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Alternative Interleukin 17A/F Locus Haplotypes Are Associated With Increased Risk to Hip and Knee Osteoarthritis

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ABSTRACT: We studied the genetic epidemiology of primary large-joint (hip and knee) osteoarthritis (OA), in order to find disease risk factors by a candidate-gene approach. We used case–control study in the Croatian Caucasian population. We genotyped 500 OA patients (260 hip, 240 knee; both with total joint replacements) and 597 healthy individuals for single-nucleotide polymorphisms (SNPs) in interleukin 17A (IL17A) (rs2275913) and IL17F (rs763780 and rs1889570) genes. On the basis of our population and allelic and genotypic frequencies haplotypes were predicted by PHASE software and compared between patients and controls. The three-SNP haplotype (rs2275913–rs763780–rs1889570) G–C–A confers predisposition to hip ($p < 0.005$) but not knee OA. The three-SNP haplotype having opposed nucleotides A–T–G was found significantly associated with 2.6 times higher risk for developing knee ($p < 0.02$) but not hip OA. The *haplotype* G–T (IL17A–IL17F; rs2275913–rs763780) is associated with protection to the disease in hip OA ($p < 0.01$). Our analyses show that two disparate haplotypes within the IL17A-F gene locus are associated with higher risk to developing hip and knee OA in the Croatian population. The data might suggest a difference in the etiology of hip OA from that of the knee OA, perhaps due to an unknown dissimilarity in vulnerability of these joints to the actions of IL17. Alternatively, other differences in genetic factors like the long non-protein coding region LINCMD1 and/or microRNA species like miR133b and miR206 found in the vicinity of the IL17 locus might be involved in the observed risk. © 2019 The Authors. *Journal of Orthopaedic Research*® published by Wiley Periodicals, Inc. on behalf of Orthopaedic Research Society. J Orthop Res 37:1972–1978, 2019

Primary osteoarthritis (OA) is a complex genetic disease of unknown origin that has close to 100 genetic loci associated with the onset of disease. Each of these might contribute by a minute part of the risk. They are distributed across various human populations in an unknown manner. Our goal is to map the risk factors and understand their role in the OA pathogenesis. We have started by studying inflammatory cytokines that seem to be associated with susceptibility to development of OA,[1–3] which could therefore be important for understanding primary OA pathophysiology. Following the same aim, the IL17 gene locus, whose products could increase predisposition to primary OA, is located on chromosome 6 (6p12.3-q13), in the area found previously to harbor such genetic risk in the UK population.[4] The genes at this locus encode interleukin 17A (IL17A) and IL17F cytokines,[5] which are involved in the innate and adaptive immune responses. Some autoimmune diseases and organ allograft rejection were reported following disruption in their production,[6] and IL17A/F cytokines could contribute to the development of OA.[7] Activated CD4^{+} T cells of the Th17 type produce the IL17. In the synovial sublining layer of

patients with OA,[8] IL17A/F has been detected. It exerts its action via IL17 receptor A (IL17RA, CD217), which is found on many cells and tissues.[7] Because T-cell reactivity against chondrocyte surface antigens was detected in patients with OA,[9] it has been surmised that IL17A plays an important role in pathogenesis of OA. Additional roles have been assigned to IL17 cytokines involving pathologic processes like connective, intestinal, and airway tissue inflammation with granulocyte mobilization, and physiologic processes including release of cartilage matrix or prevention of its synthesis.[10–12]

In chondrocytes, IL17 induces other pro-inflammatory cytokines like IL1β, tumor necrosis factor-α (TNF-α), and IL6. Chondrocytes under influence of the IL17 upregulate the production of nitric oxide and matrix metalloproteinases, and reduce proteoglycan levels.[13–15] Furthermore, in patients with OA, IL17 stimulates the release of vascular endothelial growth factor in synovial fibroblasts isolated from their joints.[16]

Several genome wide studies have investigated the risk factors in OA of different joints (hip, knee, and hand) in Iceland [17] and United Kingdom.[18] Other loci identified in these studies point to genes that perhaps have a physiologic role in joint development like Chondroadherin like (CHADL) on chromosome 22 (involved in chondrocyte differentiation) and Cartilage oligomeric matrix protein (COMP) on chromosome 19 (involved in development of noncollagenous extracellular matrix) in Icelandic population.[17] On chromosome 6, recently,[18] several loci have been significantly

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associated with risk to OA. Some have a role in bone development like BMP5 (about 3 million bp downstream from the IL17 locus), histone activity like HIST1H2AC (26 million bp upstream), adaptive immune responses like HLA-DPB1 (about 20 million bp upstream), or some other unknown feature(s) like a single-nucleotide polymorphism (SNP) (rs12154055) located approximately 8 million bp upstream of the IL17 locus.[17] Over 50 other genetic variants have been distributed over all chromosomes,[18] and their odds ratios (ORs) (which is an indicator of the relative risk) have been rather small, barely 5–10% above the normal level, indicating that the overall risk for development of OA is likely a conglomeration of all minor risks. The case complicates when many of these genetic susceptibilities are found specific for a certain population or even subpopulation.

With this in mind we set out to investigate the IL17A-F locus using haplotype analyses in our case–control subpopulation from Croatia (patients were mainly with total or partial arthroplasty of large joints—hip and knee). We asked why did SNP IL17F rs763780 show significant association with hip OA (HOA) but not knee OA (KOA) in our previous study.[19] Since haplotype analysis is known to increase the power of a study, we performed a set of additional analyses on the same data set. We made haplotype predictions using known software tools, and found indeed that the IL17 locus harbors predisposition for KOA, whose significance we discuss and suggest novel lines of research.

METHODS

The patients were diagnosed using the clinical criteria for standard HOA or KOA diagnosis by the American College of Rheumatology guidelines and Western Ontario and McMaster Universities' Osteoarthritis (WOMAC) index to assess pain, physical function, and joint stiffness.[20–22] For radiological assessment of OA changes in hip and knee joint we used Kellgren–Lawrence grading scale from 1 to 4 (4 is the worst stage) on anteroposterior and lateral joint radiographs.[23] The inclusion criteria for participation was clinical and radiological diagnosis of primary HOA or KOA, indication for total knee arthroplasty (TKA), partial (unicondylar, unicompartmental) knee arthroplasty (PKA), and total hip arthroplasty (THA) according to the National Institutes of Health Consensus Statement.[24,25] The exclusion criteria were (i) patients without indication for TKA, PKA, or THA surgery; (ii) unsigned or withdrawn informed consent; (iii) secondary form of HOA or KOA; and (iv) rheumatoid arthritis of hip or knee joint.

The study included in total 500 OA patients, with 260 HOA and 240 KOA patients, of which 67.3% (175) HOA patients were female, and 32.7% (85) were male. The mean age of HOA patients was 67.82 years (standard deviation [SD]: 9.61) with a range from 31

to 90. The mean age of female HOA patients was 68.90 years, with a range from 46 to 87, and the mean age of male HOA patients was 65.63 years, with a range from 31 to 90 years.

The 240 KOA patients included 72.5% (174) women and 27.5% (66) men. The mean age of KOA patients was 69.74 years (SD: 7.24), with a range from 47 to 86. The average age of female KOA patients was 69.68 years, with a range from 47 to 86. The average age of male KOA patients was 69.91 years, having a range from 52 to 82 years.

The control group comprised 597 healthy persons, of which the majority were voluntary blood donors and the rest were medical personnel at the clinical hospital center. Women covered 25.5% (152) of the controls, whereas 74.5% (445) were men. Their mean age was 42.64 years, (SD: 11.12), with a range from 19 to 91 years. Radiological analysis (X-rays) of knees and hips in the control group has not been done because of ethical and financial limitations.

Statistical Analyses

The estimation of haplotypes for the IL17A–IL17F gene locus was done by the software program "Phase."[26,27] First, we predicted two-SNP haplotypes (either IL17A rs2275913–IL17F rs763780 or IL17F rs763780–IL17F5′ rs1889570) and analyzed them for the association with the disease by χ^2 statistics software found on the Internet as contingency tables (http://statpages.info/ctab2x2.html).

Next, the three-SNP haplotypes were estimated (IL17A rs2275913–IL17F rs763780–IL17F5′ rs1889570) by "Phase" and similarly analyzed for the association with the disease by χ^2 statistics. The rationale for increasing the number of loci included in the haplotype analysis was to increase the power of detection of significant differences. For post hoc analysis, statistical power $1 - \beta$ was computed as a function of significance level α, sample size, and population effect size.

We used power calculators as described in the program "G*Power 3.1."[28] Using data from our previous report, the post hoc power in the single allele SNP rs763780 (IL17F) analysis was surprisingly low (5.2%) for KOA patients compared with HOA (86.4%).[19] This indicated that we could have missed a significant marker in this genetic area for KOA. Calculating a priori requirements for haplotype analyses, for 95% actual power, we would need 557 controls and at least 915 KOA patients. Since we lack the latter, we computed post hoc power, which was 49,0%. This was the achieved power with given α (0,05), sample size ($n = 502$) and effect size of 5.73% change in haplotype frequency. Similar calculations for HOA patients gave a post hoc power of 45,5%, for $\alpha = 0.05$, sample size ($n = 486$) and effect size of 2.3% increase in haplotype frequency. The calculations were done with the Fischer's exact test surmising cases and controls were two independent groups.

Table 1. Haplotype Analysis IL17A SNP (rs2275913)–IL17F SNP (rs763780) With Hip OA in Croatian Population

		Frequency (n)						
IL17A–IL17F	Haplotypes[a]	OA Hi24p— Patients	Controls	p	Odds Ratio	95% CI	χ^2 test	Association
1	G–T	0.593 (175)	0.672 (329)	6×10^{-4}	0.66	0.49–0.90	7.60	Protection
2	G–C	0.064 (26)	0.019 (11)	0.43×10^{-4}	4.06	1.89–8.90	16.73	Predisposition
3	A–T	0.311 (102)	0.304 (151)	0.43	1.13	0.83–1.53	0.61	–
4	A–C	0.031 (3)	0.005 (1)	0.30	4.86	0.50–46.95	–	–
Haplotype		$n = 306$	$n = 492$					
Samples		$n = 153$	$n = 246$					

CI, confidence interval; IL17, interleukin 17; OA, osteoarthritis; SNP, single-nucleotide polymorphisms.
[a]G/A—alleles at IL17A SNP (rs2275913); T/C—alleles at IL17F SNP (rs763780).

The assembly and prediction of haplotypes for the IL17 gene locus were done using patients and controls that were typed on all two and all three SNPs. This procedure diminished our initial genotyped populations (controls vs. HOA patients, and controls vs. KOA patients), because not all were typed for all three SNPs. This was due to a lack of either specimens or chemicals. Nevertheless, typing all SNP loci is an important prerequisite for the haplotype estimation by the software Phase. If we let the software estimate the missing links in the haplotype predictions (based on the whole population frequency calculations), the analysis once showed an aberrant result (but only in the analysis with the IL17F5′ SNP). We thus opted to predict phases only by using individuals with completely genotyped loci, the decision that left us with 190 controls, 296 HOA, and 302 KOA patients.

The IL17A SNP (rs2275913) is located at 52,186,235 on human chromosome 6 (DNA sequence assembly vs. GRCh38.p12), separated by 50,706 bp from the IL17F SNP (rs763780; 52,236,941), which is further 8,995 bp away from IL17F5′ (rs1889570) SNP.

RESULTS
Haplotype Analysis of Two SNPs: IL17A (rs2275913)–IL17F (rs763780)
The frequency and number of estimated IL17A (rs2275913)–IL17F (rs763780) haplotypes are listed in Table 1 (HOA vs. controls) and Table 2 (KOA vs. controls).

In Table 1, the statistically significant association was found for G–C (IL17A rs2275913–IL17F rs763780) haplotype in HOA patients compared with controls with $p < 0.0001$, and OR (95% CI): 4.06 (1.89–8.90). This OR suggests that there is about four times higher risk to developing HOA in the individuals carrying the G–C haplotype. In contrast, the haplotype G–T was found significantly associated with approximately 30% lower risk to developing HOA, with $p < 0.01$ and OR (95% CI): 0.66 (0.49–0.90).

In Table 2, the two-SNP haplotype analysis revealed that none of the estimated haplotypes were significantly associated with predisposition to developing KOA. Interestingly, the haplotype G–T was found significantly associated with about 30% lower risk to developing KOA, with $p < 0.03$ and OR (95% CI): 0.72 (0.53–0.97).

Haplotype Analysis of Two SNPs: IL17F (rs763780)–IL17F5′ (rs1889570)
In Tables 3 (HOA vs. controls) and 4 (KOA vs. controls) we show the frequency and number of estimated IL17F (rs763780)–IL17F5′ (rs18I9570) haplotypes by the program Phase.

In Table 3, we have found a statistically significant association for C–A IL17F (rs763780)–IL17F5′ (rs1819570) haplotype in HOA patients compared with controls with $p < 0.01$ and OR (95% CI): 73 $(1.23–97 \times 10^5)$. This OR suggests that there is about 70 times higher risk to developing HOA in the

Table 2. Haplotype Analysis IL17A SNP (rs2275913)–IL17F SNP (rs763780) With Knee OA

		Frequency (n)						
IL17A–IL17F	Haplotype[a]	OA Knee—Patients	Controls	p	OR	95% CI	χ^2 test	Association
1	G–T	0.597 (189)	0.672 (329)	0.024	0.72	0.53–0.97	5.117	Protection
2	G–C	0,031 (12)	0.019 (11)	0.20	1.70	0.69–4.20	1.615	–
3	A–T	0.363 (118)	0.304 (151)	0.07	1.32	0.97–1.80	3.35	–
4	A–C	0.009 (1)	0.005 (1)	1.000	1.54	0.10–24.70	–	–
Total haplotypes		$n = 320$	$n = 492$					
Samples		$n = 160$	$n = 246$					

CI, confidence interval; IL, interleukin; OA, osteoarthritis; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]G/A—alleles at IL17A SNP (rs2275913); T/C—alleles at IL17F SNP (rs763780).

Table 3. Haplotype Analysis IL17F SNP (rs763780)–IL17F5′ SNP (rs1889570) With Hip OA

IL17F–IL17F5′	Haplotypes[a]	Frequency (n) OA Hip—Patients	Controls	p	OR	95% CI	χ^2 test	Association
1	T–G	0.420 (118)	0.473 (164)	0.085	0.76	0.56–1.04	2.96	–
2	T–A	0.485 (150)	0.487 (174)	0.752	1.05	0.77–1.43	0.10	–
3	C–G	0.053 (22)	0.032 (14)	0.056	1.94	0.93–4.08	3.66	–
4	C–A	0.041 (5)	0.007 (0)	0.009	72.8	$1.23–97 \times 10^5$	7.170	Predisposition
Total haplotypes		$n = 296$	$n = 352$					
Samples		$n = 148$	$n = 176$					

CI, confidence interval; IL, interleukin; OA, osteoarthritis; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]T/C—alleles at IL17F SNP (rs763780); G/A—alleles at IL17F5′ SNP (rs1889570)

Table 4. Haplotype Analysis of SNPs at IL17F (rs763780) and IL17F5′ (rs1889570) With Knee OA

IL17F–IL17F′	Haplotypes[a]	Frequency (n) OA Knee—Patients	Controls	p	OR	95% CI	χ^2 test	Associations
1	T–G	0.538 (166)	0.473 (164)	0.089	1.30	0.95–1.79	2.894	–
2	T–A	0.420 (133)	0.486 (174)	0.079	0.76	0.55–1.05	3.08	–
3	C–G	0.026 (10)	0.032 (14)	0.595	0.80	0.35–1.83	0.28	–
4	C–A	0.015 (3)	0.008 (0)	–	–	–	–	–
Total haplotypes		$n = 312$	$n = 352$					
Samples		$n = 156$	$n = 176$					

CI, confidence interval; IL, interleukin; OA, osteoarthritis; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]T/C—alleles at IL17F SNP (rs763780); G/A—alleles at IL17F5′ SNP (rs1889570).

individuals carrying the C–A haplotype. The calculations were done using Fischer's exact test, which is normally used whenever a value is less than 5 in at least one of the contingency table slots.

In Table 4, the analysis of two-SNP IL17F (rs763780)–IL17F5′ (rs18I9570) haplotypes showed neither association with susceptibility to KOA nor protection to developing KOA that was statistically significant.

Haplotype Analysis of Three SNPs: IL17A (rs2275913)–IL17F (rs763780)–IL17F5′ (rs1889570)

The frequency and number of estimated three-SNP IL17A (rs2275913)–IL17F (rs763780)–IL17F5′(rs18I9570) haplotypes are shown in Tables 5 (HOA vs. controls) and 6 (KOA vs. controls).

In Table 5, the statistically significant association was found for three-marker (SNP) G–C-A (IL17A rs2275913–IL17F rs763780–IL17F5′ rs1889570)

Table 5. Haplotype Analysis of Three SNPs at IL17A-F Loci, IL17A SNP (rs2275913)–IL17F SNP (rs763780)–IL17F5′ SNP (rs1889570) in Patients With OA Hip in Croatian Population

IL17A–IL17F–IL17F5′	Haplotypes[a]	Frequency (n) OA Hip—Patients	Controls	p	OR	95% CI	χ^2 test	Association
1	G–T–G	0.353 (112)	0.422 (86)	0.104	0.74	0.51–1.67	2.64	–
2	G–T–A	0.246 (62)	0.204 (33)	0.332	1.26	0.79–2.01	0.94	–
3	G–C–G	0.033 (10)	0.005 (1)	0.057	6.61	0.84–52.05	–	–
4	G–C–A	0.027 (11)	0.000 (0)	0.004	73.3	$1.33–97 \times 10^5$	–	Predisposition
5	A–T–G	0.067 (13)	0.059 (5)	0.311	1.70	0.60–4.85	1.01	–
6	A–T–A	0.239 (81)	0.293 (62)	0.214	0.78	0.52–1.16	1.55	–
7	A–C–G	0.020 (5)	0.14 (3)	1.000	1.07	2.53–4.53	–	–
8	A–C–A	0.014 (2)	0.002 (0)	–	–	–	–	–
Total haplotypes		$n = 296$	$n = 190$					
Samples		$n = 148$	$n = 95$					

CI, confidence interval; IL, interleukin; OA, osteoarthritis; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]G/A—alleles at IL17A SNP (rs2275913); T/C—alleles at IL17F SNP (rs763780); G/A—alleles at IL17F5′ SNP (rs1889570).

Table 6. Haplotype Analysis of Three SNPs at IL17A-F Loci, IL17A SNP (rs2275913)–IL17F SNP (rs763780)–IL17F5′ SNP (rs1889570) in Patients With OA Knee in Croatian Population

IL17A–IL17F–IL17F5′	Haplotypes[a]	Frequency (n)		p	OR	95% CI	χ^2 test	Association
		OA Knee—Patients	Controls					
1	G–T–G	0.416 (134)	0.422 (84)	0.782	0.95	0.66–1.37	0.08	–
2	G–T–A	0.176 (50)	0.196 (33)	0.694	0.91	0.56–1.47	0.15	–
3	G–C–G	0.028 (10)	0.013 (3)	0.387	2.06	0.56–7.60	–	–
4	G–C–A	0.006 (1)	0.000 (0)	–	–	–	–	–
5	A–T–G	0.120 (32)	0.063 (8)	0.015	2.60	1.12–6.27	5.89	Predisposition
6	A–T–A	0.246 (83)	0.298 (61)	0.186	0.77	0.52–1.14	1.75	–
7	A–C–G	0.000 (0)	0.002 (0)	–	–	–	–	–
8	A–C–A	0.008 (2)	0.006 (1)	1.000	1.22	0.11–13.54	–	–
	Total haplotypes	$n = 312$	$n = 190$					
	Samples	$n = 156$	$n = 95$					

CI, confidence interval; IL, interleukin; OA, osteoarthritis; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]G/A—alleles at IL17A SNP (rs2275913); T/C—alleles at IL17F SNP (rs763780); G/A—alleles at IL17F5′ SNP (rs1889570).

haplotype in HOA patients compared with controls with $p < 0.005$, and (95% CI): 73 $(1.33–97 \times 10^5)$. This OR suggests that there was about 73 times higher risk to developing HOA in the individuals carrying the G–C–A haplotype. The calculations were done using Fischer's exact test. The reason for the high OR might be a consequence of statistical approximation when 0 (there were no controls with this haplotype) has been used in such calculations. Therefore, the expected relative risk is probably closer to the estimation obtained by analyzing the two-SNP haplotype (IL17A–IL17F) and might be about fourfold (see Table 1).

In Table 6, the three-SNP haplotype analysis revealed that one of the estimated haplotypes was significantly associated with predisposition to developing KOA; namely, the haplotype A–T–G was found significantly associated with approximately 2.5 higher risk to developing KOA, with $p < 0.02$ and OR (95% CI): 2.60 (1.12–6.27).

In Figure 1 we displayed the summary of haplotype analyses. The three-SNP-marker haplotype G–C–A on chromosome 6 in the IL17A-F locus (IL17A–IL17-F–IL17F5′; rs2275913–rs763780–rs1889570) confers susceptibility to HOA, but not KOA. These data extend and confirm our previously published results about
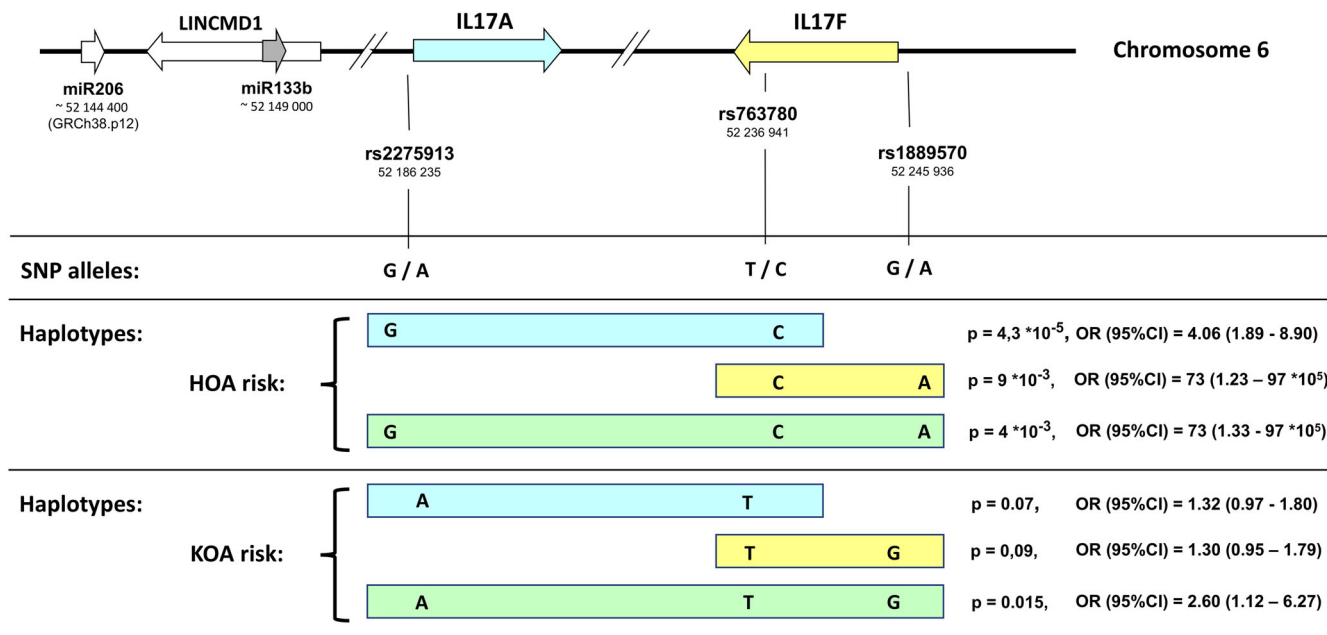
Figure 1. IL17 Locus on Chromosome 6: Summary of Haplotypes' Analyses—Hip and Knee OA Susceptibility. CI, confidence interval; HOA, hip osteoarthritis; KOA, knee osteoarthritis; IL17, interleukin 17; OA, osteoarthritis; OR, odds ratio; SNP, single-nucleotide polymorphism. [Color figure can be viewed at wileyonlinelibrary.com]

HOA risk in those carrying the IL17F rs767380 SNP minor allele C in the Croatian population.[19]

Already the two-marker haplotypes' analyses (IL17A–IL17F or IL17F–IL17F5′) showed a similar message: both are associated only with HOA, with predisposition to the disease in individuals carrying either G–C (IL17A–IL17F) or C–A (IL17F–IL17F5′) haplotypes. In addition, the *haplotype* G–T (IL17A–IL17F) is associated with protection to HOA.

Interestingly, the opposed three-SNP (IL17A–IL17-F–IL17F5′) haplotype A–T–G was found significantly associated with about 2.5 higher risk to developing KOA.

DISCUSSION

We have mapped the predisposition to HOA and KOA on the chromosome 6, which is centered on the IL17F rs763780 SNP. The minor allele of this SNP (C, which is represented by G in the coding DNA strand) encodes a missense polymorphism of the primary protein sequence of the IL17F cytokine. There is a single base exchange CAT into CGT at position 553 in the messenger RNA of the IL17F, which makes the shift in the code in amino acid sequence from His (H) into Arg (R) at position 161.

Logically, this information leads to a conclusion that such primary amino acid change might alter the IL17F protein conformation, which somehow facilitates development of HOA but not KOA. Nevertheless, there are other equally likely explanations for the found associations. Ever since the non-coding RNA genes and elements have been discovered and their influence on regulation of other genes in the genome proven, a search for such cis- and trans-acting regulatory factors is on the rise. Specifically, in the vicinity of the IL17A-F gene locus we found other DNA elements that could be involved in conferring or reducing the risk to OA. There are at least three DNA elements in the chromosome 6 (sequence assembly vs. GRCh38.p12), as shown in the Figure 1:

(1) The first is the long non-protein coding RNA (lncRNA) that harbors Muscle differentiation-1 gene, called "The long intergenic non-protein coding RNA, Muscle differentiation-1" gene (LINCMD1) located at positions 52,146,814–52,151,023 downstream (3′) from the IL17A gene in the direction of the HLA complex genes (about 20 Mb away).

(2) The second is short non-coding RNA, called microRNA133b (miR133b), located in LINCMD1 (at position ~52,149,000).

(3) Further downstream from the IL17A gene (and upstream from LINCMD1) is another microRNA - miR206 at position ~52,144,400.

Mir206 and *Mir133b* are referred to as *myomi*Rs because they regulate genes involved in adult muscle formation. However, they are also involved in the development of dopaminergic neurons and possibly craniofacial cartilage of zebrafish.[29,30] In general, long and short non-coding RNA elements are known to affect the expression of various genes, and thus might be involved in regulating the risk for OA apart from IL17 genes.

We thus used haplotype estimation and analysis to recognize such a possibility. We wished to confirm the expected findings about IL17F SNP (rs763780) for HOA and to search for association of DNA polymorphisms with KOA because haplotype analysis has a greater power to detect associations with the disease than SNP analysis alone. Indeed, we were successful in both aspects. Moreover, we confirmed that in all analyzed haplotypes, being either with two SNPs or three SNPs, the associated haplotype with the HOA always held the C allele in it (G–C in *IL17A–IL17F*, C–A in *IL17-F–IL17F5′*, and G–C–A in *IL17A–IL17F–IL17F5′*). Remarkably, despite finding none of previously analyzed SNP markers to be associated with the knee OA, we have found a haplotype A–T–G that conferred predisposition to KOA, but only when three-SNP haplotype analyses were made (*IL17A–IL17-F–IL17F5′*). Interestingly, this A–T–G haplotype carries all the alternative alleles from the one found (G–C–A) to confer higher risk to HOA.

Therefore, it is still possible that haplotypes G–C–A and A–T–G are just markers for another set of "true" risk factors for HOA/KOA, which are represented perhaps by the non-coding DNA elements mentioned above. By DNA sequencing of these genetic regions in selected affected individuals we might learn more about their role in the pathogenesis of OA in the future. If the haplotypes (found in this study) carry different miRNAs and/or lncRNAs with respect to the type of disease (HOA vs. KOA), then it could be a sign that they might be involved in the joint-specific OA pathogenicity.

In conclusion, we suggest that allelic variants coding for IL17A or IL17F cytokines, defined by the G–C–A and A–T–G haplotypes, are the culprits for increased risk for HOA and KOA, respectively, in the Croatian Caucasian population. Moreover, we cannot exclude the possibility that, instead of IL17 alleles, allelic variants of *Mir133b* and *Mir206* alone, or with the *LINCMD1 alleles* might be involved in the predisposition to hip and knee OA.

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