Inherited platelet disorders: thrombocytopenias and thrombocytopathies

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Introduction

Platelets play an important role in normal haemostasis halting blood flow immediately after injuries through four fundamental different mechanisms: adhesion, aggregation, secretion, and expression of procoagulant activity. First, in the presence of vascular damage, platelets adhere to the connective tissue and, particularly when the damage occurs in vessels with a low shear rate, to subendothelial collagen, fibronectin and laminin. On the other hand, when damage occurs in regions with a high shear rate, platelet adhesion requires the presence of subendothelial von Willebrand factor (VWF) and specific platelet receptors, such as the glycoprotein Ib/IX/V (GPIb/IX/V) complex. In any case, following initial adhesion, platelets aggregate to complete the formation of a solid haemostatic plug. Platelet aggregation requires stimulation by agonists such as ADP, thrombin, collagen, or epinephrine, as well as the presence of calcium or magnesium ions and specific plasma proteins (fibrinogen, VWF and the glycoprotein IIb/IIIa (GPIIb/IIIa) complex). Platelet stimulation results in the generation of intracellular second messengers that convey the stimulus back to the platelet surface, exposing protein binding sites on GPIIb/IIIa. Fibrinogen (or VWF) then binds to GPIIb/IIIa and cross-links adjacent platelets to produce platelet aggregates, following platelet secretion and the elaboration of platelet procoagulant activity. In platelet disorders caused by a reduction in the number of platelets (thrombocytopenia) (Table I) or by platelet function defects (thrombocytopathies) (Table II), bleeding generally occurs immediately after injury, primarily in the skin, mucous membranes, nose, and gastrointestinal and urinary tracts and, generally, does not involve joints and muscles. Congenital disorders are uncommon and sometimes may be misdiagnosed with acquired disorders, which are much more frequently encountered in clinical practice. The differential diagnosis between congenital or acquired platelet disorders is often very complex and requires medical experience and a careful assessment of the patient's personal and family history of bleeding episodes. A lifelong bleeding diathesis may suggest a congenital platelet disorder, which is often first recognised during childhood, but an onset in adulthood does not exclude a congenital defect. Because technological progress in laboratory practices and greater information about platelet functions have increased the specificity and accuracy of diagnosis of platelet disorders, a large number of cases has been identified recently. In this review, we describe current knowledge on congenital platelets disorders with regards to granule packaging, signalling and platelet coagulant function, and hereditary thrombocytopenias in order to improve information available for research and clinical practice.

Thrombocytopathies

Several blood disorders characterised by dysfunctional platelets result in prolonged bleeding time, defective clot formation, and a bleeding tendency. Inherited thrombocytopathies can be classified according to platelet function into adhesion, activation, secretion, and aggregation defects and can determine several haemorrhagic disorders with inherited transmission and marked phenotypic heterogeneity. However, the low prevalence of these diseases and the high percentage of patients with unclassified platelet disorders underline the need for the creation of a network for comprehensive care of these patients.
Platelet adhesion disorders:

Bernard-Soulier syndrome

Bernard-Soulier syndrome (BSS) is a rare and often severe bleeding disorder named after Bernard and Soulier who first described, in 1948, a young man with a prolonged bleeding time, mild thrombocytopenia and giant platelets approaching the size of lymphocytes. BSS often presents early with bleeding symptoms, such as epistaxis, ecchymosis, menometrorrhagia, gingival, gastrointestinal, muscular or visceral bleeding. Thrombocytopenia is variable in BSS, and the platelet count typically ranges from less than 30 to 200 \times 10^3/\mu L, with over 80% of them being large. The clinical symptoms range from mild to severe and only rare patients have suffered from fatal haemorrhages. The diagnosis can be confirmed by platelet aggregation studies and flow cytometry analysis. The platelets of patients with BSS do not agglutinate in response to ristocetin, but show normal aggregation in response to a variety of aggregating agents, and have a delayed response to thrombin. This failure to agglutinate cannot be corrected by the addition of normal plasma, which distinguishes BSS from von Willebrand's disease (VWD). The molecular basis of BSS has been identified as qualitative or quantitative defects of the GPIbIX/V complex on the platelet membrane; this complex is the principal receptor for VWF and mediates both agglutination and platelet adhesion.

### Table I - Classification of thrombocytopenias

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<th>Inheritance</th>
<th>Syndrome</th>
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<td>Small size</td>
<td>WAS</td>
<td>X-linked inheritance</td>
<td>Wiskott-Aldrich syndrome, X-linked thrombocytopenia</td>
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<tr>
<td>Normal size</td>
<td>c-mlp, HOXA11</td>
<td>Autosomal recessive and dominant</td>
<td>CAMT, thrombocytopenia-absent radius</td>
</tr>
<tr>
<td>Large size</td>
<td>del 22q, GPIba, MYH9</td>
<td>Autosomal dominant</td>
<td>Velocardi-facial syndrome, Mediterranean macrothrombocytopenia, von Willebrand's disease (VWD), platelet type, May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, Epstein syndrome</td>
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However, the vast majority of carriers are asymptomatic. Among 156 families, leading to a Val to Ala substitution at position 57, both occurring in the leucine-rich repeat region of the protein. The majority of mutations detected are point mutations that produce premature stop signals, slightly decreased expression of a dysfunctional receptor, or, more often, an abnormal/unstable complex with strongly decreased surface expression, such as the Bolzano variant that affects a functional domain. To date, 47 different genetic defects associated with BSS have been identified. Defects are due to mutations in GPIBA (20 mutations), which is the largest subunit and bears the binding site for VWF, in GPIBB (16 mutations), and in GP9 (11 mutations). Most of the defects are unique, having been identified in a single individual or family, with the exception of the GP9 Asn45Ser mutation, which has been found in several patients of different families from different countries, probably due to a founder effect. There is no specific treatment for BSS and bleeding episodes may require platelet transfusions.

Platelet activation disorders: ADP receptors defects

Adenosine 5'-diphosphate (ADP) receptors P2Y12, P2Y1, and P2X were identified 40 years ago and serve as activators of adhesiveness and platelet aggregation, playing a very important physiological role as paracrine mediators of haemostasis and thrombosis. The exact contribution that each of these P2 receptors makes to phenomena associated with haemostasis, platelet adhesion and aggregation still remains poorly defined but it seems that, in response to ADP, they activate unique signal transduction pathways working in a combined manner. Initially, the effect of ADP is mediated by the P2Y1 receptor, which induces mobilisation of cytoplasmic calcium followed by an initial wave of reversible and transient platelet aggregation. The P2Y12 receptor mediates a progressive amplification of the response with sustained platelet aggregation, whereas the role of the P2X receptor remains to be fully understood, although it seems to be involved in platelet shape changes.

ADP receptors defects are very rare and, to date, have been described in only five patients with severe deficiency of P2Y12, a patient with P2Y1 deficiency and one, with a severe bleeding disorder showing an autosomal dominant inheritance, carrying a P2X deficiency. P2Y12 abnormalities were found in three consanguineous Italian patients with a homozygous mutation that produced a frame-shift leading to premature truncation of the protein. A fourth patient carried an untranslated allele and a second allele silenced by an additional 2-bp deletion. More recently, a fifth patient was described who is a compound heterozygote for Arg256Gln and Arg265Trp substitutions and shows normal expression of a non-functioning receptor. Defects of platelet P2Y1 or P2Y12 receptors are inherited as an autosomal recessive trait and patients show a mild bleeding phenotype.

Platelet secretion disorders: storage pool diseases

Platelet activation involves the secretion of the contents of several subcellular organelles, each with specific properties concerning both the structure and the role played by released constituents. The subcellular organelles include α-granules (containing large-adhesive and healing proteins involved in adhesion, such as fibronectin and VWF, cell-cell interactions, such as P-selectin, and in promoting coagulation, such as factor V and platelet factor 4), dense granules (containing small non-protein molecules that recruit others platelets, such as ADP, binding proteins in the platelet cytoskeleton and the GP Ibα cytoplasmic domain. Subunits of the complex are encoded by genes located on different chromosomes: 17q12 (GPIbα), 22q11.2 (GPIbβ), 3q29 (GPV) and 3q21 (GPIX). The syndrome is very rare and to date about 100 cases have been reported, mostly in the patients from Japan, Europe, and North America. The prevalence of BSS has been estimated to be less than 1/1,000,000 although it may be higher due to misdiagnosis and underreporting. The condition is more frequent in ethnic groups such as the Iraqi and in regions where consanguineous marriages are common such as the south of India and northern Iran. BSS is transmitted in an autosomal recessive manner, patients being homozygous or compound heterozygous for the GPIBA, GPIBB or GP9 genes. The vast majority of carriers are asymptomatic. However, GPIBA gene mutations in two cases transmitted as an autosomal dominant trait have been described: a missense mutation of residue 57 and a transition, reported in different Mediterranean populations: a missense mutation of residue 57 and a transition, reported in different Mediterranean populations.
and serotonin), and lysosomes (containing hydrolases to dissolve platelet aggregates into the circulating blood and, thereby, eliminate them).

Platelet storage pool diseases (SPD)\(^{18}\) are a heterogeneous group of disorders associated with an abnormal presence or contents of intracytoplasmatic platelet granules, causing a mild to moderate bleeding diathesis characterised mainly by mucocutaneous bleeding (epistaxis, menorrhagia, and easy bruising). However, there are also patients at risk of severe bleeding, which may sometimes cause death. Inherited diseases of platelet granules are very rare and often difficult to identify due to a high variability in laboratory tests\(^{19,20}\), such as impaired aggregation in response to different agonists and abnormal platelet secretion induced by several platelet agonists. Platelet granule disorders show marked genetic and phenotypic heterogeneity\(^{21}\), and include several syndromes, such as gray platelet syndrome, Quebec platelet disorder, Hermansky-Pudlak syndrome and Chediak-Higashi syndrome.

Gray platelet syndrome (GPS)\(^{22}\) is a rare disorder of platelets and megakaryocytes, also know as α-storage pool disease, characterised by moderate thrombocytopenia (although on occasions the platelet count can be severely reduced) and large abnormal platelets with a specific or general absence of α-granules and their content (fibrinogen, VWF, thrombospondin, β-thromboglobulin and platelet factor 4). In some patients, the α-granule deficiency is associated with platelet-dense or granulocyte granule abnormalities. Platelet aggregation may be only moderately affected, being in the normal range in a large proportion of cases, indicating that a minimal amount of granules ensures signalling through tyrosine kinases. Although the molecular basis of the disease is unknown, current data suggest that α-granules fail to mature during megakaryocytic differentiation. GPS has a widespread geographical distribution and about 20 families with several affected members have been reported with an autosomal recessive\(^{23}\) or autosomal dominant\(^{24}\) pattern of inheritance, mild to moderate thrombocytopenia, and bleeding episodes. A few cases with severe deficiency of collagen-induced platelet aggregation and severe GPVI deficiency have been described\(^{25}\). The genes involved remain to be identified; mRNA expression analysis of about 4,500 genes from fibroblasts of skin biopsies from patients with GPS showed upregulated gene clusters, including those coding for cytoskeletal-related proteins fibronectin, TSP-1, MMP-2, and collagen V I α-chain. Recently, a missense mutation at position 759 in the GATA-1 gene, inducing an amino-acid change (Arg216Gln), which segregates with GPS, was identified, suggesting that the GATA-1 gene is required for platelet α-granule regulation\(^{26}\).

Quebec platelet disorder (QPD)\(^{27}\) is an autosomal dominant bleeding disorder associated with only fibrinolytic abnormalities, including increased megakaryocytic expression, increased urokinase-type plasminogen activator storage in platelets causing degradation of α-granule proteins, such as fibrinogen\(^{28}\). Initially, clinical expression was attributed to a deficiency of functional platelet factor V, and hence the designation factor V Quebec\(^{29}\). The increased urokinase plasminogen activator in QPD platelets is only partially inhibited, and, as a result, there is intraplatelet generation of plasmin, and secondary degradation of many platelet α-granule proteins. This observation suggests that the moderate to severe bleeding in patients with QPD could result from accelerated fibrinolysis within the haemostatic plug, where the concentrations of released urokinase-type plasminogen activator may overwhelm protease inhibitors. Laboratory tests reveal normal to reduced platelet counts, absent platelet aggregation responses to epinephrine (6 µM), and normal to abnormal aggregation in response to collagen and ADP. QPD is associated with “gain-of-function” abnormalities that increase the lysis of forming or preformed clots and with moderate to severe delayed bleeding that typically begins 12 to 24 hours after surgery or trauma. Haemorrhagic manifestations can be controlled with fibrinolytic inhibitors but not with platelet transfusions\(^{29}\). Measurements of platelet urokinase-type plasminogen activator and α-granule protein degradation might be important methods to assess the nature and severity of bleeding problems among affected patients.

The Hermansky-Pudlak syndrome (HPS) is a rare and heterogeneous autosomal recessive disorder, characterised by tyrosinase-positive oculocutaneous albinism, platelet dysfunction, lysosomal ceroid lipofuscin storage, and absence of platelet dense granules. Prolonged bleeding, congenital neutropenia, pulmonary fibrosis, and granulomatous colitis can also occur. The impaired function of specific organelles indicates that the causative genes encode proteins...
operative in the formation of lysosomes and vesicles. In mice, at least 16 loci are associated with HPS. Thus far, mutations in seven orthologous genes have been found in HPS patients: HPS1, HPS2 (AP3B1), HPS3, HPS4, HPS5, HPS6, and HPS7. HPS results from the abnormal formation of intracellular vesicles. Four of the genes, HPS1, AP3B1, HPS3, and HPS4 are associated with the four known subtypes of the disease (HPS-1 to HPS-4). These genes arose with the evolution of mammals and have no homologues in yeast, reflecting their specialised function; among them, HPS1 is known to play a key role in the genesis of HPS. The human HPS1 gene was mapped to chromosome segment 10q23. To date, in patients from Puerto Rico, Japan, and Europe about 18 pathological mutations have been identified, mainly affecting exons 5, 11, and 15, suggesting a few mutational spots. However, the HPS1 gene product remains to be defined. The finding that mutations in the HPS1 gene are involved in abnormalities in melanosome morphology and melanin production shed light on the role and function of the HPS1 gene product in the synthesis of melanosomes and melanin pigment.

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder that affects multiple systems of the body, was described 50 years ago and different studies suggest that vesicle fusion, fission, or trafficking in all granule-containing cells including granulocytes, lymphocytes, melanocytes, and neurons is involved in the disorder. Patients may exhibit hypopigmentation, recurrent infections, mild coagulation defects, severe immunological deficiency, a variety of neurological problems, and lack of natural killer (NK) cell function. Morbidity results from patients succumbing to frequent bacterial infections or to an "accelerated phase" of lymphoproliferation into the major organs of the body. Positional cloning and YAC complementation studies resulted in the identification of the CHS1/LYST gene, present in all eukaryotes and mapping to an 18.8 cM interval in chromosome segment 1q42-q44 in humans. This gene is highly conserved throughout evolution and shows 86.5% sequence homology to beige mouse gene, carriers of which demonstrate many characteristics similar to those of human CHS patients. The CHS1/LYST gene encodes a cytosolic protein of 429,000 Da consisting of 3801 amino acids. Clinical reports of CHS have identified only a few mutations throughout the CHS1/LYST gene, because the large size of the LYST gene has made it difficult to screen for genetic abnormalities in a large number of patients. To date, about ten mutations have been identified and all of them lead to a truncated LYST protein.

Platelet aggregation disorders: Glanzmann’s thrombasthenia

After adhesion of platelets to the injured blood vessel wall, aggregation is the major physiological function of platelets, resulting from interaction of platelets with one another.

Defects in this process may be classified as primary or secondary depending on which aggregating agent fails to stimulate platelets. Primary aggregation agents, ADP, adrenaline, and thrombin induce platelet aggregation independently of ADP contained within platelets (defects are Glanzmann’s thrombasthenia, and impairment of platelet ADP receptors). Secondary aggregation is determined by agents that induce aggregation following the release reaction of ADP or the synthesis of prostaglandin (defects are storage pool disease, impairment of platelet thromboxane receptors) (Figure 1).

Glanzmann’s thrombasthenia (GT), first described in 1918, is an inherited haemorrhagic diathesis affecting the megakaryocytic lineage. It is characterised by a prolonged bleeding time, normal platelet count and severely impaired aggregation in response to platelet agonists such as ADP, epinephrine, serotonin, thrombin and collagen. Laboratory tests show normal platelet agglutination induced by ristocetin and impaired or absent clot retraction. Abnormalities are due to a qualitative and/or quantitative deficiency of the platelet GPIIb/GPIIIa complex (also known as αIIbβ3 integrin) which is a heterodimeric calcium-dependent molecule. The GPIIb/GPIIIa complex, only exposed on the platelet membrane (normally 40,000 to 80,000 sites per cell), contains binding-sites for fibrinogen, VWF, fibronectin, and vitronectin and promotes, after stimulation, binding to plasma fibrinogen. GT is a rare congenital bleeding disorder (1 case/ 1,000,000 people), although higher prevalences have been recorded in some populations, consequent to an elevated rate of consanguineous marriages. The GT bleeding phenotype is essentially characterised by purpura, epistaxis, petechiae, gingival haemorrhage, menorrhagia during childbearing years, and subcutaneous haematomas. Few cases manifest
bleeding symptoms early after birth, although the majority of patients are diagnosed later in life. The disease is transmitted as an autosomal recessive trait with variable clinical expression, ranging from a mild to severe haemorrhagic diathesis. Sometimes, heterozygous subjects have normal aggregation tests but a decreased amount of GPIIb/GPIIIa complex. The phenotypic heterogeneity among thrombasthenic patients corresponds to the effect of different mutations or to interactions with others genes involved in platelet function. GPIIb (or $\alpha_{IIb}$) and GPIIIa (or $\beta_3$) are encoded by two distinct genes (17.5 Kb and 70 Kb, respectively), which are closely linked on chromosome 17q and encode a 3.4 and a 6.2 Kb transcript, respectively. GPIIb has a molecular weight of 13,600 Da, comprises 30 exons, and is divided into two chains: GPIIb$\alpha$ (heavy chain, m.w. 125,000 Da, 871 amino acids), and GPIIb$\beta$ (light chain, m.w. 23,000 Da, 137 amino acids). GPIIIa is smaller than GPIIb (m.w. 115,000 Da), being constituted by 15 exons, and more widespread. Indeed, it is expressed in different cell types, being also able to bind vitronectin, the $\alpha_\beta$ platelet receptor, which has often been used as a marker to differentiate patients with a genetic defect affecting the $GP2B$ (ITGA2B) or the $GP3A$ (ITGB3) gene. GT shows three phenotypic patterns of membrane abnormalities reflecting the marked underlying genetic heterogeneity. GT is classified as type 1 (severe receptor deficiency with <5% of GPIIb/GPIIIa complex, platelet aggregation and clot retraction absent), type 2 (platelets containing 10% to 20% of GPIIb/GPIIIa complex, a value sufficient to allow only for microaggregate formation, and reduced platelet aggregation) and type 3, which is a qualitative disorder (platelets containing >50% of GPIIb/GPIIIa receptor). The type 3 form of GT was first identified in 1987 and accounts for only about 5% of GT patients. GT is caused by mutations in either the $GP2B$ (ITGA2B) or $GP3A$ (ITGB3) gene and at least 100 mutations have been described and collected in an online database. The mutations identified affect post-translational processes, mRNA stability or lead to an incorrectly folded gene product in the Golgi apparatus, inducing, in the majority of cases, a type 1 phenotype. Most of the gene mutations identified are deletions, nonsense, missense and frame-shift mutations, or insertions. Splice site mutations, but not large deletions, are also common. In GT patients carrying mutations within the $GP2B$ gene, only four different mutations lead to the formation of a functionally defective receptor (type 3 GT). All of them are located in the proximity of the

**Figure 1 - Schematic representation of the main functions of platelets**
Platelet procoagulant function disorders

Platelets promote the catalysis of two sequential, calcium-dependent reactions in blood coagulation: the activation of factor X by a complex of factor IXa and factor VIIIa and the conversion of prothrombin to thrombin by a complex of factor Xa and factor Va. Platelets contribute to prothrombin activation by ensuring receptor binding sites for factor Xa, factor V, and factor Va and with specific receptors for factor IXa. Thrombogenic surfaces of activated cells expose optimal amounts of aminophospholipids (phosphatidylserine [PS] and phosphatidylethanolamine [PE]) which interact with coagulation proteins to promote assembly and catalysis of the tenase- and prothrombinase complex, which can result in a nearly million-fold increase in the rate of thrombin formation. Similar membrane surfaces are also required for the anticoagulant protein C-pathway, which restricts thrombin formation by inactivation of Va and VIIIa, cofactors of the prothrombinase- and tenase complex, respectively. The physiological importance for blood coagulation of aminophospholipid exposure has been illustrated in patients with Scott syndrome, a bleeding disorder in which activated platelets and other peripheral blood cells have a strongly impaired capacity to expose PS and PE and to stimulate prothrombinase and tenase activity.

Surface exposure of PS is also an early event in cells undergoing apoptosis, in which it functions as a recognition signal for sequestration by macrophages. Nonetheless, the aggregatory potential of platelet agonists (mediated by fibrinogen ligation to the conformationally active α₃β₃ integrin) is distinct from that linked to exposure of PS and PE. Scott syndrome is a relatively severe bleeding disorder associated with an isolated defect in the expression, in stimulated platelets, of the membrane catalytic tenase activity (complex of coagulation factors VIIIa and IXa) and prothrombinase activity (complex of coagulation factors Va and Xa).

Studies on the originally described patient showed that this defect results from impaired binding of coagulation factors Va and VIIIa to activated platelets, reflecting diminished exposure of PS on the surface, and is also associated with reduced shedding of microparticles from the platelet surface. To date, only three documented cases of Scott syndrome have been reported, not allowing for the identification of candidate genes. Likewise, the manner of inheritance is still unclear. It should be emphasised that none of these patients had clinical symptoms other than bleeding, suggesting that the rapid PS passage for an efficient platelet haemostatic response and the slower membrane remodelling occurring during apoptosis are mechanisms under different controls. In Scott syndrome, unaffected family members show a variable clinical expression suggesting the possibility of an interaction between two (or more) defective genes. Recently, considering that the ATP-binding cassette transporter A1 gene, ABCA1, has been proposed as a PS translocase regulator, it was investigated for a potential role in the pathophysiology of Scott syndrome and a novel heterozygous missense mutation (R1925Q) was found. In vitro expression studies of the R1925Q mutation showed overexpression of ABCA1 in lymphocytes suggesting that this mutation might contribute to the clinical phenotype.
**Congenital thrombocytopenias**

Congenital thrombocytopenias, once considered very rare conditions representing only a few cases of thrombocytopenia known to haematologist, are now recognised with an increasing frequency, mainly because platelet counts are now a part of routine blood testing. Nevertheless, in some cases they may still be misdiagnosed as an acquired thrombocytopenia, i.e. idiopathic thrombocytopenic purpura. In patients with a haemorrhagic disorder and persistent thrombocytopenia, a careful clinical examination and an expert collection of the medical history can be crucial for a correct diagnosis. Then, laboratory tests, chosen on the basis of clinical information previously obtained, will lead to a precise characterisation and will define the severity of the platelet disorder. Certain features, if identified in patients with persistent thrombocytopenia, suggest specific types of congenital thrombocytopenia, which can be classified in several different types differing from each other in patterns of inheritance. The clinical spectrum of congenital thrombocytopenias shows remarkable heterogeneity, ranging from a severe bleeding diathesis, recognised within the first few weeks of life, to mild conditions that may remain undetected even in adulthood. Understanding the genetic basis of inherited thrombocytopenias may improve knowledge on developing steps from pluripotent haematopoietic stem cells to platelets.

**Thrombocytopenia with reduced platelet size**

X-linked thrombocytopenia and the Wiskott-Aldrich syndrome are two rare X-linked recessive disorders with an unclear pathophysiology, characterised by small platelets and thrombocytopenia. The *Wiskott-Aldrich syndrome* (WAS), originally described in 1937, is a condition with a variable clinical expression and affects males but not females. WAS is usually a mild haemorrhagic condition, with marked or severe thrombocytopenia and smaller than normal platelets, associated with T-lymphocyte abnormalities, immunoglobulin M (IgM) deficiency, and eczema. The deficiency occurs early, during the first decade of life, and usually before the age of 3 years. The incidence of WAS is 4 cases every 250,000 live male births in the European population. In most cases, mortality, also at an early age, is due to fulminant infections. In addition, about 12% of patients developed malignancies, primarily lymphoreticular tumours, and leukaemia. WAS results from a X-linked genetic defect in a protein with multiple functions, now termed WAS protein (WASp), which plays a fundamental role in haematopoietic cell lineages where is exclusively expressed. The gene is located on the short arm of the X-chromosome, Xp11.22-23, is composed of 12 exons and encodes a 502 amino-acid protein. Although the exact functions of WASp have not been fully elucidated, this protein is involved in signalling resulting in cell activation and promotion of cell motility, through movements of actin filaments in the cytoskeleton. The phenotypic variability of WAS may be explained by the nature of the genetic abnormality. Numerous different mutations (about 300) have been identified in the WAS gene, particularly in a few mutational spots. Predominant WAS gene mutations include missense mutations, typically located in exons 1, 2, 3 and 4, those occurring in the first and third exon being associated with milder forms, and splicing site mutations, predominantly occurring downstream of intron 6. Deletions involve less than ten nucleotides and are more common in areas rich in CG nucleotides.

X-linked thrombocytopenia (XLT), a rare recessive hereditary disorder, is considered a mild allelic variant of WAS. It is characterised by isolated thrombocytopenia with small-sized platelets, easy bruising, without profound immunodeficiency and defects of white blood cell cytoskeleton, and is occasionally associated with transient eczema. The prevalence of XLT appears to be similar to that of WAS. Hereditary X-linked thrombocytopenia can be often misdiagnosed as chronic ITP, in which platelets are normal-sized or enlarged.

**Thrombocytopenia with normal platelet size**

*Congenital amegakaryocytic thrombocytopenia* (CAMT) is an autosomal recessive inherited syndrome, characterised by isolated and severe thrombocytopenia (< 10,000/µL), elevated serum levels of thrombopoietin (TPO), and absence of megakaryocytes in the bone marrow. CAMT is regularly expressed in infancy and often identified within a few days or the first month of birth. Some patients (10 to 30%) have orthopaedic or neurological abnormalities or intracranial haemorrhages. Medical treatments other than platelet transfusions are largely unsuccessful. Most patients will develop complete aplasia and many infants die because of infections or...
haemorrhage. Patients with a mild form of CAMT develop thrombocytopenia, and have decreased leucocyte and erythrocyte production towards the end of the first decade of life\(^{66}\). The defect in thrombocytopoiesis is not well understood. It is well known that TPO plays a key role in the regulation of megakaryopoiesis (Figure 2). After cloning of the c-mpl receptor, the ligand for TPO, the \textit{CMPL} gene was considered as one of the genes responsible for this disorder\(^{67}\). The hypothesis to explain the development of aplastic pancytopenia is that c-mpl is also required for stem cell maturation. A deficiency in expression or function of the TPO receptor, essential for mature megakaryocyte production, is the molecular cause of CAMT. The more severe form of CAMT, with early development of pancytopenia, is due to the complete absence of receptor expression or function, determined by frameshift or nonsense mutations, whereas a milder form results from residual function of mutant c-mpl receptor, occurring in the presence of a missense mutation.

\textit{Thrombocytopenia-absent radius} (TAR) syndrome\(^{68}\), first described in 1951, is a congenital malformation syndrome characterised by bilateral absence of the radii and hypomegakaryocytic thrombocytopenia. Other abnormalities are often present, including additional skeletal defects, such as absent or defective development of other bones of the forearm (ulnar), structural malformations of the heart, and kidney defects. The syndrome shows an autosomal recessive pattern of inheritance resulting in potentially severe bleeding episodes primarily during infancy. Among the amegakaryocytic syndromes, it is the commonest with over 50 families, with more than one affected member, having been identified. The causative gene for TAR has not been identified and the pathogenesis of this disorder remains unclear. Recent studies have suggested a role for \textit{HOXA10, HOXA11} and \textit{HOXD11}, which are homeobox genes involved in directing embryonic development\(^{69,70}\). Recently, a role for a deletion on chromosome 1q21.1 was hypothesised for 30 patients with TAR, but only in the presence of additional as-yet unknown abnormalities.

\textbf{Thrombocytopenia with increased platelet size}

Congenital macrothrombocytopenias (CMT)\(^{71}\) include a heterogeneous group of rare disorders, characterised by abnormal giant platelets, thrombocytopenia and a bleeding tendency of variable severity. Several entities, affecting platelet structure at different levels, are responsible for this phenotype. CMT is associated with GPS, velocardiofacial syndrome\(^{72}\), Mediterranean macrothrombocyto-

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Principal steps of platelet maturation and the genetic counterparts involved}
\end{figure}
Inherited platelet disorders

Mediterranean macrothrombocytopenia, also known as autosomal dominant benign BSS\(^7\), has been described in Mediterranean populations such as Greeks and Italians but is absent in the north of Europe. The syndrome is characterised by a mild form of thrombocytopenia (70,000-150,000/µL), large platelets, normal platelet functions, and a normal megakaryocyte count, in the absence of significant bleeding. Most individuals with Mediterranean macrothrombocytopenia carry heterozygous mutations in GP1BA or GP1BB genes, emphasizing that the genotype and phenotype of Mediterranean macrothrombocytopenia and BSS are similar. The most common genotypic abnormality is a GP1BA missense mutation, leading to an amino acid substitution (Val156Ala), known as the Bolzano variant\(^7\).

DiGeorge or velocardiofacial syndrome (DGS/VCFS) is characterised by conotruncal cardiac abnormalities, learning disabilities, velopharyngeal insufficiency, immunodeficiency, facial dysmorphisms and thymic hypoplasia.

A variable clinical immunodeficiency and mild macrothrombocytopenia, affecting only 20% of patients, can also be present. The syndrome shows an autosomal recessive inheritance and is due to a de novo heterozygous deletion of part of chromosome 22 (22q11.2), which includes 24-30 genes\(^6,7\). The relationships between these genes and clinical features are not clearly understood. The 22q11.2 deletion is the most common chromosomal microdeletion syndrome known and occurs with a frequency of 1 in 4,000-5,000 live births. Studies in mutant mice suggest that a single gene, TBX1 (encoding a T-box containing transcription factor), is a strong candidate gene for human VCFS/DGS\(^6,7,8\). The 22q11.2 deletion may include the GP1BB gene, which is adjacent to TBX1, giving rise to BSS in the presence of a mutation on the second allele.

Pseudo-von Willebrand's disease (pseudo-VWD) is an autosomal dominant inherited disease that has clinical and laboratory similarities with the more common type 2 VWD. A selective loss of plasma large VWF multimers, which spontaneously bind to the platelet receptor GPIb and inhibit platelet adhesion, producing bleeding events, is a useful marker for distinguishing pseudo-VWD from type 2 VWD\(^9,80\). Usually, patients with this condition also have both giant platelets and mild thrombocytopenia, although in rare families a severe macrothrombocytopenia is associated with circulating platelet aggregates. The disorder is caused by mutations in the GP1B gene (in particular in GP1BA), which probably cause a conformational change of the glycoprotein and induces the interaction with inactivated VWF molecules without any specific activation\(^81\). Only two mutations, both leading to a gain-of-function effect, have been found in the carboxy-terminal flanking region of GP1Bζ; these cause increased affinity of VWF for the GPIb/IX/V complex.

May-Hegglin anomaly (MHA), Fechtner syndrome (FTNS), Sebastian syndrome (SBS), and Epstein syndrome (ES) constitute a group of autosomal dominant related disorders characterised by thrombocytopenia, large platelets, and characteristic basophilic inclusions in peripheral blood leucocytes ("Döhle-like" bodies), associated with deafness (FTNS, SBS, ES) and nephritis (FTNS, ES). In addition to thrombocytopenia and giant platelets, MHA, the most prevalent among these uncommon disorders, is characterised by specific spindle-shaped bodies within polymorphonuclear leucocytes. Patients with FTNS have nephritis, neurosensorial hearing loss, cataracts and macrothrombocytopenia. In SBS, the renal manifestations of FTNS are usually lacking whereas interstitial or mixed nephritis associated with neurosensorial deafness occurs in ES. In MHA, thrombocytopenia is often mild (although in some cases it is very severe) and bleeding is infrequent and rarely life-threatening. Myocardial infarction has been reported in elderly patients. All these disorders are attributed to mutations in the MYH9 gene on chromosome 22 (22q12-13)\(^82-84\). This gene comprises 44 exons, and encodes a large cytoplasmic protein non-muscle myosin heavy chain peptide (NMMHC-IIA), which is expressed in many different tissues including platelets, kidney and leucocytes and is involved in cell motility, cytokine-directed cell polarity and cell architecture. It is now clear that classic MHA leucocyte inclusions are due to aggregates of the abnormal NMMHC-IIA peptide. The similarities between these disorders seem to be due to a common overlapping region of 480 kb, suggested that all the disorders are allelic. Several distinct mutations have been found in about 200 families with...
MYH9-related disorders. MYH9 mutations identified involve highly conserved residues in both the globular head and the coiled-coil domains of the protein. Mutations may affect the correct assembly and stability of myosin leading to altered platelet and leucocyte structure and functions. Types of mutations include missense, nonsense, and frameshift mutations, the majority being found in the last exon. Although all these syndromes result from mutations in the MYH9 gene, clear phenotype-genotype relationships have not been determined. In fact, the clinical phenotype is highly variable, even within kindreds. In addition, because the same mutation has been identified in different MYH9-related disorders, the diseases appear to be not truly monogenic. From a clinical point of view, these disorders may be distinguished from each other by the presence or absence of leucocyte inclusions, hearing loss, and nephritis. However, several studies have suggested that May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome are not distinct entities but rather a single disorder with a continuous clinical spectrum ranging from mild macrothrombocytopenia with leucocyte inclusions to a severe form complicated by hearing loss, cataracts, and renal failure. Jacobsen and Paris-Troussau syndromes are very rare autosomal dominant traits sharing multiple similar congenital anomalies, including thrombocytopenia and giant platelet α-granules resulting from the fusion of smaller organelles, associated with the same chromosomal deletion (11q23.3).

In all cases, thrombocytopenia is due to a dysmegakaryocytopenia associated with a mild bleeding tendency. In the Paris-Troussau syndrome, platelet dense bodies are normal in number, size, and contents whereas they are reduced in the Jacobsen syndrome, indicating the presence of a storage pool deficiency.

Therapy

Congenital platelets disorders are associated with a wide range of bleeding symptoms. However, as a rule, there is no specific therapy for the vast majority and only severe cases need to be treated. The management of patients with congenital platelet diseases usually consists of general measures aimed at avoiding bleeding and the use of supportive therapy to control haemorrhagic episodes. However, as the type and severity of bleeding vary in different patients, therapeutic approaches must be personalised.

General measures

Education of patients is of great importance. Patients and their parents must be instructed about drugs that impair platelets functions, such as acetylsalicylic acid-containing medications, regular dental care and the use of oral contraceptives to prevent menorrhagia. Local measures, such as the application of firm pressure in the case of epistaxis, will usually be sufficient to stop cases of mild bleeding.

Drugs

Desmopressin (DDAVP), a synthetic analogue of the diuretic hormone vasopressin that increases factor VIII and VWF transiently by releasing them from storage sites into the blood, is a mainstay of the therapy of patients with congenital platelet defects. Depending on the type of platelet defect, administration of DDAVP may shorten the bleeding time and its use has been suggested to be of value in some patients with congenital platelet defects, such as BSS, MHA, GPS, SPD and GT. In general, the response to DDAVP varies among patients but is constant in each patient; a test dose may be of value to identify those patients who will benefit from this treatment to prevent or control future bleeds.

The use of recombinant activated factor VII has proven useful in patients who no longer respond to platelet transfusion, usually because they have developed antibodies against platelets as a result of previous transfusions. Administration of recombinant activated factor VII has been used to stop bleeding in patients with congenital platelet disorders, such as BSS and GT. However, adverse reactions, such as venous thromboembolism and arterial ischaemia, have been described.

Platelet transfusions

Platelet transfusions, using platelets from HLA-matched donors when available, are used to control severe haemorrhage in thrombocytopenic patients or in individuals with thrombocytopenias. As the risk of post-traumatic as well as of spontaneous bleeding increases as the platelet count falls, spontaneous haemorrhage becomes common as platelet counts drop below 20,000/μL and is extremely likely at counts below 5,000/μL. Extensive clinical experience has
shown that bleeding can be controlled by an adequate increase in the circulating platelet count. However, the co-existence of platelet dysfunctions must be taken into account for a correct calculation of the dosage of platelets required. Platelet transfusions are effective in controlling bleeding but may be responsible for febrile reactions or the development of alloimmunisation. Indeed, in patients with thrombocytopathies, platelet transfusions should be employed only for the treatment of severe bleeding because of the risk of alloimmunisation. The occurrence of platelet alloimmunization is more frequent in patients lacking a membrane glycoprotein, such as those with BSS or GT. Alloantibodies develop because they recognise the endogenously deficient proteins present in the transfusion as foreign and, in turn, induce refractoriness to platelet transfusions. Care should be taken to limit the risk of alloimmunisation by limiting exposure and using pre-stored and leucodepleted platelet concentrates or HLA-matched donors for transfusion. The efficacy of prophylactic platelet transfusions in clinically stable patients remains questionable.

Other measures

Splenectomy has no effect in congenital platelet disorders except in WAS. Allogeneic haematopoietic stem cell transplantation could be effective for inherited platelet disorders, restoring normal megakaryocytopenia, but the risk of such procedures is still high. Transplantation was successful in 80% of patients below the age of 5 years and complete correction was obtained in patients with WAS and severe GT. However, because the risks associated with haematopoietic stem cell transplantation still exceed those related to bleeding, it is generally not recommended unless the patient suffers from life-threatening haemorrhages or has developed refractoriness to platelet transfusion due to antibody formation.

Concluding remarks

Over the last years, the pathogenesis of congenital platelet disorders has been better understood. The genes responsible for various congenital platelet diseases have been identified and advances are being made in molecular characterisation of these disorders. This information has allowed for a more accurate comprehension of congenital thrombocytopathies and thrombocytopenias. Careful collection of personal and family clinical data, an accurate physical examination and appropriate laboratory tests are of great value for the evaluation of a patient presenting with bleeding due to congenital platelet disorders. Using this approach, it is possible to identify the platelet defect correctly in some cases. However, despite recent gains in knowledge, the underlying molecular mechanisms remain unknown in most patients with a congenital bleeding disorder and impairment of platelet function. The challenge for the future is to increase our understanding of congenital platelet disorders in order to obtain powerful strategies for the prevention, diagnosis and therapy of bleeding.

Key words: Inherited platelet disorders, thrombocytopenias, thrombocytopathies.

References

10) Bernard-Soulier syndrome http://www.bernardsoulier.org/


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