EXTENDED REPORT

Insulin-like growth factor I gene promoter polymorphism, collagen type II α1 (COL2A1) gene, and the prevalence of radiographic osteoarthritis: the Rotterdam Study

G Zhai, F Rivadeneira, J J Houwing-Duistermaat, I Meulenbelt, C Bijkerk, A Hofman, J B J van Meurs, A G Uitterlinden, H A P Pols, P E Slagboom, C M van Duijn

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See end of article for authors' affiliations

Correspondence to: Professor Cornelia M van Duijn, Department of Epidemiology and Biostatistics, Erasmus Medical Centre, Postbus 1738, 3000DR, Rotterdam, Netherlands; c.vanduijn@erasmusmc.nl

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Objective: 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.
Methods: Individuals genotyped for IGF-I (n = 1546) and COL2A1 gene polymorphisms (n = 808) were selected from a random sample (n = 1583) derived from the Rotterdam study. 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 (n = 971), 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 non-carriers (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.

Osteoarthritis, the most common form of arthritis in the elderly,[1 2] is characterised by progressive degeneration of articular cartilage, along with subchondral bone changes leading to the formation of osteophytes.[3] Osteoarthritis is a complex disease[4] with clearly described environmental factors and a strong genetic influence. Familial aggregation has been recognised for Heberden's nodes[5] and for early onset generalised osteoarthritis in combination with Heberden's nodes.[6] 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.[7–11] Linkage to several chromosomal regions has also been reported in different studies.[12–16] 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[17 18] 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.[19] An age related decline in the ability of IGF-I to stimulate chondrocytes to produce articular matrix components has also been demonstrated.[20] Findings of the relation between osteoarthritis and either serum or synovial IGF-I concentrations are still conflicting.[21–24] 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.

We previously 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.[25] In a subsequent study, we found that absence of the 192 base pair (bp) (wild type) allele of this polymorphism was associated with lower serum IGF-I concentrations in our population,[26] suggesting that this allele has functional properties.

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. We previously 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,[27 28] although this findings was not confirmed by others.[29 30]

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

Abbreviations: BMD, bone mineral density; BMI, body mass index; PCR, polymerase chain reaction; ROA, radiographic osteoarthritis; VNTR, variable number of tandem repeats

also studied the interaction of this genetic polymorphism with the COL2A1 polymorphism that we had earlier found to be associated with osteoarthritis.[27]

METHODS

Study population

The study was part of the Rotterdam Study, a prospective population based investigation of the determinants and prognosis of chronic diseases in 7983 elderly people. The design of the study has been described elsewhere.[31] Written informed consent was obtained from each participant. The Rotterdam Study was approved by the medical ethics committee of Erasmus University Medical School. Previously, a random sample of 1583 subjects from the Rotterdam Study, aged 55 to 70 years, was assessed for ROA and disk degeneration.[10] We included in the present study 1554 individuals from that random group who had the genotype for the IGF-I polymorphism and 816 who had the genotype for COL2A1 gene polymorphism. Eight subjects were excluded because of absent scoring of radiographs at sites considered for ROA assessment. The final study population comprised 1546 individuals in the IGF-I association analysis and 808 individuals in the gene interaction analysis.

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 = 971) and cases older than 65 years with late onset osteoarthritis (n = 575). 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) as described previously.[32] Radiographic measurement and scoring techniques were done at baseline as described previously for knee and hip[33] and for hand and spine.[10] Definite ROA at a particular joint site was defined as a Kellgren score[34] 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 and May.[35] Genotypes of the VNTR polymorphism located 1.35 kb downstream of the COL2A1 gene were determined according to Berg and Olaisen[36] in 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 previously for the IGF-I gene polymorphism[26] and for the COL2A1 gene polymorphism.[27] 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 non-carriers of the 192 bp allele. For the COL2A1 gene polymorphism we used Berg and Olaisen nomenclature[36] and looked for the presence or absence of the 13R1 allele.[27] Earlier, we used heteroduplex analysis (HA)[37 38] to study the relation between the COL2A1 gene and knee osteoarthritis.[28] 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 591 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 χ^2 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 SPSS package V.10 (SPSS 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 non-carriers 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

Table 1 General characteristics of the study populations

	Association study							
	Overall study subjects		Subjects ⩽65 years		Subjects >65 years		Interaction study	
	ROA+ (n = 1355)	ROA− (n = 191)	ROA+ (n = 817)	ROA− (n = 154)	ROA+ (n = 538)	ROA− (n = 37)	ROA+ (n = 676)	ROA− (n = 132)
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*	32.4	60.8	50
Height (cm)	168.5 (8.5)	169.8 (9.4)	168.6 (8.6)	169.7 (9.4)	168.4 (8.4)	170.3 (9.5)	168.7 (8.4)	169.8 (9.1)
Weight (kg)	75.2 (12.1)†	72.0 (11.8)	75.5 (12.5)§	71.8 (12.2)	74.8 (11.3)	72.9 (9.8)	72.5 (12.4)*	72.5 (11.9)
BMI (kg/m^2)	26.5 (3.7)†	25.0 (3.3)	26.5 (3.8)†	24.8 (3.2)	26.4 (3.7)	25.2 (3.5)	26.5 (3.7†	25.1 (3.2)
BMD (g/cm^2)	0.86 (0.13)	0.84 (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+ (%)	–	–	–	–	–	–	67.0	64.0
Hand ROA (%)	60.8	–	57.3	–	66.8	–	57.3	–
Knee ROA (%)	17.6	–	15.9	–	20.5	–	18.1	–
Hip ROA (%)	10.9	–	8.0	–	15.7	–	9.3	–
Disk degeneration of spine (%)	69.2	–	63.9	–	78.0	–	61.7	–

Values are mean (SD) unless stated otherwise.
*p<0.05, †p<0.0001.
BMD, bone mineral density; BMI, body mass index; ROA, radiographic osteoarthritis.

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.[39 40] The occurrence of spurious associations in our study is unlikely[41] as cases and controls were sampled from the same (ethnically homogeneous) source population.[31] 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.[35 36]

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.[25] 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.[26] 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,[20] 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.[42] Furthermore, there is evidence that IGF-I may be concerned in ROA through a pathway involving the cartilage matrix.[43] 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

Table 2 Association analysis

	Overall study population			Subjects ⩽65 years†			Subjects >65 years		
	ROA+ (n = 1355)	ROA− (n = 191)	OR (95%CI)	ROA+ (n = 817)	ROA− (n = 154)	OR (95%CI)	ROA+ (n = 538)	ROA− (n = 37)	OR (95%CI)
Homozygous for 192 bp (%)	594 (43.8)	93 (48.7)	Reference	344 (42.1)	79 (50.3)	Reference	250 (46.5)	14 (37.8)	Reference
Heterozygous for 192 bp (%)	593 (43.8)	78 (40.8)	1.2 (0.9 to 1.5)	368 (45.0)	60 (38.2)	1.4 (1.0 to 1.8)	225 (41.8)	18 (48.6)	0.8 (0.5 to 1.3)
Non-carriers of 192 bp (%)	168 (12.4)	20 (10.5)	1.4 (0.9 to 2.4)	105 (12.9)	15 (9.6)	1.9 (1.1 to 3.3)	63 (11.7)	5 (13.5)	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.

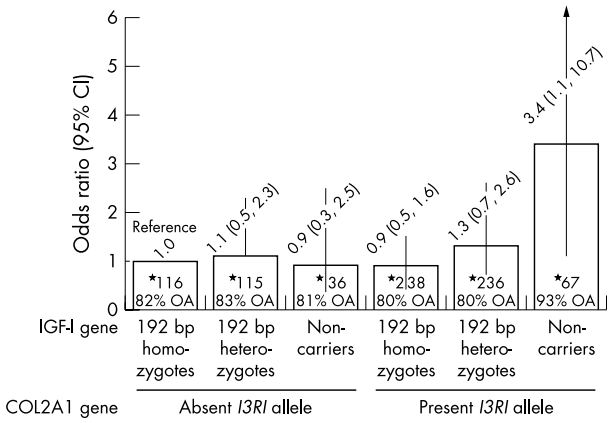

Figure 1 Interaction between the IGF-I and COL2A1 genes in the occurrence of radiographic osteoarthritis (ROA). Risk estimates adjusted for age, sex, body mass index, and bone mineral density. *Total number by genotype and percentage of subjects with ROA. bp, base pair; CI, confidence interval; OA, osteoarthritis.

were even smaller after splitting the COL2A1 13R1 carriers into two subgroups).

Conclusions

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.

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Authors' affiliations

J J Houwing-Duistermaat, A Hofman, C M van Duijn, Department of Epidemiology and Biostatistics, Erasmus Medical Centre, Rotterdam, Netherlands
J B J van Meurs, A G Uitterlinden, H A P Pols, Department of Internal Medicine, Erasmus Medical Centre
I Meulenbelt, P E Slagboom, Department of Molecular Epidemiology, Leiden University Medical Centre, Leiden, Netherlands
C Bijkerk, Department of Rheumatology, University Medical Centre St Radboud, Nijmegen, Netherlands
F Rivadeneira, Instituto de Genetica Humana, Facultad de Medicina, Pontificia Universidad Javeriana, Bogota, Colombia
G Zhai, Netherlands Institute for Health Sciences, Rotterdam, Netherlands

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ECHO

Public understanding and acceptance of randomisation

Please visit the *Annals of the Rheumatic Diseases* website [www.annrheumdis.com] for a link to the full text of this article.

Although most health professionals accept that randomisation in clinical trials is important there is evidence that patients may not understand randomisation (either the word or the concept) and may not accept its validity. A study in North Staffordshire UK has shown that, while most people understand randomisation, many do not accept it.

The participants were 130 students at five further education and leisure courses. They varied in age from 18 to 70 years (mean 32 years) and in occupation from unskilled to professional. Two thirds were women. Participants were given two scenarios, one medical (referral to a consultant nearby or far away) and one non-medical (a free trip locally or to Spain) and asked to say which of five methods of allocation were random. The methods were selection by computer with no information about individuals, toss of a coin, drawing paper slips out of a hat, individual choice, and alternate allocation in turn. They were then asked to imagine they had been asked to take part in a clinical trial comparing two drugs, each of them known to be of value, to try to find out which drug was the better. The five methods of allocation were stated again and participants were asked to decide whether each method was acceptable. (It was left open whether that meant acceptable to the participant as a patient or acceptable as a feature of the trial.) Half of the participants were given a written justification for randomisation in clinical trials and half were not.

Most participants (73–92%) considered computer allocation, coin tossing, and drawing from a hat to be random methods. Most (77–92%) considered patient choice to be non-random. Participants were divided about allocation in turn. The answers were similar for the medical and the non-medical scenario. On the whole most people did not find any method of randomisation acceptable in a clinical trial. Among those who judged the randomness of each of the five methods correctly a minority (around one third) considered randomisation acceptable. Three quarters of the group considered that asking the patients to choose their own allocation was acceptable. When written justification of randomisation was given, the proportion of participants considering computer allocation to be acceptable rose to 58% but a majority still considered tossing a coin or drawing from a hat unacceptable.

Members of the general public mostly understand randomisation but balk at its use in clinical trials. Methods such as tossing a coin or drawing from a hat are considered too frivolous for medical use but computer allocation, after explanation, is more acceptable.

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