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Original article

Interleukin-1β gene polymorphism associated with radiographic signs of osteoarthritis of the knee

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Abstract

Background. Osteoarthritis is recognized as a noninflammatory, progressive condition, the principal cause of which is regressive changes associated with aging and which pursues a chronic course. Recently, the involvement of genetic factors has been widely reported. The purpose of this study was to identify polymorphisms at particular risk of osteoarthritis of the knee for community-living middle-aged and elderly people.

Methods. Focusing on 359 participants (ages 44–86 years) of the comprehensive health examination program (CHEP), we investigated the presence/absence of radiographic osteoarthritis (ROA) of the knee, and 11 types of gene polymorphisms and their association with ROA.

Results. Interleukin-1β (IL1B) T-31C polymorphism was found to be associated with ROA. In the case of IL1B T-31C polymorphism in the ROA group, a significant difference was found between the groups combining the C/C genotype, the C/T genotype, and the T/T genotype. In particular, the genotypes with the C allele differed from the T/T genotype, with the morbidity rate being higher in the T/T group (odds ratio (OR) 2.04, 95% confidence interval (95%CI) 1.05–3.98, P = 0.036).

Conclusion. Our results confirm that in IL1B T-31C with the T/T genotype, the rate of ROA was significantly higher than that with the C/C and C/T genotypes. It might be possible to implement active preventative measures, such as avoidance of obesity and excessive exercise loads, for carriers of IL1B T/T.

Introduction

Osteoarthritis (OA) affects more than 5% of the world's adult population, including more than 7 million Japanese,[1] and is the most common disease causing impaired ambulation. OA is characterized by age-related regressive changes in cartilage, with environmental factors such as obesity, trauma, and heavy labor also considered to play a significant role.[2,3] Although OA is recognized a noninflammatory, progressive condition, the principal cause of which is regressive changes associated with aging and which pursues a chronic course, recently the involvement of genetic factors has been widely reported as well. Hitherto, polymorphisms in the vitamin D receptor gene, the estrogen receptor gene, type II and type IX collagen genes, and the proteoglycan gene, among others, have been described.[1,4–9]

An association between interleukin-1 polymorphism and OA has been reported previously.[10–13] However, genetic polymorphisms affecting OA vary between populations.[14] In this study, we assessed the genetic associations with knee OA in the Japanese population.

Joint function is maintained by achieving a balance between degradation and synthesis of the joint cartilage extracellular matrix, which is made up of type II collagen and proteoglycans. "Proteoglycans" is a general term used to describe molecules in which glycosaminoglycan is covalently bound to protein (core protein). The elasticity of cartilage is maintained by these two elements; stretching intensity is maintained by collagen, and the water content is maintained by glycosaminoglycans. When the destruction of joint cartilage extracellular matrix is accelerated, progression to OA occurs. This implies that the identification of factors that upset the balance between the breakdown and synthesis of joint cartilage extracellular matrix would be important in order to devise measures to prevent and treat OA.

In this study, we determined 11 types of gene polymorphisms in subjects in a comprehensive health examination program (CHEP), and investigated the association between OA and various gene polymorphisms with reference to the results of knee joint radiological studies.

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Patients and methods

The CHEP was in Y-town (population approximately 17000) in Hokkaido, in northern Japan, where a cohort study has been conducted for 23 years, since August 1982. We selected 359 subjects who were participating in the CHEP in 2003, and who underwent both knee joint and gene polymorphism examinations. The gene polymorphism examinations were conducted in accordance with "Ethical Guidelines for Human Genome and Gene Research." An adequate explanation was provided to the subjects beforehand, and their freely given consent was obtained in writing. The test and analysis results were handled in such a way as to guard the anonymity of the subjects.

There were 137 men and 222 women, with a mean age of 67.6 years (range 44–86 years). The knee examination focused on the physical findings and the results of standing X-ray examinations, which were performed according to Rosenberg et al.[15]

The radiological evaluation was based on the Kellgren and Lawrence classification[16,17] according to the presence/absence of a narrowing of the joint space, osteophyte formation, and osteosclerosis, with each knee joint being evaluated from grade 0 to grade 4. A knee was judged to be grade 0 when no findings of osteoarthritis (OA) were evident, grade 1 when slight or unclear osteophyte formation was present, grade 2 when clear bone proliferative changes were noted in the absence of narrowing of the joint space, grade 3 when moderate narrowing of the joint space was present, and grade 4 when more pronounced narrowing of the joint space associated with osteosclerosis of the subchondral bone was found. Grades 2–4 were defined as the "radiographic signs of knee osteoarthritis group" (ROA group) and grades 0–1 as the normal group. The subjects of the screening examinations were divided into a radiographic osteoarthritis (ROA) group and a normal group, and the frequency of each gene polymorphism in each group was determined.

Gene polymorphisms were analyzed from blood samples using the polymerase chain reaction with the confronting two-pair primers method (PCR–CTPP). The PCR–CTPP method was developed by Hamajima et al.[18,19] In contrast to polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), which is used extensively to detect single-nucleotide polymorphisms (SNP) but which, because of its use of restriction enzymes, requires about 3 h for the amplification of DNA by PCR, 3–12 h to allow for the reaction with the restriction enzymes, and about 1 h for electrophoresis, PCR–CTPP relies only on PCR and electrophoresis for SNP gene determination, requires only one extra pair of primers for a total of 4 primers, and requires no special equipment or reagents, thus making it very economical.

Eleven types of gene polymorphism were investigated: alcohol sensitivity-associated polymorphisms of alcohol dehydrogenase (ADH2) Arg47His; aldehyde dehydrogenase (ALDH2) Glu487Lys; smoking sensitivity-associated polymorphisms of glutathione S transferase (GST) M1; GSTT1; NAD(P)H quinone oxidoreductase 1 (NQO1) C609T; inflammation-associated polymorphisms of interleukin-1β (IL1B) T-31C; tumor necrosis factor α (TNFα) T-1031C; endothelial constitutive nitric oxide synthase (ecNOS) Glu298Asp; longevity-associated polymorphism of mitochondrial DNA (mtDNA) 5178 A/C; allergy-associated polymorphism of interleukin-4 (IL-4); immunity-associated polymorphism of CD14.

A statistical analysis of the data was performed using logistic regression analysis, and odds ratios (OR) were calculated with 95% confidence intervals (95%CI). The analysis was performed with the presence/absence of ROA as a dependent variable, and with each gene polymorphism and age, sex, body mass index (BMI), and bone stiffness as independent variables. The statistical software used was StatView Version 5.0 (SAS Institute, Cary, NC, USA).

Results

ROA was found bilaterally in 56 subjects, 8 men and 48 women, with a mean age of 70.7 years. There were 303 subjects in the normal group, 129 men and 174 women, with a mean age of 67.0 years. The mean BMI of both groups combined was 24.3. The mean BMI of the ROA group was 25.9, and that of the normal group was 24.0 (Table 1).

Table 1. Characteristics of the study subjects

Characteristics	Total	Normal	Radiographic signs of osteoarthritis	*P* value
Number	359	303	56	
Age (mean ± SD)	67.6 ± 6.6	67.0 ± 6.4	70.7 ± 6.9	$P = 0.001$
Sex (M/F)	137/222	129/174	8/48	$P < 0.0001$
BMI (mean ± SD)	24.3 ± 3.1	24.0 ± 3.0	25.9 ± 2.9	$P < 0.0001$
Stiffness (mean ± SD)	72 ± 16.6	73.2 ± 16.8	65.2 ± 14.5	$P = 0.0009$

BMI, body mass index; Stiffness, parameter assessed by quantitative ultrasound densitometry (see text)

Table 2. Risk effects for the occurrence of knee ROA among carriers of IL1B alleles

		Genotype (frequency)		Crude OR (95%CI)		Adjusted OR (95%CI)	
	No.	C/C, T/C	T/T	T/T	*P*	T/T	*P*
IL1B							
ROA group	56	31 (0.55)	25 (0.45)	2.45 (1.36–4.41)	0.0028	2.04 (1.05–3.98)	0.036

ROA, radiographic signs of osteoarthritis of the knee; OR, odds ratio; 95%OR, 95% confidence interval

In the bilateral ROA group, a significant difference in IL1B (T-31C) was found between the group combining the C/C genotype and the C/T genotype (namely the genotype including the C allele) (31 subjects, 55.4%) and the group containing the T/T genotype only (25 subjects, 44.6%). After adjusting for age, sex, BMI, and bone stiffness, the odds ratio for T/T was 2.04 (95%CI 1.05–3.98, $P = 0.036$). No statistically significant differences were detected in any of the other gene polymorphisms (Table 2).

Discussion

Hitherto, a number of reports have focused on the association between osteoarthritis (OA) and various gene polymorphisms.[10–13] In cartilage cells, the synthesis of matrix components is known to be decreased, and that of matrix metalloproteinases (MMPs) to be increased, by IL1B.[10] MMPs degrade cartilage extracellular matrix. Due to the presence of inflammatory cytokines, the metabolic balance of normal cartilage repair tends to tilt to substrate degradation, resulting in eventual progression to OA. Moos et al.[12] divided TNFα phenotypes into subgroups (TNFα high and TNFα low) for analysis. They noted that while both the TNFα and IL1B pathways are involved in the regressive changes occurring in cartilage, IL1B was the more significant cytokine regulating the balance between cartilage extracellular matrix synthesis and degradation. Since IL1B is a polymorphism involved in inflammation, which in turn plays an important role in the development and progression of OA, IL1B polymorphism is thought to be implicated in the development of OA.

Genetic associations between genes have been reported in some studies.[20,21] There are several genes (such as ADAM12, ESR1) which are associated with OA, and a higher OR was suggested as compared with the current study. As for these genes, their association with OA may be stronger than that of IL1B.[21]

IL1B is a 17kDa cytokine which is involved in inflammation and immunity and which has various functions. It is produced by macrophages, B cells, endothelial cells, fibroblasts, dendritic cells, and other cell types. The IL1B gene is present on chromosome 2q14, and recently a number of studies have implicated its polymorphism in individual differences in the risk of developing various illnesses. Meulenbelt et al.[10] reported finding an association between IL1B C-511T and radiographic signs of OA of the hip, but no associations between it and OA of the knee or finger. In particular, since the progression of OA in the knee is subject to the influence of various environmental factors such as trauma and BMI, the association between knee OA and IL-1 gene polymorphism has been assumed to be attenuated.[2,3] In the present results also, logistic likelihood ratio analysis revealed a significant ($P < 0.0001$) influence of BMI. In the same report, with regard to the association between hip OA and IL1B, with the T/T genotype at IL1B C-511T the OR was found to be 2.9 (95%CI 1.4–6.3).

The -511C allele of IL1B is almost always linked to the -31T allele, as is the -511T allele to the -31C allele.[22] Since the OR of T/T is high at IL1B C-511T, it is likely that the OR of C/C at IL1B T-31C is also high. However, this is not consistent with the result obtained in this study with regard to the knee joint, namely that with IL1B and -31T/T the OR was high. This discrepancy may be attributable to intrinsic site-related differences between the hip and knee joints. For example, regarding joint site-related factors, it has been reported that knee joint chondrocytes are more sensitive than ankle joint chondrocytes to stimulation by IL1B,[23] and that excessive and continuous mechanical stress induces IL1B production by chondrocytes.[24] On the other hand, it has been reported that gene associations with OA are dependent on sex and ethnicity, and are probably often site-specific.[14] The difference in ethnicity may be related. Regarding the conflict with the gene polymorphism results, in the case of OA, differences may also result from the setting of the endpoint, since some studies use artificial joint replacement as the endpoint,[11,12] while others, like the present study, define OA based on the radiographic findings.[10] In the present study, the facts that a cohort of healthy volunteers was used and early ROA was evaluated were also thought to have been factors.

At present no radically effective therapeutic intervention is available for OA, but if a predisposition to its development could be identified in a timely fashion, it might be possible to implement active preventative

measures such as the avoidance of obesity and of excessive exercise loads. Advances in pharmacological therapy, such as the development of disease-modifying OA drugs (DMOADs), are also likely to play an important role and are eagerly awaited.

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