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The Impact of ASXL1 Gene Mutations on Clinical Course and Prognosis in Myeloproliferative Neoplasms

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ABSTRACT

Objective: Chronic myeloproliferative neoplasms (MPNs) form a group of diseases characterized by clonal proliferation in all three cell lines of the bone marrow. "Philadelphia chromosome"-negative (Ph–) MPNs are primarily categorized into essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). The JAK-2 V617F mutation was found in 95–98% of PV patients and 50% of ET and PMF patients. Later, JAK2 exon-12 mutations in PV, MPL W515L/K and calreticulin mutations in PMF and ET were identified. Another mutation observed in MPN patients is the additional sex combs like 1 (ASXL1) gene mutation. This study investigated the frequency of ASXL1 gene mutations in 103 Ph– MPN patients and examined their impact on the disease's clinical course and prognosis.

Materials and Methods: A total of 103 Ph– MPN patients were included in the study. DNA sequence analysis was used to screen for ASXL1 gene mutations using blood count samples.

Results: ASXL1 gene mutations were detected in 6 patients (5.8%) in our cohort. Screening of the 12th exon of the ASXL1 gene revealed the most common mutation as c.1934dupG (p.g646TrpfsX12) and the second most frequent as c.1954G.a (p.G652S). The group with ASXL1 gene mutations showed higher rates of thrombosis. In our MPN group with an average follow-up duration of 4.1 years, non-hematologic cancer rates (solid tumors) were found to be considerably high (14.5%). 80% of these patients had the JAK-2 V617F mutation. Two patients with the JAK-2 V617F mutation also had the ASXL1 c.1954G.a (p.G652S) gene mutation.

Conclusion: In our cohort, the diagnosis of non-hematologic cancer following the use of hydroxyurea in five patients initially diagnosed with MPN suggests that hydroxyurea use might contribute to the development of non-hematologic cancer. On the other hand, the development of secondary cancer in two patients without hydroxyurea use post-MPN diagnosis indicates that factors other than hydroxyurea may also influence the development of non-hematologic cancers.

Keywords: Additional sex combs like 1, Chronic myeloproliferative neoplasms, Essential thrombocythemia, Myeloproliferative neoplasms, Polycythemia vera, Primary myelofibrosis

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INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a group of diseases resulting from mutations in pluripotent hematopoietic stem or progenitor cells, leading to persistent and progressive increases in all three cell lines of the bone marrow. Chronic myeloid leukemia (CML) in this group is characterized by the presence of the "Philadelphia chromosome" and its oncogene BCR-ABL. Ph-negative (Ph–) MPNs are categorized into essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). The discovery of the JAK-2 V617F mutation in 2005 revealed its presence in 95–98% of PV patients and 50% of ET and PMF patients.[1] It is crucial to exclude reactive erythrocytosis and thrombocytosis in the diagnosis of these diseases.[2,3]

The additional sex combs like 1 (ASXL1) gene is the human homolog of the "Additional sex combs (Asx)" gene found in Drosophila located at 20q11, ASXL1 encodes a 1084 amino acid nuclear protein. The exact function of the ASXL1 protein is not fully understood, but it is believed to control epigenetic changes through histone modification. ASXL1 gene mutations are predominantly frameshift mutations located in the 12th exon, typically resulting in the loss of the carboxy-terminal plant homeofinger domain. ASXL1 gene mutations are observed in PV at a rate of 2–7%, in ET at 0–10%, and in PMF at 13–32%. Studies suggest that ASXL1 influences gene transcription by binding to chromatin, activating certain regions while repressing others, thus potentially contributing to the development of myeloid neoplasms.[4-6] ASXL1 gene mutations have also been demonstrated in different hematologic malignancies, including MDS, AML, and CMML.[7]

In this study, conducted at the Hematology Clinic of Istanbul Bilim University Faculty of Medicine, the frequency of ASXL1 gene mutations was investigated in 103 Ph– MPN patients, along with their impact on the clinical course and prognosis of the disease. ASXL1 gene mutations were screened using DNA sequence analysis of blood count samples taken during routine clinic visits, and their effects on clinical progression and prognosis were examined.

MATERIALS AND METHODS

This study included 103 patients (48 ET, 46 PV, 9 PMF) diagnosed with Ph– MPN meeting the criteria of the World Health Organization.[8] The study received approval from the Ethics Committee of Istanbul Bilim University (Date: 17.02.2015, Number: 44140529/2015-30) and was conducted with informed consent from the patients. The study was supported by the Istanbul Bilim University Research Fund. All procedures were followed in accordance with the ethical standards of the Responsible Human Experimentation Committee (institutional and national) and with the 1964 Helsinki Declaration and its later versions.

Demographic and clinical data of the included patients, including the onset of the disease, initial hemoglobin, leukocyte, platelet levels, presence of initial splenomegaly, thrombosis and bleeding complications, malignancy, and cytogenetic examinations (JAK-2 V617F, JAK-2 EXON 12, MPL W515L/K, calreticulin [CALR]), and administered drugs were reviewed.

Venous blood samples from the included patients were collected and processed at the Molecular Hematology and Oncology Laboratory of Istanbul Bilim University, where DNA isolation was performed, followed by sequencing analysis to screen for ASXL1 gene mutations.

Genomic DNA isolation was performed from 103 patients (48 ET, 46 PV, 9 PMF) diagnosed with Ph– MPN using a DNA isolation kit (HibriGen Biotechnology, Istanbul, Türkiye). The liquid containing genomic DNA was stored at +4°C until analysis time. For mutation analysis, the 12th exon of the ASXL1 gene was amplified using polymerase chain reaction (PCR) with a designed primer pair (Table 1).

ASXL1 gene mutations were analyzed using a device operating on the principle of Sanger's dideoxy method (Applied Biosystems ABI 310xl Genetic Analyzer). The target region for examination was amplified using the PCR program outlined in Table 1. The success of amplification in PCR was verified by loading 5 µL of the product on a 2.5% (w/v) agarose gel. DNA sequencing was then performed in four stages. PCR products were purified using a column-based kit (PCR purification kit, HibriGen Biotechnology, Istanbul, Türkiye) and checked on a 2% (w/v) agarose gel. Samples not visible on the gel underwent PCR again. The purified samples were subjected to cycle sequencing on the same day. The cycle sequencing protocol was carried out according to the protocol below using the "ready reaction mix" from the kit (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems) containing fluorescently labeled four different dideoxy nucleotides.

Table 1. PCR mixture used for amplification of exons

PCR components	µL/Tube	Final concentration
Sterile ultra-pure water	16.7	-
10× PCR buffer	2.0	1X
MgCl2 (25 mM)	1.0	0.25 mM
dNTP mix (25 mM)	1.5	1.5 mM
Primer 1 (10 pmol/µL)	0.8	0.8 pmol
Primer 2 (10 pmol/µL)	0.8	0.8 pmol
Taq DNA polymerase (5U/µL)	0.2	1U
DNA	2	-
Total volume	25	-

PCR: Polymerase chain reaction.

Ready reaction mix: 8 µL Primer (3.2 pmol): 4 µL

DNA sample 2–5 µL dH2O: 3–6 µL

Total volume: 20 µL

Cycle sequencing was performed using the forward and reverse primers used in the PCR stage, and the sequencing mixture was prepared and applied according to the program below.

96°C for 10 s

50°C for 5 s 25 cycles

60°C for 4 min

4°C indefinitely

After cycle sequencing, a further purification step was conducted to clean the products from residuals. 2 µL of NaAc with pH 4.6 was added to the PCR product, followed by 50 µL of 95% (v/v) ethanol. The mixture was transferred to 1.5 mL tubes, gently mixed by tapping, and incubated on ice for 15 min. It was then centrifuged at 13,000 rpm for 20 min. The supernatant was removed, and 250 µL of 70% (v/v) ethanol was added, gently mixed, and centrifuged at 13,000 rpm for 5 min. After discarding the supernatant, the samples were left to dry at room temperature. The lyophilized DNA samples were either immediately loaded onto the analyzer (ABI Prism 310 Genetic Analyzer, Applied Biosystems) or stored at –20°C for up to 1 week for later analysis.

Statistical Analysis

Statistical analyses in this study were performed using Number Cruncher Statistical System 2007 Statistical Software (Utah, USA). Descriptive statistical methods (standard deviation, mean) were used for data evaluation, along with independent t-tests for comparing two groups, Chi-square, Fisher's exact test, odds ratio (OR) values, and Tukey's multiple comparison test for qualitative data comparisons. Results were considered significant at $p<0.05$.

RESULTS

In this study, a total of 103 patients with Ph– MPN, including 48 with ET, 46 with PV, and 9 with PMF, were examined. The gender distribution consisted of 58 female and 45 male patients. The average follow-up duration for our patient group was 4.21 years (standard deviation 3.83 years).

A statistically significant difference in gender distribution was observed among the ET, PV, and PMF groups (p=0.0001) (Table 2). There was no statistically significant difference in the average ages and diagnosis ages among the ET, PV, and PMF groups; however, PMF patients being of older age was noteworthy (respectively, p=0.632, p=0.563) (Table 2).

The presence of JAK-2 V617F was significantly higher in the PV group as expected (p=0.041). There was no statistically significant difference in the presence of CALR mutations between ET and PMF groups (p=0.067) (Table 3).

Table 2. Demographic characteristics of patients diagnosed with ET, PV, and PMF

	ET n=48	PV n=46	PMF n=9	p
Age	52.75±19.87	55.33±15.72	58.11±16.81	0.632
Age of diagnosis	48.52±19.06	50.5±16.15	55.33±19.22	0.563
Gender, (%)				0.0001
Female	32 (66.67)	26 (56.52)	0 (0.00)	
Male	16 (33.33)	20 (43.48)	9 (100.00)	

ET: Essential thrombocythemia; PV: Polycythemia vera; PV: Primary myelofibrosis.

Table 3. Presence of genetic mutations in patients diagnosed with ET, PV, and PMF

Mutation	ET n=48 (%)	PV n=46 (%)	PMF n=9 (%)	p
JAK-2 V617F	29 (60.42)	39 (84.78)	4 (44.4)	0.041
MPL W515L/K	0 (0.00)	0 (0.00)	1 (11.11)	0.004
CALR	6 (12.50)	0 (0.00)	1 (11.11)	0.067
ASXL1	3 (6.25)	2 (4.35)	1 (11.11)	0.720
c.1934dupG (p.g646TrpfsX12)	3 (6.25)	1 (2,17)	0 (0.00	0.509
c.1954g.a (p.G652S)	0 (0.00)	1 (2.17)	1 (11.11)	0.061

ET: Essential thrombocythemia; PV: Polycythemia vera; PV: Primary myelofibrosis.

Among the 6 patients with ASXL1 gene mutations, 3 had ET (50%), 2 had PV (33.33%), and 1 had PMF (16.67%). Patients with ASXL1 gene mutations tended to be older in terms of age and diagnosis age compared to those without the mutations, but this difference was not statistically significant (Table 4).

When patients diagnosed with ET, PV, and PMF were grouped based on the presence of ASXL1 gene mutations, patients with these mutations tended to have higher initial platelet and hemoglobin averages. Their initial leukocyte and neutrophil averages were lower. However, due to the small number of patients, no statistically significant difference was found between the average values of initial hemoglobin, leukocyte, platelet, and neutrophil levels. There was no statistically significant difference observed in the distribution of initial symptoms such as itching, plethora, fever, weight loss, and sweating between the two groups (respectively, p=0.304, 0.516, 0.313, 0.295, 0.547). Constitutional symptoms were notably less in patients with ASXL1 gene mutations. Similarly, no statistically significant difference was observed in the presence of splenomegaly between the two groups (p=0.286), with a higher likelihood of splenomegaly in the group without ASXL1 gene mutations (Table 5).

Table 4. Comparison of demographic characteristics of patients diagnosed with ET, PV, and PMF based on the presence of ASXL1 gene mutations

Parameters	ASXL1 (+)	ASXL1 (–)	p
Age	54.51±17.82	52.17±18.49	0.756
Age of diagnosis	50.13±17.74	47.5±20.98	0.728
Gender, (%)			
Female	3 (50.00)	55 (56.70)	0.748
Male	3 (50.00)	42 (43.30)	
Diagnosis, (%)			
ET	3 (50.00)	45 (46.39)	0.720
PV	2 (33.33)	44 (45.36)	
PMF	1 (16.67)	8 (8.25)	

ASXL1: Additional sex combs like 1; ET: Essential thrombocythemia; PV: Polycythemia vera; PV: Primary myelofibrosis.

An interesting observation in our study group was that non-hematologic (solid) cancers were more prevalent than expected. In our cohort of 103 patients, 15 (14.5%) had a total of 17 non-hematologic cancers. In 7 patients, cancer was known before the MPN diagnosis (1 sarcoma, 1 bladder cancer and renal cell cancer, 1 basal cell cancer, 1 colon cancer, 3 breast cancers), while 7 patients were diagnosed with non-hematologic cancer following an MPN diagnosis (1 basal cell cancer and parotid squamous cell cancer, 1 breast cancer, 1 pancreatic cancer, 1 cervical cancer, 1 lung cancer, 2 prostate cancers). In one patient, MPN and non-hematologic cancer were diagnosed simultaneously.

Molecular characteristics of patients with non-hematologic cancers revealed that 12 had the JAK-2 V617F mutation (12/15, 80%), and 1 had CALR mutations. Two patients had both JAK-2 V617F and ASXL1 c.1954g.a (p.G652S) mutations. Two patients were negative for all mutations, and the Ph– MPN diagnosis was made through bone marrow biopsy.

Patients were treated with hydroxyurea and interferon (IFN) for MPN. Ten patients had been diagnosed with non-hematologic cancer without having received any prior treatment. Five patients had a history of long-term hydroxyurea use. The average age of patients with non-hematologic cancer in Ph (–) MPN was 64.06, and the average age of diagnosis was 58.5.

Table 5. Comparison of clinical characteristics of patients diagnosed with ET, PV, and PMF based on the presence of ASXL1 gene mutations

Clinical feature	ASXL1 (+) n=6	ASXL1 (–) n=97	p
Initial hemoglobin (g/dL)	14.12±2.53	13.96±2.64	0.890
Initial platelets (mm^3)	733.83±559.84	629.56±392.63	0.540
Initial leukocytes (mm^3)	9486.67±3756.1	11249.37±5484.53	0.441
Initial neutrophils (mm^3)	4948±4105.79	7596.51±4302.93	0.186
Itching (%)	1 (0.9708)	39 (37.864)	0.304
Plethora (%)	1 (0.9708)	30 (29.126)	0.516
Fever (%)	0 (0.00)	15 (14.563)	0.313
Weight loss (%)	0 (0.00)	16 (15.533)	0.295
Sweating (%)	1 (0.9708)	29 (28.155)	0.547
Splenomegaly (%)	2 (1.941)	54 (52.427)	0.286

ASXL1: Additional sex combs like.

DISCUSSION

The gender distribution of the 103 included Ph(–) MPN patients showed 58 females and 45 males. In the largest study reported from Türkiye, involving 184 Ph(–) MPN patients, the female-to-male ratio was reported as 54.3% female and 45.7% male (58/49) in ET cases and 55.84% female, 44.16% male (43/34) in PMF cases.[9] Due to the small number of PMF patients in our study, it is not possible to make a definitive comment on the difference in gender distribution.

The average diagnosis age of our ET patients was 48.52±19.06; for PV patients, it was 50.5±16.15, and for PMF patients, it was 55.33±19.22. Although no statistically significant difference was found when comparing the average onset ages, our findings indicate that ET tends to occur in relatively younger individuals, whereas PMF is seen in an older patient group. The literature reports average ages of 50–57 for ET, 60 for PV, and 65 for PMF.[10] The average diagnosis ages for Ph(–) MPN subgroups in our cohort were notably lower. This may be explained by the ability to screen all patients in our laboratory for the 3 mutations (JAK-2 V617F, CALR, MPL W515L/K) considered significant markers in the diagnosis of Ph(–) MPN. Routine use of molecular diagnostic methods can be considered as facilitating factor for earlier diagnosis in patients.

When initial hematologic parameters were considered, as expected, high platelet counts in ET, high erythrocyte counts in PV, and high leukocyte and neutrophil counts in PMF were observed. There was a statistically significant difference in the distribution of splenomegaly presence among ET, PV, and PMF groups; a lower incidence of splenomegaly was observed in the ET group, while a higher tendency for splenomegaly was noted in the PMF group.

In this study of Ph– MPN patients, when evaluated for commonly occurring clonal mutations, the JAK-2 V617F mutation was detected in 71.3% of patients (72/103). Examining the distribution across subgroups, this mutation was found in 84.78% of PV patients (39/46), 60.42% of ET patients (29/48), and 50% of PMF patients (4/8). One PV patient was found to have the JAK-2 exon 12 mutation, bringing our detection rate of mutant JAK-2 molecules in PV patients to 87%. Compared to the literature, the rate of JAK-2 mutations in our PV patient group was low.[11] Several interpretations can be made for this. In some cases, the JAK-2 V617F mutation could not be demonstrated in peripheral blood cells but was positive in genetic examination from bone marrow. Unfortunately, not all patients with negative JAK-2 mutations underwent genetic examination from bone marrow, as some were diagnosed based on bone marrow biopsies performed at external centers and did not consent to a second marrow procedure.

CALR mutations were found in a total of 7 patients, with 6 in the ET group and one in the PMF group. The MPL W515L/K mutation was demonstrated in only one PMF patient.

In our study group, ASXL1 gene mutations were detected in 6 patients (5.8%). These mutations were present in 6.25% of ET patients (3/48), 4.35% of PV patients (2/46), and 11.11% of PMF patients (1/9). Looking at the distribution of these mutations by diagnosis in the literature, the frequencies are reported as 2–7% in PV, 0–10% in ET, and 13–32% in PMF.[9,12–16] Although there are different results in studies concerning ASXL1 gene mutations, the frequency is generally given as approximately 10% in Ph(–) MPN patients.[13] In a study published in 2011 by Stein et al.,[15] 166 MPN patients were evaluated for ASXL1 gene mutations. ASXL1 gene mutations were found in 2% of PV patients, 32% of PMF patients, and none in ET patients. In another study conducted in 2012 by Brecqueville et al.,[12] ASXL1 gene mutations were investigated in 149 MPN patients: found in 7% of 30 PV patients, 4% of 53 ET patients, 20% of 30 PMF patients, 50% of 4 post-PV PMF patients, and 10% of 10 post-ET PMF patients. In a study by Gelsi-Boyer et al.[16] in 2009, the frequencies were determined as 2–5% in PV, 5–10% in ET, and 13–26% in PMF. In a study conducted in Türkiye by Yonal-Hindilerden et al.,[9] the frequency of ASXL1 gene mutations in 184 MPN patients was investigated and found to be 8.4% in ET (9/107) and 24.7% in PMF (19/77).

In our Ph– MPNs cohort, the most common mutations found upon screening the 12th exon of the ASXL1 gene were c.1934dupG (p.g646TrpfsX12) in four patients, followed by c.1954G.a (p.G652S) in two patients. No other mutations were detected. Similar to another study conducted in Türkiye, only exon 12 of the ASXL1 gene was screened, identifying the same frequent mutations: c.1934dupG (p.g646TrpfsX12), c.1954G.a (p.G652S), and 1900_1922 del.[9]

Internationally, the most commonly observed mutation is also c.1934dupG (p.g646TrpfsX12). In a study by Gelsi-Boyer et al.[7] in 2012, this mutation was found in over 50% of patients with ASXL1 gene mutations. In a cohort study by Stein et al.,[15] c.1934dupG (p.g646TrpfsX12) was the most frequent mutation found, followed by c.1900_1922del and c.1954G.a (p.G652S). Tefferi et al.'s[17] study similarly highlighted the prevalence of the c.1934dupG (p.g646TrpfsX12) variant.

Due to the limited number of ASXL1 gene mutations (c.1934dupG p.g646TrpfsX12, c.1954G.a p.G652S) in our patient group, it was not possible to separately analyze these two ASXL1 variants. Statistical analysis was conducted by grouping patients based on the presence or absence of ASXL1 gene mutations. The relationship between ASXL1 gene mutations and epidemiological and clinical findings is not yet fully clarified in the literature, and there are conflicting interpretations. In

our patient group, the gender distribution among the six patients with ASXL1 gene mutations was equal (3 males and 3 females). The average age was 54.51±17.82, and the average age at diagnosis was 50.13±17.74. Although no statistically significant difference was found between patients with and without ASXL1 gene mutations in terms of average age and diagnosis age, it is notable that those with ASXL1 gene mutations tended to be older.

In a cohort study by Stein et al.[15] involving 83 patients, those carrying ASXL1 gene mutations were found to have a lower average onset age and average age, though the difference was not statistically significant (p=0.14 and 0.67, respectively). Vannuchi et al.'s[18] study covering 879 Ph(–) MPN patients found ASXL1 gene mutations more frequently in older patients. In a 2012 study by Brecqueville et al.[12] involving 127 Ph(–) MPN patients, the average age of patients with ASXL1 gene mutations was 74, significantly older than the 63 years in those without mutations (p=0.008). In a Turkish study involving 184 Ph(–) MPN patients, the diagnosis age and average age were higher in those with ASXL1 gene mutations compared to those without.[9]

The distribution of ASXL1 gene mutations and their demographic characteristics in PV, ET, and PMF are contradictory in the literature. In our study, ASXL1 gene mutations were more common in women than in men in ET (66.7%), equally distributed in PV, and only found in male patients in PMF. Due to the small number of patients and the presence of only male patients in the PMF group in our study, it is not feasible to make a definitive comment on these observations.

In our study, we investigated the impact of ASXL1 gene mutations on the initial platelet and hemoglobin averages in patients with Ph– MPNs. It was observed that patients with ASXL1 gene mutations had higher initial hemoglobin and platelet averages, but lower initial leukocyte and neutrophil averages compared to those without mutations. Due to the small number of patients, these findings were not statistically significant. Although not statistically significant, the higher platelet counts in ASXL1-positive patients may be attributed to the higher proportion of ET patients (50%) in this group. This contrasts with another study conducted in Türkiye by Yonal-Hindilerden et al.,[9] where patients with Ph(–) MPN and ASXL1 gene mutations had higher initial leukocyte values compared to the group without mutations, while the initial platelet and hemoglobin values were similar in both groups. In a cohort study by Stein et al.,[15] initial leukocyte, hemoglobin, and platelet values were found to be similar in PMF patients with and without ASXL1 gene mutations. Brecqueville et al.[12] found no difference in initial leukocyte and platelet counts between groups with and without ASXL1 gene mutations, although hemoglobin levels were lower in those with mutations. Overall, results in the literature vary significantly.

In our study, the rate of splenomegaly in patients with ASXL1 gene mutations was lower (33.33% vs. 55.67%). The relationship between ASXL1 gene mutations and splenomegaly has been highlighted in a few studies. In a cohort study by Stein et al.,[15] spleen sizes were compared in PMF patients with and without ASXL1 gene mutations, but no significant difference was found. Yonal-Hindilerden et al.'s[9] study also found no significant difference in the presence of splenomegaly and spleen sizes between ET and PMF patients with and without ASXL1 gene mutations. Unfortunately, in our study, not all patients had ultrasound data for spleen sizes, so no conclusions could be drawn in this regard.

Our study found that the development of thrombosis was 1.66 times higher in patients with ASXL1 gene mutations compared to those without. The rate of arterial thrombosis was 66.7% and venous thrombosis was 33.3% in patients with ASXL1 gene mutations. In the mutation-negative group, the rates were 14.4% for arterial thrombosis and 20.6% for venous thrombosis. Few studies in the literature investigate the relationship between ASXL1 gene mutations and thrombosis. In a 2012 study by Brecqueville et al.,[12] no significant difference was found in the development of thrombosis between patient groups with and without ASXL1 mutations. Similarly, in Yonal-Hindilerden et al.'s[9] recent study, the rates of thrombosis development were similar between groups with and without ASXL1 mutations. In contrast, our study demonstrated higher rates of arterial thrombosis in the group with ASXL1 gene mutations.

The relationship between ASXL1 gene mutations and survival has been the subject of numerous studies. In a 2012 study by Brecqueville et al.,[12] it was observed that in a group of 44 patients with PMF, those with ASXL1 gene mutations had a shorter 5-year survival rate compared to those without mutations (56% vs. 87%). A cohort study by Mayo Clinic involving 279 PMF patients found similar 5-year survival rates in groups with and without ASXL1 gene mutations.[18] A 2012 study by Gelsi-Boyer et al.[7] showed that patients with ASXL1 gene mutations had a worse prognosis. In an international study published in 2014 by Tefferi et al.,[19] involving 570 PMF patients, the presence of ASXL1 gene mutations was identified as a poor prognostic factor. It was shown that patients with these mutations were older, had higher DIPSS-Plus scores, and significantly reduced survival. The same study also indicated that CALR type 1 mutations had a favorable prognostic impact. Based on these findings, Tefferi et al.[19] suggested that ASXL1 and CALR gene mutations should be examined in all PMF patients and included in the DIPSS-Plus scoring. In a 2016 study by Alvarez Argote et al.,[20] survival was also shorter in the presence of ASXL1 mutations in PMF. In our study, the av-

erage follow-up period for PMF patients is <5 years. All 9 PMF patients in our group are still under follow-up, with no cases of death or leukemic transformation. Therefore, it is not possible to comment on survival at this stage.

The association of ASXL1 gene mutations with hematologic malignancies other than MPN has been known since its first description in the literature. ASXL1 mutations in AML are predominantly associated with patients with secondary AML.[20] A study of 40 patients with MDS/AML by Gelsi-Boyer et al.,[16] conducted during genetic research, identified ASXL1 gene mutations. In this 2009 study, ASXL1 gene mutations were found in 4 out of 35 patients with MDS (11%) and 17 out of 39 patients with chronic myelomonocytic leukemia (43%). Another study by Carbuccia reported ASXL1 gene mutations in 5 out of 64 patients with MPN (8%) and 11 out of 63 patients with AML (17%).[13,21] In a 2010 study by Boultwood et al.,[22] ASXL1 gene mutations were detected in 5 out of 79 MDS patients (6%), 17 out of 55 patients with refractory anemia with excess blasts 1 (31%), and 17 out of 67 AML patients (25%).

It is known that in Ph(–) MPNs, conversion to acute leukemia may occur during follow-up in 1–5% of patients. However, the association between MPNs and non-hematologic cancers (solid tumors) is not well documented in the literature. None of the patients in our cohort transformed to acute leukemia, which could be attributed to the relatively short average follow-up time (4.66 years). On the other hand, in our cohort, 17 non-hematologic cancers (solid tumors) were identified in 15 patients (14.5%, 15/103), which is considerably higher than what might be expected in a normal population of the same age. The increased frequency of non-hematologic cancers can be explained by several hypotheses.

Firstly, it could be speculated that mutations associated with MPN might facilitate the development of other malignancies. When we examined the molecular characteristics of our patients who developed non-hematologic cancers, MPN mutations were found in all except two patients (12 with JAK-2 V617F mutations, one with a CALR mutation). Also in two patients, both JAK-2 V617F and ASXL1 c.1954G.a (p.G652S) mutations were detected. One of the patients with a diagnosis of non-hematologic cancer before the diagnosis of MPN had an ASXL1 c.1954G.a (p.G652S) mutation. These mutations are known to activate cell proliferation pathways, but current knowledge supports that they are somatic mutations occurring only in hematopoietic stem cells. Therefore, it seems unlikely that these mutations would induce malignant transformation in other cell lineages. There is no literature evidence that JAK-2 V617F and CALR mutations occur in non-hematologic cell lines. A study by Lee et al.[23] that screened for JAK-2 V617F mutations in gastric cancers with activated JAK-STAT pathways found no positive cases.

The second possibility is the risk of developing secondary cancers due to drugs used in MPN treatment, particularly hydroxyurea. There is a significant amount of literature on the risk of secondary cancer development associated with hydroxyurea. One of the most significant studies in this regard is by Kissova et al.,[10] who investigated secondary cancer development in 172 Ph(–) MPN patients. In their study, 19.7% of the 66 patients treated with hydroxyurea developed secondary cancers, predominantly skin cancers (especially squamous cell carcinoma), which is a higher rate compared to those who used other cytoreductive treatments or did not use hydroxyurea. Another study by Hansen et al.,[24] reported an increased risk of secondary malignancies in MPN patients treated with hydroxyurea compared with patients treated with IFN-alfa 2. In a study conducted by Mathur et al.[25] in 2022, 10.2% of 324 MPN patients treated with hydroxyurea and 2% of 47 patients who did not receive hydroxyurea developed skin tumors during follow-up. In a 2020 study, Saliba et al.,[26] was found that myelofibrosis is independently associated with an increased risk of non-hematologic malignancies. In our patient group, 7 patients had a history of malignancy before MPN diagnosis. Five patients developed non-hematologic cancers following MPN diagnosis and hydroxyurea treatment. Two patients developed secondary cancers after an MPN diagnosis without using hydroxyurea or another cytoreductive drug/IFN. These findings suggest that factors other than hydroxyurea may also contribute to the development of non-hematologic cancers in our patients. Further research involving larger patient groups is planned to continue exploring this issue.

Our finding of 14.5% non-hematologic cancer frequency is notably higher than the 6.6% secondary solid cancer rate reported by Hindilerden et al.[27] in a recent multicenter Turkish study of 1013 Ph– MPN patients, though their study found similar associations with arterial thrombosis and potential protective effects of interferon-based therapy. Our finding that c.1934dupG (p.G646WfsX12) was the most common ASXL1 mutation (4/6 patients, 66.7%) is consistent with recent literature, as Yang et al.[28] similarly identified G646WfsX12 as the most frequent amino acid change (47.06%) in their cohort of 34 ASXL1-mutated AML/MDS patients, and notably demonstrated that mutations in G646W were independently associated with worse prognosis (HR=4.302, 95% confidence interval: 1.150-16.097).

This study has several limitations that should be acknowledged. First, the small sample size, particularly in the PMF group (n=9) and overall ASXL1-positive cohort (n=6), limits statistical power and generalizability. Second, we screened only exon 12 of ASXL1, potentially missing mutations in other regions. Third, we did not assess ASXL1 mutation allele burden or perform tissue-specific analysis beyond peripheral blood. Fourth, potential confounding factors for non-hematologic

cancer development, including smoking history, environmental exposures, family history, and specific hydroxyurea dosing, were not collected. Finally, our follow-up period averaging 4.21 years may be insufficient to detect all long-term outcomes including leukemic transformation. Despite these limitations, our study provides valuable preliminary data on ASXL1 mutations in a Turkish MPN cohort and generates hypotheses for future research.

CONCLUSION

In this study focusing on Ph– MPNs, ASXL1 gene mutations were screened, revealing several significant findings. ASXL1 gene mutations were detected in 6.25% of ET patients (3/48), 4.35% of PV patients (2/46), and 11.11% of PMF patients (1/9). The gender distribution among patients with these mutations was found to be equal, and patients with ASXL1 gene mutations tended to be older on average compared to those without. Furthermore, patients with ASXL1 gene mutations exhibited higher initial hemoglobin and platelet values but lower leukocyte and neutrophil values. Although not statistically significant splenomegaly rates were lower in patients with ASXL1 gene mutations, and the most common mutations identified were c.1934dupG (p.g646TrpfsX12) and c.1954G.a (p.G652S). Notably, thrombosis rates, particularly arterial thrombosis, were higher in the group with ASXL1 gene mutations.

In addition, a significant observation in our MPN cohort was the high rate of non-hematologic cancers (solid tumors), found in 14.5% of patients, with 80% of these patients carrying the JAK-2 V617F mutation. Intriguingly, two patients with JAK-2 V617F mutation also had ASXL1 gene mutations, both specifically the c.1954G.a (p.G652S) mutation. Similar to our study, a case report published by Liu et al.[29] showed that JAK2 V617F mutation was associated with myeloproliferative, lymphoproliferative and solid neoplasms. The occurrence of non-hematologic cancers following hydroxyurea treatment in five patients initially diagnosed with MPN suggests a potential link between hydroxyurea use and the development of such cancers. However, the emergence of non-hematologic cancers in two patients who had not used hydroxyurea or other cytoreductive drugs post-MPN diagnosis indicates that factors other than hydroxyurea may also influence the development of non-hematologic cancers.

DECLARATIONS

Ethics Committee Approval: The study was approved by Istanbul Bilim University Ethics Committee (No: 44140529/2015-30, Date: 17/02/2015).

Informed Consent: Informed consent was obtained from the patients.

Conflict of Interest: The authors declare that there is no conflict of interest.

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