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Polymorphism of Janus Kinase 2 and Telomerase Reverse Transcriptase Genes in Iraqi Patients with Myeloproliferative Neoplasm

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Abstract

Specific somatic mutations in any of the three genes—JAK2, CALR, or MPL—are frequently found in myeloproliferative neoplasms (MPNs), which include polycythaemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The rs2736100 in the telomerase reverse transcriptase gene (TERT) increases the risk of MPN disease in Iraqi subjects. This research attempted to investigate the prevalence of TERT rs2736100 and JAK2V617F in Iraqi patients with MPN and normal population. According to the collected data, significant correlation was indicated between the allele T of TERT rs2736100 and the prevalence of MPN (OR =3.3108, 95% CI =1.3828 to 7.9269, P =0.0072). Additionally, we have observed that the allele T of TERT rs2736100 has been correlated significantly with JAK2 V617F-positive MPN (OR = 4.0737, 95% CI (1.8347 to 10.8681) P = 0.0010). In accordance, the present work revealed a link between the TERT gene polymorphism rs2736100 and the development of MPN in an Iraqi population sample. Hence, it can be stated that the TERT rs2736100 polymorphism has a key impact over the development of MPN. Nevertheless, more investigations should be proceeded on larger population to validate this outcome.

Keywords: Myeloproliferative neoplasms; SNP; rs2736100; telomerase reverse transcriptase gene; JAK2 V617F.

تعدد الأشكال لجينات *JAK2* و *TERT* في المرضى العراقيين المصابين بالأورام النقوية التكاثرية

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الخلاصة

توجد طفرات جسدية محددة في أي من الجينات الثلاثة – JAK2 أو CALR أو MPL – بشكل متكرر في الأورام النخاعية العمرية (MPNs) ، والتي تشمل كثرة الحمر الحقيقية (PV) ، كثرة الصفيحات الأساسية (ET) ، وداء النخاع الشوكي الأولي (PMF). يزيد rs2736100 في جين تيلومريز النسخ العكسي

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(TERT) من خطر الإصابة بمرض MPN في الأشخاص العراقيين. بحثت هذه الدراسة في توزيع TERT rs2736100 و JAK2V617F في المرضى العراقيين المصابين بـ MPN والأشخاص الطبيعيين. أظهرت النتائج وجود ارتباط معنوي بين الأليل T لـ TERT rs2736100 ونسبة حدوث MPN (OR = 3.3108، 95٪ CI = 1.3828 إلى 7.9269 ، P = 0.0072). بالإضافة إلى ذلك ، وجدو أن أليل T لـ TERT rs2736100 كان أيضًا مرتبطًا بشكل كبير بـ JAK2 V617F إيجابي MPN (OR = 4.0737، 95٪ CI (1.8347 إلى 10.8681) P = 0.0010). ، أظهرت هذه الدراسة وجود علاقة بين تعدد الأشكال rs2736100 لجين TERT ونسبة حدوث MPN في عينة من السكان العراقيين. وتدعم نتائج هذه الدراسة دور تعدد الأشكال rs2736100 TERT في التسبب في MPN. وتوصي بإجراء دراسات أكبر لتأكيد هذه النتيجة.

1. Introduction

The increase of clone number of abnormally differentiated blood stem cells in the bone marrow is known as a myeloproliferative neoplasm (MPN). There are two subgroups of this condition, each defined by the existence of the Philadelphia (Ph) chromosome, Ph chromosome-positive MPNs and Ph chromosome-negative MPNs). Ph chromosome-positive MPNs are sometimes referred to as chronic myelogenous leukemia (CML). The three main types of Ph chromosome-negative MPNs are essential thrombocytosis (ET), primary myelofibrosis (PMF), and polycythemia vera (PV). An increase in granulocytes, erythroid cells, B lymphocytes, and megakaryocytes in the bone marrow marks the progression of PV, a clonal illness that starts with mutations in pluripotent hematopoietic stem cells. Typical consequences of PMF include extramedullary hematopoiesis and reactive fibrous connective tissue deposition. PMF symptoms are mostly brought on by an excess of megakaryocytes and granulocytes in the bone marrow. ET starts with a single abnormal megakaryocyte clonal development, followed by a rise in platelet count [1, 2].

Elderly people are more susceptible to developing MPNs than younger people, with the risk significantly increasing after age 60. Moreover, the incidence of MPNs is gender-selective, that it is more frequent in males than females. There are few risk factors in the literature related to the development of certain types of MPNs such as smoking, obesity, alcohol abuse, benzene anhelation, and physical activity [3]. The widespread use of JAK2 recurrent mutational anomalies has greatly improved the process of identification, classification, and understanding the progression of these medical conditions [2]. JAK2V617F mutation is the most known type of genetic mutations in MPNs, where it has been observed in 50% of ET and 95% of PMF and PV [4]. The employment of such mutations brings out an easy way to predict the progression of MPNs, but the root of the disease still a mystery. For instance, the rate of MPN occurrence differs critically according to geographic locations, extending from around 2/100,000 patient/year in China [5] to 5.8/100,000 patients/year in European Union [6, 7], which points to genetic susceptibility disparities. Up to these days, the contribution of the genetic factors cannot be decided it for sure affects the incidence rates in Caucasians and Chinese ethnicities.

Telomerase is a DNA polymerase that is RNA-dependent, with the major catalytic part being telomerase reverse transcriptase (TERT). The transcriptional suppression of the TERT gene causes the majority of normal human cells to lack telomerase, whereas malignant transformation necessitates both TERT induction and telomerase activation [8, 9]. Malignant cells have an endless proliferation capacity due to abnormal TERT expression. MPNs have also been found to have strong telomerase activity and TERT expression [10, 11].

Recent research has found a connection that linked rs2736100 A>C single nucleotide polymorphism (SNP) to the TERT gene and the chance of incidence of MPN diseases [12-16].

The rs2736100 of TERT gene is found in intron 2 of this gene and its CC genotype has been shown to increase TERT transcription and, consequently, the risk for developing cancer [17].

According to the aforementioned importance of the TERT and JAk2 genes in myeloproliferative neoplasm pathophysiology, the polymorphism of the rs2736100 in TERT gene and Jak2 gene was investigated in the present study to predict their association with the disease.

2. Materials and Methods

2.1. Subjects

Ninety-six specimens were gathered from a haematology test center located in Baghdad, Iraq, between January 2021 and April 2021. The specimens comprised sixty-four Iraqi MPN patients and thirty-two healthy participants who were chosen as a control group. They were all of comparable age (Mid-fifties for patients and early-forties for control) and sex (66% males and 34% females for patients and 69% males and 31% females for control). Each diagnosis was evaluated using the MPN categorization from the 2008 World Health Organization. The Mustansiriyah University's institutional ethics committee provided its approval for this study (Code No. 4 in 14/1/2024), which involved participating center.

2.2. DNA Extraction and SNP Genotyping

DNA samples were collected from peripheral blood participants using a DNA extraction kit (Bionear kit, Korea, Cat. No. K-3032). Sequencing was used to genotype the rs2736100 (TERT) locus. In this study, two primers were designed to amplify A 889 bp product size of the TERT gene. Twenty-five μL of the reaction mixture for PCR consisted of 12.5 μL of Promega's Hot Start Green Master mix, 1 μL of each primer, 2 μL of genomic DNA, and 8.5 μL of distilled water. The PCR conditions were five-minute starting point of denaturation at 95 ºC, which was followed by 30 cycles of denaturation at 95 ºC for 30 seconds, annealing at 60 C for 30 seconds, prolonging for 30 seconds at 72 ºC, and final extension of 7 minutes at 72 ºC. The integrity of PCR products (889 bp) was checked using 1.5 agarose gel electrophoresis (1.5 percent (w/v)), as shown in Figure 1. According to the Sanger protocol [18], all PCR products were sequenced.

2.3. Statistical Analysis

The SPSS version 11.2 program was used to examine the data. Using OR with 95% confidence intervals, the degree of connection between the rs2736100 polymorphism and MPN risk was evaluated. When $P \leq 0.05$, the changes were deemed significant.

3. Results

For rs2736100 variations, 96 MPN subjects from Iraq were genotyped in total. Table 1 summarizes the clinical characteristics, including age, sex, Hb, PCV, WBC, PLT, and mutation status (JAK2V617F mutations).

Table 1: Demographic and clinical parameters of MPN patients and healthy controls.

Variables	Patients	Control	P value
Number	64	32	-
Age	56.25 ± 16.81	41.22±17.11	0.851
Sex	Female: 22(34) Male :42(66)	Female: 10(31) Male :22(69)	-
JAK2-status, n(%)	Jak positive 48(75) Jak negative 16(25)	-	-

MPN: Myeloproliferative neoplasm, Hb: Hemoglobin, PCV: Packed cell volume, WBC: White blood cell, PLT: Platelet.

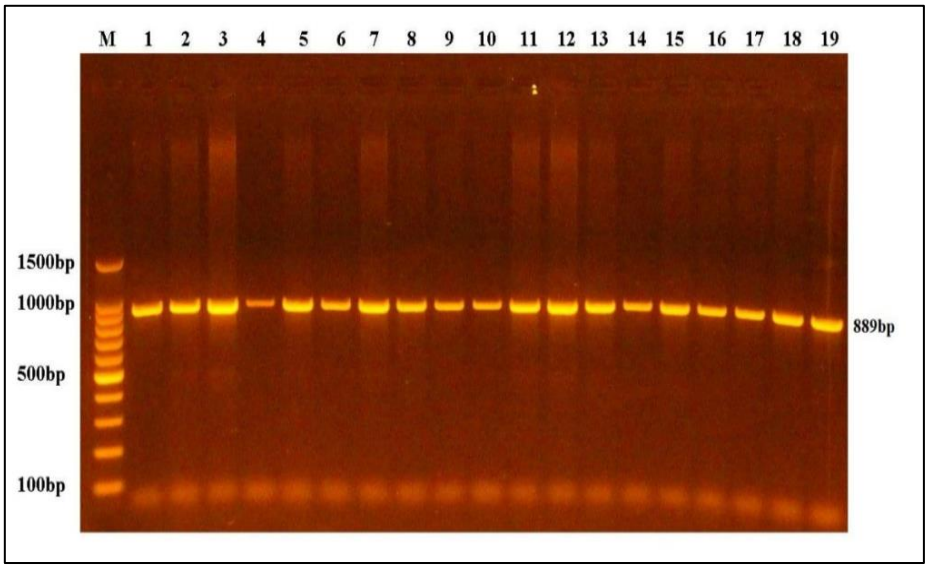

Figure 1: Amplification results of rs2736100 in human samples. The samples were fractionated on a 1.5% agarose gel electrophoresis and stained with an Ethidium bromide. M: 100bp ladder marker. The 889bp PCR products in lanes 1–19 are similar, 100 volt/ 50 mAmp for 60 min.

The TERT gene fragment (889 bp), following amplification using PCR (Figure 1), was subjected to sequencing using Sanger protocol (Figure 1). Three genotypes of rs2736100 were detected, namely GG, GT, and TT (Figure 2). The genotype frequencies for GG, GT, TT of the patients and control groups were 35, 21, 8 and, 26, 5, 1 respectively.

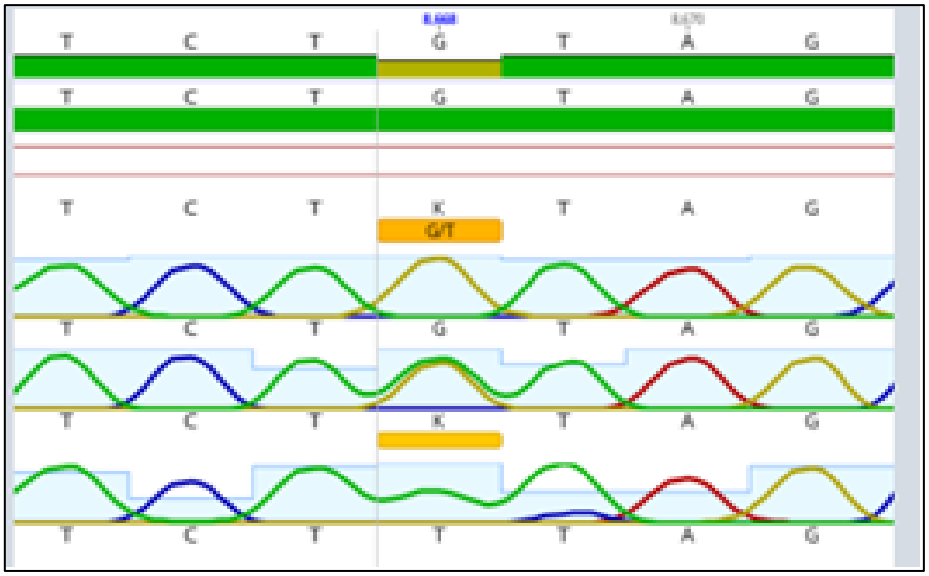

Figure 2: Analysis of rs 2736100 SNP of TERT gene using sanger sequencing. The single G peak is an indicative of a G homozygous allele. The single T peak is an indicative of a T homozygous allele. The presence of the G and T peaks indicate a G/T heterozygous allele.

The rs2736100 was statistically tested and the results demonstrated that the allele T represents a significant risk for the incidence of MPN disease (O.R =3.3108, 95% CI =1.3828 to 7.9269, P =0.0072), as shown in Table 2.

Table 2: Distribution of TERT rs2736100 in MPN patients and controls.

rs2736100	MPN N(%) 64	Control N(%) 32	OR(95%CI)
CC GG	35 (54.69)	26 (81.25)	1
AC GT	21 (32.81)	5 (15.62)	3.1200 (1.0391 to 9.3677)
AA TT	8(12.5)	1(3.13)	5.9429 (0.6993 to 50.5076)
TT+GT vs. GG	29 (45.31)	6 (18.75)	3.5905 (1.3012 to 9.9075)

OR: odds ratio, CI: confidence interval.

According to Table 3, there was no shift in the order of distribution of the rs2736100 alleles between female MPN patients and female controls. But there was a significant difference between male patients and male controls. In males, patients had significantly more T alleles (40%) than the control (14%) (OR =4.3067,95 % CI: 1.6408 to 11.3038, P = 0.0030). The findings also indicate that, corresponding to the GG genotype, the GT and TT genotypes were linked to an elevated incidence of MPNs in men [OR =4.7813, 95 % CI (1.3286 to 17.2065), P = 0.0166, for GT; OR =8.5; 95 % CI (0.9531 to 75.8068), P = 0.0552 for TT].

Table 3: Genotypes of TERT rs2736100 in women and men.

rs2736100	Men			Women		
	MPN N(%) 42	Control N(%) 22	OR (95% CI)	MPN N(%) 22	Control N(%) 10	OR (95% CI)
CC GG	16	17	-	19	9	-
AC GT	18	4	4.7813(1.3286 to 17.2065) P = 0.0166	3	1	1.4211(0.1292 to 15.6357) P = 0.7740
AA TT	8	1	8.5000(0.9531 to 75.8068) P = 0.0552	0	0	-
Allele G	50 (60)	38 (86)		41 (93)	19(95)	-
Allele T	34 (40)	6 (14)	4.3067(1.6408 to 11.3038) P = 0.0030	3(7)	1(5)	1.3902 (0.1356 to 14.2555) P = 0.7814

OR: odds ratio, CI: confidence interval.

This study evaluated the distribution of genotypes for TERT rs2736100 among V 617 – positive and negative MPN patients (Table 4). The G/T and T/T genotypes were significantly correlated with JAK2 V617Fpositive MPN [OR=4.25; 95% CI (1.3580 to 13.3293); P = 0.0129) for G/T and OR=9.4545, 95% CI (1.0958 to 81.5756), P = 0.0410) for T/T]. As well as, the allele T was also significantly associated with JAK2 V617F-positive MPN (OR= 4.0737, 95% CI (1.8347 to 10.8681), P = 0.0010).

Table 4: Distribution of TERT rs2736100 among JAK2 V617F-positive and - negative MPN patients and controls.

Genotype	JAK2 V617F-positive MPN	Control	JAK2 V617F-positive MPN vs control OR (95%)	JAK2 V617F-positive MPN	JAK2 V617F-negative MPN	JAK2 V617F-positive VS negative MPN OR (95%)
rs2736100						
G/G	22	26		22	13	
G/T	18	5	4.2545 (1.3580 to 13.3293) P = 0.0129	18	3	3.5455 (0.8730 to 14.3985) P = 0.0767
T/T	8	1	9.4545 (1.0958 to 81.5756) P = 0.0410	8	0	10.2000(0.5441 to 191.2029) P = 0.1204
Allele A	62 (65)	57 (89)	-	62 (65)	29 (91)	-
Allele T	34 (35)	7 (11)	4.0737 (1.8347 to 10.8681) P = 0.0010	34 (35)	3(9)	5.3011 (1.5035 to 18.6911) P = 0.0095

OR: odds ratio, CI: confidence interval.

4. Discussion

One of the crucial polymorphism loci connected to many human malignancies is the TERT gene [19]. The TERT gene is located on chromosome 5 (5p15.33), and it has 16 exons. The catalytic part of telomerase is encoded by the TERT gene. Complex cellular alterations caused by TERT, including TERT structural variants [20], TERT gene amplifications [21], TERT epigenetic changes [22], alternative telomere lengthening [23], and TERT promoter mutations [24], are required for preserving telomeres. Any modification to these pathways may be connected to various single-cell carcinogenesis types [20]. TERT-based telomerase activity affects telomere length and can serve as a useful biomarker for the early detection and prognosis of various cancers [25]. TERT polymorphism may be related to the early start and development of cancers because of its importance in various crucial functions throughout the cells. The TERT gene has been related to a wide range of cancers, including melanoma, as well as thyroid, esophageal, pancreatic, prostate, stomach, thyroid, and bladder cancers [26]. Other studies reported that the TERT rs2736100_C allele is linked to an elevated risk of MPNs in Caucasians [12-15]. Accordingly, it is crucial to explore the association between MPN and the TERT gene's rs2736100 SNP in our population. This is the initial genetic examination when attempting to having insights into this kind of connection in Iraq. Our findings support the hypothesis that the TERT rs2736100 G>T variant is strongly associated with MPN vulnerability in the Iraqi community. Table 2 shows that MPN patients with the TT or GT genotype had a higher risk of being affected the disease. The findings of the present study are consistent with those of Lawi *et al.* in his study on an Iraqi lung cancer sample, who found that the frequencies of allele G in controls and patients (70 and 74.5 %, respectively) were higher than those of allele T (30 and 25.5 %, respectively) [26].

However, the findings of the present study diverge from those of an earlier research in Swedish, Chinese, and Japanese populations, which found that TERT rs2736100_C is a risk factor that increases the incidence of MPN [4, 27]. According to Matsuguma *et al.*, TERT rs2736100_C and JAK2 46/1 haplotype are both risk factors for MPNs in Japanese patients [27]. This variation may be attributable to the size of the sample, geographical origin, and ethnic variety. It is possible that the rs2736100 genotype directly regulates TERT expression because the C allele of rs2736100 has been associated to longer telomeres. TERT

rs2736100_C has also been linked, albeit less strongly, to an increased risk of numerous additional malignancies [28-30]. Additionally, rs2736100_C is associated with higher blood cell counts in the Japanese population [31]. As well as, this study observed that the T alleles increase the risk of MPN only in males, as shown in Table 3. In Sweden and China, the general population's genotype distribution of rs2736100 showed no gender differences, according to Dahlström's and colleagues research [4].

The bulk of published research lack information on the relationship between gender and TERT rs2736100 allele variant in MPN patients, but a single investigation revealed an analogous variant distribution in men and women. Women have reportedly had better rates of survival in MPN patients than men. There are no gender differences in the chance of acquiring a secondary malignancy, according to one study, while another claims that male MPN patients' lower overall survival is mostly due to a higher frequency of secondary AML transformation [4]. This study also discovered a substantial correlation between JAK2 V617F-positive MPN and TERT rs2736100 SNPs. Previous research has shown that MPN patients with the 46/1 haplotype who are JAK2 V617F-positive appear to have a high load of mutant alleles. This research provides important prognostic information since the JAK2 V617F allele load at diagnosis has been associated with the phenotypic presentation and severity of MPNs, elevated risk for thrombotic factors, and worsening secondary myelofibrosis [27].

5. Conclusions

In summary, it can be concluded from this study that there is an association between rs2736100 polymorphism of the TERT gene and MPN in a sample of the Iraqi population. This finding supports the role of TERT rs2736100 polymorphism in the pathogenesis of MPN, especially in people who have JAK2 V617F-positive MPN.

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Authors' declaration

Conflict of Interest: The authors declare that they have no conflicts of interest.

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