Inherited thrombocytopenias
The evolving spectrum
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Summary
The chapter of inherited thrombocytopenias has expanded greatly over the last decade and many “new” forms deriving from mutations in “new” genes have been identified. Nevertheless, nearly half of patients remain without a definite diagnosis because their illnesses have not yet been described. The diagnostic approach to these diseases can still take advantage of the algorithm proposed by the Italian Platelet Study Group in 2003, although an update is required to include the recently described disorders. So far, transfusions of platelet concentrates have represented the main tool for preventing or treating bleedings, while haematopoietic stem cell transplantation has been reserved for patients with very severe forms. However, recent disclosure that an oral thrombopoietin mimetic is effective in increasing platelet count in patients with MYH9-related thrombocytopenia opened new therapeutic perspectives.

This review summarizes the general aspects of inherited thrombocytopenias and describes in more detail MYH9-related diseases (encompassing four thrombocytopenias previously recognized as separate diseases) and the recently described ANKRD26-related thrombocytopenia, which are among the most frequent forms of inherited thrombocytopenia.

Knowledge of inherited thrombocytopenias greatly improved during the preceding ten years, in that several new disorders have been discovered and some of them were unexpectedly found to be among the most frequent forms of genetic thrombocytopenia. This is the case of

- MYH9-related (MYH9-RD) (1) and
- ANKRD26-related thrombocytopenia (ANKRD26-RT) (2, 3).

Moreover, a better understanding of the pathogenetic mechanisms of many forms allowed us to realize that diseases previously considered separate entities derive from mutations of the same gene and are indeed different clinical expressions of a single disorder.

For instance, the identification of MYH9 as the gene whose mutations cause May-Hegglin anomaly (MIM 155100) (4) allowed us to recognize that mutations of the same gene describe the overlapping disorders

- Sebastian platelet syndrome (MIM 605249),
- Epstein syndrome (MIM 153650) and
- Fechtner syndrome (MIM 153640).

Similarly, Montreal platelet syndrome has been cancelled from the list of inherited thrombocytopenias because it has been shown that subjects with this diagnosis were actually affected by von Willebrand disease 2B (5). The same fate occurred to Mediterranean macrothrombocytopenia after demonstration that some patients carried the genetic defects of sitosterolemia (6) or had a mild form of Bernard-Soulier syndrome (BSS) (7).

Notwithstanding considerable progress, nearly half of patients with inherited thrombocytopenias remain without a definite diagnosis because their illnesses have not yet been described (8).
In this review the general aspects of inherited thrombocytopenias will be discussed briefly. MYH9-RD and ANKRD26-RT, which have been identified recently and probably represent the more frequent forms of genetic thrombocytopenia, will be described and discussed in more detail.

**Inherited thrombocytopenias**

**Classification**

Different parameters have been used to classify inherited thrombocytopenias, as the
- inheritance pattern or the
- presence of symptoms other than thrombocytopenia.

However, the inheritance pattern is not always easy to identify because sporadic cases deriving from de novo mutations occur frequently (MYH9-RD). Moreover, transmission of some disorders, as BSS, may be both dominant and recessive. Likewise, a classification based on clinical symptoms is not always reliable, as syndromic and non-syndromic forms may result from mutations of the same gene, as in MYH9-RD. Moreover, patients with isolated thrombocytopenia at birth may develop the typical picture of a syndromic form in adult life (MYH9-RD).

Among other types of classification, one of the most satisfactory is based on platelet size. It is very effective for diagnostic purposes, in that platelet size is easy to determine by microscopic observation of peripheral blood smears and represents a constant feature of each illness (9). Table 1 describes the essential features of inherited thrombocytopenias classified according to this parameter. It also reports some references to relevant papers that describe these disorders.

**Clinical picture**

The bleeding diathesis of inherited thrombocytopenias is not different from that of acquired forms and consists of
- nose bleeds,
- menorrhagia, and
- gastrointestinal bleeding.

Few patients with platelet counts lower than 20–30 × 10^9/l suffer from spontaneous haemorrhages since birth, but in most cases the degree of thrombocytopenia is mild and bleeding is occasional and/or related to trauma, surgery or invasive procedures.

However, the bleeding tendency is sometimes disproportionate to the degree of thrombocytopenia because of associated defects of platelet function.

This is the case of patients with biallelic BSS, who may suffer from severe bleeding episodes also when thrombocytopenia is relatively mild (10). Some inherited thrombocytopenias differ from acquired forms also for the presence of additional defects to low platelet count (syndromic forms). Table 1 reports syndromic forms and their clinical picture.

**Epidemiology**

Inherited thrombocytopenias are considered exceedingly rare, but their prevalence is unknown because no population-based study has been performed. The relative frequency of different forms in our database, which currently includes nearly 500 patients from 160 families, is reported in Figure 1.

In our experience, the most frequent disorder is a mild, dominant form of BSS due to monoallelic Bolzano mutation in GPIb alpha (11).

However, this is an Italian disease, since analysis of the geographic origin of affected pedigrees and haplotypes indicated that this mutation originated in southern Italy (11). Moreover, no case has been described so far in other countries.

The second, most frequent form is MYH9-RD, which has been diagnosed in 12 % of our patients. According to our database, the prevalence of ANKRD26-RT is a little lower than that of MYH9-RD.

The Italian Registry for MYH9-RD (www.registromyh9-rd.org) now includes 200 affected individuals, indicating that the prevalence of the disorder in Italy is at least 3.2 in 100,000. Based on this figure and the relative frequency of MYH9-RD in our case series, we calculate that the prevalence of inherited thrombocytopenias in Italy is at least 2.7 in 100,000. The actual prevalence of these disorders is expected to be higher because
- mild forms are discovered incidentally and
- severe forms are often misdiagnosed with other disorders.

**Pathogenesis**

Megakaryocytes (Mks) are formed from haematopoietic stem cells in the bone-proximal proliferative osteoblastic niche. During maturation, they migrate from the osteoblastic to the vascular niche, which is enriched in vascular sinusoids. Here, mature Mks bind to vessels and extend proplatelets, which protrude into the vascular lumen and release nascent platelets directly into the bloodstream (12).

Although the genes whose mutations result in thrombocytopenia have been identified for all known disorders (Tab. 1), the exact molecular mechanisms that translate mutations into low platelet counts are still a matter of debate. Figure 2 describes the current model of megakaryocytopoiesis and the steps that have been hypothesized to be affected by some genetic defects. Table 2 makes an attempt to classify inherited thrombocytopenias according to their pathogenesis. In most cases, defects in the complex mechanisms that regulate Mk maturation (for instance, in thrombocytopenias deriving from GATA1 mutations) (13, 14) and proplatelet formation (BSS and MYH9-RD) (15–17) are responsible for reduced platelet production.

More rarely, a defect of Mk commitment and/or differentiation reduces the number of bone marrow Mks. These forms are often severe and put the patients at risk of developing bone marrow aplasia (congenital amegakaryocytic thrombocytopenia, CAMT, and congenital amegakaryocytic thrombocytopenia with radio-ulnar...
<table>
<thead>
<tr>
<th>platelets' size</th>
<th>disease (abbreviation, MIM #)</th>
<th>reference</th>
<th>inheritance</th>
<th>gene (localization)</th>
<th>other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>large</td>
<td>biallelic (BSS, 231200) and monoallelic Bernard-Soulier syndrome (nd, nd)</td>
<td>(10) (11)</td>
<td>AR-AD</td>
<td>GP1BA (17p13), GPIB8 (22q11), GP9 (3q21)</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>MYH9-related disease (MYH9-RD, nd)</td>
<td>(1)</td>
<td>AD</td>
<td>MYH9 (22q12-13)</td>
<td>leukemia inclusions, cataracts, nephropathy and/or deafness</td>
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<tr>
<td></td>
<td>dyserythropoietic anaemia with thrombocytopenia (nd, 300367)</td>
<td>(13)</td>
<td>X-L</td>
<td>GATA1 (Xp11)</td>
<td>haemolytic anaemia</td>
</tr>
<tr>
<td></td>
<td>X-linked thrombocytopenia with thalassemia (XLTT, 314050)</td>
<td>(14)</td>
<td>AD</td>
<td>large deletion (11q23)</td>
<td>cardiac and facial defects, developmental delay ± other defects</td>
</tr>
<tr>
<td></td>
<td>Paris-Trousseau thrombocytopenia (TCPT, 188025/600588), Jacobsen syndrome (JSB, 147791)</td>
<td>(64)</td>
<td>AD</td>
<td>large deletion (11q23)</td>
<td>cardiac and facial defects, developmental delay ± other defects</td>
</tr>
<tr>
<td></td>
<td>gray platelet syndrome (GPS, 139090)</td>
<td>(65-68)</td>
<td>AR</td>
<td>NBEAL2 (3p21.1)</td>
<td>&quot;pale&quot; platelets, myelofibrosis, splenomegaly, high serum vitamin B12</td>
</tr>
<tr>
<td></td>
<td>sitosterolaemia (STSIL, 21025)</td>
<td>(6)</td>
<td>AR</td>
<td>ABCG5, ABCG8 (2p21)</td>
<td>stomatocytosis, possible anaemia, tendon xanthomas, atherosclerosis</td>
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<td></td>
<td>platelet-type von Willebrand disease (PT-VWD, 177820)</td>
<td>(69)</td>
<td>AD</td>
<td>GP1BA (17pter-p12)</td>
<td>The platelet count goes down under stress.</td>
</tr>
<tr>
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<td>ITGA2/ITGB3-related thrombocytopenia (nd, nd)</td>
<td>(70)</td>
<td>AD</td>
<td>ITGB3 (17q21.32)</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>TUBB1-related macrothrombocytopenia (nd, nd)</td>
<td>(71)</td>
<td>AD</td>
<td>TUBB1 (6p21.3)</td>
<td>none</td>
</tr>
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<td>FLNA-related macrothrombocytopenia (nd, nd)</td>
<td>(72)</td>
<td>X-L</td>
<td>FLNA (Xq28)</td>
<td>possible periventricular nodular heterotopia, bone dysplasia, mental retardation, and other malformations</td>
</tr>
<tr>
<td>normal</td>
<td>ANKRD26-related thrombocytopenia (THC2, 313900)</td>
<td>(2, 3)</td>
<td>AD</td>
<td>ANKRD26 (10p2)</td>
<td>risk of leukaemia?</td>
</tr>
<tr>
<td></td>
<td>congenital amegakaryocytic thrombocytopenia (CAMT, 604498)</td>
<td>(18)</td>
<td>AR</td>
<td>c-MPL (1p34)</td>
<td>reduced megakaryocytes, evolution into bone marrow aplasia</td>
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<td>congenital amegakaryocytic thrombocytopenia with radio-ulnar synostosis (CTRUS, 605432)</td>
<td>(19)</td>
<td>AD</td>
<td>HOXA11 (7p15-14)</td>
<td>reduced megakaryocytes, possible evolution into aplastic anaemia, radio-ulnar synostosis ± other defects</td>
</tr>
<tr>
<td></td>
<td>familial platelet disorder and predisposition to acute myelogenous leukaemia (FPD/AML, 601399)</td>
<td>(20)</td>
<td>AD</td>
<td>CBFA2 (21q22)</td>
<td>development of leukaemia or MDS in 40% of patients</td>
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<td></td>
<td>thrombocytopenia with absent radii (TAR, 274000)</td>
<td>(73)</td>
<td>AD</td>
<td>RBM8A (1q21.1)</td>
<td>Platelet count tends to rise and often normalizes in adult life, reduced megakaryocytes, bilateral radial aplasia ± other malformations</td>
</tr>
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<td>CYCS-related thrombocytopenia (THC4, 612004)</td>
<td>(74)</td>
<td>AD</td>
<td>CYCS (7p15.3)</td>
<td>none</td>
</tr>
<tr>
<td>small</td>
<td>Wiskott-Aldrich syndrome (WAS, 301000)</td>
<td>(75)</td>
<td>X-L</td>
<td>WAS (Xp11)</td>
<td>severe immunodeficiency</td>
</tr>
<tr>
<td></td>
<td>X-linked thrombocytopenia (XLIT, 313900)</td>
<td></td>
<td></td>
<td></td>
<td>no or mild immunodeficiency</td>
</tr>
</tbody>
</table>

AD: autosomal dominant; AR: autosomal recessive; X-L: X-linked
synostosis, CTRUS) or leukaemia (familial platelet disorder and predisposition to acute myelogenous leukemia, FDP/AML) (18–20). In a few cases, reduced platelet survival has been demonstrated or hypothesized.

Diagnosis

Differentiation between inherited and acquired thrombocytopenias

The recognition of the genetic origin of a thrombocytopenia is often hampered by several difficulties. Especially in the mild forms, thrombocytopenia is frequently identified incidentally late in infancy or in adult life, and, therefore, suspecting a congenital form is not obvious, particularly when patient have de novo mutations and no other family members are affected.

Moreover, in inherited macrothrombocytopenias both platelet count and volume are regularly underestimated by the automated cell counters that measure platelet parameters by evaluating particles within a specified volume window (e.g., 2–20 fl).

As a consequence, subjects with very large platelets (as in BSS and MYH9-RD) are at risk of misdiagnosis with severe immune thrombocytopenia and of undue medical therapy or splenectomy, as has been reported to have occurred in several cases (21).

To avoid this error, careful investigation of each patient with isolated thrombocytopenia is essential, especially in those cases with no record of previously normal platelet counts.

Morphologic examination of peripheral blood smears is the most informative single examination because it can reveal qualitative platelet abnormalities, such as platelet macro- or microcytosis, that strongly support an inherited disorder. Cut off values of platelet size for distinguishing between inherited macrothrombocytopenias and autoimmune forms have been recently proposed (22). Also medical history and physical examination have an important role in the diagnostic process since...

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they can allow to identify one of the many defects that are associated with thrombocytopenia in inherited syndromic forms.

**Identification of specific forms of inherited thrombocytopenia**

Although requiring an update to include some new forms, the algorithm proposed in 2003 by the Italian Platelet Study Group (9) and modified in 2004 by Noris et al. (8) may be useful for diagnostic purposes. In order to put forward diagnostic hypotheses it is based on:

- a first phase of clinical investigations (differentiation between syndromic and non-syndromic forms) and
- simple laboratory tests
  - evaluation of platelet size,
  - in vitro platelet aggregation and
  - flow cytometry for platelet glycoproteins.

Then, more complex studies are required to confirm the diagnostic suspicion. Since the genes responsible for nearly all known inherited forms of thrombocytopenia have been identified, mutation screening can further confirm diagnosis and, in some cases, it can also define prognosis (MYH9-RD).

Unfortunately, not all inherited forms of thrombocytopenia have been identified and about 50% of patients with (usually mild) genetic thrombocytopenias remain without a definite diagnosis because they do not fit the criteria of any known disorder.

**Therapy**

**Prevent bleeding**

The most important aspect of management of inherited thrombocytopenias is to anticipate risks and to prevent bleeding. A major measure is to instruct patients to avoid drugs that impair platelet function (above all, acetylsalicylic acid, ASA). Regular dental care is essential to prevent gingival bleeding. Hormonal contraceptives should be used to prevent menorrhagia.

**Increase platelet count**

Transfusions of platelet concentrates may be used to transiently increase platelet count. However, they expose to the risks of

- alloimmunization,
- subsequent refractoriness to platelet infusions,
- infectious diseases, and
- febrile reactions.

Thus, platelet transfusions should only be used in case of haemorrhages that cannot be otherwise managed and as prophylaxis prior to surgery or other major haemostatic stresses. When available, platelets from HLA-matched donors should be used to prevent or overcome alloimmunization. However, recent demonstration that an oral TPO mimetic increased platelet count in patients with MYH9-RD (MYH9-RD) opened new therapeutic perspectives also for other forms of inherited thrombocytopenia (23).

**Stem cell transplantation**

Allogeneic haematopoietic stem cell transplantation is an appealing therapy for restoring normal megakaryocytopoiesis. However, in most cases the risk of this procedure is higher than that induced by the bleeding tendency, and transplantation has been used so far only to cure patients with very severe forms (CAMT, BSS, and Wiskott-Aldrich syndrome, WAS) (24, 25).

**MYH9-related disease**

MYH9-RD is the autosomal dominant syndromic thrombocytopenia deriving from mutations in the gene for the heavy chain of the non-muscle myosin II A (NMHC-IIA) (4, 26, 27). The term MYH9-RD encompasses four thrombocytopenias that were previously described as distinct diseases, namely

- May-Hegglin anomaly (MHA),
- Sebastian platelet syndrome (SBS),
- Fechtner syndrome (FTNS) and
- Epstein syndrome (EPTS).

The identification of MYH9 as the gene responsible for all these conditions and the subsequent analysis of large case series of patients demonstrated that MHA, SBS, FTNS and EPTS represent different clinical presentations of the same disease. It is characterized (28, 29) by

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**Tab. 2 Pathogenetic mechanisms of inherited thrombocytopenias**

<table>
<thead>
<tr>
<th>defect</th>
<th>syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>megakaryocytic commitment and differentiation</td>
<td>congenital amegakaryocytic thrombocytopenia, amegakaryocytic thrombocytopenia with radio-ulnar synostosis, thrombocytopenia with absent radii, familial platelet disorder and predisposition to acute myelogenous leukaemia</td>
</tr>
<tr>
<td>megakaryocyte maturation and/or proplatelet formation</td>
<td>dyserthropoietic anaemia with thrombocytopenia, X-linked thrombocytopenia with thalassemia, biallelic and monoallelic Bernard-Soulier syndrome, MYH9-related disease, Paris-Trousseau thrombocytopenia and Jacobsen syndrome, CYCS-related thrombocytopenia, ANKRD26-related thrombocytopenia, ITGA2/ITGB3-related thrombocytopenia, Wiskott-Aldrich syndrome and X-linked thrombocytopenia, gray platelet syndrome</td>
</tr>
<tr>
<td>shortened platelet survival</td>
<td>platelet-type von Willebrand disease, sitosterolaemia, ITGA2/ITGB3-related thrombocytopenia, Wiskott-Aldrich syndrome and X-linked thrombocytopenia, gray platelet syndrome</td>
</tr>
</tbody>
</table>

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The non-muscle myosin IIA participates in functions requiring the production of intracellular chemomechanical force by the cytoskeleton and the sliding along actin filaments, including cytokinesis, cell motility and polarization, cell-cell and cell-matrix interaction, and maintenance of cell shape (30).

To date, at least 44 different MYH9 mutations have been reported in 218 MYH9-RD unrelated families (1). The spectrum of mutations is limited, suggesting that only specific alterations of the NMMHC-IIA molecule are compatible with the MYH9-RD phenotype. Most mutations lead to single aminoacid substitutions hitting only 19 of the 1960 residues of the protein. All the nonsense and frame-shift alterations affect the 34 residues of the C-terminal non-helical tailpiece. In rare cases, the disease derived from in frame deletions or duplications of a few nucleotides, occurring prevalently in the exon 24. A relevant aspect is the high frequency of sporadic cases (about 35%), most of them carrying mutations in the head domain (1).

**Pathogenesis**

NMMHC-IIA mutations cause thrombocytopenia by altering the late processes of platelet release by mature Mks, while differentiation and maturation of Mks are unaffected (33). Recent studies showed that myosin IIA is a negative regulator of platelet production through the inhibition of proplatelet extension by Mks (34, 35). In particular, a functional NMMHC-IIA is essential for the suppression of proplatelet formation initiated by Mks interaction with type I collagen through the α2β1 integrin. This binding activates the small GTPase Rho and the Rho-kinase ROCK, which in turn phosphorylates the regulatory light chains of NMMHC-IIA (34–37). Being an abundant extracellular protein of the osteoblastic niche of bone marrow, type I collagen prevents Mks from a premature release of platelets in this space (38). Consistent with this model, Mks cultured from blood progenitors of patients with two different MYH9 mutations extended proplatelets even in adhesion to type I collagen, suggesting that MYH9-RD thrombocytopenia derives from an ectopic platelet release that results in ineffective platelet production (17).

Moreover, proplatelets formed by MYH9-RD Mks showed evident morphological abnormalities, such as a reduced number and an increased size of proplatelet tips (17). Myh9 knock-out mice show early embryonic death, mainly due to defects in cell-cell adhesion and consequent failure to form a visceral endoderm (39). Recently, a knock-in mouse model of MYH9-RD recapitulating the phenotype of the human disorder has been developed (40). Proplatelets extended by Mks of these mice presented morphological alterations that were not seen in wild-type Mks.
remarkably similar to those observed in patients’ MKs (23, 40). However, these MKs had a reduced propensity to form proplatelets, in contrast to what would be expected from the previous achievements on the role of NMMHC-IIA in platelet production (40).

Another recent in vitro study demonstrated that NMMHC-IIA is essential for chemotaxis of a megakaryocytic cell line in response to stromal derived factor-1 (SDF-1), and that three mutations responsible for MYH9-RD result in a reduced efficiency of this process (41).

These observations suggest that an impaired SDF-1-driven migration of MKs from the osteoblastic niche to the perivascular space contributes to thrombocytopenia of MYH9-RD.

Clinical features

Congenital haematological defects

Macrothrombocytopenia and bleeding diathesis

Platelet macrocytosis is present in all MYH9-RD patients and is more marked than in immune thrombocytopenia and other forms of inherited macrothrombocytopenia (Fig. 4) (22). Giant platelets (larger than erythrocytes) are always present at examination of peripheral blood smears (42). Platelet count varies greatly from patient to patient, and usually remains stable in each individual throughout life. Because of the extreme degree of platelet macrocytosis, automated cell counters usually underestimate platelet count and mean platelet volume (22). In a case series of 108 consecutive patients, platelet count ranged

- from 3 to 178 × 10^9/l (median: 68 × 10^9/l) by microscopic counting, and
- from 3 to 130 (median: 31 × 10^9/l) by automated counters.

By microscopy evaluation, the proportion of patients with less than 50 × 10^9/l platelets was 30 % (43). Bleeding tendency is globally proportionate to platelet count and therefore varies widely among MYH9-RD patients.

Spontaneous bleeding, sometimes requiring transfusions, is observed in subjects with platelet counts less than 50 × 10^9/l (23).

Patients with higher platelet concentrations are usually asymptomatic and thrombocytopenia is often recognized only on the occasion of a blood cell count performed for unrelated clinical problems or for an anomalous bleeding after minor surgery, such as dental procedures.

Döhle-like leukocyte inclusions

Döhle-like leukocyte inclusions are detected at evaluation of peripheral blood smears upon conventional staining in 42–84 % of MYH9-RD patients (1). They appear as faint, light blue areas in the cytoplasm of neutrophils with diameter ranging from 1 to 7 mm and different shape (round, oval, spindle-shaped) (Fig. 5). Their recognition is pathognomonic for the disease but it is often difficult because of their faint appearance.

Extra-haematological manifestations

- Kidney damage occurs in about 30 % of patients and in 37–48 % of pedigrees. It is often present in only some of the affected family members (43–45). Proteinuria, with or without microscopic haematuria, usually appears before the age of 30. About two-third of patients develop renal failure and then end-stage renal disease within a few years. In the remaining cases, progression of the kidney impairment is slower with proteinuria and mild alteration of renal function stable for years (45–48).
- Sensorineural hearing loss is the most frequent extra-haematological manifestation, being reported in 60 % of patients and in 36–71 % of pedigrees (43, 44). At the onset or in mild forms, the defect is evident only for high tones, but in severe cases it extends toward the low frequencies. The age at onset is homogeneously distributed along the decades from first to sixth. Forms with onset in the childhood have severe progression and lead to deafness by the age of 30 years (47).
- Presenile cataract occurs in 16 % of patients and 14–17 % of families. The mean age at onset is 23 years, but congenital forms have been reported (49).

Diagnosis

The diagnostic suspicion of MYH9-RD is based on the recognition that

- thrombocytopenia is congenital and
- characterized by giant platelets.
Difficulties in identifying these features have been already discussed in the “general aspects” section. The most challenging points in MYH9-RD are the high frequency of sporadic cases, who therefore present with a negative familiar history, and the fact that automated cell counters underestimate platelet macrocytosis in almost all patients.

Once suspected, diagnosis can be easily confirmed by the immunofluorescence test for NMMHC-IIA distribution within neutrophils. This assay allows to identify typical aggregates of the MYH9 protein in the cytoplasm of neutrophils, which is a specific feature of MYH9-RD (27, 50). NMMHC-IIA aggregates are easily recognizable in all the neutrophils (28), with the only exception of the rare patients with mosaicisms, who have also neutrophils without aggregates (51).

In a prospective analysis of 118 consecutive unrelated subjects with suspected MYH9-RD, the assay demonstrated 100% sensitivity and 95% specificity with respect to the presence of MYH9 mutations (42) This assay can be easily centralized, as it can be effectively carried out on shipped blood films (www.registromyh9.org). Even if the immunofluorescence test has very high diagnostic power, identification of the causative mutations has great value in terms of prognostic assessment.

Genotype-phenotype correlations

In 2005, a review of 85 previously published MYH9-RD pedigrees suggested that mutations involving the Arg702, in the motor domain of NMMHC-IIA, were associated with a syndromic disease. In contrast, alterations in the C-terminal portion of the tail domain of the molecule were more frequently responsible for isolated thrombocytopenia (44).

In 2008, detailed characterization of 108 consecutive patients from 50 families confirmed and extended these findings, demonstrating that mutations affecting the head domain were associated with a significantly higher incidence of kidney damage and deafness and more severe thrombocytopenia with respect to mutations involving the tail domain. In particular, all patients with aminoacid substitutions in the head domain of NMMHC-IIA were expected to develop nephropathy and deafness before the age of 40.

Among the most frequent mutations of the tail domain, the p.R1933X alteration correlated with a negligible risk of extra-haematological complications, while the substitutions of the Asp1424 or Glu1841 were associated with an intermediate risk (43). Interestingly, pedigrees with these latter mutations were those characterized by a variability of phenotypic expression within affected family members.

The smaller case series published later on were consistent with this findings and, in particular, pointed out the rapid deterioration of kidney and hearing function correlated with the Arg702 mutations (47, 48, 52).

Therapy

The recent demonstration that eltrombopag, an orally available TPO receptor agonist, is effective in patients with MYH9-RD opened new prospects in the treatment of this disorder (23). In twelve patients with platelet counts below 50×10⁹/L, thrombocytopenia significantly improved at the end of 3–6 weeks treatment, and seven patients achieved a platelet concentration over 100×10⁹/L. Importantly, remission of bleeding tendency was obtained also in patients with minor improvements in platelet counts.

Since no important side effects have been recorded, short-term eltrombopag is a possible alternative to platelet transfusion in preparation of MYH9-RD patients to elective surgery.

ANKRD26-related thrombocytopenia

Thrombocytopenia 2 (THC2) is an autosomal dominant form of thrombocytopenia originally described in two large families, one in Italy (55), one in the US (56). The corresponding molecular defects should be carried out when indicated.
Etiology

The hypothesis that mutations in genes other than MASTL and ACBD5 may be responsible for this disorder derived from the observation that neither MASTL nor ACBD5 were mutated in four additional Italian pedigrees with an autosomal dominant thrombocytopenia mapping to the THC2 locus. Then, mutational screening of all genes, other than MASTL and ACBD5, located in this region identified nucleotide changes within the 5′-untranslated region (5′UTR) of ankyrin repeat domain 26 (ANKRD26) in three of the investigated families (3).

Subsequently, analysis of a large database recognized 12 different mutations in 5′UTR of ANKRD26 in 21 of 210 pedigrees (2), making this disorder one of the rare forms of inherited thrombocytopenia (► Fig. 1). Interestingly, ANKRD26 was mutated also in the Italian family carrying the ACBD5 change (58), which, therefore, was probably a private polymorphism rather than the causative mutation. No information is presently available on the ANKRD26 state in the American family with MASTL changes. Thus, we propose the name of ANKRD26-related thrombocytopenia (ANKRD26-RT) to identify THC2 patients with mutations in the 5′UTR of ANKRD26.

Pathogenesis

ANKRD26 is the ancestral gene for a primate-specific family of genes named POT (prostate-, ovary-, testis-, and placenta-expressed genes), which are expressed in a few normal tissues and in a large number of cancer cells (59). The functional role of ANKRD26 is still poorly known. Mice with partial inactivation of Ankrd26 have marked hypergastrinaemia, which results in extreme obesity, and an increase of body length due to a direct effect of ANKRD26 in the regulation of the food intake. They also develop an obesity-dependent diabetes in white adipose tissue and brown adipose tissue, but they do not develop an obesity-dependent diabetes in white adipose tissue and brown adipose tissue, but probably due to an increase in blood TPO levels.

Since no peculiar clinical or laboratory abnormalities have been identified so far, the diagnosis of ANKRD26-RT requires mutation screening.

Clinical and laboratory features

A thorough analysis of 78 patients from 21 families with ANKRD26-RT has been published recently (2). Bleeding diathesis was usually absent or mild; when present, patients mostly referred epistaxis, gum bleedings, petechiae, ecchymosis and menorrhagia, while only in single cases life-threatening bleedings were reported. Only a few bleeding complications developed after surgical procedures or in women who gave birth vaginally or by caesarian section.

In some patients previously diagnosed as suffering from immune thrombocytopenia (ITP), neither steroids nor splenectomy ensured a sustained increase in platelet count. However, at least four patients had an increase in their platelet count over 100 × 10⁹/l during infectious episodes, probably due to an increase in blood TPO levels.

The most important clinical remark of ANKRD26-RT patients is the association with haematological malignancies, in particular myeloid acute leukaemias (AML):

This observation raised the suspicion that ANKRD26 mutations favour the appearance of haematopoietic neoplasms.

This hypothesis is consistent with the report that ANKRD26 was mutated in the first AML patient whose entire genome has been sequenced (63), although it needs a confirmation in larger case series of ANKRD26-RT patients.

Thrombocytopenia was moderate in the majority of patients, although in few cases the platelet count was even lower than 10 × 10⁹/l. So far, only one subject carrying an ANKRD26 mutation had a platelet count at the lower limit of the normal range, suggesting an almost complete penetrance of the trait. Platelet volumes and in vitro aggregation are usually normal. In some patients platelets present reduced α-granule content and defective surface expression of GPIIb. However, these two defects are inconstant even in single pedigrees, and, therefore, they are not valuable tools for a diagnostic suggestion. Serum TPO is about sevenfold increased compared to normal subjects and up to three times compared to ITP patients with a superimposable platelet count.

In the majority of patients both haemoglobin level and leukocyte count are within the reference range. However, besides a few patients with a mild anaemia consistent with a haemorrhagic disorder, increased levels of haemoglobin and leucocytes were observed in a significant number of patients. To note, the last finding was reported also in THC2 patients carrying the MASTL mutation (56).

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Conflict of interest

The authors declare that there is no conflict of interest.

Since no peculiar clinical or laboratory abnormalities have been identified so far, the diagnosis of ANKRD26-RT requires mutation screening.
References


