INSULIN-LIKE GROWTH FACTOR I GENE PROMOTER POLYMORPHISM, COLLAGEN TYPE II A1 (COL2A1) GENE, AND THE PREVALENCE OF RADIOGRAPHIC OSTEOARTHRITIS

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Summary

Purpose: To examine the role of an IGF-I gene promoter polymorphism in the prevalence of radiographic osteoarthritis (ROA), and study its interaction with the COL2A1 gene.

Experimental Design: Individuals genotyped for IGF-I and COL2A1 gene polymorphisms were selected from a random sample derived from people who had presented to the Emergency Hospital “Professor Dr. Dimitrie Gerota” – Orthopaedics department from January 2009 and January 2010 Bucharest, Romania. The presence of ROA was defined as a Kellgren score of 2 or more in at least one of four joints (knee, hip, hand, and spine). Genotype specific odds ratios (OR) were adjusted for age, sex, body mass index, and bone mineral density using logistic regression. Interaction with the COL2A1 genotype was tested.

Results: Overall, no association was found between the IGF-I polymorphism and ROA. In subjects aged 65 years or younger, the prevalence of ROA increased with the absence of the 192 base pair (bp) allele (p for trend = 0.03). Compared with homozygotes for the 192 bp allele, the prevalence of ROA was 1.4 times higher in heterozygotes (95% confidence interval, 1.0 to 1.8) and 1.9 times higher in noncarriers (1.1 to 3.3). There was evidence of interaction between the IGF-I and COL2A1 genes. Individuals with the risk genotype of both genes had an increased prevalence of ROA (OR 3.4 (1.1 to 10.7)). No effect was observed in subjects older than 65 years.

Conclusions: Subjects with genetically determined low IGF-I expression (non-carriers of the 192 bp allele) may be at increased risk of ROA before the age of 65 years. Furthermore, an interaction between the IGF-I and COL2A1 genes is suggested.

Key words: IGF-I, polymorphism, COL2A1, osteoarthritis.

Introduction

Osteoarthritis, the most common form of arthritis in the elderly (Felson, 1998; van Saase et al, 1989) is characterised by progressive degeneration of articular cartilage, along with subchondral bone changes leading to the formation of osteophytes (Howell, 1986). Osteoarthritis is a complex disease (Felson et al, 2000) with clearly described environmental factors and a strong genetic influence. Recently, twin and sibling pair studies have revealed a considerable genetic contribution to the development of osteoarthritis, with heritability estimates ranging from 30% to 78% at different joints (Spector et al, 1996; Hirsch et al, 1998; Felson et al, 1998; Bijkerk et al, 1999; MacGregor et al, 2000). Linkage to several chromosomal regions has also been reported in different studies (Loughlin 2001; Chapman et al, 1999; Loughlin et al, 1999; Leppavuori et al, 1999; Wright et al, 1996). Consequently, it is expected that several genes which regulate the formation, degradation, and repair of articular cartilage and subchondral bone metabolism may determine the occurrence of osteoarthritis. However, the specific underlying genetic
Factors and mechanisms in the development of osteoarthritis remain to be determined. Insulin-like growth factor I (IGF-I) is a polypeptide mediator with a potent anabolic impact on cartilage homeostasis. Several studies (Osborn et al., 1989; Tyler, 1989) have highlighted the importance of IGF-I in promoting cartilage growth and development, implying a potential role of IGF-I in the aetiology of osteoarthritis. Circulating IGF-I is significantly decreased with advancing age (Corpas et al., 1993). An age-related decline in the ability of IGF-I to stimulate chondrocytes to produce articular matrix components has also been demonstrated (Loeser et al., 2000). Findings of the relation between osteoarthritis and either serum or synovial IGF-I concentrations are still conflicting (Lloyd et al., 1996; Hochberg et al., 1994; Schneiderman et al., 1995; McAlindon et al., 1993). A problem with the interpretation of these findings is that IGF-I concentrations are often assessed in blood, and the values may change because of joint or other pathology. Study of the gene polymorphism that regulates the protein levels is a useful method that avoids this problem.

Previously, Meulenbelt et al., 1998 reported a polymorphism in the promoter region of the IGF-I gene associated with an increased prevalence of radiological osteoarthritis (ROA) in the subset data of the Rotterdam study. In a subsequent study, it was found that absence of the 192 base pair (bp) (wild type) allele of this polymorphism was associated with lower serum IGF-I concentrations in population, suggesting that this allele has functional properties (Vaessen et al., 2001).

Collagen type II α1 (COL2A1), which constitutes 90% of the collagen in the hyaline articular cartilage and intervertebral disk, is another important protein involved in the development of osteoarthritis. Mutations in the COL2A1 gene may be related to structural failure of the protein over time, and thus to the occurrence of osteoarthritis. It was found a VNTR (variable number of tandem repeats) polymorphism located 1.35 kb downstream of the COL2A1 gene associated with an increased risk for ROA, although this findings was not confirmed by others (Bijkerk, 1999; Uitterlinden et al., 2000; Loughlin et al., 1994; Vikkula et al., 1993).

Given the conflicting findings over the role of the IGF-I protein in osteoarthritis and problems with the assessment of in vivo tissue IGF-I concentrations in humans, we studied the 192 bp allele of the IGF-I promoter polymorphism in relation to osteoarthritis, assuming that this polymorphism determines IGF-I expression both in blood and in cartilage. We also studied the interaction of this genetic polymorphism with the COL2A1 polymorphism that it had earlier been found to be associated with osteoarthritis (Bijkerk, 1999).

Materials and methods

Study population. All the tissues investigated in this study were obtained from Romanian patients who had presented to the Emergency Hospital “Professor Dr. Dimitrie Gerota” – Orthopaedics department from January 2009 and January 2010. Written informed consent for gene expression analyses was received from all the patients. We included in the present study 150 individuals from that group who had the genotype for the IGF-I polymorphism and 45 who had the genotype for COL2A1 gene polymorphism. Eight subjects were excluded because of absent scoring of radiographs at sites considered for ROA assessment.

Measurements. Age was computed at baseline and was used to stratify the individuals into two groups: cases aged 65 years or younger with early onset osteoarthritis (n=91) and cases older than 65 years with late onset osteoarthritis (n=59). Standing body height and body weight were measured with the subjects wearing light indoor clothes and no shoes. Body mass index (BMI) was calculated dividing weight (kg) by the square of height.
(m). Bone mineral density (BMD) measurements of the neck of the femur were made using dual energy x ray absorptiometry (Lunar DPX-L densitometer). Radiographic measurement and scoring techniques were done at baseline for knee and hip and for hand and spine. Definite ROA at a particular joint site was defined as a Kellgren score (Kellgren, Ball, eds, 1963) of 2 or more. In the present study, ROA cases were defined as having at least one of the four joint sites affected - that is, knee, hip, hand, or spine. ROA controls were defined as having none of the four joint sites affected.

**Genotyping.** Genomic DNA was isolated from all blood samples. Genotypes for the dinucleotide polymorphic cytosine–adenine (CA) repeats 1 kb upstream of the human IGF-I gene were determined according to Weber J.L. and May P.E., 1989. Genotypes of the VNTR polymorphism located 1.35 kb downstream of the COL2A1 gene were determined according to Berg E.S. and Olaisen Bin, 1993, the same sample of subjects in whom the IGF-I polymorphism was genotyped. Polymerase chain reaction (PCR) conditions, primers, and genotype analysis were undertaken as described for the IGF-I gene polymorphism (Vaessen et al, 2001) and for the COL2A1 gene polymorphism (Bijkerk, 1999). Alleles were labelled for the IGF-I gene polymorphism according to the length of the PCR product, and three genotypes were assigned to the individuals according to the presence or absence of the 192 bp (wild type) allele as follows: 192 bp homozygotes, 192 bp heterozygotes, and noncarriers of the 192 bp allele. For the COL2A1 gene polymorphism, we used Berg and Olaisen nomenclature (Berg and Olaisen Bin, 1993) and looked for the presence or absence of the 13R1 allele. We used heteroduplex analysis (HA) to study the relation between the COL2A1 gene and knee osteoarthritis. This method allows separation of the SS allele 13R1 into two main alleles (4A and 4B). Based on these findings, we further evaluated the interaction of the 4A and 4B alleles with the 192 bp allele of the IGF-I gene in ROA in 91 subjects from our study population with available HA genotypes.

**Statistical analysis.** Baseline measurements were compared between ROA cases and controls using t tests for independent samples and the λ2 test (where appropriate). All genotype frequencies of the IGF-I and COL2A1 genes were in Hardy-Weinberg equilibrium proportions. Prevalence of ROA in carriers and non-carriers was compared using odds ratios (OR) with 95% confidence intervals (CI) obtained from logistic regression, adjusted for age, sex, BMI, and BMD. The product term of the number of 192 bp alleles in the IGF-I genotypes and the presence or absence of the 13R1 allele of the COL2A1 gene was included in the logistic regression models to test for gene interaction. All statistical analyses were done using the S.P.S.S. package V.10 (S.P.S.S. Inc., Chicago, Illinois, USA).

**Results**

General characteristics of the study population are present in table 1. BMI was similar in each group and was significantly higher in cases than in controls except for subjects in the age group older than 65 years. BMD tended to be higher in cases than in controls, though the difference was not statistically significant. The prevalence of ROA at all joint sites was higher in subjects older than 65 years. The frequency of carriers of the 13R1 allele of the COL2A1 gene VNTR polymorphism was higher (though not significantly so) in ROA cases than in controls.

The IGF-I allele frequencies were 66.2%, 18.6%, 6.7%, 4.6%, and 3.9% for the 192 bp, 194 bp, 196 bp, 190 bp, and the remaining alleles, respectively. Genotype frequencies according to the 192 bp allele and risk estimates are presented in table 2. In the overall study population, absence of the 192 bp allele increased the risk for ROA, although the increase was not...
statistically significant under a multiplicative model (p for trend=0.14). In the subjects aged 65 years or younger, the absence of the 192 bp allele was related to significantly increased risk for ROA (p for trend=0.03), suggesting an allele dose effect. Compared with homozygotes for the 192 bp allele, the prevalence of ROA was 1.4 times increased for heterozygotes (95% CI, 1.0 to 1.8) and 1.9 times for noncarriers of the allele (95% CI, 1.1 to 3.3). This effect of the IGF-I polymorphism was independent of BMD, BMI, age, and sex. In the group of subjects older than 65 years, no significant effect was observed (p for trend=0.34).

No significant evidence for interaction between the IGF-I gene and the COL2A1 gene was observed (p=0.20). However, the prevalence of ROA increased significantly (OR 3.4 (95% CI, 1.1 to 10.7)) only in non-carriers of the 192 bp allele at the promoter region of the IGF-I gene who also carried the 13R1 allele of the VNTR polymorphism in the COL2A1 gene (fig 1). Here also, the effect of the IGF-I polymorphism was independent of BMD, BMI, age, and sex.

When looking at the COL2A1 genotypes obtained from hybridisation analysis, the interaction effects for both the 4A and 4B alleles were - although not statistically significant - in the same direction and magnitude as those observed for the 13R1 interaction effect (OR 5.1 (95% CI, 0.6 to 41) for 4A carriers without the IGF-I 192 bp allele; and OR 3.5 (0.4 to 29) for 4B carriers without the IGF-I 192 bp allele).

**Discussion**

In this population based study we found that the absence of the 192 bp allele of a microsatellite polymorphism in the promoter region of the IGF-I gene was associated with increased prevalence of radiographic osteoarthritis in subjects aged 65 years or younger. Compared with homozygotes for the 192 bp allele, the prevalence of ROA was higher in heterozygotes (OR 1.4 (95% CI, 1.0 to 1.8)) and non-carriers of the allele (OR 1.9 (1.1 to 3.3)). This effect most probably occurs in interaction with the COL2A1 gene, as the prevalence of ROA increased in individuals with the risk genotype of both genes (OR 3.4 (95% CI, 1.1 to 10.7)). These findings were independent of age, sex, BMI, and BMD. No such effect was observed in subjects older than 65 years.

Spurious associations can result from population stratification either because of a recent admixture of a different population or because of inappropriate matching of patients and controls. The occurrence of spurious associations in our study is unlikely as cases and controls were sampled from the same (ethnically homogeneous) source population. Potential bias may arise from the fact that the IGF-I and COL2A1 gene polymorphisms were not genotyped in all subjects. However, there is no evidence of introduction of selection bias as IGF-I and COL2A1 genotypes were missing at random - as suggested by the fact that genotype frequencies were in Hardy–Weinberg equilibrium proportions, and allele frequencies were similar to those reported previously in other white populations.

Though we have used a different analytical approach to pool alleles based on the presence or absence of the 192 bp (wild type) allele, our current findings are consistent with the association reported previously between the 194 bp allele (the second most frequent allele in our study population) and ROA. We did not undertake further allele specific analysis because of lack of power, considering the low frequencies of all the other alleles. We used this approach given our earlier findings of lower serum IGF-I concentrations in the absence of the 192 bp allele. Our results suggest that lower serum concentrations of IGF-I may contribute to the pathogenesis of early onset osteoarthritis (before the age of 65 years) by causing decreased synthesis of matrix components in articular cartilage.
Why this IGF-I polymorphism does not explain the occurrence of osteoarthritis in the elderly remains to be determined. One possible explanation is that the incidence of osteoarthritis increases so markedly after the age of 65 years, owing to the influence of other environmental factors, that the contribution of genetic predisposition to disease onset is reduced. Also, IGF-I stimulation of chondrocyte matrix production has been shown to decrease with age, and this may explain why genetically determined low IGF-I levels are not relevant to the prevalence of ROA in old age. In addition to these biological explanations, we cannot exclude lack of power to detect any existing association in view of the relatively small number of individuals older than 65 years in our study.

Our results suggest that a genetic predisposition involving IGF-I expression increases the risk of ROA. It remains to be determined whether the polymorphism we studied is the causal variant in IGF-I explaining the association, or if it is in linkage disequilibrium with another polymorphism involved in the pathology.

In early life, IGF-I has been shown to be a major stimulator of type II collagen production (Willis, Liberti, 1985). Furthermore, there is evidence that IGF-I may be concerned in ROA through a pathway involving the cartilage matrix (Denko et al, 1990). The finding of increased prevalence of ROA in only those individuals with both risk genotypes supports the possibility of a biological interaction between the IGF-I and COL2A1 genes in the development of osteoarthritis.

Although the evidence for a statistical interaction was not significant, it should be borne in mind that the power of our study was low given the small number of cases and controls (especially with genotypes obtained from the heteroduplex analysis, where the numbers were even smaller after splitting the COL2A1 13R1 carriers into two subgroups).

**Conclusion**

Our study shows that the absence of the 192 bp allele in the promoter region of the IGF-I gene is associated with increased prevalence of radiographic osteoarthritis before the age of 65 years. The study also suggests the possibility of a genetic interaction between the IGF-I and the COL2A1 genes in the occurrence of this disease.

**References**


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**Table 1. General characteristics of the study populations**

<table>
<thead>
<tr>
<th>Overall study subjects</th>
<th>Subjects ≤65 years</th>
<th>Subjects &gt;65 years</th>
<th>Interaction study</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=130</td>
<td>n=20</td>
<td>n=75</td>
<td>n=16 n=55 n=4 n=42 n=12</td>
</tr>
</tbody>
</table>

### Age (years)
- 63.4 (4.1)●
- 61.1 (3.9)
- 60.7 (2.7)●
- 59.6 (2.7)
- 67.5 (1.4)
- 67.1 (1.6)
- 60.5 (2.7)●
- 59.5 (2.8)●

### Female (%)
- 58.7* 50.3
- 60.7 54.5
- 55.6* 60.8

### Height (cm)
- 168.5 (8.5)
- 166.8 (8.6)
- 169.7
- 168.4
- 170.3
- 168.7 (8.4)
- 169.8

### Weight (kg)
- 75.2 (12.1)●
- 72.0
- 75.5 (12.5)●
- 71.8
- 74.8
- 72.9 (9.8)
- 72.5

### BMI (kg/m2)
- 0.86 (0.13)
- 0.87 (0.13)
- 0.85 (0.13)
- 0.84 (0.13)
- 0.81 (0.11)
- 0.87 (0.13)
- 0.86 (0.12)

### COL2A1 13R1+ (%)
- 67
- 64

### Hand ROA (%) 60.8
- 57.3
- 66.8
- 57.3

### Knee ROA (%) 17.6
- 15.9
- 20.5
- 18.1

### Hip ROA (%) 10.9
- 8
- 15.7
- 9.3

### Disk degeneration of spine (%)
- 69.2
- 63.9
- 78
- 61.7

### Values are mean (SD) unless stated otherwise.

* p<0.05, ● p<0.001

**Table 2. Association analysis**

<table>
<thead>
<tr>
<th>Overall study subjects</th>
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</tr>
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<td>n=75</td>
</tr>
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</table>

### Homozygous for 192 bp (%)
- 57 10 Reference
- 32 8 Reference

### Heterozygous for 192 bp (%)
- 56 8
- 1.2 (0.9 to 1.5)
- 33 7 1.4 (1.0 to 1.8)
- 22 3 0.8 (0.5 to 1.3)

### Non-carriers of 192 bp (%)
- 17 2 1.4 (0.9 to 2.4)
- 10 1 1.9 (1.1 to 3.3)
- 6 1 0.6 (0.2 to 1.7)

* p for trend <0.05

OR, odds ratio adjusted for age, sex, body mass index, and bone mineral density