RESEARCH ARTICLE

DOI: 10.4274/tjh.galenos.2024.2024.0161
Turk J Hematol 2024;41:167-174

*JAK2*V617F Mutation in Endothelial Cells of Patients with Atherosclerotic Carotid Disease

Karotis Aterosklerozu Olan Hastaların Endotel Hücrelerinde *JAK2*V617F Mutasyonu

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Abstract

Objective: It has been shown that clonal mutations occur in hematopoietic stem cells with advancing age and increase the risk of death due to atherosclerotic vascular diseases, similarly to myeloproliferative neoplasms. Endothelial cells (ECs) and hematopoietic stem cells develop from common stem cells called hemangioblasts in the early embryonic period. However, the presence of hemangioblasts in the postnatal period is controversial. In this study, *JAK2* gene variants were examined in patients with atherosclerotic carotid disease and without any hematological malignancies.

Materials and Methods: Ten consecutive patients (8 men and 2 women) with symptomatic atherosclerotic carotid stenosis were included in this study. ECs ($CD31^+CD45^-$) were separated from tissue samples taken by carotid endarterectomy. *JAK2* variants were examined in ECs, peripheral blood mononuclear cells, and oral epithelial cells of the patients with next-generation sequencing.

Results: The median age of the patients was 74 (range: 58-80) years and the median body mass index value was 24.44 (range: 18.42-30.85) kg/m^2. Smoking history was present in 50%, hypertension in 80%, diabetes in 70%, and ischemic heart disease in 70% of the cases. The *JAK2*V617F mutation was detected in the peripheral blood mononuclear cells of 3 of the 10 patients, and 2 patients also had the *JAK2*V617F mutation in their ECs. The *JAK2*V617F mutation was not found in the oral epithelial cells of any of the patients.

Conclusion: In this study, for the first time in the literature, we showed that the *JAK2*V617F mutation was found somatically in both peripheral blood cells and ECs in patients with atherosclerosis. This finding may support that ECs and hematopoietic cells originate from a common clone or that somatic mutations can be transmitted to ECs by other mechanisms. Examining the molecular and functional

Öz

Amaç: Yaşla birlikte hematopoietik kök hücrelerde klonal mutasyonların ortaya çıktığı, aynı miyeloproliferatif neoplazilerde olduğu gibi, bu mutasyonların aterosklerotik damar hastalıkları ile ölüm riskini artırdığı gösterilmiştir. Embriyonik hayatta erken dönemde endotel ve hematopoietik kök hücrenin hemangioblast adı verilen ortak bir kök hücreden geliştiği bilinmektedir. Ancak postnatal dönemde hemangioblast varlığı tartışmalıdır. Bu çalışmada karotis aterosklerozlu hastalarda *JAK2* gen varyantları incelenmiştir.

Gereç ve Yöntemler: Bu çalışmaya semptomatik aterosklerotik karotis darlığı olan ardışık 10 hasta (8 erkek, 2 kadın) dahil edilmiştir. Karotis endarterktomi operasyonu ile alınan doku örneklerinden endotel hücreleri ($CD31^+CD45^-$) ayrılmıştır. Hastaların endotel hücreleri, periferik kan mononükleer hücreleri ve ağız epitel hücrelerinde *JAK2* varyantları yeni nesil dizileme ile incelenmiştir.

Bulgular: Hastaların ortanca yaşı 74 (58-80), ortanca vücut kitle indeksi 24,44 (18,42-30,85) kg/m^2 idi. Sigara öyküsü %50, hipertansiyon %80, diyabet %70, iskemik kalp hastalığı %70 hastada mevcuttu. Genetik analizde 10 hastanın üçünde periferik kan mononükleer hücrelerinde *JAK2*V617F mutasyonu saptandı. Bu 3 hastanın ikisinde endotel hücrelerinde de *JAK2*V617F mutasyonu gösterildi. Hastaların hiç birinde ağız epitel hücrelerinde *JAK2*V617F mutasyonuna rastlanmadı.

Sonuç: Bu çalışmada, literatürde ilk defa, herhangi bir hematolojik malignitesi olmayan ateroskleroz hastalarında hem periferik kan hücrelerinde, hem de aterosklerotik plaktan izole edilen endotel hücrelerinde somatik olarak *JAK2*V617F mutasyonu bulunduğu gösterilmiştir. Bu bulgu endotel hücreleri ve hematopoietik hücrelerin ortak bir klondan kaynaklandığını veya somatik mutasyonun başka mekanizmalarla endotel hücrelerine iletilebildiğini destekleyebilir. Endotel hücrelerinde *JAK2*V617F mutasyonunun yarattığı moleküler

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Received/Geliş tarihi: May 2, 2024
Accepted/Kabul tarihi: May 25, 2024

changes caused by the *JAK2*V617F mutation in ECs may help open a new avenue for treating atherosclerosis.

Keywords: *JAK2*V617F mutation, Clonal hematopoiesis, Atherosclerosis, Myeloproliferative neoplasms

ve fonksiyonel değişikliklerin incelenmesi, ateroskleroz tedavisinde yeni bir sayfanın açılmasına yardımcı olabilir.

Anahtar Sözcükler: *JAK2*V617F mutasyonu, Klonal hematopoiezis, Aterosklerozis, Miyeloproliferatif neoplaziler

Introduction

Atherosclerosis is a chronic vascular disease characterized by the accumulation of oxidized low-density lipoproteins in the intimal layer of the arteries, leading to inflammatory reactions [1]. Atherosclerotic vascular diseases such as myocardial infarction, stroke, and peripheral artery occlusion are the leading cause of death for humans worldwide, with an estimated 17.9 million deaths each year according to the World Health Organization [2]. Apart from conventional risk factors for atherosclerosis such as obesity, smoking, hyperlipidemia, diabetes, and hypertension, atherosclerosis is further exacerbated by chronic inflammatory disorders and myeloproliferative neoplasms (MPNs) [1,3].

MPNs are a heterogeneous group of hematological malignancies with a chronic course, characterized by abnormal proliferation of myeloid precursors as a result of somatic mutations in the hematopoietic stem cells. MPNs usually develop as a result of driver mutations including *JAK2*V617F, *CALR*, and *MPL* [4]. The most important factor determining morbidity and mortality in patients with MPNs is thrombotic complications [5]. Increased red blood cell mass, activation of leukocytes and platelets, stimulation of coagulation, and inflammation have been postulated as possible causes of thrombosis in patients with MPNs. After the definition of the V617F mutation in the *Janus kinase-2* gene (*JAK2*V617F mutation), it became clear that mutation profile and allele burden also played important roles in the development of thrombosis [4,5]. The *JAK2*V617F mutation generates more active neutrophils and monocytes, increases leukocyte-endothelial cell (EC) interactions, and causes more tissue factor expression, inducing thrombosis in turn [6,7,8]. Most studies investigating the relationship between the *JAK2*V617F mutation and thrombosis have focused on functional changes in hematopoietic cells originating from the MPN clone, and there are limited studies on the ECs of MPN patients. Sozer et al. [9] in 2009 and Rosti et al. [10] in 2013 isolated hepatic and splenic vein ECs from MPN patients with splanchnic thromboses and demonstrated the presence of the *JAK2*V617F mutation in ECs. It has been suggested that ECs and hematopoietic stem cells originate from common stem cells called hemangioblasts in the early embryonic stage. Sozer et al. [9] and Rosti et al. [10] interpreted the detection of a somatic *JAK2*V617F mutation in both ECs and hematopoietic cells as evidence supporting the existence of hemangioblasts during the postnatal period in humans. However, conflicting results have been obtained in other studies conducted with circulating endothelial progenitor cells, suggesting that ECs and hematopoietic cells originate from different stem cells and that ECs do not harbor the *JAK2*V617F mutation in patients with MPNs [11,12].

Large-scale studies with peripheral blood cells from healthy populations showed that the frequency of somatic mutations increased with advancing age, being approximately 10% after 70 years of age. Interestingly, most of these variants appeared to accumulate in genes frequently implicated in hematological malignancies, such as *JAK2*, *DNMT3A* (*DNA methyltransferase 3-alpha*), and *TET2* (*Tet-methylcytosine dioxygenase-2*) [13]. Individuals harboring a clonal hematopoietic mutation without any signs of hematological malignancy are referred to as having clonal hematopoiesis of indeterminate potential (CHIP). The risk of developing a hematological malignancy during follow-up increases in individuals carrying CHIP mutations (0.5%-1% per year), as expected, but the increased risk of death (40%) largely comes from cardiovascular complications. Studies have demonstrated that the presence of CHIP mutations increases the risk of coronary artery thrombosis by 1.8-4 times, premature coronary artery disease by 4, and ischemic stroke by 2.6 [14,15,16]. Animal studies have also illustrated that these mutations cause aberrant inflammation and accelerate the atherosclerotic plaque burden [15,17].

While numerous studies have explored the role of CHIP mutations in hematopoietic cells in atherosclerosis, no data are available to date on the presence of these mutations in ECs. This study investigates the *JAK2* gene variants in the ECs of patients with atherosclerosis.

Materials and Methods

Inclusion of the Patients and Sample Collection

Patients older than 50 years of age who presented with neurological symptoms and were found to have carotid artery stenosis exceeding 70% by angiography were consecutively included. All patients were evaluated by both a cardiologist and a hematologist. Patients with a history of malignancies including MPNs, previous chemotherapy and/or immunosuppressive treatment, and the presence of thrombocytosis, erythrocytosis, or splenomegaly were excluded. Carotid endarterectomy operations were performed in Mehmet Akif Ersoy Chest and Cardiovascular Surgery Education and Research Hospital. Oral swab and blood samples of the patients were collected during

preoperative testing. Demographic and clinical data of the patients were obtained from their files.

Carotid Endarterectomy Operation

After the surgical incision, the common carotid artery (CCA) and its branches were located and an arteriotomy was performed starting from the proximal part of the CCA bifurcation and extending towards the internal carotid artery. The atheroma plaque was gently cut and removed. The CCA was closed with a primary suture or patch angioplasty. Carotid endarterectomy materials were preserved in sterile petri dishes containing tissue storage solution (MACS Tissue Storage Solution, Miltenyi Biotec, Bergisch Gladbach, Germany) (Figure 1).

Isolation of ECs

Atheroma tissue was cut into small pieces of 0.5 mm with the help of a scalpel. The mixture was dissociated with the Human Tumor Dissociation Kit and GentleMACS Octo Dissociator for 1 hour according to the manufacturer's protocols (Miltenyi Biotec). The dissociated cell solution was passed through 40-µm and 70-µm strainers (Sartorius, Bohemia, NY, USA), washed with phosphate-buffered saline (PBS), and centrifugated at 500 x g for 10 minutes, followed by an additional washing with 10 mL of PBS. To remove erythrocytes, cells were incubated with 4 mL of NH_4Cl erythrocyte lysate solution (Thermo Scientific, Waltham, MA, USA) for 10 minutes and centrifuged at 500 x g for 5 minutes. The supernatant was discarded and cells were incubated with 100 µL of PBS containing Zombie NIR viability dye (BioLegend, San Diego, CA, USA) for 10 minutes in the dark. After incubation, the cells were washed and incubated with 100 µL of staining buffer (SB; PBS + 1% bovine serum albumin) containing mouse anti-human CD31 PE-Dazzle 594 (Clone WM59, BioLegend) and mouse anti-human CD45 PE-Cy7 (Clone HI39, BioLegend) antibodies for 30 minutes in the dark and then washed at 500 x g for 5 minutes. The supernatant was discarded and pellets were resuspended with SB for sorting. Cells were sorted as live $CD45^-CD31^+$ cells using the FACSAria III device (BD Biosciences, Franklin Lakes, NJ, USA) according to the gating strategy shown in Figure 2. Cells were sorted with purity logic at a speed of ≤1500 events/second for maximum purity and efficiency. Live $CD45^-CD31^+$ cell purities were ≥95% after sorting.

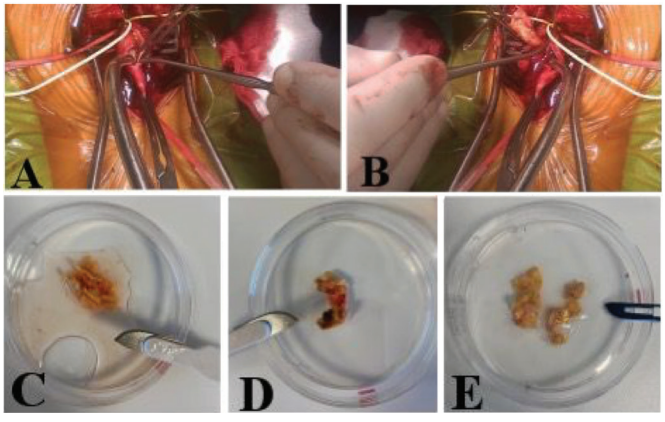

Figure 1. Carotid endarterectomy operation and atherosclerotic plaque samples: (A) exploration of the carotid artery; (B) removal of the atherosclerotic plaque; (C, D, E) atherosclerotic plaque materials were rich in lipids and calcium, and some contained necrotic areas (D).

Next-Generation Sequencing (NGS) Analysis for *JAK2* Variants

DNA was isolated from ECs, peripheral blood mononuclear cells, and oral mucosal cells. The Twist Exome 2.0 Kit and Twist Human Core Exome Enzymatic Fragmentation (EF) Multiplex Complete Kit (Twist Bioscience, South San Francisco, CA, USA) were used for library construction, and the MGIEasy FS DNA Library Prep Kit (MGI Tech, Shenzhen, China) was used for the formation of circular DNA and preparing the library for sequencing on the MGI system. The library was sequenced on the MGI-DNBSEQ-G400 instrument (MGI Tech), generating 150-bp paired-end reads with 100X mean target coverage. The NGS output was produced in the form of raw FASTQ files. Reads were aligned to the reference human genome (hg19). Variants were identified by Genemaster (İstanbul, Türkiye). Integrative Genomic Viewer software (IGV Team, San Diego, CA, USA) was used for the visualization of variants.

Bioinformatics Analysis

NGS paired-end data from 30 FASTQ files (R1.fq.gz/R2.fq.gz) were checked for quality scores across all bases using the FastQC (Galaxy Version 0.74) analysis tool (Freiburg Galaxy Team, Freiburg, Germany). Per-base sequence quality was calculated for each sample including ECs, peripheral blood, and oral epithelial cells, respectively. Phred scores were obtained greater than 30 and sequencing libraries were considered to be of good quality for subsequent NGS downstream analysis. All publicly open-source algorithms were used through the European Galaxy server. The mapping of paired-end reads against the reference human genome (*Homo sapiens*: hg38 Full) was conducted with the Bowtie2 alignment tool (Galaxy Version 2.5.3, Freiburg Galaxy Team). Outputs were generated as binary alignment map (BAM) files in BAM and BAI formats. Integrative Genomics Viewer Version 2.13.1 was used to visualize the sequence alignment data from each indexed BAM file. Pathogenic and likely pathogenic variants from the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and single-nucleotide variants, insertions, deletions, and missense mutations were analyzed for each patient for all considered cell types (i.e., ECs, blood cells, and oral epithelial cells).

This study was approved by the İstanbul Medical Faculty Clinical Research Ethics Committee (20-11-2020; 29624016-050.99-1857) and by Mehmet Akif Ersoy Chest and Cardiovascular

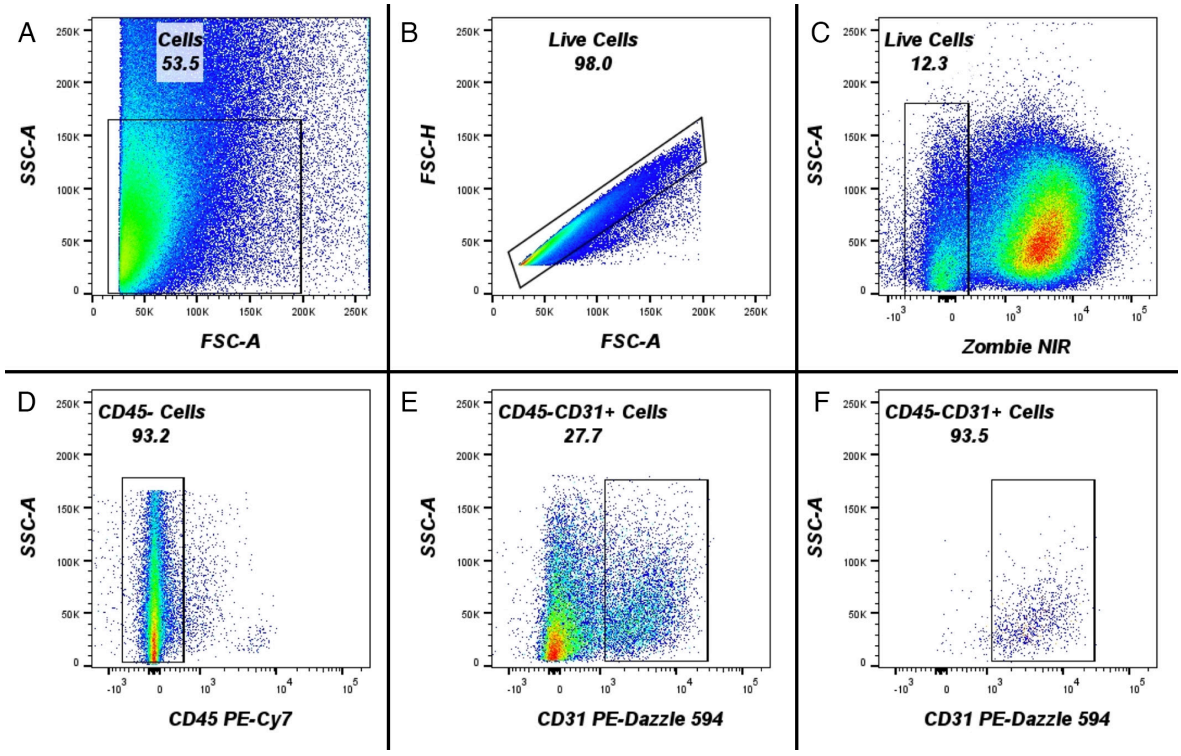

Figure 2. Isolation of endothelial cells by flow cytometry and the gating strategy for sorting the cells: (A) total cells from dissociated tissue were gated in the FSC-A x SSC-A plot and (B) doublets were discriminated using the FSC-A x FSC-H plot. (C) Live cells were gated as Zombie NIR$^-$ cells. (D) CD45$^-$ cells were selected to gate out leukocytes and (E) CD31$^+$ cells were sorted. (F) After sorting, CD45$^-$CD31$^+$ cells were analyzed to check the purity and efficiency of the sorting process.

Surgery Education and Research Hospital (22-09-2020; 10678112-000-5400). Written informed consent was obtained from all patients before any study procedures were performed.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables while evaluating the clinical and demographic data of the patients. Since statistical significance could not be achieved, eta-squared and Cohen d statistics were not applied to reveal statistical power. All statistical analyses were performed using IBM SPSS Statistics 27 (IBM Corp., Armonk, NY, USA).

Results

Demographic Characteristics of the Patients

In this study, 10 consecutive patients (8 men and 2 women) were included. The median age was 74 (range: 58-80) years (Table 1). None of these patients had splenomegaly or any signs of malignancy including hematological malignancies or MPNs (Table 2).

Risk Factors for Atherosclerosis

The median body mass index value was 24.44 (range: 18.42-30.85) kg/m^2. Five patients had a history of smoking and the median daily cigarette consumption was 15 (range: 5-30). Eight patients were diagnosed with hypertension and 7 had diabetes. Ischemic heart disease was diagnosed in 7 of the patients and 1 had peripheral artery disease (Table 1).

JAK2 Mutation Analysis

When the *JAK2* gene was examined by NGS, we found the *JAK2*V617F mutation in the peripheral blood mononuclear cells of 3 of 10 patients. We also demonstrated the *JAK2*V617F mutation in the ECs of 2 of those 3 patients. Variant allele frequencies ranged between 0.43% and 3%. We did not find the *JAK2*V617F mutation in the oral epithelial cells of any patients (Table 3). No other pathogenic variants besides the *JAK2*V617F mutation were detected in our cohort. Patients positive for the *JAK2*V617F mutation were statistically compared to those without the mutation in terms of clinical and laboratory data. However, due to the small sample size, no significant results could be obtained.

Discussion

In this study, ECs isolated from the carotid endarterectomy materials of patients who had symptomatic carotid atherosclerosis but no hematological malignancies were evaluated in terms of *JAK2* variations. We found the *JAK2*V617F mutation in the peripheral blood cells of 3 patients, and 2 of those patients also had the *JAK2*V617F mutation in ECs. Due to the small number

Table 1. Demographic features of the patients.

Patient no.	Sex	Age	BMI	Smoking	DM	HT	IHD	Cor. st.	Cor. bypass
1- R.K.	M	74	18.42	+	-	+	-	-	-
2- H.B.	F	77	30.85	-	+	+	+	-	+
3- A.T.	**M**	**76**	**27.77**	**-**	**+**	**+**	**+**	**+**	**+**
4- M.D.A.	M	67	25.82	+	+	+	+	-	+
5- R.Ö.	M	58	23.88	-	+	-	+	+	-
6- F.S.	F	73	26.96	+	+	+	+	-	+
7- O.O.	M	76	23.51	+	+	+	+	-	-
8- A.K.	**M**	**74**	**24.44**	**+**	**-**	**+**	**+**	**+**	**-**
9- İ.K.	**M**	**80**	**23.66**	**-**	**-**	**-**	**+**	**+**	**-**
10- H.E.	M	73	30.64	-	+	+	-	-	-

Bolded lines signify *JAK2*V617F-positive patients. M: Male; F: female; BMI: body mass index; DM: diabetes mellitus; HT: hypertension; IHD: ischemic heart disease; Cor. st.: coronary stenting history; Cor. bypass: coronary bypass operation history.

Table 2. Blood count values and lactate dehydrogenase levels of the patients.

Patient no.	WBC (x10^9/L)	Hb (g/dL)	HCT %	PLT (x10^9/L)	LDH (U/L)
1- R.K.	6.31	13.3	40.1	194	165
2- H.B.	6.68	13.1	41.4	319	210
3- A.T.	**5.97**	**14.2**	**43.6**	**156**	**204**
4- M.D.A.	9.84	14.3	41.7	250	177
5- R.Ö.	6.4	14.8	42.8	204	163
6- F.S.	5.03	14.1	41	247	198
7- O.O.	9.26	15.5	47.9	155	218
8- A.K.	**8.8**	**13.9**	**44.2**	**194**	**220**
9- İ.K.	**7.82**	**13.8**	**42**	**161**	**187**
10- H.E.	4.99	11.9*	36*	168	156

*: This patient had iron deficiency anemia due to a gastric ulcer. Bolded lines signify *JAK2*V617F-positive patients. WBC: White blood cells; Hb: hemoglobin; HCT: hematocrit; PLT: platelets; LDH: lactate dehydrogenase (normal reference values are 90-240 U/L).

Table 3. *JAK2*V617F variant analysis of the patients.

Patient no.	Endothelial cells: *JAK2*V617F genotype (VAF %)	Peripheral blood cells: *JAK2*V617F genotype (VAF %)	Oral epithelial cells: *JAK2*V617F genotype (VAF %)
1- R.K.	GG (100%)	GG (100%)	GG (100%)
2- H.B.	GG (100%)	GG (100%)	GG (100%)
3- A.T.	**GG (100%)**	**GG/GC (G 99%, C 1%)**	**GG (100%)**
4- M.D.A.	GG (100%)	GG (100%)	GG (100%)
5- R.Ö.	GG (100%)	GG (100%)	GG (100%)
6- F.S.	GG (100%)	GG (100%)	GG (100%)
7- O.O.	GG (100%)	GG (100%)	GG (100%)
8- A.K.	**GG/GC (G 99.56%, C 0.43%)**	**GG/GC (G 98%, C 2%)**	**GG (100%)**
9- İ.K.	**GG/GC (G 99%, C 1%)**	**GG/GC (G 97%, C 3%)**	**GG (100%)**
10- H.E.	GG (100%)	GG (100%)	GG (100%)

VAF: Variant allele frequency. Bolded lines signify *JAK2*V617F-positive patients.

of patients, it would not be appropriate to provide a rate of the *JAK2*V617F mutation in our cohort. However, recent studies have shown a high frequency of *JAK2*V617F mutation in the general population and in various groups of patients with thrombosis. In a study by Kristiansen et al. [16], the *JAK2*V617F mutation was investigated in 538 stroke patients and 19,958 controls using highly sensitive Droplet Digital PCR. The prevalence of the *JAK2*V617F mutation was found to be 11% in stroke patients and 4.4% in the control group.

In the literature, the *JAK2*V617F mutation has been shown in the ECs of the hepatic and splenic veins of MPN patients with splanchnic thromboses [9,10]. However, some researchers have suggested that these cells may be endothelial-like cells developing from MPN clones and that EC and hematopoietic cells originate from different stem cells [11,12]. In our study, we isolated $CD31^{+}CD45^{-}$ cells obtained from carotid atheroma plaques, which represent mature ECs, not monocyte-derived endothelial-like cells. While the *JAK2*V617F mutation was also detected in peripheral blood mononuclear cells, they were not present in oral epithelial cells. This finding suggests that the *JAK2*V617F mutation is not germ-line and that the somatic *JAK2*V617F mutation develops together in hematological cells and ECs. Studies in the literature examining the relationship between *JAK2*V617F or *TET2* mutations and atherosclerosis focused on monocytes and neutrophils originating from mutated hematopoietic clones [17,18]. To the best of our knowledge, our study presents the first demonstration of the *JAK2*V617F mutation in the ECs of patients with atherosclerosis but without hematological malignancies.

The occurrence of the *JAK2*V617F mutation in both ECs and hematopoietic cells can be explained by various hypotheses. It is controversial whether the hemangioblasts giving rise to ECs and hematopoietic stem cells still exist in the postnatal period because animal experiments have failed to demonstrate the postnatal presence of hemangioblasts [19,20,21]. Our results may support that hemangioblasts exist in the postnatal period in humans and that the *JAK2*V617F mutation may develop at the hemangioblast level. On the other hand, it is not known exactly when and in which stem cells the *JAK2*V617F mutation occurs. The existence of data suggesting that the *JAK2*V617F mutation may be acquired in childhood or even in utero in some rare cases may indicate that, at least in some cases, the mutation could develop during the prenatal hemangioblast stage [22]. By a third hypothesis, it is speculated that ECs and hematopoietic cells originate from different stem cells, but clonal hematopoietic mutations are transferred to ECs from clone-derived hematological cells. In both solid and hematological cancers, genetic abnormalities of the cancer cells such as aneuploidy or cancer-specific mutations have been also detected in the ECs within tumor tissues [23]. It has been suggested that ECs, which do not originate from the same source as cancer cells, acquire these genetic changes through gene transfer via cancer-derived microvesicles [23,24]. In an in vitro study conducted by Hekimoğlu et al. [25], DNA fragments carrying the *JAK2*V617F mutation were detected in microparticles secreted from *JAK2*V617F-positive ECs, indicating the potential transmission of the *JAK2*V617F mutation to neighboring and distant cells.

The current treatment strategy for atherosclerosis focuses primarily on lowering low-density lipoprotein levels, preventing platelet activation, and modifying personal risk factors. Despite this approach, atherosclerotic cardiovascular diseases continue to be the number one cause of death in all countries of the world. With the understanding of the role of inflammation in the pathogenesis of atherosclerosis, the use of anti-inflammatory drugs has garnered attention in recent years [3]. In a randomized controlled trial, canakinumab, a human monoclonal antibody that neutralizes of interleukin-1β signaling and suppresses inflammation, was administered to patients with a history of myocardial infarction and with increased C-reactive protein levels (>2 mg/L). Targeting interleukin-1β with canakinumab resulted in a significantly lower rate of cardiovascular events, regardless of lipid lowering [26]. When these patients were evaluated for somatic mutations, CHIP variants were detected in the peripheral blood of 8.6% of them. Subgroup analysis revealed that patients with *TET2* variations responded better to canakinumab in terms of reduced frequency of cardiovascular events [27]. ECs are major participants in and regulators of inflammatory reactions. Our study has revealed that in addition to hematopoietic cells, ECs can participate in atherosclerosis by harboring the *JAK2*V617F mutation. Considering the evidence indicating that the activation of the JAK-STAT signaling pathway by the *JAK2*V617F mutation in leukocytes enhances inflammatory responses [6,7,8], it is reasonable to anticipate that the presence of the *JAK2*V617F mutation in ECs will result in comparable inflammatory outcomes, potentially exacerbating the atherosclerotic process. Investigating in detail the functional changes caused by the *JAK2*V617F mutation in ECs will help in elucidating the pathogenesis of atherosclerosis and will also help identify new treatment targets.

Study Limitations

The major limitation of our study is the small number of patients. However, ECs from the carotid artery were analyzed for somatic mutations for the first time in this study. While increasing the number of the patients, we plan to screen for other CHIP variants in patients with atherosclerosis. Another limitation of this study is the lack of bone marrow biopsy evaluation in our patients. Instead, MPNs were excluded based on clinical (absence of splenomegaly) and laboratory (blood counts and serum lactate dehydrogenase levels) findings.

Conclusion

In this study, we observed that patients with atherosclerosis had the *JAK2*V617F mutation not only in peripheral blood cells but also in ECs taken from the atherosclerotic plaque. The absence of the *JAK2*V617F mutation in the oral epithelial cells of these patients supports the hypothesis that the *JAK2*V617F mutation developed somatically in the blood cells and ECs. Examining the effects of somatic mutations on ECs may open new avenues for treating atherosclerosis.

Ethics

Ethics Committee Approval: This study was approved by the İstanbul Medical Faculty Clinical Research Ethics Committee (20-11-2020; 29624016-050.99-1857) and by Mehmet Akif Ersoy Chest and Cardiovascular Surgery Education and Research Hospital (22-09-2020; 10678112-000-5400).

Informed Consent: Written informed consent was obtained from all patients before any study procedures were performed.

Authorship Contributions

Surgical and Medical Practices: R.D-K., T.İ.; Concept: R.D-K., T.G.; Design: R.D-K., T.G.; Data Collection or Processing: R.D-K.; Analysis or Interpretation: R.D-K., Ö.A., C.E.; Literature Search: R.D-K., T.G.; Writing: R.D-K., T.İ., Ö.A., C.E., T.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by the Scientific Research Projects Coordination Unit of İstanbul University (project number: 37779). It was also supported by the Turkish Society of Hematology (project number: TJH 2021-02).

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