



Study of Tumor Necrosis Factor-Alpha-Induced Protein 3 Gene Single-Nucleotide Variants in JAK2 V617F-Positive Myeloproliferative Disorders: A Case–Control Study

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Abstract

BACKGROUND: Myeloproliferative neoplasms (MPNs) are Philadelphia-negative disorders involving polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Although the JAK2 mutation is almost always involved, several other mutations are linked to MPNs risk and prognosis. Tumor necrosis factor-alpha-induced protein 3 (TNFAIP3) genetic mutations are related to several cancers and autoimmune diseases.

AIM: Our study aimed to demonstrate the effects of rs2230926_T/G and rs5029939_C/G single-nucleotide variants (SNVs) of the TNFAIP3 gene on the risk and prognosis of JAK2 V617F-positive MPNs.

METHODS: In our study, 80 MPNs cases and 130 normal healthy controls were matched in age, gender, and race, and MPNs cases were followed for 3 years. TaqMan assay probes involved in real-time polymerase chain reaction were utilized for variant analysis.

RESULTS: The rs2230926 and rs5029939 SNVs were in modest linkage disequilibrium in MPNs cases. The observed frequencies of the G allele and its genotypes of both variants were more prevalent in MPNs patients than normal controls. The bleeding symptoms and the presence of splenomegaly were more prevalent in the heterozygous genotype and the combined G involving genotypes, respectively. Overall survival was lower in G-containing genotypes of both variants, but only the rs5029939 SNV affected progression-free survival.

CONCLUSIONS: Our study revealed the association of G-containing genotypes of both rs2230926 and rs5029939 SNVs to the increased incidence as well as the poor clinical course and prognosis of JAK2 V617F-positive MPNs disorders in Egyptian ethnicity.

Edited by: Ksenija Bogoeva-Kostovska
Citation: Abdelghany WM, El Husseiny NH, Fekry GH, Korayem OH, Helmy R. Study of Tumor Necrosis Factor-Alpha-Induced Protein 3 Gene Single-Nucleotide Variants in JAK2 V617F-Positive Myeloproliferative Disorders: A Case–Control Study. Open-Access Maced J Med Sci. 2022 Jan 06; 10(B):113-119. <https://doi.org/10.3889/oamjms.2022.7934>
Keywords: TNFAIP3; MPNs; JAK2; Incidence; Prognosis
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Received: 11-Nov-2021
Revised: 09-Dec-2021
Accepted: 27-Dec-2021
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Funding: This research did not receive any financial support
Competing Interest: The authors have declared that no competing interest exists
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Introduction

Myeloproliferative neoplasms (MPNs) are BCR-ABL-negative disorders characterized by uncontrolled proliferation of hematopoietic progenitor cells. They are classified to various types including polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) [1]. They may progress to secondary acute myeloid leukemia (AML) as well PV and ET may be complicated by secondary MF [2].

Although acquired JAK2 V617F mutation is found in more than 80% of MPNs disorders, JAK2 suppression is not being achieved as a permanent curative treatment line. Signaling pathways of JAK2 are targets of researchers to develop new diagnostic and treatment lines of MPNs [3]. The JAK2 signaling cascades are either directly through signal transducer and activator of transcription (STAT3, 5) or indirectly through nuclear factor kappa light chain enhancer of

activated B cells (NF- κ B), phosphatidylinositol 3-kinase, and mitogen-activated protein kinase (MAP kinase) [4].

Tumor necrosis factor-alpha-induced protein 3 gene (TNFAIP3) also called A20 is carried on 6q23.3 chromosome. A20 is as an ubiquitin-editing enzyme; the major suppressor of NF- κ B [5]. NF- κ B is a family of transcription factors highly convenient to cellular functions, activation, and inflammatory reactions. NF- κ B disturbance due to A2 gene defect by deletion or mutations has been related to malignant and autoimmune disorders [6]. NF- κ B overexpression is noted in some classes of AML especially that with FLT3-ITD involvement [7] as well in myelodysplastic syndrome (MDS) [8].

TNFAIP3 genetic disturbance is found to be linked to B-cell non-Hodgkin's lymphoma (NHL) including follicular lymphoma, marginal zone lymphoma, diffuse large B-cell lymphoma, and primary mediastinal B-cell lymphoma, as well to HL and to T-cell lymphoma involving Sezary syndrome (SS) [9], [10].

Among TNFAIP3, single-nucleotide variants (SNVs) are *rs2230926_T/G* and *rs5029939_C/G* that were found to be in linkage disequilibrium (LD) in Caucasian population [11]. The *rs2230926* is an exonic transversion with Phe127Cys amino acid exchange [5]. This change influences the suppressor function of A20 with responding to blockers of TNF in psoriasis. Furthermore, *rs5029939*, an intronic SNV of TNFAIP3, influences A20 protein mRNA expression through NF- κ B dysfunction [11].

Due to the scarcity of information on A20 defects in MPNs disorders, the study's hypothesis was to investigate the relationships between *rs2230926_T/G* and *rs5029939_C/G* SNVs in JAK2 V617F-positive MPNs disorders in terms of MPNs incidence, clinical presentations, and behavior to suggest them as diagnostic and prognostic markers as well as therapy targets.

Materials and Methods

This case-control study involved 210 adult Egyptian participants. It was divided into two matched groups in age and gender, with 80 MPNs cases and 130 healthy controls. MPNs cases were selected among the patients attending the Hematology Outpatient Clinic of Kaser Alini Hospitals (Cairo University) from January 2017 to May 2018, followed up for 36 months. The inclusion criteria of the research involved adult Egyptian patients who were diagnosed with PRV, ET, or PMF diseases with a positive JAK2 V617F mutation. The exclusion criteria were chronic myeloid leukemia, double malignancies, JAK2 V617F-negative PRV, ET, and PMF and the other types of MPNs disorders such as mastocytosis and chronic neutrophilic leukemia. The control group was made up of people who came in for routine check-ups and had a free medical history, a normal examination, and good laboratory results.

The MPNs cases were diagnosed according to the World Health Organization (WHO) criteria for 2016 [2]. The enrolled subjects were exposed to history taking, full examinations, and laboratory analysis including complete blood count, coagulation studies, and chemistry tests with lactated dehydrogenase assay. Bone marrow (BM) aspirate and biopsy with reticulin stain were done to fulfill MPNs criteria and establish the grade of BM fibrosis from 0 to 3. This classifies BM fibrosis as mild fibrosis if <2 and overt form in grades ≥ 2 .

Radiological investigations were involved, including abdominopelvic ultrasound and Doppler assessment of thrombotic presentations. Molecular studies were assessed on peripheral blood (PB) or BM samples for BCR-ABL to ensure its negativity as well as for JAK2 V617F mutation by quantitative real-time polymerase chain reaction (PCR) with the inclusion of its positive cases.

The treatment of MPNs cases was based on their types and risk stratification according to the 2016 WHO guidelines [12]. Low-dose aspirin was given to patients with any risk of thrombosis, while hydroxyurea was only given to patients with a high risk of thrombosis [13]. PRV patients undergo phlebotomy with a hematocrit of more than 45%. Supportive treatment was administered in PMF cases according to the presenting manifestations.

All involved individuals accepted to share in the study by signing a written informed consent. The study was performed according to the human medical research ethics of the Declaration of Helsinki with the approval of the ethical committee of the Faculty of Medicine, Cairo University.

Extraction of DNA

EDTA anticoagulant-containing tubes were used to collect PB (2 mL) for DNA extraction. The tubes were kept at -20° till the process of extraction began. GeneJET mini kits (# K0781) for DNA purification were used as directed by the manufacturer.

TNFAIP3 variants genotyping

Applied Biosystems StepOne real-time PCR apparatus was utilized to analyze the investigated variants. The genotyping was done by preparing master mix cocktail of 20 μ L final volume: 3.5 μ L of the extracted DNA, 0.5 μ L of SNV probe, 10 μ L of Thermo Fisher master mix, and 6 μ L of distilled water.

SNV TaqMan probes (Thermo Fisher scientific; #4351379) were used. [VIC/FAM] GACTTGCTACTGAGGAAGGCGCTGT[G/T] CAGCAGCTCAAGGAAACAGACACA was the *rs2230926* sequence. The sequence for *rs5029939* was [VIC/FAM] GTCACCTAACTAGTTAGGAGCAGA[C/G] TTAAGCTAGAACCAAGTCCCCTGG. The PCR amplification procedure began with a 30 s pre-PCR reading at 60°C followed by a 10 min hold at 95°C . Following that, 45 amplification cycles of denaturation at 95°C and annealing/extension at 60°C for 30 s and 1 min, respectively, were formed. Finally, post-PCR holding stage at 60°C for 30 s was done. Quality control was established done by repetition of genotyping on $\sim 10\%$ of specimens and checking of the results by two observers without knowing the case-control condition.

Statistical analysis

A goodness-of-fit (χ^2) test was performed on Hardy-Weinberg equilibrium (HWE). Data were analyzed using IBM SPSS advanced statistics version 17. Quantitative variables were expressed as mean and standard deviation or median and range. Qualitative data were formulated as frequency and percentage (%). Qualitative input was

evaluated using the Chi-square test or Fisher's exact test. For quantitative variables, two group comparisons were studied by a Student's t-test while an ANOVA test was used for three group comparisons. For risk assessment, odds ratio and 95% confidence interval were calculated. LD was mathematically assessed [14], [15]. Survival rates were calculated by the Kaplan–Meier method and the log-rank test. The hazard ratio was detected by the Cox regression test. $p = 0.05$ or less was referred to as significant.

Results

Subjects criteria

The characteristics of the enrolled subjects are shown in Table 1. Regarding age and gender, no statistically significant difference between the cases and controls was detected.

Table 1: Demographic and clinical data of cases and controls

Characteristics	Cases (n=80)	Controls (n=130)	p-value*
Age (years)			
Mean \pm SD	52.68 \pm 10.899	54.37 \pm 11.387	0.611
Range	29–75	29–76	
Gender, n (%)			
Male	45 (56.25)	58 (44.6)	0.101
Female	35 (43.75)	72 (55.4)	
Type of MPN, n (%)			
PRV	42 (52.5)		
ET	20 (25)	-	
PMF	18 (22.5)		
Clinical presentations, n (%)			
Bleeding	10 (12.5)	-	
Thrombosis	17 (21.3)		
Organomegaly, n (%)			
Hepatomegaly	15 (18.8)	-	
Splenomegaly	52 (65)		
Laboratory data, median (Range)			
Hb (g/dL)	15.1 (5.01–23)	-	
RBCs (million/mm ³)	5.75 (2.4–8.9)		
HCT (%)	46.5 (18.5–70)		
TLC ($\times 10^3$ /mm ³)	12.30 (2.10–58)		
PLTs ($\times 10^3$ /mm ³)	673 (30–1525)		
JAK2 (%)	32.60 (3.125–96.1)		
Grades of fibrosis at diagnosis, n (%)			
0	23 (28.7)	-	
I	24 (30)		
II	21 (26.3)		
III	12 (15)		
Progression, n (%)			
Progression to MF	9 (11.3)		
Progression to acute leukemia	2 (2.5)		
Death, n (%)	10 (8)		

* $p \leq 0.05$ is statistically significant. HCT: Hematocrit, JAK2: Janus kinase 2 gene, PLT: Platelet, RBCs: Red blood cells, SD: Standard deviation, TLC: Total leukocytic count.

The MPNs cases were classified into PRV, ET, and PMF. Bleeding and thrombotic manifestations were present in all three categories of MPNs. As bleeding symptoms were presented in 10 cases, in the form of epistaxis in one case, bleeding per gum in five patients, and four cases manifested by bleeding per rectum. Thrombotic events were present in 17 patients, as portal vein thrombosis in five cases, gangrene of the toes in nine cases, stroke in two patients, and myocardial infarctions were detected in only one case (Table 1).

Organomegaly was variable in our cases, in the form of hepatomegaly and/or splenomegaly. Grades of BM fibrosis by reticulin stain on BMB were assessed at

presentation as well as on follow-up. Progression of the disease occurred to secondary MF and acute leukemia. In 3 years of follow-up, about 8% of MPNs cases died (Table 1).

Genotyping of rs2230926_T/G and rs5029939_C/G in cases and controls

In concordance to HWE, the observed genotypic frequencies were not statistically different than the expected ones in both cases and controls ($p > 0.05$). Both variants were in modest LD in the MPNs cases ($D' = 0.5$ and $r^2 = 0.2$).

The G allele and its containing genotypes of both variants were of high risk for MPNs incidence with $p < 0.05$ (Table 2).

Correlation of rs2230926_T/G and rs5029939_C/G to patients' characteristics

The G-containing genotypes of both variants were associated with worse clinical manifestations and poor prognostic value in MPNs disorders. The TG genotype of rs2230926 and the CG genotype of rs5029939 were statistically significant and associated with the existence of bleeding symptoms. As well, the TG+GG genotypes of rs2230926 and the GG+CG genotypes of rs5029939 were linked to the presence of splenomegaly (Tables 3 and 4).

For both variants, no significant difference was found regarding age, sex, type of MPNs, grades of BM fibrosis, thrombosis occurrence, presence of hepatomegaly, or the progression of disease to MF or acute transformation in their different genotypic modalities (Tables 3 and 4).

Overall survival (OS)

The number of deaths was significantly higher in G-containing genotypes of both variants with $p < 0.05$ (Tables 3 and 4). The mean OS of MPNs cases was higher in the TT genotype than in the TG and GG genotypes for rs2230926, 35.430, 32.595, and 27.940 months, respectively, with $p = 0.002$. The TG+GG genotype was associated with an increased risk of death (HR = 8.826, $p = 0.006$, and 95% CI = 1.873–41.594) (Figure 1).

Regarding rs5029939, the OS was prolonged in the CC genotype more than that found in CG and GG genotypes, with a mean of 35.632, 32.387, and 29.283 months, respectively; $p = 0.001$. The CG+GG genotypes carried a higher risk for mortality than the CC genotype (HR = 16.935, $p = 0.007$, and 95% CI = 2.144–133.768) (Figure 1).

Progression-free survival

For rs2230926, the mean of progression-free survival (PFS) was not different between TT, TG, and GG, being 34.457, 33.593, and 27.620 months, respectively, with $p = 0.2$. In rs5029939, the mean PFS in G-containing genotypes GG and CG was

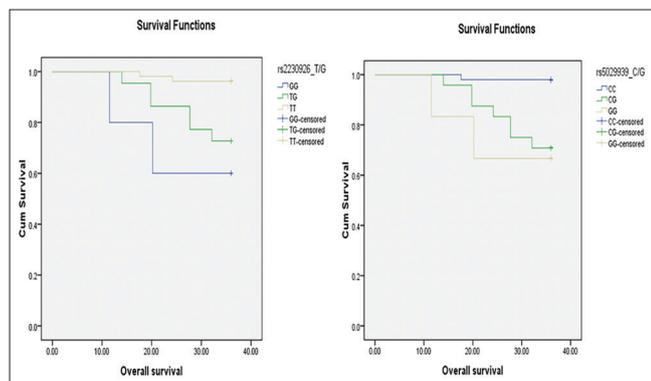


Figure 1: OS of rs2230926_T/G and rs5029939_C/G

lower than in CC genotypes at 27.367, 32.982, and 34.918 months, respectively ($p = 0.011$). As well, the CG+GG genotypes carried a higher risk for progression than the CC genotype (HR = 3.3, $p = 0.037$, and 95% CI = 1.077–10.110) (Figure 2).

Table 2: Genotyping of rs2230926_T/G and rs5029939_C/G in the studied groups

Characteristics	Cases (n=80) n (%)	Controls (n=130) n (%)	p-value*	OR (95% CI)
rs2230926_T/G				
Genotypes				
TT	53 (66.3)	106 (81.5)	Reference	
TG	22 (27.5)	23 (17.7)		
GG	5 (6.3)	1 (0.8)	0.011*	
TG+GG	27 (33.8)	24 (18.5)	0.012*	2.250 (1.185–4.272)
P ^{HWE}	0.453	0.458		
Alleles				
T	128 (80)	235 (90.4)		
G	32 (20)	25 (9.6)	0.003*	2.350 (1.335–4.138)
rs5029939_C/G				
Genotypes				
CC	50 (62.5)	103 (79.2)	Reference	
CG	24 (30)	26 (20)		
GG	6 (7.5)	1 (0.8)	0.005*	
CG+GG	30 (37.5)	27 (20.8)	0.008*	2.289 (1.231–4.255)
P ^{HWE}	0.979	0.898		
Alleles				
C	124 (77.5)	232 (89.2)		
G	36 (22.5)	28 (10.8)	0.001*	2.406 (1.402–4.127)

* $p \leq 0.05$ is statistically significant, HWE: Hardy–Weinberg equilibrium.

Table 3: Relations of rs2230926_T/G genotypes to demographic and clinical data in MPNs cases

Characteristics	TT (n=53) n (%)	TG (n=22) n (%)	GG (n=5) n (%)	p-value*	TT (n=53) n (%)	TG+GG (n=27) n (%)	p-value*
Age							
Mean \pm SD	53.66 \pm 11.109	51.27 \pm 11.05	48.40 \pm 7.335	0.462	53.66 \pm 11.109	50.74 \pm 10.402	0.260
Range	30–75	29–67	41–58		30–75	29–67	
Sex							
Male	30 (56.6)	13 (59.1)	2 (40)	0.737	30 (56.6)	15 (55.6)	0.929
Female	23 (43.4)	9 (40.9)	3 (60)		23 (43.4)	12 (44.4)	
Type of MPN							
PRV	30 (56.6)	12 (54.5)	0 (0)	0.150	30 (56.6)	12 (44.4)	0.487
ET	13 (24.5)	5 (22.7)	2 (40)		13 (24.5)	7 (25.9)	
PMF	10 (18.9)	5 (22.7)	3 (60)		10 (18.9)	8 (29.6)	
Clinical presentations							
Thrombosis	11 (20.8)	5 (22.7)	1 (20)	0.980	11 (20.8)	6 (22.2)	0.879
Bleeding	3 (5.7)	7 (31.8)	0 (0)	0.005*	3 (5.7)	7 (25.9)	0.010*
Organomegaly							
Hepatomegaly	11 (20.8)	3 (13.6)	1 (20)	0.770	11 (20.8)	4 (14.8)	0.520
Splenomegaly	29 (54.7)	18 (81.8)	5 (100)	0.019*	29 (54.7)	23 (85.2)	0.007*
Grades of fibrosis at diagnosis, n (%)							
Mild (0+I)	32 (60.4)	13 (59.1)	2 (40)	0.676	32 (60.4)	15 (55.6)	0.679
Overt (II+III)	21 (39.6)	9 (40.9)	3 (60)		21 (39.6)	12 (44.4)	
Progression							
Progression to MF	7 (13.2)	1 (4.5)	1 (20)	0.158	7 (13.2)	2 (7.4)	0.467
Progression to acute leukemia	1 (1.9)	1 (4.5)	0 (0)	0.745	1 (1.9)	1 (3.7)	0.623
Deaths	2 (3.77)	6 (27.27)	2 (40)	0.003*	2 (3.77)	8 (29.63)	0.001*

* $p \leq 0.05$ is statistically significant. SD: Standard deviation.

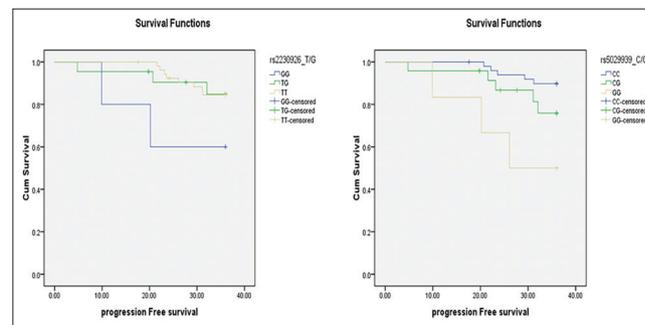


Figure 2: PFS of rs2230926_T/G and rs5029939_C/G

Discussion

Philadelphia-negative MPNs are characterized by myeloid clonal proliferation with the JAK2 mutation as one of their major diagnostic criteria [1]. The inflammatory deregulation genes are also included in MPNs' pathogenesis and progression: BCL6, CXCR1, TP53, CD40LG, and TNFAIP3 [16]. The TNFAIP3 gene is a major genetic control gene for the inflammatory signaling pathway through NF- κ B suppression and apoptosis inhibitor induced by TNF [17].

Our study revealed the association of rs2230926 and rs5029939 SNVs of the TNFAIP3 gene to MPNs incidence and prognosis in the Egyptian population. To the best of our knowledge, it is the first research concerned with investigating A20 SNVs in MPNs. In this study, we analyzed both variants by real-time PCR; the sequencing of results for confirmation was done in various previous researches. As Zhou *et al.* confirmed the results of both variants by sequencing after PCR-restriction fragment length polymorphism analysis in relation to immune thrombocytopenia (ITP) of the Chinese population [18].

For rs2230926, the TG and GG genotypes were more common in cases (27.5% and 6.3%,

Table 4: Relations of rs5029939_C/G genotypes to demographic and clinical data of MPNs cases

Characteristics	CC (n=50) n (%)	CG (n=24) n (%)	GG (n=6) n (%)	p-value*	CC (n=50) n (%)	CG+GG (n=30) n (%)	p-value*
Age							
Mean ± SD	52.24 ± 11.231	53.17 ± 11.343	54.33 ± 6.501	0.878	52.24 ± 11.231	53.40 ± 10.467	0.648
Range	29–75	33–71	45–63		29–75	33–71	
Sex							
Male	29 (58)	14 (58.3)	2 (33.3)	0.5	29 (58)	16 (53.3)	0.684
Female	21 (42)	10 (41.7)	4 (66.7)		21 (42)	14 (46.7)	
Type of MPN							
PRV	30 (60)	11 (45.8)	1 (16.7)	0.108	30 (60)	12 (40)	0.209
ET	10 (20)	6 (25)	4 (66.7)		10 (20)	10 (33.3)	
PMF	10 (20)	7 (29.2)	1 (16.7)		10 (20)	8 (26.7)	
Clinical presentations							
Thrombosis	9 (18)	5 (20.8)	3 (50)	0.194	9 (18)	8 (26.7)	0.359
Bleeding	1 (2)	9 (37.5)	0 (0)	< 0.001*	1 (2)	9 (30)	< 0.001*
Organomegaly							
Hepatomegaly	12 (24)	2 (8.3)	1 (16.7)	0.268	12 (24)	3 (10)	0.120
Splenomegaly	27 (54)	21 (87.5)	4 (66.7)	0.018*	27 (54)	25 (83.3)	0.008*
Grades of fibrosis at diagnosis, n (%)							
Mild (0+I)	30 (60)	13 (54.2)	4 (66.7)	0.821	30 (60)	17 (56.7)	0.769
Overt (II+III)	20 (40)	11 (45.8)	2 (33.3)		20 (40)	13 (43.3)	
Progression							
Progression to MF	4 (8)	3 (12.5)	2 (33.3)	0.340	4 (8)	5 (16.7)	0.319
Progression to acute leukemia	1 (2)	1 (4.2)	0 (0)	0.787	1 (2)	1 (3.3)	0.712
Death	1 (2)	7 (29.2)	2 (33.3)	0.001*	1 (2)	9 (30)	< 0.001*

*p ≤ 0.05 is statistically significant. SD: Standard deviation.

respectively) than in controls (17.7% and 0.8%, independently), while the TT genotype was more common in controls (81.5%) than in cases (66.3%); $p = 0.01$. The combined (TG+GG) genotypes carried a 2.25 higher risk of MPN occurrence than the TT genotype ($p = 0.012$, OR = 2.250, and 95% CI = 1.185–4.272). The G allele was more prevalent in cases (20%) than controls (9.6%) to be at a risk for MPN incidence (OR = 2.350 and 95% CI = 1.335–4.138).

For rs5029939, CG+GG genotype frequencies were 37.5% in cases and 20.8% in controls, increasing the risk of MPNs incidence ($p = 0.008$, OR = 2.289, and 95% CI = 1.231–4.255). As well, the G allele was found to raise MPNs occurrence ($p = 0.001$, OR = 2.406, and 95% CI = 1.402–4.127).

In agreement with our study, Kato *et al.* [9] study provided a wide genomic analysis, including A20 genetic variants in relation to malignant tumors, NHL, and HL. They found that TNFAIP3 genetic variants were prevalent in 21.8% of mucosa-associated tissue lymphoma as well as in 33.3% of the nodular sclerosis type of HL. In addition, NF- κ B pathway genetic disruption was detected in 40% of B-cell lymphoma.

Our study documented the presence of LD between rs2230926 and rs5029939 in Egyptian MPNs cases ($D = 0.5$ and $r^2 = 0.2$). This is in agreement with Bates *et al.*'s meta-analysis that confirmed the presence of LD between both variants in Caucasian ethnicity ($r^2 = 0.99$) [11]. In addition, Zhou *et al.* detected their linkage in Chinese with $r^2 = 0.108$ and $D' = 0.359$ [18].

Our results for rs2230926 genotypic modalities to clinical manifestations of MPNs revealed that TG and GG genotypes were more frequent in cases presented with splenomegaly than those found with the TT genotype ($p = 0.019$). Bleeding symptoms were more frequent in cases with TG genotype (31.8%) than in those with TT genotype (5.7%) ($p = 0.005$). Regarding OS of MPNs cases, it was more prolonged in the TT genotype

than that calculated for TG and GG genotypes ($p = 0.002$). The combined TG+GG genotypes had an 8.826 hazard ratio for mortality compared to the TT genotype ($p = 0.006$ and 95% CI = 1.873–41.594). There was no statistically significant relationship between rs2230926 genetic types and PFS.

Regarding rs5029939, 37.5% of cases with the CG genotype had bleeding symptoms while they were present in only 2% of the GG genotype ($p < 0.001$). Splenomegaly was detected in 54%, 87.5%, and 66.7% of CC, CG, and GG genotypes, respectively ($p = 0.018$). The OS was reduced in cases with CG and GG genotypes than in those with CC genotypes ($p = 0.001$). The CG+GG genotypes had a higher mortality risk than the CC genotype (HR = 16.935, $p = 0.007$, and 95% CI = 2.144–133.768). PFS was also lower in CG+GG genotypes compared to CC ones (HR = 3.3, $p = 0.037$, and 95% CI = 1.077–10.110).

No statistical significance was found regarding the different genotypes of both variants to age, sex, different types of MPNs, occurrence of thrombosis, presence of hepatomegaly, grades of BM fibrosis, or progression of disease to either MF or acute leukemia.

Concomitant to our results, Braun *et al.* [10] studied the association of A20 genetic deletion/mutation with subsequent NF- κ B deregulation in SS prognosis. They found that dysfunction of A20, either mono or biallelic, was linked to malignant T-cell proliferation and was more frequent in SS cases (6/13). The amount of mRNA of A20 and its protein expression in SS cells with A20 deletion was examined to be found not detected in biallelic deletion and weakly expressed in monoallelic deletion. No cellular toxicity in an A20 deficient cell line (SeAx A20) after induction of A20 was detected in comparison to the reference empty cell line (SeAx MOCK). The S phase of SeAx A20 was less than that found in SeAx MOCK with $p = 0.01$. As well, SeAx A20 was more in G1/G0 and less in G2/M than what was found in SeAx MOCK ($p = 0.006$ and 0.004, respectively).

In addition, Zhou *et al.* [19] discussed the NF- κ B deregulation in AML as a target for therapy. Bortezomib as an NF- κ B inhibitor has entered into various clinical trials in the treatment of AML, either alone or in combination with other drugs, with marked improvement in remission rates.

Similarly, Skov *et al.* [16] examined by microarray the genetic expression of patients diagnosed as PRV, ET, and PMF who were receiving interferon-alpha 2 (IFN- α 2) therapy. IFN- α 2 reduces the inflammatory immune reactions by regulation of its responsible genes. Skov *et al.* documented the induction of TNFAIP3 gene expression in PRV and PMF cases receiving IFN- α 2 treatment to confirm that TNFAIP3 appeared to play an important role in MPNs' pathogenesis and prognosis.

Inflammation is one of the factors promoting MPNs development and affects its prognosis. PMF is found to be correlated with inflammatory cytokines, including IL-6, C-reactive protein, and TNF [20]. MPL W515L and JAK2 V617F-positive MPNs have been linked to inflammatory pathway signaling mediators such as PI3 kinase, MAP kinase, and NF- κ B [21], [22].

The *rs2230926_T/G* is the most common variant of A20 gene in autoimmune disorders such as ITP, systemic lupus erythematosus (SLE), and rheumatoid arthritis [23], [24]. It leads to phenylalanine to cysteine amino acid exchange at position 127 in exon 3 of the A20 gene [25]. This substitution dampens the anti-inflammatory functions of TNFAIP3, which is also involved in lymphoma of B- and T-cell origin [5]. The *rs5029939_C/G* in intron 2 of A20 disrupts the expression protein level of the TNFAIP3 gene with activation of the NF- κ B signaling pathway and is related to SLE and systemic sclerosis pathogenesis and behavior [26].

The present study is the initial research of SNVs of the A20 gene in MPNs disorders. It gives the first step in clarifying the role of TNFAIP3 genetic variants in MPNs. It represents a key toward further studies for more investigations into their roles in the diagnosis and management of MPNs.

The limitations of our study were due to the absence of financial support, which will be fulfilled in the future research. It is presented in the lack of a confirmatory method for our results, but we depended on its verification by sequencing in other researches of different populations.

Conclusions

Our study provides the first analysis of TNFAIP3 SNVs in MPNs incidence and prognosis. The *rs2230926_T/G* and *rs5029939_C/G* SNVs were found to be in LD in Egyptian MPNs cases. The G-containing

genotypes of both variants were consistent with bleeding symptoms, the presence of splenomegaly, and poor survival rates. The PFS was lower in G involved genotypes of *rs5029939*, which was *not* observed in *rs2230926* genotypes.

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