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Original Article

DNA Methylation of JAK2 Gene in Intestinal Biopsy and Peripheral Blood Samples of Patients with Ulcerative Colitis

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Abstract

Background: Comprised of two main subtypes (Ulcerative Colitis (UC) and Crohn), inflammatory bowel disease is caused by an interaction between genetic and environmental factors. As of the important role of innate immunity and JAK/STAT signaling pathway, the current study was designed to investigate the methylation status JAK2 gene in blood and tissue samples of patients with UC.

Methods: Genomic DNA was extracted from blood and intestinal biopsy samples of 28 UC patients and 28 controls. After bisulfite DNA conversion, real-time quantitative multiplex methylation specific PCR (QM-MSP) method was applied in order to assess JAK2 promotor methylation status.

Results: The JAK2 promotor in the intestinal biopsy samples was significantly hypermethylated in UC as the mean of unmethylated DNA was 1.255±1.865 in the patients group, while it was 1.292±4.726 in control group.

Conclusion: Hypermathylation of JAK2 gene may play a part in pathophysiology of UC which could result in gene silencing.

Keywords: Ulcerative Colitis; JAK2; Methylation; Epigenetics

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Introduction

Characterized by recurrent periods of relapse and remission, inflammatory bowel disease (IBD) is generally classified into two main subtypes: Ulcerative Colitis (UC) and Crohn's disease (CD) (1). Pathologically different from CD, UC would mainly affect the mucosal layer of intestine with continuous involvement form rectum to proximal (2). The incidence rate of UC has been higher in Europe, Asia, Middle East, and North America respectively, showing an enhancing trend over the past decades (3).

Caused by inappropriate response of innate immunity to intestinal normal flora, the failure to tolerance against normal flora would result in chronic inflammation which would finally result in tissue damage and functional disturbance (4, 5). In addition to genetic and environmental factors, the epigenetic mechanisms including DNA methylation have been proposed to have determinative role in etiology of this disease though changing the cells' phenotype without any genotype alteration (6, 7). As of the most important mechanisms of epigenetics, DNA methylation has been closely found in association with autoimmune diseases. The mechanism mainly involves methylation of CpG dinucleotides (known as CpG islands) which are mostly found in the promotor of the gene. As of the important role of promotor in gene transcription, and requirement of transcription factors to CpG islands in order to adhere to DNA, the hypermethylation of CpG islands in the promotor region may result in gene suppression (8).

JAK2 as a protein in the category of tyrosine kinase family, participates in pathophysiology of UC through IL23 signaling pathway, which involves JAK2/STAT3 as well (9). JAK2 may also cause an abnormal cytokine-related inflammatory response which would finally confer with normal function of the intestine (9).

To date, several variants, expression level and epigenetics of JAK/STAT pathway members have been investigated in IBD, and some associations have been found already. However, as the controversy still remain regarding the exact role of each factor and their interactions, the current study was designed to elucidate the role of epigenetics and JAK/STAT in pathophysiology of UC. In this study, the methylation status of JAK2 promotor was of interest in peripheral blood and intestinal samples of patients with UC.

Materials and Method

Study design

In order to investigate the DNA methylation status of JAK2 gene in blood and intestinal biopsy of UC patients, the current case-control study was designed according to STROBE guideline for observational studies. The study was approved by Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1395.1253).

Patient Selection

Over the 18-month patient recruitment period, adult patients over 18 years of age and both sexes with clinical presentation of UC whose diagnoses were confirmed through H&E stained sections of intestinal biopsy were eligible to enter the study. As of the different proposed etiology for pediatric and adult IBD, patients under 18 were excluded from this study. Moreover, patients with concomitant or previous history of inflammatory or immune-based diseases were excluded as well. The control group were selected among adult healthy subjects who have been referred for screening with no clinical presentation of IBD and their colonoscopy results were in normal ranges. Entering the study was voluntary for all participants; and they have been all requested to fill informed consent form prior to sampling.

Sampling and DNA extraction

Peripheral blood and intestinal biopsy samples were obtained from all patients and controls. After collecting in EDTA-covered tubes, the genomic DNA from 5 cc peripheral blood samples was extracted using Phenol-Chloroform method (10). The DNA extraction from intestinal biopsy samples was performed using High Pure PCR Template Preparation Kit. Prior DNA conversion and Polymerase Chain Reaction (PCR) tests, the quality of extracted DNA was assessed using optical density and 260/280 ratio.

DNA Treatment and Bisulfite Conversion

The bisulfite conversion of DNA is required before methylation assessment which would result in conversion of unmethylated cytosine residues to uracil without affecting the methylated parts of DNA (5-methylcytosine). In the PCR amplification, the uracil will be converted to thymine. This process was performed using MethylEdge™ Bisulfite Conversion System (Promega, Madison, WI).

DNA methylation assessment using Msp (methyl specific primer) Real Time PCR

The promotor methylation of JAK2 gene was assessed using real-time quantitative multiplex methylation specific PCR (QM-MSP) (11) which includes two steps:

1) First Step: Also known as multiplex step, external forward and reverse primers (Table 1) were used to amplify a genomic section containing different alleles of interest. Containing total amount of 25 µl mixture in each reaction-well, the reactions included 1 cycle of 95°C for 5 minutes, and then 30 cycles 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. The final cycle of this step was 72°C for 5 minutes.

2) Second Step: Specific nested primers (**Table 1**) were used in order to quantify specific methylated alleles from the products of multiplex step. Methylation status of CpG islands in gene were checked though the UCSC database, and the primer blasting was performed via MethBlast tool. This part of PCR reactions for 10 µl mixture in each reaction-well included in 1 cycle of 95°C for 1 minute, and then 30 cycles of 94°C for 30 seconds, 60°C for 1 minute, 72°C for 30 seconds, and the final cycle of 72°C for 5 minutes.

Converted methylated human plasmid DNA with 100% methylation was used to determine the methylation percentage of samples.

The amount of unmethylated DNA was calculated using the following formulae:

$\Delta\Delta Cq = \Delta Cq$ sample $- \Delta Cq$ plasmid

ΔCq sample = Cq MCP – Cq BSP

ΔCq plasmid = Cq MCP – Cq BSP

Statistical analysis

Using PASS11 software, Group sample sizes of 25 and 25 achieve 80% power to detect equivalence when the margin of equivalence is from -1.0 to 1.0 and the actual mean difference is 0.0. The significance level (alpha) is 0.050 using two one-sided Mann-Whitney Tests. These results are based on 2000 Monte Carlo samples from the null distributions: Normal (M0 S) and Normal (M1 S), and the alternative distributions: Normal (M0 S) and Normal (M0 S). Mean ± SD was used for reporting the amount of unmethylated DNA in each group; and the difference between groups was assessed using Mann-Whitney Test.

Results

Generally, 28 patients and 28 controls were recruited in this study. The patients included 20 males (71.4%) and 8 females (28.6%), while the controls included 15 males (53.6%) and 13 females (46.4%). The difference between two groups were not significant (P=0.17)

The mean age of patients was 34.96±14.06 years and the mean of age control group was 42.75±11.36 years, which was significantly higher than patients (P=0.04)

JAK2 Methylation Status in Peripheral Blood and Intestinal Biopsy Samples Promotor methylation status of JAK2 gene is describe in detail in **Table 2** and shown in **Figures 1 and 2**.

Generally, the difference between JAK2 methylation in blood samples of UC patients and controls was not significant (0.21). The mean unmethylated DNA in UC group was 0.0005±0.0003 and it was 0.0015±0.0058 in control group.

Interestingly, the JAK2 gene was found hyper methylated in the intestinal biopsy samples of UC patients which was statistically significant when compared to controls (P=0.002). Accordingly, the mean of unmethylated DNA was 1.255±1.865 in the patients group and it was 1.292±4.726 in control group.

Table 1. Comparison of Unmethylated DNA in JAK2 Gene in Blood and Tissue Samples of UC patients and Healthy Controls

Primer	Sequence
External Forward Primer	TAAGGTGGTTGATGGGAGTTAG
External Reverse Primer	TAACTCACCCTAACTAACTCCCC
Nested Methylation-Specific Forward	CGGGTTTGTTGTATTCGG
Nested Methylation-Specific Reverse	AAACCGAACTACCTCCGC

Table 2. Comparison of Unmethylated DNA in JAK2 Gene in Blood and Tissue Samples of UC patients and Healthy Controls

Sample	Group	N	Min	Max	Mean	SD	P-value
JAK2 Blood	UC	22	0.00023	0.00196	0.00054	0.00039	0.21
	Control	25	0.00014	0.02966	0.00154	0.00586	
JAK2 Tissue	UC	24	0.039418	8.60395	1.25599	1.86508	0.002
	Control	25	0.02711	23.75237	1.29243	4.72625	

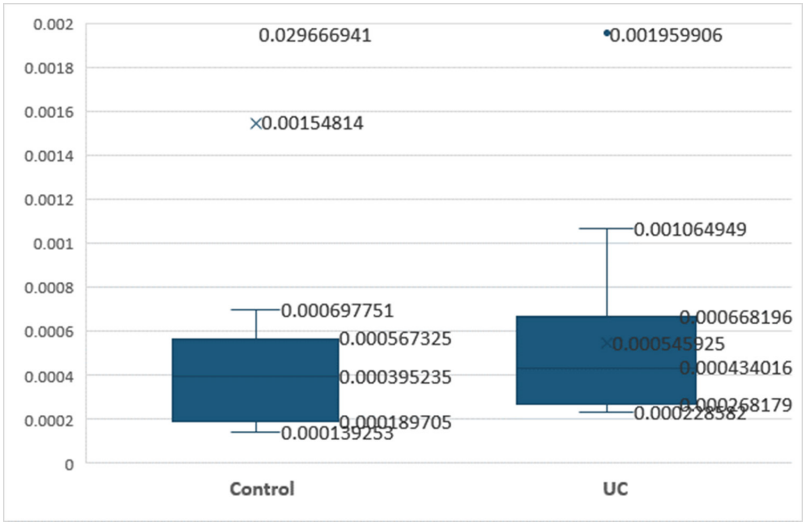

Figure 1. Comparison of Unmethylated DNA in JAK2 Gene in Blood Samples of UC Patients and Healthy Controls

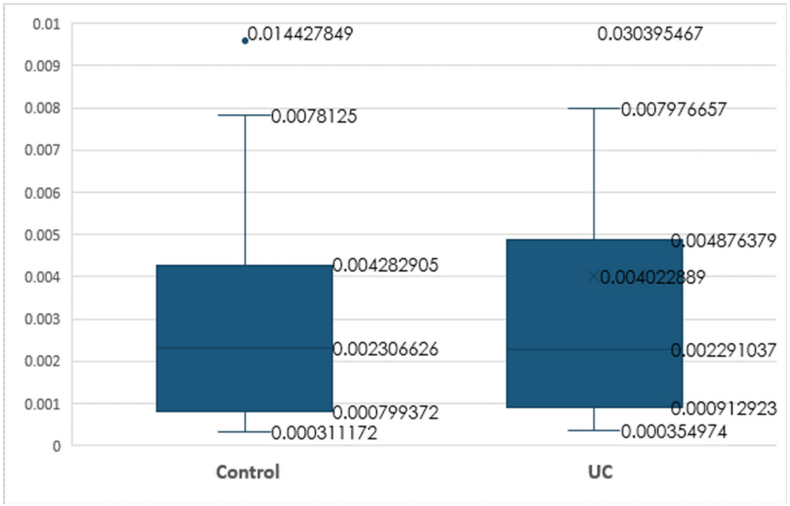

Figure 2. Comparison of Unmethylated DNA in JAK2 Gene in Tissue Samples of UC Patients and Healthy Controls

Discussion

The current study was conducted in order to better understand the role of epigenetics in JAK/STAT pathway member, JAK2, in pathophysiology of Ulcerative Colitis (UC). As one of the most important mechanisms of epigenetics, the DNA methylation status of the aforementioned gene was investigated using Quantitative MSP method.

In general, the current investigation revealed that although no remarkable association was found between methylation status of JAK2 gene in the blood samples, the JAK2 gene was significantly hyper methylated in the intestinal biopsy of UC patients, which could possibly result in gene suppression.

On the other hands, JAK2, located at 9p24.1 (12) is one of the members of JAK protein family which consists of 3 JAK members and tyrosine kinase 2. Produced by various types of cells in the immune system, the whole family is responsible for cytokine receptor-induced pathways. As the result of cytokine production imbalance in immune-based diseases and as inspired by discovery of some specific JAK2 mutations in disease, inhibitory therapies on JAK signaling were proposed for some of these diseases including IBD and UC (13).

As the crucial role of JAK2 in pathophysiology of IBD, the polymorphisms and expression of this gene have been investigated in this disease. The single nucleotide polymorphism (SNP), JAK2 rs10758669 were remarkably more frequent in patients with CD, while no similar association were found in UC patients of that population (9) Importantly, although the rs10758669 was not independently correlated to disease in a Caucasian ethnic group, there was an increased susceptibility to disease when the wild type of rs10758669 was accompanied with rs744166 of STAT3 (14). This SNP and also rs8074524 were found in close association with immunosuppressive medication need in UC patients in Turkish population. While the "CG" genotype of rs2293152 was more frequently associated with health, the homozygote "C" genotype made the individuals more susceptible for UC. Besides, patients who possessed rs11209026 of this gene, more frequently required surgical intervention in the treatment process of their disease (15). Located near to JAK2 gene, the rs1830610 (16) and also rs10975003 (17) variants made the Korean population more prone to this disease in a recent genetic investigation (16, 17).

Taking the limitations of the current study to account, co-assessment of gene expression level would be highly recommended in order to better understand the effect of DNA methylation on the final expression and function of the gene. Moreover, and as the analyzes in this study were all non-parametric, larger sample size would provide a more powerful statistical results. Meanwhile, an age-matched group could reduce the selection bias in the study as well. Although this study was a case-control investigating methylation status of JAK2 gene in two groups, prospective studies would be also advantageous in determining the relationship between methylation status and development of malignancy years after primary disease onset. and finally, as the IBD is considered a multifactorial disease with interaction of genetic and environmental risk factors, assessment of these probable factors and investigating their correlation would more precisely elucidate the role and weight of each factor in pathophysiology of this disease.

Conclusion

The promotor region of JAK2 gene was significantly hyper methylated in the intestinal biopsy of patients suffering from ulcerative colitis. The interaction between JAK2 and other members in JAK/STAT signaling pathway could possibly play a role in pathophysiology of UC through alteration of gene expression levels.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

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Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. Hanaei S, Sadr M, Rezaei A, Shahkarami S, Daryani NE, Bidoki A, et al. Association of NLRP3 single nucleotide polymorphisms with ulcerative colitis: A case-control study. Clin Res Hepatol Gastroenterol. 2017.
2. Zaki MH, Lamkanfi M, Kanneganti T-D. The Nlrp3 inflammasome in IBD and colorectal tumorigenesis. Trends Immunol. 2011;32(4):171.
3. Molodecky NA, Soon S, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012;142(1):46-54. e42.
4. Satsangi J, Grootscholten C, Holt H, Jewell D. Clinical patterns of familial inflammatory bowel disease. Gut. 1996;38(5):738-41.
5. Aghazadeh R, Zali MR, Bahari A, Amin K, Ghahghaie F, Firouzi F. Inflammatory bowel disease in Iran: a review of 457 cases. J Gastroenterol Hepatol. 2005;20(11):1691-5.
6. Scarpa M, Stylianou E. Epigenetics: concepts and relevance to IBD pathogenesis. Inflammatory bowel diseases. 2012;18(10):1982-96.
7. Van Vliet J, Oates N, Whitelaw E. Epigenetic mechanisms in the context of complex diseases. Cell Mol Life Sci. 2007;64(12):1531.
8. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, et al. Regulation of HP1–chromatin binding by histone H3 methylation and phosphorylation. Nature. 2005;438(7071):1116.
9. Prager M, Büttner J, Haas V, Baumgart DC, Sturm A, Zeitz M, et al. The JAK2 variant rs10758669 in Crohn's disease: altering the intestinal barrier as one mechanism of action. Int J Colorectal Dis. 2012;27(5):565-73.

10. Loparev VN, Cartas MA, Monken CE, Velpandi A, Srinivasan A. An efficient and simple method of DNA extraction from whole blood and cell lines to identify infectious agents. J Virol Methods. 1991;34(1):105-12.
11. Lo P-K, Watanabe H, Cheng P-C, Teo WW, Liang X, Argani P, et al. MethySYBR, a novel quantitative PCR assay for the dual analysis of DNA methylation and CpG methylation density. J Mol Diagn. 2009;11(5):400-14.
12. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), 2018. World Wide Web URL: https://omim.org/ [
13. Hedl M, Proctor DD, Abraham C. JAK2 disease-risk variants are gain of function and JAK signaling threshold determines innate receptor-induced proinflammatory cytokine secretion in macrophages. J Immunol. 2016;197(9):3695-704.
14. Polgar N, Csongei V, Szabo M, Zambo V, Melegh B, Sumegi K, et al. Investigation of JAK2, STAT3 and CCR6 polymorphisms and their gene–gene interactions in inflammatory bowel disease. Int J Immunogenet. 2012;39(3):247-52.
15. Can G, Tezel A, Gürkan H, Tozkır H, Ünsal G, Soylu AR, et al. Investigation of IL23R, JAK2, and STAT3 gene polymorphisms and gene–gene interactions in Crohn's disease and ulcerative colitis in a Turkish population. Turk J Gastroenterol. 2016;27(6):525-36.
16. Ye BD, Choi H, Hong M, Yun WJ, Low H-Q, Haritunians T, et al. Identification of ten additional susceptibility loci for ulcerative colitis through immunochip analysis in Koreans. Inflamm Bowel Dis. 2015;22(1):13-9.
17. Yang S-K, Jung Y, Kim H, Hong M, Ye BD, Song K. Association of FCGR2A, JAK2 or HNF4A variants with ulcerative colitis in Koreans. Dig Liver Dis. 2011;43(11):856-61.