Research Article

Gonca Gulbay*, Elif Yesilada, Mehmet Ali Erkurt, Harika Gozukara Bag, Irfan Kuku and Emin Kaya

Evaluation of the JAK2 V617F gene mutation in myeloproliferative neoplasms cases: a one-center study from Eastern Anatolia

Miyeloproliferatif neoplazilerde JAK2 V617F gen mutasyonunun değerlendirilmesi: Doğu Anadolu'dan tek merkez deneyimi

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Abstract

Objective: Detection of *JAK2* V617F in myeloproliferative neoplasms (MPNs) is very important in both diagnosis and disease progression. In our study, we investigated the frequency of *JAK2* V617F mutation in patients with myeloproliferative disorders.
Methods: We retrospectively reviewed the records of 720 patients (174 females and 546 males) who were tested for JAK2 V617F mutation from January 2007 to December 2017.
Results: In our patients were determined 22.6% *JAK2* V617F mutation. 33.3% in women, 19.2% in men have been positive for *JAK2* V617F mutation. In our study *JAK2* V617F present in 48.6% of essential thrombocythemia, 80.5% of polycythemia rubra vera (PV), 47.5% of primary myelofibrosis, 10% of MPNs, unclassifiable, 0.8% of others. We also investigated the difference in hematological parameters [white blood cell, hemoglobin (Hb), hematocrit (HCT), red blood cell distribution widths (RDW) and platelets count (PLT)] between *JAK2* V617F positive and *JAK2* V617F negative patients.
Conclusions: Investigation of the JAK2 V617F mutation is very important in cases of MPNs. In our study JAK2 V617F mutation was higher in PV, essential thrombocythemia, and primary myelofibrosis patients. However, there were significant differences in Hb, HCT, RDW and PLT levels in mutation-positive patients.

Keywords: Janus Kinase2; JAK-STAT pathway; Myeloproliferative disorders; *JAK2* V617F mutation; Real-time polymerase chain reaction.

Öz

Amaç: Miyeloproliferatif neoplazilerde (MPN) *JAK2* V617F mutasyonunun taranması hastalığın hem tanısında hem de ilerlemesi aşamalarında oldukça önemlidir. Çalışmamızda miyeloproliferatif hastalarda *JAK2* V617F mutasyonunun sıklığını araştırdık.
Metod: Ocak2007-Aralık 2017 tarihleri arasında JAK2 V617F mutasyonu istemi yapılan 720 hastanın (174 kadın ve 546 erkek) laboratuvar sonuçları retrospektif olarak incelendi.
Bulgular: Hastalarımızda %22.6 oranında *JAK2* V617F mutasyonu tespit edilmiştir. Bu mutasyon oranının %33.3'ü kadın, %19.2'si erkeklerden oluşmaktadır. Çalışmamız %48.6 esansiyel trombositemi, %80.5 polisitemi rubra vera, %47.5 primer myelofibrozis, %10 sınıflandırılamayan tip kronik miyeloproliferatif hastalık ve %0.8 oranında da diğerlerinden oluşmaktadır. Aynı zamanda

***Corresponding author: Gonca Gulbay,** Department of Medical Biology and Genetics, Faculty of Medicine, Inonu University, Malatya 44280, Turkey, Fax: +90 422 341 00 36, e-mail: gonca.gulbay@inonu.edu.tr.
https://orcid.org/0000-0001-5201-6352
Elif Yesilada: Department of Medical Biology and Genetics, Faculty of Medicine, Inonu University, Malatya, Turkey, e-mail: elif.yesilada@inonu.edu.tr
Mehmet Ali Erkurt, Irfan Kuku and Emin Kaya: Department of Hematology, Faculty of Medicine, Inonu University, Malatya, Turkey, e-mail: erkurtali@hotmail.com (M. A. Erkurt); irfan.kuku@inonu.edu.tr (I. Kuku); emin.kaya@inonu.edu.tr (E. Kaya)
Harika Gozukara Bag: Department of Biostatistics, Faculty of Medicine, Inonu University, Malatya, Turkey, e-mail: harika.gozukara@inonu.edu.tr

bu çalışmada *JAK2* V617F pozitif and *JAK2* V617F negatif hastalarda hematolojik parametreler (WBC, Hb, HCT, RDW ve PLT) arasındaki farklılıklar da incelenmiştir.
Sonuç: *JAK2* V617F mutasyonu MPN'li olgularda oldukça önemlidir. Çalışmamızda *JAK2* V617F mutasyonu sırası ile PV, ET ve PMF hastalarında daha yüksektir. Bununla birlikte mutasyon pozitif hastalarda Hb, HCT, RDW and PLT düzeylerinde belirgin farklılıklar tespit edilmiştir.

Anahtar Kelimeler: Janus Kinaz2; JAK-STAT yolağı; Myeloproliferatif Hastalıklar; *JAK2* V617F Mutasyon; Gerçek Zamanlı Polimeraz Zincir Reaksiyonu.

Introduction

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders characterized by an increased number of mature myeloid peripheral blood cells [1]. In 1951, William Dameshek defined chronic myelogenous leukemia (CML), polycythemia vera (PV), primary myelofibrosis and essential thrombocythemia as myeloproliferative disorders (MPDs) [2, 3]. Currently, the classification suggested by the World Health Organization (WHO) is most widely used and describes eight entities. According to this classification, MPNs include CML, BCR-ABL1 positive, chronic neutrophilic leukemia, PV, primary myelofibrosis (PMF), essential thrombocythemia (ET), chronic eosinophilic leukemia, not otherwise specified, mastocytosis and MPNs, unclassifiable (MPN-U) [4]. The reported incidence of MPNs worldwide is 0.5–2 cases per 100,000 individuals [5]. Diagnostic criteria include clinical features, bone marrow histopathology and genetic alterations [6–8].

Janus kinase (JAK) is a family of non-receptor tyrosine kinases [2]. JAKs perform their effect by further phosphorylating transcription factors called signal transducer and activator of transcription (STAT) in the JAK-STAT pathway. The JAK-STAT pathway is particularly important for hematopoiesis. It permits cell proliferation, cell differentiation and cell apoptosis regulation [2, 6, 8, 9]. JAKs include JAK1, JAK2, JAK3 and tyrosine kinase 2 (Tyk2). JAK3 expression is limited to hematopoietic cells, while JAK1, JAK2 and Tyk2 are expressed in mammalian cells [2, 9].

The *JAK2* gene, encoded by the gene region localized in 9p24, consists of 25 exons and encodes a protein with 1132 amino acids [2]. In 2005, research groups defined the somatically acquired point mutation *JAK2* in exon 14 of the *JAK2* gene [10–13]. In BCR-ABL1 negative MPN patients, the *JAK2* V617F mutation emerges as a result of a valine to phenylalanine substitution at codon 617 [14]. The *JAK2* V617F mutation was observed in 95% of patients with PV, in approximately 50–60% of patients with ET and in about 50% of patients with PMF [11, 12, 15]. Less commonly *JAK2* exon 12 also result in JAK-STAT pathway activation in MPNs [16, 17].

Our study evaluated the frequency of the *JAK2* V617F mutation in a group of individuals with MPNs.

Materials and methods

Study groups

This study investigated the presence of the *JAK2* mutation (V617F) in patients with myeloproliferative disorders from Inonu University Turgut Ozal Medical Center Outpatient Hematology. Participants were diagnosed with MPNs according to the WHO criteria. Demographic and clinical features of 720 cases diagnosed with MPNs were evaluated retrospectively. Accordingly, our study groups consists of ET (70 cases), PV (113 cases), PMF (61 cases), CML (73 cases), MPN-U (10 cases), others (393 cases). However, the cases consisted of 174 women and 546 men.

JAK2 V617F mutation detection

Genomic DNA was extracted from ethylenediamine tetraacetic acid anticoagulated venous blood using the EZ1 DNA Blood 200 μL kit and the BioRobot EZ1 Workstation (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Samples were stored at –20°C until analysis.

After extracting DNA isolated from peripheral blood, a semi-quantitative Ipsogen JAK2 MutaScreen RS kit (Qiagen, Hilden, Germany) was used to detect JAK2 V617F mutations. Protocols for the *JAK2* V617F study followed the manufacturer's specifications.

BCR-ABL1 mutation detection

Real-time polymerase chain reaction method used for quantitative analysis of BCR-ABL1 fusion transcripts. RNA isolation from isolated from peripheral blood, were performed with QIAamp RNA Blood Mini (Qiagen, Germany) kit. RNA quality was analyzed by spectrophotometry in the range of 260–280 nm. After cDNA synthesis (RT-DX

kit, Qiagen, Hilden, Germany) BCR-ABL1 mutations was detected with a Ipsogen BCR-ABL1 Mbcr IS-MMR Kit and BCR-ABL1 mbcr kit (Qiagen, Hilden, Germany).

Statistical analysis

Normal distribution of quantitative data was assessed by Kolmogorov-Smirnov test. Since the data was not normally distributed, quantitative data were summarized by median (interquartile range) and Mann Whitney U-test was used for comparisons. Qualitative data were expressed as count (percent) and comparisons were made by Pearson chi-square test. In all analysis level of significance was considered as 0.05.

Results

The study group consisted of 720 patients, 174 women and 546 men, who were referred to the Inonu University Turgut Ozal Medical Center Outpatient Clinic of Hematology. The participants were diagnosed with MPNs according to the WHO criteria. The patients clinical and hematological characteristics are summarized in Table 1.

We first determined that the *JAK2* V617F mutation positive rate was 22.6%, occurring in 33.3% of women and 19.2% of men (Table 2). In our study, the *JAK2* V617F mutation was present in 48.6% of patients with ET, 80.5% of patients with PV, 47.5% of patients with PMF, 10% of patients with MPN-U and 0.8% of other patients (Table 3). Also, five patients (6.8%) were BCR-ABL1 negative. We found that the proportion of *JAK2* V617F allele burden in patients with PV was higher than in other patients (Table 3). In our study ET (27%), PV (18.4%) and PMF (17.2%) were more common in females ($p < 0.001$) (Table 4).

Next, we investigated the difference in hematological parameters between *JAK2* V617F positive and *JAK2* V617F negative cases (Table 1). There was a significant difference in white blood cell (WBC) counts ($p < 0.001$), hemoglobin (Hb) values ($p < 0.001$), hematocrit (HCT) values ($p = 0.004$), red blood cell distribution widths (RDW) ($p < 0.001$) and platelet counts ($p < 0.001$) in the *JAK2* V617F positive patients. The median WBC count was 10.6 10^9/L (range 4.3–10.3), the median Hb value was 15.1 g/dL (range 13.6–17.2), hematocrit (HCT) levels (median 46.6%)

Table 1: Clinical characteristics stratified by *JAK2* V617F mutation status in all patients.

Parameters	*JAK2* V617F		p-Value
	Positive (n = 163) Median (IQR)	Negative (n = 557) Median (IQR)	
WBC (10^9/L)	10.6 (5.7)	8.3 (3.8)	<0.001*
RBC (10^{12}/L)	5.53 (1.79)	5.58 (1.03)	0.579
Hb (g/dL)	15.1 (4.1)	16.4 (3.5)	<0.001*
HCT (%)	46.6 (11.9)	49.7 (9.9)	0.004*
RDW (%)	15.9 (5.5)	13.7 (2.0)	<0.001*
PLT (10^9/L)	450.0 (295.0)	241.0 (118.5)	<0.001*

*Statistically significant. In table median [interquartile range(IQR)] values are given. White blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), red blood cell distribution width (RDW), platelets count (PLT).

Table 2: *JAK2* V617F mutation positivity rates by sex.

Sex	*JAK2* V617F		Total n (%)	p-Value
	Positive n (%)	Negative n (%)		
Female	58 (33.3)	116 (66.7)	174 (100.0)	
Male	105 (19.2)	441 (80.8)	546 (100.0)	<0.001*
Total	163 (22.6)	557 (77.4)	720 (100.0)	

*Statistically significant.

Table 3: In MPNs patients *JAK2* V617F mutation rates.

MPNs	*JAK2* V617F	
	Positive n (%)	Negative n (%)
ET	34 (48.6)	36 (51.4)
PV	91 (80.5)	22 (19.5)
PMF	29 (47.5)	32 (52.5)
CML	5 (6.8)	68 (93.2)
MPN-U	1 (10.0)	9 (90.0)
Others	3 (0.8)	390 (99.2)
Total	163 (22.6)	557 (77.4)

Myeloproliferative neoplasms (MPNs), essential thrombocythemia (ET), polycythemia rubra vera (PV), primary myelofibrosis (PMF), chronic myelogenous leukemia (CML), myeloproliferative neoplasms, unclassifiable (MPN-U).

Table 4: *JAK2* V617F mutation positivity rates by sex and MPNs.

Sex	ET n (%)	PV n (%)	PMF n (%)	CML n (%)	MPN-U n (%)	Others n (%)
Female	36 (20.7)	32 (18.4)	30 (17.2)	15 (8.6)	3 (1.7)	58 (33.3)
Male	34 (6.2)	81 (14.8)	31 (5.7)	58 (10.6)	7 (1.3)	335 (61.4)

The distribution of disease groups in females and males is different ($p < 0.001$).

(range 39.5–50.3) and the median RDW and platelet counts were 15.9% (range 11.8–14.3) and 450.0 10^9/L (range 150–400), respectively. In our study, there was no difference between *JAK2* V617F positive cases and *JAK2* V617F negative cases in terms of red blood cell (RBC) (median 5.5 10^{12}/L) (range 4.38–5.77) ($p = 0.579$).

In addition to the *JAK2* V617F mutation, we also examined the BCR-ABL1 mutation. Patients who were BCR-ABL1 mutation negative were divided into groups based on whether the *JAK2* V617F mutation was positive or negative. Out of 73 patients, five (6.8%) had a positive *JAK2* mutation and a negative BCR-ABL1 mutation. We analyzed the difference in hematological parameters between patients with positive *JAK2* V617F mutations/negative BCR-ABL1 mutations and negative *JAK2* V617F mutations/negative BCR-ABL1 mutations. However, no significant difference in terms of hematological values were found (Table 5).

In our study, at the same time, we evaluated the clinical parameters of our study groups according to the *JAK2* V617F mutation status (Table 6). The number of cases diagnosed with ET is 70. *JAK2* V617F mutated ET cases occurred RBC count ($p < 0.001$), HCT level ($p < 0.001$) and RDW count ($p = 0.046$) were higher than *JAK2* V617F negative ET cases (Table 6). When compared with PV cases (113 cases), median WBC counts, RDW counts and platelet

Table 5: Relationship with demographic characteristics and complete blood count parameters of patients according to *JAK2* V617F and BCR-ABL1 mutation.

Parameters	*JAK2* V617F/BCR-ABL1		p-Value
	JAK2 V617F (+)/BCR-ABL1 (–) (n = 5) Median (IQR)	JAK2 V617F (–)/BCR-ABL1 (–) (n = 68) Median (IQR)	
WBC (10^9/L)	10.4 (4.1)	7.55 (2.2)	0.208
RBC (10^{12}/L)	5.96 (1.99)	5.53 (1.19)	0.559
Hb (g/dL)	16.7 (4.65)	16.25 (3.27)	0.531
HCT (%)	52.4 (15.05)	49.15 (9.25)	0.618
RDW (%)	13.6 (2.5)	13.8 (2.97)	0.633
PLT (10^9/L)	167.0 (56.0)	197.0 (60.5)	0.157

In table median [interquartile range (IQR)] values are given. White blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), red blood cell distribution width (RDW), platelets count (PLT).

Table 6: Comparison of hematological parameters in cases *JAK2* V617F positive and *JAK2* V617F negative.

MPNs	JAK2 V617F	Median (IQR)					
		WBC (10^9/L)	RBC (10^{12}/L)	Hb (g/dL)	HCT (%)	RDW (%)	PLT (10^9/L)
ET	Positive	9.35 (6.93)	5.71 (2.40)	15.15 (3.65)	47.45 (10.72)	18.2 (7.3)	640.0 (473.5)
	Negative	10.15 (5.42)	4.32 (1.0)	12.75 (3.25)	37.7 (6.47)	15.55 (3.15)	659.0 (757.75)
	p-Value	0.851	<0.001*	0.005*	<0.001*	0.046*	0.581
PV	Positive	11.82 (5.9)	5.67 (1.63)	15.6 (3.4)	48.4 (10.3)	15.7 (5.4)	401.0 (178.0)
	Negative	9.4 (2.92)	5.88 (0.72)	17.1 (2.85)	51.2 (7.32)	14.05 (1.82)	292.0 (180.25)
	p-Value	0.022*	0.842	0.037*	0.140	0.003*	0.006*
PMF	Positive	9.4 (4.75)	4.9 (0.9)	13.5 (2.75)	40 (6.6)	14.8 (4.15)	530.0 (271.5)
	Negative	8.75 (4.3)	4.49 (1.41)	12.6 (4.42)	39.2 (15.0)	15.2 (4.95)	586.5 (176.5)
	p-Value	0.840	0.448	0.845	0.902	0.506	0.340
CML	Positive	10.4 (4.1)	5.96 (1.99)	16.7 (4.65)	52.4 (15.05)	13.6 (2.5)	167.0 (56.0)
	Negative	7.55 (2.2)	5.53 (1.19)	16.25 (3.27)	49.15 (9.25)	13.8 (2.97)	197.0 (60.5)
	p-Value	0.208	0.559	0.531	0.618	0.633	0.157
MPN-U	Positive	5.1 (0)	5.92 (0)	17.0 (0)	50.2 (0)	13.3 (0)	204.0 (0)
	Negative	8.5 (5.65)	5.61 (1.33)	16.5 (4.25)	49.8 (11.05)	13.8 (2.85)	286.0 (199.0)
	p-Value	NA	NA	NA	NA	NA	NA
Others	Positive	27.8 (12.6)	3.19 (1.43)	9.7 (1.9)	30.0 (6.5)	21.8 (6.9)	39.0 (226.0)
	Negative	8.35 (3.7)	5.67 (0.75)	16.6 (2.22)	50.45 (6.12)	13.6 (1.6)	232.5 (88.25)
	p-Value	0.004*	0.007*	0.007*	0.011*	0.006*	0.121

*Statistically significant. In table median [interquartile range (IQR)] values are given. White blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), red blood cell distribution width (RDW), platelets count (PLT).

counts were found to be significantly greater in cases with the *JAK2* V617F mutation (p, 0.022, 0.003, and 0.006, respectively). However, the Hb value (p = 0.037) was found to be high in the *JAK2* V617F mutation negatives. When we examined PMF (61 cases) and CML (73 cases) cases for hematological parameters, there was no significant difference between positive and negative cases in terms of *JAK2* V617F mutation (Table 6). Our MPN-U study group consisted of 10 cases. In the MPN-U group, the number of *JAK2* V617F mutation positive cases (one case) was not sufficient and no statistical evaluation was performed. When a similar comparison was made among others cases (393 cases including 10 *JAK2* V617F mutation positive, 390 *JAK2* V617F mutation negative), median WBC (p = 0.004) and RDW (p = 0.006) was higher for the JAK2 V617F positive group and median RBC (p = 0.007), Hb (p = 0.007), HCT (p = 0.011) was greater for the *JAK2* V617F negative group. There was no significant differences among the two groups in terms of platelets count (PLT) (p = 0.121) (Table 6).

Discussion

MPNs are clonal hematopoietic stem cell malignancies characterized by hypersensitivity of hematopoietic progenitors to numerous cytokines and by clonal proliferation of one or more myeloid blood cell lineages [1]. Additionally, thrombotic or hemorrhagic complications or leukemic transformations can occur [18]. *JAK2* was found in the majority of MPNs patients. The *JAK2* V617F assay is a very important test in the assessment of MPNs [19]. This is the first molecular *JAK2* gene study of patients with MPNs from the Malatya Province in Turkey.

In our study, we detected the *JAK2* V617F mutation in exon 14 of the *JAK2* gene in 163 (22.6%) patients. We found that the frequency of *JAK2* V617F mutations was 48.6% in patients with ET, 80.5% in patients with PV, 47.5% in patients with PMF, 6.8% in patients who were BCR-ABL1 negative and in 10% of patients with MPN-U.

Based on a study population of 174 women and 546 men, we found that the *JAK2* V617F mutation was positive in 58 women (33.3%) and 105 men (19.2%). Of the female participants, 20.7% were diagnosed with ET, 18.4% were diagnosed with PV, 17.2% were diagnosed with PMF and 1.7% were diagnosed with MPN-U. PV is defined as a disease seen predominantly in men [6, 20, 21], although there are PV groups reported to be predominantly female [22–24]. Our PV group was also predominantly female. Female predominance in ET and male predominance in PMF has been reported in the literatüre [20, 25, 26]. *JAK2* V617F mutation positive patients were predominantly female in our study. However, it should be noted that the number of men and women participants was not equal.

The *JAK2* V617F mutation was found in the vast majority (80.5%) of patients with PV [6]. Two other studies from Turkey have declared the frequency of the *JAK2* V617F mutation to be 80% and 95% of patients with PV, respectively [24, 27, 28]. Our results are compatible with the literature. We found no *JAK2* V617F mutation in 22 patients with PV (19.5%). A *JAK2* exon 12 mutation screening should be performed on these patients. Mutations in exon 12 of *JAK2* has also been found in one-third of V617F negative PV cases [29].

Up to 50–60% of ET and PMF cases do not have mutations in *JAK2* [6]. In our study, we found the *JAK2* V617F mutation in 48.6% of patients with ET and in 47.5% of patients with PMF. The frequencies of the *JAK2* V617F mutation in an Iranian study were 63% in patients with ET and 53.3% in patients with PMF [30]. In a study by Karkucak et al. the *JAK2* V617F mutation was found in 42% of patients with ET [27]. Our results were similar to the majority of studies of the *JAK2* V617F mutation in patients with ET and PMF found in the literature [27, 30, 31].

CML is from MPDs, and increased tyrosine kinase activity due to BCR-ABL1 translocation is observed in generally patients with CML. The resulting fusion gene is observed in 90% of patients with CML and differentiates this disease from other myeloproliferative diseases [4, 32]. In this study, only five (6.8%) out of 73 patients had positive *JAK2* mutation/negative BCR-ABL1 mutation. Therefore, no significant difference in terms of hematological parameters was found between patients with positive *JAK2* V617F mutation/negative BCR-ABL1 mutation and negative *JAK2* V617F mutation/negative BCR-ABL1 mutation.

Although most of the patients with chronic MPNs can be classified as having CML, PV, ET, or PMF, it is sometimes impossible to classify patients. This is referred to as unclassified chronic MPNs [33]. Our study found that 10% of MPNs were unclassifiable.

In the present study, we also researched the relationship between *JAK2* V617F and hemogram variation in MPNs. This examination provided significant information in understanding the role of *JAK2* V617F in influencing hematological values. They display higher WBC counts, HCT and hemoglobin levels and they have increased RDW and platelet counts [34]. However, we did not find a significant difference between the two groups in terms of RBC values. The JAK2 V617F mutation is known to cause erythroide lineages proliferation. Our results are consistent

with the literature [27, 35, 36]. Other studies have found elevated levels of different hematological parameters in patients carrying mutations [27, 35–37].

Cases with the *JAK2* V617F mutation were shown to have a tendency for higher WBC counts, and higher RDW count, and lower Hb levels. In ET, several studies have found that *JAK2* V617F mutated cases presented with, higher Hb level and WBC count, but lower PLT count [24, 38, 39]. In our ET group, we found that cases with the *JAK2* V617F mutation were with higher Hb value, RBC level, HCT value and RDW count compared with cases *JAK2* V617F mutation negative. In literature research, *JAK2* V617F positive in PV patients was shown to be correlated with leukocytosis, greater cell count [40, 41]. We found WBC, PLT and RDW counts to be greater *JAK2* V617F positive in PV patients. In the group of others, three cases are *JAK2* V617F positive. The number of *JAK2* V617F negative patients is 390. Studies on larger population groups are needed.

In conclusion these molecular assays have become a helpful tool, making the diagnosis faster and more exact. The assessment of genetic factors implicated in the improvement of MPDs helps to detect acquired mutations, such as *JAK2* V617F. The demonstration of the presence of this mutation is important in terms of making a differential diagnosis. Longer follow-up and larger case populations are needed to investigate the effects of clinical and laboratory parameters in progression myeloproliferative diseases.

Conflicts of interest: The authors have no conflicts of interest to declare.

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