
	Exon 20	MYH9_20F	Forward	TTGAGGACAAGACCAGGACTGTTA	68^{0}C	261
		MYH9_20R	Reverse	ACAAACAATTAGCCAGGTATGTATGG	68^{0}C	
	Exon 21	MYH9_21F	Forward	CCACCACAGCGTGTCTTCTTGCC	68^{0}C	349
		MYH9_21R	Reverse	AAACTTCCAGCATGCCGTGCCTAC	68^{0}C	
	Exon 22	MYH9_22F	Forward	TGGAAGGTACCTGGAAGCTTCAGAGC	68^{0}C	479
		MYH9_22R	Reverse	GAGGAGCAGCCTCCTTGGACCCTAA	68^{0}C	
	Exon 23	MYH9_23F	Forward	CCTTCGGACCTTGCTGCCTTCAC	68^{0}C	288
		MYH9_23R	Reverse	CCCTGCAAGGGTGACCACACTC	68^{0}C	

	Exon 24	MYH9_24F	Forward	CCGGGCGAGTCATGCTTTGA	68^{0}C	294
		MYH9_24R	Reverse	CTCGGTGTTCCGGTCAGACA	68^{0}C	
	Exon 25	MYH9_25F	Forward	TGCGAGTGTCTGTGTGTTTGTGATG	68^{0}C	416
		MYH9_25R	Reverse	GTGGAAAGAATGCTCACAGCTCACTA	68^{0}C	
	Exon 26	MYH9_26F	Forward	TCAGGCCTGTCCTGCAAACTCTGCT	68^{0}C	423
		MYH9_26R	Reverse	TTCCATGCCTGCTGGTGCCTAAGAG	68^{0}C	
	Exon 27	MYH9_27F	Forward	AGAAAAGCTGCCTGGAGTGCCTGTG	68^{0}C	347
		MYH9_27R	Reverse	GCTCTGCAGGACTGGTTTGGATTCTG	68^{0}C	
	Exon 28	MYH9_28F	Forward	GGTCCAGTGATGATAGACCAGCCA	68^{0}C	390
		MYH9_28R	Reverse	GCCAGTTTGAGAAGAGAGAGAGACAG	68^{0}C	
	Exon 29	MYH9_29F	Forward	CTGTCTCTCTCTCTTCTCAAACTGGC	68^{0}C	329
		MYH9_29R	Reverse	GGCTCTGAAGCTAATGTTGCGTGG	68^{0}C	
	Exon 30	MYH9_30F	Forward	TCCCTCTCCTCAAGGGTGTGGGGTT	68^{0}C	394
		MYH9_30R	Reverse	CCTTGAGAGCACTGATGTGGGAGAGCA	68^{0}C	
	Exon 31	MYH9_31F	Forward	GGTTTCATAACTGGGCAGATCCCT	68^{0}C	530
		MYH9_31R	Reverse	AGCCTGAGGGTCCTCTAAGCACTG	68^{0}C	
	Exon 32	MYH9_32F	Forward	ACTGTGTGTATTGTCCTGGGC	68^{0}C	504
		MYH9_32R	Reverse	AAGTCAGGAGCAAAGGGACT	68^{0}C	
	Exon 33	MYH9_33F	Forward	GGAGGACCTTATGAGCTCCAAG	68^{0}C	479
		MYH9_33R	Reverse	CAGGTGGAAGGAGAGAACAGAA	68^{0}C	
	Exon 34	MYH9_34F	Forward	CCATGGATCCTGCAGAACT	68^{0}C	385
		MYH9_34R	Reverse	GGACCTTCCCAGGAGGTG	68^{0}C	
	Exon 35	MYH9_35F	Forward	ATACAGCATTGAGTGGAGCACCAGC	68^{0}C	324
		MYH9_35R	Reverse	CCTGTCCTCAGCTGAAAGCCCCA	68^{0}C	
	Exon 36&37	MYH9_36 &37F	Forward	GTGAGCTAGAGGGTTTCTGGAGGAA	68^{0}C	575
		MYH9_36 &37R	Reverse	GGTGCCTGGACATTTTCCCCTAAG	68^{0}C	
	Exon 38	MYH9_38F	Forward	TTCTGGGAGACCCAAGACTCTGGAC	68^{0}C	442
		MYH9_38R	Reverse	TCAGGAGACAGAGAGCTGGTTGTGG	68^{0}C	
	Exon 39	MYH9_39F	Forward	TGGGTGGTCCTGGTTAGGGCTTGTT	68^{0}C	362
		MYH9_39R	Reverse	CTTGAGCTGCTTCAGGCGGGTAGAT	68^{0}C	
	Exon 40	MYH9_40F	Forward	GAGCGGAGGAACGCCGAGCAGTACA	68^{0}C	432
		MYH9_40R	Reverse	CGTGCCTTGCTTGTGGGCTCTGGTTGA	68^{0}C	

	Exon 41	MYH9_41F	Forward	TTGAGATGTGTGGGCTGTGCTG	68^{0}C	301
		MYH9_41R	Reverse	TCACAGCAGTCCCAAGAAGGTG	68^{0}C	
ABCG5	Exon 1	ABCG5_1F	Forward	CCAACTGAAGCCACTCTGG	58^{0}C	293
		ABCG5_1R	Reverse	AAGAGTGAAGAAAGGCAGCA	58^{0}C	
	Exon 2	ABCG5_2F	Forward	CACAGGTAGGATCAATGCTG	58^{0}C	305
		ABCG5_2R	Reverse	CAAACCTGTGGCTTTCTTGT	58^{0}C	
	Exon 3 & 4	ABCG5_3&4F	Forward	CACAGAGGGTCTCGGGAAG	60^{0}C	499
		ABCG5_3&4R	Reverse	GAGTGACGAGCAAAGGGAAG	60^{0}C	
	Exon 5	ABCG5_5F	Forward	GTGTGCTGCCTCTTTCATGT	60^{0}C	283
		ABCG5_5R	Reverse	TGCACACACACAGAAGATGC	60^{0}C	
	Exon 6	ABCG5_6F	Forward	GTTTACTTCCCACCGCACACT	60^{0}C	322
		ABCG5_6R	Reverse	GATTCCCAGCTCAACACACCA	60^{0}C	
	Exon 7	ABCG5_7F	Forward	CCAGAGACATTCAAAGTGCA	58^{0}C	267
		ABCG5_7R	Reverse	TCCAGGCAGAAGTCTGAGAT	58^{0}C	
	Exon 8	ABCG5_8F	Forward	GGCCAGTACTCCTGTACCAA	58^{0}C	361
		ABCG5_8R	Reverse	GTTATTGGGGGATGGCTAAA	58^{0}C	
	Exon 9	ABCG5_9F	Forward	TAGCCATCCCCCAATAACAAT	60^{0}C	300
		ABCG5_9R	Reverse	GAGAAAGAGGTGCACCTCCAG	60^{0}C	
	Exon 10	ABCG5_10F	Forward	AGACCTCACATTCAGCTTGG	60^{0}C	283
		ABCG5_10R	Reverse	TCCCACTAGCTCCATGACTC	60^{0}C	
	Exon 11	ABCG5_11F	Forward	TCACAGAGGCAAGTGCAGTA	60^{0}C	348
		ABCG5_11R	Reverse	TCTGGTATTCCTTTACTTCAGTCAT	60^{0}C	
	Exon 12	ABCG5_12F	Forward	TTGCCTTTCTTTTCATTTGG	58^{0}C	246
		ABCG5_12R	Reverse	CCAAGAAATTGCTTCCTCAG	58^{0}C	
	Exon 13	ABCG5_13F	Forward	ACCTGAGATAAACCACACCTG	60^{0}C	298
		ABCG5_13R	Reverse	TCAGAGCAGTCATGCACAGT	60^{0}C	
ABCG8	Exon 1	ABCG8_1F	Forward	GCAAGGAATGCTGGGAGAG	60^{0}C	286
		ABCG8_1R	Reverse	AGGCTCCTGAGGGAAGAGAG	60^{0}C	
	Exon 2	ABCG8_2F	Forward	GCCCACCCTTTTATTTCCAC	60^{0}C	270
		ABCG8_2R	Reverse	GCCCACCCTTTTATTTCCAC	60^{0}C	
	Exon 3	ABCG8_3F	Forward	TGAAGCCCTCTGAACCATTC	60^{0}C	256
		ABCG8_3R	Reverse	TCCCAGGAGAGAAACCATTG	60^{0}C	

	Exon 4	ABCG8_4F	Forward	GGAGAGTGTATGGGGAGCAG	60^{0}C	449
		ABCG8_4R	Reverse	GGAAGGCAAGCTGAGTTGTT	60^{0}C	
	Exon 5 & 6	ABCG8_5&6F	Forward	CCTTTATCCTTGGGGTCACA	60^{0}C	667
		ABCG8_5&6R	Reverse	AAGCTTGGGCAGGGTTTAAG	60^{0}C	
	Exon 7 & 8	ABCG8_7&8F	Forward	GGTGATCAGCATTGTGAGCTG	62^{0}C	596
		ABCG8_7&8R	Reverse	CTGGGATTACAGGCATAAGCC	62^{0}C	
	Exon 9	ABCG8_9F	Forward	CCCCATTTTGCATAGGAGAA	62^{0}C	369
		ABCG8_9R	Reverse	AGGAACACAGCTTGGAGGTG	62^{0}C	
	Exon 10	ABCG8_10F	Forward	AGTCTCCAAAACAGAAGCACTG	64^{0}C	223
		ABCG8_10R	Reverse	TGTAGCAACGTTTTCTCCACA	64^{0}C	
	Exon 11	ABCG8_11F	Forward	AGTGAAGGTGCTGGCTTCAT	60^{0}C	379
		ABCG8_11R	Reverse	AGCAGGCTTCATCCAGTCAC	60^{0}C	
	Exon 12 & 13	ABCG8_12&13F	Forward	CGAATATGGGGAAACCATGA	60^{0}C	467
		ABCG8_12&13F	Reverse	TTGAAGGGTCTGCTCAGGTC	60^{0}C	
TUBB1	Exon 1	TUBB1_EX1F	Forward	AACCGAAGCTCTGGATTCTG	60^{0}C	269
		TUBB1_EX1R	Reverse	AAGCCCAAAGGCATTGTCTG	60^{0}C	
	Exon 2	TUBB1_EX2F	Forward	TTTCTCTGTGGTTAACACAGC	55^{0}C	283
		TUBB1_EX1R	Reverse	CTGAGCATAGACATCACTGC	55^{0}C	
	Exon 3	TUBB1_EX3F	Forward	TGGACCAGTATCACAAAGTTC	55^{0}C	726
		TUBB1_EX3R	Reverse	CTCATGGTCAAGGACACTAG	55^{0}C	

Table 2: Phenotypic and genotypic features of Congenital Macrothrombocytopenia cases from India

Patient ID	Sex	AOD (Yrs)	Clinical Manifestations/ WHO Bleeding Score	Family History of Bleeding	Platelet Count ($X10^9$/L) NR:150-450	MPV (fl) NR: 7.8-10.2	Mutation		
							Gene	cDNA[a]	Protein
BM1.01	M	19	0/0	No	91	12	*GP9*	c.185 G>C	*p.Ser62Thr*
BM2.01	M	25	0/0	No	66	12.5	*GP9*	c.146 T>G	*p. Leu49Arg*
BM3.01	M	30	0/0	No	145	13	NMD		
BM4.01	M	20	0/0	No	81	13	NMD		
BM5.01	F	28	4/2	Yes	107	12	NMD		
BM6.01	M	30	1/1	No	51	13.9	*GP9*	c.290 G>C	*p. Arg97Pro*
BM7.01	F	32	3/3	No	54	13	*ABCG5*	c.1653 C>G	*p. Asn551Lys*
BM8.01	F	67	0/0	No	65	12.9	*GP9*	c.115 A>G	*p. Arg39Gly*
BM9.01	M	70	0/0	No	73	12	NMD		
BM10.01	F	37	1,4/2	yes	77	12.5	*GP1BB*	c.285 C>G	*p.Cys95Trp*
BM11.01	F	60	0/0	No	90	12	NMD		
BM12.01	M	42	0/0	No	74	>18.5*	NMD		
BM13.01	M	40	0/0	No	109	13.1	NMD		
BM14.01	F	17	2, 4/2	No	140	13.3	*ABCG5*	c.148 C>T	p.Arg50Cys[k]
BM15.01	M	30	5/4	Yes	27	14.1	NMD		
BM16.01	M	55	0/0	No	52	12.9	NMD		
BM17.01	F	15	3/3	No	34	>18.5*	*GP9*	c.115 A>G	*p. Arg39Gly*
BM18.01	M	47	5/3	Yes	47	>18.5*	*GP9*	c.83C>G	*p.Ala28Gly*
BM19.01	F	27	0/0	No	55	>18.5*	*GP9*	c.466G>C	*p. Ala156Pro*

BM20.01	M	25	4/2	No	140	12	NMD		
BM21.01	M	30	4/2	No	122	13.2	NMD		
BM22.01	F	45	0/0	No	106	12	NMD		
BM23.01	F	40	0/0	No	73	14	NMD		
BM24.01	F	23	0/0	No	103	13.9	NMD		
BM25.01	F	25	3/3	No	51	13	NMD		
BM26.01	M	35	0/0	No	52	15.5	*GP1BA*	c. 28 G>C	*p.Leu10 Val*
BM27.01	F	18	3/4	yes	30	14.5	NMD		
BM28.01	F	52	0/0	No	36	16.5	NMD		
BM29.01	M	40	0/0	No	45	16	NMD		
BM30.01	F	9	4/2	No	83	16	*ABCG5*	c.293 C>G	p.Ala98Gly[k]
BM31.01	M	40	0/0	No	92	14	NMD		
BM32.01	M	35	4,5/3	Yes	107	13	NMD		
BM33.01	M	21	6/3	No	133	13	NMD		
BM34.01	M	22	0/0	No	95	13.9	NMD		
BM35.01	M	29	6/3 Tx	No	42	>18.5*	NMD		
BM36.01	F	24	3/2	No	97	13.6	NMD		
BM37.01	M	18	0/0	No	71	14	NMD		
BM38.01	F	45	0/0	No	51	15.6	*ABCG5*	c.293 C>G	p.Ala98Gly[k]
BM39.01	F	42	2/1	No	77	14	*GP1BB*	c.320 G>C	p.Arg107Pro
BM40.01	F	19	6/3	Yes	65	13.9	*GP9*	c.527 T>G	p.Leu176Arg
BM41.01	M	52	2/1	No	54	14.7	*GP9*	c.203 C>G	*p.Pro68Arg*
BM42.01	M	25	0/0	No	113	13.4	NMD		
BM43.01	M	30	0/0	No	128	12.9	NMD		
BM44.01	M	29	0/0	No	113	13.9	NMD		

BM45.01	F	25	2/1	No	125	13.3	NMD		
BM46.01	M	38	0/0	No	40	14.9	*MYH9*	c.5797 C>T	p. Arg1933*[k]
BM47.01	F	9	2,5/3	No	45	14	NMD		
BM48.01	M	30	0/0	No	67	13	NMD		
BM49.01	M	52	0/0	Yes	72	12	*GP9*	c.115A>G	*p. Arg39Gly*
BM50.01	M	38	6/3	No	71	14.2	*ABCG5*	c.1653 C>G	*p.Asn551Lys*
BM51.01	F	45	0/0	No	137	13.2	NMD		
BM52.01	F	48	0/0	No	103	13.9	NMD		
BM53.01	F	25	4/2	No	118	14.5	NMD		
BM54.01	M	57	6/3	No	65	>18.5*	*GP9*	c.115A>G	*p. Arg39Gly*
BM55.01	M	50	4/2	Yes	115	14.3	NMD		
BM56.01	F	26	1,3/3	Yes	38	14.2	NMD		
BM57.01	F	34	4/2	No	147	13	*GP9*	c.290 G>C	*p.Arg97Pro*
BM58.01	M	59	0/0	No	131	14.2	NMD		
BM59.01	M	55	0/0	No	72	12.5	*GP9*	c.290 G>C	*p.Arg97Pro*
BM60.01	M	25	0/0	No	101	13	NMD		
BM61.01	M	23	0/0	No	110	14.2	*GP9*	c.185G>C	*p.Ser62Thr*
BM62.01	M	21	0/0	No	140	12	NMD		
BM63.01	M	24	0/0	No	105	15.3	*GP9*	c.185G>C	*p.Ser62Thr*
BM64.01	M	27	0/0	No	121	12	NMD		
BM65.01	F	28	0/0	No	78	13.8	NMD		
BM66.01	F	25	0/0	No	82	13.5	NMD		
BM67.01	F	25	5/3	Yes	67	13.9	*GP1BA*	c.1360 C>T	*p.Pro454Ser*
BM68.01	M	40	4/2	No	83	>18.5*	*ABCG5*	c.148C>T	p.Arg50Cys[k]
BM69.01	M	44	4/2	No	91	>18.5*	*GP1BB*	c.338A>T	*p.Tyr113Phe*

BM70.01	F	18	0/0	No	107	13.8	NMD		
BM71.01	M	21	0/0	No	88	12.8	*GP9*	c.290 G>C	*p. Arg97Pro*
BM72.01	F	34	1,3/3	Yes	80	13.9	NMD		
BM73.01	M	32	0/0	No	36	>18.5*	NMD		
BM74.01	M	30	0/0	No	63	12.4	*GP9*	c.185G>C	*p.Ser62Thr*
BM75.01	M	19	5/3	No	53	>18.5*	NMD		
BM76.01	F	8	0/0	No	49	>18.5*	NMD		
BM77.01	M	30	0/0	No	131	14	*GP9*	c.228G>C	*p.Gln76His*
BM78.01	F	15	3,5,6/4 Tx	No	47	>18.5*	*GP1BA*	c.638T>G	*p. Leu213Arg*
BM79.01	M	33	0/0	No	51	>18.5*	NMD		
BM80.01	F	30	3,4/4	No	101	14.8	NMD		
BM81.01	M	17	2,4/2	No	140	13	NMD		
BM82.01	F	16	0/0	No	70	16.1	*GP1BA*	c.1463T>G	*p.Leu488**
BM83.01	M	42	0/0	No	80	12.8	*ABCG5*	c.148C>T	p.Arg50Cys[k]
BM84.01	M	40	0/0	No	125	13.6	NMD		
BM85.01	F	70	0/0	No	109	13	NMD		
BM86.01	M	45	0/0	No	132	12	NMD		
BM87.01	F	30	3/3	No	90	13	NMD		
BM88.01	F	32	4/2	No	105	12.1	NMD		
BM89.01	F	11	2/1	No	120	12.6	*ABCG5*	c.148C>T	p.Arg50Cys[k]
BM90.01	F	15	0/0	No	139	13.8	*ABCG5*	c.148C>T	p.Arg50Cys[k]
BM91.01	F	23	0/0	No	89	14	NMD		
BM92.01	F	16	0/0	No	90	13.1	NMD		
BM93.01	F	23	0/0	No	123	12	NMD		
BM94.01	F	19	0/0	No	78	>18.5*	NMD		

BM95.01	M	12	0/0	No	98	>18.5*	NMD		
BM96.01	F	20	0/0	No	110	14	*ABCG5*	c.148C>T	p.Arg50Cys[k]
BM97.01	F	14	0/0	No	136	12.9	NMD		
BM98.01	M	47	0/0	No	148	12.3	NMD		
BM99.01	M	58	0/0	No	134	12	NMD		
BM100.01	M	45	0/0	No	90	13	NMD		
BM101.01	F	48	0/0	No	83	13.5	*MYH9*	c.5836G>A	*p.Glu1946Lys*
BM102.01	F	36	2/1	No	67	13.1	*MYH9*	c.5797C>T	p.Arg1933*[k]
BM103.01	F	50	0/0	No	89	12.8	*MYH9*	c.5842G>A	*p.Asp1948Asn*
BM104.01	F	49	0/0	No	112	12.9	*MYH9*	c.5842G>A	*p.Asp1948Asn*
BM105.01	F	17	3/2 Tx	No	150	12.5	*MYH9*	c.5842G>A	*p.Asp1948Asn*
BM106.01	M	15	0/0	No	198	>18.5*	*MYH9*	c.5836G>A	*p.Glu1946Lys*
BM107.01	F	09	0/0	No	101	12.9	*MYH9*	c.5842G>A	*p.Asp1948Asn*
BM108.01	M	25	0/0	No	80	13.5	*MYH9*	c.5842G>A	*p.Asp1948Asn*
BM109.01	F	25	0/0	No	115	13.1	*ABCG5*	c.211G>A	*p.Asp71Asn*
BM110.01	F	35	4/2	No	76	14.1	*ABCG5*	c.211G>A	*p.Asp71Asn*
BM111.01	F	25	4/2	No	75	14	*MYH9*	c.5842G>A	*p.Asp1948Asn*
BM112.01	M	20	4/2	No	75	13.5	*MYH9*	c.5842G>A	*p.Asp1948Asn*

AOD: age of diagnosis**; *:** instrument did not measure MPV above 18.5fl; **k :** known mutation **; a:** nucleotide A of the ATG translation initiation start site of the *GP1BA* (NM_000173.5), *GP1BB* (NM_000407.4), *GP9* (NM_000174.3), *ABCG5* (NM_022436.2) and *MYH9* (NM_002473.4) is indicated as nucleotide +1, (mutations are mapped to the immature protein structure of the gene); **Tx:** transfused; **NR:** normal range; **NMD:** no mutation detected. **Clinical manifestations:** 0. Asymptomatic 1. Easy Bruisability 2. Echymoses 3. Menorrhagia 4. Epistaxis 5. Prolonged bleeding after trauma 6. Frequent gum bleed. **WHO (World Health Organisation): grade 0**: no bleeding; **grade 1:** petechiae; **grade 2:** mild blood loss; **grade 3:** gross blood loss; **grade 4:** debilitating blood loss.

Table 3: **Demographic and laboratory findings of included cases**

Parameters	**Median (IQR)**
Age (yrs) (n=112)	30 (8-70)
Age Females (yrs)(n=55)	25(8-70)
Age Males (yrs) (n=57)	30 (8-70)
Platelet Count ($X10^9$/L) (n=112)	89 (27-198)
MPV (fl) (n=96)	13.25 (12-16.5)
WHO Bleeding Score	0 (0-4)
Screening Coagulation Assays (secs) (n=112)	
Prothrombin Time (PT) (NR: 12-14)	12.5 (11-13.9)
Activated Partial Thromboplastin Time (APTT) (NR:28-33)	28.6 (25-32)
Thrombin Time (TT)(NR: 15-19)	15.1 (12.9-18)
Receptor Study (%) (n=112)	
GP1b (CD42b) (NR: 50-150)	75.3 (42.9-98.6)
GPIIb/IIIa (CD41a) (NR: 50-150)	80.5(34.6-98.8)
GPIX (CD42a) (NR: 50-150)	79.35 (27.3-98.0)
Genes	Platelet Count ($X10^9$/L)
GP1BA (n=4)	59.50 (47-70)
GP1BB (n=3)	77.0 (77-91)
GP9 (n=18)	65.50 (34-147)
ABCG5 (n=12)	83.0 (51-140)
MYH9 (n=11)	83.0 (40-198)

IQR denotes the 25^{th} and 75^{th} interquartile range and n denotes no. of patients; NR: Normal Range

Table 4: Statistical Analysis of known Single Nucleotide Variants in cases with congenital macrothrombocytopenia and normal controls from India

Polymorphic Marker		Alleles	Odds ratio for risk allele (95% confidence Interval)	P Value
Gene	**Mutation/Polymorphism**	**Risk/Non risk**		
GP1BA	rs6065	T/C	1.300 (0.3126 to 5.406)	1.00
	rs2243093	C/T	0.4318 (0.1350 to 1.381)	1.00
GP9	**rs3796130**	A/G	0.1270 (0.03156 to 0.5109)	0.0034*
	rs6069	A/G	0.1598 (0.06936 to 0.3681)	< 0.0001*
ABCG5	rs6720173	C/G	1.263 (0.6450 to 2.473)	0.6095
	rs56200894	C/G	1.000 (0.06164 to 16.224)	1.0000
TUBB1	p.Gln43Pro	P/Q	1.111 (0.7432 to 1.659)	0.682

*Statistically Significant

Table 5: Prediction of the nature of novel missense mutations in Congenital Macrothrombocytopenia cases from India

	Heterozygous Variations in Congenital Macrothrombocytopenia Cases				
Gene	**Protein**	**POLYPHEN2 (Score: 0-1)**	**SIFT**	**ALIGN GVGD**	**MUPRO/I-Mutant 2.0 (Stability of Protein Structure)**
GP1BA	p. Leu10Val	Dam (0.99)	Int	Del	Decreases stability
	p.Pro454Ser	Dam(0.99)	Int	Del	Decreases stability
	p.Leu213Arg	Dam (1)	Int	Del	Decreases stability
GP1BB	p.Cys95Trp	Dam (0.99)	Int	Del	Decreases Stability
	p.Arg107Pro	Dam (0.99)	Int	Del	Decreases stability
	p.Tyr113Phe	Dam (0.99)	Int	Del	Decreases stability
GP9	p.Ser62Thr	Dam (0.99)	Int	Del	Decreases stability
	p.Leu49Arg	Dam (0.99)	Int	Del	Decreases stability
	p.Arg97Pro	Dam(0.99)	Int	Del	Decreases stability
	p.Arg39Gly	Dam (0.67)	Int	Del	Decreases stability
	p.Ala28Gly	Dam (0.89)	Int	Del	Decreases the stability
	p.Ala156pro	Dam(0.95)	Int	Del	Deceases the stability
	p.Leu176Arg	Benign(0.00)	Bord	Uncla	Decreases the stability
	p.Pro68Arg	Dam(0.99)	Int	Del	Decreases the stability
	p.Gln76His	Benign(0.208)	Bord	Uncla	Decreases the stability
	p.Ala156Thr	Dam (0.688)	Bord	Uncla	Decreases the stability
ABCG5	p.Asn551Lys	Dam(0.92)	Int	Del	Decreases the stability
	p.Arg50Cys	Dam(0.99)	Int	Del	Decreases the stability
	p.Ala98Gly	Dam (0.99)	Int	Del	Decreases the stability
	p.Asp71Asn	Dam (0.99)	Int	Del	Decreases the stability
MYH9	p.Glu1946Lys	Benign(0.208)	Bord	Uncla	Decreases the stability
	p.Asp1948Asn	Dam (0.98)	Int	Del	Decreases the stability

Dam: Damaging; **Int:** Intolerant; **Uncla**: Unclassified; **Del:** Deleterious; **Bord:** Borderline

Figure 1: Distribution of Congenital Macrothrombocytopenia cases across India, most concentrated towards Eastern India (West Bengal, Assam, Bihar, Jharkhand, and Orissa)

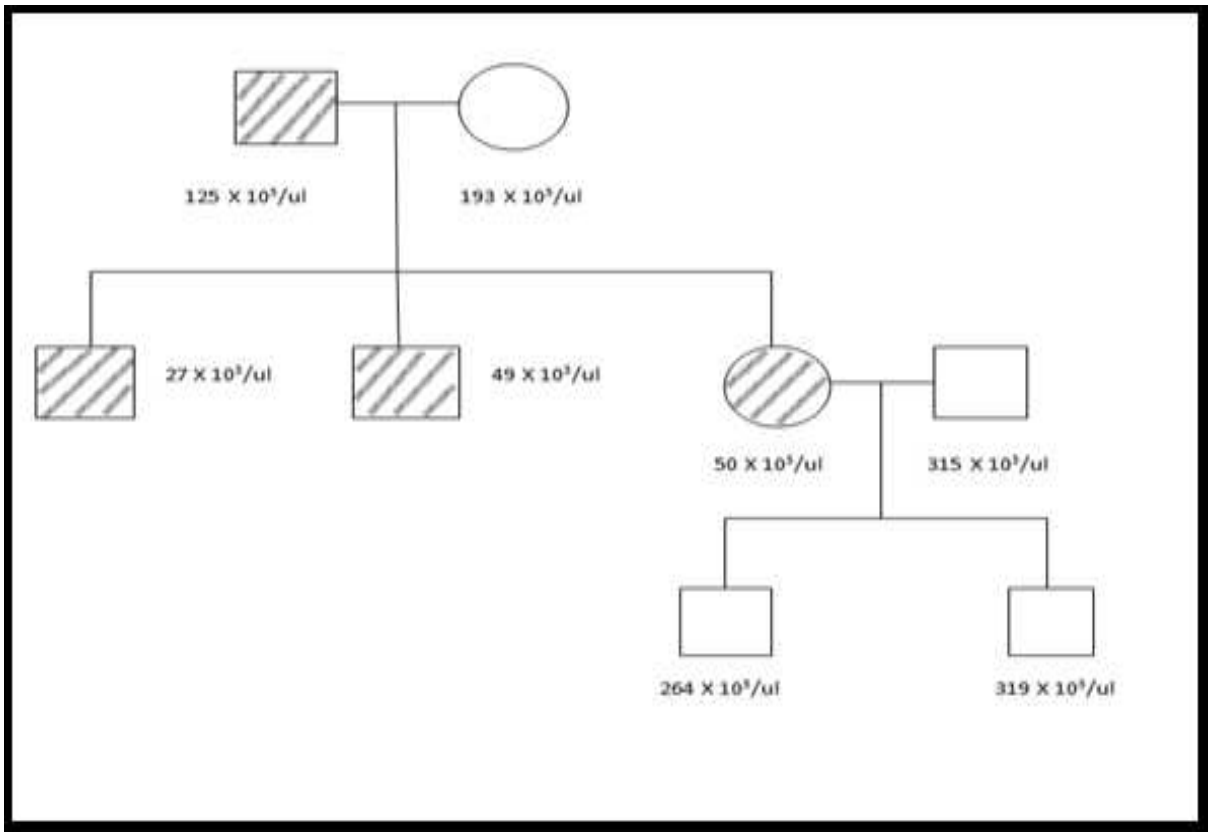


Figure 2: A family tree showing dominant mode of inheritance in a Bengal Macrothrombocytopenia (BM15.01) family

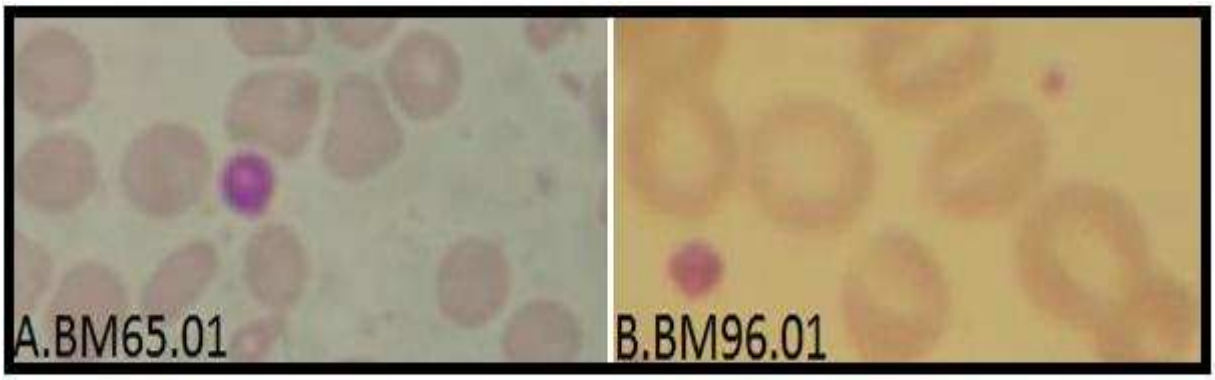
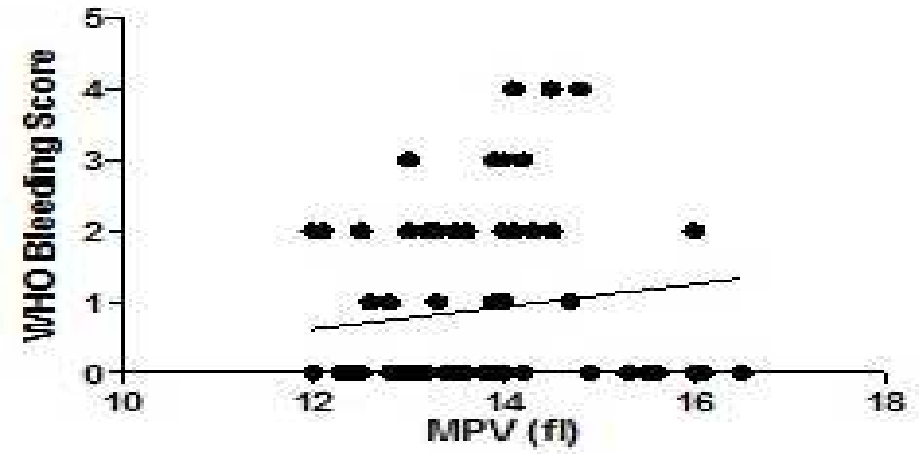
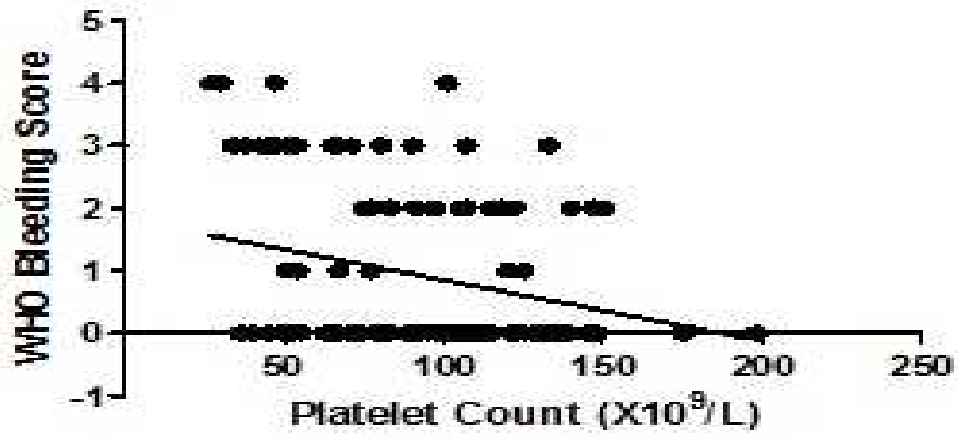


Figure 3: Blood films of case A. showing giant platelets and Case B. showing giant platelets and stomatocytes in their RBCs



(A)



(B)

Figure 4: WHO Bleeding Score in relation to the degree of (A). Mean Platelet Volume (MPV) and (B). Thrombocytopenia in congenital macrothrombocytopenia cases from India