

Mutation status of essential thrombocythemia and primary myelofibrosis defines clinical outcome

For the majority of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF), clinical diagnosis can be aided by mutational findings in *JAK2*, *CALR* or *MPL*. The *JAK2* V617F mutation is found in about half of the cases and has been included as a major criterion for diagnosis in the WHO 2008 classification.¹ A minor proportion has instead been reported to harbor mutations in the *MPL* gene. The most frequently reported mutations, W515L and W515K, have been found in up to 5% of ET and 10% of PMF patients.² In remaining cases, mutations in exon 9 of the *CALR* gene have filled a substantial part of the molecular diagnostic gap.^{3,4} Although there is a diagnostic value of mutations in the three genes *JAK2*, *CALR* and *MPL*, the impact of the mutation type correlated to the clinical outcome for patients has not been fully elucidated. Reports indicate that the outcome varies with different mutations. The clinical course of patients with *CALR*-mutated disease seems to be more indolent with a lower risk of thrombosis in ET and better overall survival (OS) in PMF compared to *JAK2* mutated patients.⁵ It has been suggested that myeloproliferative neoplasms (MPNs) might be diagnosed based on mutation type to better match prognosis, rather than the classical phenotypical division.⁶ Moreover, a small group of ET and PMF patients are triple-negative (TN), i.e. lacking any of these mutations. This indicates that mutations in other genes might be important for the development of MPN. If so, these mutations could be useful as diagnostic or prognostic markers. In this study, we searched for additional mutations in the TN group and compared the findings and clinical data to patients with mutations in *JAK2*, *CALR* or *MPL*, to study if gene mutations could diversify diagnosis and serve as prognostic markers. The type of mutation was found to have prognostic implications, and TN ET and PMF are uncommon but indicate inferior survival.

91% of all patients diagnosed with ET or PMF between 2008-2013 in Western Sweden were analyzed for mutations in *JAK2*, *CALR* and *MPL* (Figure 1). Among ET patients, 12% were TN while 19% were TN in the PMF group. The heterogeneous nature and lack of recurrent mutations led us to critically review the diagnosis of all TN patients to make sure the initial diagnosis was correct. Two ET patients with TN disease were assigned as PMF instead. Four patients (two ET and two PMF) were excluded because they could not be classified as MPN according to the current diagnostic criteria.

To search for other mutations, a sequencing panel including genes associated with myeloid malignancies was used. All TN patients and controls in the ET group harboring *JAK2*, *CALR* or *MPL* mutations were analyzed (*Online Supplementary Methods*). Interestingly, the number of patients harboring *MPL* mutations doubled after extended analysis. One ET case had a S505N mutation. This mutation was originally associated with familial thrombocythemia⁷, but has since been identified as also being a somatic mutation.⁸ The other mutations affected the W515 codon, but led to alternative amino acid substitutions. One ET and one PMF patient harbored a W515A mutation, one ET patient had a W515R mutation and one had a W515R mutation in combination with a Q516E mutation. Both the W515R and W515A mutations have been reported as rare, pathogenic mutations.⁹ In the current patient cohort, however, these were as frequent as the W515L mutation. This finding warrants more extensive screening for *MPL* mutations in a diagnostic setting, e.g. with a sequencing panel or high-resolution melting technique.

The discovery of additional *MPL* mutations in combination with a review of the diagnosis led to a regrouping of the patients which decreased the TN ET to 6% (Figure 1). Thus, it is relatively uncommon with ET without mutations in *JAK2*, *CALR* or *MPL*.

To investigate if the type of mutation could serve as a prognostic factor, correlation to clinical outcome was performed for the four groups (*JAK2*, *CALR*, *MPL* and TN)

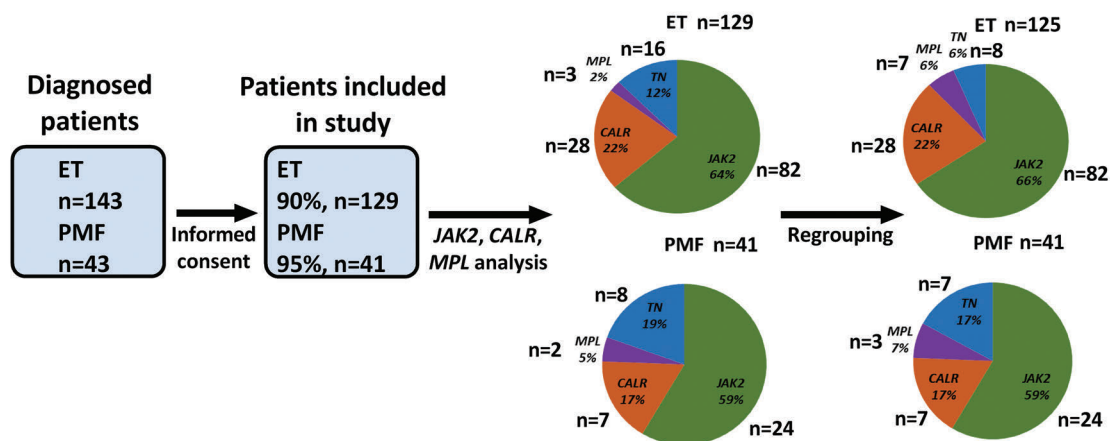


Figure 1 Study design and distribution of mutations. Patients diagnosed with ET or PMF between 2008 and 2013 in Western Sweden. All patients were initially analyzed for the *JAK2* V617F mutation. Since mutations in *JAK2*, *CALR* and *MPL* have been reported to be mutually exclusive in MPN, patients without *JAK2* mutations were further analyzed for mutations in *CALR* exon 9 and, if negative, also for the *MPL* W515K/L mutations as described in *Online Supplementary Methods*. After a critical review of the diagnosis of TN patients and the findings of additional *MPL* mutations, patients were regrouped and excluded if they could not be classified as MPN.

after regrouping (Online Supplementary Table S1 and S2). This revealed fewer vascular complications ($P=0.035$) and a milder clinical course, in spite of significantly higher platelet levels ($P=0.0002$) in ET patients with CALR mutations than in patients in the other mutation groups. ET cases with *JAK2* V617F mutation had features similar to polycythemia vera (PV), but importantly without meeting the diagnostic criteria for this disease. These patients had significantly higher hemoglobin and hematocrit values and lower EPO values ($P=0.0007$, $P=0.0081$ and $P<0.0001$ respectively) compared to non *JAK2*-mutated patients. This strengthens the idea that the phenotypical division in PV and ET should be substituted for a classification based on the type of mutation status to better match clinical prognosis.⁶ ET patients harboring a *MPL* mutation had significantly lower hemoglobin and hematocrit values and consequently higher EPO values ($P=0.0002$, $P=0.0004$ and $P=0.0094$ respectively). These patients also had a significantly higher proportion of transformation to secondary MF and acute myeloid leukemia (AML, $P=0.031$) compared to the other mutation groups. Two out of seven ET patients with a *MPL* mutation had transformed (both to AML), in contrast to

two out of all the remaining 118 patients. PMF patients with TN disease also had a significantly higher proportion of transformation to AML ($P=0.024$) compared to the other groups of mutations. Similar findings were found in the PMF patient group as in the ET group, but no other differences regarding either clinical data or vascular complications were significant in this smaller group of patients.

Survival analysis of the different mutation groups was performed on all patients. Both ET and PMF patients showed a significant difference in OS based on mutation (Figure 2). Patients with a *CALR*-mutated disease had a superior OS rate. Among the ET patients, there was a significantly inferior OS rate for patients with *MPL*-mutated disease. Only two patients were still alive at the end of the study. Both surviving patients had the W515L mutation, which previously has not been associated with inferior survival.⁸ However, since the median age of patients with *MPL* mutations was significantly higher than in the other groups, survival was also analyzed in patients aged 70 and over. This still showed a pattern of inferior survival of patients with *MPL* mutated ET, although no

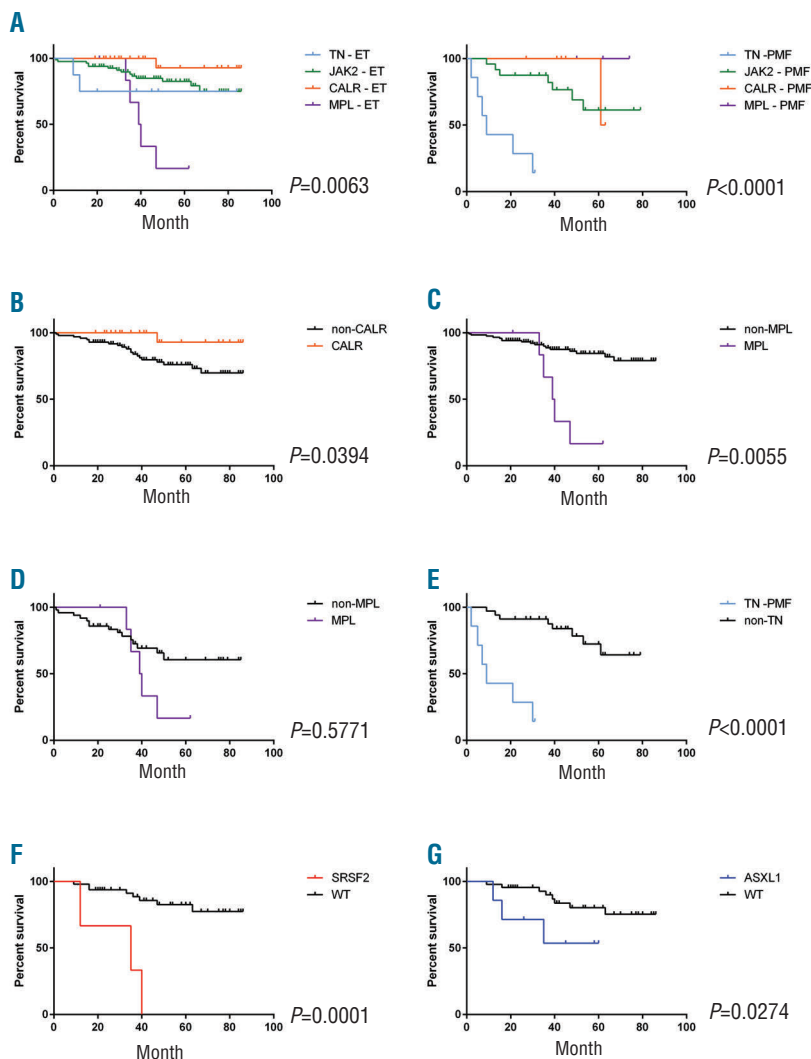


Figure 2. Survival correlated to gene mutations.

Survival curves of patients according to mutation profile. (A) Survival of all ET patients ($n=125$) and PMF patients ($n=41$) based on mutation group (*JAK2*, *CALR*, *MPL* or *TN*). (B) Survival of ET patients with *CALR* mutation ($n=28$) compared to ET patients without *CALR* mutation ($n=97$). (C) Survival of ET patients with *MPL* mutation ($n=7$) compared to ET patients without *MPL* mutation ($n=118$). (D) Survival of ET patients with *MPL* mutation aged 70 and above. Patients with *MPL* mutation ($n=7$) compared to patients without *MPL* mutation ($n=49$). (E) Survival of *TN* PMF patients ($n=7$) compared to PMF patients with mutation in *JAK2*, *CALR* or *MPL* ($n=34$). (F) Survival of ET patients with *SRSF2* mutation ($n=3$) compared to ET patients without *SRSF2* mutation ($n=48$). (G) *ASXL1* mutated ET ($n=7$) compared to ET patients without *ASXL1* mutation ($n=44$).

longer statistically significant. No significant difference in survival was seen among ET patients in the *JAK2* or TN groups compared to the rest of the patients ($P=0.8537$ and $P=0.1759$ respectively). In the PMF group TN patients had a significantly shorter survival time. This indicates a different clinical course with worse prognosis for this subgroup.

In addition to mutations in *JAK2*, *CALR* and *MPL*, several other genes have been found to be mutated in MPN, e.g. *TET2*, *DNMT3A* and *ASXL1*.¹⁰ In the current study, mutations were found in 35 additional genes (Figure 3 and *Online Supplementary Table S3*). All but one patient with *MPL* mutated ET had additional mutations, while in 39% of ET cases with *JAK2* mutation ($n=7$) and 28% with *CALR* mutation ($n=5$) no additional mutations were found. This was also the case in three ET and one PMF patient with TN disease. TN patients could have mutations affecting the same signaling pathways as do muta-

tions in *JAK2*, *CALR* or *MPL*, although not included in our gene panel. A more extended search for additional mutations, e.g. with exome sequencing, could be warranted to unravel the molecular mechanism behind their disease.

TET2 was the most frequently mutated gene, mutated in one third of analyzed cases. The large number of different mutations ($n=19$) makes it difficult to use *TET2* as a molecular marker, and *TET2* mutations have also been reported in other hematological malignancies.¹¹ Moreover, *TET2* mutations, in addition to *DNMT3A* and *ASXL1* mutations, have been reported in apparently healthy individuals (i.e. clonal hematopoiesis) especially in the elderly.¹² Herein, mutations in *DNMT3A* were found in 14% of analyzed samples and *ASXL1* in 12% of analyzed samples. When correlating these mutations to survival in the analyzed ET cohort ($n=51$), patients with *ASXL1* mutation were found to have worse OS (Figure 2).

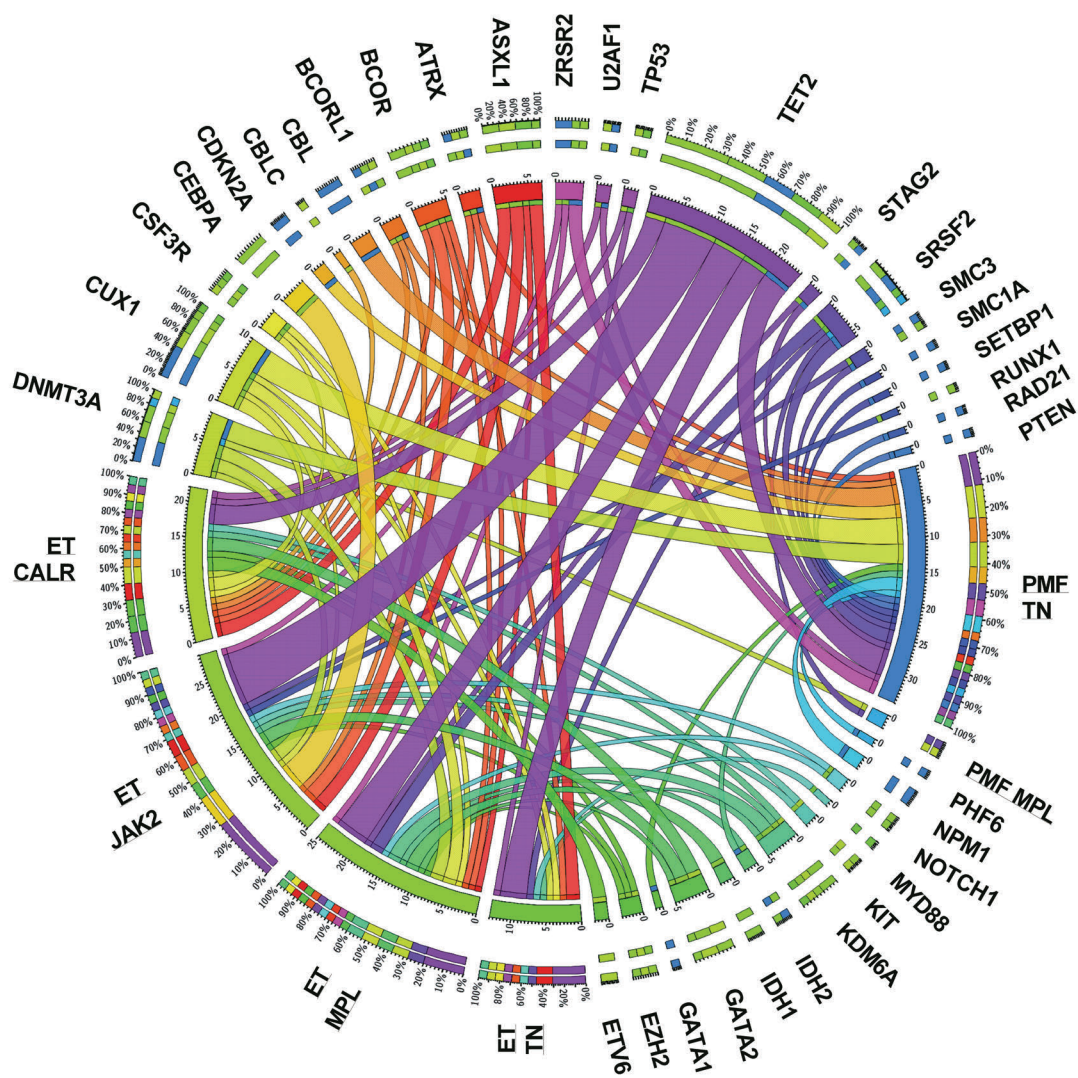


Figure 3. Mutations in additional genes. Mutations detected by TruSight myeloid sequencing panel in TN patients (ET and PMF) and in ET patients with mutation in *JAK2*, *CALR* or *MPL*. On average two and four additional genes were mutated in ET and PMF, respectively (range 0-11). No clear differences were seen between the mutation groups, neither regarding the number ($P=0.10$) nor the type of identified mutations.

This has recently been reported for PMF, but no significant difference for survival in ET was seen.¹³ In addition, mutation in *SRSF2* significantly correlated to inferior OS in ET patients in the current study. Three different mutations affecting the mutational hotspot P95 were found in three ET and three PMF patients. All patients with *SRSF2* mutated ET were deceased (median survival 35 months). Only one PMF patient harboring *SFSR2* mutation was still alive. Notably, however, the *MPL* and *TN* groups harbored most of the mutations in *ASXL1* and *SRSF2*. No significant difference in survival was seen for patients with other mutations. However, survival analyses for mutations in additional genes were performed only on a subset of cases and need to be confirmed in larger patient cohorts.

In conclusion we show that mutations in *JAK2*, *CALR* and *MPL* in ET and PMF also have prognostic implications besides diagnostic value. ET and PMF without mutations in *JAK2*, *CALR* and *MPL* seem to be a rare circumstance, and thus an extended analysis for mutation in these genes is the first priority in diagnosis. Additional gene screening gave limited extra prognostic information, with the exception of *SRSF2* and *ASXL1*. A more extended genetic analysis of *TN* ET and PMF patients will reveal the validity of these entities.

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