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GENETICS OF ESSENTIAL THROMBOCYTHEMIA - A CHRONIC MYELOPROLIFERATIVE NEOPLASM

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Summary

Essential thrombocythemia (ET) is one of the three non-Philadelphia-chromosome myeloproliferative neoplasms (MPN). It is considered to be of relatively benign clinical course with expected overall survival for patients approaching the one of general population. However, as a clonal disease of hematopoietic stem it can progress to myelofibrosis or transform into leukemia. It shows increased risk of hemorrhage (when platelet counts over 1 mil/ul), or propensity for thrombosis. In the last 10 years tremendous advances in detecting molecular genetic defects responsible for MPN, including ET were made. The most important are acquired valine to phenylalanine substitution at aminoacid 617 of the JAK2 (Janus Kinase 2) protein (JAK2V617F) that leads to constitutive activation of the tyrosine kinase, increased kinase activity and hyper-responsiveness to cytokine signaling and cell proliferation. This mutation is present in 60% of ET, >97% of PV and in around 50% of patients with PMF. The thrombopoietin receptor (*c-MPL*) is another oncogene in ET with W515L mutation that induces constitutive, cytokine-independent activation of the JAK-STAT pathway. MPLW515L or MPLW515K mutations are present in patients with PMF or ET at a frequency of approximately 8% and 3%, respectively, not found in PV. The recent important genetic discovery is description of Calreticulin (*CALR1*) mutation in 30-38% of non-*Jak2*/non-*c-MPL* mutated ETs. The del/ins mutation in the gene C-terminus or this intracellular protein chaperone and calcium controller is clinically associated with favorable prognosis if not occurring with other secondary hits, e.g. *ASXL1* mutation.

Key words: thrombocythemia genetics, chronic myeloproliferative neoplasms

INTRODUCTION

In the spectrum of chronic myeloproliferative neoplasms (MPNs) only t(9;22)(q34;q11) diagnostic for chronic myelogenous leukemia (CML) is specific for a single disease. There is also a relative strong association for the del(13)(q12;q14) and

primary myelofibrosis (PMF), the rearrangements of *PDGFRβR* on 5q33 with various partner genes from 7q11, 10q21 and 17p13 are linked to chronic eosinophilia, as well as del(20)(q11;q13), +8, +9 is associated with polycythemia vera (PV). However, Essential thrombocythemia (ET) is the disorder for which no single recurrent chromosomal abnormality has been described and only relatively unspecific structural and numerical karyotipic pathology is observed (see Table 1, Fig. 1).

Table 1. Chromosome anomalies and genes associated with essential thrombocythaemia

Chromosome anomalies or candidate genes	**Associated MPN**	**Other associated hematologic malignancy**	**Comment**
del(13)(q12q14)	PV, PMF	AML, CLL, MM, MDS, NHL	*RB1* gene locus
del(20)(q11q13)	PV, PMF, CNL	AML, MDS	40 genes in the CDR
+8	all MPNs	AML, ALL, MDS, LPD	in ~10% cases
+9	PV, PMF	AML, MDS	~ 2%
+21	PV, PMF	AMl, ALL, MDS, t-AML, t-MDS	~ 3%
-7	PV, PMF, CMML, JMML		~ 8 %
-Y	PV	AML, ALL, LPD, MDS, MM, NHL	~ 15% male pts
JAK2^{V617F} at 9p24	PV, PMF	MDS, AML	40-55%
del(17)(p13)	PMF, PV	LPD, other hematopoietic npl.	leukemic transformation
+1q			leukemic transformation
t(1;7)(q10;p10)	-	-	leukemic transformation (ref. 4)
TPO gene	-	-	herediatry thrombocythemia
c-MPL$^{W515L/K}$			~ 1%
CALR1 del/ins mutation	ET, PMF	-	driver mutation good prognosis

Abbreviations: PV, polycythaemia vera; PMF, primary myelofibrosis; CNL, chronic neutrophilic leukemia; MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia, ALL, acute lymphoblastic leukemia; LPD, chronic lymphoproliferative disease; MM, multiple myeloma; CDR, critical deleted chromosomal region; CLL, chronic lymphocytic leukemia; NHL, Non-hodgkin lymphoma; JMML, juvenile mylomonocytic leukemia.

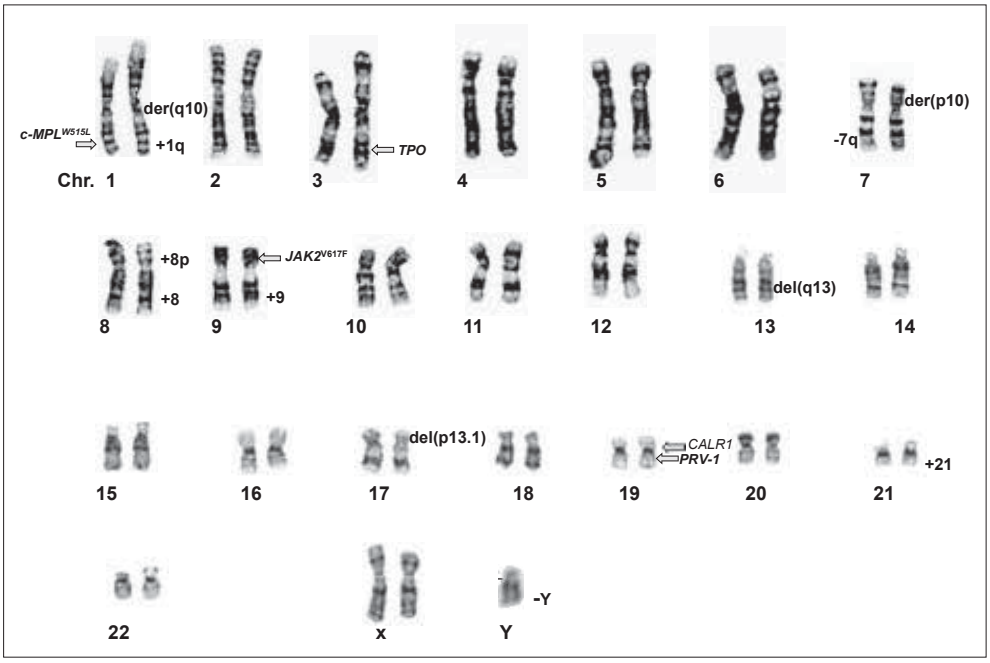

Figure 1. Chromosomal map with depicted regions of numerical and structural aberrations and loci of candidate genes for ET

In the literature reported and summarized there have been approximately 400 ET patients cytogenetically examined at diagnosis and some of them were later followed, some examined in the progression to acute leukemia [1-3]. Frequency of chromosomal anomalies was ranging from 5 to 7% at diagnosis and is the lowest among other MPNs. Generally, if found, observed chromosomal aberrations are stable in the course of the disease except for cases that transform and progress to acute leukemia where transformation can be associated with del(13)(q22) or appearance of translocations such are: t(2;17), t(3;17)(p24;q12), t(1;7)(q10;p10), t(6;11)(p24;q13) or chromosome 1 long arm trisomy, monosomy 7q, del (17p): the site of TP53 gene (4,4,6). While initial karyotyping of ET seldom reveals pathology and is not included in the standard diagnostic criteria it is not considered mandatory in the first phase of the disease, but it may reveal clonal evolution when performed at the time of signs of hematological dynamics for individual patient in the course of the disease.

Some of the apparent ET patients may harbor a masked Ph chromosome and this could lead to erroneous diagnosis of ET rather than CML. For this reason either molecular cytogenetics (FISH) of molecular test for *bcr-abl* fusion gene is recommen-

ded for the diagnostic workup of primary thrombocytosis. Thrombocytosis is also seen in some other myeloid malignancies, particularly myelodysplastic syndromes where del(5q-), t(3;3)(q21;q26) or inv(3)(q21;q26) can be expected. 8-15% of patients with the isolated interstitial deletion of the long arm of chromosome 5 and characteristic clinical features e.g. female sex, refractory anemia with mild leucopenia have also increased platelets, most likely due to haploinsufficiency of miR-145 and miRNA 146a expression from the remaining 5q allele.

The *JAK2* V617F and *CALR1* mutations in ET

The discovery of a recurrent, acquired point mutation G to T, with valine to phenylalanine substitution at aminoacid 617 of the JAK2 (Janus Kinase 2) protein (*JAK2*^{V617F}) leads to constitutive activation of the tyrosine kinase, increased kinase activity and hyper-responsiveness to cytokine signaling and cell proliferation [6] (Fig 2).

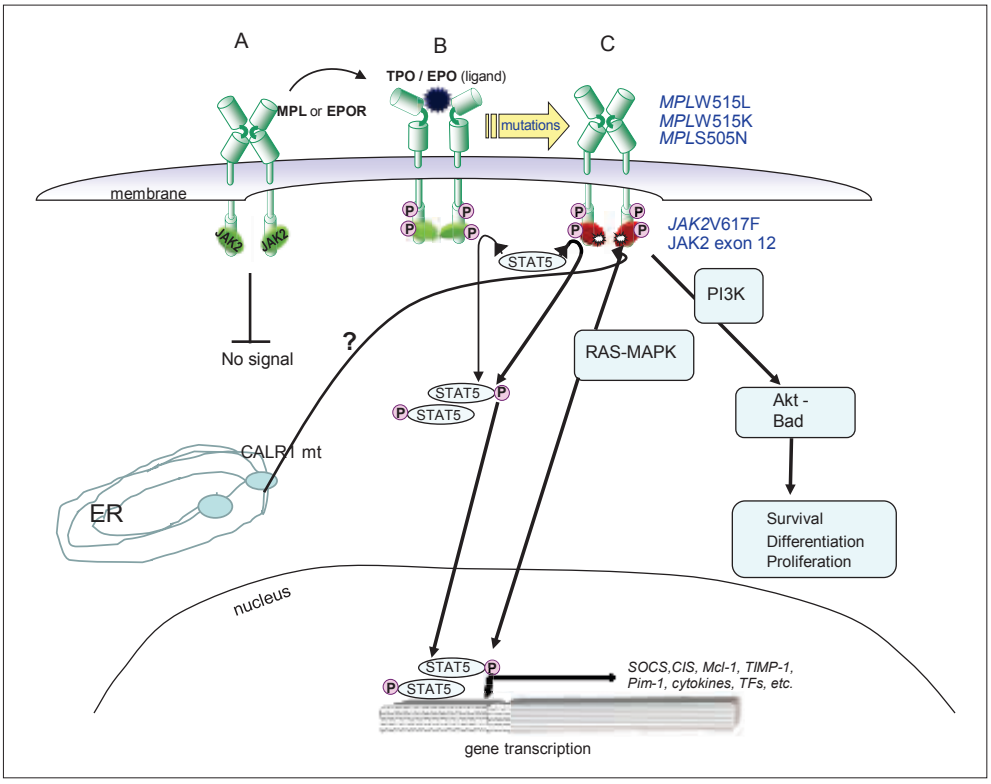

Figure 2. Erythropoietin receptor (EPOR) and thrombopoietin receptor (MPL) signaling

A and B: binding of ligand eg. erythropoietin or thrombopoietin to the receptor induces conformational changes resulting in phorphorylation (P) of JAK2 and the cytoplasmic tail of the receptor. In C: mutations either in JAK2 or cMPL lead to constitutive activation and increased signaling through pathways (JAK-STAT, PI3K, RAS-MAPK) even in the absence of the cytokine. CALR1 is a protein transporting chaperone from endocytoplasmic reticulum whose pathogenetic mechanism is not yet fully understood.

This mutation is present in most of the patients with PV and in around 50% of patients with PMF. The frequency of JAK2 mutation in ET patients published ranges from 30% to 57% [7-9]. A substantial proportion of PV and PMF patients are homozygous for the mutant allele due to uniparental disomy resulting from mitotic recombination [9], but this phenomenon is rarely detected in ET [10]. The higher mutated allele burden increasing in the percentage from PV to PMF might reflect a biological continuum, where phenotypic presentation in part is influenced by mutated allele burden [11]. It is proposed that the low level of mutated JAK2 allele is sufficient for activation o MPL and that low level signaling is sufficient to induce thrombocytosis, but with the raise in the mutated alleles EPOR is activated with the induction of thrombocytosis associated with polycythemia and even further activation is leading to polycythemia and finally very strong JAK V617F expression and signaling will be responsible for myelofibrosis [12]. The $JAK2^{V617F}$ mutant allele burden in ET contributes to determining the clinical phenotype in patients with ET in terms of involvement of myeloid lineage proliferation and higher risk or incidence of thrombosis or microvascular complications [13,14]. Patients with JAK2 mutation have multiple features resembling polycythemia vera, with significantly increased hemoglobin level and neutrophil counts, an increased risk of venous thrombosis and a higher rate of transformation to PV compared with patients without the mutation [13,15]. Furthermore, *JAK2* V617F positive patients had lower serum ferritin and Epo concentrations, than did mutation-negative patients. Response to therapy also proved different in terms of having or not the mutation. *JAK2* V617F-positive ET patients are more sensitive to therapy with hydroxyurea, but not anagrelide, compared with mutation-negative patients [13,16]. However, regarding the rate of thrombosis in ET patients according to *JAK2* mutational status, discrepant results have also been reported stating that thrombotic events were similar between mutation-positive and mutation-negative patients [14]. Recently, a deletion/insertion mutation in calreticulin gene (*CALR1*) has been described in some 16-33% of ET and 21-25% of MF leaving only approximately 10-15% of ETs and MFs without known mutation. Two most frequent types of *CALR1* mutations are deletion of 52bp and insertion of 5bp accounting for some 80% of all *CALR1* mutations. Phenotypically *CALR1* mutated patients have higher platelet counts when compared to JAK2 mutated, have lower leukocytes and better prognosis in terms of EFS and OS [17,18].

TPO and *c-MPL* mutations

Thrombopoietin (TPO) is the primary cytokine regulator of thrombopoiesis and it has been reported elevated in patients with ET compared to controls, but similar has been found in patients with other MPNs and in patients with reactive thrombocytosis, so measurement of TPO is not diagnostically useful. Mutations of the TPO gene were reported in some but not all kindreds with familial thrombocytosis, but they are not seen in sporadic ET [19,20]. The thrombopoietin receptor (c-MPL) is another candidate oncogene in ET. Reduced expression of c-MPL in platelets from patients with ET has been reported [21]. A highly penetrant gain-of-function MPL mutation in the transmembrane domain (*MPL*S505N) has been described for hereditary thrombocythemia [22]. Recently, an acquired mutation in c-MPL, *MPL*W515L, has been described that induces constitutive, cytokine-independent activation of the JAK-STAT pathway. Mutation is located in the sequence important for keeping the receptor inactive [23]. (Fig2). Furthermore, the expression of *MPL*W515L in murine bone marrow resulted in an MPD phenotype. *MPL*W515L or *MPL*W515K mutations are present in patients with PMF or ET at a frequency of approximately 5% and 1%, respectively, but are not observed in patients with polycythemia vera (PV) or other myeloid disorders. *MPL* mutations may occur concurrently with the *JAK2*V617F mutation, suggesting that these alleles may have functional complementation in myeloproliferative disease [24].

Other molecular markers in ET

By using microarray technology to study the malignant megakaryocytopoiesis of ET the expression of genes involved in the apoptotic pathway was found impaired in ET CD34-derived megakaryocytes (MK). Moreover, CD34-derived MKs of patients with ET were more resistant to apoptosis than their normal counterparts. Proapoptotic genes *BAX*, *BNIP3*, and *BNIP3L* were down-regulated in ET MKs together with genes that are components of the mitochondrial permeability transition pore complex, a system with a pivotal role in apoptosis. Conversely, antiapoptotic genes such as *IGF1-R* and *CFLAR* were up-regulated in the malignant cells, as was the *SDF1* gene, which favors cell survival [25]. Overexpression of polycythemia rubra vera-1 (*PRV-1*) gene was found in PV, PMF and ET. Overexpression of PRV-1 may be associated with a subset of patients who may be initially defined as ET by diagnostic criteria but may later evolve in PV [14,27]. Another potential candidate molecule able to distinguish between frequently overlapping phenotypes or PV and ET is the expression of angiotensin receptor II (AT2R1) that is highly expressed on hematopoietic cells in PV but not in ET and this irrespective of the JAK2 mutational status of ET [28].

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Sažetak

Genetički aspekti mijeloproliferativnih neoplazmi

Esencijalna trombocitemija je jedna od tri Filadelfija–kromosom-negativne kronične mijeloproliferativne neoplazme (MPN). Drži se za relativno benignog tijeka s preživljenjem pacijenata sličnom onom opće populacije, Ipak, kao klonalna bolest matične stanice može napredovati u sekundarnu fibrozu ili transformirati u akutnu leukemiju. Pokazuje povišeni rizik za hemoragiju (osobito ako su brojevi Tr viši od milijun u mikrolitru) ili trombozu. U posljednjih 10-tak godina postignut je veliki napredak u razumijevanju genetičke osnove ove bolesti. Najvažnija su otkriće točkaste mutacije V617F gena *Janus kinaza 2* (JAK2V617F) koja postoji u 90-tak % ET, > 97% PV te 50% MF. Mutacija *trombopoetinskog receptora* je također važna pri čemu W515L mutacija potiče konstitucijsku aktivnost, nezavisnu od citokina za aktivaciju JAK-STAT signalnog puta. MPLW515L ili MPLW515K su prisutne u otprilike 3% slučajeva. Nedavno je otkrivena mutacija gena za protein endocitoplazmatskog retikuluma, *kalretikulina 1* (*CALR1*). Riječ je o ins/del mutaciji koja je prisutna u 30-38% ET i 21-25% MF, JAK2/MPL-mutacija negativnih. Klinički je ova mutacija povezana s povoljnom prognozom, u koliko nema dodatnih mutacija poput *ASXL1*, a koja onda donosi nepovoljan prognostički trend.

Ključne riječi: genetika trombocitemije, kronične mijeloproliferativne neoplazme

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