Candidate Gene Association Study of Magnetic Resonance Imaging-based Hip Osteoarthritis (OA): Evidence for *COL9A2* Gene as a Common Predisposing Factor for Hip OA and Lumbar Disc Degeneration

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ABSTRACT. ***Objective.*** To study whether gene variants associated with lumbar disc degeneration (LDD) phenotypes are also associated with hip osteoarthritis (OA).

Methods. Magnetic resonance imaging (MRI)-based hip OA changes for 345 twins were assessed and 99 single-nucleotide polymorphisms (SNP) were analyzed.

Results. Variants in the *COL9A2* (rs7533552, p = 0.0025) and *COL10A1* (rs568725, p = 0.002) genes showed association with hip OA.

Conclusion. The associating G allele in *COL9A2* changes a glutamine to arginine or to tryptophan and may predispose to both hip OA and LDD, making it a candidate for degenerative connective tissue diseases. (J Rheumatol First Release Dec 15 2010; doi:10.3899/jrheum.100080)

Key Indexing Terms:
OSTEOARTHRITIS HIP JOINT COLLAGEN TYPE IX GENES

Osteoarthritis (OA) is a degenerative joint disease common in the elderly. Based on twin studies, the heritability of hip OA is over 50%[1]. It is likely that there are common genetic factors behind hip OA and disc degeneration, since hip OA has been shown to predict the progression of disc space narrowing and the presence of anterior vertebral osteophytes of the discs[2]. Many different genes including collagens and collagen-breaking factors have been suggested to have an effect on OA as well as with other connective tissue and skeletal diseases[3].

In our previous study, we monitored for association of 25 candidate genes with the lumbar disc degeneration (LDD) traits disc bulging, disc narrowing, and a quantitative measure of water content, using magnetic resonance imaging (MRI). We studied 99 single-nucleotide polymorphisms (SNP) in aggrecan 1 (*AGC1*, *ACAN*) and 12 collagen, 8

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interleukin, and 4 matrix metalloproteinase genes[4]. In our current study, we investigated whether the same genes were also involved in another connective tissue disease, hip OA, using the same twin study material and the same SNP panel.

MATERIALS AND METHODS

The study sample was the part of the population-based Twin Spine Study material[4] that has been investigated by MRI. It consists of 588 Finnish monozygotic (MZ) and dizygotic (DZ) male twins aged 35–70 years. The studied phenotype was MRI-based calcified structure changes also visible in radiographs. Imaging of the hips was done using Siemens Vision 1.5 T scanning, both hips at the same time, using a body matrix coil. Analysis of the scans was done using Siemens MRI workstation at the Puijon Magneetti Oy company, Kuopio, Finland. The grading scale was based on Li, *et al*[5] and the Kellgren-Lawrence radiological assessment of OA[6]: Individuals with 2 normal MRI images were considered as healthy, and individuals with at least small local osteophytes in caput or acetabulum in 1 hip were considered affected, yielding a phenotype allowing for early-onset OA (Table 1).

Polychoric correlations for MZ and DZ twins and heritability using quantitative genetic models allowing for additive genetic, common, and unique environmental variance in the liability were estimated using Mx software (M. Neale, Virginia Commonwealth University; available from: http://www.vcu.edu/mx/). Lifestyle factors (body mass index, mean age, smoking pack-years, and exercise frequency) were compared between the healthy and affected subjects (Mann-Whitney U test).

Genotyping and ethical approval is described in Videman, *et al*[4]. One individual from each MZ twin pair was used in the SNP association analysis (n = 345, 216 unrelated). Evidence for association of the studied SNP was evaluated using the Pseudomarker program that can use various pedigree structures including combined data of individuals and sibling pairs. The program is able to separate the linkage evidence from evidence for association. The test statistic for association linkage was used with dominant and recessive modes[7]. Association was also calculated for unrelated individuals using Fisher's exact test and the size of genetic effect for unrelated individuals was analyzed. The Haploview program[8] was used to calculate the linkage disequilibrium (LD) between the markers.

To define variation at the codon level, we sequenced the flanking region of SNP rs7533552 in all samples (ABI3730xl DNA Analyzer, BigDye Terminator v3.1 Cycle Sequencing Kit, Life Technologies Corp., Carlsbad, CA, USA). Genotype call rate for rs7533552 was 97.5% and Hardy-Weinberg equilibrium was 0.826.

To correct for multiple testing, the SNP spectral decomposition (SpD) method[9] with modifications by Li and Ji[10] was used to calculate the p value threshold for 5% significance (0.00073; Genetic Epidemiology Laboratory, Queensland Institute of Medical Research; available from: http://genepi.qimr.edu.au/general/daleN/SNPSpD).

RESULTS

The differences in lifestyle factors were nonsignificant between the healthy and affected subjects (Table 1). The polychoric correlation for hip OA was 0.71 (SE 0.11) for MZ twins and 0.40 (SE 0.13) for DZ twins. A quantitative genetic model allowing for additive genetic and unique environmental variance estimated the heritability of hip OA to be 0.72 (95% CI 0.49–0.87).

One variant in the *COL9A2* gene (rs7533552; location chr1:40545736, hg18, second base in the codon for Gln^{326}), which has previously shown association with disc bulging in the lumbar spine (L1 to L4)[4], also showed association with hip OA (p = 0.0025; Table 2). The G allele was the predisposing allele in both diseases, with a frequency of 30.8% in the hip OA cases and 17.5% in the healthy subjects. The G allele changes a polar negatively charged glutamine (Gln^{326}) to a polar positively charged arginine (Arg). The same amino acid will be further changed to a nonpolar hydrophobic tryptophan (Trp), if the rare SNP in the first position of the same codon is a T allele (rs12077871; location chr1:40545737, hg18; Figure 1). Since the genotyping method used was only designed to monitor variation at rs7533552, we further sequenced the locus to define the genotypes of both the variants in order to reveal the carried amino acid for each individual. Eleven individuals carried a Trp allele as a combination of C/T heterozygote for SNP rs12077871 (first base of the codon) together with A/G heterozygote or G/G homozygote for SNP rs7533552 (second base of the codon; Table 3). This indicated that the minor alleles T and G of the 2 studied SNP were inherited together (D' LD = 1.00, r^2 LD = 0.04, calculated using the Haploview program)[8].

The size of genetic effect for unrelated individuals was analyzed by comparing amino acid counts of the wild-type Gln allele with the predisposing Arg or Trp alleles between hip OA cases (45 predisposing alleles, 101 wild-type alleles) and controls (50 predisposing alleles, 236 wild-type alleles). The disease odds ratio for carrying either of the predisposing variant alleles Arg or Trp versus the wild-type allele Gln was 2.10 (95% CI 1.66–2.67; p = 0.0021, Fisher's exact test).

Table 1. Subject characteristics by hip OA status (data are mean ± SD).

Disease Status	n (%)	Age, yrs	BMI	Smoking†	Endurance Exercise††	Resistance Exercise††	Sport Exercise††
Healthy*	251 (72.8)	49.7 ± 7.4	25.8 ± 3.1	13.7 ± 16.7	1.94 ± 2.72	0.07 ± 0.32	2.54 ± 2.83
Affected**	94 (26.4)	51.2 ± 7.3	25.8 ± 3.3	12.8 ± 16.6	1.73 ± 2.04	0.05 ± 0.35	2.34 ± 2.28
Total	345 (100)	50.1 ± 7.4	25.8 ± 3.2	13.4 ± 16.6	1.88 ± 2.55	0.06 ± 0.32	2.48 ± 2.68

* MRI images in both hips normal (grade 0). ** At least 1 hip graded as 1–4: 1 = small local osteophytes in caput or acetabulum; 2 = osteophytes in 2 or more locations and subchondral signal loss and/or individual subchondral changes; 3 = same as class 2 with incipient deformation of caput/acetabulum deformation, wider or several cysts; 4 = significant deformation of bone structure, malposition, and subchondral or paraarticular changes (increase in T2 signal intensity, loose bodies). † Pack-years at baseline. †† Mean weighted frequency per week of exercise after age 20 years. BMI: body mass index; MRI: magnetic resonance imaging.

Table 2. Association of the studied SNP with hip OA using the Pseudomarker program.

Gene	rs Number	MAF	Disc Degeneration	p values (hip OA)* Dominant	Recessive	Gene	rs Number	MAF	Disc Degeneration	p values (hip OA)* Dominant	Recessive
AGC1	rs939587	0.280	X	0.888	0.920	*COL11A1*	rs1415359	0.400		0.374	0.332
	rs4932424	0.280	X	0.480	0.488		rs1337185	0.180	X	0.439	0.362
	rs3825996	0.490	X	1.000	1.000		rs1463035	0.180	X	0.258	0.230
	rs1042631	0.230	X	0.498	1.000		rs3753841	0.320		0.597	0.498
	rs1516797	0.340	X	0.115	0.082	*COL11A2*	rs2072915	0.240	X	0.124	0.106
COL1A1	rs1800012	0.120		0.171	0.168		rs9277933	0.240	X	0.308	0.277
	rs2075555	0.130	X	0.202	0.229		rs2076311	0.240	X	0.237	0.207
	rs1007086	0.270	X	0.888	0.888		rs2855432	0.300		0.399	0.299
	rs909102	0.140		0.037	0.032		rs2257126	0.360		0.862	0.806
COL1A2	rs3763468	0.050		0.078	0.091		rs734181	0.180		0.191	0.142
	rs388625	0.400		0.920	0.920	*ILA*	rs2071375	0.350	X	0.916	0.923
	rs412777	0.420		0.045	0.048	*ILB*	rs1143634	0.260		0.071	0.068
	rs400218	0.350		0.383	0.351	*IL1R1*	rs1465325	0.200		0.512	0.617
	rs1034620	0.200		0.777	0.841		rs956730	0.280		1.000	0.920
OL2A1	rs1859443	0.180		0.365	0.390		rs3917225	0.410		0.517	0.502
	rs1635529	0.200		0.303	0.299		rs2287047	0.250		0.538	0.532
	rs2276453	0.390		0.920	0.920		rs3771200	0.430		0.036	0.033
	rs917055	0.200		0.354	0.330	*IL1R2*	rs740044	0.160		0.718	0.791
	rs2276458	0.360		0.431	0.396		rs4141134	0.260		0.450	0.450
	rs6823	0.470		0.371	0.340		rs719250	0.190		0.284	0.299
COL3A1	rs1878199	0.290		0.823	1.000		rs3218984	0.410		0.269	0.245
	rs2056156	0.470		0.663	0.603		rs1008394	0.410		0.269	0.265
	rs2203601	0.470		0.450	0.446	*IL1RL1*	rs1420089	0.130		0.054	0.040
	rs1800255	0.230		0.383	0.488		rs1997466	0.490		1.000	1.000
COL5A1	rs4842138	0.120		0.233	0.271		rs1041973	0.240		0.142	0.142
	rs4341231	0.490		0.180	0.221		rs12905	0.270		0.242	0.194
	rs3128619	0.480		0.498	0.498	*IL1RL2*	rs2241132	0.130		0.157	0.080
	rs10858281	0.450		0.273	0.368		rs870684	0.400		0.095	0.062
	rs7357740	0.490		0.127	0.177		rs1922290	0.400		0.067	0.049
COL5A2	rs1983318	0.110		0.578	0.560		rs1922295	0.400		0.069	0.051
	rs13005821	0.340		0.920	0.920		rs1997502	0.350		0.232	0.216
	rs2138374	0.310		0.740	1.000		rs2302612	0.140		0.572	0.590
	rs12693527	0.200		0.888	0.888		rs1558626	0.460		0.888	0.888
	rs1131518			0.663	0.680	*IL18R1*	rs2287037	0.390		0.752	0.920
COL9A1	rs696990	0.160	X	0.322	0.406		rs2270298	0.270		0.202	0.148
	rs564031	0.400		0.192	0.192		rs1035130	0.270		0.320	0.294
	rs592121	0.320		0.327	0.359		rs1420096	0.480		0.084	0.053
	rs2076816	0.400		0.213	0.187	*IL18RAP*	rs1420106	0.210	X	0.240	0.245
	rs1200564	0.070		1.000	1.000		rs1420100	0.480	X	0.110	0.068
	rs997953	0.380		1.000	1.000		rs917997	0.200	X	0.191	0.244
COL9A2	rs449541	0.340		0.158	0.196	*MMP3*	rs645419	0.390		0.192	0.238
	rs364281	0.370		0.920	0.888		rs646910	0.250		0.322	0.380
	rs7533552	0.175	X	0.0033	0.0025	*MMP8*	rs1940475	0.470		0.791	1.000
COL9A3	rs3891033	0.450		0.127	0.134		rs2509013	0.380		1.000	1.000
	rs1046789	0.270		1.000	1.000		rs1276283	0.330		0.777	0.764
COL10A1	rs549332	0.470		0.178	0.125	*MMP9*	rs3918241	0.170		0.152	0.081
	rs1064583	0.340		0.030	0.025		rs2664538	0.380		0.271	0.322
	rs3812111	0.340		0.024	0.011		rs20544	0.490		0.087	0.091
	rs568725	0.320		0.002	0.004	*MMP13*	rs2252070	0.400		0.028	0.063
							rs3819089	0.110		0.192	0.227

* Noncorrected (significance threshold = 0.00073). Disc degeneration: X denotes SNP associated with any of the disc degeneration phenotypes in our previous study[4] ($p < 0.05$ after 1000 permutations). Dominant, Recessive: Dominant and recessive mode of inheritances in the association analysis using the Pseudomarker program. MAF: minor allele frequency in Finnish population; SNP: single-nucleotide polymorphism; OA: osteoarthritis.

Also, 3 SNP in LD with each other ($r^2 > 0.63$, $D' > 0.83$) in the *COL10A1* gene showed association with hip OA in the present study ($p = 0.0015$) but were not associated with any of the lumbar disc degeneration traits in our previous study (Table 2).

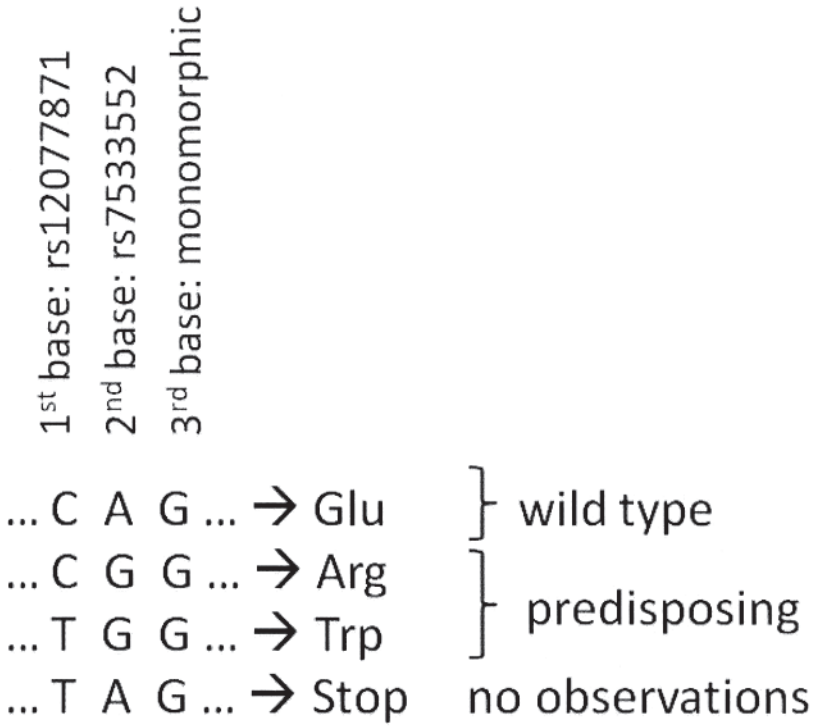

Figure 1. Single-nucleotide polymorphisms in the amino acid Gln^{326}.

Table 3. Amino acid combinations for variants in Gln^{326} codon in unrelated individuals.

SNP Combination			Corresponding Amino Acid	Function	Unrelated Carriers*	
1st base: rs12077871	2nd base: rs7533552	3rd base: monomorphic			Controls, n (%)	Hip OA, n (%)
C	A	G	Gln	WT	96 (67.1)	36 (49.3)
C	A	G	Gln	WT		
C	A	G	Gln	WT	41 (28.7)	28 (38.4)
C	G	G	Arg	P		
C	A	G	Gln	WT	3 (2.1)	1 (1.4)
T	G	G	Trp	P		
C	G	G	Arg	P	3 (2.1)	7 (9.6)
C	G	G	Arg	P		
C	G	G	Arg	P	0 (0.0)	1 (1.4)
T	G	G	Trp	P		
Total					143 (100)	73 (100)

* Unrelated individuals used in chi-square test: one twin of each monozygotic twin pair was randomly selected for genotyping. From dizygotic pairs, affected individual from discordant pairs and random individual from concordant pairs were selected. SNP: single-nucleotide polymorphism; OA: osteoarthritis; WT: wild-type allele; P: predisposing allele; Gln: glutamine; Arg: arginine; Trp: tryptophan.

DISCUSSION

We monitored for association between hip OA and 99 SNP that were previously studied with MRI-based lumbar disc degeneration phenotypes[4]. One SNP (rs7533552, also known as rs2228564) in the *COL9A2* gene was found to be associated with hip OA ($p = 0.0025$) and also with greater disc bulging in the L1-L4 lumbar region ($p = 0.036$; p value for the within-family component of multivariate regression analysis of disc bulging in L1-L4 discs using the quantitative transmission disequilibrium test program version 2.4.3, calculated with 1000 Monte Carlo permutations for correction of the skewed phenotype)[4]. It is possible that either one of the observed SNP associations is affected by the other trait, or that the SNP independently affects both traits.

Hip OA has previously been shown to associate with growth differentiation factor 5 (*GDF5*) with genome-wide

significance ($p = 1.8 \times 10^{-13}$)[11,12], while variants in SMAD family member 3[13] (*SMAD3*), acidic (leucine-rich) nuclear phosphoprotein 32 family member A[14] (*ANP32A*), deiodinase, iodothyronine type II[15] (*DIO2*), and frizzled-related protein[16,17] (*FRZP)* genes have shown association in replicated studies. Our finding is, to our knowledge, the first to suggest that the change from Gln^{326} to Arg or Trp in *COL9A2* gene predisposes to hip OA. Neither of the 2 SNP in the codon nor any SNP in high LD with them in European populations were represented on the Illumina HumanHap 610 genome-wide chip used in the genome-wide association studies. The Gln^{326} to Arg change has previously been reported to be more frequent in patients with severe and chronic back pain surgically treated for intervertebral disc herniation, although the association did not reach statistical significance[18]. The Gln^{326} to Trp change ("Trp2 allele") has been shown to be associated with unilateral back and radiating leg pain[19] and with degenerative lumbar spinal stenosis[20].

COL9A2 encodes one of the 3 alpha chains of type IX collagen, having an important role in load-bearing cartilages stabilizing the collagen fibrils, usually found in tissues containing type II collagen. Lack of type IX collagen can increase the susceptibility of the matrix to mechanical damage[21]. According to the PolyPhen protein structure prediction program, the change from glutamine to arginine is predicted not to cause major structural damage for the protein, but the change from glutamine to tryptophan is predicted as possibly damaging (Division of Genetics, Department of Medicine, Brigham and Women's Hospital – Harvard Medical School, Cambridge, MA, USA; available from: http://genetics.bwh.harvard.edu/pph/).

Association was also observed with the *COL10A1* gene that encodes 3 alpha 1 chains in type X collagen homotrimer. It is expressed by hypertrophic chondrocytes in a correlation with endochondral ossification and thus can be considered as a marker for new bone formation in articular cartilage[22]. Additionally, Boos, *et al*[23] found type X collagen in osteoarthritic cartilage of the hip when absent from normal adult cartilage.

We focused on a strictly narrowed measurable phenotype instead of on symptoms such as joint pain potentially caused by various biological mechanisms. The phenotype for bone changes was selected because of its known reliability/validity in OA grading on the basis of radiographic studies, but the lack of information on measurement reliability is a limitation. The high MZ correlation suggests that reliability must be high. The age range used also allows cases of early-onset hip OA to be included in the group of affected individuals. However, including the younger, seemingly healthy individuals, who may later develop the disease, in the group of unaffected individuals could decrease the power of detecting a true association. Also, the associations did not exceed the 5% significance level ($p = 0.00073$, according to the SNP SpD method)[9] when multiple testing was taken into account, and thus the results need to be interpreted carefully. Analysis in larger materials and functional studies is needed to validate the finding by excluding the possibility of a false-positive finding.

Ninety-nine SNP were analyzed in a population-based male twin cohort to study if there were common variants affecting hip OA and lumbar disc degeneration. One SNP (rs7533552) changing Gln^{326} to Arg or Trp in the *COL9A2* gene predisposed to hip OA and lumbar disc degeneration, making type IX collagen an interesting target for future studies.

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