Frequency of Calreticulin (CALR) Mutation and Its Clinical Prognostic Significance in Essential Thrombocythemia and Primary Myelofibrosis: A Meta-analysis

Hao Kong, Yancheng Liu, Sai Luo, Qiaoqiao Li and Qinglu Wang

Abstract

Objective As the calreticulin (CALR) mutation frequency is significantly associated with essential thrombocythemia (ET) and primary myelofibrosis (PMF), this mutation may be an important biomarker in patients with ET and PMF.

Methods We performed a literature search until April 2015 and obtained 21 relevant studies. The outcome was pooled as the effect size by using the Stata software program.

Results The CALR mutation frequencies in patients with ET and PMF were 19% and 22%, respectively. The CALR mutation ratio in Asian patients with ET was 23% and higher than that in European-American patients (16%). Moreover, the mutation ratio in Asian patients with PMF was lower (21%) than that in European-American patients (23%). A slight trend toward fibrotic transformation was found in ET with CALR mutations, whereas leukemic transformation was not significant in patients with ET or PMF with CALR mutations.

Conclusion CALR mutations significantly influence the incident of ET as demonstrated by the meta-analysis.

Key words: CALR mutation, essential thrombocythemia, primary myelofibrosis, meta-analysis


Introduction

Myeloproliferative syndrome is characterized by relatively specific molecular markers, such as JAK2 and MPL mutations, which are found in approximately 60% of patients with PMF or ET (4). As a recent study identified calreticulin (CALR) mutation in patients with ET and PMF lacking JAK2 and MPL mutations (5, 6), we performed a meta-analysis to estimate the frequency of CALR gene mutations in patients with ET or PMF.

In a previous study which identified CALR mutation in patients with ET or PMF, the results of a multivariate Cox regression analysis of the overall survival (OS) showed that patients with CALR mutation had a lower risk of death than those with JAK2 (V617F) or MPL mutation (5). Therefore, in the present study, we investigated whether the OS of patients with ET or PMF was associated with CALR mutation. The International Prognostic Score for Essential Thrombocythemia (IPSET) (7), International Prognostic Scoring Sys-
Materials and Methods

Literature search

We searched the PubMed, Embase, and Web of Science databases until April 2015 using the following terms: “Calreticulin,” “CALR,” “thrombocythemia,” “myelofibrosis,” “Myeloproliferative neoplasms,” and any combinations thereof. The references of the potential studies, as well as article reviews and bibliographies, were manually checked for additional eligible studies.

Inclusion and exclusion criteria

The inclusion criteria for the meta-analysis included the following: (1) the paper must involve cases; (2) the relationship between the frequency of CALR mutation and ET or PMF must be assessed; (3) the paper must provide the number of patients with CALR mutation and wild-type CALR; (4) the paper must be written in English; and (5) the research must provide sufficient information to estimate the RR and its 95% confidence intervals (CIs).

The exclusion criteria for the analysis included the following: 1) reviews, conference abstracts, and case reports; 2) articles without sufficient data to calculate the RR of the relationship between the frequency of CALR mutation and ET or PMF; 3) articles published in languages other than English; and 4) overlapping/redundant articles.

Data extraction and study quality assessment

Two investigators (Y.L. and S.L.) reviewed each eligible study and extracted the following data: the name of the first author, publication year, nationality, number of patients, number of patients with CALR mutation, number of patients without CALR mutation, and disease type (ET or PMF). Controversies were left to the arbitration of a third investigator (Q.W.). The Newcastle-Ottawa quality assessment scale was used to evaluate the quality of each study (12). Finally, we selected 21 articles, which included two articles for the meta-analysis of fibrotic transformation, leukemic transformation, and risk analysis of patients with CALR mutation.

Statistical analysis

The prevalence estimate (%), RR, and their 95% CIs were determined to assess the relationship of CALR gene mutation with ET or PMF, fibrotic transformation, and leukemic transformation and analyze high risks for patients with CALR and JAK2 mutations. The prevalence estimate (%) and its 95% CI were included in the summary statistics for the pooled analysis of the effect of CALR gene mutation on patients with ET or PMF. The RR and its 95% CI were also mentioned in the summary statistics for the pooled analysis of the effect of CALR gene mutation on fibrotic transformation, leukemic transformation, and high risks. Statistical variables were directly pooled if they were described in the literature; otherwise, the variables were calculated based on the available numerical data in the articles according to the methods described by Parmar et al. (13). An observed RR=1 implies unfavorable parameters for the group without a significant effect of CALR gene mutation on fibrotic and leukemic transformations and high risks for patients with CALR and JAK2 mutations. The effects of CALR gene mutation on fibrotic transformation, leukemic transformation, and high risks were statistically significant if the 95% CIs did not overlap with 0. Heterogeneity (I²) across studies was assessed using the Chi-square-based Q statistical test. The F statistic was measured to quantify the total variation proportion, which is ascribed to inter-study heterogeneity rather than sampling error and measured from 0 to 100%. A p > 0.10 for the Q statistical test indicated an absence of heterogeneity among the studies, and the pooled effect size (ES) or RR estimate of each study was calculated using the fixed-effects model (Mantel-Haenszel method). Egger’s plot was used to evaluate the probability of publication bias. Statistical analyses were conducted using the Stata/SE 12.0 software program for Windows. All p values presented were obtained from two-tailed tests and considered to be statistically significant at p<0.05.

Results

Description of the studies

A total of 21 studies (including two studies for the meta-analysis of fibrotic and leukemic transformations and risk analysis of patients with CALR mutation) were identified from the database search (Fig. 1, Table 1). The abstracts and full texts of the identified studies were evaluated. A total of 19 eligible studies were selected for the meta-analysis of the prevalence estimates (5, 6, 14-30) (Table 1). Among these studies, four studies were selected for the meta-analysis of fibrotic transformation (15, 19, 31, 32) (Table 2), five studies for the meta-analysis of leukemic transformation (15, 16, 29, 31, 33) (Table 3), and five studies for the meta-analysis of risks for patients with CALR mutation (15, 16, 18, 23, 31, 33) (Table 4). The clinical features of the 21 studies published from 2013 to 2015 are summarized in Tables 1-4. A total of 3,141 patients with ET and 1,605 patients with PMF were enrolled to determine the prevalence estimates for patients with CALR mutation. A total of 1,664 patients...
were enrolled to investigate ET fibrotic transformation, 1,972 patients for leukemic transformation, and 2,324 patients for a high risk analysis of ET and PMF. Sample sizes of the included studies ranged from four to 614 patients. The 21 studies included were conducted in Italy, USA, Austria, UK, Spain, Romania, Hungary, Mexico, Israel [because...
that of European-American patients (e.g., 23% of patients population, the ratio of patients with ET was higher than 23% of European-American patients with PMF. In the Asian population, we found in 16% of European-American patients with ET and 17-20% (95% CI: 20-24%), whereas that of Asia patients with ET and EMF was 18% (95% CI: 17-20%), as shown in Fig. 2A. The pooled CALR mutation prevalence estimate of European-Americans rather than Asians (34) and Poland (European-American population); and Japan, China, and Korea (Asian population).

**Meta-analysis results**

The pooled CALR mutation rates differed among patients with ET (ES=19%, 95% CI: 17%-20%) and PMF patients (ES=22%, 95% CI: 20%-24%), as shown in Fig. 2A. The pooled CALR mutation prevalence estimate of European-American patients with ET and EMF was 18% (95% CI: 17-20%), whereas that of Asian patients with ET and EMF was 19% (95% CI: 17%-20%).

### Table 2. Characteristics of Studies Included for the Meta-analysis of Fibrotic Transformations for ET with CALR Mutation.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Disease</th>
<th>Genomic DNA obtained from</th>
<th>Leukemic transformations for ET with CALR mutation (transformation number/patients number)</th>
<th>Leukemic transformations for ET without CALR mutation (transformation number/patients number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumi**</td>
<td>2014</td>
<td>USA/Italy/Austria</td>
<td>ET</td>
<td>bone marrow biopsy peripheral blood or bone marrow biopsies</td>
<td>7/176</td>
<td>5/466</td>
</tr>
<tr>
<td>Rotunno</td>
<td>2014</td>
<td>Italy</td>
<td>ET</td>
<td>bone marrow biopsy peripheral blood or bone marrow biopsies</td>
<td>4/89</td>
<td>15/487</td>
</tr>
<tr>
<td>Chen</td>
<td>2014</td>
<td>China</td>
<td>ET</td>
<td>bone marrow biopsy</td>
<td>1/33</td>
<td>5/114</td>
</tr>
<tr>
<td>Telleri</td>
<td>2014</td>
<td>USA</td>
<td>ET</td>
<td>bone marrow biopsy</td>
<td>11/95</td>
<td>17/204</td>
</tr>
</tbody>
</table>

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### Table 3. Characteristics of Studies Included for the Meta-analysis of Leukemic Transformations for Patients with CALR Mutation.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Disease</th>
<th>Genomic DNA obtained from</th>
<th>Leukemic transformations for patients with CALR mutation</th>
<th>Leukemic transformations for patients without CALR mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumi**</td>
<td>2014</td>
<td>Italy/USA</td>
<td>PMF</td>
<td>bone marrow biopsy</td>
<td>6/69</td>
<td>25/208</td>
</tr>
<tr>
<td>Tefferi#</td>
<td>2014</td>
<td>USA</td>
<td>PMF</td>
<td>bone marrow biopsy</td>
<td>8/95</td>
<td>10/204</td>
</tr>
<tr>
<td>Rumi**</td>
<td>2014</td>
<td>Italy/USA</td>
<td>ET</td>
<td>bone marrow biopsy</td>
<td>2/176</td>
<td>12/466</td>
</tr>
<tr>
<td>Park</td>
<td>2015</td>
<td>Korea</td>
<td>ET</td>
<td>bone marrow biopsy</td>
<td>0/12</td>
<td>0/26</td>
</tr>
</tbody>
</table>

*Blood, 124(7): 1062-1069; **Blood, 123(10): 1544-1551; #Leukemia; *Am J Hematol

### Table 4. Characteristics of Studies Included for the Meta-analysis of Risks for Patients with CALR Mutation.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Disease</th>
<th>Genomic DNA obtained from</th>
<th>High risk for patients with CALR mutation</th>
<th>High risk for patients without CALR mutation</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumi**</td>
<td>2014</td>
<td>Italy/USA</td>
<td>PMF</td>
<td>bone marrow biopsy</td>
<td>8/140</td>
<td>16/399</td>
<td>IPSS</td>
</tr>
<tr>
<td>Tefferi#</td>
<td>2014</td>
<td>USA</td>
<td>PMF</td>
<td>bone marrow biopsy</td>
<td>11/61</td>
<td>51/146</td>
<td>DIPSS-plus</td>
</tr>
<tr>
<td>Tefferi*</td>
<td>2014</td>
<td>USA</td>
<td>ET</td>
<td>bone marrow biopsy peripheral blood or bone marrow biopsies</td>
<td>16/114</td>
<td>52/227</td>
<td>IPSET</td>
</tr>
<tr>
<td>Rumi**</td>
<td>2014</td>
<td>Italy/USA</td>
<td>ET</td>
<td>bone marrow biopsy</td>
<td>6/176</td>
<td>63/466</td>
<td>IPSET</td>
</tr>
<tr>
<td>Qiao</td>
<td>2014</td>
<td>China</td>
<td>ET</td>
<td>bone marrow biopsy peripheral blood samples</td>
<td>1/69</td>
<td>40/153</td>
<td>IPSET</td>
</tr>
<tr>
<td>Qiao</td>
<td>2014</td>
<td>China</td>
<td>PMF</td>
<td>bone marrow biopsy peripheral blood samples</td>
<td>0/4</td>
<td>0/29</td>
<td>DIPSS-plus</td>
</tr>
<tr>
<td>Li</td>
<td>2014</td>
<td>China</td>
<td>PMF</td>
<td>bone marrow biopsy</td>
<td>7/70</td>
<td>27/270</td>
<td>DIPSS-plus</td>
</tr>
</tbody>
</table>

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the closest genetic neighbors of the Israeli population are Europeans rather than Asians (34) and Poland (European-American population); and Japan, China, and Korea (Asian population).
heterogeneity was significantly reduced ($I^2=62.8\%$). The heterogeneity of the PMF and ET data significantly reduced to 59.3% and 48.6%, respectively. The reason for these data enhancing the heterogeneity was unclear.

Four studies reported the effect of CALR mutation on fibrotic transformation of patients with ET (15, 19, 31, 32), and these data were pooled for the meta-analysis. The results (Fig. 4A) indicated that CALR mutation slightly affected fibrotic transformation in patients with ET, and the RRs of the influence of CALR mutation was 1.60 (95% CI: 0.97-2.65).

The RR of patients with CALR mutation in five studies (15, 16, 29, 31, 33) was pooled using the Stata software program to analyze leukemic transformation of patients with CALR mutation. The meta-analysis results (Fig. 4B) showed that CALR gene mutation did not influence leukemic transformation as evidenced by the pooled RR of 1.05 (95% CI: 0.72-1.53).

CALR gene mutation has been reported to be closely associated with patients with PMF and ET (25, 35). In the
present meta-analysis (Fig. 4C), CALR gene mutation significantly affected the high risk of all patients with ET and PMF as evidenced by the pooled RR of 0.53 (95% CI: 0.40-0.71). CALR gene mutation also more strongly influenced patients with ET (RR=0.37, 95% CI: 0.24-0.57) than patients with PMF (RR=0.83, 95% CI: 0.56-1.25).

Publication bias

Egger’s plot was used to determine the publication bias of all studies included in this review. The shape of Egger’s plot did not suggest any obvious asymmetry (Fig. 2B).

Discussion

Mutation rate analysis

The meta-analysis of the different populations (including Asian, European, North American and Australian) showed that CALR mutation may increase the prevalence of patients with ET (19%) and PMF (22%). The Asian population with CALR gene mutation presented a higher risk of ET, but a lower risk of PMF than European-American patients. Future case-control investigations must focus on continental groups (especially for south Americans and Africans) to reveal whether CALR mutation is associated with an increased risk of ET or PMF and whether it exhibits ethnicity-specific effects. Our meta-analysis estimated that the use of CALR mutation as a specific molecular marker, exclusive of JAK2 and MPL mutations, is a novel powerful tool for studying ET and PMF.

Clinical prognostic significant analysis

Fibrotic transformation in ET

Rumi et al. (19, 33) hypothesized that CALR mutation increased the prevalence of fibrotic transformation, however, Chen et al. reported a different conclusion. A trend toward higher fibrotic transformation was observed in patients with CALR-mutant ET compared with the controls (20). By using these four studies, we compared fibrotic transformation between ET with and without CALR mutation to systematically determine whether CALR mutations may serve as a prognostic factor in the prediction of fibrotic transformation. The meta-analysis results showed a trend toward higher fibrotic transformation in patients with CALR-mutant ET compared with those in patients without CALR-mutant ET (RR=1.60, 95% CI: 0.97-2.65).

The different frequency of genetic mutation has some clinical significance, such as the different frequency of genetic variation may predict specific treatment effects. Ge and colleagues found that genetic variation in IL28B exists with a geographical discrepancy, and this phenomenon also explains some of the difference in the response rates between African-Americans and patients of European ancestry (36). Thus, CALR mutation appears to have a geographical discrepancy that must be investigated in further studies.

Leukemic transformations in ET and PMF

Tefferi et al. (15, 16) speculated that CALR-mutant ET presented with a higher leukemic incidence than wild type CALR-mutant (wtCALR-mutant) ET, however, this conclusion was invalid for CALR-mutant PMF. By contrast, Rumi et al. presumed that CALR-mutant PMF may have a higher leukemic incidence than wtCALR-mutant PMF, however, these results were not applicable for CALR-mutant ET (31, 33). Thus, the possibility that CALR-mutant ET or PMF would have higher leukemic transformation has not yet been confirmed. We further compared leukemic transformation in patients with and without CALR mutation to analyze their possible association. The meta-analysis results of five reports showed a negative association (RR=1.05, 95% CI: 0.72-1.53), however, some studies confirmed that the high expression of the CALR gene is related to the pathogenesis of acute myeloid leukemia (37). Thus, a greater accumulation of patients is needed in future studies to confirm this conclusion.

Risks for patients with CALR mutation

Four of the six eligible studies showed that CALR mutant-ET or PMF presented significantly higher OS rate than wtCALR ET or PMF as assessed through IPSET, IPSS, and DIPSS-plus (15, 16, 23, 31). Our meta-analysis according to these six studies suggested significantly lower numbers in the high-risk groups (RR=0.53, 95% CI: 0.40-0.71). Thus, CALR mutation presented a significant association with improved clinical features and the OS. This result may indicate that ET or PMF with CALR mutation is associated
with a higher hemoglobin level, higher platelet count, and lower leukocytes than ET or PMF without CALR mutation. Moreover, patients with ET or PMF with CALR mutation were younger than those without CALR mutation. These findings demonstrate that CALR may cause myeloproliferative syndrome, thereby presenting a new direction toward the understanding of the pathogenesis of myeloproliferative syndrome.

**Conclusion**

There are some limitations associated with this study. First, only published studies in English were included in the data analysis; hence, other studies published in different languages or unpublished materials could have been overlooked. Second, the included studies involved Asian, European, North American, and Australian populations. In this regard, further studies on South American and African populations must be conducted to investigate the association and clinical prognostic significance. Third, the lack of large statistical power existed due to the small size of each subgroup analysis. Finally, we did not assess the types of CALR mutation, which may be a major limitation of our study.

As some studies hypothesized that Ph-negative myeloproliferative neoplasms (MPN)-related gene variation could be a sequel to the structural instability of the nucleotide sequence, impairment of the DNA repair pathway may also play a role in the early pathogenesis of myeloproliferative syndrome (37). CALR mutants have altered subcellular localization (6), and the overexpression of the most frequent CALR deletion in Ba/F3 cell lines can cause interleukin-3 independent growth and STAT5 phosphorylation (5). Therefore, CALR mutations can influence cell adhesion, phagocytosis, signal transduction, modulation of the STAT pathway activity, and thereby cause the occurrence of diseases.

The discovery of CALR mutations is another milestone in our understanding of the new pathogenesis of myeloproliferative syndrome. Additional raw data and detailed subgroup analyses are therefore needed to reveal the functional relevance of CALR mutation in the pathogenesis of ET and PMF and the influence of these mutations on the disease phenotype.

The authors state that they have no Conflict of Interest (COI).

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