Autosomal Dominant Hereditary Essential Thrombocythemia due to a Gain of Function Mutation in the Thrombopoietin (TPO) and JAK2 Gene as the Cause of Congenital Aspirin-Responsive Sticky Platelet Syndrome: Personal Experiences and Review of the Literature

Jan Jacques Michiels1*, Jan Stasko2, Peter Kubish3, Achille Pich3 and Hendrik De Raeve4

1Multidisciplinary Internist & Investigator, Goodheart Institute & Foundation, Rotterdam and International Collaborations and Research on Myeloproliferative neoplasms (ICARMPN). Erasmus Tower, Veeninos 13 3069 AT Rotterdam, The Netherlands
2Department of Hematology, University Hospital Maria, Jessenius Faculty of Medicine in Martin of the Comenius University in Bratislava, Slovakia
3Department of Molecular Biotechnology and Health Sciences, Section of Pathology, University of Turin, Via Santena 7, I-10126 Torino, Italy
4Departments of Pathology,OLV Hospital Aalst and University Hospital of Brussels, Laarbeeklaan 101, B-1020; Brussels, Belgium

Abstract

Autosomal dominant hereditary Essential Thrombocythemia (ET) due to the gain of function mutation C→G transversion in the splice donor of intron 3 in the TPO gene on chromosome 3q27 in a Dutch and Polish family is associated with marked increased TPO levels and Aspirin-responsive Sticky Platelet Syndrome (SPS). SPS is featured by typical clinical manifestations of aspirin responsive microvascular circulation disturbances including erythromelalgia and atypical transient ischemic attacks. Increase of large platelets in blood smears and large mature megakaryocytes with hyperploid nuclei in a normal cellular bone marrow were diagnostic for autosomal dominant hereditary ET (HET). The spectrum of platelet-mediated thrombophilia in HET is comparable to the aspirin responsive SPS in acquired JAK2V617F positive ET. The affected members of the Dutch and Polish HET families showed no endogenous erythroid colony (EEC) formation. The first generation of the Dutch HET family, two females and one male had stable increased platelet counts, no features of PV, no splenomegaly during life-long follow-up. Three of four elderly family members in the Dutch HET family developed pancytopenia due to myelofibrosis at the age of 71 and 73 years in two, and evolution in acute myeloid leukemia at age 60 in one. These 3 HET patients were on long-term low dose aspirin to prevent SPS manifestations and not treated cytoreductive agents indicating that evolution of ET into myelofibrosis (MF) and leukemia belong to the natural history of TPO-induced HET. The congenital HET caused by gain of function mutation in the TPO and the JAK2 gene (JAK2V617F and JAK2V617G) the responses of mutated CD33 and CD34+ cells to EPO are increased, but the responses to EPO were normal thereby explaining why HET caused by heterozygous germline TPO and JAK2 mutations are associated with the biological characteristics of ET without PV features.

Keywords: Autosomal dominant hereditary essential thrombocythemia; Myeloproliferative neoplasm; WHO classification; JAK2 mutation; TPO gene mutation; Myelofibrosis

Introduction

In the 1990s, studies on murine leukemia and oncogenes led to the recognition of a new member of the hematopoietin receptor superfamily [1-5]. The murine myeloproliferative leukemia (MPL) gene is the normal cellular homologue of the oncogene v-MPL and responsible for a pannmyeloid transformation capacity [1]. This was followed by the molecular cloning and charaterisation of MPL, the human homologue of the c- and v-MPL [2-4]. The receptor MPL was then rapidly recognized as being the thrombopoietin receptor (TpoR) by the demonstration that antisense oligonucleotides of c-MPL inhibited the colony-forming of megakaryocyte progenitors by Wending et al. [5]. The MPL ligand became the key to the identification of TPO and was cloned in 1994 by five independent groups [6-10]. The MPL ligand is identical to thrombopoietin and labeled as megakaryocyte growth and development factor (MDGF) [9-12]. Human TPO has all the functions ascribed to MDGF, and all MDGF-like activity can be neutralized by soluble recombinant MPL [13]. TPO stimulates hematopoietic stem cell division and differentiation, and plays a central role in the development and maturation of megakaryocytes and platelet formation.
Clinical data of Dutch HET patients and non-HET subjects

<table>
<thead>
<tr>
<th></th>
<th>HET (%)</th>
<th>No HET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Age years, range</td>
<td>11-58</td>
<td></td>
</tr>
<tr>
<td>Vaso-occlusive symptoms: erythromelalgia (E)</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Early E. tip paresthesia</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Red congested erythromelalgia</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>E complicated by cold tip feeling-arocyanosis/gangrene</td>
<td>20 / 10</td>
<td>0 / 0</td>
</tr>
<tr>
<td>Transient ischemic attack: TIA</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow histology data of ten HET patients[14] (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megakaryopoiesis -increased</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Megakaryocyte clustering of 2-4 cells</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Erythropoiesis increased</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Reticulin-content increased grade 1</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Storage iron present</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Chromosome abnormalities</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Note: While on low dose aspirin since 1986 according to Michiels et al. [17], all affected HET patients in the Dutch family were free of microvascular ischemic circulation disturbances and major thrombosis at a stable platelet count between 400x10^9/L and around 1000x10^9/L during life long follow up without the need of myelosuppressive treatment [17,37].

Table 1. Clinical manifestations and main bone marrow histology data in the Dutch family with Sticky Platelet Syndrome in autosomal hereditary essential thrombocythemia (HET)[14,15].

Figure 2: Pedigree of the Dutch family with hereditary essential thrombocythemia (HET)[14,15,38]. TPO serum TPO concentrations (pg/ml) from the available Dutch family members with autosomal hereditary essential thrombocythemia (HET) caused by a gain of function mutation in the TPO gene. The numbering of generations and individuals in the pedegree is the same as in the original publication by Schlemper et al. [14]. Filled in symbols affected individuals, open symbols normal individuals. Numbers below symbols indicate TPO serum concentrations in pg/ml, the mean of triplicate ± SEM is given; numbers in italics represent platelet counts x10^9/L.

Hereditary Essential Thrombocythemia (HET): Personal Experiences

The propositus of the Dutch family with hereditary essential thrombocythemia (HET) presented in 1986 with typical erythromelalgia complicated by acrocyanosis of a few toes followed by gangrene and amputation of toe (Table 1) [14,15]. Recurrent erythromelalgia and acrocyanosis in 1986 typically responded to low dose aspirin but not to vitamin K antagonist Aspirin responsive platelet-mediated microvascular disturbances or Sticky platelet syndrome (SPS) [16] was first described by Michiels et al. one year before in 1985 [17] in patients with essential thrombocythemia (ET) and polycythemia vera (PV). The histopathological findings in bone marrow biopsies of the propositus (case II 3, Figure 2) in the Dutch family with HET was compatible with ET very similar to the bone marrow findings in our patients with acquired ET) and PV complicated by erythromelalgia caused by platelet-mediated arteriolar inflammation and thrombosis in thrombocythemia [17].

The propositus case II3 of the Dutch HET family was referred to Dr Michiels at the Erasmus University Medical Center (Academic Hospital Dijkzigt Rotterdam) for expert evaluation. At time of diagnosis of familial ET the histopathology from bone marrow biopsy material from the propositus in 1986 and in 1991 was diagnostic for ET as described by Michiels et al. [17] (Figures 3-5). All features according to the Rotterdam Clinical and Pathological (RCP) diagnostic criteria for ET [17] were present:

1. Increase of platelet count in excess of 400 x10^9/L in the absence of any cause or sign of reactive thrombocytosis (Figure 3).
2. Typically clustering and increase of enlarged megakaryocytes showing mature cytoplasm and hyperploid nuclei in a normocellular bone marrow (Figures 4 and 5).
3. No preceding or allied other subtype of myeloproliferative disorder (MPD) or myelodysplastic syndrome (MDS).
4. Normal cellularity of the bone marrow with only slight increase of fine reticulin fibers.

Based on the publications in the 1990s on the discovery of thrombopoietin (TPO) and its receptor MPL (= TpoR) on megakaryocytes and platelets [1-13], Wiestner et al. [15] discovered in 1998 that the Dutch HET was caused by a gain of function mutation in the thrombopoietin (TPO) gene with the co-segregation of G to C in the splice donor site of intron 3 in the TPO gene. This gain of function mutation in the TPO gene caused increased TPO levels and microvascular manifestations of affected member of the Dutch HET family (Figure 2 and Table 1, Dutch HET pedigree) [14,15]. While on low dose aspirin since 1986 according to the Rotterdam recommendation in 1985 by Michiels et al. [17], all affected HET patients in the Dutch family were free of microvascular ischemic circulation disturbances and major thrombosis by treatment with low dose aspirin at platelet count between 400x10^9/L and around 1000x10^9/L during life long
features of the 11 affected family members of the Polish HET family are summarized in Table of Figure 6. The serum TPO levels in most affected members were increased or even in the upper range of normal. Five of eleven affected family members had thrombocytopenia related symptoms including headaches, acrocyanosis, limb paresthesias, venous thrombosis, transient ischemic attacks, miscarriage and thrombo-angiitis obliterans consistent with SPS similar as has been described in acquired thrombocytopenia in ET and PV by Michiels et al. [17]. SPS typically responds to low dose aspirin (75 mg/day) and attempts to relieve microvascular ischemic symptoms by cytoreduce therapy with hydroxyurea in the proposita (PL09) were ineffective. The bone marrow histology features in 6 affected members of the Polish HET family were similar to early prefibrotic stage of MPN: increase and loose to dense clustering as reflected by normal myeloid to erythroid ratio of bone marrow nucleated cells of normal to medium sized mature megakaryocytes with normal to slight increased cellularity according to age, no increase of erythropoiesis, no increase of reticulin fibrosis (RF grade 0 to 1). As compared to controls, the clustered megakaryocytes were more compact, of normal to increased size with slightly hyperlobulated nuclei, but less pronounced as compared to JAK2

Molecular Etiology and Pathophysiology of TPO-Induced Autosomal Dominant HET

Analysis of the TPO gene in his Dutch family with an autosomal inherited ET was initiated by us and performed by Dr Skoda [15]. The CG transversion in the splice donor of intron 3 of the TPO gene co-segregated with the affected autosomal dominant hereditary ET (HET) in the family. This mutation destroys the splice donor site in intron 3 and results in exon 3 skipping. The shortened 5'UTR gene resulted in a gain of function which leads to overproduction of thrombopoietin (TPO) by a mechanism of increased efficiency of the TPO mRNA translation. The in vivo increased TPO levels are responsible for the etiology of hereditary ET by stimulating megakaryocyte production both in vitro and in vivo. Size, number, and mean geometric ploidy of megakaryocytes are increased by TPO as compared with other cytokines with hematopoietic activity. Evidence for a decisive role of deregulated TPO in ET comes from observations in mice overexpressing a TPO transgene where increased TPO production resulted in a fatal myeloproliferative disorder [20]. Lethally irradiated mice grafted with

follow up without the need of myelosuppressive treatment. Kondo et al. [18] independently described a second HET family and Liu et al. [19] a third HET family with a similar gain of function mutation in the thrombopoietine (TPO) gene. The laboratory, clinical and bone marrow
bone marrow cells infected with a retrovirus carrying the murin TPO cDNA induced high TPO levels in mice (TPO\textsuperscript{high} mice), who developed thrombocytopenia followed by a lethal myeloproliferative disorder of megakaryocytic granulocytic myeloproliferation (MGM) with reduced erythropoiesis in the spleen and bone marrow [21]. In this study, platelets of normal size and morphology in control mice (A) and large platelets in TPO\textsuperscript{high} mice were observed initially at time of the ET picture [21]. The continuous forced expression of TPO, (TPO\textsuperscript{high} mice) in mice induces megakaryocyte proliferation and differentiation and subsequently develop myelofibrosis [21,22]. TPO\textsuperscript{high} mice engineered to overexpress TPO in their liver and those that received transplants of marrow cells infected with a TPO containing retrovirus develop thrombocytopenia due to massive hyperplasia of megakaryocytes and granulocytes and hypoplasia of erythropoiesis in the bone marrow followed by myelofibrosis and extramedullary hematopoiesis within 2 to 3 months and die from myelosclerosis and myelofibrosis thereafter [21]. TGF-Beta-1 has been implicated in the pathobiology of myelofibrosis by the observation that megakaryocytes from TPO\textsuperscript{high} rats and mice express high levels of TGF-Beta in marrow extracellular fluids and plasma [22]. Another growth factor produced by megakaryocytes, platelet derived growth factor (PDGF), was found to be upregulated in a fashion similar to TGF-Beta1. High levels of TGF-Beta1 mRNA in bone marrow and spleen cells in TPO\textsuperscript{high} mice were associated with high levels of TGF-beta1 protein in extracellular fluids from these organs [22]. Evolution of ET into transient myelofibrosis has been observed in rats receiving recombinant TPO [21]. Mice respond to TPO treatment by increasing the number of platelets in the circulation and megakaryocytes in the spleen at day 7 to 10 and returned to pretreatment values at day 14 [22]. In wild type mice, TPO treatment increases platelet counts 2.3 fold and the number of CPU-megakaryocytes. TPO treatment had profound effects on the morphology of megakaryocytes in wild type mice. The overall morphology of the megakaryocytes in the spleen became less mature as revealed by reduced localization of P-selectin and von Willebrand factor on the alpha granules. In addition, a significant portion of these megakaryocytes had heavy-electron dense para-apoptotic morphology and contained neutrophils embedded in the cytoplasm, as confirmed by myeloperoxidase immunostaining. In wild mice TPO treatment decreased GATA-1 content in megakaryocytes, and the development of myelofibrosis is associated with high levels of transforming growth factor beta-1 (TGF-Beta-1) expression in bone marrow and spleen [22].

Congenital gain of function mutation in the TPO gene on chromosome 3q27 results in increased levels of plasma TPO levels, which induce a physiological activation of the TPO-sMPL signalling pathway. This results in hyperproliferation of large mature megakaryocytes (Figure 2) and increased platelet count complicated by platelet-mediated cutaneous complications (Table 1). Platelets do contain many constituents of the TPO signalling machinery. Apart from MPL, platelets contain JAK2-PI-3 kinase, Stat-3 and Stat-5 p38, MAPK and signalling elements downstream of those regulators [23-27]. Platelet stimulation with a supra-physiological plasma concentration of TPO initiates aggregation and secretion, illustrating that TPO act as an independent inducer of platelet responses [28-30]. Pre-incubation of platelet with 20 ng/ml TPO for 5 minutes increases the amount of serotonin secretion by low a low dose of the agonist thrombin (0.1 U/mL) [23]. Activation of platelets induced by TPO and thrombin leads to the subsequent activation of the enzyme cPLA2, which liberates arachidonic acid from membrane phospholipids, which is the source for platelet cyco-oxygenase (COX-1), thereby causing spontaneous platelet aggregation with formation of platelet thrombi in the end-arterial circulation at places of high shear rate consistent with aspirin responsive platelet sticky syndrome (SPS) [17,23,30-33]. In addition to the activation of platelets free in suspension (plasma) by soluble agonist such as thrombin, platelets bind to each other mediated by the von Willebrand factor (VWF) as well as to subendothelial and specific surface bound VWF protein that become accessible in the damaged vessel wall [23]. Perfusion models mimicking platelet adhesion in flowing blood show that platelets (including VWF-platelet aggregates) bind to these surface-bound adhesive protein in the absence of a soluble platelet activator. Very low concentrations of plasma TPO (0.01 to 1.0 ng/mL) enhance this platelet-VWF adhesion and VWF-platelet aggregation by more than 50%. Adhesion to VWF is preceded by a rolling phase until the interaction with VWF is sufficiently strong. In the presence of 1 ng/mL TPO, firm platelet attachment to subendothelial surface is almost immediate. Subsequently, the formation of circulating VWF-platelet aggregates/thrombi mediated by the high shear stress occurs that are formed by platelets attaching to the subendothelium appears to be very characteristic for SPS [23]. These findings show that increased plasma TPO concentrations causes hypersensitive platelets in circulating plasma and thereby have a major effect on the initiation of the clinical manifestations of spontaneous platelet-mediated arteriolar inflammation and thrombosis at high shear rate in the end-arterial circulation including aspirin erythromelalgia and its ischemic complications [17] or Sticky Platelet Syndrome\textsuperscript{16}, which is preventable by inhibition of platelet cyclo-oxygenase (COX-1) by low dose aspirin but not by Coumadin (vitamin K antagonist, Table 1). The clinical picture of aspirin responsive SPS in HET is similar as has been described in 1984/1985 for aspirin responsive erythromelalgia caused by arteriolar inflammation and thrombosis in the endarteriolar circulation in myeloproliferative thrombocytocmya of ET and PV patients [17]. Since the discovery of the molecular etiology of myeloproliferative neoplasms (MPN), the association of aspirin responsive SPS (ASPS) and ET could be confirmed to occur in JAK2\textsuperscript{V617F} positive ET, PV, MPL and calreticulin (CALR) mutated ET as well [34,35]. Briefly, aspirin responsive microvascular episodes of fainting and dizziness typical for APS are the predominating clinical feature in 23 affected family members of the Dutch and Polish HET families. Disease onset in HET occur already in childhood in patients with HET and in adults or elderly patients in acquired ET and PV. The nature and frequencies of aspirin responsive erythromelalgic thrombotic vascular manifestations and hemorrhagic events were similar in congenital HET and acquired ET [17,34].

**Natural History of TPO-Induced Autosomal Dominant HET in the Dutch Family**

The initial bone marrows histologic findings in the Dutch HET family were blindly and independently evaluated in 2014 by Drs Piche and De Raeve directly from the pictures without any knowledge of the clinical and laboratory data and not aware of the diagnosis of congenital familial thrombocytocmya caused by a gain of function mutation in the TPO gene. The first bone marrow biopsy in 1986 of case I13 is consistent with ET according to the 2014 WHO clinical molecular and pathological (2014 WHO-CMP, Figures 3 and 4) [35]. A second bone marrow biopsy in 1991 showed dense clustering of large megakaryocytes megakaryocytes (Figure 5). Bone marrow cellularity is normocellular to slightly increased showing slight dyserythropoiesis and marked megakaryocytic hyperplasia. Most megakaryocytes are large with abundant cytoplasm with slightly increased reticulin stain grade 1 (Figure 5). The histology picture of the third follow-up bone marrow biopsy in 1996 was dramatically changed (Figures 7 and 8) and showed a hypocellular bone marrow with a few focal dense clustered dysplastic megakaryocytes and reticulin grade 3 to 4 according to PVSG criteria.
with dysplastic megakaryopoiesis, granulopoiesis and erythropoiesis, and increase of blasts (10%). The peripheral blood showed leukoerythroblastosis, macrothrombocytes, and teardrop cells. She died 3 months after diagnosis of myelofibrosis. Case II8 of the Dutch HET pedigree had a history of diabetes, hypertension and transient ischemic attack in 1989, and was referred in 2008 because of fatigue, anemia and fever (hemoglobin 6.1 mmol/L, leukocytes 4.8x10⁹/L, platelets 90x10⁹/L, 4% blasts and increased LDH, 1509 U/L [37]. Bone marrow cytology showed 45% myeloid blasts (CD34/117/13/33 and HLA-DR positive) and complex cytogenetic abnormalities: 47, add(2) (p213), del 5q; i(8)(q10) + i (21)(q10), -18, -20, i(21)(q10), =2-5 (cp5), and no JAK2V617F mutation. The diagnosis was consistent with AML with maturation not otherwise classifiable according to WHO criteria. The AML was refractory to treatment and the patient died at the age of 71 years [37]. Patients II2, II8 and III3 of the Dutch HET family were treated with low dose aspirin to effectively prevent microvascular ischemic attacks including erythromelalgia and did not receive cytoreductive agents [17,37]. Consequently, the evolution of normocellular ET into myelofibrosis or AML in the second generation at ages around 70 years of the Dutch HET family is part of the natural history of polycythaemic TPO-induced HET caused by a gain of function mutation in the TPO gene. Increased plasma TPO levels produced by liver cells caused by a gain of function mutation in the TPO gene on chromosome 3q27 in the Dutch and Polish HET families do not affect the erythroid and granulocytic hematopoietic stem cells but selectively induced proliferation and differentiation of polyclonal megakaryopoiesis and platelets leading to the disease called hereditary essential thrombocytopenia (HET), which after life-long follow-up evolved into pancytopenia associated with myelofibrosis or blastic transformation of hematopoietic stem cells at the age around 70 years in the Dutch HET family. 

Pathophysiology of Clinical and Bone Marrow Features in Congenital TPO-Induced HET

The frequencies of aspirin sensitive microvascular circulation disturbances (Sticky Platelet Syndrome [16]) were similar in the Dutch and Polish HET families. Platelet counts were above 400x10⁹/L to around 1000x10⁹/L was associated with normal leukocyte and erythrocyte counts, no or slight splenomegaly, and increase of clustered megakaryocytes in a normocellular bone marrow with normal myeloid/erythroid ratio and absence of EEC [34-36]. Kralovics et al. compared cMPL expression in the acquired MPDs ET and PV versus HET [38]. He examined 44 patients with MPD (23 PV, 15 ET, and 6 MF) and 18 healthy individuals. Decreased expression of c-MPL protein was found in 30% of patients with PV (7 of 23), 40% of ET (6 of 15) and 67% of IMF (4 of 6). Thus, c-MPL cannot be used as a diagnostic test for PV [38]. To assess whether lower expression of c-MPL is specific for MPD Kralovics et al. studied the Dutch HET family in which thrombocytopenia is caused by elevated TPO serum levels due to a splice donor mutation in the TPO gene [40]. They found lower expression of c-MPL protein in 7 of 8 affected HET individuals (88%), despite normal c-MPL mRNA levels. These findings could be confirmed in the Polish HET family (Table in Figure 6) [19]. Hence, decrease of c-MPL protein also can occur in patients who display sustained monolinar thrombocytopenias (HET) caused by a molecular mechanism different from sporadic MPD. Importantly, EECs were negative in all affected Dutch and Polish family members indicating the absence of PV features at the biological bone marrow level. EECs remain the most reliable auxiliary diagnostic assay for PV.

Liu et al. [19] analysed in collaboration with the molecular genetic laboratory of Dr Skoda the large Polish and Dutch families with HET caused by the identical mutation C>G transversion in the splice donor.

**Figure 7:** Bone marrow histology of HET (1996). Hypocellular bone marrow with decreased granulopoiesis and erythropoiesis, marked periosteal fibrosis and focal osteosclerosis (left). Hypercellular bone marrow with increased dysmorphic megakaryocytes (right). Two atypical megakaryocytes with small irregularly shaped, hyperchromatic nuclei. (right).

**Figure 8:** Bone marrow fibrosis in HET (1996). Diffuse and dense reticulin fibrosis grade 3 (RF 3) (WHO: MF 2/3) (Left). Diffuse heavy increased of reticulin fibers with numerous cross-over (RF 3) and focal increase of the bone trabeculae thickness indicative of osteosclerosis (WHO: MF 2/3).
normal controls. Similar clinical, laboratory and findings were found in the compactness of their nuclei was significantly higher in HET than in mutation in the TPO gene (Figure 6). The size of megakaryocytes and Polish families with TPO-induced HET caused by the gain of function medium sized enlarged and strikingly compact and clustered in the of intron 3 of the THPO gene in 11 affected family members with erythropoiesis in the bone marrow typical for PV were not seen in the affected members of the Dutch and Polish HET patients caused by aspirin-responsive platelet sticky syndrome (ASPS) and frequently show evolution into myelofibrosis [35,40].

Pathophysiology of Clinical and Bone Marrow Features in Congenital JAK2-Induced HET

There are two novel molecular variants of congenital polyclonal HET caused by a gain of function mutation in the JAK2 gene: HET caused by a gain of function mutation JAK2V617F and JAK2V617I in the JAK2 gene [41-43]. Mead et al. described the germline mutation JAK2V617F as the sole genetic abnormality, sufficient to induce the ET phenotype of MPN in a family with autosomal dominant HET complicated by microvascular ischemic events in some of the them [41,42]. Peripherial blood and bone marrow histology are consistent with normocellular ET without features of PV [41,42]. The authors demonstrated that JAK2V617F is the sole driver in JAK2V617F-positive individuals with typical ET peripheral blood and bone marrow features and completely normal values for haemoglobin, haematocrit, erythrocytes TPO and EPO levels [41,42]. There was a non-significant trend to increased (mean 2.4-fold) numbers of phenotypic hematopoietic stem cells (HSCs) relative to controls in the JAK2V617F-positive persons. There were no significant differences in the numbers of other myelo-erythroid progenitor populations as determined by fluorescence-activated cell sorting, in both peripheral blood and bone marrow of the JAK2V617F-positive ET cases. Compared with controls, however, CFU-GM were increased in the BM of JAK2V617F-positive ET cases. BFUEs were not affected, in agreement with the lack of erythroid phenotype in these patients, whereas CFU-Mks were slightly increased in the BM. After stimulation with granulocyte colony-stimulating factor (G-CSF), TPO and EPO of peripheral blood CD34+ myeloid and CD34+stem and progenitor cells, significant differences in congenital JAK2V617F and acquired JAK2V617F mutated cells as compared to controls [42]. The response to G-CSF was increased in JAK2V617F, and more pronounced JAK2V617F mutated HSC. In signalling and transcriptional experiments assays, JAK2V617F showed more activity than wild type JAK2, but substantially less than JAK2V617F. After cytokine stimulation, JAK2V617F resulted in markedly increased downstream signalling compared to JAK2 wild type and comparable with JAK2V617F. The responses to TPO were equally increased in JAK2V617F and JAK2V617I and the response to EPO was normal in congenital JAK2V617I but increased in acquired JAK2V617I. These findings demonstrate that heterozygous congenital JAK2V617I mutation induces sufficient cytokine hyperresponsiveness of the HSC to induce a homogeneous ET phenotype in blood and bone marrow without PV features [42]. In congenital HET caused by the heterozygous JAK2V617I mutation the responses to TPO are equally increased in germline JAK2V617I and somatic JAK2V617I mutated CD33 and CD34+ cells, but the responses to EPO are normal in germline mutated JAK2V617I and increased in acquired somatic JAK2V617I mutated hematopoietic progenitor cells (Figure 10), thereby explaining why the heterozygous JAK2V617I germline mutation is associated with ET without PV features [42].

Another novel heterozygous JAK2V617E mutation has been identified in another family with autosomal dominant HET [43]. The...
growth promoting effects of JAK2V617F were much milder than those of acquired JAK2V617F mutation. The authors found higher levels of STAT1 and STAT3 in cells expressing acquired JAK2V617F, compared to congenital JAK2V617F. Total STAT1 levels were increased with acquired JAK2V617F and with congenital JAK2V617F expression as compared to wild type JAK2 but this effect was more prominent with the somatic R564Q mutation and subject to western blot analysis. Phosphorylation of JAK2 was increased in the JAK2V617F-positive family members (R564Q1 and R564Q2) compared with the father, who is negative for the mutation (WT). The growth characteristics of the JAK2-expressing cell lines in response to TPO treatment were then determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide assays. Cells expressing JAK2Y107F, either with or without JAK2V617F, were factor-independent, and proliferation was significantly increased from wild type JAK2-expressing cells in the absence of, and at all concentrations of, TPO. JAK2V617F-expressing cells also showed significantly increased proliferation compared with wild type JAK2 cells, although cell proliferation was much less striking than with acquired JAK2V617F, thereby confirming the findings in JAK2V617F HET and explaining why heterozygous JAK2V617F and JAK2V617F are associated with ET without PV features (Figure 10). These differences do explain why acquired JAK2V617F mutated ET frequently shows features of PV with low serum EPO and increased bone marrow cellularity due to increased erythropoiesis (Figure 11) [35,37].

Conclusion

Increased plasma TPO levels produced by liver cells caused by a gain of function mutation in the TPO gene on chromosome 3q27 do not affect the hematopoietic stem cells but selectively induced proliferation and differentiation of polyclonal large megakaryocytes and large platelets leading to the platelet-mediated arterial thrombophilia in hereditary essential thrombocythemia (HET) without features of PV. While not treated with myelosuppressive agents spontaneous evolution of normocellular HET into myelofibrosis in two patients and acute myeloid leukemia in a third case at ages around 70 years. Congenital HET caused by heterozygous gain of function mutations in the JAK2 gene is also associated with the clinical picture of ET without any feature of PV during long-term follow-up. Acquired JAK2V617F ET typically shows features of PV with low serum EPO, the presence of EEC and increased erythropoiesis and poikilocytosis in the bone marrow.

References

Mpl ligand, is essential for full megakaryocyte development. Proc Natl Acad Sci U S A 92: 3234-3238.


