Published in final edited form as:

Curr Hematol Malig Rep. 2014 December ; 9(4): 311–318. doi:10.1007/s11899-014-0232-3.

Back to Biology: New Insights on Inheritance in Myeloproliferative Disorders

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Abstract

The myeloproliferative disorders (MPDs) are a group of hematologic diseases with significant overlap in both clinical phenotype and genetic etiology. While most often caused by acquired somatic mutations in hematopoietic stem cells, the presence of familial clustering in MPD cases suggests that inheritance is an important factor in the etiology of this disease. Though far less common than sporadic disease, inherited MPDs can be clinically indistinguishable from sporadic disease. Recently, germline mutations in Janus kinase 2 (*JAK2*) and *MPL*, two genes frequently mutated in sporadic MPD, have been shown to cause inherited thrombocytosis. Study of the function of these mutant proteins has led to a new understanding of the biological mechanisms that produce myeloproliferative disease. In this review, we summarize the data regarding inherited mutations that cause or predispose to MPDs, with a focus on the biological effects of mutant proteins. We propose that defining inherited MPDs in this manner has the potential to simplify diagnosis in a group of disorders that can be difficult to differentiate clinically.

Keywords

Familial myeloproliferative disorder; Hereditary thrombocytosis; Hereditary erythrocytosis

Introduction

The development of a hematologic cancer is dependent on environmental factors, aging, and host genetics. Inheritance modifies risk at every step of the cancer process, from acquisition of cancer to its progression. The Philadelphia chromosome negative myeloproliferative disorders (MPDs) are hematologic malignancies characterized by clonal autonomous expansion of at least one myeloid lineage, development of myelofibrosis, and a risk of transformation

to acute myeloid leukemia. Essential thrombocythemia (ET) and polycythemia vera (PV) are characterized by hyperproliferation of platelets and red blood cells, respectively, while primary myelofibrosis (PMF) is characterized by bone marrow fibrosis, extramedullary hematopoiesis, and hematopoietic progenitor expansion. Like other cancer syndromes, host genetics play a large role in both the development and course of MPD.

The majority of MPD cases are acquired; however, inherited cases are being reported with increasing frequency. Inherited MPDs have traditionally been defined by their clinical phenotype, resulting in two broad categories of disease [1]. The first category, termed “true” familial MPD, describes a malignant disorder that mimics sporadic MPD in its risk of progression to acute myeloid leukemia and occurs in two or more family members. The term familial is used to describe an unknown germline lesion that predisposes the acquisition of an MPD (Fig. 1). This disease is characterized by clonal hematopoiesis, incomplete penetrance, and, often, multilineage proliferation. The second category encompasses the MPD-like disorders hereditary thrombocytosis and erythrocytosis, which are considered non-malignant diseases characterized by proliferation of only a single hematopoietic cell lineage, polyclonal hematopoiesis, and complete disease penetrance. In this context, the term hereditary is used to define a known germline lesion that causes an MPD phenotype and carries a Mendelian pattern of inheritance (Fig. 1). In recent years, mostly due to advances in next-generation sequencing techniques, our understanding of the genetic etiology of hereditary, familial, and sporadic MPDs has greatly expanded. With this knowledge, however, has come a blurring of the division between these diseases. To avoid confusion, we will use the term MPD to refer to all myeloproliferative disease in which the phenotype is cell autonomous. This review will summarize the recent developments in the genetics of the MPDs, and re-evaluate the distinction between hereditary, familial, and sporadic disease.

Genetics of the MPDs

The 2005 discovery of the Janus kinase 2 (JAK2) V617F mutation not only defined the most common acquired genetic lesion specific to the MPDs but it also paved the way for the identification of other MPD-specific lesions in the JAK signaling pathways. The subsequent identification of acquired somatic lesions in myeloproliferative leukemia virus oncogene (*MPL*), lymphocyte-specific adapter protein (*LNK*), *JAK2* exon 12, colony-stimulating factor 3 receptor (*CSF3R*), and calreticulin (*CALR*) in MPD patients reinforced the central role of cytokine receptor/signal transduction lesions in promoting MPD phenotypes. In surveys of large MPD cohorts, *JAK2*, *CALR*, and *MPL* mutations comprise the vast majority of phenotype driver lesions specifically associated with sporadic ET, PV, and PMF (Table 1) [2•, 3•]. The genomes of sporadic MPD are also characterized uniquely by the development of acquired uniparental disomy (aUPD), which, when encompassing a signal transduction mutation, generates homozygosity of the driver lesion. aUPD most commonly involves chromosome 9p generating homozygosity for *JAK2* V617F, but it has also been observed less frequently across the genome and has been reported to involve many other myeloid cancer alleles, including *MPL* and *JAK2* exon 12 [4, 5]. aUPD introduces gene dosage as another factor which modifies phenotype in sporadic MPD and effectively delivers a double dose of activating lesions in stem cell sub-clones.

Familial MPD

Familial MPD is defined as the presence of two or more individuals who acquire an MPD in the same family. Based on a large Swedish study, the risk of developing an MPD in first-degree relatives of affected patients is five- to sevenfold higher than that in the general population [6]. Further, two recent studies following a large population of MPD patients in Italy found the prevalence of inherited disease to be 7–11 % [7, 8]. Multiple cohort studies have found that families with MPD display an inheritance pattern most consistent with autosomal dominance with incomplete penetrance [7, 9, 10]. Clinically, familial MPD is indistinguishable from sporadic MPD, with identical risk for disease complications and progression to acute leukemia [10]. This is likely due to the fact that the pathologic mutations that drive the disease phenotype in familial MPD are acquired and are identical to the mutations found in sporadic disease (Table 1). Accordingly, the JAK2 V617F mutation is the most frequent pathologic abnormality seen in familial MPD; however, mutations in *JAK2* exon 12, *TET2*, and, most recently, *CALR* have also been observed [8, 11–14]. This implies that the somatic mutations seen in familial MPD are responsible for the proliferative advantage and subsequent clonality observed in this disease, while the inherited component simply predisposes to the acquisition of somatic mutations. This is supported by the presence of disparate disease phenotypes and acquired mutations within the same family. For example, the development of PV or PMF in a first-degree relative of an individual with ET has been documented in multiple studies [7, 15]. Similarly, one affected individual in a family may be positive for the JAK2 V617F mutation while another affected relative may be JAK2 V617F negative or has a JAK2 exon 12 mutation [11, 16].

While the constitutional genetic variation(s) predisposing to familial MPD have yet to be ascertained, there is a wellknown association between development of disease and a particular *JAK2* allele. Several studies have shown that the JAK2 V617F mutation occurs more frequently on a specific *JAK2* gene haplotype, referred to as the GGCC or 46/1 haplotype [17–19]. However, this haplotype is seen with high frequency in European populations, most of whom do not develop disease. Thus, the JAK2 46/1 haplotype has a very low penetrance and cannot be used to predict disease development. Furthermore, a direct comparison of familial and sporadic MPD revealed no difference in the presence of this allele, indicating that other inherited factors likely contribute to familial MPD [8].

Hereditary MPD

Hereditary erythrocytosis and thrombocytosis are extremely rare disorders with only a small number of families reported in the literature (see [20, 21] for review). Genetic transmission of both disorders is autosomal dominant with complete penetrance, and the clinical phenotype of erythrocytosis or thrombocytosis is usually discovered early in life. These disorders are primary, or cell autonomous, meaning that the inherited mutation leads to abnormalities in the cells that produce the clinical phenotype. Secondary erythrocytosis caused by defects in oxygen sensing (due to mutations in the *VHL* or *HIF1a* genes) or altered hemoglobin affinity is a distinct disorder and will not be discussed here.

Hereditary Erythrocytosis

Hereditary erythrocytosis (also called primary familial and congenital polycythemia, PFCP) is caused by heterozygous gain of function mutations in the erythropoietin receptor (*EPOR*) [22]. Multiple different mutations have been described, all resulting in truncation of the cytoplasmic region of the EPOR protein [23]. The mutations do not lead to constitutively active receptor signaling but, instead, produce a hypersensitivity to erythropoietin (EPO) due to lack of a crucial inhibitory domain in the receptor [24]. All patients with EPOR mutations display suppressed EPO levels but do not produce endogenous (EPO-independent) erythroid colonies (EECs) due to the lack of constitutive receptor activation [25]. Further, the disease course is generally benign. While patients may have mild increased risk of vascular complications, transformation to acute leukemia has never been reported [20]. Notably, however, EPOR mutations have been found in only about 15 % of hereditary erythrocytosis cases, and no mutations in the *EPO* gene have been reported [26]. Thus, additional disease genes must exist that have yet to be discovered.

Hereditary Thrombocytosis

To date, mutations in three genes have been shown to cause hereditary thrombocytosis: thrombopoietin (*TPO*), its receptor (*MPL*), and its immediate signaling effector (*JAK2*). All of these genes are known to have crucial functions in megakaryocyte development. Similar to hereditary erythrocytosis, mutations are heterozygous and gain of function, leading to increased signaling in the thrombopoietin pathway. Mutations in the *TPO* gene are not located in the protein coding region but rather in the splice donor site of the third exon (which contains the translational start site) or in the 5' untranslated region (UTR). The mechanism by which these mutations cause disease is due to the distinctive structure of the *TPO* mRNA transcript, which contains seven short upstream open reading frames (ORFs) in its 5' UTR. The presence of serial ORFs just prior to the translational start site inhibits efficient ribosomal translation of the TPO protein [27]. Mutations in this region either shift the translational start site up or delete an inhibitory ORF, leading to more efficient translation and increased protein production [28]. Accordingly, all patients with *TPO* mutations have thrombocytosis secondary to elevated TPO levels [21].

The germline mutations in *MPL* and *JAK2* discovered thus far are all localized to coding regions and cause single amino acid missense changes. The first to be described was a serine to asparagine mutation at position 505 in the transmembrane domain of the MPL protein. This mutation is unique in that it results in ligand-independent dimerization and subsequent downstream activation of MPL signaling [29]. Thus, it is the only MPL mutation currently known that causes a constitutively active mutant. Two other MPL mutations (K39N and P106L) have been described; however, the resulting gain of function is much weaker compared to S505N. When present in the heterozygous state, both K39N and P106L cause mild thrombocytosis, whereas homozygous individuals have chronic, severe thrombocytosis [30, 31]. In fact, these variants can be defined as polymorphisms, as they are present in approximately 7 % of African American and 6 % of Arabic individuals, respectively. Both polymorphisms are located in the extracellular domain of the MPL protein and likely function by altering the ability of TPO to bind and activate its receptor.

Multiple germline *JAK2* mutations have been reported recently, providing additional insight into the function of this protein in disease states. Four mutations have been reported in the pseudokinase domain of the *JAK2* protein (R564Q, V617I, H608N, and S755R), while two others were found in the kinase domain (R876Q and R938Q). All are associated with a phenotype of thrombocytosis with complete penetrance [32, 33, 34•, 35]. However, the S755R mutation was found in cis together with the R938Q mutation in the same family and showed limited activity in functional studies, making its pathogenicity unclear [34•]. Interestingly, overexpression of these mutants in cell lines produced increased kinase activity compared to wild type but significantly weaker activity compared to the *JAK2* V617F mutation [34•, 36•]. This is potentially explained by the ability of the V617F mutation to overcome the function of inhibitory proteins such as suppressor of cytokine signaling 3 (SOCS3), while the germline mutations lack this property [35]. Regardless, these mutants provide an example of how the biology of a mutation impacts disease phenotype.

Differentiating Between Inherited Diseases

Familial MPD and hereditary erythrocytosis/thrombocytosis were originally defined by their respective clinical phenotypes, creating different categories of disease. The most important distinction between them being that familial MPD can affect multiple hematopoietic lineages and carries a risk of transformation to acute leukemia, while hereditary erythrocytosis and thrombocytosis affect only a single lineage and have a benign course. Further genetic characterization described familial MPD as a clonal disorder with incomplete penetrance, while hereditary erythrocytosis and thrombocytosis are polyclonal and display complete penetrance. While these descriptions held true for many years, the elucidation of many of the genetic lesions found in both sporadic MPD and hereditary thrombocytosis have obscured the distinctions between these diseases.

Protein Function

It is well established that *JAK2* V617F is the most common genetic lesion driving disease in MPD [2•, 37]. However, as detailed above, we now know that inherited mutations in *JAK2* can cause hereditary thrombocytosis as well. Furthermore, using the guidelines above, a mutation at the exact same amino acid residue in the *JAK2* protein can cause a different disease depending on the amino acid change (phenylalanine in MPD versus isoleucine in hereditary thrombocytosis) (Table 2). Biological examination of each of these mutations revealed that while both are gain of function, V617I produces weaker kinase activity, termed cytokine hyper-responsiveness, compared to the strong constitutive activation of V617F [36•]. These genotypic changes correlate with disease phenotype, as the V617F mutation manifests predominately with erythrocytosis and/or thrombocytosis depending on gene dosage, while V617I manifests as thrombocytosis. This data implies that the disease phenotype in inherited MPD is produced by the relative strength of mutation, and in the case of *JAK2*, weak gain of function mutations give rise to thrombocytosis. One can infer from this that a constitutional *JAK2* V617F mutation is “too strong” to be viable, thus explaining why it has not been found to date.

Similar parallels can be drawn with the mutations found in the *MPL* protein. Of the known mutants, *MPL* S505N produces the strongest gain of function by inducing receptor

activation via dimerization. It likely functions similarly to the MPL W515L/K mutants identified in sporadic MPD [38]. A recent Italian study examined a patient cohort with thrombocytosis and a family history of ET and found germline MPL S505N mutations in 24 out of 44 patients [39]. Clinically, patients with the mutation had no difference in platelet counts, thrombotic complications, or splenomegaly compared to those with acquired JAK2 V617F mutations. This indicates that the MPL S505N mutant acts as a driver of disease in a similar manner as somatic mutations in sporadic MPD. Indeed, a screen for acquired MPL mutations in a large cohort of ET patients identified an S505N mutation in 3 out of nearly 800 samples (Table 2) [40]. While clearly not a common reoccurring mutation, the fact that MPL S505N occurs in both sporadic ET and hereditary thrombocytosis further clouds any distinction between these two disorders.

Clonality

Another characteristic that has been used to distinguish between familial MPD and hereditary thrombocytosis/erythrocytosis is the presence of clonality. Clonal hematopoiesis is universally present in sporadic MPD, and familial MPD is also thought to be a clonal disorder [41]. Conversely, several studies have demonstrated polyclonality in hereditary thrombocytosis and erythrocytosis based on X-chromosome inactivation [25, 33, 34•, 35, 42]. However, this distinction is problematic for multiple reasons.

First, this assay can be misleading, particularly in ET, where cases of sporadic disease have been indicated to be polyclonal [25, 43]. Second and more important, constitutional mutations alleviate the drive for clonal selection. In inherited disorders, every cell in the body contains the pathologic mutation predisposing to disease; thus, there is no need for any cell to “out-compete” another in a cell population such as hematopoietic stem cells (HSCs). In fact, evidence suggests that the JAK2 V617I mutation acts to expand the entire bone marrow HSC population [36•]. The clonal hematopoiesis seen in familial MPD is likely a consequence of the secondary somatic mutations occurring in hematopoietic stem cells. Many of these mutations have yet to be identified, as it is well established that clonality can exist in sporadic MPD prior to the occurrence of known mutations in genes such as JAK2 and TET2 [44, 45]. Analysis of clonality in a cohort study of families with inherited MPD manifesting as PV further supports this notion. As expected, affected individuals with elevated hemoglobin and EECs were found to have clonal hematopoiesis by X-inactivation. However, multiple asymptomatic family members with normal hemoglobin were also found to have EECs but were identified as polyclonal [46]. These “pre-malignant” individuals were likely to develop symptomatic, clonal disease upon acquisition of additional somatic mutations. This was recently shown to occur in AML, where mutations in DNA methyltransferase 3 A were found in populations of pre-leukemic HSCs [47].

Disease Complications

Hereditary thrombocytosis and erythrocytosis have classically been described as benign disorders with minimal complications and no risk of disease progression. Familial MPD, conversely, carries the same risk of disease progression as sporadic MPD [23, 48]. While this remains true in general, it has become clear that the risk for complications and/or transformation in inherited disease is dependent on the relative strength of the germline

mutation and the expression pattern of the mutant protein (Table 1). As stated earlier, the EPOR mutations seen in hereditary erythrocytosis cause a relatively weak gain function and fail to manifest with complications such as splenomegaly or thrombosis [20]. Expression of EPOR is lineage restricted to erythroid progenitors, and longstanding hyperactivity in these cells is not sufficient to induce progression to myelofibrosis or acute leukemia [49]. To date, no EPOR mutations have been seen in sporadic MPD [2••]. This is distinct from proteins expressed on both hematopoietic progenitors and differentiated cells, such as MPL. A “stronger” mutation, such as MPL S505N, can produce symptomatic disease indistinguishable from sporadic ET, including risk of progression to myelofibrosis [39, 43].

Furthermore, disease transformation in hereditary thrombocytosis has been reported. One large family was diagnosed with an inherited thrombocytosis segregating as autosomal dominant with high penetrance, most consistent with hereditary thrombocytosis [50]. Multiple family members presented with symptomatic disease, and two were found to have somatic JAK2 V617F mutations. Linkage analysis strongly supported a causative mutation in *TPO* or a nearby gene; however, no mutation was found. Another family with a documented mutation in the splice donor site of exon 3 in *TPO* gene was reported to have two affected individuals with disease transformation [51]. One family member developed acute myeloid leukemia, while another developed myelofibrosis with increased blasts; neither patient had been treated with cytoreductive therapy. Thus, patients with inherited thrombocytosis must be treated based on their clinical symptoms and cannot be reassured they have a benign disease.

Inherited MPD—a Unified Disease

Inherited disorders are by definition genetic in etiology, and it makes sense to classify them by their causative genetic lesions when possible. In the past few years, mutations that cause hereditary thrombocytosis have been discovered in both *MPL* and *JAK2*, genes that are also found to be mutated in sporadic MPD. Analysis of these mutations has provided tremendous insight into the function of these genes in disease states and clearly illustrated that disease phenotype is a function of the biology of the mutation. The mutations in inherited disease are gain of function but generally “less pathologic” or weaker than acquired mutations. Rare mutations, such as MPL S505N, appear to straddle this line and can be found in both inherited and sporadic disease. Disease phenotype, risk of complications, and prognosis are all a function of the mutational biology and should be managed no different than in sporadic disease.

Thus, attempts to distinguish hereditary thrombocytosis/erythrocytosis and familial MPD no longer appear valid. These disorders should instead be classified as one unified disorder, termed inherited MPD, with the biology of the mutation determining subtype (Fig. 1). For example, a patient with erythrocytosis found to have a germline *EPOR* mutation should be diagnosed with an inherited MPD but can be reassured that his or her disease course will almost certainly be benign and never progress to a more severe disease. Alternatively, a more cautious approach should be taken in a patient with thrombocytosis who is found to have a germline *JAK2* mutation. Since *JAK2* is expressed in the hematopoietic stem cells that can potentially give rise to neoplastic disease, chronic hyperactivity likely poses some

(albeit small) risk of disease progression. While the potential to acquire additional somatic mutations that could drive more severe disease appears to be low, the same is true for sporadic ET, which is associated with the lowest risk of disease progression of the three types of MPD [2•]. The benefits of this type of classification will simplify diagnosis and avoid confusion over attempts to place patients into distinct categories.

The majority of patients, however, will fall into the category of inherited MPD with no known germline mutation. These patients appear to have a higher risk of disease progression and most often have a long latency period prior to onset of disease, as patients do not present with clinical symptoms until after acquisition of at least one somatic driver mutation. The nature of the inherited factor in these cases remains unknown; however, it is likely a gain of function mutation that confers a small increase in DNA instability. If this hypothesis is true, one would expect to see an increased incidence of all cancer types, not only hematopoietic disease, in families with inherited MPD. This was examined in a recent study that included patients with either familial or sporadic MPD. While the overall incidence of malignancies was the same between cases of familial and sporadic MPD, malignancies were more frequent in patients with familial disease aged between 50 and 70 years compared to aged matched controls [8]. This is consistent with a weak gain of function mutation that takes many years to exert an effect. In addition, one might expect the average age of disease onset in familial MPD to be lower than sporadic disease. Indeed, this appears to be the case in studies of multiple families with familial MPD and no known germline mutation, with some even suggesting the presence of anticipation [7, 9, 15]. However, the data remain inconclusive, as the largest population-based study comparing both familial and sporadic MPD patients showed no difference in age of onset [6].

Conclusion

The past decade has seen a revolution in our ability to identify important mutations in genetic diseases, largely due to advances in next-generation sequencing techniques [52]. Rare inherited disorders frequently offer an opportunity to discover disease genes, often providing insight into broad developmental processes. This is exemplified by the recent identification of both *MPL* and *JAK2* mutations in patients with inherited thrombocytosis. Exome and whole-genome sequencing of individuals with familial MPD will likely allow for the identification of additional lesions that drive disease acquisition and progression. The study of the biological function of these mutations will lead to improvements in diagnosis and provide targets for new therapeutic interventions.

Acknowledgments

Compliance with Ethics Guidelines: **Conflict of Interest** Dr. Evan M. Braunstein is supported by NIH grant #K12HL087169.

Dr. Alison R. Moliterno reports personal fees from Incyte Inc.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Skoda RC. Hereditary myeloproliferative disorders. *Haematologica*. 2010; 95(1):6–8. [PubMed: 20065077]
- 2••. Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood*. 2014; 123(14): 2220–8. Used targeting next-generation sequencing to search for mutations in a large cohort of MPD patients. Found that the overall mutation rate in MPD is low and correlated survival to number of somatic mutations. [PubMed: 24478400]
- 3••. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*. 2013; 369(25):2379–90. Identified CALR as a frequently mutated gene in MPD. [PubMed: 24325356]
4. Makishima H, Maciejewski JP. Pathogenesis and consequences of uniparental disomy in cancer. *Clin Cancer Res*. 2011; 17(12):3913–23. [PubMed: 21518781]
5. Kralovics R. Genetic complexity of myeloproliferative neoplasms. *Leukemia*. 2008; 22(10):1841–8. [PubMed: 18754034]
6. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Bjorkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 firstdegree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. *Blood*. 2008; 112(6): 2199–204. [PubMed: 18451307]
7. Rumi E, Passamonti F, Della Porta MG, Elena C, Arcaini L, Vanelli L, et al. Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. *J Clin Oncol*. 2007; 25(35):5630–5. [PubMed: 17998545]
8. Olcaydu D, Rumi E, Harutyunyan A, Passamonti F, Pietra D, Pascutto C, et al. The role of the JAK2 GGCC haplotype and the TET2 gene in familial myeloproliferative neoplasms. *Haematologica*. 2011; 96(3):367–74. [PubMed: 21173100]
9. Bellanne-Chantelot C, Chaumarel I, Labopin M, Bellanger F, Barbu V, De Toma C, et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. *Blood*. 2006; 108(1):346–52. [PubMed: 16537803]
10. Malak S, Labopin M, Saint-Martin C, Bellanne-Chantelot C, Najman A. French Group of Familial Myeloproliferative Disorders. Long term follow up of 93 families with myeloproliferative neoplasms: life expectancy and implications of JAK2V617F in the occurrence of complications. *Blood Cells Mol Dis*. 2012; 49(3–4):170–6. [PubMed: 22818858]
11. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. *Blood*. 2008; 111(3):1686–9. [PubMed: 17984312]
12. Saint-Martin C, Leroy G, Delhommeau F, Panelatti G, Dupont S, James C, et al. Analysis of the ten-eleven translocation 2 (TET2) gene in familial myeloproliferative neoplasms. *Blood*. 2009; 114(8):1628–32. [PubMed: 19564637]
13. Maffioli M, Genoni A, Caramazza D, Mora B, Bussini A, Merli M, et al. Looking for CALR mutations in familial myeloproliferative neoplasms. *Leukemia*. 2014; 28(6):1357–60. [PubMed: 24441291]
14. Lundberg P, Nienhold R, Ambrosetti A, Cervantes F, Perez-Encinas MM, Skoda RC. Somatic mutations in calreticulin can be found in pedigrees with familial predisposition to myeloproliferative neoplasms. *Blood*. 2014; 123(17):2744–5. [PubMed: 24764562]
15. Rumi E, Passamonti F, Pietra D, Della Porta MG, Arcaini L, Boggi S, et al. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. *Cancer*. 2006; 107(9):2206–11. [PubMed: 16998940]
16. Pardanani A, Lasho T, McClure R, Lacy M, Tefferi A. Discordant distribution of JAK2V617F mutation in siblings with familial myeloproliferative disorders. *Blood*. 2006; 107(11):4572–3. [PubMed: 16717134]

17. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat Genet.* 2009; 41(4):455–9. [PubMed: 19287384]
18. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet.* 2009; 41(4):450–4. [PubMed: 19287385]
19. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet.* 2009; 41(4):446–9. [PubMed: 19287382]
20. Gordeuk VR, Stockton DW, Prchal JT. Congenital polycythemia/erythrocytoses. *Haematologica.* 2005; 90(1):109–16. [PubMed: 15642677]
21. Teofili L, Larocca LM. Advances in understanding the pathogenesis of familial thrombocythaemia. *Br J Haematol.* 2011; 152(6):701–12. [PubMed: 21303356]
22. Prchal JT. Pathogenetic mechanisms of polycythemia vera and congenital polycythemic disorders. *Semin Hematol.* 2001; 38(1 Suppl 2):10–20. [PubMed: 11242597]
23. Rumi E. Familial chronic myeloproliferative disorders: the state of the art. *Hematol Oncol.* 2008; 26(3):131–8. [PubMed: 18484677]
24. Kralovics R, Prchal JT. Genetic heterogeneity of primary familial and congenital polycythemia. *Am J Hematol.* 2001; 68(2):115–21. [PubMed: 11559951]
25. Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF, Prchal JT. Discrimination of polycythemia and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-E response to erythropoietin. *Blood.* 2003; 101(8):3294–301. [PubMed: 12515724]
26. Rives S, Pahl HL, Florensa L, Bellosillo B, Neusuess A, Estella J, et al. Molecular genetic analyses in familial and sporadic congenital primary erythrocytosis. *Haematologica.* 2007; 92(5):674–7. [PubMed: 17488692]
27. Ghilardi N, Wiestner A, Skoda RC. Thrombopoietin production is inhibited by a translational mechanism. *Blood.* 1998; 92(11):4023–30. [PubMed: 9834204]
28. Wiestner A, Schlemper RJ, van der Maas AP, Skoda RC. An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. *Nat Genet.* 1998; 18(1):49–52. [PubMed: 9425899]
29. Ding J, Komatsu H, Iida S, Yano H, Kusumoto S, Inagaki A, et al. The Asn505 mutation of the c-MPL gene, which causes familial essential thrombocythemia, induces autonomous homodimerization of the c-mpl protein due to strong amino acid polarity. *Blood.* 2009; 114(15):3325–8. [PubMed: 19483125]
30. El-Harith, eHA.; Roesl, C.; Ballmaier, M.; Germeshausen, M.; Frye-Boukharriss, H.; von Neuhooff, N., et al. Familial thrombocytosis caused by the novel germ-line mutation p.Pro106Leu in the MPL gene. *Br J Haematol.* 2009; 144(2):185–94. [PubMed: 19036112]
31. Moliterno AR, Williams DM, Gutierrez-Alamillo LI, Salvatori R, Ingersoll RG, Spivak JL. Mpl baltimore: a thrombopoietin receptor polymorphism associated with thrombocytosis. *Proc Natl Acad Sci U S A.* 2004; 101(31):11444–7. [PubMed: 15269348]
32. Rumi E, Harutyunyan AS, Casetti I, Pietra D, Nivarthi H, Moriggl R, et al. A novel germline JAK2 mutation in familial myeloproliferative neoplasms. *Am J Hematol.* 2014; 89(1):117–8. [PubMed: 24142793]
33. Mead AJ, Rugless MJ, Jacobsen SE, Schuh A. Germline JAK2 mutation in a family with hereditary thrombocytosis. *N Engl J Med.* 2012; 366(10):967–9. [PubMed: 22397670]
34. Marty C, Saint-Martin C, Pecquet C, Grosjean S, Saliba J, Mouton C, et al. Germ-line JAK2 mutations in the kinase domain are responsible for hereditary thrombocytosis and are resistant to JAK2 and HSP90 inhibitors. *Blood.* 2014; 123(9):1372–83. Identified novel germline JAK2 mutations and showed that they are biologically less active than the V617F mutation. [PubMed: 24398328]
35. Etheridge SL, Cosgrove ME, Sangkhae V, Corbo LM, Roh ME, Seeliger MA, et al. A novel activating, germline JAK2 mutation, JAK2R564Q, causes familial essential thrombocytosis. *Blood.* 2014; 123(7):1059–68. [PubMed: 24381227]

36. Mead AJ, Chowdhury O, Pecquet C, Dusa A, Woll P, Atkinson D, et al. Impact of isolated germline JAK2V617I mutation on human hematopoiesis. *Blood*. 2013; 121(20):4156–65. Found that the V617I mutation induces cytokine hyperresponsiveness and may expand the bone marrow stem cell population. [PubMed: 23535062]
37. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005; 352(17):1779–90. [PubMed: 15858187]
38. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006; 108(10):3472–6. [PubMed: 16868251]
39. Teofili L, Giona F, Torti L, Cenci T, Ricerca BM, Rumi C, et al. Hereditary thrombocytosis caused by MPLSer505Asn is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. *Haematologica*. 2010; 95(1):65–70. [PubMed: 19713221]
40. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood*. 2008; 112(1):141–9. [PubMed: 18451306]
41. Prchal JT. Polycythemia vera and other primary polycythemia. *Curr Opin Hematol*. 2005; 12(2):112–6. [PubMed: 15725900]
42. Liu K, Martini M, Rocca B, Amos CI, Teofili L, Giona F, et al. Evidence for a founder effect of the MPL-S505N mutation in eight Italian pedigrees with hereditary thrombocytosis. *Haematologica*. 2009; 94(10):1368–74. [PubMed: 19608689]
43. Teofili L, Giona F, Martini M, Cenci T, Guidi F, Torti L, et al. Markers of myeloproliferative diseases in childhood polycythemia vera and essential thrombocytosis. *J Clin Oncol*. 2007; 25(9):1048–53. [PubMed: 17369568]
44. Kralovics R, Teo SS, Li S, Theodorides A, Buser AS, Tichelli A, et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood*. 2006; 108(4):1377–80. [PubMed: 16675710]
45. Schaub FX, Looser R, Li S, Hao-Shen H, Lehmann T, Tichelli A, et al. Clonal analysis of TET2 and JAK2 mutations suggests that TET2 can be a late event in the progression of myeloproliferative neoplasms. *Blood*. 2010; 115(10):2003–7. [PubMed: 20061559]
46. Kralovics R, Stockton DW, Prchal JT. Clonal hematopoiesis in familial polycythemia vera suggests the involvement of multiple mutational events in the early pathogenesis of the disease. *Blood*. 2003; 102(10):3793–6. [PubMed: 12829587]
47. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014; 506(7488):328–33. [PubMed: 24522528]
48. Harutyunyan AS, Kralovics R. Role of germline genetic factors in MPN pathogenesis. *Hematol Oncol Clin N Am*. 2012; 26(5):1037–51.
49. Skoda R, Prchal JT. Lessons from familial myeloproliferative disorders. *Semin Hematol*. 2005; 42(4):266–73. [PubMed: 16210040]
50. Higgs JR, Sadek I, Neumann PE, Ing VW, Renault NK, Berman JN, et al. Familial essential thrombocythemia with spontaneous megakaryocyte colony formation and acquired JAK2 mutations. *Leukemia*. 2008; 22(8):1551–6. [PubMed: 18496561]
51. Posthuma HL, Skoda RC, Jacob FA, van der Maas AP, Valk PJ, Posthuma EF. Hereditary thrombocytosis not as innocent as thought? Development into acute leukemia and myelofibrosis. *Blood*. 2010; 116(17):3375–6. [PubMed: 21030572]
52. Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. *Cell*. 2013; 155(1):27–38. [PubMed: 24074859]

Fig. 1.

Guidelines for the work-up of patients with a suspected inherited myeloproliferative disorder (MPD). The traditional model (*left*) attempts to distinguish between hereditary erythrocytosis and thrombocytosis versus familial MPD based on disease penetrance and the presence of a known germline lesion. Identification of a germline lesion correlates with the absence of disease transformation. In the proposed new model (*right*), all patients with a suspected inherited MPD should be screened for the most common acquired mutations. If no mutation is found, screening for known germline mutations should be performed. Only those individuals with EPOR mutations can be assured of a benign disease course. *Bolded arrows* indicate the more common pathway. *EPO* erythropoietin, *EPOR* erythropoietin receptor, *TPO* thrombopoietin, *MPL* thrombopoietin receptor, *CALR* calreticulin, *JAK2* Janus kinase 2

Table 1
Signal transduction lesions in the MPD

Gene	Tissue expression	Somatic phenotype	Germline phenotype
JAK2	HSC, lymphoid, and myeloid stem cells	ET, PV, and PMF	Thrombocytosis
MPL	HSC, myeloid progenitors, and megakaryocytes	ET, PMF	Thrombocytosis, myelofibrosis
CALR	Ubiquitous	ET, PMF	
CSF3R	HSC, myeloid progenitors, and neutrophils	Atypical CML, CNL	
EPOR	Erythroid progenitors		Erythrocytosis
TPO	Liver		Thrombocytosis

Table 2
Mutations associated with thrombocytosis

Gene	Allele	Sporadic/familial MPD	Inherited MPD	AML risk
JAK2	V617F	X		X
	V617I		X	
	H608N		X	
	R876Q		X	
	R938Q		X	
MPL	K39N		X	
	P106L		X	
	S505N	X	X	X
	W515K/L	X		X
TPO			X	X