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Title: Association of the C-285T and A5954G Polymorphisms in the DNA repair gene OGG1 with the Susceptibility of Rheumatoid Arthritis.

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Abstract: Rheumatoid arthritis (RA) is a chronic autoimmune disease and can lead to deformities and severe disabilities, due to irreversible damage of tendons, joints, and bones. Previous study indicated that DNA repair system was involved in the pathology of RA. In this study, we investigated the association of two 8-oxoguanine glycosylase 1 (OGG1) gene polymorphisms (rs159153 and rs3219008) with the susceptibility to RA in 384 Taiwanese individuals (192 RA patients and 192 controls). Our data showed that statistically significant difference in genotype frequency distributions was found at rs3219008 SNP between RA patients and control groups (p = 5.6E-0.5). Our data also indicated that individuals with the AG genotype at rs3219008 SNP may has a higher risk of developing RA. We did not observe any statistically significant association of OGG1 haplotype frequencies (rs159153 and rs3219008) with RA progression. The study suggested that OGG1 polymorphisms (rs159153 and rs3219008) are associated with RA progression and that these may be used as molecular markers of RA.

Association of the C-285T and A5954G Polymorphisms in the DNA repair gene *OGG1* with the Susceptibility of Rheumatoid Arthritis.

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease and can lead to deformities and severe disabilities, due to irreversible damage of tendons, joints, and bones. Previous study indicated that DNA repair system was involved in the pathology of RA. In this study, we investigated the association of two 8-oxoguanine glycosylase 1 (*OGG1*) gene polymorphisms (rs159153 and rs3219008) with the susceptibility to RA in 384 Taiwanese individuals (192 RA patients and 192 controls). Our data showed that statistically significant difference in genotype frequency distributions was found at rs3219008 SNP between RA patients and control groups (*p* = 5.6E-0.5). Our data also indicated that individuals with the AG genotype at rs3219008 SNP may has a higher risk of developing RA. We did not observe any statistically significant association of *OGG1* haplotype frequencies (rs159153 and rs3219008) with RA progression. The study suggested that *OGG1* polymorphisms (rs159153 and rs3219008) are associated with RA progression and that these may be used as molecular markers of RA.

KEY WORDS:

Rheumatoid Arthritis (RA) · 8-oxoguanine glycosylase 1 (*OGG1*) · Single nucleotide polymorphisms (SNPs) · Haplotypes.

Introduction

Rheumatoid arthritis (RA) is a joint inflammation disease combining several peripheral inflammatory conditions [1]. It could involve chronic synovitis, inducing the demolition of joint tissue, especially containing bone and cartilage, and therefore, joint function is seriously impaired. The prevalence is about 1% with some variation among ethnic groups [1-3]. Its genetic contribution was well documented by multiple family studies, and multiple whole-genome sib-pair linkage studies have been reported with limited consistency among them [5, 6]. The previous studies on RA-susceptible genes were published from a group based on a high-throughput single nucleotide polymorphism (SNP) genotyping facility that adopts case-control linkage disequilibrium (LD) mapping on a large scale as an initial survey method without using subjects that were used for preceding linkage studies [7-9]. One of them identified functionally relevant polymorphisms of peptidylarginine deiminase 4, an enzyme that catalyzes the post-translational citrullination of proteins, as a RA gene [10, 11]. Besides, the major histocompatibility (MHC) class II region is an important susceptibility factor, and the human leukocyte antigen (HLA)–DR4 has been associated with serious disease courses [12, 13]. Recently, Shao et al. reported that

DNA repair system was involved in the pathology of RA [14]. It had been implicated that T cells of RA patients failed to produce sufficient transcripts and protein of the DNA repair kinase ataxia telangiectasia (AT) mutated (ATM). Therefore, we decided to study the effect of genetic polymorphism of DNA repair genes as modifiers of the risk for RA as well in this study.

Moreover, DNA repair enzymes modulate free-radical production after DNA damage. Among such enzymes, 8-oxoguanine glycosylase 1 (*OGG1*) seems to be most important since *OGG1* is primarily responsible for removing 8-oxoguanine in DNA, which is a major product of DNA damage formed by free radicals, and can mispair with adenine residues instead of the usual cytosine residues, leading to an increased frequency of G:C to T:A transversion mutations [15, 16].

In the present study, we aimed to identify genetic polymorphisms in potential candidate genes for RA, and we therefore investigated the association of *OGG1* gene polymorphisms with RA in a Taiwanese population. Our findings are expected to help us understand the role of *OGG1* gene polymorphisms in RA disease and its progression; this knowledge can point us toward possible management strategies for this common nephropathy.

Materials and Methods

Study Population

The study subjects including a total of 192 patients with RA and 192 healthy subjects were recruited from China Medical University Hospital in Taiwan. Patients with RA according to the revised America College of Rheumatology criteria [17] were enrolled. Nephelometry was used to detect rheumatoid factor (RF). Values ≧ 30 IU/ml were defined as positive. The presence or history of extra-articular manifestations in patients with RA was recorded [18]. The healthy control from the general population was selected from health examination. All individuals' samples were collected by venipuncture for genomic DNA isolation. Informed consent was from all participants and was approved by the local Ethics Committee.

Genomic DNA Extraction and Genotyping

Genomic DNA was prepared from peripheral blood according to standard protocols of the DNA extraction kit (Qiagen, Valencia, CA, USA). The two *OGG1* polymorphisms (rs159153 and rs3219008) were detected by restriction enzyme (RE) digestion. PCRs for *OGG1* gene polymorphisms were carried out in a 50-μL reaction mixture containing 50 ng of genomic DNA, 2 to 6 pmole of each primers, 1× Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 U of AmpliTaq DNA polymerase (Applied Biosystems). The primers, PCR conditions, and RE cutting sites used to determine *OGG1* polymorphisms were listed in Table 1.

Statistical Analysis

Chi-square test or Fisher's exact tests will be used to determine statistically significant differences in allele/genotype frequencies between case and control groups. Allelic frequencies will be expressed as percentage of the total number of alleles. The Hardy–Weinberg equilibrium will be tested for each marker using χ^2-test. The haplotype combination at rs159153 and rs3219008 in *OGG1* gene was estimated using Haploview version 4.1 [19]. The differences in the distribution of the haplotype frequencies between the two groups were assessed with a χ^2-test. Odds ratios [ORs] and 95% confidence intervals (95% CIs) were obtained using logistic regressions to determine associations between *OGG1* alleles/genotypes/haplotypes and RA susceptibility. The Kaplan-Meier method was used to estimate cumulative survival. Differences in survival were analyzed with the log-rank test. All data were analyzed with SPSS Version 15.0 software (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

The genotypic and allelic frequencies of rs159153 and rs3219008 are shown in Table 2. Genotype distributions were in Hardy-Weinberg equilibrium. We observed the T

allele to be the major one at the rs159153 polymorphism both in RA patients (90.6%; 348/384) and controls (89.6%; 344/384). The A allele was the major one at the rs3219008 polymorphism in RA patients (55.7%; 214/384) and controls (51.8%; 199/384). When we compared the genotype distribution between RA patients and control groups, statistically significant differences in genotype frequency distributions were noted for the rs3219008 SNP in RA patients and controls (p = 5.6E-05). Our data indicated that individuals with the AG genotype at rs3219008 SNP may have a higher risk of developing RA (Table 2).

Haplotype frequencies were estimated using the rs159153 and rs3219008 SNPs. Three haplotypes of the *OGG1* were present in the study population. The TG and TA were the common haplotypes both in RA patients (42.9% and 47.7%, respectively) and health control (47.4% and 42.2%, respectively) groups. Subsequently, we constructed haplotypes and considered haplotype with wild-type alleles of the two polymorphisms as reference (TG) and analyzed the risk of RA in individuals with other haplotypes. The trend of frequency distribution of three haplotypes (TG, TA and CA) obtained in control individuals. Regression analysis revealed that there was no association with combined effect of these two polymorphisms with RA risk (Fig. 1).

Comparisons of the clinical features of RA patients with the different genotypes were shown in Table 3. There were no differences in gender distribution,

rheumatoid factor accompaniment, and incidence of extra-articular. We observed the percentage of bone erosion occurrence in RA patients with the AA genotype at rs3219008 SNP was much higher than RA patients with non-AA genotype (p = 0.008). Briefly, our data indicated that individuals with the AA genotype at rs3219008 SNP may have a higher risk of developing RA (Table 3).

Discussion

Currently, RA is considered to be a chronic inflammatory disease with tissue-destructive potential that occurs in genetically susceptible individuals. Polymorphic gene sequences of cytokines known to be involved in the pathogenesis of RA are potential markers of disease susceptibility. Previous studies have examined the relationship between cytokine gene polymorphisms and the incidence of RA, including urokinase, IL-6, IL-8, CD4 cells [20-22]. Moreover, One effect involves a "snow-balling" mechanism of increased levels of cellular damage and death leading to more inflammation, which in turn produces more ROS [23]. The question remains whether the defect in DNA damage repair functions to render individuals susceptible to RA or is a consequence of disease. Accumulation of DNA damage may also have broader implications on impairing diverse cellular functions.

In this study, we focused on the variants of the *OGG1* gene that had previously been investigated for breast cancer, bladder cancer, and Alzheimer's disease [24-26]. We found a statistically significant association between RA and the rs3219008 polymorphism. The AG genotype frequency at rs3219008 was significantly higher in RA than in the control participants (Table 1). Our results also indicated that the T-A haplotype of the *OGG1* gene was estimated to be present in approximately 47.7% of RA patients. We observed that the T-A haplotype seems appeared to be an “at-risk” haplotype for RA progression, although the difference was not statistically significant (Fig. 1).

The treatment strategies for RA patients are also an important issue. We observed the percentage of bone erosion occurrence in RA patients with the AA genotype at rs3219008 SNP was much higher than RA patients with non-AA genotype (p = 0.008). Despite the similar mode of treatment given to our patients, greater rheumatoid factor accompaniment and incidence of extra-articular were observed in the AA subgroup than in the subgroups with non-AA genotype at rs3219008, although the difference was not statistically significant (Table 3). These data suggest that a dose readjustment in the drugs given may be required according to the different genotype. In addition, more specific drugs that interact with *OGG1* could be given in addition to regular DNA repair regimens, especially in patients with the AA genotype

at rs3219008.

In conclusion, our findings strongly suggest an association between *OGG1* (rs3219008) genetic variants and RA disease susceptibility; further, we indicated that these polymorphisms contribute to the genetic background of RA pathogenesis. Moreover, the absence of the AA genotype at rs3219008 is associated with the bone erosion occurrence in RA patients. The findings should prompt specific considerations for the treatment of RA patients with the AA genotype at rs3219008.

Acknowledgements

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Table 1 Characteristics of the *OGG1* genetic polymorphisms and PCR condition for genotyping analysis.

Gene	SNPs	rs number	Position	Alleles	Primers	PCR product size (bp)	PCR conditions (annealing temperature)	Restriction enzyme	DNA fragment size
OGG1	OGG1 C-285T	rs159153	3:9729875	C/T	Forward:5'-AGGGCAAAGGGGATACAAAG-3 Reverse:5'-CTGGTTGAAGAGCCAGGTTT-3'	347	57°C	DdeI	C：154+113+80 T：267+80
	OGG1 A5954G	rs3219008	3:9735543	A/G	Forward:5'-ATTCACCTCTTCCGGCTTCT-3' Reverse:5'-CCACCTCAGCCTCCTGAGTA-3'	320	60°C	MspI	A: 12+308 G: 12+119+189

Table 2 Genotypic and allelic frequencies of *OGG1* genetic polymorphisms in the patients with RA and controls.

dbSNP ID		Patients with RA (N=192)	Controls (N=192)	*p* value
rs159153				
	Genotype			
	TT	158 (82.3)	153 (79.7)	0.629[a]
	TC	32 (16.7)	38 (19.8)	
	CC	2 (1.0)	1 (0.5)	
	CC + TC	34 (17.7)	39 (20.3)	
	Allele frequency			
	T	348 (90.6)	344 (89.6)	0.717
	C	36 (9.4)	40 (10.4)	
rs3219008				
	Genotype			
	AA	22 (11.5)	25 (13.0)	5.6E-05[a]
	AG	170 (88.5)	149 (77.6)	
	GG	0 (0)	18 (9.4)	
	GG + AG	170 (88.5)	167 (87.0)	
	Allele frequency			
	A	214 (55.7)	199 (51.8)	0.311
	G	170 (44.3)	185 (48.2)	

[a]Genotype distribution between patients and control were calculated by 2 x 3 chi-square test

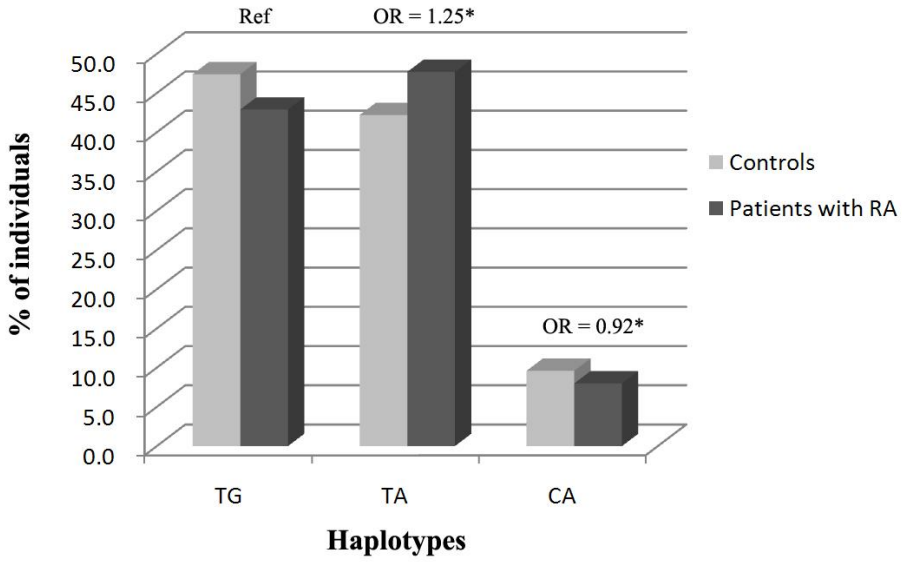

Fig. 1 Association of *OGG1* gene haplotypes with rheumatoid arthritis risk. *$p > 0.05$

Table 3 Association between clinical feature and genotype distribution of OGG-1 polymorphisms in the RA patients.

	Patient with RA					
	rs159153			rs3219008		
Clinical Parameters	**TT (n=158)**	**non TT (n=34)**	***p* value**	**AA (n=22)**	**non AA (n=170)**	***p* value**
Gender						
male	35 (22.2)	6 (17.6)	0.561	2 (9.1)	39 (22.9)	0.173
female	123 (77.8)	28 (82.4)		20 (90.9)	131 (77.1)	
Rheumatoid factor						
with	115 (72.8)	26 (76.5)	0.659	18 (81.8)	123 (72.4)	0.344
without	43 (27.2)	8 (23.5)		4 (18.2)	47 (27.6)	
Extra-articular						
with	72 (45.6)	19 (55.9)	0.275	13 (59.1)	78 (45.9)	0.243
without	86 (54.4)	15 (44.1)		9 (40.9)	92 (54.1)	
Bone Erosion						
with	80 (50.6)	17 (50.0)	0.947	17 (77.3)	80 (47.1)	0.008[a]
without	78 (49.4)	17 (50.0)		5 (22.7)	90 (52.9)	

[a]With significant differences and $p<0.05$

Dear Editor,

Attacheded please find a copy of our manuscript entitled, "**Association of the C-285T and A5954G Polymorphisms in the DNA repair gene *OGG1* with the Susceptibility of Rheumatoid Arthritis.**" to be considered for publication in *Rheumatology International.*

This manuscript comprised a study of the human Rheumatoid Arthritis (RA). We tested a hypothesis that the *OGG1* genetic polymorphism confers RA susceptibility. Study participants were Taiwanese RA patients and a healthy control group. Our data indicated that *OGG1* genetic polymorphism (rs3219008) contributes to the susceptibility of RA.

"None of the authors has any potential financial conflict of interest related to this manuscript."

Thank you for your time and consideration. We look forward to hearing from you in due time. Should you have any questions, please feel free to contact me.

Very truly yours,

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