

PLATELETS

Giant platelet syndrome

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Introduction

Platelets are small, disk-shaped, anuclear cells with a mean diameter of 2 to 3 μm . They are derived from cytoplasmic fragmentation of megakaryocytes, released into the circulation and survived for 7–10 days. Megakaryocyte development and platelet formation are regulated by thrombopoietin and other cytokines. It is now generally assumed that platelets are released by extension and fragmentation of cytoplasmic processes (proplatelet) of megakaryocytes through sinus endothelial cells in the bone marrow. It is not known, however, how the number and the size of the platelet are controlled at this step [1].

Giant platelet syndrome is a group of unique disorders characterized by the presence of abnormally large platelets, and is usually accompanied by thrombocytopenia. Thus, it is also called macrothrombocytopenia. Giant platelets are occasionally observed as an incidental finding in routine blood smear examinations. Most of them are due to acquired disorders such as idiopathic thrombocytopenic purpura (ITP) and myelodysplastic syndrome (MDS) (Table I). In contrast, inherited giant platelet disorders are rare. The mechanisms of giant platelet formation and thrombocytopenia are not fully understood in both inherited and acquired disorders. It is important from a clinical standpoint that congenital disorders are distinguished from acquired disorders, especially ITP, to avoid unnecessary treatments. We will discuss the molecular basis, diagnosis, and management of some major inherited giant platelet syndromes.

Inherited giant platelet syndrome

In recent years we have seen remarkable progress in the molecular understanding of some giant platelet syndromes. The Table I lists some major inherited

giant platelet syndromes according to the possible underlying cause: abnormalities in the platelet cytoskeleton, GPIb/IX/V, and transcription factors. Clinical and laboratory features as well as responsible genes and chromosomal localizations are also shown. There are still many inherited disorders in which the underlying genetic abnormality has not yet been elucidated.

Autosomal dominant macrothrombocytopenias with leukocyte inclusions (*MYH9* disorders)

May-Hegglin anomaly (MHA), first described a century ago, is the prototype of these disorders (Figure 1) [2]. Sebastian (SBS), Fechtner (FTNS), and Epstein (EPS) syndromes belong here. All four disorders have macrothrombocytopenia however, each of these disorders is distinguished from others by the presence or absence of granulocyte inclusion bodies and the presence or absence of a variable combination of Alport manifestations, including nephritis, deafness and cataracts (Table II). Although these four disorders were previously considered to be separate clinical entities, a recent positional cloning approach disclosed that these disorders are caused by mutations in the same gene, *MYH9*, which encodes the nonmuscle myosin heavy chain-A (NMMHCA) [3–5]. Thus, they appear to represent the same entity with different genetic penetrance and variable phenotypic expression. The bleeding tendency is usually mild.

The diagnosis of macrothrombocytopenia with leukocyte inclusions has been conventionally made on the basis of hematomorphological examinations. It is, however, not always easy to detect granulocyte inclusions on Wright stained smear. Immunofluorescence analysis of neutrophil NMMHCA localization has revolutionized the diagnosis of *MYH9* disorders [6]. Abnormal NMMHCA aggregates and accumulates in the neutrophil cytoplasm, and this abnormal

Table I. Characteristics of Giant Platelet Syndromes

Disease	Inheritance	Gene	Chromosome	Clinical and laboratory features
Acquired				
ITP				almost normal RBC and WBC
MDS				anemia, abnormal WBC
Inherited				
Abnormalities in platelet cytoskeleton				
Autosomal dominant macrothrombocytopenia with leukocyte inclusions/ <i>MYH9</i> disorders*	AD	<i>MYH9</i>	22q12-13	Macrothrombocytopenia, granulocyte inclusions with/without Alport manifestations (Table II)
Abnormalities in GPIb/IX/V				
Bernard-Soulier syndrome	AR	<i>GP1BA</i> <i>GP1BB</i> <i>GP9</i>	17pter-p12 22q11 3q21	No ristocetin-induced platelet agglutination
Mediterranean macrothrombocytopenia/ Bernard-Soulier syndrome carrier	AD	<i>GP1BA</i> <i>GP1BB</i>	17pter-p12 22q11	No bleeding tendency Mild thrombocytopenia with normal ristocetin-induced platelet agglutination
DiGeorge/Velocardiofacial syndrome	AD	<i>GP9</i> <i>GP1BB</i>	3q21 22q11	Contiguous gene syndrome due to chromosome 22q11 microdeletion Parathyroid and thyroid hypoplasia, cardiac abnormalities, cleft palate, mental retardation
Abnormalities in transcription factors				
X-linked macrothrombocytopenia with dyserythropoiesis	XL	<i>GATA1</i>	Xp11	Dyserythropoiesis with/without β -thalassemia trait
Paris-Trousseau thrombocytopenia/ Jacobsen syndrome	AD	<i>FLI1</i>	11q23	Contiguous gene syndrome due to chromosome 11q23 microdeletion Growth and psychomotor retardation Dysmegakaryopoiesis associated with giant α -granules
Unknown cause				
Gray platelet syndrome	AR, AD	unknown		Gray or colorless platelets due to absent α -granules

*See Table II for details.

AD: autosomal dominant, AR: autosomal recessive, XL: X-linked.

subcellular localization of NMMHCA is present in every neutrophil from individuals with *MYH9* mutation. The localization pattern of neutrophil NMMHCA in *MYH9* disorders can be classified into three groups according to the number, size, and shape of the fluorescence-labeled NMMHCA granules: type I, II and III (Figure 2). In type I, NMMHCA forms one or two large and intensely stained cytoplasmic foci. Type II neutrophils consist

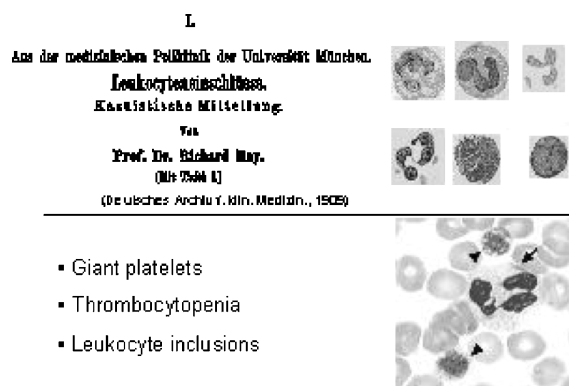


Figure 1. May-Hegglin anomaly.

of several cytoplasmic spots with circular to oval shape. Type III or speckled staining is detected in patients with EPS and isolated macrothrombocytopenia, in which Wright or May-Grünwald-Giemsa (MGG)-stained inclusion bodies have never been identified [6].

An *MYH9* mutation is strictly associated with the hematological abnormalities. Although the molecular mechanism of the production of giant platelets has not been elucidated, it is suggested that abnormal NMMHCA, by interfering with the formation of myosin thick-filament, affects proper proplatelet formation in megakaryocytes. Genetic analysis of many kindred with *MYH9* disorders revealed that there is no clear relationship between clinical phenotypes and the sites of the *MYH9* mutations. It is likely that the *MYH9* mutation alone does not cause associated Alport manifestations and that unknown genetic and/or epigenetic factors might influence the phenotypic consequences of *MYH9* mutations [7,8].

We have recently generated and analyzed *MYH9* knock-out mice [9]. No homozygous mice were born, suggesting that *MYH9* expression is required for

Table II. Macrothrombocytopenia with leukocyte inclusions/*MYH9* disorders

Phenotype	Macrothrombocytopenia	Leukocyte inclusions	Alport syndrome*
May-Hegglin anomaly (MHA)	+	+	-
Sebastian syndrome (SBS)	+	+	-
Fechtner syndrome (FTNS)	+	+	+
Epstein syndrome (EPS)	+	-	+

*Nephritis, deafness and cataracts.

embryonic development. By an antisense oligonucleotide-directed suppression of transcription, NMMHCA is involved in a rearrangement of the actin cytoskeleton and loss of cell adhesion [10]. *MYH9* $-/-$ embryos, therefore, appeared to fail to develop a normal patterned embryo. Such fetal lethality may partly explain the absence of naturally occurring homozygous mutations in human subjects. In contrast, heterozygous mice (*MYH9* $+/-$) were viable and fertile without gross anatomical, hematological, and nephrological abnormalities. Immunofluorescence analysis showed the normal cytoplasmic distribution of NMMHCA. Interestingly, we found that some but not all mice have hearing loss. The distribution of *MYH9* expression in the inner ear has been studied in the developing fetal, neonate, adult mice [11], suggesting that *MYH9* may have important roles in the development and maintenance of auditory function. On the other hand, the unconventional myosins, Myosin VIIA is localized in the stereocilia and cell body of hair cells and is critical in differentiation, formation, and/or maintenance of sensory hair cell structure [12–14]. Myosin VIIA have been linked to nonsyndromic and syndromic hearing loss. Therefore, the requirement of NMMHCA in the mammalian auditory system might be limited and could be compensated by other conventional myosins. Thus heterozygous loss of NMMHCA might result in the phenotype that does not appear uniformly, or the severity of phenotype might be unrecognizable.

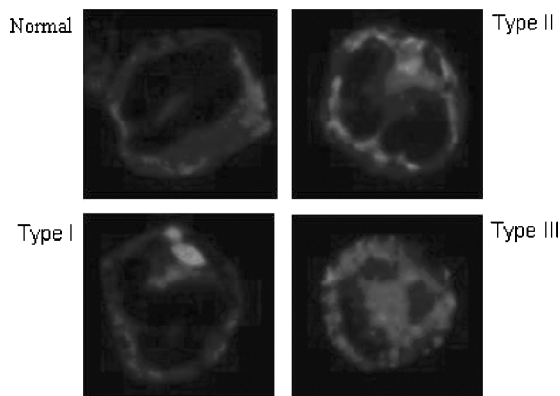


Figure 2. Subcellular localization of neutrophil NMMHCA.

Bernard-Soulier syndrome

Bernard-Soulier syndrome (BSS) is an autosomal recessive bleeding disorder characterized by giant platelets, thrombocytopenia and prolonged bleeding time (Figure 3), originally described by Bernard and Soulier in 1948 [15]. BSS is caused by quantitative or qualitative abnormalities in the glycoprotein (GP) Ib/IX/V complex, the platelet receptor for von Willibrand factor [16,17]. As a result of the absence of GPIb/IX/V complexes on the platelet membrane, platelets cannot stick to the damaged blood vessel walls, and consequently patients bleed. In the central cytoplasmic domain, GPIb associates with the actin cross-linking protein, filamin A. Thus, the defective linkage between GPIb/IX/V and cytoskeleton is the proposed molecular cause of the giant platelets.

The classical diagnostic features are a prolonged bleeding time, moderate to severe thrombocytopenia, and giant platelets. Especially, giant platelets and the absence of ristocetin-induced platelet agglutination are the laboratory hallmarks of BSS (Table I). Flow cytometric determination of platelet GPIb/IX expression is a convenient method for diagnosis of BSS in a clinical laboratory.

Thus far, 39 mutations in the genes for GPIb α , GPIb β , and GPIX have been described. Approximately half of the mutations were found in the GPIb α gene (17 mutations), and the remaining were found in the GPIb β (12 mutations) and GPIX genes (10 mutations). A majority of the mutations correspond to single-base substitutions or small base deletion and insertion mutations. Recent studies have shown that the phenotypes caused by mutations in the subunits of GPIb/IX span a wide spectrum, from

- Giant platelets
- Thrombocytopenia
- Prolonged bleeding time
- Absent ristocetin-induced platelet aggregation
- Deficiency of platelet GPIb/IX/V complex
 - Confirmed by flow cytometry and immunoblotting
 - Homozygous mutations in *GP1BA*, *GP1BB*, *GP9*

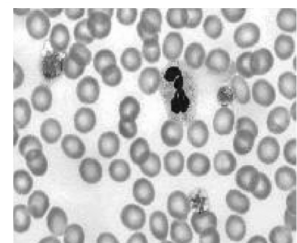


Figure 3. Bernard-Soulier syndrome.

the normal phenotype to isolated macrothrombocytopenia with normal platelet function, to full-blown BSS and platelet-type von Willebrand disease. It is important to note that although heterozygous BSS carriers are generally asymptomatic with subnormal platelet number and function, they have giant platelets. Several individuals with heterozygous GPIb/IX mutation have been initially identified as having undifferentiated thrombocytopenia or refractory ITP. In Italy, a mutation in GPIb α (Ala156Val) was found to be a founder mutation responsible for the autosomal dominant macrothrombocytopenia previously known as a Mediterranean macrothrombocytopenia [18]. In addition, patients with DiGeorge/velo-cardio-facial syndrome due to a heterozygous chromosome 22q11.2 microdeletion, which includes the GPIb β gene, have macrothrombocytopenia [19] (Table I).

X-linked macrothrombocytopenia with dyserythropoiesis

Recently, several unrelated families with X-linked macrothrombocytopenia with mild to moderate dyserythropoiesis have been found to have mutations in the GATA-1 gene. GATA-1 is a megakaryocyte- and erythroid-specific transcription factor required for normal growth and differentiation of both lineages. Defective GATA-1 function due to missense mutations causes reduced transcription and thus protein expression of its target genes, including GPIb α , GPIb β , GPIX, and GPV. The concomitant decrease in the expression of GPIb/IX/V and other platelet-specific gene products is related to the macrothrombocytopenia and bleeding tendency in this disorder [20].

Paris-Trousseau syndrome/Jacobsen syndrome

Paris-Trousseau syndrome/Jacobsen syndrome is a contiguous gene syndrome characterized by mental retardation, and facial and cardiac abnormalities due to a heterozygous 11q23 deletion. The platelets contain giant α granules on peripheral blood smears, and in the bone marrow megakaryocytes are increased with many micro megakaryocytes. Hemizygous deletion of the transcription factor Fli1 contributes to the hematopoietic defects in this disorder [21].

Inherited macrothrombocytopenias of unknown cause

Gray platelet syndrome

Gray platelet syndrome (GPS) is characterized by thrombocytopenia and abnormal giant platelets with absent platelet α -granules. Patients with GPS have a bleeding tendency of variable severity. The gene(s)

responsible for the disease are currently not known. The most characteristic feature and thus the laboratory hallmark of the syndrome is agranular platelets. On Wright- or MGG- stained peripheral blood smears, platelets appear gray or colorless due to the absence of platelet α -granules and their constituents. Because platelet α -granule proteins such as platelet-derived growth factor are synthesized but not properly stored in the granules and released from megakaryocytes into the bone marrow, myelofibrosis is present in most cases [22].

Type 2B von Willebrand disease

Patients have a prolonged bleeding time, decidedly low vWF activity measured as ristocetin cofactor activity, a mild deficiency of vWF antigen level, and enhanced ristocetin-induced platelet aggregation at low concentrations of ristocetin. Although the molecular mechanisms remain to be elucidated, some patients with type 2B vWD have been reported to have giant platelets [23].

Approach to patients with macrothrombocytopenia [24]

First of all, acquired causes of macrothrombocytopenia, including ITP and myelodysplastic syndromes, should be ruled out. Complete history and physical examination should be carefully performed. In syndromic forms, patients show complications of physical abnormalities such as facial, cardiac, skeletal anomalies and/or mental retardation. If the patient previously had normal platelet counts, acquired rather than congenital conditions are more likely to be the underlying cause. In inherited macrothrombocytopenias, platelet counts are constantly decreased, ranging from as low as $10 \times 10^9/l$ to near normal $150 \times 10^9/l$. On a peripheral blood smear, the majority of platelets are large, being similar to or larger than red blood cells or small lymphocytes. In contrast, in patients with the much more common ITP, large platelets are present but the majority are of normal size. Because routine automated blood cell counting systems differentiate blood cells by their size and do not recognize giant platelets as platelets, these instruments underestimate platelet counts in patients with macrothrombocytopenia. The mean platelet volume, usually calculated as a parameter of the complete blood count, also does not reflect actual platelet size in the case of giant platelets. Platelet count should therefore be determined manually in a calculating chamber or on peripheral blood smears. Careful examination of a smear also allows morphological assessment of leukocytes and erythrocytes. If granulocyte inclusion bodies are obscure or absent, immunofluorescence analysis for neutrophil NMMHCA localization is helpful to make a clear distinction. Flow cytometric analysis of

platelet GPIb/IX expression can differentiate BSS heterozygotes from patients with “true” isolated macrothrombocytopenia.

Patients with congenital macrothrombocytopenia generally do not respond to standard ITP treatments, including corticosteroids, intravenous immunoglobulin, and splenectomy. If treatment for bleeding is clinically indicated, the administration of antifibrinolytic agents such as ϵ -aminocaproic acid or tranexamic acid and recombinant activated factor VII may transiently improve the episodes [25]. Transfusion of platelets is effective for serious bleeding and as prophylaxis prior to major surgery, but may be complicated by the development of alloantibodies. In certain instances, hematopoietic stem cell transplantation (SCT) may be a curative therapeutic option [26]. It is important to make a proper diagnosis to avoid unnecessary treatment. Affected families should be educated about their diagnosis to avoid unnecessary medications and potentially dangerous treatments for presumed ITP. When evaluating patients with refractory ITP or undifferentiated thrombocytopenia, congenital macrothrombocytopenias should be included in the differential diagnosis.

Conclusions

Inherited giant platelet syndromes are rare conditions, yet the study of them has been instrumental in elucidating the structure and function of normal platelets as well as the mechanisms of thrombopoiesis. Further research is needed to understand the pathogenesis of many congenital disorders with unknown causes.

Acknowledgements

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