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ORIGINAL ARTICLE

Genetic polymorphisms of the DNA repair gene *UNG* are associated with the susceptibility of rheumatoid arthritis

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Abstract The involvement of uracil-DNA glycosylase (UNG) in the pathogenesis of cancer is well documented. In contrast, the role of this protein in rheumatoid arthritis (RA) development is not well defined, although previous studies suggest a possible link between autoimmune diseases and malignancy. Therefore, we aimed to examine whether there is a link between UNG genetic polymorphisms and RA. Our present study investigated the effects of UNG (*rs3219218* and *rs246079*) single nucleotide polymorphisms (SNPs) on RA among Taiwan's Han Chinese population. Polymorphism of the UNG gene was analyzed in 192 controls and 183 RA patients. Genotyping for UNG SNPs was performed by restriction fragment length polymorphism assay. Our data confirmed statistically significant variations in genotype frequency distributions at *rs246079* SNP between RA patients and controls ($P = 3.05 \times 10^{-4}$). The G allele at *rs246079* SNP is a high-risk factor in developing RA (odds ratio [OR] = 1.77; 95% confidence interval [CI] = 1.290–2.42). A comparison of haplotype frequencies between the case and the control revealed that RA patients with the *Ht2* haplotype are at additional risk for RA development ($P = 0.042$). Our data yielded new information on UNG polymorphisms associated with RA development and as RA molecular markers. The polymorphisms revealed by the present study merit further investigation.

Keywords Uracil-DNA glycosylase · Gene polymorphism · Rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic disease characterized by joint inflammation along with multiple organ system involvement [1, 2]. Broad regulatory abnormalities of the immune system in RA have been suggested [2, 3]. Increased risk of certain cancers, particularly hematologic cancers, has been found in autoimmune diseases such as RA and systemic lupus erythematosus compared with the general population [4–6]. The exact etiology of these associations, however, is unknown. DNA damage [7] and deficiencies of the DNA repair system [8] have been implicated in the development of autoimmune disorder and cancer. The deficiency of the DNA repair enzyme in RA [9] and association of autoantibodies with DNA repair proteins in connective tissue diseases [10] were previously reported. In human, approximately 150 DNA repair genes involving several repair pathways, including base excision repair (BER), nucleotide excision repair (NER), mismatch repair, and double-strand break repair, have been reported [11]. Among these repair enzymes, BER was found to be associated with cancer risk [12, 13] and immunological abnormalities [14, 15]. There are several BER proteins dedicated to the recognition and removal of specific forms of damage. Among them, the uracil-DNA glycosylase (UNG) excise misincorporated uracil from DNA, preventing the formation of U/G mispairs, which may result in C/G to T/A transition mutations on DNA replication [16]. Excision of deaminated cytosine by the action of UNG initiates BER to prevent this promutagenic event. Ung-deficient mice were found to have an increased incidence of B-cell lymphoma [17], as well as aberrant somatic hypermutation (SHM) and class-switch recombination (CSR) in B cells [18]. The recent success of the B-cell depletion therapy with rituximab [19] brings back the important role of B cells in the pathogenesis of RA, whereas previous studies focused on T cells in the development of RA [3, 20]. Considering these findings concerning the importance of UNG in the pathogenesis of autoimmune diseases, we hypothesized that UNG gene polymorphism will confer the risk of RA development. To verify our hypothesis, we investigated the effects of UNG (*rs3219218* and *rs246079*) single nucleotide polymorphisms (SNPs) on RA among Taiwan's Han Chinese population.

Materials and methods

Patient selection

The study subjects included 183 RA patients and 192 healthy subjects, recruited from China Medical University Hospital in Taiwan. Patients with RA according to the revised America College of Rheumatology criteria [21] were enrolled. Nephelometry detected rheumatoid factor (RF), values ≧30 IU/ml defined as positive. Presence or history of extra-articular manifestations in RA patients was recorded [22]. The healthy controls from the general population were selected by health examination. All blood samples were collected by venipuncture for genomic DNA isolation, with informed consent obtained from participants and approved by the local Ethics Committee.

Polymerase chain reaction

Polymerase chain reaction (PCR) was used to identify the UNG polymorphisms including rs3219218 and rs246079. Polymerase chain reaction was carried out in a total volume of 50 μl, containing genomic DNA 50 ng, 2–6 pmol of each primer, 1× Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.5 units of AmpliTaq DNA polymerase (Perkin Elmer; Foster City, CA, USA). In the study of the UNG rs3219218 SNP, the primers used were upstream 5′-TTA GCACCTGCTGACACTGG-3′ and downstream 5′-GGTT TTTGAGCATTCCCTCA-3′. For the UNG rs246079 SNP, the primers used were upstream 5′-CCAAGATGTTAACC CCATCC-3′ and downstream 5′-TTAAGACCCTGTGC GATTCC-3′. Polymerase chain reaction amplification was performed in a programmable PCR thermal cycler (GeneAmp PCR System 2400, Perkin Elmer). The PCR cycling conditions for UNG rs246079 SNP examination were as follows: one cycle at 95°C for 5 min; 35 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 45 s; one final cycle of extension at 72°C for 7 min, then holding at 25°C. The UNG rs246079 SNP was analyzed by PCR amplification followed by restriction enzyme analysis with AciI. Two fragments of 109 and 198 bp were present if the product was excised (AA homozygote). The uncut band showed up as a 307-bp length on the gel. The reaction was then incubated for overnight at 37°C, and then 10 μl of the products was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The UNG rs246079 SNP was categorized as excisable (AA homozygote), non-excisable (GG homozygote), and AG heterozygote. The PCR cycling conditions for UNG rs3219218 SNP examination were as follows: one cycle at 95°C for 5 min; 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s; one final cycle of extension at 72°C for 7 min, then holding at 25°C. The UNG rs3219218 SNP was analyzed by PCR amplification followed by restriction enzyme analysis with MnlI. Three fragments of 55, 269 and 25 bp were present if the product was excised (AA homozygote). The uncut band showed up as four fragments of 55-, 48-, 221-, and 25-bp length on the gel. The reaction was then incubated for overnight at 37°C, and then 10 μl of the products was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The UNG rs3219218 SNP was

categorized as excisable (AA homozygote), non-excisable (GG homozygote), and AG heterozygote.

Statistical analysis

The genotypic and allelic frequencies of UNG SNPs (rs3219218 and rs246079) for the RA patients and controls were compared using the chi-square test. Among the RA patients, genotype groups with different clinical variables were also compared using chi-square test. When one cell had an expected count of <1, or >20% of the cells had an expected count of <5, Fisher's exact test was used. Results were considered statistically significant when P values are <0.05. The odds ratios (OR) were calculated from the genotypic frequency and allelic frequency with a 95% confidence interval (95% CI) for the UNG SNPs (rs3219218 and rs246079). The statistical analysis was performed by using the SPSS version 11.

Results

Table 1 depicts the genotypic and allelic frequencies of *rs3219218* and *rs246079*. Genotype distributions were in Hardy–Weinberg equilibrium. We observed A allele as the major form at *rs3219218* polymorphism, both in RA patients (74.6%; 273/366) and controls (78.6%; 302/384), and A allele as the major form at *rs246079* polymorphism in patients (63.9%; 234/366) and controls (75.8%; 291/384). A comparison of allele and genotype distribution yielded significant variations for the *rs246079* SNP in RA patients and controls ($P = 4.02 \times 10^{-4}$ and 3.05×10^{-4}, respectively). Data indicated that individuals with G allele or G carrier (GG + AG) genotype at *rs246079* SNP were at higher risk for RA.

Haplotype frequencies were estimated via *rs3219218* and *rs246079* SNPs. Four haplotypes of UNG emerged in the study population, wherein *Ht1 (AA)* was the most common form in both RA patients (42.9%) and healthy controls (56.2%). Significant differences of *Ht1* and *Ht2* haplotype frequencies between RA patients and controls were observed when the overall distribution of the haplotype frequencies between RA patients and healthy controls were compared, and such differences remain significant for *Ht1* haplotype after Bonferroni correction (Table 2). Our data showed that the *Ht1* haplotype was a significant protective haplotype compared with the other haplotypes (OR: 0.59, 95% CI: 0.39–0.89; $P = 0.011$; $P_{\text{corrected}} = 0.023$). Moreover, the *Ht2* haplotype may play a risky role for RA development ($P = 0.042$, $P_{\text{corrected}} = 0.085$). Comparisons of the clinical features of RA patients with the various genotypes and haplotypes were also performed (data not shown). However, there were no significant variations in the rheumatoid factor accompaniment, incidence of extra-articular manifestations, and bone erosion occurrence with respect to the various genotypes and haplotypes.

Table 1 Genotypic and allelic frequencies of UNG genetic polymorphisms in the patients with RA and controls

dbSNP ID	Patients with RA $N = 183$ (%)	Controls $N = 192$ (%)	P value	OR (95% CI)
rs3219218				
Genotype				
AA	99 (54.1)	117 (60.9)	1.00 (ref)	
AG	75 (41.0)	68 (35.4)	–	
GG	9 (4.9)	7 (3.7)	–	
AG + GG	84 (45.9)	75 (39.1)	0.180	1.32 (0.88–2.0)
Allelic frequency				
Allele A	273 (74.6)	302 (78.6)	1.00 (ref)	
Allele G	93 (25.4)	82 (21.4)	0.189	1.25 (0.89–1.76)
rs246079				
Genotype				
AA	67 (36.6)	106 (55.2)	1.00 (ref)	
AG	100 (54.6)	79 (41.1)	–	
GG	16 (8.7)	7 (3.6)	–	
AG + GG	116 (63.4)	86 (44.8)	3.05E−04	2.13 (1.41–3.23)
Allelic frequency				
Allele A	234 (63.9)	291 (75.8)	1.00 (ref)	
Allele G	132 (36.1)	93 (24.2)	4.02E−04	1.77 (1.29–2.42)

CI confidence interval, *OR* odds ratio

Table 2 Distribution of UNG haplotype frequencies in the patients with RA and controls

Haplotype	rs3219218	rs246079	Patient with RA (%) ($N = 183$)	Control (%) ($N = 192$)	OR (95% CI)	P value	$P_{corrected}$ value*
Ht1	A	A	42.9	56.2	0.59 (0.39–0.89)	0.011	0.023
Ht2	A	G	31.7	22.5	1.61 (1.01–2.55)	0.042	0.085
Ht3	G	A	21.0	19.6	1.06 (0.64–1.76)	0.815	1.629
Ht4	G	G	4.4	1.7	2.88 (0.75–11.03)	0.107	0.214

* Data with Bonferroni correction

Discussion

A great variety of DNA-damaging agents cause genome instability, which would be an overwhelming problem for cells and organisms if the damaged DNA are not repaired [23]. Such DNA-damaging agents [7] and deficiencies of the DNA repair system [8] have been implicated in the development of autoimmune disorder and cancer. Although the DNA repair enzymes are generally perceived to maintain the integrity and stability of the whole genome by correctly repairing mutagenic DNA intermediates, they can also induce mutations to generate antibodies through SHM and CSR [24]. SHM of immunoglobulin variable genes is a process used to introduce a large number of point mutations into a small region of the genome to produce antibodies with increasing affinity for various antigens [25]. CSR is a process involving the recombination of the variable region of immunoglobulin genes with the various constant region genes to produce various antibody isotypes with identical antigen specificity, but distinct effector functions [26]. Both processes occur by a shared pathway induced by targeted DNA deamination by a B-cell-specific factor, and such activation-induced cytidine deaminase is only expressed in antigen-activated germinal center B cells [27]. Although BER proteins play an important role in antibody diversification, not all of them act similarly during the process. Given that UNG has a distinguished important role in SHM and CSR [18, 28], UNG gene polymorphism may possibly affect antibody diversification and subsequent immunological function to confer the risk of RA development. Our present study supports such hypothesis by determining significant genotypic and allelic variations for the UNG SNP polymorphism at *rs246079* between RA patients and controls ($P = 4.02 \times 10^{-4}$ and 3.05×10^{-4}, respectively). Our data indicated that individuals with G allele or G carrier (GG + AG) genotype at *rs246079* SNP are at higher risk for RA. Further comparison of haplotype distribution between patients and control subjects found that *Ht1*(*AA*) is a significant protective haplotype, whereas *Ht2*(*AG*) may be a risky haplotype for RA. These associations of the UNG gene polymorphism with RA development are not related to the clinical and biochemical manifestations, considering that the comparison of these variables with respect to the UNG genotypes or haplotype distributions yielded no significant variations.

Our present study is the first to determine that genetic polymorphisms of the UNG gene are associated with the susceptibility of RA. The present study also established that SNP at an intron region confers the risk of RA, which qualifies the present work as a novel research in the study of SNP polymorphism in RA, in contrast to the previous studies concerning promoter, coding region, or 3′UTR polymorphisms. Although the exact function of the UNG SNP polymorphism at *rs246079* cannot be illustrated in our present study, it is possible that it is in linkage disequilibrium with other functional variants and serves as a genetic marker of susceptibility. Another possibility is that this UNG SNP polymorphism at *rs246079* influences splicing and regulation, and affects UNG expression. In conclusion, our data yield new information on UNG polymorphisms (*rs3219218* and *rs246079*), which are associated with RA development and as RA molecular markers. The polymorphisms revealed by the present study merit further investigation.

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