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The role of CYP2C9 gene polymorphism in rheumatoid arthritis

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Ethics Committee Approval
The study was approved by the Mersin University Clinical Research Ethics Committee (date: November 21, 2008 and no: 2008/111). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: The inflammatory disorder rheumatoid arthritis (RA) affects quality of life and worsens with symptoms in the extra-articular tissues and systemic joints. The most significant member of the Cytochrome P450 enzyme family, Cytochrome P450 2C9 (CYP2C9), plays an essential role in the alkylation, demethylation, and hydroxylation of a variety of substances. Insufficient studies as to whether the susceptibility to rheumatoid arthritis is genetic exists. Therefore, our study presents new information on whether CYPC9 is a genetic risk factor. In this study, we sought to determine whether rheumatoid arthritis and the CYP2C9 gene polymorphism are related.

Methods: This study was conducted as a prospective case-control study. Fifty patients with RA and 50 healthy individuals were included in our study group. Blood from the controls and patients was drawn into ethylenediaminetetraacetic acid (EDTA)-containing tubes, and using a DNA isolation kit, DNA was isolated from leukocytes. Real-time polymerase chain reaction (RT-PCR) was used to assess the genotypes of CYPC9*2 and CYP2C9*3 with the LightCycler-CYP2C9 mutation detection kit.

Results: The heterozygous CYP2C9*2 genotype was found to carry a 2.85-fold risk when compared with the controls (odds ratio [OR]=2.85, 95% confidence interval [CI]: 0.52–15.50; P=0.22); however, this risk was not statistically significant. It was found that people with the CYP2C9*3 heterozygous genotype had a statistically significant 2.79-fold higher risk compared to the controls (OR=2.79, 95% CI: 1.13–7.00 P=0.04).

Conclusion: The heterozygous genotype of CYP2C9*3 may contribute to the onset of RA.

Keywords: CYP2C9, polymorphism, rheumatoid arthritis

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Introduction

The prevalent systemic inflammatory autoimmune disease known as rheumatoid arthritis (RA) causes painful joint inflammation that produces a significant impact on the quality of a patient's life. RA patients are at greater risk for cancer, respiratory and cardiovascular diseases, osteoporosis, early mortality, and serious infections than found in the general population. RA is also defined as a disease that is affected by both genetic and environmental factors [1,2]. In recent years, close interactions between newly identified genes in RA, genetic factors, and epigenetic mechanisms have attracted attention. In addition, the effect of environmental factors on the progression of this disease and new mechanisms of the innate and adaptive immune system, which are thought to be effective in different phases of the disease, are also being investigated [1–3]. Based on epidemiological studies, it has been suggested that some hormonal events in addition to hereditary and environmental factors may contribute to RA onset. It is thought that concentrations of sex-specific steroid hormones and circulating immune complexes may contribute to the promotion of inflammatory responses in synovial fluid and cartilage. While only a small proportion of rheumatoid arthritis patients experience spontaneous remission, it is known that approximately 20% of the disease progresses chronically despite treatment [4,5].

The heme protein superfamily, known as cytochrome P450, catalyzes numerous and various processes, primarily hydroxylation. These cytochromes play an essential role in the oxidative metabolism of substances produced in the body naturally, such as steroids and fatty acids. They also take part in the oxidative metabolism of many other structural molecules, including exogenous substances, such as medicines, carcinogens, and environmental factors [6]. In distinct gene families and subfamilies, CYP genes are categorized based on commonalities in their sequences. The CYP2C gene subfamily consists of four genes and is arranged on chromosome 10q24 in a specific order: CYP2C8–CYP2C9–CYP2C19–CYP2C18, and around 82% of the amino acids in these follow the same pattern. The CYP2C9 gene, which has nine exons in total and a length of about 55 kb, encodes a protein of 490 amino acid residues. CYP2C9 has been found to have two widely distributed variant alleles, the CYP2C9*2 allele (R144C) and CYP2C9*3 allele (I359L), which cause a 30% and 80% decrease in enzymatic activities, respectively [7–9]. Other possible genes that may predispose a person to RA have not yet been identified even though the Human Leukocyte Antigen (HLA) system, in particular the HLA-DRB1 molecule, is crucial for the diagnosis of RA [10]. A central feature of RA is inflammation, and DNA and lipids are components of the inflammatory response. Inflammation produces reactive oxygen species (ROS) via oxidation, a process that leads to various cytotoxic products, including lipids, alkenes, and DNA hydroperoxides [11]. Evidence implicating ROS and its products has been identified in RA pathology. During hypoxia–reperfusion, phagocytes in the pannus and synovial fluid in addition to synovial endothelial cells produce ROS [11,12].

Individual differences are also important in the detoxification process of the products resulting from ROS activity. It is thought that polymorphism in enzymes, especially CYP2C9, that detoxify ROS and its products may also play a role in joint damage and functional deterioration [11–13]. Therefore, the purpose of this investigation was to ascertain how CYP2C9 polymorphism contributed to RA etiology.

Materials and methods

Study subjects

This study was conducted as a prospective case-control study. The research group consisted of 50 (18 men, 32 women) unrelated healthy individuals and 50 (18 men, 32 women) RA patients from Mersin University Hospital, Department of Physical Therapy and Rehabilitation. The control group consisted of individuals with no rheumatic illness or known comorbidities. Patients with infectious diseases, hematological, kidney, and/or liver diseases, and/or malignancies were excluded from the study.

G*Power 3.0.10 for Windows was used for power analysis. To detect a difference in the CYP2C9 polymorphism between the patient and control groups, at least 60 patients in each group are required with a Type I error of 0.05 and 80% power. Five subjects who did not meet the inclusion criteria and five subjects with no DNA sample available for analysis in both groups were excluded from the study. The Mersin University Clinical Research Ethics Committee granted approval for the current study in compliance with the requirements of the Declaration of Helsinki (Approval number: 2008/111, Date: November 21, 2008). After being made aware of the study's goals, all participants signed a consent form.

DNA isolation and genotyping

Peripheral blood was obtained and added to tubes with ethylenediaminetetraacetic acid (EDTA) for use in genetic analyses. DNA extraction was performed from circulating leukocytes by utilizing a highly pure polymerase chain reaction (PCR) template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany, catalog no: 1 796 828). Using a CYP2C9 mutation detection kit and real-time PCR (qPCR), the CYPC29*2 and CYP2C9*3 alleles were identified. (Roche Diagnostics GmbH, Mannheim, Germany; catalog number: 3113914).

A CYP2C9 reaction mixture containing a dNTP mix, DNA polymerase reaction buffer, enzyme solution (Taq polymerase), control template, hybridization probes 3 and 4, sterile H_2O, and CYP2C9 reaction mixture was used for PCR.

Statistical analysis

The distribution of CYP2C9 genotypes between the RA and control groups was assessed using a chi-squared test. By generating odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models, the connection between CYP2C9 genotypes and RA patients was assessed. SPSS software demo version 20 was used to do all statistical calculations (IBM SPSS Inc. Free Download, Chicago, Illinois, USA). Every test was run with a significance threshold of 0.05.

Results

The study included 50 RA patients (32 females and 18 men) and 50 controls (32 females and 18 males). The mean (SD) age was 52.86 (8.00) in patients and 50.48 (8.92) in controls. Between the patients and the controls, no statistically significant age difference was found (P=0.21). In the patient, the frequency of the wild and heterozygous CYP2C9*2 genotypes was found to be 84% and 10%, respectively, while it was 96% and 4% in the controls. The prevalence of the CYP2C9*2 mutant genotype was 6%; however, the mutant genotype in the controls could not be identified, making it impossible to calculate the odds ratio (OR). The risk of RA was 2.85 times higher in people with the CYP2C9*2 heterozygous genotype (OR=2.85, 95% CI: 0.52–15.50 P=0.22) compared to the control; however, this higher risk was not statistically significant (Table 1). Wild and heterozygous CYP2C9*3 genotype frequencies in patients were 62% and 38%, respectively, whereas they were 82% and 18% in controls, respectively. The risk of RA was shown to be significantly higher in individuals with the CYP2C9*3 heterozygous genotype when compared with controls (OR=2.79, 95% CI: 1.13–7.00, P=0.04) as shown in Table 1. The patient and the CYP2C9*2 genotype did not show a significant correlation.

Table 1: CYP2C9 genotypes and risk of RA

Variable	Genotype	Patient n=50 n (%)	Control n=50 n (%)	OR (95% CI)*	*P*-value
CYP2C9*2	wild	42 (84)	48 (96)	1 (reference)	0.22
	heterozygote	5 (10)	2 (4.0)	2.85 (0.52–15.50)	
	mutant ‡	3 (6.0)	-	-	
CYP2C9*3	wild	31 (62)	41 (82)	1 (reference)	0.044
	heterozygote	19 (38)	9 (18)	2.79 (1.11–7.00)	

CYP2C9: Cytochrome P_{450} subtype, RA: rheumatoid arthritis, *OR: odds ratio, CI: confidence interval from logistic regression, ‡ Odds ratio cannot be calculated, wild alleles were used as references.

Discussion

It would be clinically beneficial to accurately target more appropriate therapy based on early and reliable identification of at-risk RA patients for poor long-term outcomes. Finding genetic indicators of clinical outcomes is therefore of tremendous interest [3,10]. According to studies, xenobiotic metabolizing enzymes including CYP, glutathione S-transferase (GST), and N-acetyltransferase 2 (NAT2) genes may affect the prevalence and course of RA [13]. An essential cytochrome P450 enzyme, known as cytochrome P450 2C9, is crucial for the oxidation of both xenobiotic and endogenous substances [14]. Due to an imbalance between the pro and antioxidant systems brought on by inherited deficits of this enzyme activity, excessive ROS might be produced. ROS are crucial mediators of inflammatory and immunological responses because they produce oxidative cellular damage *in vivo* [15]. RA pathophysiology includes the involvement of reactive oxygen species [16]. Patients with RA experience oxidative damage to joint lipids, DNA, and other cellular components [16–18]. Synovial phagocytes and synovial superoxide can be made by chondrocytes and endothelial cells [14–19]. In several studies, RA and xenobiotic metabolizing enzymes have been linked to deletion polymorphism of glutathione S- transferase mu 1 (GSTM1), which is thought to increase vulnerability to RA, according to Morinobu and colleagues [20]. CYP1A1 4887A appears to help inhibit the emergence of RA according to a study by Yen et al. [21]. Pawlik et al. [22] suggest that polymorphism of NAT2 may be a hereditary risk factor for joint injury. They discovered that the probability of getting RA was 4.39 times higher in slowvNAT2 acetylators than in rapid acetylators. However, no research on the relationship between CYP2C9 gene polymorphisms and RA is available. The candidate gene development study shows that the existence of single nucleotide polymorphism (SNPs) or haplotypes for estrogen receptors 1 and 2 (ESR1 and ESR2, respectively), CYP1B1, CYP2C9, low-affinity immunoglobulin gamma Fc region IIIA (FcR3A), and sex-hormone binding globulin (SHBG) affects the probability of developing bone erosion in RA. This research also proves that genotyping of hormone-related SNPs can help accurately predict the course of disease in seropositive patients and that the influence of most SNPs or haplotypes is dependent on the rheumatoid factor (RF) status [5]. A case-control study was conducted to determine whether 47 possibly functional SNPs in 16 genes related to steroid hormones are connected to the prevalence of RA and the responsiveness to anti-tumor necrosis factor (TNF) medication. That study demonstrated how the CYP2C9 SNP 1799853 affects the body's reaction to anti-TNF medications and drugs that block the effects of estrogen. Exonic variant CYP2C9 rs1799853 modifies the amino acid sequence, lowers the enzyme's activity, and subsequently seems to slow down the metabolism of some medicines. The response to anti-TNF medications is therefore believed to be related to the CYP2C9 gene. It is currently unclear whether the gene's impact on drug response is directly caused by flaws in the metabolism of anti-TNF medications or, alternatively, whether it is caused by variations in the metabolism of steroid hormones [23]. Our findings imply that genes implicated in protection from oxidative stress may have an impact on the RA disease process. In our analysis, the CYP2C9*2 genotype was not linked to a higher risk of contracting RA. However, heterozygous CYP2C9*3 individuals with the genotype were shown to have a 2.79 times higher risk of developing RA. Therefore, it is hypothesized that CYP2C9*3 will act as a catalyst for the onset of RA.

Limitations

The main limitation of our study is the small number of participants, which should be supplemented by larger investigations. Despite these limitations, this study is still an important preliminary study and forms the basis for future studies.

Conclusion

In conclusion, it is possible that genetic polymorphisms in xenobiotic metabolizing enzymes have a role in the progression of the disease by influencing food and environmental factors. An evaluation of the literature indicates that this study is the first to describe the connection between CYP2C9 polymorphism and RA. The incidence of CYP2C9 polymorphisms in RA patients and whether they represent an essential risk factor for the onset of RA require further research in larger populations.

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