

p53 Codon 72 Polymorphism and Rheumatoid Arthritis

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ABSTRACT. Objective. To investigate whether the p53 codon 72 polymorphism is associated with susceptibility to rheumatoid arthritis (RA) and its clinical features.

Methods. A polymerase chain reaction of genomic DNA-restriction fragment length polymorphism was used to determine genotypes of the p53 codon 72 in 114 patients with RA and 114 healthy controls. Clinical/serological manifestations were analyzed in each patient and correlated with the genotypes.

Results. The genotype distribution of the p53 codon 72 did not differ between patients with RA and controls (Arg/Arg, Arg/Pro, Pro/Pro genotypes 38, 58, 18 vs 37, 60, 17 controls, respectively; chi-square = 0.08, 2 df, $p = 0.96$). No significant difference was found in allele frequencies between the groups. Clinically there was no significant difference in age at onset, functional class, physician's global assessment, ESR, CRP, RF titer, extraarticular and cervical spine involvement, frequencies of joint operation, and admission in RA patients according to the p53 codon 72 genotypes. However, the number of patients within each group was extremely small, for example only 5 patients with cervical spine involvement. No firm conclusions could safely be reached about clinical manifestations from this study.

Conclusion. No association was found between the p53 codon 72 polymorphism and RA. Studies are needed to clarify the role of the p53 polymorphism in the pathogenesis of RA. (J Rheumatol 2001;28:2392-4)

Key Indexing Terms:
p53 POLYMORPHISM

RHEUMATOID ARTHRITIS

The p53 tumor suppressor gene plays a central role in cell proliferation and death¹. The 2 common polymorphic variants of the wild-type p53 have been identified². This polymorphism arises from a single base-pair substitution at codon 72, where either CCC encodes proline (Pro) or CGC encodes arginine (Arg). Studies have shown that 2 polymorphic variants of wild-type p53 differ biochemically and biologically³ and are associated with several kinds of cancers^{4,5}.

Rheumatoid arthritis (RA) is an autoimmune disease of unknown cause, and RA synovium resembles properties of malignant tumors⁶. Recent studies show that the p53 tumor suppressor protein is overexpressed and that somatic mutations previously identified in human tumors are present in RA synovium⁷⁻¹⁰.

Studies implicate p53 dysfunction in the pathogenesis of RA, but no study has been reported concerning the associa-

tion of p53 codon 72 polymorphism with RA. We investigated whether the p53 codon 72 polymorphism is associated with susceptibility to RA and its clinical features.

MATERIALS AND METHODS

Patients and controls. One hundred fourteen Korean patients with RA (93 women, 21 men, mean age 45 yrs, range 16-75 yrs) and 114 ethnically matched healthy controls (92 women, 22 men, mean age 46 yrs, range 20-76 yrs) were enrolled in this study. All patients were recruited from the Rheumatology Clinic of the Korea University Hospital and all fulfilled the classification criteria of the American College of Rheumatology (ACR) for RA¹¹. The control group consisted of healthy individuals with no autoimmune disease. Clinical manifestations were determined in each patient: age at onset, functional status class according to the ACR criteria¹², physician global assessment (PGA), erythrocyte sedimentation rate (ESR, Westergren), C-reactive protein (CRP), rheumatoid factor (RF), history of total joint replacement (TJR) surgery, history of hospitalization for RA for reasons other than TJR, extraarticular manifestations such as nodule, vasculitis, eye and lung involvement, and cervical spine involvement. The PGA was the physician's subjective opinion of disease severity on a visual analog scale of 0 (not severe) to 10 (most severe). CRP and RF were measured by nephelometry.

DNA preparation. Blood samples from all subjects were obtained for DNA extraction. Blood was collected in EDTA tubes and DNA was extracted using the method of proteinase K treatment and phenol/chloroform extraction.

Polymorphism typing of the p53 codon 72. The polymorphism at codon 72 of the p53 was defined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was carried out using a forward primer 5'-ATCTACAGTCCCCCTTGCCG-3' and a reverse primer 5-GCAACTGACCGTGCAAGTC-3'. The following conditions were applied: initial denaturation for 4 min at 95°C, followed by 25 cycles (60 s 95°C, 60 s 60°C, 60 s 72°C), and a final extension of 4 min at 72°C. The

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Supported by Korea University Medical Science Research Center.

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Submitted October 16, 2000; revision accepted May 15, 2001.

Arg coded allele has a single AccII site in the amplified fragment. PCR products were further subjected to RFLP analysis with the enzyme AccII(CGCG) and separated on a 2% agarose gel. The fragment of homozygote of Pro gave only a single undigested band at 296 bp, and the fragment of homozygote of Arg gave bands of digested DNA at 169 and 127 bp, while the fragment of heterozygote gave 3 bands at 296, 169, and 127 bp (Figure 1).

Statistical analysis. The genotype and allele frequencies in RA patients were compared to those in controls using the chi-square test. An association of clinical features with each genotype was analyzed by chi-square or one way ANOVA test. A p value < 0.05 was considered significant.

RESULTS

P53 codon 72 polymorphism in patients with RA and controls. Samples from 114 patients with RA and 114 controls were successfully genotyped for the p53 codon 72 polymorphism. Figure 2 shows representative results of the p53 codon 72 genotypes from patients and controls. The genotypes of the p53 codon 72 polymorphism in RA patients and controls did not deviate from the Hardy-Weinberg predictions.

The genotype distribution of the p53 codon 72 did not differ between RA patients and controls (Arg/Arg, Arg/Pro, Pro/Pro genotypes 38, 58, 18 vs 37, 60, 17 controls, respectively; chi-square = 0.08, 2 df, p = 0.96). No significant difference was found in allele frequencies between the groups (Table 1).

Clinical analysis of patients with RA based on the p53 codon 72 genotypes. Clinically there was no significant difference in age at onset, functional class, PGA, ESR, CRP, RF titer, extraarticular and cervical spine involvement, frequencies of joint operation, and admission in RA patients according to the p53 codon 72 genotypes (Table 2).

DISCUSSION

RA is a chronic inflammatory disease, characterized by hyperplasia of the synovial lining cells, excessive infiltration of mononuclear cells, and extensive destruction of the

Table 1. P53 codon polymorphism in patients with RA and controls.

	RA n=114 (%)	Controls n=114 (%)
Genotype frequencies*		
Arg/Arg	38 (33)	37 (32)
Arg/Pro	58 (51)	60 (53)
Pro/Pro	18 (16)	17 (15)
Allele frequencies**		
Arg	134 (59)	134 (59)
Pro	94 (41)	94 (41)

* Chi-square test of heterogeneity between RA and controls. Chi-square = 0.08, 2 degrees of freedom; p=0.96.

** Chi-square test of heterogeneity between RA and controls. Chi-square = 0.01, 1 degree of freedom; p=0.92.

articular cartilage. RA synovium shows aggressive invasiveness of rheumatoid pannus, occurrence of newly formed blood vessels, and pleomorphic fibroblast-like cells with large nuclei and prominent nucleoli¹³. These features resemble those of preneoplastic conditions.

Because of its role as a tumor suppressor gene, the p53 gene is one of the most intensely studied human genes, and mutations of the p53 gene are the most common genetic alteration in human cancer.

Besides studies on mutations of the p53 gene, several studies have shown that common p53 polymorphisms are associated with some cancers, including human papillomavirus associated cancer⁴ and lung cancer⁵, with respect to susceptibility or prognosis.

There have been increasing data that implicate p53 dysfunction with RA⁷⁻¹⁰, but the role of the p53 gene in RA is not clearly understood^{14,15} and there has been no report on the association of the p53 polymorphism with RA. To our knowledge, this is the first study to investigate an association between the p53 codon 72 polymorphism and RA.

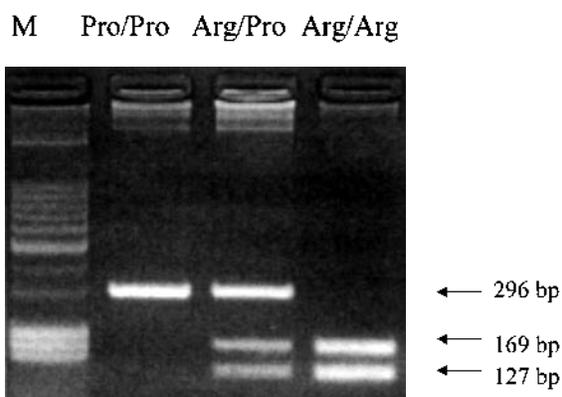


Figure 1. p53 codon 72 genotypes by PCR-RFLP. The genotypes Pro/Pro (296 bp), Arg/Pro (296, 169, 127 bp), and Arg/Arg (169, 127 bp) are shown. M: DNA size marker.

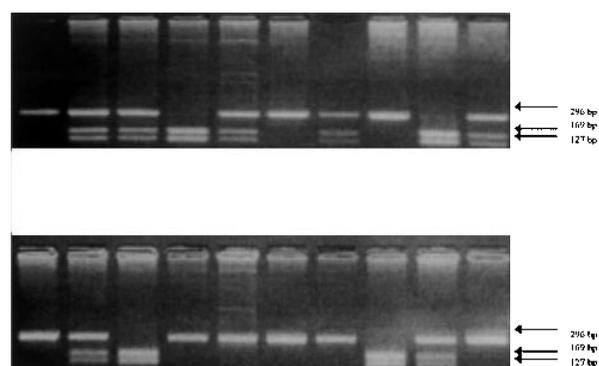


Figure 2. Representative results of p53 codon 72 genotypes from 10 patients with RA (A) and 10 controls (B).

Table 2. Clinical analysis of patients with RA according to p53 codon 72 polymorphism.

	P53 genotypes			Chi-square or F	
	Arg/Arg, n = 38	Arg/Pro, n = 58	Pro/Pro, n = 18		
Age at onset (yrs)	45.9 ± 14	45.7 ± 12	44.9 ± 13	0.033	0.967
Extraarticular involvement, n	8	6	2	2.647	0.266
Admission, n	4	7	5	3.929	0.140
Joint operation, n	4	2	3	4.373	0.112
Cervical spine involvement, n	1	2	2	2.616	0.270
Functional class (≥ 3), %	35.1	28.3	35.3	7.346	0.119
Physician Global Assessment	6.6 ± 1.5	6.4 ± 1.3	6.4 ± 1.9	0.224	0.800
ESR mm/h	40.7 ± 28	44.0 ± 25	35.1 ± 23	0.801	0.452
CRP, U/ml	39.9 ± 32	45.2 ± 68	29.5 ± 16	0.378	0.686
RF, IU/ml	410 ± 783	291 ± 497	134 ± 122	1.097	0.338

Data are mean ± SD

Our study showed that the p53 polymorphism did not confer susceptibility to RA and was not associated with its clinical/serological manifestations considered for this study. Extraarticular involvement and RF were about twice as high in homozygous Arg patients as in homozygous Pro patients. Some of the clinical manifestations appear to be affected based on the p53 codon 72 genotypes, but they did not reach statistical significance. However, the standard deviations shown for ESR, CRP, and RF seemed excessive and the number of patients within each group was extremely small, for example only 5 patients with cervical spine involvement. No firm conclusions could be reached about clinical manifestations from this study and geographical and ethnic differences in the distribution of p53 polymorphism. Therefore, we cannot rule out the possibility that the p53 codon 72 polymorphism might be associated with RA in other ethnic populations.

Our data showed no association between the p53 codon 72 polymorphism and RA. Further studies are needed to clarify the role of the p53 polymorphism in the pathogenesis of RA.

REFERENCES

1. Donehower LA, Bradley A. The tumor suppressor p53. *Biochim Biophys Acta* 1993;1155:181-205.
2. Matlashewski G, Tuck S, Pim D, Lamb P, Schneider J, Crawford L. Primary structure polymorphism at amino acid residue 72 of human p53. *Mol Cell Biol* 1987;7:961-3.
3. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* 1999;19:1092-9.
4. Storey A, Thomas M, Kalita A, et al. Role of a p53 polymorphism in the development of human papilloma-virus-associated cancer. *Nature* 1998;393:229-34.
5. Wang YC, Chen CY, Chen SK, Chang YY, Lin P. p53 codon 72 polymorphism in Taiwanese lung cancer patients: Association with lung cancer susceptibility and prognosis. *Clin Cancer Res* 1999;5:129-34.
6. Hamilton JA. Hypothesis: in vitro evidence for the invasive and tumor-like properties of the rheumatoid pannus. *J Rheumatol* 1983;10:845-51.
7. Firestein GS, Echeverri F, Yeo M, et al. Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. *Proc Natl Acad Sci USA* 1997;94:10895-900.
8. Firestein GS, Nguyen K, Aupperle KR, et al. Apoptosis in rheumatoid arthritis: p53 overexpression in rheumatoid arthritis synovium. *Am J Pathol* 1997;149:2143-51.
9. Tak PP, Smeets TJ, Boyle DL, et al. p53 overexpression in synovial tissue from patients with early and longstanding rheumatoid arthritis compared with patients with reactive arthritis and osteoarthritis. *Arthritis Rheum* 1999;42:948-53.
10. Reme T, Travaglio A, Gueydon E, Adla L, Jorgensen C, Sany J. Mutations of the p53 tumour suppressor gene in erosive rheumatoid synovial tissue. *Clin Exp Immunol* 1998;111:353-8.
11. Arnett FC, Edworthy SM, Kaplan SR, et al. The American Rheumatism Association 1987 revised criteria for classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
12. Hochberg MC, Chang RW, Dwosh I, et al. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. *Arthritis Rheum* 1992;35:498-502.
13. Bromley M, Woolley DE. Histopathology of the rheumatoid lesion: Identification of cell types at sites of cartilage erosion. *Arthritis Rheum* 1984;27:857-63.
14. Kitasato H, Okamoto R, Kawai S. Absence of p53 mutation in Japanese patients with rheumatoid arthritis. *Arthritis Rheum* 2000;42:469-70.
15. Lee YH, Ji JD, Kim A, Kim CH, Song GG. Expression of p53 protein in rheumatoid arthritis synovium. An immunohistochemical analysis. *Korean J Intern Med* 1999;14:59-65.