No Association Between Myeloperoxidase Gene G-463A Polymorphism And Rheumatoid Arthritis

Miyeloperoksidaz Geni G-463A Polimorfizmi İle Romatoid Artrit Arasında Bir İlişki Yoktur

[1]Doç.Dr. Sacide PEHLİVAN
[2]Yrd.Doç.Dr. Ali AYDENİZ
[1]Yüksek Biyolog. Tuğçe SEVER
[2]Yrd.Doç.Dr. Özlem ALTINDAĞ
[1]Doç.Dr.Sibel OĞUZKAN-BALCI
[2]Dr.Türkan HARUNLAR

[1]Gaziantep University Faculty of Medicine, Department of Medical Biology and Genetics
[2]Gaziantep University Faculty of Medicine, Department of Physical Medicine and Rehabilitation

Gaziantep Tıp Dergisi 2009;15(3):14-16.

Özet

Miyeloperoksidaz (MPO) ve genetik polimorfizmleri Romatoid Artrit (RA) gibi reaktif oksijen türlerinin aşırı arttığı çeşitli hastalıkların patogenezinde rol oynamaktadır. Bu çalışmada MPO G463A polimorfizminin RA ile ilişkisinin olup olmadığı araştırıldı. Çalışma kapsamında 75 hasta ve 90 sağlıklı kontrol birey analiz edildi. Genotiplendirme polimeraz zincir reaksiyonu ve restriksiyon enzimi uzunluk polimorfizmi (PCR-RFLP) yöntemi ile yapıldı. Bu tek nükleotid polimorfizmi (SNP) ve RA arasındaki ilişki ki-kare testi ve de-finetti programları kullanılarak analiz edildi. Kontrol grubu ve RA hasta grubu arasında genotip dağılımı ve allel sıklıkları bakımından istatistiksel olarak anlamlı bir fark yoktu. Bunun yanı sıra gruplarda "Hardy-Weinberg Dengesi"nden sapmanın olmadığı saptandı (p>0.05). MPO geni G-463A polimorfizmi ile RA arasındaki ilişkinin ilk defa analiz edildiği bu çalışma sonucunda Türk RA hastalarında bu ilişkinin olmadığı sonucuna varıldı. Bir sonraki aşamada MPO genindeki diğer polimorfizmlerin çalışılması planlanmaktadır.

Anahtar kelimeler: MPO geni, G-463A Promotör polimorfizmi, Romatoid Artrit, PCR-RFLP.

Abstract

Myeloperoxidase (MPO) has been involved in the pathogenesis of several diseases such as rheumatoid arthritis (RA) through excessive production of reactive oxygen species (ROS) as well as through its genetic polymorphism. We examined whether G-463A polymorphism of Myeloperoxidase (MPO) gene was associated with RA. Exactly, 75 patients with RA and 90 healthy control subjects were included in this study. The genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism method. The association between these single nucleotide polymorphism (SNP) and RA was analyzed using chi-square test and de-Finetti program. Genotype distributions and allele frequency of RA patients were not significantly different from healthy controls. In addition, it was also determined that there was no deviation from Hardy-Weinberg Equilibrium in any groups (p>0.05). Whether there was an association between MPO gene G-463A gene polymorphism and RA was investigated for the first time in this study in literature and it was demonstrated that it did not exist in the Turkish RA patients. It was planned to investigate the other polymorphisms of MPO gene in the future.

Key words; MPO gene, G-463A Promoter polymorphism, Rheumotaid Artritis, PCR-RFLP.

Introduction

Rheumatoid Arthritis (RA) is a chronic, inflammatory disease of the joints that affects 0.5-1.0% of the adult population. It is estimated, that at least 50% of the risk to develop RA is determined by genetic factors. Considerable efforts have been made to elucidate these genetic factors to better understand the disease. However, even after the advent of genome wide association studies, only somewhat more than half of the estimated genetic risk for RA has been assigned to specific genetic determinants. Genetic and environmental factors seem to be involved in the onset of RA (1-4).

Myeloperoxidase (MPO) is a myeloid-specific enzyme present in high levels in neutrophils, monocytes, and some classes of macrophages, including microglia. MPO is a haeme-containing peroxidase expressed and stored in neutrophils and monocytes, which, during cellular activation and degranulation, is released into phagocytic vacuoles as well as into the extracellular space.

MPO catalyses a reaction between hydrogen peroxide and chloride to generate hypochlorous acid (HOCl), a potent oxidant and chlorinating agent that can cause vascular damage when released by activated cells at inflammatory sites. The MPO gene encodes myeloperoxidase, a lysosomal hemoprotein located in the azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes (5,6). MPO has been involved in the pathogenesis of several diseases such as RA through excessive production of reactive oxygen species (ROS) as well as through its genetic polymorphism (6,7).

We examined whether G-463A polymorphism of Myeloperoxidase (MPO) gene was associated with RA.

Doç. Dr. Sadice PEHLİVAN, Gaziantep Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji ve Genetik AD
Adres: : Gaziantep Üniversitesi Tıp Fakültesi Tıbbi Biyoloji ve Genetik AD, Şehitkamil / GAZİANTEP
Tel: 0342 360 60 28 **Fax:** 0342 360 60 28 **E-mail:** psacide@hotmail.com
Bu çalışma, 2009 yılı 23-26 Mayıs tarihleri arasında Avusturya'nın Viyana kentinde düzenlenmiş olana "European Human Genetics Conference" isimli toplantıda poster bildirisi olarak sunulmuştur.

Geliş Tarihi: 04.08.2009 **Kabul Tarihi:** 28.08.2009

Table 1. Genotype distribution and allele frequencies of MPO gene -463G/A polymorphism in RA patients and healthy controls (*HWE: Hardy-Weinberg Equilibrium).

MPO (-463G/A)	Control N %	RA N %	OR	CI 95%	p
Genotype					
GG	62 (68.8)	48 (64.0)	1.135	0.685-2.643	0.388
AG	24 (26.6)	25 (33.3)	2.083	0.349-12.446	0.413
AA	4 (4.4)	2 (2.6)	0.646	0.114-3.675	0.619
Total	**90**	**75**			
Allele					
A	32 (17.7)	121 (80.6)	1.108	0.635-1.935	0.716
G	148 (82.3)	29 (19.4)	0.902	0.517-1.575	0.716
Total	**180**	**150**			
HWE*p	**0.404**	**0.551**			

Materials and Methods

Seventy-five patients with RA consecutively enrolled at physical medicine and rehabilitation outpatient clinic and 90 healthy control subjects matched for age and sex were studied. The genotyping was determined by PCR-RFLP. Genomic DNA was extracted from peripheral blood by using a salting out method (8). PCR was performed as follows: 0.6 μmol/L MPO(F) 5′'3fACAGGTGAATCGCTGACATGCTGCCT-3′'3f and MPO(R) 5′'3f- GAGACTCCCTGGAGGAAGAAGTTGAG-3′'3f, 0.5 mmol/ L dNTP, 3 mmol/LMgcl2, and 1.25 U Taq DNA polymerase, using the following temperature profile: 94ºC for 2 min, followed by 35 cycles of 94°C for 1 min, 57,3°C for 30 s, and 72°C for 5 min, and a final extension at 72°C for 10 min.

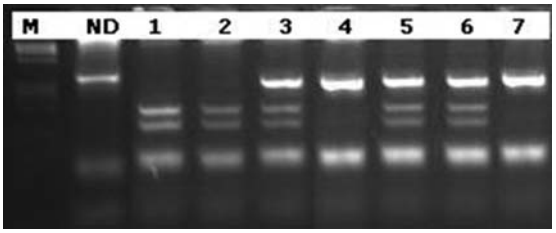

Figure 1. MPO gene G -463A polymorphism digestion products:1,2: G/G homozygous genotype. 3,5,6: A/G heterozygous genotype 4,7: A/A homozigot. M—DNA molecular weight marker. ND- Non Digest-PCR Product

Results

The distribution of the MPO gene G-463A polymorphism were evaluated in RA patients and healthy control populations by using PCR-RFLP method. Digestion of PCR product by AciI enzyme generated three fragments for homozygous -463G/G (168, 121, and 61 bp), four fragments for heterozygote -463G/A (289, 168, 121 and 61 bp) and two fragments for homozygous -463A/A (289 and 61 bp) (Figure 1).

The distribution of genotypes were obtained in patients and control groups The overall distribution of genotypes did not significantly differ between controls and RA patients ($p>0.05$). There was no difference in frequencies of alleles when comparing the patient groups and the control individuals ($p>0.05$). The observed genotype counts was not deviated significantly from those expected according to the HWE. All data were shown in (Table 1).

Discussion

Rheumatoid arthritis is a multifactorial autoimmune disease that affects many organs of the body. The myeloperoxidase (MPO) is an endogenous oxidant enzyme that generates reactive oxygen species.

MPO system of activated phagocytes is central to normal host defense mechanisms, and dysregulated MPO contributes to the pathogenesis of inflammatory disease states ranging from atherosclerosis to cancer (9). For example a single nucleotide polymorphism (SNP) G-463A in the promoter region has been associated with a decrease in risk of breast cancer (10). In a recent study Polonikov described a possible association between common polymorphism G-463A in the promoter of MPO gene, is associated with the risk of bronchial asthma. (11).

Atzeni investigated the potential association the-463 G/A in the promoter polymorphism and susceptibility to Behçet's disease in Italian patients, but couldn't find any association (12). In the present study we compared MPO gene G-463A genotype distributions and allele frequency of RA patients and healthy controls. We aim to investigate whether there was an association between MPO gene G-463A gene polymorphism and RA. We didn't found any significant difference between two groups.

As far as we searched, this is the first time in literature and it was demonstrated that no association was found when MPO gene G-463A promoter polymorphism is considered in Turkish RA patients. However, further studies are required for the role of MPO gene and related molecules in RA.

References

1.Kochi Y, Suzuki A, Yamada R, Yamamoto K. Genetics of rheumatoid arthritis: Underlying evidence of ethnic differences. J Autoimmun. 2009;32:158–62.

2.Plenge RM. Recent progres in rheumatoid arthritis genetics: one step towards improved patient care. Curr Opin Rheumatol. 2009;21:262-71.

3.Pratt AG, Isaacs JD, Mattey DL. Current concepts in the pathogenesis of early rheumatoid arthritis. Best Prac Res Clin Rheumatol. 2009;23:37–48.

4.Coenen MJ, Gregersen PK. Rheumatoid arthritis: a wiew of the current genetic landscape. Genes Immun. 2009;10:101-11.

5.Cascorbi I, Henning S, Brockmöller J, Gephart JMeisel C, Müler JM, Loddenkemper R. Subtantially reduced risk of cancer of the aerodigestive tract in subjects with variant -463A of the myeloperoxidase gene. Cancer Res. 2000;60:644-49.

6.Hoy A, Trégouët D, Leininger-Muller B, Poirier O, Maurice M, Sass C, et al. Serum myeloperoxidase concentration in a healthy population: biological variations, familial resemblance and new genetic polymorphisms. Eur J Hum Genet. 2001;9:780-6.

7.Baskol G, Demir H, Baskol M, Kilic E, Ates F, Karakukçu Ç, et al. Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis. Cell Biochem Funct. 2006;24:307–11.

8.Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from nucleated cells. Nucleic Acids Res. 1988;16:1215.

9.Minota S, Horie S, Yamada A, Iwamoto M, Yoshio T, Mimori A, et al. Circulating myeloperoxidase and anti-myeloperoxidase antibody in patients with vasculitis. Scand J Rheumatol. 1999;28:94-9.

10.He C, Tamimi RM, Hankinson SE, Hunter DJ, Han J. A prospective study of genetic polymorphism in MPO, antioxidant status, and breast cancer risk. Breast Cancer Res Treat. 2009;113:585-94.

11.Polonikov AV, Solodilova MA, Ivanov VP. Genetic variation of myeloperoxidase gene contributes to atopic asthma susceptibility: a preliminary association study in Russian population. J Asthma. 2009;46:523-8.

12.Atzeni F, Boiardi L, Casali B, Farnetti E, Sarzi-Puttini P, Pipitone N, et al. Lack of association of the -463 G/A myeloperoxidase promoter polymorphism with Behcet's disease in Italian patients. Rheumatology. 2007;46:1547-50.