DOI: 10.4274/terh.galenos.2021.55899
J Tepecik Educ Res Hosp 2023;33(1):45-9

CLINICAL RESEARCH / KLİNİK ARAŞTIRMA

The Investigation Frequency of *JAK2* Gene Mutation in Glomerulonephritis

Glomerulonefritlerde *JAK2* Gen Mutasyonu Sıklığının Araştırılması

Tuba Demirci Yıldırım[1], Utku Erdem Soyaltın[2], Altuğ Koç[3], Serkan Yıldız[4], Mehmet Tanrısev[5], Yaşar Bekir Kutbay[6], Ferhat Ekinci[7], Süleyman Yıldırım[8], Harun Akar[9], Cengiz Ceylan[9]

[1]Dokuz Eylül University Faculty of Medicine, Department of Rheumatology, İzmir, Turkey
[2]University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital, Clinic of Endocrinology, İstanbul, Turkey
[3]Dokuz Eylül University Faculty of Medicine, Department of Medical Genetics, İzmir, Turkey
[4]Dokuz Eylül University Faculty of Medicine, Department of Nephrology, İzmir, Turkey
[5]University of Health Sciences Turkey, İzmir Tepecik Education and Research Hospital, Clinic of Nephrology, İzmir, Turkey
[6]University of Health Sciences Turkey, İzmir Tepecik Education and Research Hospital, Clinic of Genetic Diagnostic Center, İzmir, Turkey
[7]Manisa Celal Bayar University Faculty of Medicine, Department of Medical Oncology, Manisa, Turkey
[8]University of Health Sciences Turkey, Dr. Suat Seren Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Intensive Care Unit, İzmir, Turkey
[9]University of Health Sciences Turkey, İzmir Tepecik Education and Research Hospital, Clinic of Internal Medicine, İzmir, Turkey

Cite as: Demirci Yıldırım T, Soyaltın UE, Koç A, Yıldız S, Tanrısev M, Kutbay YB, Ekinci F, Yıldırım S, Akar H, Ceylan C. The Investigation Frequency of *JAK2* Gene Mutation in Glomerulonephritis. J Tepecik Educ Res Hosp 2023;33(1):45-9

Abstract

Objective: Glomerulonephritis (GN) a heterogeneous group disease and the pathophysiology of GN is not understood yet. The aim of the study, the investigation frequency of *Janus kinase (JAK)2* gene mutation in GN.

Methods: The study was conducted in University of Health Sciences Turkey, İzmir Tepecik Education and Research Hospital, Nephrology Clinic, and Dokuz Eylül University Faculty of Medicine Nephrology Clinic, between May 2015 and October 2015. Sixty-seven patients with GN and 100 healthy participants as a control group were included in the study. *JAK2* gene mutation was investigated on peripheral venous blood.

Results: Thirty-three (49%) of the participants in the GN group were male and the mean age was 45.8±12.4 years. Thirty-seven (37%) of the participants in the control group were male and the mean age was 40.1±14.7 years. There were no *JAK2* V617F mutations in both the study and control groups.

Conclusion: In our study, no direct relationship was found between the *JAK2* mutation and GN. To evaluate the relationship between the JAK-signal transducers and activators of transcription (STAT) pathway and GN, further studies are needed, especially at the tissue level.

Keywords: Glomerulonephritis, *JAK2* gene mutation, JAK-STAT pathway

Address for Correspondence/Yazışma Adresi: Tuba Demirci Yıldırım, Dokuz Eylül University Faculty of Medicine, Department of Rheumatology, İzmir, Turkey
Phone: +90 506 269 72 71 **E-mail:** tubademirci87@mynet.com
ORCID ID: orcid.org/0000-0003-3186-0591

Received/Geliş tarihi: 08.08.2021
Accepted/Kabul tarihi: 13.12.2021

Öz

Amaç: Glomerulonefritler (GN) patofizyolojisi tamamen anlaşılamamış bir grup heterojen hastalıktır. Bu çalışmanın amacı GN hastalarında *Janus kinase (JAK)2* mutasyon sıklığının araştırılmasıdır.

Yöntem: Bu çalışma Mayıs 2015 ve Ekim 2015 tarihleri arasında Sağlık Bilimleri Üniversitesi İzmir Tepecik Eğitim ve Araştırma Hastanesi Nefroloji Kliniği ve Dokuz Eylül Üniversitesi Tıp Fakültesi Nefroloji Bilim Dalı'nda yapılmıştır. GN tanılı 67 hasta ve kontrol grubu olarak 100 sağlıklı gönüllü çalışmaya dahil edilmiştir. JAK2 mutasyonu genetik analizi periferik venöz kanda çalışılmıştır.

Bulgular: GN grubundaki katılımcıların 33'ü erkekti (%49) ve yaş ortalaması 45,8±12,4'tü. Kontrol grubundaki katılımcıların 37'si erkekti (%37) ve yaş ortalaması 40,1±14,7'ydi. Her iki gruptaki katılımcıların hiçbirinde JAK2 mutasyonu tespit edilmedi.

Sonuç: Çalışmamızda JAK2 mutasyonu ve GN arasında doğrudan bir ilişki tespit edilememiştir. JAK-sinyal transdüserleri ve transkripsiyon aktivatörleri (STAT) yolağı ile GN arasındaki ilişkinin değerlendirilmesi için özellikle doku düzeyinde olmak üzere daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Glomerulonefrit, JAK2 gen mutasyonu, JAK-STAT yolağı

Introduction

Glomerulonephritis (GN) are a heterogenous group of diseases with multifactorial etiology. Infections, autoimmune diseases, malignancies, and drugs may cause GN[1]. The pathophysiology of GN is not understood yet. Janus kinase-signal transducers and activators of the transcription (JAK-STAT) pathway is the principal signaling mechanism for many cytokines and growth factors. JAK activation stimulates cell proliferation, differentiation, migration, and apoptosis[2]. The JAK family consists of four members: JAK1, JAK2, JAK3 and tyk2[3]. Mutation in the JAK-STAT pathway may lead to various diseases such as hematologic malignancies, solid cancers, and rheumatologic diseases[4]. *JAK2* gene mutation is used as a diagnostic test for myeloproliferative neoplasms (MPNs) such as essential thrombocythemia and polycythemia vera. GN could be seen as a late complication of MPN also known MPN-related glomerulopathy[5]. There are several case reports about the association between GN and MPN[6-9]. The Mechanism for the development of GN in MPN is not known, but JAK-STAT may well be an ultimate common pathway. Proinflammatory cytokines such as interleukin-6 (IL-6) play a central role in the pathogenesis of GN[10]. Experimental studies suggest that IL-6 contributes to renal injury in GN and an increased level of IL-6 is associated with poor prognosis[11]. IL-6 exerts its effects via JAK-STAT pathway. ILs contribute to renal injury in GN and exert their actions via JAK-STAT pathway. Therefore, the *JAK2* gene mutation may have a role in the development of GN. We hypothesized that the *JAK2* gene mutation has a role in the development of GN in various diseases and settings. We explored the *JAK2* gene mutation frequency in GN.

Materials and Methods

Study Centers and Participants

The study was conducted in University of Health Sciences Turkey, İzmir Tepecik Education and Research Hospital, Nephrology Clinic, and Dokuz Eylül University Faculty of Medicine Nephrology Clinic, between May 2015 and October 2015. These centers are tertiary care facilitys which located in Izmir, the third largest city of Turkey. These centers can manage GN. Patients 18 years and older who were diagnosed with GN by kidney biopsy were included in the study. Thrombocyte counts, and hemoglobin levels were recorded retrospectively at the time of diagnosis. Participants 18 years and older without any nephrological or hematological disease were included in the control group. The study was approved by the Ethics Committee of İzmir Katip Çelebi University (ethical approval number: 100, date: 03.06.2015). Participants were included in the study after being informed in detailed about the study, and written consent was taken in accordance with the Helsinki Declaration principles.

Genetic Analyze

Five milliliter blood drawn from the patient and control group into purple top ethylenediaminetetra-acetic acid contained tube. Blood samples were obtained at the nephrology clinic each center and immediately transferred to the genetic laboratory. Genomic DNA was extracted from peripheral whole blood using MagPurix Blood DNA ExtractionKit using MagPurix 12 (Zinexts Life Science Corp., Taiwan). The genotyping procedure consisted of polymerase chain reaction (PCR) amplification and SNP detection of the V617F mutation (rs77375493) of the *JAK2* gene using the following pair of primers: Forward 5'-TGCTGAAAGTAGGAGAAAGTGCAT-3' Reverse 5'-TCCTACAGTGTTTT CAGTTTCAA-3' by direct

sequencing. PCR products are purified as follows: 5 5 mL PCR products are treated with 2 2 mL of ExoSAP-IT enzyme (USB Affymetrix, USA) at 37 °C for 30 min and at 85 °C for 15 min. Products of sequence PCR were purified (secondpurification) by spin colon (ZR DNA Sequencing Clean-up Kit™, Zymo Research, USA). Sanger sequencing was performed by capillary electrophoresis after 5 min denaturation (3500 Genetic Analyzer, Lifetechnologies, USA). The obtained sequences are analyzed using SeqScape® Software v3.0.

Statistical Analysis

We used the "Statistical Package for Social Science (SPSS) 20.0" program for the analyses. While performing the analyses, we showed continuous numeric variables with normal distribution as mean ± standard deviation, and those without normal distribution as median, minimum and maximum values. Since our study was a descriptive study, a statistical comparison was not made.

Results

Sixty-seven participants in the patient group and 100 participants in the control group were included in the study. The average age was 45.8±12.4 years and 33 (49.3%) of 67 were male in the study group. The demographic characteristics of the participants are shown in Table 1. Twenty-one (31.3%) of the patients were diagnosed with IgA nephritis, 19 (28.4%) with focal sclerosing glomerulosclerosis (FSGS), 14 (20.9%) with membranous GN, and 13 (19.4%) with membranoproliferative GN (Table 2). Thrombocyte count and hemoglobin levels were in the normal range. The mean age was 40.1±14.7 years and 37 (37%) of 100 were male in the control group. Thrombocyte count, hemoglobin level, and hematocrit were also normal range in the control group. The JAK2 gene mutation was detected in the patient group nor the control group.

Discussion

A better understanding of disease pathogenesis will contribute to the development of new treatment options. GN are a heterogenous group of diseases with multifactorial etiology. Regardless of the etiology, inflammatory processes play an important role in the pathophysiology of GN. JAK-STAT pathway is one of the most important pathways responding to inflammatory signals. Although this response is well defined in lymphoid cells, it has also been described in kidney parenchymal and mesangial cells[12]. JAK-STAT pathway is highly activated in certain kidney diseases such as diabetic nephropathy, autosomal dominant polycystic kidney disease, human immunodeficiency virus-associated nephropathy[13]. An increase in systemic and intrarenal JAK-

Table 1. Demographic characteristics of the participants

Patient characteristics	Study group (n=67)	Control group (n=100)
Age, years ± SD	45.8±12.4	48.4±14.9
Male gender, n (%)	33 (49%)	53 (53%)
BMI, kg/m^2, (IQR)	29 (26-35)	30 (25-34)
Smoking status, n (%)		
None	30 (45%)	46 (46%)
Active smoker	22 (33%)	36 (36%)
Former smoker	15 (22%)	18 (18%)
Smoking timeand years (IQR)	18 (10-40)	15 (13-25)
Comorbidities, n (%)		
COPD	4 (6%)	6 (6%)
Hypertension	35 (52%)	25 (25%)
DM	10 (15%)	13 (13%)
CAD	4 (6%)	4 (4%)
CHF	1 (1%)	3 (3%)
Hyperlipidemia	18 (27%)	22 (22%)

BMI: Body mass index, CHF: Congestive heart failure, COPD: Chronic obstructive pulmonary disease, CAD: Coronary arterial disease, DM: Diabetes mellitus, IQR: Interquartile range, SD: Standard deviation

Data are shown as n (%), mean ± SD and median (25th-75th percentiles)

Table 2. Type of glomerulonephritis of the participants		
The type of glomerulonephritis	**Study group**	**JAK2 gene mutation**
IgA nephritis, n (%)	21 (31.3%)	0
FSGS, n (%)	19 (28.4%)	0
MGN, n (%)	14 (20.9%)	0
MPGN, n (%)	13 (19.4%)	0
FSGS: Focal sclerosing glomerulosclerosis, JAK-2: Janus Kinase 2, MGN: Membranous glomerulonephritis, MPGN: Membranoproliferative glomerulonephritis		

STAT activation was shown in a study conducted on patients with biopsy prove-focal sclerosing glomerulosclerosis (FSGS) [14]. In our study, 19 patients had FSGS and none of them had *JAK2* gene mutations. FSGS is a histological lesion, characterized by sclerosis in parts (segmental) of some (focal) glomeruli on light microscopic examination. Although the *JAK2* gene mutation is detected sporadically in FSGS, JAK-STAT signaling is increased in FSGS patients[14-16]. The association between GN and MPN is shown in a small series[17]. Myeloproliferative-related glomerulopathy is described as a late complication of MPN[5]. *JAK2* mutation is frequent in MPN and causes dysregulation of the JAK-STAT pathway[18]. We speculated that dysregulation due to *JAK2* mutation may cause the development of GN. Our study did not support an association between the *JAK2* mutation and GN. There are some reasons why the study failed: (1) the activation of JAK-STAT pathway is not only mediated by the *JAK2* mutation, (2) assessment of JAK-STAT pathway activation at the tissue level, and (3) *JAK2* mutation prevalence is too low, 0.2% in Denmark, and it can be difficult to detect in a small study population[19].

Study Limitations

This study had some limitations. We did not investigate the association between activation of JAK-STAT pathway and GN in tissue level. The low number of participants to demonstrate the relationship between the JAK2 mutation and GN. Although there is no relationship between the JAK2 mutation and GN, the activation of JAK-STAT pathway is involved in the pathophysiology of many kidney diseases. JAK inhibitors may be an alternative for treating kidney disease.

Conclusion

Although there is no direct relationship between the *JAK2* mutation and GN development, the JAK-STAT pathway can play a role in the pathogenesis of kidney diseases. To evaluate the association between the activation of JAK-STAT pathway and GN, well-designed studies are needed especially at tissue levels.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of İzmir Katip Çelebi University (ethical approval number: 100, date: 03.06.2015).

Informed Consent: Participants were included in the study after being informed in detailed about the study, and written consent was taken in accordance with the Helsinki declaration principles.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.D.Y., U.E.S., M.T., H.A., C.C., Design: T.D.Y., U.E.S., H.A., C.C., Data Collection or Processing: A.K., S.Y., M.T., Y.B.K., F.E., S.Y., Analysis or Interpretation: T.D.Y., U.E.S., A.K., S.Y., Y.B.K., F.E., H.A., Literature Search: T.D.Y., U.E.S., A.K., S.Y., M.T., Y.B.K., F.E., S.Y., C.C., Writing: T.D.Y., U.E.S., S.Y., H.A.

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

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Chadban SJ, Atkins RC. Glomerulonephritis. Lancet 2005;365:1797-806.
2. Harrison DA. The Jak/STAT pathway. Cold Spring Harb Perspect Biol 2012;4:a011205.
3. Rawlings JS, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. J Cell Sci 2004;117:1281-3.
4. O'Shea JJ, Schwartz DM, Villarino AV, Gadina M, McInnes IB, Laurence A. The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med 2015;66:311-28.
5. Said SM, Leung N, Sethi S, et al. Myeloproliferative neoplasms cause glomerulopathy. Kidney Int 2011;80:753-9.
6. Haraguchi K, Shimura H, Ogata R, Inoue H, Saito T, Kondo T, Nagata M, Kobayashi T. Focal segmental glomerulosclerosis associated with essential thrombocythemia. Clin Exp Nephrol 2006;10:74-7.
7. Demirci Yıldırım T, Soyaltın UE, Komaç A, Ekinci F, Akar H. Polycythemia Vera Associated with IgA Nephropathy: A Case Report. Turkish Nephrol Dial Transplant 2016;25:211-4.
8. Chen H, Zhang B, Li M, Hu R, Zhou C. Polycythemia vera associated with IgA nephropathy: A case report and literature review. Exp Ther Med 2015;10:555-60.
9. Wang R, Tong H, Wang H, Chen Z, Wang L, Chen J. Nephrotic syndrome related to chronic neutrophilic leukemia. Intern Med 2014;53:2505-9.

10. Su H, Lei CT, Zhang C. Interleukin-6 Signaling Pathway and Its Role in Kidney Disease: An Update. Front Immunol 2017;8:405.
11. Horii Y, Muraguchi A, Iwano M, et al. Involvement of IL-6 in mesangial proliferative glomerulonephritis. J Immunol 1989;143:3949-55.
12. Berthier CC, Zhang H, Schin M, et al. Enhanced expression of Janus kinase-signal transducer and activator of transcription pathway members in human diabetic nephropathy. Diabetes 2009;58:469-77.
13. Brosius FC 3rd, He JC. JAK inhibition and progressive kidney disease. Curr Opin Nephrol Hypertens 2015;24:88-95.
14. Tao J, Mariani L, Eddy S, et al. JAK-STAT signaling is activated in the kidney and peripheral blood cells of patients with focal segmental glomerulosclerosis. Kidney Int 2018;94:795-808.
15. Bohra GK, Meena DS, Bajpai N, Purohit A. Focal segmental glomerulosclerosis in a patient with prefibrotic primary myelofibrosis. BMJ Case Rep 2018;2018:bcr2017223803.
16. Kaygusuz I, Koc M, Arikan H, et al. Focal segmental glomerulosclerosis associated with idiopathic myelofibrosis. Ren Fail 2010;32:273-6.
17. Au WY, Chan KW, Lui SL, Lam CC, Kwong YL. Focal segmental glomerulosclerosis and mesangial sclerosis associated with myeloproliferative disorders. Am J Kidney Dis 1999;34:889-93.
18. Kaushansky K. On the molecular origins of the chronic myeloproliferative disorders: it all makes sense. Blood 2005;105:4187-90.
19. Nielsen C, Birgens HS, Nordestgaard BG, Kjaer L, Bojesen SE. The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. Haematologica 2011;96:450-3.