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

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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-acetyltransferase-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


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Introduction

Ulcerative colitis (UC) and Crohn’s disease (CD) are 2 of the most important chronic inflammatory bowel diseases (IBDs). UC leads to diffuse mucosal inflammation of the large intestine but CD affects the digestive tract extending from the mouth to the anus. In recent decades, incidence of IBDs has substantially increased. Interaction between individual genetic backgrounds, defects in the immune system and environmental factors such as food habits contribute to incidence and prevalence of IBD. Evidence has shown that defect in cytochrome (CYP) P450 and other drug-metabolizing enzymes and transporters may trigger development of inflammatory conditions. The CYP450 enzymes are responsible for detoxification of a large number of compounds including drugs, carcinogens, toxins, steroids, fatty acids and prostaglandins. Numerous isoforms of P450s have been documented in humans. Trzcinski et al reported that CYP1, CYP2, and CYP3 families are primarily associated with the phase 1 metabolism of exogenous compounds. 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. 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. 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). 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
compounds in their body, Trzcinski et al showed that the EM genotype may increase IBD susceptibility. 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). 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. 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.

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. Lee et al reported that MDR1 knockout mice develop spontaneous colitis under specific pathogen free conditions. Thiopurine drugs (Azathioprine [AZA], 6-mercaptopurine [6-MP], and thioguanine [TG]) are prescribed as an immunosuppressant for various disorders including IBD. 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. TPMT activity is allele dependent going from high to virtually undetectable among people. 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. 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.” 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. Primer design and restriction enzyme analysis for MDR1 point mutation C3435T was performed according to previous studies with minor modifications.

The following primers were used to determine CYP2D6 *4 and NAT2*7 genotypes: CYP2D6*4, forward 5' TGTTAAAACGACGGCAGTATCTCTGACGTTGGA-TAGGAGGT 3' and reverse 5' CAGGAAACGCTAT-GACCTGATGGCCAGAAGGCACAA 3'; NAT2*, forward 5' GCTGTTGGTCGTAAGCTCCTC-3 and reverse 5' TTGGGTATCATACTACAAAGGG-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.

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...
Statistical Analysis

The allelic frequencies were calculated by the gene counting method. The χ² 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 χ² 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 = 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 AG and AG genotypes of NAT2*7 gene were significantly lower in comparison with the control group (Table 4).

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

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UC Patients (n = 210)</th>
<th>Control Subjects (n = 212)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>35.9 ±13.2</td>
<td>34 ±14.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>83/127</td>
<td>96/127</td>
<td>0.27</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.8 ± 1.8</td>
<td>14.6 ± 1.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPMT activity (mU/L)</td>
<td>1.09 ± 20.4</td>
<td>1.1 ± 20.6</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 3.97</td>
<td>24.1 ± 4.51</td>
<td>0.87</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>CYP2D6*4 genotypes</th>
<th>UC Patients (n = 210)</th>
<th>Control Subjects (n = 212)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/A vs. G/G</td>
<td>39 (18.6%) vs. 164 (78.1%)</td>
<td>30 (14.2%) vs. 179</td>
<td>(84.4%)</td>
</tr>
<tr>
<td>(g² = 1.75, df = 1, P = 0.18)</td>
<td>OR = 1.42 (0.84-2.4), P = 0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A vs. G/G</td>
<td>7 (3.3%) vs. 164 (78.1%)</td>
<td>3 (1.4%) vs. 179</td>
<td>(84.4%)</td>
</tr>
<tr>
<td>(g² = 1.9, df = 1, P = 0.16)</td>
<td>OR = 1.60 (0.83-2.4), P = 0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A + A/G vs. G/G</td>
<td>46 (21.9%) vs. 164 (78.1%)</td>
<td>33 (15.6%) vs. 179</td>
<td>(84.4%)</td>
</tr>
<tr>
<td>(g² = 2.8, df = 1, P = 0.095)</td>
<td>OR = 1.6 (0.83-3.1), P = 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g² = 3.42, df = 2, P = 0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>NAT2*7 genotypes</th>
<th>UC Patients (n = 210)</th>
<th>Control Subjects (n = 212)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/A vs. G/G</td>
<td>239 (11%) vs. 187 (89%)</td>
<td>20 (9.4%) vs. 192</td>
<td>(90.6%)</td>
</tr>
<tr>
<td>(g² = 0.27, df = 2, P = 0.6)</td>
<td>OR = 1.18 (0.63-2.2), P = 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs. G</td>
<td>397 (94.5%)</td>
<td>20 (4.7%) vs. 404</td>
<td>(95.3%)</td>
</tr>
<tr>
<td>(g² = 23, df = 1, P = 0.6)</td>
<td>OR = 1.08 (0.85-3.1), P = 0.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>CYP2D6*4 genotypes</th>
<th>UC Patients (n = 164)</th>
<th>Control Subjects (n = 179)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>13.86±1.83</td>
<td>14.6±1.76</td>
<td>0.006</td>
</tr>
<tr>
<td>TPMT activity (mU/L)</td>
<td>108.6±19.8</td>
<td>111.2±19.5</td>
<td>0.23</td>
</tr>
<tr>
<td>AG+GG (n = 46)</td>
<td>AG+GG (n = 33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.6±1.9</td>
<td>14.8±1.5</td>
<td>0.006</td>
</tr>
<tr>
<td>TPMT activity (mU/L)</td>
<td>110±21.6</td>
<td>114.3±21</td>
<td>0.34</td>
</tr>
<tr>
<td>NAT2*7 genotypes</td>
<td>G/G (n = 187)</td>
<td>G/G (n = 192)</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.8±1.9</td>
<td>14.6±1.7</td>
<td>0.01</td>
</tr>
<tr>
<td>TPMT activity (mU/L)</td>
<td>108.7±20.3</td>
<td>112±19</td>
<td>0.37</td>
</tr>
<tr>
<td>G/A (n = 23)</td>
<td>G/A (n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.6±1.9</td>
<td>14.8±2.1</td>
<td>0.046</td>
</tr>
<tr>
<td>TPMT activity (mU/L)</td>
<td>110±19</td>
<td>109±25</td>
<td>0.86</td>
</tr>
</tbody>
</table>
(Table 5). We observed that combination of T allele of MDRI, A allele of CYP2D6*A 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 MDRI 3435 T, CYP2D6*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. Detrimental and foreign substances are metabolized by various xenobiotic-metabolizing enzymes such as cytochrome P450 and N-acetyl-transferase. One of the important cytochrome P450 isoenzymes is CYP2D6, 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.

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. The frequencies of CYP2D6 phenotype, UM, EM, 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. 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 MDRI, CYP2D6*A 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 MDRI, A CYP2D6*A 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*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*A allele was higher in patients from Poland with CD compared to healthy subjects (15.0% vs. 25.5%, P = 0.030). Up to now, only 2 case control studies have investigated the association between the CYP2D6*A genotype and susceptibility to IBD and according to these studies there is no association between CYP2D6*A genotypes and susceptibility to IBD.

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.

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*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. However, the NAT2 genotype apparently is not a risk factor for IBD in Caucasians and North America patients. 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. 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.

Anti-inflammatory drugs and immunomodulators such as sulfasalazine, aminosalicylates, corticosteroids, and azathioprine are prescribed for treatment of IBD. 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%). 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.

With respect to previous results, we detected that the T allele of MDRI, A allele of CYP2D6*A and A allele of NAT2*7 strongly increased the risk of UC in a population from west of Iran. MDRI 3435 C/T genotypes were in correlation with UC disease in populations from New Zealand, Great Britain, Germany, Iran and Scotland.
The main outcome of this study, for the first time, is that tyle and medication information of the participants.

Inhibitors have been used to overcome poor response to corticosteroids therapy and P-gp methylators.

Interestingly, increased expression of P-gp leads to poor response to immunosuppressant therapy in patients with UC and CD.

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 genotypes and alleles between patients and controls from western Iran.

The main outcome of this study, for the first time, is that tyle and medication information of the participants.

Inhibitors have been used to overcome poor response to corticosteroids therapy and P-gp methylators.

Interestingly, increased expression of P-gp leads to poor response to immunosuppressant therapy in patients with UC and CD. 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. 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).

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References


