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## Can Heterogenic Patterns of JAK2, MPL, and CALR Genes Predict Specific Clinical Characteristics of Myeloproliferative Disorders?

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| Submitted: 2023-08-16   | Abstract:   |
|---|---|
| Accepted: 2023-08-28  | Myeloproliferative neoplasm (MPN) is a neoplasm with three categories; essential  |
| Keywords:<br>JAK2<br>MPL<br>CALR<br>MPD<br>Myeloproliferative Neoplasm<br>©2023.Personalized Medicine Journal | thrombocythemia (ET), primary myelofibrosis (PMF), and polycythemia vera (PV),<br>and it usually is diagnosed through mutation analysis in several essential genes;<br>JAK2, MPL, CALR. The mutations of the mentioned genes in 50 patients with MPN<br>and 50 healthy volunteers were determined via allele-specific PCR and sequencing.<br>Based on the results, MPN and its subtypes have a significant relation with mutations<br>(p<0.05). JAK2 (exon 14) mutation was related to MPN and its subtypes except for<br>ET and CALR (exon 9) type 1 was merely related to ET, but CALR (exon 9) type 2<br>mutation was more prevalent in MPN and PV (p<0.05). None of the mutations co-<br>occurred. There was no evidence of mutation in JAK2 (exon 12) and MPL (exon 9<br>and 10) in our study, so they are unsuitable diagnostic candidates. So, mutations in<br>JAK2 (exon 14), and CALR (exon 9) type 1 and 2 are essential in MPN diagnosis<br>in Iranians. |

## INTRODUCTION

Primary myelofibrosis (PMF) also named myeloproliferative neoplasm (MPN) is divided into Philadelphia chromosome-negative [Ph(-)] and -positive [Ph(+)], composed of a non-homologous category of hematopoietic stem cell dysfunction characterized by anemia, overproduction of erythroid, myeloid, and/or megakaryocytic cells in peripheral blood as well as the bone marrow  $(\underline{1})$ . The classification of these neoplasms can occur based on bone marrow morphology as well as technical and cytogenetic characteristics. One of the most important hallmarks of the Ph(-) MPNs is the presence of proliferated large heterogenous megakaryocytes in the bone marrow. The other one is related to the absence of ring sideroblasts (RS) in the MPNs at the initial stages (2). Furthermore, in Ph(-) MPN, in contrast with JAK2, MPL, or CALR mutations, SF3B1 mutations are relatively rare with a rate of 2-10%,  $(\underline{3}-\underline{6})$ . With the technology of next-generation sequencing (NGS), the mutational profiles of MPN have been discovered which help provide more efficient therapeutic guidelines (2). As some of the non-germ line mutations are observed

in most cases of MPN in the JAK2, MPL, and, CALR genes, they can be considered the common diagnostic agents of Ph(-) MPN (7).

In general, WHO divided MPN into several categories; essential thrombocythemia (ET), primary myelofibrosis (PMF), and polycythemia vera (PV) based on clinical outcome, morphology, and disease presentation, especially at early stages (<u>8</u>). For example, a mutation in JAK2 V617F, MPLW515L/K, and CALR is one of these morphologic variations. As early PV and JAK2 -mutant ET, as well as pre-fibrotic PMF present common molecular features, molecular tests can be considered a diagnostic test for MPN (<u>9</u>).

As PMN is considered one of the life-threatening cancers among Iranians due to its weak diagnosis, introducing the most important gene mutation can play an essential role in better diagnosis which can lead to longer overall survival and more satisfying therapeutics outcomes. On the other side, diagnostic tests are expensive, especially for patients without insurance. So, determining the important diagnostic tests will help patients financially.

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## MATERIALS AND METHODS

This study was performed as a cross-sectional one over two years with the approved etic number of IR.IAU.PS.REC.1399.058 by the Tehran Medical Sciences, Islamic Azad University, and was done based on the declaration of Helsinki. In this study, 50 patient data was gathered from the Hematology Laboratory of Dr. Masih Daneshvari Hospital in Tehran, Iran. The inclusion criteria of this study contained; 35 years old and above at the time of data collection and diagnosis of MPN and its subtypes confirmed by Hematologists based on the 2016 WHO classification guidelines. Fifty healthy controls (without Myeloproliferative, systemic, Hematologic, and genetic disorders). The demographic, clinical, and laboratory data entailed; gender, age, white blood cell (WBC), platelet (Plt), and hemoglobin (Hb). The mutational evaluations (JAK2, MPL, and CALR) were done on the DNA samples which were extracted from the whole blood by using the commercial FavorPrep<sup>™</sup> Blood / Cultured Cell Genomic DNA Extraction Mini Kit (Favorgen Company, Taiwan), following the manufacturer protocol. The mutation of JAK2, MPL, and CALR was characterized via

designing several allele-specific oligonucleotide primers polymerase chain reactions (PCR) for the 5' and 3' ends of the following sequences; JAK2 (Exon 12 and 14), MPL (S505N and W515L), and CALR (Exon 9). The related specific primers were checked by the NCBI nucleotide BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi)after evaluation. The chromosome location, accession numbers of genes, products' length, related specific primers, and primers' sequences were presented in Table 1. The PCR reaction with its reaction volume is shown in table 2. The PCR was performed by a Veriti PCR thermal cycler based on the specific program obtained after setting for each reaction (Table 3). Then, the PCR products were assessed after visualization by SYBR<sup>TM</sup> Safe DNA Gel Stain (Thermo Fisher Scientific) and electrophoresis on 2% agarose gel (Figure 1). Then, the JAK2 (Exon 12) and CALR (Exon 9) gene mutations were evaluated by Sanger sequencing via Genetic - Analyzer ABI3130XL sequencer figure 2. With regards to the literature review, various kinds of mutations can lead to the JAK2 (Exon 12) and CALR (Exon 9) gene mutations (10, 11), and allele-specific PCR will not be an adequate method

Table 1. Chromosome location, accession numbers, products' length, and primers' sequences for screening of JAK2 (exon 12 and 14), CALR (exon 9), and MPL (exon 9 and 10) mutations

| Genes'<br>names | Chromosome<br>Location | accession<br>numbers | Products'<br>length | Primers'<br>names              | Primers' sequences                   |
|-----------------|------------------------|----------------------|---------------------|--------------------------------|--------------------------------------|
| JAK2<br>(exon   | 9p24.1                 | NG_009904.1          | 496bp               | Forward                        | 5'-CTCCTCTTTGGAGCAATTCA-3'           |
| 12)             |                        |                      |                     | Reverse                        | 5'-GAGAACTTGGGAGTTGCGATA-3'          |
| JAK2<br>(exon   | 9p24.1                 | NG_009904.1          | 364bp               | Forward                        | 5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3' |
| 14)             |                        |                      | 203bp               | Forward<br>(mutant)            | 5'-AGCATTTGGTTTTAAATTATGGAGTATAT-3'  |
|                 |                        |                      |                     | Reverse                        | 5'-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3' |
| CALR<br>(exon   | 19p13                  | NG_029662.1          | 921bp               | Forward                        | 5'-TAACAAAGGTGAGGCCTGGTC-3'          |
| 9)              |                        |                      |                     | Reverse                        | 5'-ACCACTGCTGGGTTTCCTT-3'            |
| MPL<br>(exon    | 1p34                   | NG_007525.1          | 211bp               | Forward                        | 5'-TGGGCCGAAGTCTGACCCTTT-3'          |
| 9)              |                        |                      |                     | Reverse                        | 5'-CAGAGCGAACCAAGAATGCCTGT-3'        |
|                 |                        |                      | 123bp               | Forward<br>(Allel<br>specific) | 5'-GGCCTGCTGCTGCTGAGAT-3'            |
| MPL<br>(exon    | 1p34                   | NG_007525.1          | 211bp               | Forward                        | 5'-TGGGCCGAAGTCTGACCCTTT-3'          |
| 10)             |                        |                      | -                   | Reverse                        | 5'-CAGAGCGAACCAAGAATGCCTGT-3'        |
|                 |                        |                      | 94bp                | Reverse<br>(Allel<br>specific) | 5'-CAGGCCCAGGACGGCG-3'               |

## Table 2. Constituents of PCR reactions with their volumes

| Genes' names   | Constituents             | Concentration | Volume (µL)     |
|----------------|--------------------------|---------------|-----------------|
| JAK2 (exon 12) | Master Mix RED           | 2X            | 10 µl           |
|                | GENOMIC DNA              | 5 µl          | 8 µl            |
|                | Forward Primer (JAK2 -F) | 0.1 pmol/µl   | 1 µl            |
|                | Reverse Primer (JAK2 -R) | 0.1 pmol/µl   | 1 µl            |
|                | Master Mix RED           | 2X            | 10 µl           |
|                |                          |               | Total: 20 µl    |
| JAK2 (exon 14) | Master Mix RED           | 2X            | 10 µl           |
|                | GENOMIC DNA              | 5 µl          | 7 μl            |
|                | Forward Primer (JAK2 -F) | 0.1 pmol/µl   | 1 μl            |
|                | Reverse Primer (JAK2 -R) | 0.1 pmol/µl   | 1 μl            |
|                |                          |               | Total: 20 µl    |
| CALR (exon 9)  | Master Mix RED           | 2X            | 10 µl           |
|                | GENOMIC DNA              | 5 µl          | 8 µl            |
|                | Forward Primer (CALR-F)  | 0.1 pmol/µl   | 1 µl            |
|                | Reverse Primer (CALR-R)  | 0.1 pmol/µl   | 1 µl            |
|                |                          |               | Total: 20 µl    |
| MPL (exon 9)   | Master Mix RED           | 2X            | 10 µl           |
|                | GENOMIC DNA              | 5 µl          | 8 µl            |
|                | Forward Primer (MPL-F)   | 0.1 pmol/µl   | 1 μl            |
|                | Reverse Primer (MPL-R)   | 0.1 pmol/µl   | 1 μl            |
|                |                          |               | Total: 20 µl    |
| MPL (exon 10)  | Master Mix RED           | 2X            | 10 µl           |
|                | GENOMIC DNA              | 5 μ1          | 8 μl            |
|                | Forward Primer (MPL-F)   | 0.1 pmol/µl   | 1 µl            |
|                | Reverse Primer (MPL-R)   | 0.1 pmol/µl   | 1 µl            |
|                |                          |               | <b>T</b> 1 20 1 |

Total: 20 µl

for evaluation of their mutations. However as we did not detect any mutations for JAK2 (Exon 12), no sequencing was available for it. Finally, the raw data was analyzed by Chromas software, and compared with the reference sequences in the NCBI database via CLC software.

In the end, the statistical analyses were performed on the data by Statistical Package for the Social Sciences (SPSS) Statistics for Windows (Version 22.0; IBM Corp., Armonk, NY, USA). The chi-square test was performed for categorical data and the independent t-test for the parametric numerical variables. Moreover, Fisher's exact test and Mann-Whitney were used for qualitative and non-parametric data, respectively. In all analyses, p-values less than 0.05 showed statistically significant differences.

## RESULTS

The demographic and clinical features of healthy controls and patients suffering from MPN are presented in Table 1. Fifty patients participated in this study; 26 (51%) males and 24 (49%) females, MPN was categorized into 21 (42%) ET, 17 (34%) PV, and 12 (24%) PMF with mean and standard deviation (SD)

#### Table 3. Thermal cycler program for JAK2 (exon 12 and 14), CALR (exon 9), and MPL (exon 9 and 10)

| Genes' names    | Cycles               | Temperatures (oC) | Time     | numbers of cycles |
|-----------------|----------------------|-------------------|----------|-------------------|
|                 | Initial denaturation | 95                | 2 min    | 1                 |
|                 | Denaturing           | 95                | 34 s     |                   |
| LAK2 (over 12)  | Annealing            | 56                | 34 s     | 35                |
| JAK2 (exon 12)  | Extension            | 72                | 34 s     |                   |
|                 | Final extension      | 72                | 7 min    | 1                 |
|                 | Hold                 | 4                 | 00       | 1                 |
|                 | Initial denaturation | 95                | 3 min    | 1                 |
|                 | Denaturing           | 95                | 33 s     |                   |
| IAK2 (evon 14)  | Annealing            | 55                | 35 s     | 39                |
| JAK2 (CAOII 14) | Extension            | 72                | 35 s     |                   |
|                 | Final extension      | 72                | 7 min    | 1                 |
|                 | Hold                 | 4                 | 00       | 1                 |
| CALR (exon 9)   | Initial denaturation | 95                | 2 min    | 1                 |
|                 | Denaturing           | 95                | 30 s     | 35                |
|                 | Annealing            | 56                | 30 s     |                   |
|                 | Extension            | 72                | 30 s     |                   |
|                 | Final extension      | 72                | 7 min    | 1                 |
|                 | Hold                 | 4                 | $\infty$ | 1                 |
|                 | Initial denaturation | 95                | 2 min    | 1                 |
|                 | Denaturing           | 95                | 35 s     |                   |
| MPL (exon 9)    | Annealing            | 55                | 30 s     | 38                |
|                 | Extension            | 72                | 30 s     |                   |
|                 | Final extension      | 72                | 10 min   | 1                 |
|                 | Hold                 | 4                 | œ        | 1                 |
|                 | Initial denaturation | 95                | 2 min    | 1                 |
|                 | Denaturing           | 95                | 35 s     |                   |
| MPL (exon 10)   | Annealing            | 55                | 30 s     | 38                |
|                 | Extension            | 72                | 30 s     |                   |
|                 | Final extension      | 72                | 10 min   | 1                 |
|                 | Hold                 | 4                 | 00       | 1                 |

ages of 55.95  $\pm$  8.70 (ET), 59.71  $\pm$  8.58 (PV), and 58.58  $\pm$  8.55 (PMF) years. The mean and SD of leukocyte, hemoglobin, and platelet counts are shown in Table 1. The first group of MPN was ET with 21 patients 11 of them were male and 10 were female, with an average age of 55.95 (8.58) years (35–71 years). The second

group was PV with 17 patients 8 of them were male and 9 were female, with an average age of 59.71 (8.58) years (35–78 years). The last group was PMF with 12 patients 7 of them were male and 5 were female, with an average age of 58.58 (8.55) years (35–78 years). The average age of MPN was 58.04 (8.62) years (35 to 78).



Fig 1. PCR products of MPL, JAK2, and CARL after agarose gel electrophoresis. Electrophoresis of PCR products to recognize mutations of MPL (1 and 2) and (4) JAK2 V617F genes; N: normal, M: mutant, +: positive, -: negative controls, and 100 bp Ladder: 100 bp DNA Ladder Thermo Fisher







2) CALR-5 ins



4) Non mutant Jak2 Exon 12.

| JACI Even 12 Seg.<br>JACI Even 13 Reference Brog.<br>Consense           |            |            | TOTATITTOA | AUTGATATAT |              |           |
|---|------------|------------|------------|------------|--------------|-----------|
| Conservation  |            |            | mmm        | -          |              |           |
| EUMC2 Exem 12 Sec.<br>UMX 2 Exem 12 Reference Dep<br>Conservation       | TTTTTTCADA | TAAATCAAAC | CTYCTAGTCT | FCAGAACGAA |              | 100       |
| Conservation  | mmm        |            |            |            |              |           |
| JANE & Loss 12 Same<br>JANE & Empire 12 References (See<br>Conservation | GATGTACEAA | COTEACGAAG | ATTACAGASS | COTACTCATA | TGAACCAAD    | 100<br>78 |
| Conservation  |            |            | -          | TERMINET   | TOTO TOTO TO |           |



Furthermore, in Table 4 the prevalence of five mutant genes has been presented in different subgroups of MPN diseases. None of mutations was observed in control group, whereas mutations in JAK2, exon 14 (V617F), CALR (exon 9) type 1 (52bp; 1092-1143 del), and CALR (exon 9) type 2 (1154 -1155 in; 5bp ins TTGTC) were reported in 29 (58%), 6 (12%), and 2 (4%) patients, respectively. The JAK2 V617F mutation occurred in 9 of ET, 13 of PV, and 7 of PMF volunteers. CALR (exon 9) type 1 mutation was detectable in 4 of ET and 2 of PMF patients, but CALR (exon 9) type 2 mutation was only detected in ET subgroup. Merely 13 of MPN patients reported as triple-negative. None of the evaluated mutations was detected simultaneously.

The correlation between sex, age, WBC, Hb, platelets, and mutations based on MPN and its subtypes are summarized in Table. 5.

## DISCUSSION

In general, the mutations of three genes of the JAK2, CALR, and MPL have been considered the

Table 4. The mean of sex, age, WBC, Hb, platelets, and mutations based on MPN and its sub-types

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most important screening factors in the (Philadelphia) Ph-MPN diagnosis, especially JAK2 mutants for PV. Though in the newer editions of the WHO guidelines, more precise criteria in all MPN subgroups have been introduced as the diagnostic criteria for this cancer, as nearly a quarter of MPN patients are triplenegative, and ones without JAK2 V617F mutation are problematic  $(\underline{12})$ . Therefore, the mutant analysis is not sufficient for sure diagnosis, but it can be used for initial diagnosis. In this study, the frequency of two genders between patients and healthy controls does not differ significantly which along with other studies, a small gender bias is visible in MPNs, with a bigger number of males. However, the different MPN subtypes show little inconsistency similar to the Heppner et al results (13). It seemed that gender did not affect the on the frequency of mutations in JAK2 (exon 14) and CALR (exon 9) type 1 or 2. In this study, the mean of WBC, Hb, and Platelet is bigger in males in comparison with females but merely Hb difference is significant. Moreover, Age was the same between healthy controls and MPN patients, as well

|  |                            | Control        | MPN                    | ET                   | PV                    | PMF             |
|--|----------------------------|----------------|------------------------|----------------------|-----------------------|-----------------|
| Sex M                                      | lale                       | 25 (50%)       | 26(52%)                | 11(44%)              | 8(32%)                | 7(28%)          |
| F  | emale                      | 25 (50%)       | 24(48%)                | 10(40%)              | 9(36%)                | 5(20%)          |
| Age  | Age                        |                | 58.04 (8.62)           | 55.95 (8.58)         | 59.71 (8.58)          | 58.58 (8.55)    |
| WBC (×10 <sup>9</sup> /L) (Range)          |                            | 6962 (1825.77) | 9225.49<br>(5869.1)    | 8352.38<br>(4082.72) | 10252.94<br>(6479.11) | 9550 (7821.36)  |
| Hb (g/dL) (Ranş                            | ge)                        | 14.14 (1.63)   | 14.55882<br>(3.413513) | 13.57 (1.88)         | 18.19 (1.61)          | 10.97 (2.45)    |
| Platelets<br>(×10 <sup>9</sup> /L) (Range) |                            | 295.1 (70.44)  | 543.1569<br>(523.3259) | 881.19 (650.42)      | 342.41 (198.74)       | 261.92 (206.69) |
| Triple negative                            |                            | 50 (100%)      | 13 (26%)               | 6 (12%)              | 4 (8%)                | 3 (6%)          |
|  | JAK2<br>(exon 12)          | 0 (0%)         | 0 (0%)                 | 0 (0%)               | 0 (0%)                | 0 (0%)          |
|  | <i>JAK2</i> (exon 14)      | 0 (0%)         | 29 (58%)               | 9 (18%)              | 13 (26%)              | 7 (14%)         |
|  | CALR<br>(exon 9)<br>type 1 | 0 (0%)         | 6 (12%)                | 4 (8%)               | 0 (0%)                | 2 (4%)          |
|  | CALR<br>(exon 9)<br>type 2 | 0 (0%)         | 2 (4%)                 | 2 (4%)               | 0 (0%)                | 0 (0%)          |
| Maria                                      | MPL<br>(exon 9)            | 0 (0%)         | 0 (0%)                 | 0 (0%)               | 0 (0%)                | 0 (0%)          |
| mutations                                  | MPL<br>(exon 10)           | 0 (0%)         | 0 (0%)                 | 0 (0%)               | 0 (0%)                | 0 (0%)          |

Table 5. The P-value of sex, age, WBC, Hb, platelets, and mutations based on MPN and its sub-types

|                             |                            | Sex   | MPN   | ET    | PV    | PMF   | <i>JAK2</i><br>(exon 14) | CALR<br>(exon 9)<br>type 1 | CALR<br>(exon 9)<br>type 2 |
|-----------------------------|----------------------------|-------|-------|-------|-------|-------|--------------------------|----------------------------|----------------------------|
| Sex                         |                            | -     | 0.841 | 0.94  | 0.94  | 0.94  | 0.827                    | 0.977                      | 0.27                       |
| Age                         |                            | -     | 0.86  | 0.487 | 0.447 | 0.75  | 0.033                    | 0.34                       | 0.97                       |
| WBC (×10 <sup>9</sup> /L)   | (Range)                    | 0.396 | 0.159 | 0.632 | 0.119 | 0.815 | 0.21                     | 0.919                      | 0.874                      |
| Hb (g/dL) (Ran              | nge)                       | 0.03  | 0.465 | 0.13  | 0     | 0     | 0.015                    | 0.07                       | 0.295                      |
| Platelets                   |                            | 0.725 | 0.002 | 0     | 0.463 | 0.008 | 0.135                    | 0.054                      | 0.058                      |
| (×10 <sup>9</sup> /L) (Rang | ge)                        |       |       |       |       |       |                          |                            |                            |
| Mutation posit              | ive                        | 0.957 | 0     | 0.001 | 0.001 | 0.008 | 0                        | 0.002                      | 0.135                      |
|                             | <i>JAK2</i> (exon 14)      | 0.827 | 0     | 0.174 | 0     | 0.036 | -                        | 0.827                      | 1                          |
|                             | CALR<br>(exon 9)<br>type 1 | 0.977 | 0.495 | 0.07  | 0.607 | 0.151 | 0.827                    | -                          | 1                          |
| Mutation                    | CALR<br>(exon 9)<br>type 2 | 0.27  | 0.002 | 0.474 | 0.031 | 0.773 | 0.27                     | 1                          | -                          |

as within each MPN sub- groups. Whereas the age of JAK2 (exon 14)-mutated patients were significantly more than non-mutated ones, it was almost the same in patients with and without type 1 or 2 mutations of CALR (exon 9). This can represent that this mutation was acquired at higher ages and may be related to the aging process. WBC showed few non-significant inconsistencies, but Hb and platelets were significant in some groups, maybe more future studies on bigger populations confirm them as the main symptoms of this disease or the mutations. The subtypes of ET, PV, and PMF, were 42%, 34%, and 24% of MPN, respectively, which were different from prospective cohort studies performed on German and Asian populations (14, 15). In Wong et al. study, ET, PV, and PMF were 30.52%, 24.21%, and 45.26%, respectively (9). In our study, ET was the most prevalent cancer. And JAK2 (exon 14) mutation was the most significant one in MPN, as more than half of MPN patients contained this mutation. The rates of the JAK2 V617F mutation in patients with ET, PMF, and PV were 18%, 14%, and 26%, respectively. Our findings were not similar to counterpart ones in Chinese and Sudanese populations because this mutation was observed more in PV, then PMF, and at last in ET, maybe because of differences in population.

According to the WHO edition of 2016, the JAK2 mutations play a key role in the autonomous RBC production and the myeloid and megakaryocytic lineages' stimulation. As valine to a phenylalanine point mutation at the 617 position of the gene activates many

of the cytokine receptors, like thrombopoietin and granulocyte colony-stimulating factors continuously, resulting in an increased level of platelets and leukocytes (16).

The other key gene in this cancer is CALR, which can usually undergo two types of mutation. In the present study, 12% of CALR type 1 mutation and 4% of type 2 mutation were detected within MPN patients with higher platelets (p < 0.05) 8% of them were diagnosed as ET, and 4% with PV without any types of JAK2 and MPL mutations. None of the patients diagnosed with PV had type 1 or 2 CALR mutations. These data were not similar to frequency of CALR mutations among MPN Slovenian (4.4%) (17). This could probably be observed because of using various analytical tests, different sample sizes, and the geographical distribution of studied populations. Our findings also were similar to Chinese, Korean, and American studies, because ET and PMF patients had more CALR mutations (18-20). Our frequent type of CALR mutation was Type 1 (75%), very similar to Zulkeflee et al. study (77.78%) (12, 20). Just like Zulkeflee et al. study, in our research, none of the MPN patients showed MPL mutations. Also in Lieu and Eldeweny et al. study MPL W515L/K was not reported in 60 MPN patients from Egypt and 88 from Taiwan (18, 21), as well as merely 1-4% of MPL mutations, were observed in studies on Korean and Chinese people (19, 20). Our results were in contrast with previous studies on the MPL mutation in Iranian and Turkish populations which was 4-6% of ET and PMF (22, 23). This discrepancy can be because of sample size differences. Furthermore, no double mutations for JAK2, MPL, and CALR genes were found in the current study, just like Lang et al. who reported no concurrent mutations in MPL and CALR genes (17). The frequency of triple-negative mutations in this research were 26%, which was very similar to a Korean study (20%) (20) and a Malaysian one (27.7%) (12). While this frequency were lower in Indian (10–15%) and Slovenia PMN patients (17, 24). In our investigation, Mutations were detected with allele-specific tests which merely evaluate hotspot mutations (25) and also gene sequencing for our target genes with more types of mutations like JAK2 (exon 12) and CALR (exon 9).

## CONCLUSION

Despite of a small population, the data of current report is important as the first investigation of the frequency of JAK2, CALR, and MPL mutations related to the MPN biological and clinical characteristics and its subtypes among Iranians. To sum up, the results of this study, as none of the MPN patients showed JAK2 (exon 12), or MPL (exon 9 and 10) mutational status, it seems that they are not suitable diagnostic candidates.

## Clinical practice points

In summary, we do not recommend analyses of JAK2, CALR, and MPL mutations for diagnosis of MPN and its subtypes among Iranians.

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## Compliance with ethical standards

All protocols were carried out based on the outlines of the Helsinki Convention and approved by the research ethics committee of Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.

#### Statements and Declarations

There is no statements and declarations.

#### Conflict of interest statement

There is no conflict of interest.

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