MPL Y252H and *MPL F126fs* mutations in essential thrombocythemia: Case series and review of literature

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Abstract

Essential thrombocythemia (ET) is a clonal bone marrow disease, characterized by increased production of platelets along with other clinical and bone marrow findings. Most patients with ET will have a somatic mutation in one of the known gene locations of *JAK2, CALR* , or *MPL* that can upregulate the JAK-STAT pathway. *MPL* mutation is present in 5% of cases with the most common mutations being *W515L* and *W515K.* In this report we describe 2 cases of patients with clinical and laboratory picture of ET. One patient carried *MPLY252H* mutation which is previously unreported in the adult population but has been shown to be a gain-of-function mutation. The other patient carried *MPL F126fs* mutation which is not known to be of clinical importance and has not been previously reported.

Introduction

Essential thrombocythemia (ET) is a clonal bone marrow disease, characterized by increased production of platelets. Most patients with ET will have a somatic mutation in Janus Kinase 2 (*JAK2*), Calreticulin (*CALR*), or myeloproliferative leukemia virus oncogene (*MPL*) with subsequent upregulation of the JAK-STAT pathway. *JAK2 V617F* activating mutation is present in 50-60% of ET cases, *CALR* mutation is present in 20-25% of cases, while *MPL* mutation is present in 5% of cases. Patients who lack all three mutations are usually called triple negative. The most common mutations in *MPL* are *W515L* and *W515K.*[1] Other mutations were also reported such as *S505N*, *W515A* and *W515R*. We hereby report 2 cases of patients with clinical and laboratory picture of ET who carried 2 mutations that are previously unreported in the adult population.

Case Report #1

A 55-year-old female with a past medical history of hypertension, osteoarthritis, neuropathy, and hyperlipidemia presented in consultation for thrombocytosis. She has had thrombocytosis for 8 years prior to presentation. Her laboratory exam showed a platelet count of 558k, white blood cell counts of 9.2k, and hemoglobin of 12.9 gm. She reported having occasional headaches and blurry vision, but was otherwise asymptomatic. Bone marrow biopsy showed mildly hypercellular bone marrow (70%) showing trilineage hematopoiesis with mildly increased megakaryocytes. She had adequate reticuloendothelial iron, with no ringed sideroblasts. There was no fibrosis on reticulin stain. No evidence of causes of secondary thrombocytosis was found. Molecular studies showed *MPLY252H* mutation. Other variants found were *EP300, MAP3K7, NTRK1, and YY1AP1.*

Case Report #2

A 50-year-old female with a past medical history of diabetes, rheumatoid arthritis, and COPD was referred for thrombocytosis. Further review of laboratory data revealed that her platelet count has been elevated for 4 years prior to her referral. Platelet counts had been ranging from 518-600k. Patient has been asymptomatic and denied any history of thrombotic event. Patient declined bone marrow biopsy so molecular studies were sent from peripheral blood. Results revealed multiple abnormalities. *MPL F126fs*5* mutation was detected. Other variants detected included *CSF1R, ERBB4, GPR124, KIT, MUTYH, NFE2L2, PIM1, PTPN6, and RNF43.* Patient was prescribed Aspirin 81 mg and has been asymptomatic with stable counts after 1 year of follow up.

Discussion and Conclusions

ET is a clonal disease characterized by marked thrombocytosis, prominent large to giant megakaryocytes in bone marrow, and absence of other identifiable causes of thrombocytosis. World Health Organization (WHO) diagnostic criteria includes absence of other clonal bone marrow disorders such as chronic myeloid leukemia and other myeloproliferative neoplasms. The demonstration of clonal markers favors the diagnosis. *JAK2*, *CALR* and *MPL* mutations are the most common. Subsequently the JAK-STAT pathway gets upregulated with further stimulation to cell growth and hematopoiesis. Some of the triple negative cases will have an unusual mutation in *JAK2*, *CALR* or *MPL* on whole gene sequencing.[2] Other mutations in different genes such as *ASXL1*, *TET2,* and *CBL* have been reported in patients with MPN. These can exist with other more common gene mutations and are helpful to establish clonality.

The *MPL* gene encodes for a transmembrane receptor that is highly expressed in CD34+ hematopoietic cells and in the megakaryocytic lineage. The murine *v-MPL* gene was discovered in 1990. Shortly afterwards its human homolog *c-MPL* was cloned.[3] The human c-*MPL* gene contains 12 exons. The two cytokine receptor domains are encoded for by eight exons (2-9), the cytoplasmic domain is encoded for by two exons (11-12), and the trans-mem-

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brane domain is encoded for by exon 10. Exon 1 encodes for signal peptide.[3] The gene encodes for a 635 amino acid transmembrane domain (CD 110). Binding of thrombopoietin to the extracellular domain for MPL leads to dimerization of the receptor and activation of the JAK-STAT pathway. Figure 1 illustrates the structure of human MPL receptor.

Disruption of the juxtamembrane region of the thrombopoietin receptor MPL leads to receptor activation in the absence of receptor binding by thrombopoietin.[4] Mutations have been reported in the *MPL* gene. The *MPL W515L* mutation has been linked to ET. Transplanting bone marrow cells that express this mutation into mice leads to marked thrombocytosis and splenomegaly, as well as other features associated with MPN.[5] The *MPL W515K* mutation was discovered several months after *MPL W515L*, and was shown to be linked to the clinical picture of myeloproliferative neoplasms.[1]

Beer *et al*. performed an analysis of molecular data from a large retrospective cohort of unselected patients with ET and Primary myelofibrosis (PMF). The entire *MPL* coding region in 18 patients with ET and 2 patients with PMF was sequenced. No mutations were found outside exon 10 in this study. Subsequently, *MPL* exon 10 in granulocyte DNA from 200 patients was sequenced. Of these, 88 had ET and 112 had PMF. Three patients with ET were found to have mutations. One had the *MPL S505N* mutation and two carried *MPL W515L* mutations. Eight out of 112 PMF patients were found to have mutations. One carried the *MPL S505N* mutation, five carried *MPL W515L* mutations, and two carried *MPL W515K* mutations.[6]

MPL W515A and *MPL W515R* mutations are rare, but they are thought to function like *MPL W515K* and *L*. Given the extremely low mutation rate, the clinical significance is not confirmed.[7] Other mutations described are *A506T*, *L510P,* and *A519T*. The clinical significance of these mutations is still unclear.[8]

MPL S505N can occur of somatic origin in sporadic cases of ET, but has also been reported along with other MPL mutations in cases with hereditary thrombocythemia (HT). In such cases it is a germline mutation that is inherited in an autosomal dominant pattern.[9] *MPL* S505N was found to be associated with an increased risk of thrombosis and subsequently splenomegaly and bone marrow fibrosis.[10]

Further mutations associated with HT include *MPL K39N* and *MPL P106L*. *MPL K39N* is a polymorphism reported in African Americans. Screening of more than 400 patients and controls revealed that approximately 7% of African Americans are heterozygous for this polymorphism and that patients affected had a significantly higher platelets count than controls without the polymorphism.[11] *MPL P106L* is another mutation that was associated with familial thrombocytosis. It was discovered in an Arab family when two siblings presented with thrombocytosis. Molecular studies in a sample of 213 people revealed that the prevalence of this mutation is 3.3% in this cohort of unrelated individuals of Arabic descent. The control group of 193 healthy German individuals had no mutation detected.[12] The clinical outcome of individuals with these 2 mutations (*MPL K39N* and *MPL P106L*) is still unknown. Other germline mutations that are felt to be associated with HT are *MPL V285E* and *MPL R321W*. These are 2 activating germline mutations in exon 6 of *MPL* identified in 2016 in cases initially diagnosed as ET (*R321W*) and PMF (*V285E*).[13]

MPL T119I, *MPL S204F*, *MPL S204P*, and *MPL E230G* were reported in 2016. All of these mutations lead to constitutive activation of JAK-STAT signaling indicating that they are gain of function mutations.[13] Interestingly, all these mutations are outside of exon 10. The same report identified a new somatic mutation *MPL Y591D* in exon 12 in a patient who was initially diagnosed with triple negative ET. This patient, however, developed *JAK2 V617F* mutation at 5.5 years follow-up. *MPLS204P and MPLY591N* were reported in 2016 in patients with triple negative ET. Both mutations

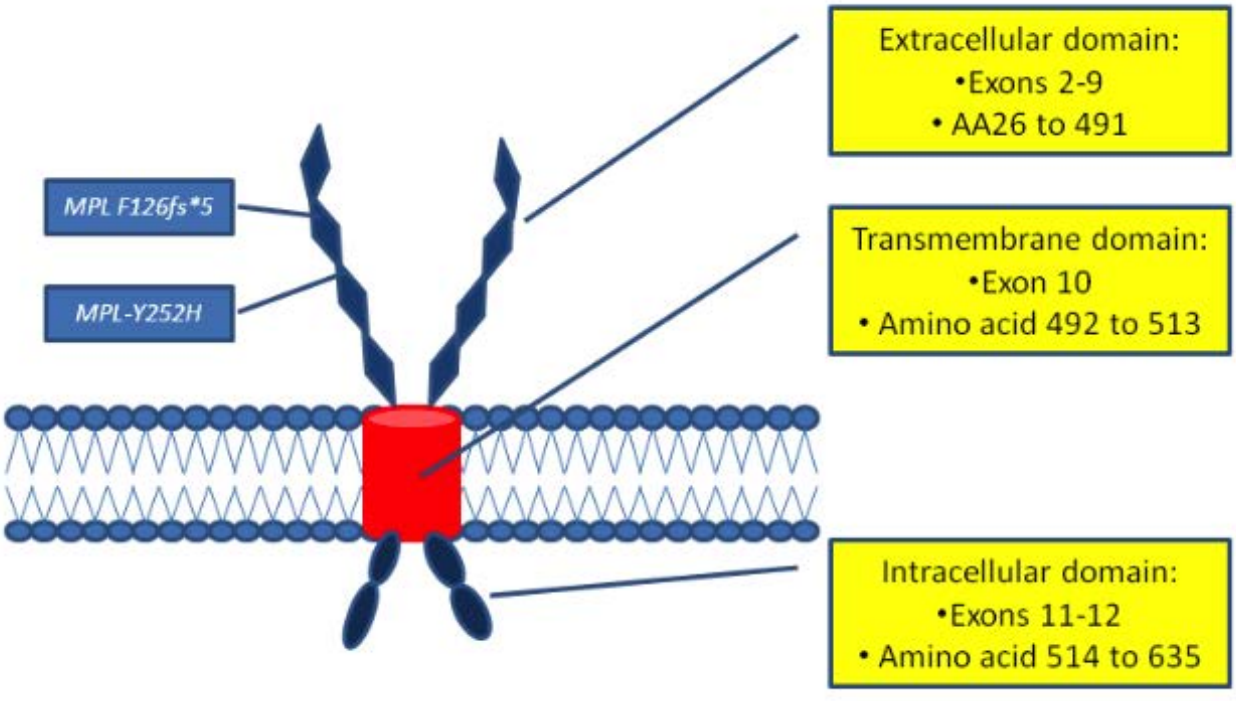

Figure 1. Structure of MPL receptor and location of the 2 reported mutations. Both mutations are in the extracellular domain.

appeared to be gain of function mutations via in vitro studies.[14] Table 1 summarizes the mutations reported in *MPL* gene.[13-17] The *MPL Y252H* mutation was first described in 2011 in a three-year-old African American female with a JAK2 mutation-negative ET. Exposing bone marrow cells from this patient to thrombopoietin lead to generation of megakaryocyte colonies *in vitro*. BaF3 cells with the mutation were found to have increased thrombopoietin mediated cell growth and increased cell survival upon cytokine withdrawal.[15]

The *MPL Y252H* mutation in our case has not been reported in adults before. Based on the work of Lambert et al, this mutation was shown to be a gain of function mutation in the extracellular domain of MPL.[15] Our case supports this finding and documents the first reported adult case with clinical picture of ET.

Our second case documents the first clinical case report of a patient with *MPL F126fs*. This is a frame shift mutation that results in a change in the amino acid sequence of the MPL protein beginning at amino acid 126 of total of 635. This is

Table 1. Mutations reported in MPL gene.

Mutation	type	Clinical effect	Comments [Ref.]
K39N	Polymorphism	Familial thombocytosis.	Approximately 7% of African Americans are heterozygous for this polymorphism [11]
P106L	Germline	Familial thrombocytosis	Prevalence was 3.3% in a cohort of unrelated individuals from Arabic descent [12]
T119I	Somatic	Gain-of-function when analyzed in functional assays	[13]
*F126fs*5*	Somatic		Described in this report in a patient with clinical picture of ET
S204F	Somatic	Gain-of-function when analyzed in functional assays	[13]
S204P	Somatic	Weak gain-of-function mutation	[14]
E230G	Somatic	Gain-of-function when analyzed in functional assays	[13]
Y252H	Somatic	Described in 2011 in a three year old African American female with a JAK2 mutation-negative ET	Described in an adult patient with ET in this report [15]
V285E	Germline	Felt to be associated with HT	Activating germline mutations in exon 6 identified in 2016 in a case initially diagnosed as primary myelofibrosis [13]
R321W	Germline	Felt to be associated with HT	Activating germline mutations in exon 6 identified in 2016 in a case initially diagnosed as ET [13]
T487A	Somatic	Acute megakaryoblastic leukaemia	Induces myeloproliferative disorder in mice [16]
T496-A497 ALVI ins	Somatic	Reported in a patient with PMF	[17]
V501L		Reported in a patient with JAK2 negative MPN	Patient had also *S505N* mutation [17]
S505N	In most cases germline mutation	Hereditary thrombocythemia	Inherited in an autosomal dominant pattern, but reported as a somatic mutation in sporadic cases as well [9,10]
A506T	Somatic	Not gain-of-function mutation based on *in vitro* studies	Both *A506T* and *A519T* mutations were found in a patient with PMF in association with *JAK2 V617F* [8]
V507I		Reported in a patient with JAK2 negative MPN	[17]
L510P	Somatic	Not gain-of-function mutation based on *in vitro* studies	[8]
R514K		Reported in a patient with JAK2 negative MPN	[17]
W515L W515K	Somatic	Essential thrombocythemia	Most common mutations in MPL
W515A W515R	Somatic	Essential thrombocythemia	Given the extremely low mutation rate of W515A/R, the clinical significance is not confirmed but they are reported to function like W515K/L
W515S W515G	Somatic	Detected in patient with JAK2 negative MPN	[17]
W515-P518 del/ins KT	Somatic	MPN	Patient had MPN not otherwise specified [17]
A519T	Somatic	Not gain-of-function mutation based on *in vitro* studies	Both *A506T* and *A519T* mutations were found in a patient with PMF in association with JAK2 V617F [8]
A519V		Reported in a patient with JAK2 negative MPN	[17]
*R525C fs*14*	Somatic	Patient had confirmed chronic MPN not otherwise specified	Exon 11 mutation involving a deletion of 2 nucleotides (AG) and insertion of T with subsequent frameshift and a stop codon after 13 amino acids [17]
D545G D545N	Somatic	Reported in a patient with JAK2 negative MPN	Exon 11 [17]
Y591D	Somatic	ET	Found on exon 12 in a patient who was initially diagnosed with triple negative ET. This patient, however, developed *JAK2 V617F* mutation at 5.5 years follow-up [13]
Y591N	Somatic	Weak gain-of-function mutation	[14]

expected to result in a premature truncation of the functional protein leading to loss of functional domains, which would lead to loss of function. It is interesting that our case documents the occurrence of a clinical picture consistent with ET in a patient with this mutation.

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