Arch Iran Med. November 2018;21(11):530-535

http www.aimjournal.ir

ARCHIVES OF IRANIAN MEDICINE

Open Access

Original Article

Cytochrome P450 (*CYP450,2D6*A*), N-Acetyltransferase-2 (*NAT2*7, A*) and Multidrug Resistance 1 (*MDR1 3435 T*) Alleles Collectively Increase Risk of Ulcerative Colitis

Farzaneh Lotfi, MSc[1]; Fariborz Bahrehmand, PhD[2]; Asad Vaisi-Raygani, PhD[3*]; Reza Khodarahmi, PhD[2]; Maryam Tanhapour, PhD[3]; Amir Kiani, PhD[4]; Zohreh Rahimi, PhD[2]; Tayebeh Pourmotabbed, PhD[5]

[1]Department of Clinical Biochemistry, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4]Regenerative Medicine Research Center (RMRC), Kermanshah University of Medical Sciences, Kermanshah, Iran
[5]Department of Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA

Abstract

Background: Discovering the association between genetic variations of metabolizing enzymes with idiopathic diseases such as ulcerative colitis (UC) may not only be an auxiliary agent in diagnosis but also could be an effective pharmacotherapy for inflammatory bowel disease (IBD). The aim of the present case-control study was to determine the association of cytochrome *P450 2D6* (*CYP2D6 *4*), N-acteyltransferase-2 (*NAT2*7*) and multidrug resistance 1 (*MDR1*) 3435 C/T genotypes with UC susceptibility and thiopurine methyltransferase (TPMT) enzyme activity.

Methods: *TPMT* activity was measured by high performance liquid chromatography (HPLC) and genotypes for the 3 mentioned polymorphisms were determined in 215 unrelated UC patients and 212 unrelated healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in a Kurdish population from Iran.

Results: *CYP2D6*4 A* allele, *NAT2*7 A* and *MDR1* 3435 C/T alleles act synergistically to increase the risk of UC by 3.49 times. The frequency of the A allele of *CYP2D6*4* was significantly higher in UC patients (12.6%) compared to control subjects (8.5%, $P = 0.046$) that significantly increased the risk of UC by 1.56-fold ($P = 0.047$). The frequencies of *NAT2*7* genotypes and alleles were similar in both studied groups.

Conclusion: The most important outcome of this study is that for the first time we demonstrated the simultaneous presence of *TMDR1*, A *CYP2D6*4* and A *NAT2*7* alleles robustly increased the risk of developing UC by 3.49-fold. The current study suggests that *CYP2D6*4* and *MDR1 3435 C/T* gene polymorphisms may be risk factors for UC susceptibility.

Keyword: Cytochrome P450, *MDR1*, *NAT 2*, Thiopurine methyltransferase (TPMT), UC

Cite this article as: Lotfi F, Bahrehmand F, Vaisi-Raygani A, Khodarahmi R, Tanhapour M, Kiani A, et al. Cytochrome P450 (*CYP450,2D6*A*), N-acetyltransferase-2 (*NAT2*7, A*) and multidrug resistance 1 (*MDR1 3435 T*) alleles collectively increase risk of ulcerative colitis. Arch Iran Med. 2018;21(11):530-535.

Received: April 24, 2018, Accepted: October 7, 2018, ePublished: November 1, 2018

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are 2 of the most important chronic inflammatory bowel diseases (IBDs).[1] UC leads to diffuse mucosal inflammation of the large intestine but CD affects the digestive tract extending from the mouth to the anus.[2] In recent decades, incidence of IBDs has substantially increased.[3] Interaction between individual genetic backgrounds, defects in the immune system and environmental factors such as food habits contribute to incidence and prevalence of IBD.[4] Evidence has shown that defect in cytochrome (*CYP*) P450 and other drug-metabolizing enzymes and transporters may trigger development of inflammatory conditions.[5] The *CYP* P450 enzymes are responsible for detoxification of a large number of compounds including drugs, carcinogens, toxins, steroids, fatty acids and prostaglandins.[6] Numerous isoforms of P450s have been documented in humans.[7] Trzcinski et al reported that *CYP1*, *CYP2*, and *CYP3* families are primarily associated with the phase 1 metabolism of exogenous compounds.[6] The *CYP2D6* has been mapped on chromosome 22q13.1 and is one of the highly polymorphic regions of *CYP 450* with over 100 allelic variants.[8] Alleles related with *CYP2D6* gene are determined by combinations of variants located on the chromosome and are demonstrated using a star (*) for nomenclature. Moreover, single-nucleotide polymorphism (SNP) creates subfamilies in which each variant is described alphabetically (e.g., *2A, *2B, and *2C) as reported by Lyon et al.[9] People are classified in 4 groups with regard to allelic variants that affect the enzyme activity: poor metabolizers (PMs), ultra-rapid metabolizers (UMs), extensive metabolizers (EMs) and intermediate metabolizers (IMs).[10] Individuals who carry EMs genotype may be capable of faster detoxification of xenobiotics and carriers of PMs genotype may be exposed to accumulation of toxic

***Corresponding Authors:** Fariborz Bahrehmand, PhD; Assistant professor in Clinical Biochemistry, Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran, Daneshgah Avenue, P.O. Box 6714869914, Kermanshah, Iran. Email: fariborzbahrehmand@gmail.com
Asad Vaisi-Raygani, PhD; Professor in Clinical Biochemistry, Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Daneshgah Ave., P.O. Box 6714869914, Kermanshah, Iran. Email: avaisiraygani@gmail.com and asadvaisiraygani@kums.ac.ir

compounds in their body.[11] Trzcinski et al showed that the EM genotype may increase IBD susceptibility.[7] However, to the best of our knowledge, there is no study related to PM and UC susceptibility.

One of the most important enzymes contributing to metabolism of toxic and detrimental materials with aromatic amines, hydrazine, sulphonamides and aliphatic amines in their structures is N-acetyl-transferase (*NAT, 612182*).[12] There are 2 *NAT* isoforms, *NAT1* and *NAT2*, which are encoded by 2 different genes on chromosome 8. *NAT 2* increases solubility of toxic compounds through acetyl group transformation to the hydrophobic substrates. Individuals' genotype can reduce the enzyme activity, thus, 2 populations or groups may be known as slow and fast acetylators.[13] The effect of mutant alleles of *NAT2* on various disorders such as cancers and autoimmune diseases has been substantially investigated, but the role of *NAT2*7* polymorphism in IBD susceptibility is not well studied.[14,15]

Clinical trial evidence support the role of multidrug resistance 1 (*MDR1,* 171050), a drug transporter P-glycoprotein [P-gp] in protecting cells against xenobiotics and foreign compounds.[16] Lee et al reported that *MDR1* knockout mice develop spontaneous colitis under specific pathogen free conditions.[17] Thiopurine drugs (Azathioprine [AZA], 6-mercaptopurine [6-MP], and thioguanine [TG]) are prescribed as an immunosuppressant for various disorders including IBD.[17] Inactivation of these drugs via methylation of aromatic and heterocyclic sulfhydryl groups is catalyzed by thiopurine methyltransferase (TPMT). The TPMT is a cytosolic enzyme expressed in many cells including liver and brain.[18] TPMT activity is allele dependent going from high to virtually undetectable among people.[18] In addition, we recently demonstrated that UC patients with dominant mutant genotypes of *MDR1* C3435T significantly increased the risk of UC and significantly reduced TPMT activity.[19] There is one available study in this field that has examined just the association of genetic polymorphism with IBDs as "significance of the genetic polymorphism of CYP2D6 and NAT2 in patients with IBDs".[5] However, we tested whether *CYP2D6*4*, *NAT2*7* and *MDR1* 3435 C/T polymorphisms increased the risk of UC susceptibility in a population from west of Iran. Up to now, to the best of our knowledge, there is no study which has assessed the association between CYP450 enzymes as well as other drug metabolizing enzymes such as NAT and also drug transporter MDR1 with IBD in a population from Iran. In the present study, for the first time, we investigated the association of *CYP2D6 *4*, *NAT2*7* and *MDR1* 3435 C/T genotypes with UC susceptibility and TPMT enzyme activity in the Kurdish population from west of Iran.

Materials and Methods

Patients

This is a case-control study with a definite time period. Five milliliters peripheral blood in ethylene diamine tetra acetic acid (EDTA) (0.5 mM) was obtained from 215 unrelated Iranian UC patients and 212 unrelated healthy individuals as control group in Mahdieh Clinic of Kermanshah Medical University in the period between Februarys to June 2015. Sampling was done from patients who were referred to the university clinic under the supervision of a gastroenterologist based on diagnostic criteria for verification of UC disease. Also, samples were collected from a control group which were sex- and age-matched with patients under the supervision of a gastroenterologist and subjects with any gastrointestinal discomfort which were suspected to have IBD were excluded from the study. Since, other ethnic groups such as Lur and Turk live in Kermanshah, to confirm the ethnic background of all individuals, their identification documents were requested and also were asked questions about their origin. Those individuals who were born in Kermanshah province and were residents of this province for at least 2 generations and had Kurdish background were selected for the study.

CYP2D6, *NAT2* and *MDR1* Genotyping

Genomic DNA was extracted from 3 mL peripheral blood using the phenol chloroform extraction method.[20] Primer design and restriction enzyme analysis for *MDR1* point mutation C3435T was performed according to previous studies with minor modifications.[19]

The following primers were used to determine *CYP2D6 *4* and *NAT2*7* genotypes: *CYP2D6*4*, forward 5′ TGTAAAACGACGGCCAGTATCTCTGACGTGGA-TAGGAGGT 3′ and reverse 5'- CAGGAAACAGCTAT-GACCTGATGGGCAGAAGGGCACAA 3′; NAT2*7, forward 5′-GCTGGGTCTGGA AGCTCCTC-3′ and reverse 5′-TTGGGTGATACATACACAAGGG-3.

A 355-bp PCR product was generated from amplification of the *CYP450D*6* gene with following cycling condition: DNA denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 63°C for 45 sec and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. Then PCR products were digested with the restriction enzyme *MvaI*. Wild-type *CYP2D6*4* GG genotype showed 2 fragments of 250- and 105-bp, whereas homozygous mutant AA genotype showed a single band at 355-bp and heterozygous GA genotype showed 3 fragments of 355-, 250- and 105-bp.

The 547-bp PCR product obtained from *NAT2*7* gene amplification using the cycling condition of: DNA denaturation at 95°C for 5 minutes, followed by 33 cycles at 94°C for 1 min, 59.5°C for 30 seconds and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. PCR products were digested with the restriction enzyme *BamHI*. Accordingly, wild-type GG genotype showed a single fragment of 547-bp; homozygous mutant AA genotype showed 2 fragments of 490- and 57-bp and heterozygous GA genotype showed 3 fragments of 547-, 490- and 57-bp.[21,22]

TPMT Activity

TPMT activity was measured in whole blood by using a non-extraction high performance liquid chromatography (HPLC) system (Agilent Technologies 1200 Series, Agilent Corp., Germany) using EC 250/4.6 Nucleodur 5 µm C18 column (Macherey-Nagel, Duren, Germany), as previously

described.[23]

Statistical Analysis

The allelic frequencies were calculated by the gene counting method. The χ^2 test was used to verify the agreement of the observed genotype frequencies with those expected according to the Hardy–Weinberg equilibrium. The genotype frequencies in UC patients were compared to controls using the χ^2 test. Data were analyzed first for normality of distribution by using the Kolmogorov–Smirnov test. Results were expressed as mean ± SD for normally distributed data, median and interquartile range (IQR) for non-normally distributed data, and percentages for categorical data.

Statistical significance was assumed at $P<0.05$. The SPSS statistical software (SPSS for Windows 16; SPSS Inc, Chicago, IL, USA) was used for the statistical analysis.

Results

Details of the clinical, laboratory and demographic characteristics of patients and the control group are summarized in Table 1. Except Hb concentration in UC patients that was significantly lower than the control group (13.8 ± 1.85 vs 14.6 ± 1.73, $P \leq 0.001$), there were no significant difference between age, BMI and gender of the 2 study groups.

Frequency of *CYP2D6*4* alleles and genotypes in UC patients and the control group are demonstrated in Table 2. The frequency of *CYP2D6*4* A allele was significantly higher in UC patients (12.6%) compared to healthy subjects (8.5%, $P = 0.046$). Also, the presence of A allele significantly increased the risk of UC by odds ratio (OR) =1.56-fold ($P = 0.047$). No significant difference was observed in the overall distribution of *CYP2D6*4* genotypes between controls and UC patients.

As presented in Table 3, we found that the overall distribution of *NAT2*7* genotypes and alleles in UC patients were not significantly different from that of the control group. The effect of *CYP2D6*4* and *NAT2*7* genotypes on Hb concentration (g/dL) and TPMT activities (mU/L), in UC patients and the control group are shown in Table 4. As shown in Table 4, Hb concentration in UC patients with *CYP2D6*4* AG+GG and GG genotypes and GG and AG genotypes of *NAT2*7* gene were significantly lower in comparison with the control group (Table 4).

We also investigated the interaction between *MDR1* 3435 T, *CYP2D6*4* A and *NAT2*7* A alleles in UC patients

Table 1. The Demographic Characteristics in UC Patients and Control Group

	UC Patient (n = 210)	Control Subjects (n = 212)	*P* Values
Age (y)	35.9 ±13.2	34 ±14.2	0.58
Sex (M/F)	83/127	96/116	0.27
Hb (g/dL)	13.8 ± 1.8	14.6 ± 1.73	<0.001
TPMT activity (mU/L)	1.09± 20.4	1.1 ± 20.6	0.2
BMI (kg/m^2)	24 ± 3.97	24.1 ± 4.51	0.87

Abbreviations: UC, ulcerative colitis; BMI, body mass index; TPMT, thiopurine methyltransferase.

Table 2. Odd Ratio and Distribution of *CYP2D6*4* Genotypes and Alleles in Patients With UC and Control Subjects

	UC Patients (n = 210)	Control Subjects (n = 212)
*CYP2D6*4* genotypes		
G/A vs. G/G	39 (18.6%) vs. 164 (78.1%) ($\chi2 = 1.75$, $df = 1$, = 0.18) OR = 1.42 (0.84-2.4), P = 0.18	30(14.2%) vs. 179 (84.4%)
A/A vs. G/G	7(3.3%) vs. 164 (78.1%) ($\chi2 = 1.9$, $df = 1$, P=0.16) OR = 1.6(0.8-3.2), P=0.18	3 (1.4%) vs. 179 (84.4%)
A/A + A/G vs. G/G	46 (21.9%) vs. 164 (78.1%) ($\chi2 = 2.8$, $df = 1$, P=0.095) OR = 1.6 (0.8 -3.1), P=0.1 ($\chi2 = 3.42$, $df = 2$, P=0.18)	33 (15.6%) vs. 179 (84.4%)
*CYP2D6*4* alleles		
G	367(87.4%)	388 (91.5%)
A	53(12.6%) ($\chi2 = 4.1$, $df = 1$, P=0.046) OR = 1.56 (1.01-1.6, P=0.047)	36 (8.5%)

UC, ulcerative colitis; *CYP2D6*4*; OR, Odd ratio is an estimate relative risk for disease that was calculated and 95% CI was obtained by using $\chi2$ regression binary logistic analysis.

Table 3. Odd Ratio and Distribution of *NAT2*7* Genotypes and Alleles in Patients With UC and Control Subjects

	UC Patients (n = 210)	Control Subjects (n = 212)
*NAT2*7* genotypes G/A vs. G/G	23 (11%) vs. 187 (89%) $\chi2 = 0.27$, $df = 2$, $P = 0.6$) (OR = 1.18 (0.63–2.2, $P = 0.6$)	20 (9.4%) vs. 192 (90.6%)
*NAT2*7* alleles A vs. G	23 (5.5%) vs. 397(94.5%) 397 (94.5%) 23 (5.5%) ($\chi^2 = 0.26$, $df = 1$, $P = 0.6$) (OR = 1.08 (0.8–1.5, $P = 0.61$)	20 (4.7%) vs. 404 (95.3%)

UC, ulcerative colitis; *NAT2*7* ; OR, Odd ratio is an estimate relative risk for disease that was calculated and 95% CI was obtained by using $\chi2$ regression binary logistic analysis.

Table 4. Comparison of TPMT Activities in mU/L, Hb Concentration and BMI Between Dominant Models of *CYP2D6*4* AG+GG and *NAT2*7* Genotypes in UC Patients With Control Subjects

	UC Patients	Control Subjects	*P*
***CYP2D6*4* genotypes**	**G/G (n = 164)**	**G/G (n = 179)**	
Hb (g/dL)	13.86±1.83	14.6±1.76	0.006
TPMT activity (mU/L)	108.6±19.8	111.2±19.5	0.23
	AG+GG (n = 46)	**AG+GG (n = 33)**	
Hb (g/dL)	13.6±1.9	14.8±1.5	0.006
TPMT activity (mU/L)	110±21.6	114.3±21	0.34
***NAT2*7* genotypes**	**G/G (n = 187)**	**G/G (n = 192)**	
Hb g/dL	13.8±1.9	14.6±1.7	0.01
TPMT activity (mU/L)	108.7±20.3	112±19	0.37
	G/A (n = 23)	**G/A (n = 20)**	
Hb (g/dL)	13.6±1.9	14.8±2.1	0.046
TPMT activity (mU/L)	110±19	109±25	0.86

Tables 5. Carrier odds Ratios Interaction Among *MDR1, CYP2D6*4* and *NAT2*7* Allele in UC Patients Compared With Control Group

	MDR1	*CYP2D6*	*NAT2*7*	No. of UC Patients	No. of Control	OR (95% CI)	*P* Value
1	C	G	G	N=29 (0.138)	N=45, (0.212)	1.00	-
2	T	G	G	N=121 (0.576)	N=119, (0.561)	1.58 (0.93–2.7)	0.092
3	T	A	G	N=29 (0.138)	N=24, (0.113)	1.86 (0.96–2)	0.083
4	T	G	A	N=12 (0.0572)	N=11, (0.052)	1.7 (0.87–1.6)	0.27
5	C	A	G	N=8 (0.038)	N=4, (0.019)	3.1 (0.97–1.62)	0.085
6	T	A	A	N=9(0.043)	N=4, (0.019)	3.49 (1.02–6.84)	0.049

Global haplotype association ($\chi 2 = 5.9$, $df = 1$, $P = 0.27$).

(Table 5). We observed that combination of T allele of *MDR1*, A allele of *CYP2D6*4* and A allele of *NAT2*7* strongly increased the risk of UC by OR = 3.49 ($P = 0.04$). In addition, we analyzed the effect of *MDR1* 3435 T, *CYP2D6*4* A and *NAT2*7* A alleles on TPMT activities (mU/L). However, difference in TPMT activity between UC and control was not statistically significant (data not shown).

Discussion

CD and UC are idiopathic disorders influenced by genetic, geographic and ethnic variations. In addition, xenobiotic compounds such as drugs, drug metabolites, and other toxic environmental agents may exacerbate IBD pathology.[24] Detrimental and foreign substances are metabolized by various xenobiotic-metabolizing enzymes such as cytochrome P450 and N-acetyl-transferase.[25] One of the important cytochrome P450 isoenzymes is *CYP2D6*4* which is also known as debrisoquine hydroxylase. One study indicated that the expression of both multiple CYP P450 enzymes and other drug metabolizing enzymes are influenced by infectious diseases leading to inflammation.[5]

Changes that affect the structure of coded proteins may be due to genetic variations. Individual variability of responses to the same dose of a drug and difference in drug metabolism as a result of genetic variation has attracted a lot of attention.[26] The frequencies of *CYP2D6* phenotype, UMs, EMs, intermediate metabolizers, and PMs had been reported to approximately have the following distribution; 3%–5%, 70%–80%, 10%–17% and 5%–10%, respectively among Caucasians population.[27] In the present case-control study, we have assessed the potential effects of *CYP2D6*4* and *NAT2*7* polymorphisms on the risk of UC disease in a population from west of Iran.

To the best of our knowledge, there is no report regarding the association of *MDR1*, *CYP2D6*4* and *NAT2*7* genotypes with IBD susceptibility and its correlation with TPMT activity. The most important outcome of this study is that, for the first time, we demonstrated simultaneous presence of T *MDR1*, A *CYP2D6*4* and A *NAT2*7* alleles robustly increased the risk of developing UC by 3.49-fold in our studied population.

We found that the frequency of *CYP2D6*4* A allele was significantly higher in UC patients (12.6%) compared to the control group (8.5%) and it increased the risk of UC by 1.56-fold. This is consistent with the Dudarewicz report which showed that the frequency of the *CYP2D6*4* A allele was higher in patients from Poland with CD compared to healthy subjects (15.0% vs. 25.5%, $P = 0.030$).[28] Up to now, only 2 case control studies have investigated the association between the *CYP2D6*4* genotype and susceptibility to IBD and according to these studies there is no association between *CYP2D6*4* genotypes and susceptibility to IBD.[7,28] In a meta-analysis performed by Lee et al, it has been reported that the *CYP2D6* 1934 A allele was in correlation with autoimmune diseases in Caucasians.[29]

In this study, we assessed the impact of *NAT2*7* polymorphism on UC susceptibility. Neither *NAT2*7* genotype nor its allele was associated with UC in our studied population. In addition, there was no interaction between the presence of *NAT2*7* A and *CYP2D6*4* A alleles with increased risk of UC in our studied population. A significant increased risk of UC and CD was observed in carriers of the *NAT2*7* allele in a population from Poland.[30] However, the *NAT2* genotype apparently is not a risk factor for IBD in Caucasians and North America patients.[30,31] Machida et al in their report concerning the association of the *NAT2* polymorphism with UC and CD, mentioned that it seems the *NAT2* gene is one of the determinants for CD in Japanese. Alternatively, a new CD determinant may exist in the 8p22 region, where *NAT2* is located.[32] In another study, Ricart et al failed to indicate a direct association between *NAT1* and *NAT2* genotypes and response to mesalamine or sulfasalazine, or toxicity to sulfasalazine in patients with UC.[33]

Anti-inflammatory drugs and immunomodulators such as sulfasalazine, aminosalicylates, corticosteroids, and azathioprine are prescribed for treatment of IBD.[34] Chen et al have reported that Chinese patients with slow acetylator genotypes and without the *NAT2*4* allele more frequently suffered from adverse effects of sulfasalazine and sulfapyridine compared to those with the fast acetylator genotypes and at least one *NAT2*4* allele (36% vs 11%), although their results did not reach to statistical significance. In addition, they reported patients with slow acetylator genotypes were exposed to more sulfapyridine (SP) side effects than those with fast acetylator genotypes (36% versus 8%).[35] Side effects of sulfasalazine consisting of nausea, vomiting, diarrhea, anorexia, headache, rash and fever have been observed in 10 to 45% of patients with UC.[35]

With respect to previous results, we detected that the T allele of *MDR1*, A allele of *CYP2D6 *4* and A allele of *NAT2*7* strongly increased the risk of UC in a population from west of Iran. *MDR1* 3435 C/T genotypes were in correlation with UC disease in populations from New Zealand, Great Britain, Germany, Iran and Scotland.[36]

Previous studies revealed that *MDR1* variants contributed to the exacerbation of both CD and UC disease.[37] We recently reported that *MDR1* 3435 C/T polymorphism in dominant and codominant genetic models significantly increased the risk of UC by 1.45- and 1.46-fold, respectively.[19] One cohort study including 199 CD patients, 109 UC patients, and 120 matched healthy controls indicated that both CD and UC were associated with *MDR1* polymorphism in the Croatian population. Interestingly, they demonstrated that the heterozygous genotype of *MDR1* had a protective role against risk of CD.[38]

Previously, Bahrehmand et al reported that IBD patients with *MDR1* mutant genotypes of C3435T had lower TPMT activities and haemoglobin (Hb) concentrations. The carriers of mutant C3435T *MDR1* are not good TPMT methylators.[19] Interestingly, increased expression of P-gp leads to poor response to corticosteroids therapy and P-gp inhibitors have been used to overcome poor response to immunosuppressant therapy in patients with UC and CD.[39] However, one of the limitations of this study is lack of lifestyle and medication information of the participants.

Conclusion

The main outcome of this study, for the first time, is that the simultaneous presence of T *MDR1*, A *CYP2D6*4* and A *NAT2*7* alleles robustly increased the risk of developing UC by 3.49-fold in our studied population. We also detected that *CYP2D6*4* A allele was significantly involved in susceptibility to UC. No significant difference was found in the frequency of *NAT2*7* genotypes and alleles between patients and controls from western Iran.

Authors' Contribution

AVR: study design, data analysis and writing the manuscript. MT: writing draft of manuscript. FL: sampling and testing. FB: sampling and testing. ZR: revising of the manuscript. AK: sampling and testing. RK: study design, introduce of patients and collected blood. TP: scientific revising and English language.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

The human subject study protocol was approved by the Ethics Committee of the Kermanshah University of Medical Sciences (KUMS), Iran and was in accordance with the principles of the Declaration of Helsinki II and all subjects provided written informed consent (No:1394.140).

Funding

This study was funded by Kermanshah University of Medical Sciences, Kermanshah, Iran (grant No. 94140)

Acknowledgments

This work was performed in partial fulfillment of requirements for an M.Sc. degree in Clinical Biochemistry, Kermanshah University of Medical Sciences, Kermanshah, Iran (Farzaneh Lotfi). All authors contributed equally to this study.

References

1. Ye L, Jiang Z, Liu J, Zhou W, Cao Q. Is There a New Classification of Inflammatory Bowel Disease (IBD)-Adult-Onset Mixed IBD. Inflamm Bowel Dis. 2018;24(5):e19-e20. doi: 10.1093/ibd/izx093.
2. Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol. 2010;105(3):501-23; quiz 24. doi: 10.1038/ajg.2009.727.
3. Baskaran K, Pugazhendhi S, Ramakrishna BS. Association of IRGM gene mutations with inflammatory bowel disease in the Indian population. PLoS One. 2014;9(9):e106863. doi: 10.1371/journal.pone.0106863.
4. Ghobadi F, Vaisi-Raygani A, Bahrehmand F, Tanhapour M, Kiani A, Rahimi Z, et al. Genetic Variants of Pre-microRNAs A-499G(rs3746444) and T-196a2C(rs11614913) with Ulcerative Colitis (UC) and Investigated with Thiopurine-S-Methyltransferase (TPMT) Activity. Clin Lab. 2017;63(10):1683-90. doi: 10.7754/Clin.Lab.2017.170502.
5. Morgan ET. Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. Clin Pharmacol Ther. 2009;85(4):434-8. doi: 10.1038/clpt.2008.302.
6. Badyal DK, Dadhich AP. Cytochrome P450 and drug interactions. Indian J Pharmacol. 2001;33(4):248-59.
7. Trzcinski R, Skretkowicz J, Dziki A, Rychlik-Sych M, Baranska M. Genetic polymorphisms of CYP2D6 oxidation in patients with inflammatory bowel disease. Dig Dis Sci. 2010;55(4):1037-43. doi: 10.1007/s10620-009-0816-2.
8. Bernard S, Neville KA, Nguyen AT, Flockhart DA. Interethnic differences in genetic polymorphisms of CYP2D6 in the U.S. population: clinical implications. Oncologist. 2006;11(2):126-35. doi: 10.1634/theoncologist.11-2-126.
9. Lyon E, Gastier Foster J, Palomaki GE, Pratt VM, Reynolds K, Sabato MF, et al. Laboratory testing of CYP2D6 alleles in relation to tamoxifen therapy. Genet Med. 2012;14(12):990-1000. doi: 10.1038/gim.2012.108.
10. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev. 2009;41(2):89-295. doi: 10.1080/03602530902843483.
11. Baranska M, Skretkowicz J. [Genetic polymorphisms of oxidation and acetylation in cancers of digestive system]. Pol Merkur Lekarski. 2009;26(152):145-7.
12. Baranska M, Trzcinski R, Dziki A, Rychlik-Sych M, Dudarewicz M, Skretkowicz J. The role of N-acetyltransferase 2 polymorphism in the etiopathogenesis of inflammatory bowel disease. Dig Dis Sci. 2011;56(7):2073-80. doi: 10.1007/s10620-010-1527-4.
13. Wormhoudt LW, Commandeur JN, Vermeulen NP. Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: relevance to xenobiotic metabolism and toxicity. Crit Rev Toxicol. 1999;29(1):59-124. doi: 10.1080/10408449991349186.
14. McKay JD, Hashibe M, Hung RJ, Wakefield J, Gaborieau V, Szeszenia-Dabrowska N, et al. Sequence variants of NAT1 and NAT2 and other xenometabolic genes and risk of lung and aerodigestive tract cancers in Central Europe. Cancer Epidemiol Biomarkers Prev. 2008;17(1):141-7. doi: 10.1158/1055-9965.epi-07-0553.
15. Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet. 2005;366(9486):649-59. doi: 10.1016/s0140-6736(05)67137-1.
16. Senhaji N, Kassogue Y, Fahimi M, Serbati N, Badre W, Nadifi S. Genetic Polymorphisms of Multidrug Resistance Gene-1 (MDR1/ABCB1) and Glutathione S-Transferase Gene and the Risk of Inflammatory Bowel Disease among Moroccan Patients. Mediators Inflamm. 2015;2015:248060. doi: 10.1155/2015/248060.

17. Lee BI, Choi KY, Lee KM, Chung WC, Kim BW, Choi H, et al. [Is C3435T polymorphism of MDR1 related to inflammatory bowel disease or colorectal cancer in Korean?]. Korean J Gastroenterol. 2006;47(1):22-9.
18. Zeglam HB, Benhamer A, Aboud A, Rtemi H, Mattardi M, Saleh SS, et al. Polymorphisms of the thiopurine S-methyltransferase gene among the Libyan population. Libyan J Med. 2015;10:27053. doi: 10.3402/ljm.v10.27053.
19. Bahrehmand F, Kiani A, Vaisi-Raygani A, Bashiri H, Zobeiri M, Tanhapour M, et al. Pharmacogenetics of drug metabolizing enzyme: thiopurine methyl transferase phenotypes and multidrug resistance 1 gene polymorphism in inflammatory bowel disease. Cell Mol Biol (Noisy-le-grand). 2016;62(7):102-9.
20. Bahrehmand F, Vaisi-Raygani A, Kiani A, Rahimi Z, Tavilani H, Navabi SJ, et al. Matrix metalloproteinase-2 functional promoter polymorphism G1575A is associated with elevated circulatory MMP-2 levels and increased risk of cardiovascular disease in systemic lupus erythematosus patients. Lupus. 2012;21(6):616-24. doi: 10.1177/0961203312436857.
21. Anwarullah, Aslam M, Badshah M, Abbasi R, Sultan A, Khan K, et al. Further evidence for the association of CYP2D6*4 gene polymorphism with Parkinson's disease: a case control study. Genes Environ. 2017;39:18. doi: 10.1186/s41021-017-0078-8.
22. Osian G, Procopciuc L, Vlad L, Iancu C, Cristea PG, Mocan T, et al. NAT2 polymorphisms and sporadic colorectal cancer survival. J Gastrointestin Liver Dis. 2010;19(4):361-8.
23. Bahrehmand F, Vaisi-Raygani A, Kiani A, Bashiri H, Zobeiri M, Tanhapour M, et al. Whole-Blood Thiopurine S-Methyltransferase Genotype and Phenotype Concordance in Iranian Kurdish Ulcerative Colitis (UC) Patients. Clin Lab. 2017;63(5):947-54. doi: 10.7754/Clin.Lab.2017.161201.
24. Lemos MC, Cabrita FJ, Silva HA, Vivan M, Placido F, Regateiro FJ. Genetic polymorphism of CYP2D6, GSTM1 and NAT2 and susceptibility to haematological neoplasias. Carcinogenesis. 1999;20(7):1225-9.
25. Das A, Srinivasan M, Ghosh TS, Mande SS. Xenobiotic Metabolism and Gut Microbiomes. PLoS One. 2016;11(10):e0163099. doi: 10.1371/journal.pone.0163099.
26. Rychlik-Sych M, Baranska M, Waszczykowska E, Torzecka JD, Zebrowska A, Skretkowicz J. Genetic polymorphisms of CYP2D6 oxidation in patients with autoimmune bullous diseases. Postepy Dermatol Alergol. 2013;30(4):211-7. doi: 10.5114/pdia.2013.37030.
27. Gopisankar MG. CYP2D6 pharmacogenomics. Egypt J Med Hum Genet. 2017;18(4);309-13.
28. Dudarewicz M, Rychlik-Sych M, Baranska M, Wojtczak A, Trzcinski R, Dziki A, et al. Significance of the genetic polymorphism of CYP2D6 and NAT2 in patients with inflammatory bowel diseases. Pharmacol Rep. 2014;66(4):686-90. doi: 10.1016/j.pharep.2014.04.002.
29. Lee YH, Bae SC. Association between Functional CYP2D6 Polymorphisms and Susceptibility to Autoimmune Diseases: A Meta-Analysis. Immunol Invest. 2017;46(2):109-22. doi: 10.1080/08820139.2016.1226898.
30. Ernst A, Andersen V, Ostergaard M, Jacobsen BA, Pedersen IS, Drewes AM, et al. Common polymorphisms in the microsomal epoxide hydrolase and N-acetyltransferase 2 genes in association with inflammatory bowel disease in the Danish population. Eur J Gastroenterol Hepatol. 2011;23(3):269-74. doi: 10.1097/MEG.0b013e3283438a44.
31. Mahid SS, Colliver DW, Crawford NP, Martini BD, Doll MA, Hein DW, et al. Characterization of N-acetyltransferase 1 and 2 polymorphisms and haplotype analysis for inflammatory bowel disease and sporadic colorectal carcinoma. BMC Med Genet. 2007;8:28. doi: 10.1186/1471-2350-8-28.
32. Machida H, Tsukamoto K, Wen CY, Shikuwa S, Isomoto H, Mizuta Y, et al. Crohn's disease in Japanese is associated with a SNP-haplotype of N-acetyltransferase 2 gene. World J Gastroenterol. 2005;11(31):4833-7.
33. Ricart E, Taylor WR, Loftus EV, O'Kane D, Weinshilboum RM, Tremaine WJ, et al. N-acetyltransferase 1 and 2 genotypes do not predict response or toxicity to treatment with mesalamine and sulfasalazine in patients with ulcerative colitis. Am J Gastroenterol. 2002;97(7):1763-8. doi: 10.1111/j.1572-0241.2002.05838.x.
34. Mulder CJ, van den Hazel SJ. Drug therapy: dose-response relationship of oral mesalazine in inflammatory bowel disease. Mediators Inflamm. 1998;7(3):135-6. doi: 10.1080/09629359891027.
35. Chen M, Xia B, Chen B, Guo Q, Li J, Ye M, et al. N-acetyltransferase 2 slow acetylator genotype associated with adverse effects of sulphasalazine in the treatment of inflammatory bowel disease. Can J Gastroenterol. 2007;21(3):155-8.
36. Ho GT, Nimmo ER, Tenesa A, Fennell J, Drummond H, Mowat C, et al. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. Gastroenterology. 2005;128(2):288-96.
37. Potocnik U, Ferkolj I, Glavac D, Dean M. Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. Genes Immun. 2004;5(7):530-9. doi: 10.1038/sj.gene.6364123.
38. Brinar M, Cukovic-Cavka S, Bozina N, Ravic KG, Markos P, Ladic A, et al. MDR1 polymorphisms are associated with inflammatory bowel disease in a cohort of Croatian IBD patients. BMC Gastroenterol. 2013;13:57. doi: 10.1186/1471-230x-13-57.
39. Farrell RJ, Menconi MJ, Keates AC, Kelly CP. P-glycoprotein-170 inhibition significantly reduces cortisol and ciclosporin efflux from human intestinal epithelial cells and T lymphocytes. Aliment Pharmacol Ther. 2002;16(5):1021-31.