Original Article

The Lack of Correlation between the Increased Frequency of Allele IL-1RN*2 of Interleukin-1 Receptor Antagonist Gene in Czech Patients with Knee Osteoarthritis and the Markers of Cartilage Degradation

(knee osteoarthritis / cytokine / *IL-1RN* gene / VNTR polymorphism / susceptibility)

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Abstract. Primary OA is a common multifactorial disease with not fully clarified molecular factors influencing the development of the disease. Among factors disturbing the cartilage integrity are cytokines, such as IL-1, which can stimulate proteinases, resulting in the cartilage destruction. In this regard, IL-1RA competing with IL-1 for binding to its receptor may act as an inhibitor of cartilage breakdown. Because of the possible functional implications, we tested VNTR polymorphism in the second intron of the *IL-1RN* gene as a putative factor of susceptibility to knee OA. Fifty patients with primary knee OA (diagnosed according to ACR criteria) and 170 healthy controls were included into the study. PCR using primers flanking the VNTR region containing variable numbers of an 86-bp tandem repeat was employed to test the hypothesis. An increased frequency and carriage rate of the IL-1RN*2 allele was found in OA patients in comparison with controls (28 % vs. 15 %, P = 0.0013, OR = 2.97; 95% CI 1.55–5.68 for frequency; 52.5 % vs. 25.3 %, P = 0.0019, OR = 2.95; 95% CI 1.54–5.68 for carriage rate). In addition, a higher frequency of genotype IL-1RN*1/*2 in OA patients was observed as compared with controls (42 % vs. 20.6 %, P = 0.0032, OR = 2.79; 95% CI 1.42–5.48). These results suggest that the IL-1RN*2 allele might represent a factor of susceptibility to OA; however, no correlation between this allele and the markers of cartilage degradation was found.

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Abbreviations: ACR – American College of Rheumatology, CI – confidence interval COMP – cartilage oligomeric matrix protein, HA – hyaluronic acid, IL-1 – interleukin 1, IL-R – IL-1 receptor, IL-1RA – IL-1R antagonist protein, IL-1RN – IL-1R antagonist gene, JSN – joint space narrowing, MMP – matrix metalloproteinase, OA – osteoarthritis, OR – odds ratio, PCR – polymerase chain reaction, PEN – pentosidine, TIMP-1 – tissue inhibitor of matrix metalloproteinases 1, VNTR – variable number tandem repeat.

Introduction

Primary osteoarthritis (OA) is one of the most common aging diseases characterized by degeneration of joint articular cartilage and remodelling of the subchondral bone (Di Cesare et al., 2005). The aetiopathogenesis of the disease is not yet fully understood; however, population-based studies and genetic linkage studies in OA families suggest that OA is a multifactorial disease with genetic contribution. Positive association of OA and human chromosome 2q13-32 region was found in genome-wide studies of families with OA (Wright et al., 1996; Leppavuori et al., 1999, Loughlin et al., 2000, 2002; Gillaspy et al., 2002). This region contains two genes, *IL-1α* and *IL-1β*, and the *IL-1RN* (interleukin-1 receptor antagonist) gene coding for protein (called IL-1RA) which competes with IL-1 for the binding site on the IL-1 receptor (IL-1R) and inhibits activation of this receptor (Nicklin et al., 1994, Schreuder et al., 1997).

The chondrocytes of OA cartilage and OA synovial fluid were shown to produce IL-1 and IL-RA cytokines, suggesting their local autocrine and paracrine effects on joint physiology (Moos et al., 1999, Richette et al., 2008). Moreover, IL-1 is a potent inducer of metalloproteinases (e.g. matrix metalloproteinases (MMP) 1, 3 and 9) as the mediators of the cartilage destruction and thus is directly responsible for the joint pathology in OA, especially under the condition of local IL-1/IL-RA imbalance (Tetlow et al., 2001, Inoue et al., 2005). Therefore, the inhibitory effect of IL-RA on IL-1 mediated joint degeneration is of physiological relevance and indeed, progression of experimental OA in dogs was prevented by IL-RA administered by intraarticular injections of recombinant protein (Caron et al., 1996), *ex vivo* gene

therapy (Pelletier et al., 1997), or *in vivo* gene therapy (Fernandes et al., 1999). The polymorphic region containing an 86-bp variable tandem repeat (VNTR) occurring in five allelic variants was found in the second intron of the *IL-1RN* gene (Tarlow et al., 1993). Although allele 2 (IL-1RN*2) was found to be more frequent among patients with autoimmune diseases such as systemic lupus erythematosus, Sjögren's syndrome or juvenile idiopatic arthritis (Blakemore et al., 1994; Vencovský et al., 2001), the data on its association with OA are still contradictory. Nevertheles, an association of the IL-1RN*2 allele with the occurrence of radiographic OA in hips, knee, hand or discs was confirmed by several groups (Moos et al., 2000, Meulenbelt et al., 2004). However, the genotypes were correlated to measurements of radiological progression as the criterion of joint destruction. To our knowledge the association of *IL-1RN* genotypes has not been related to markers of synovitis and cartilage destruction such as hyaluronic acid (HA), pentosidine (PEN), cartilage oligomeric matrix protein (COMP) and metalloproteinases, having a direct role in the final joint degeneration.

The objective of our pilot study was therefore to determine whether the IL-1RN*2 allele could be a factor predisposing to development of knee OA in general and/or whether it could correlate with the markers of cartilage destruction, additionally affecting the course and severity of the disease.

Material and Methods

Patients and biochemical markers

A cohort of 50 patients with rapidly progressing primary knee OA and 170 controls were included into the study. The patients with clinically symptomatic primary knee OA were diagnosed according to classification criteria for OA (Altman et al., 1986). Their joint space narrowing (JSN) was > 0.2 mm/year in target knee in the last year before inclusion into the study. The measurements of radiographic progression were performed in patients standing with extended knees. The JSN in the narrowest point of medial compartment of the tibiofemoral joint of signal knee was measured manually using a magnifying glass according to the Lequesne method. Serum concentrations of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) as well as markers of synovitis and cartilage destruction (HA, PEN and COMP) were evaluated as described previously (Vilim et al., 2003; Pavelka et al., 2004; Šenolt et al., 2005; Table 1).

Isolation of DNA and analysis of VNTR IL-1RN polymorphism

Peripheral blood was collected based on informed consents obtained from patients and anonymous controls, and DNA was isolated from the peripheral blood cells using QIAmp DNA isolation Maxi Kit(50) (Quiagen, Hilden, Germany). The purity and integrity of DNA was verified spectrophotometrically and electrophoretically.

Table 1. The concentrations of MMP-9, TIMP-1, HA, PEN and COMP in the serum of patients with primary knee OA

	Range	Median ± SD
MMP-9 (ng/ml)	28–974	108 ± 161
TIMP-1 (ng/ml)	428–1204	804 ± 176
HA (ng/ml)	7.2–255	22.8 ± 40.7
PEN (nmol/l)	62.6–545	109.8 ± 89
COMP (ng/ml)	109–5195	974 ± 846

IL-1RN VNTR polymorphism was analysed by PCR as described (Tarlow et al., 1993). PCR products were separated in 1% agarose gel, visualized by ethidium bromide, and particular alleles were determined using a 100-bp size standard (Roche, Mannheim, Germany).

Statistical analysis

The differences in frequencies of the IL-1RN*2 allele between patients with knee OA and controls were calculated using GraphPad Prism software and Fisher's exact test (GraphPad, San Diego, CA).

Results

A cohort of 50 patients with knee OA and a control group of 170 healthy individuals were analysed. In the group of OA patients there were 34 (68 %) females and 16 (32 %) males. The age of patients ranged from 28.3 to 69.4 years (median 54.1 ± SD 1.5) and the average duration of the disease was 3.5 years (0.4–9.0; median 3.3 ± SD 1.8) with the onset of OA after 50 years of age in 70 % patients (35/50). The observed frequencies of individual *IL-1RN* genotypes, the frequencies of *IL-1RN* alleles and their carriage rates are shown in Table 2 and they were found to be in accordance with the Hardy-Weinberg-Castle equilibrium.

An increased frequency of the IL-1RN*2 allele was found in patients with knee OA in comparison with controls (28 % vs. 15 %, P = 0.0013, OR = 2.97; 95% CI 1.55–5.68; Table 2, Fig. 1a). A significantly higher carriage rate of allele IL-1RN*2 was observed in the group of patients as compared to controls (52.5 % vs. 25.3 %, P = 0.0019, OR = 2.95; 95% CI 1.54–5.68; Table 2, Fig. 1b).

In addition, a similar pattern was seen for the frequency of the IL-1RN*1/*2 genotype, which was significantly increased in OA patients as compared to controls (42 % vs. 20.6 %, P = 0.0032, OR = 2.79; 95% CI 1.42–5.48; Table 2, Fig. 1c). The frequency of the IL-1RN*2/*2 genotype was slightly higher in patients with knee OA as compared to controls (6 % vs. 4.7 %; Table 2, Fig. 1c).

No correlation between the 1RN*1/*2 genotype and/or the carriage rate of allele IL-1RN*2 and clinical course or serum concentrations of TIMP-1, MMP-9, HA, PEN as well as COMP was found (Fig. 2a-e, Fig. 3a-e). Also, correlation analysis did not reveal any substantial significant difference in the behaviour of patients homozygous for allele IL-1RN*2, although

Table 2. The frequencies of IL-1RN genotypes (a) and the frequencies of IL-1RN alleles and their carriage rates (b) in patients with primary knee OA and controls

a)

	Number (%)				
***IL-1RN* genotype**	**IL-1RN*1/*1**	**IL-1RN*1/*2**	**IL-1RN*1/*3**	**IL-1RN*2/*2**	**IL-1RN*2/*3**
patients (N = 50)	22 (44.0)	21 (42.0)	3 (6.0)	3 (6.0)	1 (2.0)
controls (N = 170)	123 (72.4)	35 (20.6)	4 (2.3)	8 (4.7)	0 (0.0)

b)

	IL-1RN*1		**IL-1RN*2**		**IL-1RN*3**	
	frequency No. (%)	**carriage rate No. (%)**	**frequency No. (%)**	**carriage rate No. (%)**	**frequency No. (%)**	**carriage rate No. (%)**
patients (N = 50)	68 (68.0)	46 (92.0)	28 (28.0)	25 (50.0)	4 (4.0)	4 (8.0)
controles (N = 170)	285 (83.8)	162 (95.3)	51 (15.0)	43 (25.3)	4 (1.2)	4 (2.3)

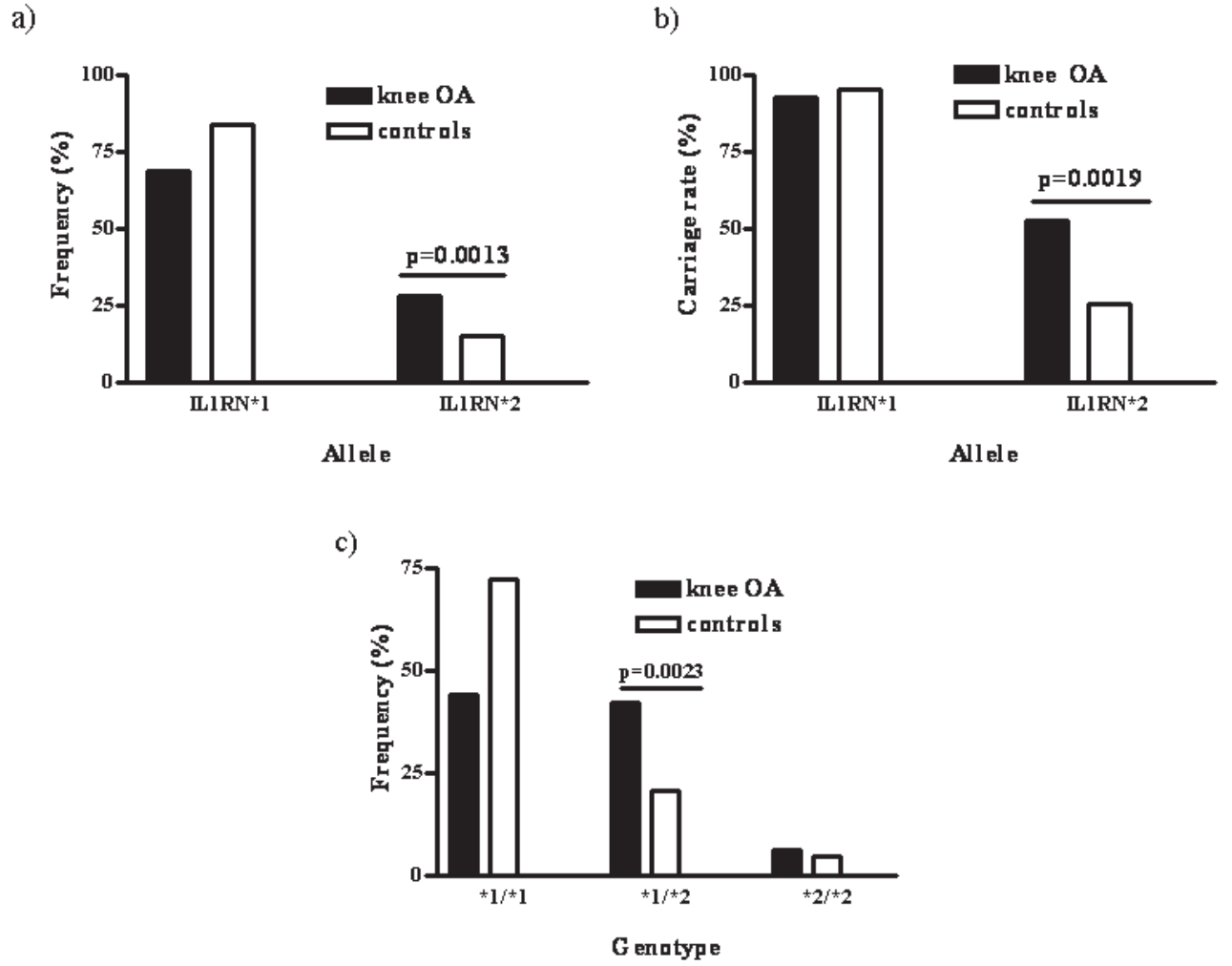

Fig. 1. a) The frequency of the IL-1RN*2 allele in patients with knee OA and controls, b) the carriage rates of the IL-1RN*2 allele in patients with knee OA and controls and c) the frequency of the IL-1RN*1/*2 genotype in patients with knee OA and controls.

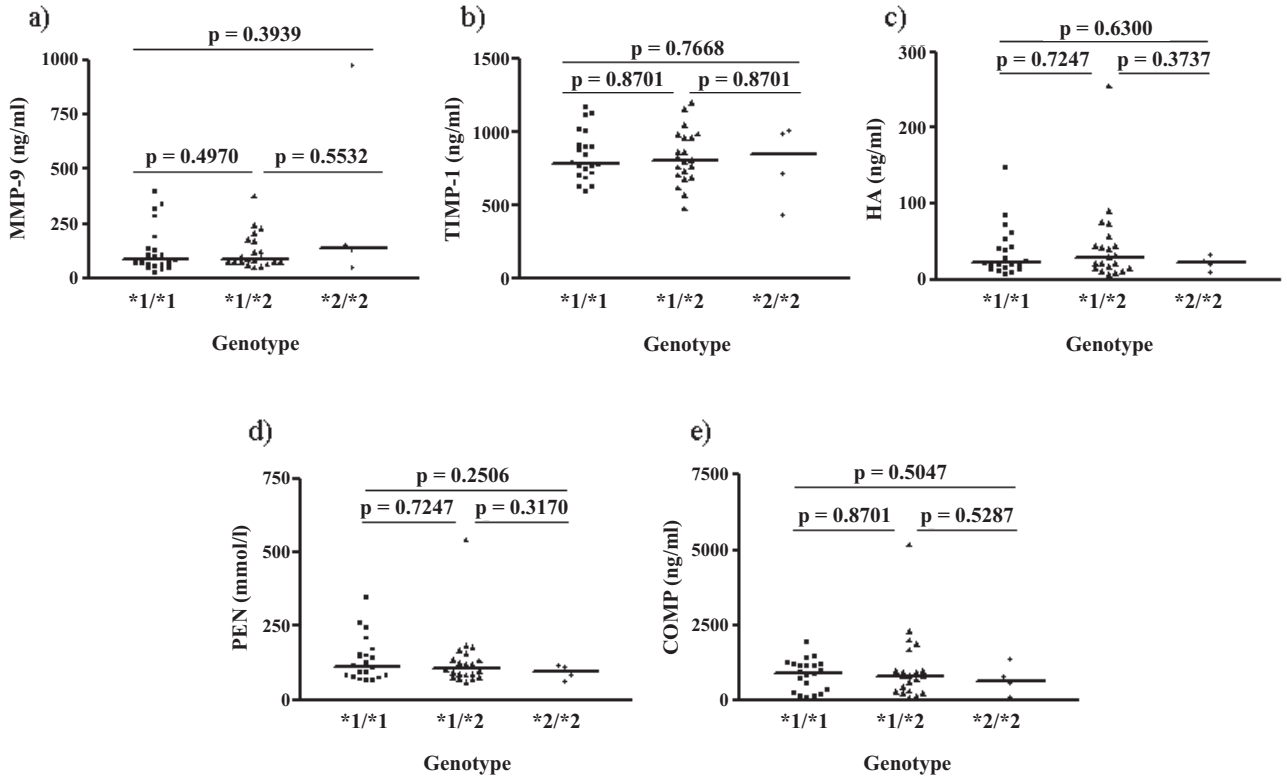

Fig. 2. IL-1RN genotypes and serum concentrations of MMP-9 (a), TIMP-1 (b), HA (c), PEN (d) and COMP (e). Medians are indicated.

MMP-9 and TIMP-1 serum titres seemed to be slightly higher in comparison to those having the IL-1RN*1/*2 genotype (Fig. 2a,b).

Discussion

Both the frequency and the carriage rate of the IL-1RN*2 allele were found to be significantly increased in the group of patients with knee OA in comparison with controls, and the genotype IL-1RN*1/*2 occurred in a higher frequency in OA patients. On the other hand, the analysis of JSN, biochemical markers, clinical parameters, and severity of the disease did not reveal any significant correlation to either the frequency or the carriage rate of the IL-1RN*2 allele or its homozygosity. Such a correlation was shown for patients with hip OA, in whom a positive association between radiographic progression and allele IL-1RN*2 was demonstrated (Meulenbelt et al., 2004).

The possible mechanisms of association of the IL-1RN*2 allele to the knee OA are still unclear. The functional significance of the IL-1RN*2 allele is not known yet, particularly with respect to disturbances of the IL-1/IL-1RA balance, which influences physiological and pathophysiological effects of IL-1. IL-1 represents one of the most important local mediators of cartilage destruction. Articular chondrocytes are very sensitive both to IL-1β stimulation and to induction of IL-1β production by long-lasting mechanical stress (Fujisawa et al., 1999; Cole and Kuettner, 2002). In this regard, the IL-1RA protein (encoded by the *IL-1RN* gene) might serve as a potent local inhibitor of cartilage degradation, which restores IL-1/IL-1RA balance directly within the affected joint. In this context, the very mild increase of MMP-9 and TIMP-1 serum titres detected in patients with knee OA carrying the IL-1RN*2/*2 genotype (Fig. 2a, b) could suggest that the balance between IL-1 and IL-1RA might be disturbed, possibly due to the presence of the IL-1RN*2 allele. However, a similar tendency should then also be seen between patients with IL-1RN*1/*2 and IL-1RN*1/*1 genotypes, which is not apparent in this case. One possible explanation of this discrepancy could be that the analysis of biochemical markers of cartilage degradation in serum cannot reflect the subsequent events resulting from disturbed local IL-1/IL-1RA balance in the joint and therefore it would be better to perform such analysis of synovial fluid/tissue of OA knees to delineate local joint conditions directly. Further, in the case of MMP-9, the increase was given rather by the highest value of 974 ng/ml found in one of the tested patients homozygous for IL-1RN*2 and the number of such patients is low. In addition, the average duration of the disease, which ranged from 0.4 to 9 years, and variations in the age of OA patients cannot be excluded as a factor responsible for the lack of any correlation. Finally, there are no data in the literature which would correlate the *IL-1RN* genotype, IL-1/IL-RA local joint ratio, and joint/serum levels of the above-mentioned biomarkers in detail. Thus, it seems necessary to find more patients with identical

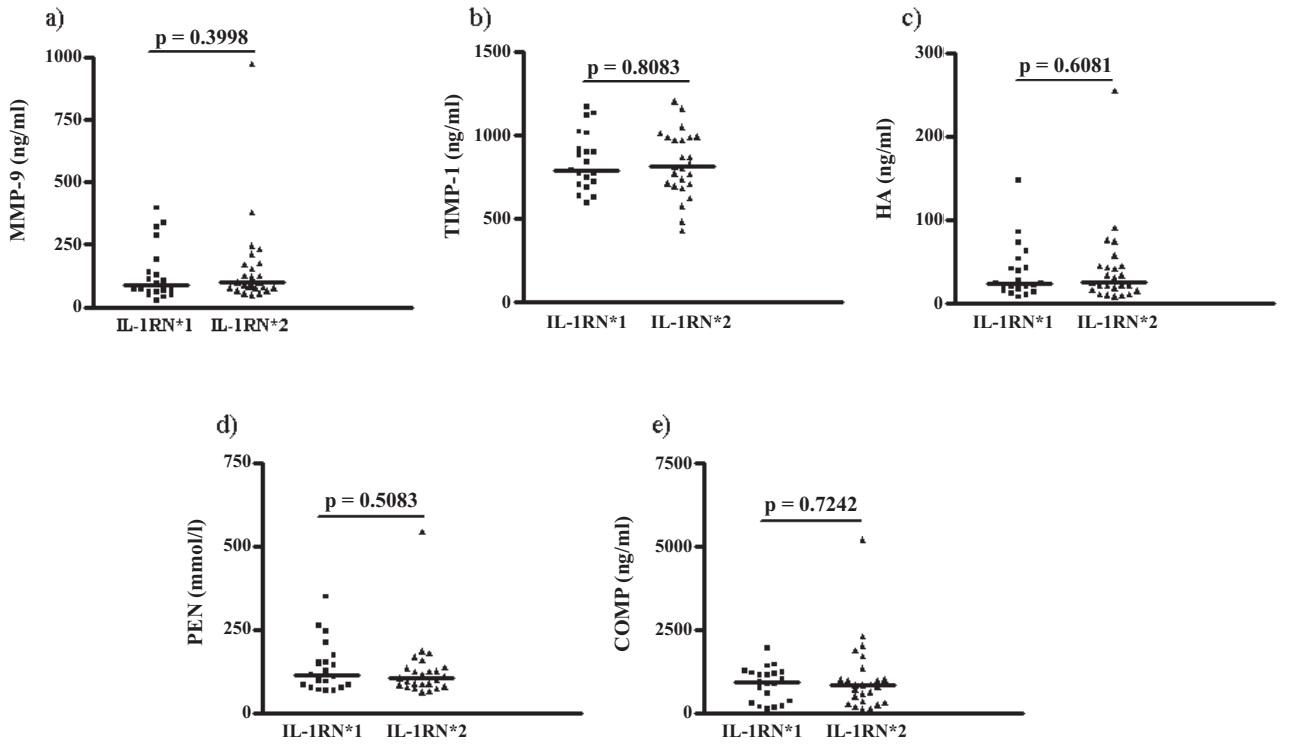

Fig. 3. IL-1RN*2 allele carriage and serum concentrations of MMP-9 (a), TIMP-1 (b), HA (c), PEN (d) and COMP (e). Medians are indicated.

genotype compositions in earlier stages of the disease and to analyse them in order to address this question more thoroughly.

The IL-1RA itself is produced in the form of several isoforms, secreted form of protein (sIL-1RA) and three intracellular isoforms (icIL-1RA 1, 2 and 3) arising from alternative splicing/translation (Arend and Gabay, 2000). Many contradictory data have been reported so far concerning the impact of the IL-1RN*2 allele on production of both IL-1 and secreted IL-1RA. In the presence of this allele, increased IL-1RA secretion and reduced IL-1α production were observed in healthy individuals (Danis et al., 1995). In contrast, decreased levels of total IL-1RA protein in monocytes of patients with ulcerative colitis and controls with allele IL-1RN*2 were found (Santilla et al., 1998). In addition, two of the key players in the cartilage homeostasis, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-3 (MMP-3), were induced in human articular chondrocytes and synoviocytes by IL-1β *in vitro* and this effect was completely abolished by recombinant IL-1RA (Inoue et al., 2005). Similarly, suppression of early experimental OA after locally enhanced production of IL-1RA was shown in the rabbit OA model (Zhang et al., 2004). These findings, together with the demonstration of high expression of IL-1 in cartilage of OA patients (Hulejová et al., 2007), suggest that rather the local than the peripheral ratio between IL-1 and IL-1RA is likely more critical for the outcome of effects of these two cytokines. However, in this regard, the effect of the IL-1RN*2 allele on the expression of intracellular IL-1RA isoforms and their contribution to the local IL-1/IL-1RA balance and cartilage turnover in the joint under pathological conditions remains unclear.

In conclusion, an increased frequency and carriage rate of the IL-1RN*2 allele, as well as the frequency of the IL-1RN*1/*2 genotype, were found in Czech patients with knee OA. However, no correlation between genetic data, biochemical markers and clinical course of the disease were observed, which might rather suggest a purely predisposing role of this allele in the development of knee OA.

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