CONGENITAL CYTOPENIAS

The molecular basis of congenital thrombocytopenias: Insights into megakaryopoiesis

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Thrombocytopenia, defined as a platelet count less than 150,000 \( \mu l^{-1} \), is a common problem in clinical hematology. In most patients, the cause is secondary to an acquired autoimmune disorder, a systemic illness or infection, or an adverse drug effect. Inherited thrombocytopenias are relatively rare, but the identification of genetic mutations in patients with these disorders has contributed significantly to our understanding of the molecular basis of megakaryopoiesis and platelet production. Although laboratory models based on engineered cell lines or transgenic mice are useful in the evaluation of genetic changes that affect megakaryopoiesis, they are not always faithful representations of the human conditions. Therefore, studies from affected patients continue to be vital for our understanding of these diseases.

The list of recognized syndromes of inherited thrombocytopenia is growing, reflecting our increased understanding of the variety of genes that regulate megakaryocyte development and the formation of platelets. Individual syndromes can be classified conceptually by grouping them into disorders with amegakaryocytosis, impaired megakaryocyte maturation, or more distal defects in platelet formation. Low platelets are usually the dominant feature, but abnormalities in other blood cell lineages may be present. In addition, features outside of the hematopoietic system may be associated, such as skeletal defects, hearing abnormalities, or renal dysfunction. Importantly, several of the inherited thrombocytopenias carry a risk of progression to aplastic anemia or leukemia and may occasionally present with these features if the preceding thrombocytopenic phase has not been detected.

Disorders associated with amegakaryocytosis include congenital amegakaryocytic thrombocytopenia (CAMT), thrombocytopenia with absent radii (TAR), and amegakaryocytic thrombocytopenia with radio-ulnar synostosis (ATRUS). These children typically present at birth with severe thrombocytopenia, platelets of normal size, and a paucity of bone marrow megakaryocytes. If plasma levels of thrombopoietin (TPO), the primary cytokine regulating platelet production, are obtained they are elevated due to decreased uptake by the decreased megakaryocyte mass [1]. The prototypical disorder in this category is CAMT, in which patients have severely reduced megakaryocytes and platelets due to mutations in the TPO receptor c-Mpl [2]. Because of the complete lack of receptor-mediated uptake, elevated TPO levels are particularly prominent in CAMT. Although infants present with low platelets, they usually progress to complete bone marrow aplasia within a few years, providing evidence for a critical role for TPO in the maintenance of the hematopoietic stem cell [3]. Mice engineered to have homozygous deletion of c-Mpl are thrombocytopenic and have a reduced number of stem cells [4,5], but the mice do not develop pancytopenia even with prolonged monitoring (unpublished observation). The basis for this difference between humans and mice deficient in c-Mpl is not understood. Identified mutations of c-Mpl are scattered throughout the gene and are inherited in an autosomal recessive pattern, as heterozygous expression of a wild type receptor is adequate for normal thrombopoiesis. Mutations can be classified as type I, in which there is complete loss of the receptor due to a truncation or nonsense mutation, or type II, in which an amino acid substitution results in a receptor with some partial function [2]. This distinction is clinically significant as it appears that patient with type II mutations tend to have a higher initial platelet count and a slower course of progression to aplasia. Because of the expected development of marrow failure, it is recommended that patients with CAMT be trans-
planted if a matched related stem cell donor is available.

Infants with TAR syndrome may resemble CAMT in that they present with severe thrombocytopenia from birth; however, they are distinguished by characteristic forearm abnormalities in which the radii are absent [6]. This abnormality is not typically seen in Fanconi anemia (FA), in which the thumbs may be missing but the radii are present. TAR usually exhibits autosomal recessive inheritance, though families with apparently autosomal dominant transmission have been reported [7]. Bone marrow megakaryocytes in TAR are reduced and may appear immature [8]. Curiously, the thrombocytopenia in TAR syndrome spontaneously improves after the first year of life, and unlike CAMT there is not a progression to marrow aplasia; however sporadic cases of late transformation to leukemia have been reported [9–11]. The genetic basis of TAR is not understood; investigations into members of the homeobox family genes known to be involved in limb development have not revealed any mutations [12]. Although platelets from individuals with TAR have defective signaling and do not show the expected phosphorylation of intracellular proteins in response to TPO stimulation, the lack of signaling is not due to a mutation in c-Mpl or its associated Jak2 kinase [8,13,14]. Elucidation of the genetic defect in TAR may therefore provide new insights into the mechanisms of TPO signaling.

Like TAR, ATRUS is characterized by amegakaryocytic thrombocytopenia from birth and the presence of a forearm defect, with proximal radioulnar fusion. This may be difficult to detect on exam and forearm radiographs are helpful. Other skeletal abnormalities including clinodactyly and shallow acetabulae may be present. In two pedigrees with ATRUS, autosomal dominant inheritance of mutations in the homeobox family gene HoxA11 have been described [15,16]. Unlike TAR, the thrombocytopenia in ATRUS does not improve over time, and case reports have indicated that these children may be at risk of progression to aplastic anemia. The molecular mechanism by which HoxA11 leads to thrombocytopenia is not clear because although both HoxA10 and HoxA11 are expressed in hematopoietic stem cells, only HoxA10 has been found in megakaryocytes and the HoxA11 knockout mouse does not have thrombocytopenia [17,18]. Target genes of HoxA11 that may be candidates for its role in megakaryopoiesis have not been identified. Mutations involving TPO in clinical syndromes of thrombocytopenia are notable for their absence; despite the fact that TPO deletion in knock-out mice cause a phenotype nearly identical to that of c-Mpl mutations [5], defects in this growth factor have not been reported in patients with low platelets or with aplastic anemia.

Disorders in which megakaryocyte maturation is impaired frequently involve mutations affecting specific transcription factors, such as RUNX1 (AML1), GATA1, and FLI1. These disorders are usually associated with a moderate thrombocytopenia and normal or large platelet size. Evaluation of the bone marrow reveals megakaryocytes that are small and may be hyperproliferative. If ploidy analyses are performed, megakaryocytes in the higher ploidy classes are reduced. Mutations in RUNX1, a transcription factor with a critical role in definitive hematopoiesis [19,20] and leukemogenesis [21], have been described in familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) [22,23]. Such families are characterized by autosomal dominant inheritance of mutations in RUNX1 either leading to haploinsufficient expression of the transcription factor or its inability to bind DNA. Because individuals with FPD/AML may be candidates for bone marrow transplantation (BMT) for the treatment of leukemia, it is important to screen potential sibling donors for the presence of this disorder. The molecular description of FPD/AML has provided new insight into the significant role of RUNX1 in normal megakaryopoiesis. The critical targets of RUNX1 in megakaryopoiesis are not known, but RUNX1 interacts with the megakaryocytic transcription factor GATA1 [24] and this interaction is likely important for its role in platelet production. GATA1 and its co-factor Friend of GATA1 (FOG1) are essential in both thrombopoiesis and erythropoiesis [25–27]. Mutations in the X-linked GATA1 gene have been found in 5 families with thrombocytopenia, often with large hypogranular platelets and accompanied by dyserythropoiesis or thalassemia [28–30]. All mutations to date have been described in the N-terminal zinc finger of GATA1 and either disrupt its interaction with FOG1 or with DNA [25]. Although mutations in FOG1 have not been described, they would be predicted to have a similar phenotype but autosomal inheritance. Another critical transcription factor that may interact with RUNX1 and GATA1 in megakaryopoiesis is the Ets family member FLI1 [31,32]. In murine models, deletion of FLI1 leads to defects in vascular development megakaryocyte maturation [33], and consensus sites for FLI1 are found in proximity to GATA1 sites in the promoters of many megakaryocyte-specific genes [32,34]. Although no specific mutations in FLI1 have been described in humans, children with the Paris-Trousseau syndrome have a constitutive deletion of 11q24 that encompasses FLI1 [33,35]. FLI1 is therefore a candidate as the critical gene in this disorder. Paris Trousseau is a variant of the Jacobsen syndrome, in which congenital heart defects, facial dysmorphism, and mental retardation are accompanied by thrombocytopenia with small marrow megakaryocytes [35–37]. Platelets in this syndrome demonstrate giant, fused alpha granules and may be large. In addition to these disorders, GATA1 may
regulate the expression of the heterodimeric transcription factor NF-E2 [38,39]. In mouse models, NF-E2 has been demonstrated to regulate a number of megakaryocyte-specific genes and to be required for thrombopoiesis [40–43]. Despite evidence of its importance as a transcription factor directing megakaryocytic differentiation, mutations in NF-E2 have not been described as a cause of inherited thrombocytopenia in humans. Not all mutations leading to impaired megakaryocyte maturation have been found in transcription factors; a large kindred with autosomal dominant thrombocytopenia linked to chromosome 10 led to the identification of a novel kinase, MASTL (previously FLJ14813), that appears to be important for megakaryocytic maturation and polyploidization [44]. The function of this kinase is still unknown. Although the risk for progression to leukemia associated with mutations in RUNX1 is clear, it is not known if mutations involving GATA1, FLI1, or MASTL confer a similar predisposition to leukemic transformation. However, mutations in GATA1 are uniformly found in the megakaryoblasts of patients with Down syndrome [45], and mouse models of GATA1 knockout are associated with the development of leukemias [46] whereas mice models with lineage-specific GATA1 deletion are associated with the development of myelodysplasia [47]. In the clinical setting, patients with constitutive defects in megakaryocyte maturation should be monitored closely.

Thrombocytopenia syndromes in which platelet production is primarily affected are often marked by abnormal platelet size. The mutations described generally involve regulators of the cytoskeleton. Small platelets in males with moderate to severe thrombocytopenia are suggestive of Wiskott Aldrich Syndrome (WAS). WAS is an X-linked syndrome characterized by microthrombocytopenia, T-cell deficiency, and immune dysregulation resulting in infections, eczema and a predisposition to lymphoid malignancy [48]. Platelet function in WAS is also impaired, leading to bleeding out of proportion to the degree of thrombocytopenia. The mutated gene, encoding WAS protein (WASP), encodes a hematopoietic specific actin regulating protein that activates Arp2/3 to nucleate actin polymerization [49,50]. Binding domains within WASP mediate interactions with WASP-interacting protein (WIP), phospholipids, SH3-containing signaling proteins, and the active GTP-bound form of cdc42 [49,51]. Nonsense mutations or truncations that result in the absence of WASP lead to a more severe phenotype, whereas substitution mutations in which the protein retains some partial function may only result in an X-linked microthrombocytopenia without the features of immune dysfunction [52–55]. The diagnosis of WAS can be confirmed by flow cytometry of lymphocytes to evaluate levels of WASP protein and by sequencing for WASP mutations. Disruption of specific protein–protein interactions may account for some variability in the phenotype. Young boys with the full WAS syndrome are generally transplanted if they have a suitable stem cell donor, due to the risk of life threatening infection and development of malignancy. It is thought that peripheral destruction contributes to the thrombocytopenia in WAS and XLT [56], and in many cases platelet counts may be significantly improved by splenectomy [48,57]. In contrast to WAS, Bernard Soulier syndrome (BSS) is characterized by macrothrombocytopenia, often with giant platelets. In addition to their large size, platelets in BSS are severely impaired in their function. This disorder can be attributed to mutations in GPIb or GPIX [58], which can be detected by flow cytometry as reduced expression of the GPIb/IX/V complex, or by platelet function testing as absent ristocetin-induced platelet aggregation (RIPA). Originally thought to be a recessive disorder, it is now appreciated that patients heterozygous for BSS mutations often have a partial phenotype with mild macrothrombocytopenia [59]. In addition, patients with DiGeorge syndrome may also have a mild macrothrombocytopenia due to the location of the GPIb beta gene within the deleted region of chromosome 22q11 [60,61]. The reason for the large platelet size in BSS is not clear but may be due to loss of the interaction between GPIb and filamin which serves to anchor the platelet membrane to the underlying actin cytoskeleton [62,63]. Management of bleeding in patients with homozygous BSS syndrome is problematic as platelet transfusion can result in allosensitization and the development of platelet refractoriness. DDAVP may have some benefit in improving bleeding times in affected patients, probably through a von Willebrand factor-independent mechanism [64,65]; alternatively recombinant factor VIIa has been used [66]. In addition to BSS, macrothrombocytopenia is also seen in the autosomal dominant May Hegglin anomaly and Sebastian’s, Fechtner’s, Epstein’s, and Alport’s-like syndromes, now collectively recognized as MYH9-related disorders [67,68]. MYH9 encodes the non-muscle myosin heavy chain IIA, which is expressed in platelets, neutrophils, the kidney and cochlear cells. Precipitates of myosin can be detected as neutrophilic inclusions, appearing like Dohle-bodies on a peripheral smear, which may facilitate diagnosis in this disorder. The macrothrombocytopenia in the MYH9-related disorders is associated with the variable expression of glomerulonephritis, hearing loss, and cataracts, and patients should be monitored for these complications. Recently a mouse model of MYH9-deficiency was reported; although the heterozygous mice show variable hearing deficits they do not manifest macrothrombocytopenia, neutrophil inclusions, or renal disease [69]. The reason for this finding is not understood but it may relate to the production of a
mutant myosin in most cases of the human disorder, whereas in the mouse model there is only a reduced amount of the normal protein.

In addition to platelet number, platelet structure can be affected by inherited mutations. Abnormal platelet granules may have been noted as a feature of TAR, GATA1 mutations, Paris-Trousseau syndrome, and WAS. The gray platelet syndrome is marked by autosomal dominant inheritance of thrombocytopenia in which the platelets are deficient in $\alpha$-granules, which is visualized on a Wright-Giemsa stain as gray appearing platelets and can be confirmed by electron microscopy. The genetic cause of gray platelet syndrome is not known, although it is thought to involve a transport or storage process rather than defective synthesis of a specific alpha-granule component [70]. Hermansky-Pudlak, Griscelli and Chediak-Higashi syndromes exhibit deficiencies of dense granules and platelet dysfunction [71]. Thrombocytopenia is variable in these disorders. Because the pathways regulating the formation and trafficking of dense granules are also involved in the trafficking of lysosomes and melanosomes, pigmentation abnormalities frequently accompany the platelet defects. Platelet dysfunction associated with abnormal granule release and defective platelet aggregation can also cause significant bleeding in the absence of thrombocytopenia [72]. In addition, inherited defects extrinsic to hematopoietic cells may lead to increased platelet consumption and thrombocytopenia; these include congenital TTP, in which a deficiency of ADAMTS 13 leads to ultralarge von Willebrand multimers and episodic thrombocytopenia and anemia, type IIb von Willebrand disease, in which a mutation in von Willebrand factor causes increased affinity for GPIb and platelet destruction, and platelet type von Willebrand disease, in which a mutation in GPIb causes increased affinity for von Willebrand factor and platelet destruction. Familial ITP has been reported but the genetic basis of this is not clear and it may represent a manifestation of an inherited immunodeficiency. Because these syndromes are not primarily associated with impaired platelet formation, for brevity these disorders will not be addressed here and the reader is directed to other recent reviews [73–78].

Once artifact and acquired causes have been excluded, identifying the genetic basis for chronically low platelets in an individual or family remains difficult. Proper evaluation often requires a combination of clinical and research investigations, due to the rarity of these disorders and a lack of standardized testing. An algorithm has been developed by the Italian Gruppo di Studio delle Piastrine to facilitate the diagnosis of the major identified syndromes of inherited thrombocytopenia [79–81]. This algorithm utilizes generally available parameters such as platelet size, inheritance, and associated features to direct the evaluation for known platelet disorders. Specific testing is then applied to confirm the diagnosis (Figure 1). Using this algorithm and extensive laboratory evaluation, approximately half of the patients in that registry with could be diagnosed with a known platelet disorder.

In clinical practice, the diagnostic workup for inherited thrombocytopenia should first exclude the more common phenomena of immune and non-immune mediated platelet destruction, drug effects, infections, and malignancy. In addition, spurious thrombocytopenia, or pseudothrombocytopenia, due to clumping of platelets collected in EDTA based anticoagulants can be ruled out by examining the smear and repeating the platelet count on a specimen collected in citrated buffer. The patient’s history should be reviewed for duration and degree of thrombocytopenia, symptoms of bleeding and the presence of associated abnormalities involving the skin, kidneys, or hearing. A careful family history will help to establish the mode of inheritance and the presence of associated features such as nephropathy, hearing loss, or a predisposition to leukemia. The patient should be examined for growth deficiency, the presence of skeletal or other congenital abnormalities, and skin or pigmentation defects. A complete blood count, leukocyte differential, and peripheral smear should be carefully reviewed for additional cytopenias, red cell indices, red cell morphology, neutrophilic

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Figure 1. Algorithm for evaluation of candidate disorders of inherited thrombocytopenia.
inclusions, and platelet size and granularity. It is important to remember that automated cell counters may not give an accurate platelet count if the platelets are very small. Patients should have a bone marrow aspiration and biopsy to assess the frequency and appearance of the megakaryocytes in the bone marrow and to exclude alternative diagnoses; in addition, cytogenetics should be sent on the bone marrow specimen. Forearm radiographs will help to exclude subtle defects such as radioulnar fusion. If suspected, FA should be ruled out by chromosomal breakage analysis. Von Willebrand disease should be excluded by standardized testing and multimer analysis if the history suggests it; a low dose RIPA using patient plasma will show increased platelet aggregation if the patient has type IIb von Willebrand disease, whereas a low dose RIPA using patient platelets is used to detect platelet-type von Willebrand disease. Although theoretically useful, platelet aggregation studies are often difficult to standardize in patients with platelet counts of less than 100,000 μl⁻¹. In syndromes of large platelets, flow cytometry to evaluate surface expression of GPIb/IX/V is clinically available and can be used to diagnose BSS, although reduced expression of GPIb/IX/V has been reported in MYH9-related disease and GATA1 deficiency and therefore this may test may not be specific [82]. Similarly, flow cytometry tests have been developed to assay for WASP in lymphocytes. A few clinical laboratories will provide diagnostic sequencing for candidate genes including c-Mpl and WAS. However, many potentially useful diagnostic tests are available only in the research setting and have not been developed for clinical use, including reticulated platelets, TPO levels, hematopoietic progenitor assays, slide-based immunofluorescence for myosin inclusions, and sequencing for the majority of candidate genes known to be associated with inherited thrombocytopenia. Once the diagnostic workup has been completed, it is important to follow the clinical course of patients with constitutive thrombocytopenia, especially those with amegakaryocytosis, impaired megakaryocytic maturation or without a definitive diagnosis, given the unclear risk for progression to aplasia or leukemia. Where possible, these patients should be entered into one of the several registries for inherited thrombocytopenia that exist in Europe, Canada, and in the US so that information regarding these rare but informative individuals can be captured.

References


