MINI-REVIEW

Coexisting *JAK2*V617F and *CALR* Exon 9 Mutations in Myeloproliferative Neoplasms - Do They Designate a New Subtype?

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Abstract

The classic *BCR-ABL1*-negative myeloproliferative neoplasm is an operational sub-category of MPNs that includes polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The *JAK2*V617F mutation is found in ~95% of PV and 50-60% of ET or PMF. In most of the remaining *JAK2*V617F–negative PV cases, *JAK2* exon 12 mutations are present. Amongst the *JAK2*V617F-negative ET or PMF 5-10% of patients carry mutations in the *MPL* gene. Prior to 2013, there was no specific molecular marker described in the remaining 30-40% ET and PMF. In December 2013, two research groups independently reported mutations in the gene *CALR* found specifically in ET (67-71%) and PMF (56-88%) but not in PV. Initially *CALR* mutations were reported mutually exclusive with *JAK2* or *MPL*. However, co-occurrence of *CALR* mutations with *JAK2*V617F has been reported recently in a few MPN cases. Many studies have reported important diagnostic and prognostic significance of *CALR* mutations in ET and PMF patients and *CALR* mutation screening has been proposed to be incorporated into WHO diagnostic criteria for MPN. It is suggestive in diagnostic workup of MPN that *CALR* mutations should not be studied in MPN patients who carry *JAK2* or *MPL* mutations. However *JAK2*V617F and *CALR* positive patients might have a different phenotype and clinical course, distinct from the *JAK2*-positive or *CALR*-positive subgroups and identification of the true frequency of these patients may be an important factor for defining the prognosis, risk factors and outcomes for MPN patients.

Keywords: Calreticulin- essential thrombocythemia - myeloproliferative neoplasms-JAK2V617F

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Introduction

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of chronic myeloid neoplasms with the potential to progress to acute leukemia. All MPNs are clonal disorders with an initial hit in the HSCs resulting in an excessive production of blood cells because of hypersensitivity or independence from normal cytokine regulation.

According to WHO classification system 2008, MPNs comes under eight clinicopathological entities: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic eosinophilic leukemia-not otherwise specified, mastocytosis and MPN-unclassifiable (Vardiman et al., 2009). MPNs involve the three main myeloid lineages but predominate in one of them: the erythroid lineage for PV, the megakaryocyte (MK)/platelet lineage for ET, and the MK/granulocytic lineages for PMF (Vainchenker et al., 2011). Majority of clinical features of MPNs is shared to a variable degree among the three MPN entities (Kralovics, 2008). Routine diagnostic tests for MPNs include red cell mass determination, bone marrow aspirate and trephine biopsy, arterial oxygen saturation and carboxyhaemoglobin level, neutrophil alkaline phosphatase level, vitamin B12 and serum urate. Although these tests strongly support the diagnosis of MPNs, the disease could still not be well discriminated from the reactive hyperplasia. Moreover, misdiagnosis could occur when the symptoms of disease are atypical (Wu et al., 2014).

In addition to morphologic evaluation, the genetic abnormality is a major diagnostic criterion for MPNs and considerable attention has been devoted in the past to explaining the genetic basis of clonal hematopoiesis in MPNs. Discovery of the Philadelphia chromosome in 1960 and the subsequent identification of the BCR-ABL fusion protein in CML was the landmark (Kralovics, 2008). The year 2005 was the time when four seminal studies published defined the molecular pathogenesis of classic"*BCR-ABL1*-negative" chronic MPNs PV, ET and PMF by reporting the existence of a recurrent V617F

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point mutation in exon 14 of Janus kinase 2 (*JAK2*) gene in these three disorders (Guglielmelli et al., 2014). The *JAK2*V617F mutation is found in the vast majority (~95%) of PV patients and 50-60% of those with ET or PMF (ET~55%, PMF~65%) (Klampfl et al., 2013; Nangalia et al., 2013; Guglielmelli et al., 2014; Lin et al., 2015). In most of the remaining *JAK2*V617F–negative PV cases, *JAK2* exon 12 mutations were first described in 2007. These *JAK2* exon 12 mutations, such as insertions or deletions, are relatively specific to *JAK2*V617F-negative PV and not found in ET and PMF. Amongst the *JAK2*-negative ET or PMF patients 5-10% of patients carry mutations in exon 10 at codon 515 of the gene *MPL* encoding the thrombopoietin receptor. Both *JAK2* and *MPL* are gain of function mutations and promote the over activation of STAT signaling largely mediated by abnormal and sustained phosphorylation of *JAK2* (Tefferi et al., 2010; Vainchenker et al., 2011; Klampfl et al., 2013; Nangalia et al., 2013; Guglielmelli et al., 2014; Lin et al., 2015). The close association of *JAK2*V617F mutation with MPNs led to a revision of the WHO diagnostic criteria in 2008, where *JAK2*V617F, *JAK2* exon 12 and *MPL*515 mutations were elected to represent new major diagnostic criteria for the three classic MPN (Guglielmelli et al., 2014). Prior to 2013, there was no specific molecular marker described in the remaining 30-40% of ET and

Table 1. JAK2V617F Positive MPN Cases with *CALR*/*MPL*

S. No	Disease	Population	*CALR* mutation	*MPL*	Method	Reference
1	1 PMF	Caucasian (USA)	c.1099_1150del;L367fs*46		Sanger sequencing	Tefferi et al., 2014a (Epub 9 Jan, 2014)
2	1 ET	Caucasian (Switzer-land)	c.1154_1155insTTGTC;p. K385fs*47		Allele specific PCR	Lundberg et al., 2014 (Epub 29 Jan, 2014)
3	1 ET 1 ET	Asian (China)	c.1099_1150del;L367fs*46 c.997 C>T (arginine>tryptophan)		Sanger sequencing	Fu et al., 2014 (Epub 15 Apr, 2014)
4	1 ET	Caucasian (UK)	c.1094_1139del;p.Q365fs*50		PCR Fragment analysis and Sanger sequencing	McGaffin et al., 2014 (Epub 17 Jun, 2014)
5	1 ET 1 PV	Asian (China)	c.1099_1150del; p.L367fs*46 c.1095_1097del;p.E371fs*49		Sanger sequencing	Xu et al., 2015 (Epub 5 Nov, 2014)
6	1 ET 1 ET	Asian (Japan)	c.1131_1152del;p.E378fs*45 W515L		Fragment analysis and deep sequencing	Shirane et al., 2015 (Epub 14 Nov, 2014)
7	1 ET	Asian (Korea)	c.1099_1150del;L367fs*46		Sanger sequencing	Ha and Kim, 2015 (Epub 8 Dec, 2014)
8	1 PMF	Caucasian (Spain)	c. 1142_1144del; E380del		6-FAM and Sanger sequencing	Zamora et al., 2015 (11 Mar, 2015)
9	13 ET	Asian (Taiwan)	c.1095_1140del;p.L367fs*48 c.1142A>C; p.E381A c.1154_1155insTTGTC;p. K385fs*47 c.1108del;p.E370fs*60 c .1111del;p.E371fs*59 c.1110_1112del;p.E371del c.1132_1134del;p.E378del c.1188_1190del;p.E396del c.1120A>T;p.E374X c.1138G>T;p.E380X c.1171A>T;p.K391X c.1115A>G;p.E372G c.1139A>G;p.E380G		High Resolution Melting analysis and Sanger sequencing	Lim et al., 2015 (Mar, 2015)
10	1 ET	Caucasian (Belgium)	c.1153_1154insTCTGT;K385fs*47		Sanger sequencing	Al Assaf et al., 2015 (Epub 1 May, 2015)
11	2 ET 2 ET 1 PMF 1 MPN-U 1 ET 1 PMF	Asian (China)	c.1099_1150del;L367fs*46 c.1153_1154insTCTGT;K385fs*47 c.1120_1125AAGAAA>TGCGT; K374fs*56 c.1092_1143del;L367fs*46	W515L W515S	Sanger sequencing	Lin et al., 2015 (Jul, 2015)
12	1 ET	Asian (Pakistan)	c.1214_1225del;p.E405_D408del		Sanger sequencing	Rashid et al., 2015 (September, 2015)

Epub=E publication

PMF patients that has now largely been resolved by the discovery of mutations in the gene *CALR*eticulin (*CALR*) (Klampfl et al., 2013; Nangalia et al., 2013; Guglielmelli et al., 2014; Lin et al., 2015). In December 2013 two research groups independently reported mutations in the gene (*CALR* found specifically in ET (67-71%) and PMF (56-88%) but not in PV (Klampfl et al., 2013; Nangalia et al., 2013). Many studies have reported important diagnostic and prognostic significance of *CALR* mutations in ET and PMF patients and *CALR* mutation screening has been proposed to be incorporated into WHO diagnostic criteria of MPNs (Tefferi et al., 2014c).

It was found initially that *CALR* mutations were mutually exclusive with *JAK2* or *MPL* (Klampfl et al., 2013; Nangalia et al., 2013). However, co-occurrence of both the mutations has been reported recently in few MPN cases (Table 1) across different ethnic groups. Until now 25 different types of *CALR* mutation have been reported to coexist with *JAK2*V617F mutation in MPN, all in exon 9. In 2014 Tefferi et al. (2014a) first reported the co-existence of *JAK2*V617F and *CALR* mutations in a patient of PMF. In an ET patient it was first reported by Lundberg et al., in 2014 whereas in PV it was also reported first in 2014 by Xu et al., Though the frequency of this co-occurrence was usually below 1%, Lim et al., (2015) reported it with a higher 6.8% frequency. Majority applied Sanger sequencing to detect *CALR* exon 9 mutations. Lim et al., (2015) applied a sensitive HRMA method to detect *CALR* mutations that enabled them to detect even low allele burden mutants which might be the reason of detecting higher frequency. According to Zmora et al., (2015) the low percentage of *JAK2*V617F and *CALR* positive patients could be underestimated as the majority of groups only test *CALR* mutations in *JAK2* V617F negative patients.

The phenotypic manifestations of *CALR* and *JAK2*V617F differ in ET and PMF. It has been observed in many studies that patients with *CALR* mutation often had a low risk of thrombosis, with lower hemoglobin and leukocyte counts, higher platelet count and longer survival in comparison to those who had *JAK2*V617F mutation (Klampfl et al., 2013; Nangalia et al., 2013; Fu et al., 2014; Rotunno et al., 2014; Tafferi and Pardanani, 2014; Tefferi et al., 2014a; 2014b; 2014c). Lundberg et al. (2014) analyzed a cohort of MPN patients to detect somatic mutations by targeted NGS of 104 genes and found that patients with two or more somatic mutations formed a high-risk category, with increased risk of transformation into AML and reduced survival. Though *CALR* and *JAK2*V617F co-existence has been reported in few MPN cases across different ethnic groups but unfortunately there are not many reports on the phenotype and clinical course of those patients. Patient reported in the study of McGaffin et al., (2014) was a 79 year old female who was well and had no history of bleeding, thrombosis, cerebrovascular disease or any other occlusive symptoms. Xu et al., (2015) provided the first data in detail regarding the phenotype and clinical outcomes in *CALR* and *JAK2*V617F positive patients and they have more patients with both mutations under observation. Their ET patient was a 63 year old female who was well and without history of bleeding, thrombosis, cerebrovascular disease, or any other occlusive symptoms and was in complete hematologic remission at the time of publication whereas the other one was a 65 year old male PV patient who was still in complete remission without cytoreductive therapy. Both patients also reported a positive response to interferon alfa therapy. In the study of Zmora et al., (2015) the double positive patients was 86 year old male PMF patient who died of acute myocardial infarction. Lim et al., (2015) reported the high frequency (6.8%) until now of patients with both mutations. They studied 92 ET patients and found 13 patients with both mutations. *CALR* and *JAK2*-mutated ET patients in their study were found to be associated with oldest age, higher thrombotic events and higher major arterial thrombotic events after diagnosis and more patients were in the high-risk group for thrombohemorrhagic complications. Rashid et al., (2015) reported 55 year old female ET patient who was in complete remission without cytoreductive therapy at the time of publication.

It is suggestive in diagnostic workup of MPN that *CALR* mutations should not be studied in those MPN patients who are already known to carry *JAK2* or *MPL* mutations (Tafferi and Pardanani, 2014). Either it is the difference of methods to detect *CALR* mutations in *JAK2*V617F positive samples or a different mutation spectrum in Caucasian and Asian population or the fact that majority of groups follow the suggestive diagnostic workup for MPN but these double positive patients might represent a new subtype in MPNs. As suggested by Lim et al. (2015) *JAK2*V617F and *CALR* positive patients make a specific sub group of patients and require a careful follow-up and management. McGaffin et al. (2014) and Zmora et al. (2015) proposed that double-mutant patients might have a different phenotype and clinical course, distinct from the *JAK2*-positive or *CALR*-positive subgroups and identification of the true frequency of these patients may be an important factor for defining the prognosis, risk factors and outcomes for MPN patients when mutation status is the criterion to allocate subgroups. In current scenario when inclusion of *CALR* is expected in revision of the WHO diagnostic criteria it is still to decide what should be the workup for MPN with respect to *CALR* and *JAK2*V617F.

Conclusion

Many studies have suggested that *CALR* mutations have important diagnostic and prognostic significance in ET and PMF patients and should be incorporated into WHO diagnostic criteria of MPN. Identification of the true frequency of patients with *JAK2*V617F and *CALR* mutation and findings of their clinical course and phenotype is necessary to define the prognosis, risk factor, and outcomes for those MPN patients carrying both mutations.

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