Hip Osteoarthritis Susceptibility Is Associated with IL1B −511(G>A) and IL1 RN (VNTR) Genotypic Polymorphisms in Croatian Caucasian Population

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Received 28 October 2010; accepted 11 January 2011
Published online 24 February 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.21378

ABSTRACT: Among the predisposing factors to osteoarthritis (OA), a frequent destructive joint disease, is the complex genetic heritage including the interleukin-1 family members like the IL1β (IL1B) and the IL1 receptor antagonist (IL1RN) genes. The aim of this study was to investigate allelic and genotypic frequencies of the IL1B gene single nucleotide polymorphism (SNP) at −511(G>A) and the variable number tandem repeat (VNTR) in the IL1RN gene in a Croatian Caucasian population of hip OA (HOA) cases and healthy controls. A total of 259 HOA patients with total hip replacement (THR) and 518 healthy blood donors as controls were genotyped for IL1B gene SNP −511(G>A) and the VNTR in the IL1RN gene associated with HOA. The genotype G/A (1/2) at IL1B was significantly associated with the protection of the HOA ($p < 0.036$, OR = 0.72, 95% CI = 0.52–0.99). The genotype G/G (1/1) had only a trend towards the susceptibility ($p = 0.053$, OR = 1.35, 95% CI = 0.98–1.86) to disease. None of the haplotypes IL1B −511(G>A) and IL1RN (VNTR) were found associated with the HOA. The haplotype 1–2 at these loci had only a trend to susceptibility ($p = 0.065$). Haplotype 1–3 had a significant male bias in diseased. Furthermore, genotype comprising 2–1/2–2 haplotypes was found significantly associated with predisposition to HOA ($p = 0.027$, OR = 2.23, 95% CI = 1.03–4.88), whereas genotype 1–1/2–2 with protection to disease ($p = 0.028$, OR = 0.65, 95% CI = 0.43–0.97). Our findings suggest that HOA in Croatian population might have a different genetic risk regarding the IL1 locus than has been reported for other Caucasian populations previously. J Orthop Res 29:1137–1144, 2011

Keywords: genetic susceptibility; osteoarthritis; hip

Osteoarthritis (OA) is the most frequent joint pathology associated with health problems for middle aged and older people. It is a debilitating, chronic, progressive, and multifactorial disease of joints. It is important to research into pathogenesis, clinical aspects, and treatment of the disease because it has high incidence, prevalence and significant medical, social and economic influence on a society. Due to a wide clinical heterogeneity, it is unclear whether OA is a single disease or a mix of distinct disorders regarding which tissues were principally involved in the initiation and progression of the disease. OA has two main types: (1) primary, with the late onset and still uncertain cause, and (2) secondary, which is distinguished by the early onset with the known cause like developmental abnormalities, trauma, or the like.[1]

The reasons for the development of the primary disease have not been completely understood and many factors were implied to influence the risk of OA like age, sex, genetics, ethnicity, behavioral influences, obesity, and occupation.[2,3] Concerning genetic linkage, many family studies have suggested a complex (polygenic) hereditary influence in predisposition to OA.[1,4–18] In particular, both sex-specific and anatomic site-specific genes are likely to influence an individual's risk of developing OA. Furthermore, it is known that complex genetic diseases might appear to be caused by various genes in different populations. Several loci on various chromosomes were identified to date to be associated with disease, and thus thought to modify the risk to OA. Four genome wide studies revealed several chromosomal loci linked with the disease (for a review see Peach et al.[1]). One of them, interleukin-1 gene cluster on chromosome 2q13, includes genes belonging to a functional group of pro-inflammatory cytokines (IL1, TNF, and IL6).[19] However, a candidate gene approach would also identify the IL1 family of pro-inflammatory genes as a candidate in changing the risk for OA since the IL1 cytokine has been implicated in joint damage due to stimulation of production of proteases that can degrade the extracellular matrix proteins of cartilage.[9]

Several case/control genetic studies concerning susceptibility to OA gave controversial results. The IL1 gene cluster was found to be associated with knee OA (but not with hip OA, HOA) in UK population.[19] By contrast, a study by Moos et al.[20] analyzing IL1RN variable number tandem repeat (VNTR) in total hip replacement (THR) patients revealed a higher risk for development of HOA, and Meulenbelt et al.[21] corroborated that, albeit in radiographically diagnosed OA (ROA) patients. However, Smith et al.[22] showed that OA of knee joints, but not of the hip, is associated with IL1 gene cluster using additional polymorphic genetic

All authors disclose any potential conflicts of interest, including specific financial interests relevant to the subject of their manuscript.
Ethics approval: The study was approved by the Medical ethics committees of the Clinic for Orthopaedic Surgery Lovran, and School of Medicine, University of Rijeka, Croatia.
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markers within the cluster and combining them into extended-risk haplotypes. A study by Moxley et al.[23] showed that the extended-risk IL1 cluster haplotype conferred a higher risk also for hand OA.

Frazer et al.[24] noted that associations of the single nucleotide polymorphism (SNP) identified in one population are rarely transferable to members of the other populations, if the risk is divided over a larger gene pool. To ascertain this, we investigated the association to primary HOA in Croatian Caucasians with a severe HOA that have undergone THR using two polymorphic markers for the IL1 region: IL1B (−511 G>A) and IL1RN (VNTR).

PATIENTS AND METHODS

Subjects

The clinical criteria for admission to this case–control study were the American College of Rheumatology (ACR) guidelines for the classification and reporting of HOA[25] and Western Ontario and McMaster Universities' Osteoarthritis (WOMAC) index to assess pain, joint stiffness and physical function in patients with HOA.[26] Indications for primary THR used in our study were according to the National Institutes of Health (NIH) Consensus Statement on THR, that include: joint pain, functional limitation, and radiographic evidence of joint damage (in our case: grade range 55–75, average 66).[27] The study included Croatian (259) patients with HOA (mean age = 67.82 years, standard deviation (SD) = 9.61, range: 31–90). Control individuals were 518 blood donors (as; mean age = 41.58 years, SD = 11.72, range: 19–91), who were healthy according to criteria in admitting blood donors by the Department of Transfusiology, University of Rijeka, Croatia. The consequences of comparing such controls (some of which might develop OA later in life) with OA patients are that our results might be more conservative. We excluded from the study patients with primary HOA without indication for THR (i.e., less severe OA) or assigned informed consent. Similarly, patients with any secondary form of HOA and rheumatoid arthritis were excluded too. The study was approved by the Medical ethics committees of the Clinic for Orthopaedic Surgery Lovran, and School of Medicine, University of Rijeka, Croatia.

Radiography and Interpretation

On the day of admission to the hospital, a supine anteroposterior pelvic radiograph including both hips was obtained for every patient included in the study using the same protocol. Patient was positioned in the supine position on the X-ray table with both lower extremities oriented in 15° of internal rotation. The focus-to-film distance was 100 cm. The X-ray beam was oriented perpendicular to the table and centered approximately 2 cm above the superior aspect of the symphysis pubis. Each radiograph was assessed for five individual radiographic features (IRFs) of HOA: (1) Kellgren–Lawrence (*K/L*) grading scale (0–4),[28] (2) superomedial and superolateral joint space narrowing (JSN 0–4), (3) lateral (femoral and acetabular) and medial (femoral and acetabular) osteophytes (for each location 0–3, for all four locations 0–12), (4) subchondral cysts, sclerosis, and femoral head deformity (0 for absent and 1 for present), and (5) minimal joint space (MJS) from acetabular roof to the femoral head using digital Vernier caliper. During the readings of the radiographs, we were consulted atlas figures to improve reliability.[29] We assigned to each hip, based on IRFs, a summary grade (modified Croft grade) for radiographic HOA (RHOA) severity (0–4).[30–32] We further characterized all of our patients in three distinct RHOA phenotypes (composite, osteophytic, and atrophic), according to previously described phenotypic definitions for RHOA phenotypes.[31] All radiographs were evaluated by two experienced readers (BS and RM) to reach consensus score. Intra-rater reliability for the radiographic readings was evaluated from a random sample of 54 radiographs. The kappa coefficients were for *K/L* grading scale 0.92, for MJS 0.87, for JSN in any location 0.81, for definite osteophytes in any location 0.69, and for summary grade 0.63.

DNA Isolation

For extraction of genomic DNA, 200 μl of whole blood was mixed with 400 μl of sucrose buffer (0.32 M sucrose, 10 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 1%, v/v, Triton X-100) and incubated for 1 min at room temperature. To collect white cell nuclei, samples were centrifuged 2 min at 5,000*g*. Precipitated nuclei were washed twice with 800 μl of sucrose buffer and centrifuged (2 min at 5,000*g*). After the second wash the nuclei were resuspended in 400 μl DNAzol (Invitrogen Corporation, Carlsbad, CA) and incubated at room temperature for 5 min. Genomic DNA was precipitated with 200 μl of 100% ethanol and collected by centrifugation (2 min at 5,000*g*). The precipitate was washed twice with 1 ml of 75% ethanol and centrifuged (1 min at 5,000*g*). Genomic DNA was resuspended in 100 μl of 8 mM NaOH and allowed to solubilize for 15 min at room temperature. HEPES buffer (16 mM HEPES in 8 mM NaOH, pH 7.5) was used to adjust the pH 7–8.4. The average concentration of genomic DNA was 30 μg/ml with 260/280 OD ratio higher than 1.7.

SNP Analysis

Allele discrimination assays were performed by the PCR method. In short, the alleles of the IL1B gene at −511(G>A) (rs16944) were detected by Taqman method using the primers and probe as reported previously.[33] The IL1 receptor antagonist (IL1RN) VNTR were amplified by primers as described previously[33] and then detected by 1.5% agarose gel electrophoresis. The VNTR PCR detects variability in the repetition of 89 nucleotides in genomic DNA close to the IL1RN gene. The individuals of Caucasian origin have one and four copies of the repeats in 95% of cases. Their alleles were denoted as follows: allele 1 = 4 repeats; allele 2 = 2 repeats; allele 3 = 5 repeats, and allele 4 = 3 repeats.

Statistical Analysis

Statistical analyses were done by chi-square method (Statcalc program, Acastat software) comparing the allelic and genotypic setup of the SNP loci between cases and controls. The assembly and prediction of haplotypes for the IL1 cluster was done by the software program "Phase."[34,35] The Hardy–Weinberg and linkage disequilibrium analyses for the SNP and VNTR was assessed by the Arlequin software ver. 3.5 (Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland). These two loci were in the Hardy–Weinberg equilibrium and were in linkage disequilibrium. A statistically significant difference was defined when p was <0.05, and a trend to association and a significant association were cases when statistical significance of a difference was approaching 0.05 (0.1–0.05) or when $p < 0.05$ but 95% confidence index (CI) limits spanned values that crossed the value of 1. We did not use Bonferroni correction because the two polymorphic markers were in linkage disequilibrium ($r^2 > 0.83$). This means that these two

genetic markers are inherited as a block. We used the haplotype analysis because the genetic area between these two loci harbors six more IL1- and IL1RN-like genes.

RESULTS

Table 1 summarizes frequencies of allelic/genotypic variants of the IL1β (IL1B) and the IL1 receptor antagonist (IL1RN) genes in our case–control study. Statistical analysis was done by comparison of the frequencies in the healthy blood donor group with those of patients that have surgically replaced their hip joint.

In the Table 1, allelic frequencies for IL1B SNP −511(G>A) do not differ between patients and controls. However, the genotype frequencies for G/A (1/2) at IL1B was significantly associated with the protection of the HOA in Croatian population ($p = 0.036$) with odds ratio (OR) = 0.72 and 95% CI limits = 0.52–0.99. However, the homozygous genotype G/G showed only a trend to significant difference ($p = 0.053$, Table 1) and did not convincingly support association. A similar analysis of IL1RN VNTR locus showed differences between patient and control populations that were not statistically significant (Table 1).

As presented in Table 2, the frequencies of various haplotypes comprising IL1B −511(G>A) and IL1RN (VNTR) differ slightly among controls and patients, but none of them were found statistically significant. Despite a finding that a haplotype 1–2 showed a trend towards significance ($p = 0.065$), the two marker combination haplotype analysis revealed no association with the HOA in this population.

However, as shown in Table 3, a genotype comprising 2–1/2–2 haplotypes were found significantly associated with predisposition to HOA with $p = 0.026$ (OR = 2.23, 95% CI = 1.03–4.88). Interestingly, another genotype (1–1/1–1) was found statistically significantly different between cases and controls with $p = 0.047$, but due to large 95% CI limits (0.99–1.97) it was considered a trend, and thus not significantly associated with predisposition to OA (Table 3). Similarly, a genotype 1–2/1–2 was also found to have a trend toward statistically significant difference between cases and controls ($p = 0.06$). Importantly, a genotype 1–1/2–2 was significantly associated with protection to disease ($p = 0.028$, OR = 0.65, 95% CI = 0.43–0.97).

The analysis for gender differences revealed great variations at genotypic (Table 4) and haplotypic levels (Table 5).

Genotypes 1–1/1–3 and 1–1/2–1 were significantly associated with a bias to higher incidence of OA in either males ($p < 0.04$, OR = 0.11, 95% CI = 0.00–1.19) or females ($p < 0.03$, OR = 2.45, 95% CI = 1.05–6.41), respectively (Table 5).

The haplotype 1–3 (Table 5) was significantly associated with a bias towards male population in acquiring HOA ($p = 0.006$, OR = 0.13, 95% CI = 0.01–0.70).

Considering radiographic features and radiographic phenotypes of HOA patients, all of our patients have a *K*/*L* score of ≥3, superomedial and superolateral JSN was ≥2 (>90% of our patients had a JSN score of 3 or 4 in one of both locations), overall osteophytes score in all four locations was >8, and MJS was <1 mm (average 0.92 mm). Regarding the RHOA phenotypes, we have observed just two different phenotypes. Composite phenotype was present in 66% and atrophic phenotype in 34% of our patients. In female patients we have observed in 78% of cases composite phenotype, while in male patients we recorded the same radiographic phenotype in 57% of cases. We have not observed osteophytic RHOA phenotype in our patients with end-stage HOA.

Table 1. Association Analysis of IL1B SNP and IL1RN VNTR With Hip OA in the Croatian Population

		Allele Frequency[a]					Genotype Frequency[a]			
IL1B[b]	SNP −511	Patients, $n = 518$	Controls, $n = 916$	p-Value (OR)	IL1B[c]	SNP −511	Patients, $n = 259$	Controls, $n = 458$	p-Value	OR (95% CI)
1	G	0.678 (351)	0.644 (590)	0.20 (1.16)	1/1	G/G	0.467 (121)	0.393 (180)	0.053	1.35 (0.98–1.86)
2	A	0.322 (167)	0.356 (326)		1/2	G/A	0.421 (109)	0.502 (230)	0.036	0.72 (0.52–0.99)
					2/2	A/A	0.112 (29)	0.105 (48)	0.77	

		Allele Frequency[a]					Genotype Frequency[a]		
IL1RN[b]	VNTR	Patients, $n = 164$	Controls, $n = 974$	p-Value (OR)	IL1RN[c]	VNTR	Patients, $n = 232$	Controls, $n = 487$	p-Value
					1/1	(4/4)	0.509 (118)	0.509 (248)	0.99
1	(4)	0.694 (322)	0.703 (685)	0.72	1/2	(4/2)	0.336 (78)	0.350 (173)	0.62
2	(2)	0.282 (131)	0.272 (265)	0.68	1/3	(4/5)	0.035 (8)	0.031 (15)	0.79
3	(5)	0.024 (11)	0.024 (23)	0.99	1/4	(4/3)	0	0.002 (1)	1.00
4	(3)	0	0.001 (1)	1.00	2/2	(2/2)	0.108 (25)	0.090 (44)	0.46
					2/3	(2/5)	0.013 (3)	0.008 (4)	0.69
					3/3	(5/5)	0	0.004 (2)	1.00

[a]Frequency (number). [b]Allele designations. The copy numbers of the VNTR are in the parenthesis. [c]Genotype designations. The copy numbers of the VNTR are in the parenthesis.

Table 2. Predicted IL1B–IL1RN Haplotype Frequencies and Haplotype Association Analysis in Hip OA

IL1B −511/IL1RN VNTR		Total Population			Controls (C)			Patients (P)			C vs. P
Haplotypes[a]	Other Designations[b]	E (freq)	SE	N = 1436	E [Freq(0)]	SE (0)	N = 918	E [Freq(1)]	SE (1)	N = 518	p-Value
1–1	G(4)	0.53238	0.00493	797	0.53060	0.00566	507	0.53555	0.00723	290	0.78
1–2	G(2)	0.09984	0.00480	111	0.08927	0.00557	62	0.11857	0.00683	49	0.065
1–3	G(5)	0.02302	0.00124	33	0.02280	0.00124	21	0.02342	0.00260	12	0.97
1–4	G(3)	0.00074	0.00017	1	0.00112	0.00019	1	0.00006	0.00033	0	1.00
2–1	A(4)	0.17039	0.00501	230	0.17957	0.00567	151	0.15411	0.00692	79	0.55
2–2	A(2)	0.17025	0.00498	261	0.17474	0.00564	175	0.16229	0.00689	86	0.25
2–3	A(5)	0.00336	0.00093	3	0.00187	0.00083	1	0.00600	0.00194	2	0.30
2–4	A(3)	0.00001	0.00010	0	0.00002	0.00015	0	0.00000	0.00000	0	—

E, estimated frequency; SE, standard error. [a]IL1B −511 SNP major allele-1 or G; 2 or A-minor. [b]VNTR copy number in parenthesis. Hapotype 2–4 or A(3) was not found in our samples.

We did not notice significant differences neither in the severity grade nor in the kind of the OA of women and men that carry genotypes 1–1/2–2 and 2–1/2–2, which were found associate with protection and susceptibility to OA, respectively. The sex differences were found significantly different in genotype 1–1/2–1.

DISCUSSION

The controversial role of the IL1 gene cluster in affecting the genetic risk to HOA was examined in seven previous studies (Table 6) comprising at least three ethnic populations (German, United Kingdom, and Dutch).[19–22,36,37,40]

Moos et al.[20] pointed to an association between the IL1β polymorphism and the TNFα^{high} phenotype and between the IL1Ra polymorphism and the TNFα^{low} phenotype found in 61 (German) patients with end-stage hip and knee OA who had undergone joint replacement surgery. Previously, the same group[38] suggested that IL1β may be more important than TNFα for the regulation of cytokine and growth factor expression in articular chondrocytes. Also, they suggested that the balance between IL1β and IL1Ra regulates cartilage synthesis and degradation. Their results suggested that the deregulated cytokine expression promoted by the respective genotypes leads to an enhanced expression of matrix-degrading metalloproteinases and a reduced synthesis of matrix components, and thus contributes to the development of OA.[39] Loughlin et al.[19] genotyped eight common polymorphic variants (seven SNPs and one VNTR) located in the IL1 ligand gene cluster in a study on 557 patients with hip and knee OA (almost 400 patients with HOA who had underwent THR) and showed that the IL1 gene cluster is associated with susceptibility to knee OA but not to HOA.

Table 3. IL1B–IL1RN Genotype Association Analysis in Hip OA

Genotypes		Controls (C), $N = 459$	Patients (P), $N = 259$	C vs. P, p-Value	Odds Ratio (95% CI)	Association
1–1	1–1	122	87	0.047	1.4 (0.99–1.97)	Trend to susceptibility
1–1	1–2	38	22	0.92		
1–1	1–3	12	5	0.56		
1–1	1–4	1		1.00		
1–1	2–1	104	46	0.12		
1–1	2–2	108	43	0.028	0.65 (0.43–0.97)	Protection
1–2	1–2	4	7	0.06	3.16 (0.79–14.84)	Trend to susceptibility
1–2	1–3	1		1.00		
1–2	2–2	15	13	0.24		
1–3	1–3	2		0.54		
1–3	2–1	2	2	0.62		
1–3	2–2	2	3	0.36		
1–3	2–3		2	0.13		
2–1	2–1	15	7	0.67		
2–1	2–2	14	17	0.026	2.23 (1.03–4.88)	Susceptibility
2–1	2–3	1		1.00		
2–2	2–2	18	5	0.15		

Table 4. Hip OA: Male–Female Analysis of IL1B–IL1RN Genotypes

Genotypes		Female pt, $N = 170$	Male pt, $N = 81$	Female vs. Male pt, p-Value	Odds Ratio (95% CI)	Association
1–1	1–1	61	25	0.43		
1–1	1–2	14	7	0.91		
1–1	1–3	1	4	0.039	0.11 (0.00–1.19)	Male bias
1–1	2–1	36	8	0.028	2.45 (1.05–6.41)	Female bias
1–1	2–2	28	14	0.87		
1–2	1–2	5	2	1.00		
1–2	2–2	9	4	1.00		
1–3	2–1		1	0.32		
1–3	2–2	1	2	0.24		
2–1	2–1	3	4	0.22		
2–1	2–2	10	7	0.42		
2–2	2–2	2	3	0.33		

Moxley et al.[40] made IL1 region meta-analysis on 1,238 European-descent cases with various OA phenotypes and 1,269 European-descent controls from four study centers. For HOA, data from three centers showed heterogeneity of the extended-risk-haplotype effect, two haplotypes showing trend toward risk and another haplotype showing protection. The heterogeneity fell partly along control ascertainment lines, chiefly between controls ascertained as spouses of arthroplasty patients and controls identified through population radiographic survey. They concluded that meta-analysis data do not confirm but only suggest that some hand and HOA risk could be associated with the IL1 region, particularly centered in IL1B and possibly also IL1RN. However, we believe that genetic studies from ethnically defined populations have a potential in identifying certain low-risk genetic loci of complex hereditary diseases. Because many genes could work together to convey a phenotypic trait like, for example, a protection against OA, a mutation in any of such genes would pose a threat to their combined action thus lowering perhaps the robustness of joints to mechanical injury. As various mutations in genes happen over time, mutations would tend to separate and accumulate in different human populations. A particular human population would then carry a slightly different genetic risk to disease compared to other populations. Hence, pooling data for meta-analysis might blur such putative genetic connection and fail to discover a variety of alleles conferring a low risk to OA.

For this reason we studied HOA in Croatian population using two polymorphic markers from these two loci. First, concerning allelic frequencies, we found that the heterozygous (1/2 or G/A) genotype of IL1B (−511 G>A) SNP was significantly associated with the protection to HOA in Croatian population. Second, with regard to haplotypic frequencies, none of the haplotypes were significantly associated with HOA in our study. Third, a genotype comprising 2–1/2–2 haplotypes was found significantly associated with predisposition to HOA. On the other hand, a genotype 1–1/2–2 was significantly associated with protection to disease. Lastly, regarding the sex bias, a haplotype 1–3 was significantly associated with a bias towards male population in acquiring HOA, and genotype 1–1/2–1 was significantly associated with a bias to higher incidence of OA in females.

The two genotypes 1–1/2–2 and 2–1/2–2 had frequencies of 21% and 4.3% in total population (16.6% and 6.6% in diseased and 23.5% and 3.1% in controls), respectively. Thus, in approximately one-quarter of our study we have found markers associated with modulated risk of developing OA in this population. It is generally assumed that the power of a case–control study is comparable to the frequency of a genotype (or haplotype) in general population. The higher the frequency, the higher the power it

Table 5. Hip OA: Sex Differences in IL1B–IL1RN Haplotype Analysis

IL1B −511/IL1RN VNTR							
Haplotypes[a]	Other Designations[b]	Total, $N = 502$	Female (F), $N = 340$	Male (M), $N = 162$	F vs. M, p-Value	OR (95% CI)	Association
1–1	G(4)	284	201	83	0.096		
1–2	G(2)	48	33	15	0.87		
1–3	G(5)	9	2	7	0.006	0.13 (0.01–0.70)	Male bias
2–1	A(4)	76	52	24	0.89		
2–2	A(2)	85	52	33	0.16		

[a]IL1B −511 SNP major allele-1 or G; 2 or A-minor. [b]VNTR copy number in parenthesis.

Table 6. Associations of the IL1 Gene Cluster Polymorphisms With Hip OA

Study	Geographic Ancestry	No. of Hip OA Cases	Genotyped Markers of IL1 Region			Association
			IL1A	IL1B	IL1RN	
Moos et al.[20]	German	61[a]	—	3953	VNTR	Positive
				3953		
Loughlin et al.[19]	United Kingdom	390	−889	5810	VNTR	Negative
				−31	9589	
				−511	11100	
Meulenbelt et al.[21]	Dutch	70	—	3953	VNTR	Positive
				−511		
				3953	VNTR	
Smith et al.[22]	United Kingdom	44	−889	−31	8006	Positive
				−511	11100	
				3953	VNTR	
Meulenbelt et al.[36]	Dutch	70	−889	−31	8006	Positive
				−511	11100	
Chapman et al.[37]	United Kingdom	370	—	3953	9589	Negative
				−511		
Moxley et al.[40]	United Kingdom and Dutch	252	−889	3958	VNTR	Some hip OA risk could be associated with the IL1 region
				−31	8006	
				−511	11100	
This work (Jotanovic et al.)	Croatian	259	—	−511	VNTR	Positive

[a]Patients with end-stage knee or hip OA.

would have, provided predisposition to OA has a Mendelian character. Since OA belongs to a multifactorial nonmendelian class of hereditary diseases, a particular associated genotype (or haplotype) has potentially a greater power in the search for predisposing factors than anticipated by its frequency. For example, if our associated genotypes represent markers for putative risk factor(s), the latter could be spread to some extent over other genotypes. Such genotypes would appear not to be linked to disease due to variability of typed markers and especially because of the lack of other hypothetical risk factors. Therefore, the other genotypes might still harbor the putative risk factors detected in genotypes 1–1/2–2 and 2–1/2–2 to an unknown extent.

In our study with 259 patients with end-stage HOA, we have observed only two RHOA phenotypes. A possible explanation of these results is advanced stage of HOA and high average age of our patients (almost 68 years). Namely, in all our patients, superomedial and superolateral JSN was $\geq$2, and that is the reason why we could not classify any of those patients in the group of osteophytic phenotype. The comparison of radiographic phenotype prior to surgery with genotyping indicated that there were neither significant difference in the severity grade nor in the kind of the OA of women and men that carry genotypes 1–1/2–2 and 2–1/2–2 which were found associated with protection and susceptibility to OA, respectively. In fact, there were equal numbers of either sex in these haplotypes. The sex differences were found significantly different in genotype 1–1/2–1, which would additionally point to complex susceptibility of the disease. In this group, women patients had more either severe (grade 4) or atrophic kind of radiographic phenotype than men.

Our results conform to a view that a combination of predisposing genetic factors may play a role in developing OA. The steps to be taken to decipher them in the future would be the candidate gene approach based on our current study and literature.[41] These include genes that regulate chondrocytes differentiation and survival like the IL1 and IL4 cytokines, the secreted frizzled-related protein 3 gene, and the asporin gene. It is important to establish their global relevance by genotyping OA cohorts from different ethnic backgrounds. We would also select the control population matching the age of the cases allowing less conservative approach in the analyses, which could help in identifying low-risk factors. In addition, stratification along the severity grades based upon radiological profiling might help in searching for new associations and susceptibility factors.

It is plausible that IL1 can affect the chondro-homeostasis by aberrant dynamics of its production. Hence, it is possible that an epigenetic event (like promoter methylation or histone acetylation) causing increased IL1 production could raise the risk for developing OA. Whether OA risk can be modulated solely by epigenetic event(s) or by a combination of genetic and epigenetic ones, remains to be investigated.

Our study is the first of this type in the Croatian (Caucasian) population. Regarding the role of the IL1 gene cluster in affecting the genetic risk to HOA, our results seem to be in agreement with studies by Moos et al.[20] and Meulenbelt et al.[21], and are in disagreement with the study by Loughlin et al.[19] One of the weaknesses of the study relates to the prevalence of HOA in the general population (by radiography and clinical examination), which increases sharply with age. Henceforth, a group of seemingly healthy subjects at the age of 40 will contain more of those who will develop HOA by the age of 70. The consequences of comparing the random population controls (as we did) with OA patients with higher age than controls are that the potential differences might have been blurred, and that the trends for associations we observed might have been more pronounced. It means that our results were on the conservative edge, and perhaps that with an age-matched control group, our results might have been more persuasive if not statistically significant. Other weaknesses consider the group size of patients, which if increased, could perhaps alleviate the possible type 1 error in statistical analyses regarding the female–male ratio differences and/or haplotype analyses. To our defense, our study is the third largest amongst previously reported (Table 6).

The aggressiveness of treatment of the OA will hopefully depend on the molecular profile of patients in the future. If it would be such that it would point to a low risk of progression, a treatment would be conservative. In the case of a high risk of progression, the treatment would be more aggressive. We would thus be able to treat patients with new therapies including novel active small molecules, proteins, genes, and/or cells. If the nonsurgical treatment of patients with OA will have failed or there will have been progression of the disease, the molecular profile of patients would be helpful in choosing the type of surgical treatment for each patient individually, such as selection of the most favorable type of prosthesis for an individual requiring total joint replacement.[42] The use of molecular orthopedics will enable better prevention in the future, various modalities of biological treatment, more nonsurgical and less surgical treatment, fewer surgical complications, and an individualized approach to each patient with OA.

ACKNOWLEDGMENTS

This work was supported by grants from the University of Oslo.

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