INHERITED THROMBOCYTOPAENIAS: BEYOND THE BLEEDING

***Patrizia Noris**

Department of Internal Medicine, IRCCS San Matteo Foundation; University of Pavia, Pavia, Italy
**Correspondence to p.noris@smatteo.pv.it*

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ABSTRACT

The improvement of molecular biology technologies and the increasing number of researchers interested in inherited thrombocytopaenias (ITs) has led to a significant expansion in knowledge of the genetic basis and clinical features of the different types of IT. In particular, it is now well known that an inherited 'low platelet count' can be the unique abnormal laboratory finding of the disease, combined with a bleeding tendency usually proportionate to the extent of the thrombocytopaenia, or can associate with different congenital and/or acquired signs and symptoms, or with predisposition to other diseases. Therefore, recognising syndromic forms and their main features is crucial for the proper management of patients, who rely on appropriate follow-up and treatments beyond those for simply preventing and/or treating bleeding. Among all syndromic ITs, this review aims to describe those predisposing to hematological malignancies (such as the familial platelet disorder with predisposition to acute myelogenous leukaemia and the ANKRD26-related thrombocytopaenia), and to bone marrow aplasia (with particular regard to congenital amegakaryocytic thrombocytopaenia), as well as to summarise the main characteristics of *MYH9*-related diseases, one of the most frequent forms of IT, which expose patients to the risk of developing additional features such as cataract, sensorineural deafness, alteration of liver enzymes, and/or a glomerulonephritis that usually evolves into end-stage renal failure.

Keywords: Inherited thrombocytopaenias, congenital platelet disorder, bleeding, hematological malignancies, bone marrow aplasia.

INTRODUCTION

Until the end of the last century, inherited thrombocytopaenias (ITs) were considered exceedingly rare and only a few forms were known. The prototypical IT was Bernard-Soulier syndrome, which is characterised by both quantitative and qualitative platelet defects that are responsible for recurrent spontaneous hemorrhages, often severe and endangering the lives of patients. As a consequence, affected subjects often need repeated transfusions of blood products and develop refractoriness to platelet transfusions, making bleeding episodes or invasive procedures hard to manage. Based on the picture of ITs as severe bleeding disorders, the concern of physicians was to prevent bleeding and stop them.

With the beginning of the new century, advances in technologies for genetic analyses and a growing number of researchers interested in ITs led to the identification of many new forms and changed our view of these disorders. Now we know about 20 diseases and realised that in most cases the degree of thrombocytopaenia is mild and the tendency to spontaneous bleeding is low or even absent. We also realised that the genetic defects resulting in thrombocytopaenia may affect other cell lines or tissue, and that the risks deriving from these additional changes are sometimes larger than those induced by platelet deficiency. Table 1 reports the ITs with other defects in addition to thrombocytopaenia. It is important to note that many of these abnormalities are not present at birth but develop later in childhood or even in adult life. So, early recognition of these disorders is essential for personalising the management of affected subjects.

Table 1: Inherited thrombocytopaenias associated with other congenital defects or predisposed to other diseases.

Disease (abbreviation in this paper, phenotype MIM number)	Inheritance	Gene (chromosome localisation)	Other features
ITs predisposing to hematological malignancies			
FPD/AML, 601399	AD	*CBFA2* (21q22)	Possible development of leukaemia or MDS. Normal-sized platelets.
ANKRD26-RT, 188000	AD	*ANKRD26* (10p2)	Possible development of leukaemia or MDS. Normal-sized platelets.
ITs predisposing to BMA			
CAMT, 604498	AR	*MPL* (1p34)	Evolves into bone marrow aplasia in infancy. Normal-sized platelets.
ATRUS, 605432	AD	*HOXA11* (7p15-14)	Radio-ulnar synostosis +/- other defects. Possible evolution into aplastic anaemia. Normal-sized platelets.
ITs associated with other defects			
MYH9-RD, nd	AD	*MYH9* (22q12-13)	Cataracts, nephropathy, and/or deafness. Liver enzymes may be elevated. Giant platelets.
GPS, 139090	AR	*NBEAL2* (3p21.1)	Evolutive myelofibrosis and splenomegaly. Giant platelets.
GFI1B-RT, nd	AD	*GFI1B* (9q34_13)	Red blood cell anisocytosis. Large platelets. Myelofibrosis.
GATA1-RDs, DAT, 300367 - XLT with thalassaemia, 314050	XL	*GATA1* (Xp11)	Hemolytic anaemia, possible unbalanced globin chain synthesis, possible congenital erythropoietic porphyria. Large platelets.
WAS, 301000	XL	*WAS* (Xp11)	Severe immunodeficiency leading to death in infancy. Small platelets. Increased risk of lymphoma.
XLT, 313900			Mild immunodeficiency. Small platelets.
TAR, 274000	AR	*RBM8A* (1q21.1)	Platelet count tends to rise and often normalises in adulthood. Reduced megakaryocytes. Normal-sized platelets. Bilateral radial aplasia +/- other malformations.
TCPT, 188025; JBS, 147791	AD	Large deletion (11q23-ter)	Cardiac and facial defects, developmental delay +/- other defects. Large platelets.

ITs: inherited thrombocytopaenias; FPD/AML: familial platelet disorder and predisposition to acute myelogenous leukaemia; AD: autosomal dominant; MDS: myelodysplastic syndrome; ANKRD26-RT: ANKRD26-related thrombocytopaenia; AR: autosomal recessive; BMA: bone marrow aplasia; CAMT: congenital amegakaryocytic thrombocytopaenia; ATRUS: amegakaryocytic thrombocytopaenia with radio-ulnar synostosis; MYH9-RD: MYH9-related disease; nd: not defined; XL: linked to chromosome X; GPS: grey platelet syndrome; GFI1B-RT: GFI1B-related thrombocytopaenia; GATA1-RDs: GATA1-related diseases; DAT: dyserythropoietic anaemia with thrombocytopaenia; WAS: Wiskott-Aldrich syndrome; XLT: X-linked thrombocytopaenia; TAR: thrombocytopaenia with absent radii; TCPT: Paris-Trousseau thrombocytopaenia; JBS: Jacobsen syndrome.

For illustrative purposes, we discuss here some of these complex genetic disorders that have been identified or fully characterised recently. Of note, some of them, such as *MYH9*-related disease (*MYH9*-RD) and *ANKRD26*-related thrombocytopaenia (*ANKRD26*-RT), have been found to be among the most frequent forms of ITs. We refer the readers to recent, comprehensive reviews for a description of the other disorders.[1]

ITS PREDISPOSING TO HEMATOLOGICAL MALIGNANCIES

Two forms of IT that are usually present with mild-to-moderate thrombocytopaenia and inconspicuous bleeding tendency threaten patient life because of their tendency to develop acute leukaemia or myelodysplastic syndromes (MDS): familial platelet disorder with predisposition to acute myelogenous leukaemia (FPD/AML) and *ANKRD26*-RT.

FPD/AML

FPD/AML is an autosomal dominant (AD) disorder that has been described in 30 families since the discovery of its aetiology in 1999.[2] It was previously considered very rare, but the observation that 10 of these 30 families have been identified in the last 2 years suggests that its prevalence is higher than previously thought. An important feature of FDP/AML is that >40% of patients developed hematological malignancies; MDS, AMLs, and, more rarely, T-acute lymphoblastic leukaemia have been reported with a median age of onset of 33 years.[3]

Megakaryocytes cultured from progenitor cells of patients had profound defects in maturation and polyploidisation, and reproduced the bone marrow picture observed in patients, which was characterised by dysmegakaryopoiesis with small elements, hypolobulated nuclei, and a little amount of mature cytoplasm. Based on these features and the isolated thrombocytopaenia of affected patients, as well as the propensity to develop hematological malignancies, FPD/AML could also be classified as an inherited form of 'refractory cytopaenia with unilineage dysplasia' (refractory thrombocytopaenia), as defined in the 2008 revision of the World Health Organization classification of myeloid neoplasms and acute leukaemias.[4]

FPD/AML is associated with alterations of *RUNX1*, a gene encoding the DNA-binding subunit of the core binding factor (CBF) transcription complex. Heterodimerisation to its partner protects *RUNX1* from degradation and enhances its affinity for DNA. CBF regulates expression of multiple hematopoiesis-specific genes and is essential for the establishment of definitive hematopoiesis.[5] Most *RUNX1* mutations result in haploinsufficiency, while a few missense mutations exert a dominant-negative effect and expose to a higher risk of hematological malignancies.[6] Interestingly, *RUNX1* mutations have been described also in *de novo* AML, chronic myelomonocytic leukaemia, and MDS.[7]

The mechanism by which *RUNX1* mutations predispose to hematological malignancies is unknown, although target molecular processes of *RUNX1* in normal and malignant hematopoiesis have been revealed (see below *ANKRD26*-RT). Anyway, inherited *RUNX1* mutations are insufficient by themselves to cause overt MDS/AML, as shown in FPD/AML by the incomplete penetrance for malignancy, as well as the additional karyotype abnormalities identified at the time of MDS/AML diagnosis.

ANKRD26-RT

Mutations in the 5'UTR of *ANKRD26* have been identified as responsible for an AD form of IT in 2011.[8] Since then, 153 patients have been described in the literature,[9] making *ANKRD26*-RT one of the most reported forms of IT. The degree of thrombocytopaenia is usually moderate, and the bleeding tendency is absent or mild. Bone marrow examination in a few patients who received this analysis showed evidence of dysmegakaryopoiesis, with small and dystrophic megakaryocytes.[10] As usual for mild forms of IT, *ANKRD26*-RT is often misdiagnosed with ITP, as shown by the observation that nearly 20% of patients described so far received steroids, intravenous immunoglobulin, cyclosporin, or even splenectomy.

Patients with *ANKRD26*-RT are at risk of developing hematological malignancies, which have been described in 8% of reported subjects and consisted of AML and MDS. This unfavourable outcome occurred in 12 of 44 families, indicating that the penetrance for malignancies was incomplete and other genetic and/or environmental factors contributed to their occurrence. *ANKRD26*-RT and FPD/AML are therefore similar in this respect, although the risk of transformation seems to be much higher for the latter condition. A possibility is that this discrepancy is caused by differences in the selection criteria used to identify subjects with mutations in *RUNX1* and *ANKRD26*. In

fact, FPD/AML has been so far suspected in pedigrees with thrombocytopaenia and hematological malignancies,[11] while the search for mutations in *ANKRD26* has been performed extensively in all families with an inherited form of thrombocytopaenia of unknown origin, regardless of the occurrence of other disorders. Thus, it is possible that the incidence of malignancies in FPD/AML has been overestimated because families without this complication have been excluded from the analysis. Using the same criterion for *ANKRD26*-RT, 29 of the 44 pedigrees reported so far would have been excluded from mutation screening, and the percentage of subjects with hematological malignancies would rise to 24.7%, a figure not very far from that observed in FPD/AML.

The similarity between *ANKRD26*-RT and FPD/AML is not limited to the mode of inheritance, the moderate severity of thrombocytopaenia, and the increased risk of neoplastic transformation, but includes other aspects as well. Platelet size is usually normal in both disorders, which is in sharp contrast to the majority of ITs that have abnormally large or small platelets.[12] Moreover, in both conditions bone marrow examination display dysplastic features independently of the occurrence of MDS or leukaemia. An appealing hypothesis to explain the similarities is that mutations in *RUNX1* or *ANKRD26* affect a common pathway whose derangement predisposes to thrombocytopaenia and hematological malignancies. Some evidences supporting this hypothesis have been recently presented, in that it has been shown that mutations in the 5'UTR of *ANKRD26*-RT patients result in loss of *RUNX1* binding, and this prevents the *ANKRD26* silencing that physiologically occurs during the late stages of megakaryopoiesis and makes possible proplatelet formation.[13] Thus, both *ANKRD26* and *RUNX1* mutations are expected to result in persistent *ANKRD26* expression and defective platelet biogenesis. An interesting hypothesis is that inappropriate expression of *ANKRD26* is involved also in propensity of patients with *ANKRD26*-RT and FDP/AML to develop hematological malignancies.

ITS PREDISPOSING TO BONE MARROW APLASIA

Important information gained by the study of ITs is that genetic changes causing thrombocytopaenia in newborns may result in bone marrow aplasia (BMA) later in life. Congenital amegakaryocytic thrombocytopaenia (CAMT) and amegakaryocytic thrombocytopaenia with radio-ulnar synostosis (ATRUS) are the two forms with this characteristic, but we will discuss briefly only CAMT, because ATRUS is exceedingly rare and poorly characterised.

CAMT

CAMT is an autosomal recessive thrombocytopaenia characterised by absent or much reduced bone marrow megakaryocytes and very low platelet counts. It evolves into BMA within the first years of life and results in death whenever patients do not receive hematopoietic stem cell transplantation. More than 50 families have been reported since the identification of the aetiology of this disorder in 1999.[14]

41 different mutations of MPL, the gene coding for the thrombopoietin (TPO) receptor (MPL), have been identified so far in homozygous or double heterozygous subjects.[15,16] Nonsense mutations and deletions introducing premature stop codons result in complete loss of function of the TPO receptor, while other mutations maintain some residual function of the receptor. Genotype/phenotype correlations have been identified: patients with complete loss of the MPL function have permanent thrombocytopaenia, while those with residual activity may present a transient improvement of thrombocytopaenia within the first year of life.[15] However, BMA develops before adulthood in all cases and the search for a bone marrow donor should be therefore started as soon as the diagnosis is made.

The observation that bone marrow megakaryocytes are absent or severely reduced in CAMT clearly indicates that TPO is essential for commitment-differentiation of hemopoietic stem cells to the megakaryocyte lineage, while the constant onset of BMA later in life indicates that TPO is required also for erythrocyte and white blood cell production. The asynchrony between the occurrence of thrombocytopaenia and that of anaemia and leukopaenia suggests that hematopoietic lineageshave different dependencies on MPL signalling although BMA and the loss of trilineage hematopoiesis in CAMT derive from stem cell failure.

ITS WITH EXTRA-HEMATOLOGICAL DEFECTS

Table 1 reports the essential features of ITs with extra-hematological defects. Although *MYH9*-RD

has been identified in this century, hundreds of cases have been described, and this disorder is presently considered one of the most frequent forms of IT.

MYH9-RD

MYH9-RD is an AD disorder presenting at birth with thrombocytopaenia, giant platelets, and characteristic inclusions bodies in the cytoplasm of neutrophils containing mutant protein. However, it may subsequently turn into a syndromic form because it exposes patients to the risk of developing the additional features of cataract, sensorineural deafness, alteration of liver enzymes, and/or a glomerulonephritis that usually evolves into end-stage renal failure. Moreover, the study of 75 patients recently revealed that an elevation of liver enzymes is another feature of the clinical spectrum of *MYH9*-RD that is observed in about 50% of cases. This alteration is benign, with no evolution towards clinically relevant liver damage or dysfunction.[16]

MYH9-RD results from mutations of *MYH9*, encoding for the heavy chain of non-muscle myosin IIA (myosin-9). More than 50 different mutations affecting either the head or tail of the molecule have been identified, and the study of 255 cases identified strong correlations between genotypes and phenotypes.[17] In general, mutations in the head result in lower platelet counts and higher risk of kidney failure, while mutations in the tail are responsible for a milder phenotype. Thus, identification of causative mutations defines patients' prognosis and makes genetic counselling more informative. Making a definite diagnosis also improves patient prognosis because effective treatments for *MYH9*-RD have been recently identified. Concerning kidney damage, pharmacological blockade of the renin-angiotensin system abolished or stably reduced proteinuria in a few reported patients who received this treatment,[18] suggesting that this approach could modify the natural history of the disease. Moreover, a small clinical trial revealed that the TPO mimetic eltrombopag was effective in increasing platelet count in patients with *MYH9*-RD.[19] Of note, this drug has been recently used instead of platelet transfusions to prepare patients for surgery.[20,21]

Pathogenetic studies in human cells suggested that different mechanisms translate defective myosin-9 into thrombocytopaenia: impaired migration of megakaryocytes from the stem cell niche to the vascular niche, malfunction of the mechanism which prevents the release of platelets before megakaryocytes reach the vascular niche, and defective formation of the long megakaryocyte extensions (proplatelets) that release platelets directly in the lumen of bone marrow vessels.[22,23] Less information is available on the pathogenesis of the other defects of *MYH9*-RD. However, animal studies have suggested that an impaired podocyte function deriving from defective myosin-9 is the critical determinant of kidney damage.[24]

FINAL CONSIDERATIONS

The forms of IT discussed here are clear examples of how these disorders can worsen the quality of life of patients or even cause their death by mechanisms other than bleeding. Fortunately, treatments for all these conditions are available and an early diagnosis is therefore mandatory because it makes possible follow-up regimens appropriate to the specific risks of each disorder and allow patients to receive prompt and effective treatments of complications. For ITs predisposing to hematological malignancies, a close follow-up should be planned for the study of the parameters of peripheral blood counts and differential blood count.

Making a 'generic' diagnosis of IT is therefore no longer adequate and acceptable because this leaves the patients' prognosis undefined. Therefore, we must do our best to identify the specific disease that affects each patient. The diagnostic algorithm for ITs proposed by the Italian Platelet Study Group several years ago,[25] and recently updated[12] can provide valuable support in this regard. Unfortunately, making a definitive diagnosis is not possible despite intensive and appropriate investigation in nearly half of patients because they do not fit the criteria for any known disorder. Identifying these 'new' forms of IT and reducing the number of subjects without a diagnosis at the end of the diagnostic workup is therefore a major challenge for the coming years.

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