

**SUSCEPTIBILITY TO ULCERATIVE COLITIS AND GENETIC
POLYMORPHISMS OF A251G SOD1 AND C-262T CAT**PODLOŽNOST ULCEROZNOG KOLITISU I GENSKI POLIMORFIZMI
A251G SOD1 I C-262T CATGadier El-Kheshen¹, Maryam Moeini², Mostafa Saadat¹Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran
Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran**Summary**

Background: Reactive oxygen species can attack and damage almost every molecule found in living cells, including proteins, carbohydrates, lipids, and DNA. For this reason, their production is normally tightly controlled. Among the most important defenses against these radicals are the superoxide dismutase (SOD) enzymes and catalase (CAT). Increasing attention has been given to the role of reactive oxygen metabolites in the pathogenesis of ulcerative colitis (UC), which is defined as an idiopathic and chronic intestinal inflammation. Accordingly, we hypothesized a relation between genetic polymorphisms in the two antioxidant enzymes SOD1 A251G (rs2070424) and CAT C-262T (rs1001179) and the risk of UC.

Methods: The present case-control study included 109 UC patients (46 males and 50 females) and 186 (67 males and 119 females) gender-matched healthy controls. Genotyping was done by the PCR-RFLP method.

Results: After adjusting for age and gender, a significant association was observed between the AG+GG genotypes of SOD1 A251G polymorphism (vs. AA genotype) and risk of UC (OR=0.29, 95% CI: 0.10–0.86, P=0.025) after adjusting for age and gender. Our statistical analysis revealed that the CAT C-262T polymorphism did not associate with the risk of UC before and/or after adjusting for age and gender.

Conclusions: Based on the present statistical analysis, the G allele of the SOD1 A251G polymorphism decreases the risk of UC, thus it might be assumed that the G allele has a protective role.

Keywords: CAT, inflammatory bowel disease, SOD1, ulcerative colitis

Kratak sadržaj

Uvod: Reaktivne kiseonične vrste mogu napasti i oštetiti gotovo svaki molekul u živim ćelijama, uključujući proteine, ugljene hidrate, lipide i DNK. Zbog toga je njihova produkcija obično strogo kontrolisana. Najvažniju odbranu od ovih radikala predstavljaju enzimi superoksid-dismutaze (SOD) i katalaze (CAT). Sve veća pažnja pridaje se i ulozi reaktivnih kiseoničnih metabolita u patogenezi ulceroznog kolitisa (UK), koji se definiše kao idiopatska hronična inflamacija creva. U skladu sa ovim, iznosimo hipotezu o odnosu između genskih polimorfizama u dva antioksidantna enzima SOD1 A251G (rs2070424) i CAT C-262T (rs1001179) i rizika od UK.

Metode: Ova anamnestička studija obuhvatila je 109 obolelih od UK (46 muškaraca i 50 žena) i 186 (67 muškaraca i 119 žena) zdravih kontrolnih subjekata odgovarajućeg pola. Genotipizacija je izvršena metodom PCR-RFLP.

Rezultati: Posle prilagođavanja za starost i pol, uočena je značajna povezanost između genotipova AG+GG polimorfizma SOD1 A251G (vs. AA genotip) i rizika od UK (OR=0,29, 95% CI: 0,10–0,86, P=0,025). Naša statistička analiza je pokazala da polimorfizam CAT C-262T nije bio povezan sa rizikom od UK pre i/ili posle prilagođavanja za starost i pol.

Zaključak: Na osnovu statističke analize, G alel polimorfizma SOD1 A251G smanjuje rizik od ulceroznog kolitisa, zbog čega se može pretpostaviti da G alel ima zaštitnu ulogu.

Ključne reči: CAT, zapaljenska bolest creva, SOD1, ulcerozni kolitis

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Introduction

Inflammatory bowel disease (IBD) is defined as an idiopathic and chronic intestinal inflammation. Studies show that IBD is a multifactorial disease (1, 2). It develops in genetically susceptible individuals due to the influence of environmental factors (3, 4). IBD has two main forms, ulcerative colitis (UC) and Crohn's disease (CD). UC is characterized by inflammation that is limited to the colon. By contrast, CD involves any part of the gastrointestinal tract from the mouth to the anus—most commonly the terminal ileum. Reactive oxygen species (ROS) are free radicals that can attack and damage almost every molecule found in living cells, including proteins, carbohydrates, lipids, and DNA. For this reason, their production is normally tightly controlled. In recent years, increasing attention has been given to the role of reactive oxygen metabolites (ROMs) in the pathogenesis of IBD (5–8).

Among the most important defenses against ROS are the superoxide dismutase (SOD) and catalase (CAT) enzymes. Superoxide dismutase 1 (cytosolic Cu/ZnSOD; SOD1; OMIM: 147450) is the major intracellular SOD (9). It catalyzes the dismutation of the toxic superoxide to hydrogen peroxide and molecular oxygen. The human SOD1 is 9,309 bp in length on chromosome 21q22.11 and consists of five exons interrupted by four introns (10). A genetic polymorphism of A251G (rs2070424) is located in intron 3 of the SOD1 gene. The association between the SOD1 A251G polymorphism and several multifactorial diseases has been reported (11–16).

Catalase (CAT; OMIM: 115500) is an antioxidant enzyme responsible for hydrogen peroxide (H₂O₂) conversion into oxygen and water. Its gene is located on chromosome 11p13, 34 kb long and is split into 13 exons (17). A C/T polymorphism located 262 bp upstream to the CAT transcription site (C-262T, rs1001179) has been described (18). The T allele is associated with lower levels of red blood cell CAT activity (18, 19) which may increase the risk of oxidative stress. Several studies investigated the association between CAT C-262T polymorphism and multifactorial traits (20–25).

Considering that it is well established that IBD is an inflammatory condition influenced by oxidative stress (6–8), it is hypothesized that the CAT C-262T and SOD1 A251G polymorphisms may affect the risk of UC. Therefore, the present case-control study was carried out.

Materials and Methods

Participants

A total of 96 UC patients (46 males and 50 females) and 186 healthy controls (67 males and 119 females) were included in this case-control study. The

controls and patients were sex matched ($P=0.71$), but there was a significant difference in mean age between the patients (SD: 36.4 ± 12.8) and controls (SD: 49.9 ± 9.0) so they were not matched ($P<0.001$). Based on the several studies investigating numerous genetic polymorphisms, it is concluded that the Iranian population is a heterogeneous population (26–28). Also, it has been reported that the allelic frequency of the CAT C-262T and SOD1 A251G polymorphisms is influenced by ethnicity (21, 25, 28, also see: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2070424, and http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1001179). Taken together, our patients and controls were selected among Persian (Caucasian) Muslims living in Shiraz (Fars province, south-west Iran). Informed consent was obtained from each subject before the study. This study was approved by the Shiraz University ethics committee.

Genotyping

Blood samples were obtained from patients and controls. Immediately after collection, whole blood was stored at $-20\text{ }^{\circ}\text{C}$ until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples. Genotypic analysis for the CAT C-262T and SOD1 A251G polymorphisms was determined as described previously (11, 22).

Statistical analysis

For the control group, the observed frequencies of the genotypes in both polymorphisms were assessed for Hardy–Weinberg equilibrium using the χ^2 statistic. The odds ratio (OR) and 95% confidence intervals (CIs) were calculated to estimate the strength of the association between polymorphisms and disease. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of $P<0.05$ was considered statistically significant.

Results

Table I shows the prevalence of the genotypes of SOD1 A251G polymorphism in IBD patients and healthy control subjects. Hardy–Weinberg equilibrium was determined. The distribution of genotypic frequencies of SOD1 A251G polymorphism was in Hardy–Weinberg equilibrium in the control group ($\chi^2=0.03$ df=1, $P=0.851$). There was no significant association between the AG+GG genotypes (vs. AA genotype) and risk of UC (OR=0.45, 95% CI: 0.17–1.14, $P=0.093$). However, after adjusting for age and gender, a significant association was observed (OR=0.29, 95% CI: 0.10–0.86, $P=0.025$). This means that the G allele decreases the risk of UC and thus has a protective role.

Table I Risk of ulcerative colitis associated with SOD1 A251G and CAT C-262T genetic polymorphisms.

Genotypes	Controls	Cases	OR	95% CI	P	OR*	95% CI	P
<i>SOD1</i> A251G polymorphism								
AA	162	90	1.0	–	–	–	–	–
AG	23	6	0.47	0.18–1.19	0.113	0.30	0.10–0.89	0.031
GG	1	0	–	–	–	–	–	–
AG+GG	24	6	0.45	0.17–1.14	0.093	0.29	0.10–0.86	0.025
<i>CAT</i> C-262T polymorphism								
CC	107	63	1.0	–	–	1.0	–	–
CT	79	32	0.76	0.45–1.28	0.314	0.83	0.45–1.53	0.557
TT	8	1	0.21	0.02–1.73	0.148	0.54	0.06–4.76	0.586
CT+TT	79	33	0.70	0.42–1.18	0.189	0.81	0.44–1.48	0.499

*Adjusted OR for age and gender.

Table I also shows the frequency of the genotypes of *CAT* C-262T polymorphism in the patients and healthy control subjects. The distribution of genotypic frequencies of *CAT* C-262T polymorphism was in Hardy-Weinberg equilibrium in the control group ($\chi^2=1.97$, $df=1$, $P=0.158$). Our statistical analysis revealed that this polymorphism did not associate with the risk of UC before and/or after adjusting for age and gender (Table I).

Discussion

Our present study has indicated that there is a significant association between the AG+GG genotypes of *SOD1* gene and the risk of UC (Table I). The AG+GG genotypes, therefore, reduce the risk of UC. Further, the AG+GG genotypes seem to play a protective role and thus decrease the risk of UC. To our best knowledge, no studies have investigated the association of *SOD1* A251G polymorphism and the risk of UC. The *SOD1* A251G polymorphism has been found to be associated with several diseases either as a risk factor (11) or as a protective factor (12).

As mentioned in the Introduction section, studies have shown that oxidative stress plays an important role in the development of IBD (6–8). The genetic variation of C-262T in the *CAT* gene is a functional polymorphism (18). There is evidence that the T allele is associated with a lower expression level of the gene (18, 19). Taken together, it is hypothesized that this allele may increase the risk of UC. However, our present findings do not support the hypothesis (Table I). Only one published study investigated the association between the *CAT* C-262T polymorphism and susceptibility to UC (29). In that study, the authors reported that the CT genotype of the *CAT* C-262T polymorphism increased the risk of IBD. Our present

findings are not consistent with the abovementioned report. As strongly recommended by Strengthening the Reporting of Genetic Association studies (STREGA), the observed frequencies of the genotypes in control group should show no significant deviation from the expected values based on the Hardy-Weinberg equilibrium (30, 31). However, in the study of Khodayari et al. (29), although the CT genotype was observed in 69.2 percent of control persons, there was no healthy control subject having the TT genotype; therefore, it is worthwhile to consider that there is significant difference between the observed genotypic frequencies and the expected ones based on the Hardy-Weinberg equilibrium ($P<0.001$). It is suggested that errors in the genotyping procedures may have occurred in these situations. Taken together, the results of previous study should be interpreted with caution.

The three main limitations of our current study were the limited number of samples, the non-matching of age between cases and controls, and the fact that we studied only one polymorphism for *CAT* and *SOD1* genes. There are several other single nucleotide polymorphisms for these genes in humans, which were not studied in the present work. Simultaneous study of these polymorphisms (including the haplotype analysis) should be further researched. Further studies are recommended for more definitive conclusions.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

- Okou DT, Kugathasan S. Role of genetics in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2014; 20: 1878–84.
- Halme L, Paavola-Sakki P, Turunen U, et al. Family and twin studies in inflammatory bowel disease. *World J Gastroenterol* 2006; 12: 3668–72.
- Nunes T, Fiorino G, Danese S, Sans M. Familial aggregation in inflammatory bowel disease: Is it genes or environment. *World J Gastroenterol* 2011; 17: 2715–22.
- Bernard K, Agnès G, Ramnik JX. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011; 474: 307–17.
- Dincer Y, Erzin Y, Himmetoglu S, et al. Oxidative DNA damage and antioxidant activity in patients with inflammatory bowel disease. *Dig Dis Sci* 2007; 52: 1636–41.
- Lih-Brody L, Powell SR, Collier KP, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Dig Dis Sci* 1996; 41: 2078–86.
- Palvick KP, Laroux S, Fuseler J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radic Biol Med* 2002; 33: 311–22.
- Rezaie A, Parker RD, Abdollahi M. Oxidative stress and pathogenesis of Inflammatory Bowel Disease: An epiphenomenon or the Cause? *Dig Dis Sci* 2007; 52: 2015–21.
- Panduru NM, Cimponeriu D, Cruce M, et al. Association of copper-zinc superoxide dismutase (SOD1) and manganese superoxide dismutase (SOD2) genes with non-syndromic myelomeningocele. *Rom J Morphol Embryol* 2010; 51: 37–41.
- Miao L, Clair DKS. Regulation of superoxide dismutase genes: Implications in diseases. *Free Radic Biol Med* 2009; 47: 344–56.
- Zhang Y, Zhang L, Sun D, et al. Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. *Mol Vis* 2011; 17: 2325–33.
- Guržanova-Durnev Lj, Zisovska E, Đošić-Markovska B. Cord blood superoxide dismutase and glutathione peroxidase activity in premature infants *J Med Biochem* 2014; 33: 208–15.
- Liu YM, Li XD, Guo X, et al. Association between polymorphisms in SOD1 and noise-induced hearing loss in Chinese workers. *Acta Otolaryngol* 2010; 13: 477–86.
- Spisak K, Klimkowicz-Mrowiec A, Pera J, et al. rs2070424 of the SOD1 gene is associated with risk of Alzheimer's disease. *Neurol Neurochir Pol* 2014; 48: 342–5.
- Kim SH, Kim SH, Lee JH, et al. Superoxide dismutase gene (SOD1, SOD2, and SOD3) polymorphisms and antituberculosis drug-induced hepatitis. *Allergy Asthma Immunol Res* 2015; 7: 88–91.
- Ebrahimpour S, Saadat I. Association of CAT C-262T and SOD1 A251G single nucleotide polymorphisms susceptible to gastric cancer. *Mol Biol Res Commun* 2015; 4: 223–9.
- Quan F, Korneluk RJ, Tropak MB, Gravel RA. Isolation and characterization of the human catalase gene. *Nucleic Acids Res* 1986; 14: 5321–35.
- Forsberg L, Lyrenas U, De Faire R, Mortgenstern A. A common functional C-T substitution in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radical Biol Med* 2001; 30: 500–5.
- Ambrosone CB, Ahn J, Singh KK, et al. Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. *Cancer Res* 2005; 65: 1105–11.
- Saadat M, Safaie S, Saadat I. Genetic polymorphism of C-262T catalase and susceptibility to schizophrenia. *Maced J Med Sci* 2014; 15: 74–7.
- Hu J, Feng F, Zhu S, et al. Catalase C-262T polymorphism and risk of prostate cancer: Evidence from meta-analysis. *Gene* 2015; 558: 265–70.
- Azizian-Farsani F, Rafiee G, Saadat M. Impact of sodium arsenite on chromosomal aberrations with respect to polymorphisms of detoxification and DNA repair genes. *Int J Toxicol* 2014; 33: 518–22.
- Jančić I, Šefik-Bukilica M, Živojinović S, Damjanov N, Spasovski V, Kotur N, Klaassen K, Pavlović S, Bufan B, Arsenović-Ranin N. Influence of promoter polymorphisms of the TNF- α (-308G/A) and IL-6 (-174G/C) genes on therapeutic response to etanercept in rheumatoid arthritis. *J Med Biochem* 2015; 34: 414–21.
- Zarei N, Saadat I, Farvardin-Jahromi M. The relationship between NQO1 C609T and CAT C-262T genetic polymorphisms and the risk of age-related cataracts. *Mol Biol Res Commun* 2015; 4: 143–9.
- Saadat M, Saadat S. Genetic polymorphism of CAT C-262 T and susceptibility to breast cancer, a case-control study and meta-analysis of the literatures. *Pathol Oncol Res* 2015; 21: 433–7.
- Rafiee L, Saadat I, Saadat M. Glutathione S-transferase genetic polymorphisms (GSTM1, GSTT1 and GSTO2) in three Iranian populations. *Mol Biol Rep* 2010; 37: 155–8.
- Saadat M. Distribution of ACE insertion/deletion (I/D) polymorphism in Iranian populations. *Mol Biol Res Commun* 2015; 4: 63–6.
- Saadat M, Mousavi-Kazerooni, Fallahzadeh-Abarghoeei L. Distribution of genetic polymorphism of CAT C-262T in three Iranian populations. *Turk J Biochem* 2015; 40: 386–9.
- Khodayari S, Salehi Z, Fakhrieh Asl S, et al. Catalase gene C-262T polymorphism: importance in ulcerative colitis. *Gastroenterol Hepatol* 2013; 28: 819–22.
- Little J, Higgins JP, Ioannidis JP, et al. Strengthening the Reporting of Genetic Association studies (STREGA) – An extension of the STROBE statement. *Eur J Clin Invest* 2009; 39: 247–66.
- Saadat M. Significance of the Hardy-Weinberg equilibrium in genetic association studies. *Psychiatry Res* 2011; 190: 165.

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