Calreticulin Mutations in Philadelphia Chromosome Negative Myeloproliferative Neoplasms

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ABSTRACT

Calreticulin (CALR) is a multifunctional protein. CALR gene mutations are one of the driver mutations in cases with essential thrombocythemia (ET) and primary myelofibrosis (PMF). The aim of this study is to comprehend the functional relationship of CALR type1 and type2 mutations in the pathogenesis of Phi-ladelphia Chromosome Negative Myeloproliferative Neop-lasms (MPNs) by emphasizing the incidence, biological and clinical features of CALR mutations in Janus Kinase2 (JAK2) V617F mutation negative and thrombopoietin receptor gene (MPL) mutation negative ET and PMF cases, and to determine their effect on the disease phenotype. The laboratory results of cases analyzed with essential throm-bocythemia and primary myelofibrosis were analyzed retros-pectively. In our study of the ET cases, 18.4% CALR exon9 mutation car-ried, 5.1% a thrombopoietin receptor gene (MPL) mutation, and 57.1% JAK2 V617F mutation. 19.4% of our cases do not carry any of these three mutations. Our ET patients with CALR muta-tion positive, 61.1% have type1, 27.8% have type2 and 11.1% have mutations other than type1 and type2. In our study of the PMF cases, 27.7% CALR exon9 mutation carried, 3.6% a MPL mutation, and 47% JAK2 V617F muta-tion. 21.7% cases are triple negative. Our PMF patients with CALR mutation positive, 69.6% have type1, 30.4% have type2 mutations. CALR mutations are a new and important molecular marker for Philadelphia chromosome negative myeloproliferative neoplasm cases. Longer follow-up and larger case populations are required to investigate the effects of clinical and laboratory pa-rameters of diseases.

Keywords: Calreticulin gene, Mutation, Philadelphia chromosome negative myeloproliferative neoplasm

INTRODUCTION

Calreticulin (CALR) is a molecular chaperone and plays a role in glycoprotein folding mechanism and calcium binding mechanism in endoplasmic reticulum with calnexin.[1] In addition, CALR is involved in proliferation, apoptosis and the immune system.[2]

The CALR gene is located on chromosome 19 (19p13.2) and includes 9 exons and 8 introns.[3,4] More than 50 mutations have been identified and these mutations are in exon 9.[5,6] These mutations lead to the loss of the lizin (K), aspartik asit (D), glutamik asit (E), lösin (L) (KDEL) motif located in the C-terminal domain. Mutations in the CALR gene were determined by successive Nangalia J. et al and Klampfl T. et al.[6,7]

The two most common mutations in 80% of cases are del52 (CALRdel52/Type1;c.1092_1143del; L367fs*46) and ins5 (CALRins5/Type2;c.1154_1155insTTGTC;K385fs*47).[8] CALR mutation is found in approximately 15-25% of cases with essential thrombocythemia (ET), primary myelofibrosis (PMF) and ET transformed myelofibrosis without Janus Kinase2 (JAK2) V617F and thrombopoietin receptor gene (MPL) exon 10 mutations.[6,7]

The aim of our study is to comprehend the functional link of CALR type1 and type2 mutations in the pathogenesis of Philadelphia chromosome negative myeloproliferative neoplasms (PhNeg MPNs) by emphasizing the incidence, biological and clinical features of CALR mutations in JAK2 V617F negative and MPL negative essential thrombocythemia (ET) and primary myelofibrosis (PMF) cases, and to determine their effect on the disease phenotype.

PATIENTS AND METHODS

Study Groups

This study retrospectively researched the presence of the CALR mutation in ceses with PhNeg MPNs in the Hematology clinic of Turgut Ozal Medical Center, Inonu University, between January 2018 and June 2020. Participants were diagnosed with MPNs according to the World Health Organization classification criteria. Demographic and clinical features of 181 cases diagnosed with PhNeg MPNs were evaluated retrospectively. Accordingly, our study groups consists of ET (98 cases), PMF (83 cases). However, the cases consisted of 88 women and 93 men.

CALR Mutation Detection

Genomic DNA was isolated from ethylenediamine tetraacetic acid (EDTA) anticoagulated venous blood using the EZ1 DNA Blood 200 μL kit and the BioRobot EZ1 Workstation [Qiagen, Hilden, Germany], according to the manufacturer's instructions. Samples were stored at -20^{0}C until analysis.

After extracting DNA isolated from peripheral blood, Ipsogen CALR RGQ PCR kit (Qiagen, Hilden, Germany) was used to detect CALR mutations. Protocols for the CALR study followed the manufacturer's specifications. Ethics Committee Approval: The Ordu University, Faculty of Medicine Institutional Review Board Ethics Committee granted approval for this study (date:15.04.2021 number: 2021/101).

Statistical Analysis

The conformity of numerical data to normal distribution was analyzed using Shapiro-Wilk or Kolmogorov-Smirnov tests, depending on the number of observations. The data were summarized with median and interquartile width (IQR) as they did not conform to normal distribution. Mann-Whitney U test was utilized for comparisons of two groups, Kruskal-Wallis test for comparisons of more than two groups and Conover pairwise comparison method was used. Categorical variables were represented as numbers and percentages, and comparisons were made with Pearson chi-square, continuity-corrected chi-square or Fisher's exact chi-square tests, depending on the number of observations. Two-sided significance level was accepted as 0.05 in all tests. Statistical analysis was applied using IBM SPSS for Windows version 22.0 (New York, USA).

RESULTS

Clinico-Hematologic Features

The age and white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), platelets (PLT) counts of ET and PMF cases are given in Table 1. Accordingly, a statistically significant difference

Table 1. Clinical features of ET and PMF groups

	ET (n= 98) Median (IQR)	PMF (n= 83) Median (IQR)	p
AGE (years)	60.5 (24)	60 (25)	0,217
WBC (10^9/L)	8.9 (4.1)	10.6 (6)	0.005
RBC (10^{12}/L)	5.655 (1.03)	4.68 (0.89)	< 0.001
Hb (g/dL)	16.2 (2.7)	13 (2.1)	< 0.001
HCT (%)	50.3 (7.4)	41.5 (12.6)	< 0.001
PLT (10^9 /L)	651.5 (325)	259 (158)	< 0.001
F n (%)	49 (50)	37 (44.6)	0.467
M n (%)	49 (50)	46 (55.4)	

In table median [interquartile range(IQR)] values are given. WBC= White blood cell count, RBC= red blood cell count, Hb= hemoglobin, Hct= hematocrit, PLT= platelets count, F= Female, M= Male

Table 2. Mutation profile in ET cases

	CALRType (n= 11) Median (IQR)	CALRType (n= 5) Median (IQR)	Other CALR mutations (n= 2)	MPL mutated (n= 5) Median (IQR)	JAK2 mutated (n= 56) Median (IQR)	Triple negative (n= 19) Median (IQR)	p
AGE (years)	54 (10)	54 (8)	49 (6)	62 (35)	66.5 (26.5)	63 (26)	0.253
WBC (10^9/L)	8.1 (4.3)	8.9 (1.5)	8.9 (3.2)	9.9 (3.8)	8.95 (4.85)	8.3 (4.6)	0.979
RBC(10^{12}/L)	5.3 (2.1)	5.47 (1.05)	6.4 (0.9)	5.1 (0.9)	5.7 (0.9)	5.5 (1.5)	0.200
Hb (g/dL)	11.9 (6.6)	14.8 (2.5)	16.6 (0.6)	16.2 (2.2)	16.4 (1.6)	16.7 (3.9)	0.074
PLT (10^9 /L)	978 (91)[a]	912 (34)[a.b]	1289.5 (793)[a.b]	782 (114)[a.b]	601.5 (133.5)[b]	575 (123)[b]	< 0.001
F n (%)	4 (36.4)	1 (20)	1 (50)	2 (40)	33 (58.9)	8 (42.1)	0.429
M n (%)	7 (63.6)	4 (80)	1 (50)	3 (60)	23 (41.1)	11 (57.9)	

was found between the numbers of WBC (p= 0.005), RBC (p< 0.001), HB (p< 0.001) and PLT (p< 0.001) between ET and PMF cases (Table 1).

Median age in cases of ET is 54 years in CALR type 1 and type 2 and 49 years in other CALR mutations (Table 2). There is no statistically significant difference between the median age of ET patients with JAK2 V617F (median age 66.5), MPL mutations positive (median age 62) and triple negative (median age 63) and ET patients with CALR mutations (p= 0.253) (Table 2).

Median platelet (PLT) levels (range 150-400 × 10^9/L) in CALR mutation-positive ET patients are type1, type2, and in other CALR mutation carriers, 978 × 10^9/L, 912 × 10^9 /L, 1289.5 × 10^9 /L respectively (Table 2). PLT levels is higher in CALR type1 mutation positive ET patients than JAK2 V617F and triple neg ET patients (p< 0.001) (Table 2).

The median age of our patients with in PMF is younger age in CALR type2 mutation positive cases (33 years) than in MPL (65 years), JAK2 V617F (years 64) and triple negative (years 64) positive cases (p< 0.001) (Table 3). In addition, in cases with PMF, the median age in CALR type1 mutation-positive cases (46.5 years) is younger than the age of JAK2 V617F (64 years) and triple-negative cases (64 years) (p< 0.001) (Table 3).

There is no statistical difference between PMF and ET patients in terms of type1 and type2 mutations (p= 1.000) (Table 4).

Frequencies of Molecular Mutations

In our study of the ET cases, 18.4% (18 cases) CALR exon9 mutation carried, 5.1% (5 cases) a MPL mutation, and 57.1% (56 cases) JAK2 V617F mutation. 19.4% (19 cases) of our cases do not carry any of these three mutations (Table 2).

Our ET patients with CALR mutation positive, 61.1% (11 cases) have type1, 27.8% (5 cases) have type2 and 11.1% (2 cases) have mutations other than type1 and type2 (Table 2).

Table 3. CALR mutation profile in PMF cases

	CALR Type 1 (n= 16) Median (IQR)	CALR Type 2 (n= 7) Median (IQR)	MPL mutated (n= 3) Median (IQR)	JAK2 mutated (n= 39) Median (IQR)	Triple negative (n= 18) Median (IQR)	p
AGE (years)	46.5 (10.5)[a.b]	33 (17)[a]	65 (24)[b.c]	64 (15)[c]	64 (13)[c]	< 0.001
WBC (10^9/L)	11.7 (2.6)	10.4 (4.2)	14 (1.5)	9.4 (8.2)	8.6 (4)	0.154
RBC (1012/L)	4.6 (0.7)	4.5 (0.6)	4.7 (0.9)	4.7 (0.9)	4.7 (0.7)	0.779
HB (g/dL)	13.2 (2.7)	12.5 (1.3)	13.5 (2.6)	13.2 (2.1)	13.1 (3.3)	0.587
PLT (10^9 /L)	281.5 (201)	391 (112)	318 (158)	248 (129)	249 (114)	0.112
F n (%)	7 (43.8)	2 (28.6)	2 (66.7)	18 (46.2)	8 (44.4)	0.860
M n (%)	9 (56.2)	5 (71.4)	1 (33.3)	21 (53.8)	10 (55.6)	

Table 4. Comparison of CALR mutation types in ET and PMF cases

	CALR mutation Type			
	Type1	Type2	Total	P
Group				
ET (n/%)	11 (68.8%)	5 (31.3%)	16 (100.0%)	
PMF (n/%)	16 (69.6%)	7 (30.4%)	23 (100.0%)	
Total	27 (69.2%)	12 (30.8%)	39 (100.0%)	1.000

In our study of the PMF cases, 27.7% (23 cases) CALR exon9 mutation carried, 3.6% (3 cases) a MPL mutation, and 47% (39 cases) JAK2 V617F mutation. 21.7% (18 case) cases are triple negative (Table 3).

Our PMF patients with CALR mutation positive, 69.6% (16 cases) have type1, 30.4% (7 cases) have type2 mutations (Table 3).

In this study, there was no statistically significant difference between ET and PMF patients in terms of CALR mutation types (Table 4).

DISCUSSION

The discovery of the JAK2 V617F mutation in 2005 led to the emergence of mutations in other genes related to hematopoietic growth signaling pathways such as JAK2 exon 12, MPL and CALR.[9,10,11]

In this study, Turkey's Eastern Anatolia to understand the differences in the clinical presentation of ET and PMF patients from different province of offers clinical and molecular profiles.

To date, CALR mutations have mostly been determined in cases of ET and PMF negative for JAK2 V617F and MPL. In this study, we found CALR, MPL or JAK2 V617F mutations, 80.6% in cases with ET and 78.3% with PMF. In a study, the JAK2, MPL or CALR mutations 85.5% in cases with ET and 85.2% with PMF.[12]

In our study, we found frequency of CALR (18.4-27.7%), JAK2 V617F (57.1-47%), MPL (5.1-3.6%) and triple-negative (19.4-21.7%), between ET and PMF cases respectively. Mara Jogelina Ojeda et al. Defined as JAK2 V617F (51.2%), CALR (21.5%), MPL (2.8%) and triple negative MPN (14.5%) in 214 ET cases.[12] A similar study conducted by Li et al in 357 Chinese patients with PMF found that, 50% of patients carried JAK2 V617F, 21% had a CALR mutation, 3% carried an MPL mutation, and 27% were triple-negative PMF.[13] In another study, they found JAK2 V617F 53%, CALR 33%, MPL 3% and triple negatives 11% in ET patients. For PMF, the distribution of mutations were as follows: JAK2 V617F mut: 57%, CALR mut: 25%, MPL mut: 7%, triple negative: 11% cases (14). In a study have reported the frequency of the CALR mutation to be 11.3% and 9.1% of cases with ET and PMF, respectively.[5] In a study conducted in Turkey, JAK2 V617F was reported in 47.5% and 48.6% of cases with PMF and ET, respectively.[15] In another study conducted in Turkey, JAK2 mutation was determined as 54.8% in ET patients.[16] Our results were similar to the majority of studies of the mutations (CALR, JAK2 V617F and MPL) in cases with ET and PMF found in the literatüre.

Mutant CALR in ET and PMF is caused by insertions or deletions in exon 9. These mutations; The type1 variant, a 52-bp deletion (c.1179_1230del), and type2 variant, is a 5-bp TTGTC insertion (c.1234_1235insTTGTC).[3,7]

In our study, the CALR mutation was found in 18.4% of ET, 27.7% of PMF, respectively. Of the ET cases with CALR mutations, 61.1% had type1 mutation and 27.8% had type2 mutation. In addition, mutations other than type1 and type2 were detected in 11.1% of ET cases. Tefferi et al. detected 16% type1 and 14% type2 CALR mutations in their study.[17] Zaidi et al. in ET cases, 59.1% type1 mutations and 40.9% type2 mutations were detected.[18]

In this study, we found 69.6% type1 and 30.4% type2 mutations in PMF cases with CALR mutations. In a similar study, 52.6% found type1 CALR mutation and 47.7% type2 CALR mutation.[18]

In our study, PLT values were found to be higher in ET cases carrying type1 CALR mutations compared to JAK2 and triple negative groups. Ayalew Tefferi et al also found that PLT values were higher in patients with CALR mutation ET and their ages were younger.[17] In another study, patients with CALR mutation ET have high PLT counts.[12]

In this study, the ages of the cases carrying CALR type1 mutations in PMF patients were younger than the ages of JAK2 and triple negative cases. In the same patient group, cases with CALR type2 mutations were found to be younger than MPL, JAK2 and triple negative groups. Bing Li, et al. also identified CALR mutated PMF cases as younger in their study.[19]

In this study, there was no statistical difference between ET and PMF patients in terms of CALR mutation types. The prognostic value of Type 1 and Type 2 mutations has been discussed in various studies. Tefferi et al. indicated that cases who carry the Type 1 CALR mutation had significantly longer survival than the cases with all other mutations.[18,20]

This study had a few limitations. First, our study was retrospective. Second, for the purpose of our study, disease transformations and secondary malignancies were not evaluated.

Conclusions

As a result, CALR mutations are a new and important molecular marker for ET and PMF cases after JAK2 V617F mutation. Cases with the del52 (CALRdel52/Type1;c.1092_1143del;L367fs*46) and ins5 (CALRins5/Type2;c.1154_1155insTTGTC;K385fs*47) mutations have shown different clinical characteristics. Showing of this mutation is very significant for differential diagnosis. Longer follow-up and larger case populations are required to investigate the effects of clinical.

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