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ORIGINAL ARTICLE

Impact of the gene-gene interactions related to the HIF-1α signaling pathway with the knee osteoarthritis development

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Abstract

Introduction/objectives Articular cartilage is the target tissue of osteoarthritis (OA), and because it lacks capillary networks, the microenvironment is hypoxic. Hypoxia inducible factor-1 alpha (HIF-1α) regulates the homeostasis of this tissue. The aim of this study was to investigate whether genetic polymorphisms of the HIF-1α signaling pathway are involved in the development of knee OA.

Method We performed a case-control association study and genotyped 134 knee OA patients and 267 healthy controls. All participants were genotyped in order to evaluate 42 SNPs from 22 genes involved in the HIF-1α signaling pathway using the OpenArray technology. Gene-gene interactions (epistasis) were analyzed using the multifactor dimensionality reduction (MDR) method.

Results The MDR analysis showed epistasis between *AKT2* (rs8100018) and *IGF1* (rs2288377), *AKT2* (rs8100018) and *IGF1* (rs35767), *IGF1* (rs35767) and *COL2A1* (rs1793953), and between *GSK3B* (rs6438552) and *IGF1* (rs35767) polymorphisms, with information gain values of 21.24%, 8.37%, 9.93%, and 5.73%, respectively. Additionally, our model allowed us to identify high- and low-risk genotypes among *COL2A1* rs1793953, *GSK3B* rs6438552, *AKT2* rs8100018, and *IGF1* rs35767 polymorphisms.

Conclusions Knowing the interactions of these polymorphisms involved in HIF-1α signaling pathway could provide a new diagnostic support tool to identify individuals at high risk of developing knee OA.

Keywords Gene-gene interaction · Hypoxia inducible factor-1α · Multifactor dimensionality reduction · Osteoarthritis · Single nucleotide polymorphism

Introduction

Primary osteoarthritis (OA) is a disorder involving movable joints characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation, and loss of normal joint function that can culminate in illness [1]. Worldwide estimates indicate that 9.6% of men and 18% of women ≥ 60 years old suffer from symptomatic OA

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[2–4]. Similar to different ethnic groups, OA is the most common form of human arthritis among Mexicans; its incidence is increasing steadily due to the current demographic, epidemiological, and social transitions along with the pandemic of overweight and obesity in this ethnic group [5].

Articular cartilage is the target tissue of OA, and because it lacks capillary networks, the microenvironment is hypoxic [6]. In physiological conditions, oxygen concentration in articular cartilage varies from 0.5 to 10%. Hypoxia inducible factor-1α (HIF-1α) plays a fundamental role in maintaining the homeostatic conditions of articular cartilage [7–11]. Under normoxia, its specific proline residues 402 and 564 are hydroxylated in the oxygen-dependent degradation domain by prolyl-hydroxylases (PHDs) to form a complex with the von Hippel-Lindau (VHL) factor; in turn, this complex is subsequently degraded in the proteasome [12, 13]. However, under hypoxic conditions, the activity of PHDs decreases, stabilizing HIF-1α, which accumulates in the cytoplasm and is phosphorylated by MAPK [11, 14–16]. On the other hand, it has been that the inhibition or depletion of GSK-3 induces HIF-1α, while the overexpression of GSK-3β reduces the expression of HIF-1α [17]. Upon phosphorylation, HIF-1α translocates to the nucleus and binds to specific DNA sequences (5′ TAGCGTGH3′) present in promoter regions of genes for their subsequent expression [18, 19]. Among many others, these target genes include *NOS2*, *VEGF*, *EPO*, *GLUT1*, *IGF2*, *SOX9*, and *COL2A1*. Transcription of such target genes has the potential role of maintaining the chondroprotective functions that are challenged by the detrimental conditions occurring in the OA joint environment [20–23].

From a genetic standpoint, several studies suggest associations between single-nucleotide polymorphisms (SNPs) and knee OA [24, 25]. Nevertheless, most of them were assessed individually, in contrast to joint assessments through gene-gene interactions (epistasis), which could provide more information regarding their role [26]. The identification and characterization of gene-gene and gene-environment interactions have been limited primarily due to a lack of powerful statistical methods, and particularly because of small sample sizes, which has been a challenge for geneticists. In this sense, the multifactor dimensionality reduction (MDR) method does not require a model as such, given that no genetic models are assumed, neither is it parametric, as no parameters are estimated [27, 28]. The generalized MDR (GMDR) method is an extension from MDR and allows an adjustment for discrete and quantitative covariables and can be applied to both dichotomous and continuous phenotypes in several study designs based on population [29].

Interactions between multiple *loci* of different genes could be the foundation of the knee OA genetic origin. Therefore, this study is focused on evaluating whether interactions between several genetic variants of HIF-1α signaling pathway are associated with knee OA in the Mexican population.

Materials and methods

Study design and population

Four hundred and one unrelated Mexican-Mestizo individuals were recruited from September 2013 to September 2016 period for this case control-study. One hundred thirty-four of them were primary knee OA patients: 94 from the Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra" (INRLGII), and 40 from the Rheumatology Department of the Hospital Civil de Guadalajara "Fray Antonio Alcalde" (Ref. J45703-M CONACYT). The knee OA diagnosis was based on the American College of Rheumatology criteria [30], which included primary OA with any symptoms, and radiographic signs of OA according to the Kellgren-Lawrence (KL) score (≥ 2); the clinical examination and radiographic evaluation were performed by a qualified radiologist-rheumatologist. One hundred and fifty healthy employees from INRLGII and 117 healthy subjects from Guadalajara with no symptoms or signs of knee OA, other types of arthritis, or any painful condition of the joint were recruited as controls. The control subjects were selected among individuals with no personal and family history of OA. Knee radiographs from controls were obtained consecutively to rule out subclinical OA, and those who were grade one or less were considered. Other etiologies causing knee diseases, such as inflammatory arthritis (rheumatoid arthritis -RA- or any other autoimmune disease), post-traumatic or post-septic arthritis, poliomyelitis, and skeletal dysplasia, were excluded. This study meets all criteria contained in the Declaration of Helsinki and was approved by the Ethics and Research Committee of the Instituto Nacional de Rehabilitación (Ref. INR-18/13). All participants signed an informed consent letter; additionally, information on age, gender, weight, body mass index (BMI), and birth place was obtained. All participants were > 40 years and were geographically matched (Mexico City and neighboring states), and to have parents and grandparents born in the same geographical region.

SNPs selection and genotyping

Using a case-control design, we sought to assess the contribution of SNPs involved in the HIF-1α signaling pathway previously reviewed [31]; in addition, we include SNPs that have not been studied in order to know their involvement in OA. A total of 42 SNPs were genotyped in cases and controls and with a population frequency greater than 1% in Mexico population. SNPs selection was supported on information from the http://browser.1000genomes.org/index.html, http://www.ncbi.nlm.nih.gov/projects/SNP/ and http://www.genome.jp/kegg-bin/show_pathway?hsa04066 sources. The selection order of the SNPs was first in promoter regions, followed by exons and introns; also, these SNPs should not be in linkage

disequilibrium (LD). Seven SNPs in genes that activate the HIF-1α system, 13 SNPs in genes that interact directly with HIF-1α, and 22 SNPs from genes that are induced by HIF-1α were selected for this study (Table 1). Since the Mexican-Mestizo population is admixed, ancestry informative markers (AIMs) were used to assess whether any association could be confounded due to population stratification (Table 1). A panel of nine AIMs distinguishing mainly Amerindian, African, and European ancestry ($\delta > 0.44$) were genotyped [32, 33].

Genomic DNA was isolated from peripheral blood white cells using a commercial kit based on the salt fractionation method (QIAmp 96 DNA Blood Kit, Qiagen, Hilden, Germany). Genotyping was performed using the OpenArray technology in a QuantStudio 12 K flex System (Thermo Fisher Scientific). Genomic DNA samples were normalized at 50 ng/μl, and 2.5 μl of DNA were mixed with 2.5 μl of TaqMan OpenArray Genotyping Master Mix (Thermo Fisher Scientific) on 384-well plates. Mixes were loaded onto genotyping OpenArray plates previously loaded with the genotyping primers and probes, using the AccuFill System (Thermo Fisher Scientific). Amplification was carried out following the manufacturer's protocol. Results were analyzed using the TaqMan Genotyper v1.2 software.

Statistical analysis

The clinical variables were evaluated with Student's *t* test or Fisher's exact test, when appropriate, and values were expressed as mean ± SD. Gene and allele frequencies of all polymorphisms were calculated and compared between cases and controls using Fisher's exacts test. In order to control the global false positive rate, only SNPs with a statistically significant *p* value on Fisher's exact test were considered in the multivariate analysis. Associations of each SNP with OA risk were assessed with logistic regression models adjusted by age, gender, BMI, and ancestry, taking into account a co-dominant inheritance model for the SNP. Hardy-Weinberg equilibrium (HWE) was evaluated by calculating the inbreeding coefficient (F_{is}) using the Genetix v4.05.2 (Université de Montpellier) program with 1000 permutations each *loci* in both study groups.

The ancestry was analyzed by STRUCTURE software v2.3.4 (Pritchard Lab, Stanford University, USA), to evaluate the effect of population stratification on the associations found of each population *k* ($k = 3$) with the genotypes of the nine AIMs mentioned above. This information was included in the logistic regression models to adjust the associations found between the studied polymorphism and OA by individual mix. In addition, we performed a haplotype analysis to determine the joint effect of variants of the same gene on OA development. All the statistical analyses were performed using the statistical package STATA v14.0 (Stata Corp, Texas USA), and considering an $\alpha = 0.05$ significance level. Finally, in order to study the effect of epistasis, we used the MDR v3.0.2 and GMDR v0.9 statistical packages according the Ritchie's algorithm [27].

Results

Characteristics of the study population

Demographic and clinical characteristics of knee OA patients and controls are shown in Table 2. In the study groups, cases were significantly older than controls individuals ($P < 0.0001$, 51.3 ± 13.5 vs 43.6 ± 11.3 years, respectively). Most of the patients were female in both study groups (88.0% in cases and 70.0% in controls, $P < 0.0001$). The mean BMI of the OA group was significantly higher than the control group ($P < 0.0001$, 29.2 ± 4.8 vs 26.1 ± 4.8, respectively). There was no difference among patients and controls regarding the place of birth ($P = 0.146$). The distribution of the studied polymorphisms was consistent with HWE except for *HIF1AN* rs11292, *HIF1A* rs11549465, and *EGLN1* rs1339894 polymorphisms (Supplementary Table 1).

Association of SNPs of the HIF-1α signaling pathway with OA

After adjusting by age, gender, BMI, and admixture in a logistic regression model, the genotype and allele frequencies of ten SNPs significantly associated are presented in Table 3. Genotypes and alleles with low risk against OA were C/C genotype and C allele of *AKT2* rs8100018 (OR = 0.17, 95% CI = 0.05–0.55, $P = 0.003$, and OR = 0.58, 95% CI = 0.38–0.87, $P = 0.009$, respectively), C/T genotype and T allele of *AGER* rs2070600 (OR = 0.05, 95% CI = 0.00–0.47, $P = 0.008$, and OR = 0.23, 95% CI = 0.08–0.64, $P = 0.005$, respectively), A/G genotype of *HIF1AN* rs11292 (OR = 0.37, 95% CI = 0.14–0.96, $p = 0.04$), A/A genotype and A allele of *EGLN1* rs1339894 (OR = 0.05, 95% CI = 0.00–0.45, $P = 0.007$, and OR = 0.39, 95% CI = 0.22–0.70, $P = 0.001$, respectively), A/A genotype of *VEGFA* rs1570360 (OR = 0.31, 95% CI = 0.10–0.93, $p = 0.03$), and G/A genotype of *COL2A1* rs1793953 (OR = 0.48, 95% CI = 0.28–0.82, $P = 0.008$). On the other hand, genotypes and alleles with high risk to development OA wer: A/G genotype of *GSK3B* rs6438552 (OR = 2.58, 95% CI = 1.16–4.45, $P = 0.01$), C/T genotype and T allele of *HIF1A* rs11549465 (OR = 3.14, 95% CI = 1.82–5.42, $P = 0.000$, and OR = 2.07, 95% CI = 1.33–3.23, $P = 0.001$, respectively), A/T genotype and T allele of *IGF1* rs2288377 (OR = 1.86, 95% CI = 1.08–3.20, $P = 0.02$, and OR = 1.63, 95% CI = 1.01–2.63, $P = 0.04$, respectively), and G/A genotype and A allele of *IGF1* rs35767 (OR = 2.00, 95% CI = 1.17–3.42, $P = 0.01$, and OR = 1.51, 95% CI = 1.02–2.25, $P = 0.03$, respectively).

Table 1 Single-nucleotide polymorphisms (SNPs) studied

Gene	db SNP rs ID	Chromosome position	Location	MAF	Most severe consequence
Genes that activate the HIF-1α system					
PIK3R1	rs3730089	Chr.5:67588148	Intron	*A*	Missense variant
AKT2	rs8100018	Chr.19:40752023	Intron	*C*	Intron variant
GSK3B	rs6438552	Chr.3:119631814	Intron	*A*	Intron variant
IL6	rs1474347	Chr.7:22768124	Exon	*C*	Non coding transcript
AGER	rs2070600	Chr.6:32151443	Intron	*T*	Missense variant
AGER	rs1800624	Chr.6:32152387	5′ UTR	*T*	Upstream gene variant
AGER	rs1035798	Chr.6:32151222	Intron	*T*	Splice region variant
Genes that interact with HIF-1α					
HIF1A	rs2057482	Chr.14:62213848	3′UTR	*T*	3′UTR variant
HIF1A	rs11549465	Chr.14:62207557	Exon	*T*	Pro582Ser
HIF1A	rs11549467	Chr.14:62207575	Exon	*A*	Ala588Thr
EGLN1	rs12406290	Chr.1:231559226	5′UTR	*G*	5′UTR variant
EGLN1	rs1339894	Chr.1:231560557	5′UTR	*A*	5′UTR variant
EGLN1	rs2739513	Chr.1:231515201	Intron	*T*	Intron variant
EGLN1	rs2009873	Chr.1:231499236	3′UTR	*T*	Downstream gene variant
VHL	rs779805	Chr.3:10183337	5′UTR	*G*	5′UTR variant
VHL	rs1678607	Chr.3:10188428	Intron	*T*	Intron variant
VHL	rs1642742	Chr.3:10191943	3′UTR	*G*	3′UTR variant
HIF1AN	rs1054399	Chr.10:102312565	3′UTR	*T*	3′UTR variant
HIF1AN	rs11190613	Chr.10:102313997	3′UTR	*C*	3′UTR variant
HIF1AN	rs11292	Chr.10:102313607	3′UTR	*G*	3′UTR variant
Genes induced by HIF-1α					
VEGFA	rs699947	Chr.6:43736389	5′UTR	*A*	Upstream gene variant
VEGFA	rs1570360	Chr.6:43737830	5′UTR	*A*	Upstream gene variant
VEGFA	rs3025039	Chr.6:43752536	3′UTR	*T*	3′UTR variant
VEGFA	rs729761	Chr.6:43804571	Intergenic	*T*	Regulatory region variant
EPO	rs1617640	Chr.7:100317298	5′UTR	*C*	Upstream gene variant
NOS2	rs1060826	Chr.17:26089867	–	*T*	Synonymous variant
NOS2	rs2297518	Chr.17:26096597	Intron	*A*	Missense variant
NOS3	rs2070744	Chr.7:150690079	Intron	*C*	Intron variant
IGF1	rs35767	Chr.12:102875569	5′UTR	*A*	Upstream gene variant
IGF1	rs2288377	Chr.12:102874762	5′UTR	*T*	Upstream gene variant
EGF	rs4444903	Chr.4:110834110	5′UTR	*A*	5′UTR variant
EDN1	rs1800541	Chr.6:12289219	5′UTR	*G*	Upstream gene variant
EDN1	rs5370	Chr.6:12296255	Exon	*T*	Lys198Asn
MMP1	rs2239008	Chr.11:102661080	3′UTR	*A*	3′UTR variant
MMP3	rs679620	Chr.11:102713620	–	*G*	Coding region
MMP13	rs2252070	Chr.11:102826539	5′UTR	*C*	Upstream gene variant
MMP13	rs12792912	Chr.11:102801303	–	*G*	Transversion substitution
CA	rs1703290	Chr.5:4062706	Intergenic	*G*	Intergenic variant
COL2A1	rs2276454	Chr.12:48376291	–	*A*	Synonymous variant
COL2A1	rs1793953	Chr.12:48393526	Exon	*A*	Non coding transcript
COL3A1	rs1800255	Chr.2:189864080	Exon	*A*	Missense variant
COL3A1	rs2138533	Chr.2:189837212	5′UTR	*T*	Upstream gene variant
AIMs					
rs2695	rs2695	Chr.9:82884577	Intergenic	*T*	Intergenic variant
rs2862	rs2862	Chr.15:35145553	5′UTR	*C*	Upstream gene variant
SAP30L	rs3340	Chr.5:153831867	Intron	*C*	Intron variant

Table 1 (continued)

Gene	db SNP rs ID	Chromosome position	Location	MAF	Most severe consequence
CKM	rs4884	Chr.19:45810035	–	*A*	Synonymous variant
rs722098	rs722098	Chr.21:16685598	Intergenic	*A*	Intergenic variant
CA10	rs203096	Chr.17:50011769	Intron	*T*	Intron variant
rs223830	rs223830	Chr.16:57451971	3′UTR	*C*	Downstream gene variant
DRD2	rs1800498	Chr.11:113291588	Intron	*A*	Intron variant
PRKCE	rs2814778	Chr.1:159174683	5′UTR	*C*	5′UTR variant

MAF, minor allele frequency; *AIMs*, ancestry informative markers; Missense variant, a sequence variant that changes one or more bases, resulting in a different amino acid sequence, but where the length is preserved; Intron variant, a transcript variant occurring within an intron; Non coding transcript exon variant, a sequence variant that changes non-coding exon sequence in a non coding transcript; Upstream gene variant, a sequence variant located 5′ of a gene; Splice region variant, a sequence variant in which a change has occurred within the region of the splice site, either within 1–3 bases of the exon or 3–8 bases of the intron; Downstream gene variant, a sequence variant located 3′ of a gene; Regulatory region variant, a sequence variant located within a regulatory region; Synonymous variant, a sequence variant where there is no resulting change to the encoded amino acid; Intergenic variant, between genes

Evaluation of gene-gene interactions: MDR

Table 4 summarizes the results of exhaustive MDR analysis, which analyzes all possible combinations of the studied polymorphisms. According to the MDR analysis, the best models include the *AKT2* (rs8100018) and *IGF1* (rs2288377) polymorphisms. This model had a balanced accuracy test of 0.7678, a consistency of cross-validation of 10/10, and an interaction *P* value = 0.0010. Figure 1 shows the interaction map of the studied polymorphisms, based on entropy measures among individual variables. A strong interaction effect was observed between *AKT2* (rs8100018) and *IGF1* (rs2288377), *AKT2* (rs8100018) and *IGF1* (rs35767), *IGF1* (rs35767) and *COL2A1* (rs1793953), and between *GSK3B* (rs6438552) and *IGF1* (rs35767) polymorphisms with information gain values of 21.24%, 8.37%, 9.93%, and 5.73%, respectively. The gene-gene interaction of the ten associated polymorphisms is shown in the interaction dendogram (Supplementary Fig.1). Moreover, our model allowed us to identify interactions in high-risk genotypes of the *COL2A1* (rs1793953), *GSK3B* (rs6438552), and *IGF1* (rs35767) polymorphisms, and the most representative were (GA + AG + GA), (GA + GG + GA), and (GG + AG + GG), respectively; and low-risk genotypes [(GA + AA+GA), (GA + AG + GG), and (GA + GG + GG)], respectively. Likewise, we identify interactions in high-risk genotypes of the *AKT2* (rs8100018) and *IGF1* (rs2288377) polymorphisms [(GG + AA) and (GC + AT)], respectively; and low-risk genotypes [(GC + AA) and (GG + AT)], respectively (Fig. 2).

Haplotype analysis

In regard to the haplotype analysis, we observed that the CTG (rs2057482, rs11549465, and rs11549467, respectively) and AT (rs35767 and rs228377, respectively) haplotypes of the *HIF1A* and *IGF1* genes, respectively, were found to be associated with an increased risk of developing (OR = 2.59, *P* = 0.004, 95% CI = 1.36–4.94 and OR = 1.69, *P* = 0.038, 95% CI = 1.02–2.80, respectively) (Supplementary Table 2).

Table 2 Characteristics of the study population

Parameter	Total (*n* = 401)	OA (*n* = 134)	Controls (*n* = 267)	*P*
Age (years)	46.1 ± 12.6	51.3 ± 13.5	43.6 ± 11.3	*< 0.0001*
BMI (Kg/cm^2)	27.2 ± 5.0	29.2 ± 4.8	26.1 ± 4.8	*< 0.0001*
Gender				
Female (%)	305 (76.0)	118 (88.0)	187 (70.0)	*< 0.0001**
Male (%)	96 (24.0)	16 (12.0)	80 (30.0)	
Place of birth				
Mexico City	192 (47.8)	71 (52.9)	121 (45.3)	0.146*
Others states of Mexico	209 (52.2)	63 (47.1)	146 (54.7)	

Data are expressed as mean ± SD. *P* values were estimated using Student's *t* test, $\alpha = 0.05$; **P* values were estimated using Fisher's exact test, $\alpha = 0.05$. *BMI*, body mass index, normal, 18.5–24.9; overweight, 25.0–29.9; obesity, ≥ 30.0. Significant *P* values are in italic

Table 3 Association of the HIF-1α signaling pathway polymorphisms in knee OA patients and controls

Gene	SNP rs ID	OA *N* (%)	Controls *N* (%)	OR*	(95% CI)	*P*
AKT2	rs8100018					
	G/G	70 (58.3)	80 (46.5)	1.00	Reference	
	G/C	46 (38.3)	61 (35.5)	0.91	(0.53–1.57)	0.74
	C/C	4 (3.40)	31 (18.0)	*0.17*	(0.05–0.55)	*0.003*
	G	186 (77.5)	221 (64.2)	1.00	Reference	
	C	54 (22.5)	123 (35.8)	*0.58*	(0.38–0.87)	*0.009*
GSK3B	rs6438552					
	A/A	37 (35.9)	57 (50.9)	1.00	Reference	
	A/G	49 (47.6)	34 (30.4)	*2.28*	(1.16–4.45)	*0.01*
	G/G	17 (16.5)	21 (18.7)	1.35	(0.58–3.11)	0.474
	A	123 (59.7)	148 (66.1)	1.00	Reference	
	G	83 (40.3)	76 (33.9)	1.37	(0.89–2.12)	0.15
AGER	rs2070600					
	CC	94 (96.9)	179 (84.0)	1.00	Reference	
	CT	1 (1.00)	26 (12.2)	*0.05*	(0.00–0.47)	*0.008*
	TT	2 (2.10)	8 (3.80)	0.56	(0.10–3.07)	0.50
	C	189 (97.4)	384 (90.1)	1.00	Reference	
	T	5 (2.60)	42 (9.90)	*0.23*	(0.08–0.64)	*0.005*
HIF1A	rs11549465					
	C/C	51 (40.5)	105 (67.3)	1.00	Reference	
	C/T	75 (59.5)	50 (32.0)	*3.14*	(1.82–5.42)	*< 0.001*
	T/T	0 (0.00)	1 (0.70)	–	–	–
	C	177 (70.2)	260 (83.3)	1.00	Reference	
	T	75 (29.8)	52 (16.7)	**2.07**	(1.33–3.23)	*0.001*
HIF1AN	rs11292					
	A/A	87 (76.3)	106 (65.4)	1.00	Reference	
	A/G	9 (7.90)	26 (16.1)	*0.37*	(0.14–0.96)	*0.04*
	G/G	18 (15.8)	30 (18.5)	0.90	(0.43–1.85)	0.77
	A	183 (80.3)	238 (73.5)	1.00	Reference	
	G	45 (19.7)	86 (26.5)	0.78	(0.49–1.24)	0.30
EGLN1	rs1339894					
	G/G	103 (84.4)	123 (73.6)	1.00	Reference	
	G/A	18 (14.8)	22 (13.2)	1.07	(0.51–2.22)	0.85
	A/A	1 (0.80)	22 (13.2)	*0.05*	(0.00–0.45)	*0.007*
	G	224 (91.8)	268 (80.2)	1.00	Reference	
	A	20 (8.20)	66 (19.8)	*0.39*	(0.22–0.70)	*0.001*
VEGFA	rs1570360					
	G/G	68 (61.3)	125 (53.0)	1.00	Reference	
	G/A	38 (34.2)	78 (33.0)	0.97	(0.56–1.67)	0.92
	A/A	5 (4.50)	33 (14.0)	*0.31*	(0.10–0.93)	*0.03*
	G	174 (78.4)	328 (69.5)	1.00	Reference	
	A	48 (21.6)	144 (30.5)	0.69	(0.46–1.05)	0.08
COL2A1	rs1793953					
	G/G	53 (40.5)	66 (25.5)	1.00	Reference	
	G/A	54 (41.2)	145 (56.0)	*0.48*	(0.28–0.82)	*0.008*
	A/A	24 (18.3)	48 (18.5)	0.67	(0.34–1.34)	0.26
	G	160 (61.1)	277 (53.5)	1.00	Reference	
	A	102 (38.9)	241 (46.5)	0.76	(0.54–1.07)	0.11

Table 3 (continued)

Gene	SNP rs ID	OA *N* (%)	Controls *N* (%)	OR*	(95% CI)	*P*
IGF1	rs2288377					
	A/A	71 (61.2)	176 (75.2)	1.00	Reference	
	A/T	44 (37.9)	56 (23.9)	*1.86*	(1.08–3.20)	*0.02*
	T/T	1 (0.90)	2 (0.90)	1.57	(0.12–19.8)	0.72
	A	186 (80.2)	408 (87.2)	1.00	Reference	
	T	46 (19.8)	60 (12.8)	*1.63*	(1.01–2.63)	*0.04*
IGF1	rs35767					
	G/G	57 (47.5)	137 (59.8)	1.00	Reference	
	G/A	55 (45.8)	72 (31.4)	*2.00*	(1.17–3.42)	*0.01*
	A/A	8 (6.70)	20 (8.70)	1.43	(0.55–3.71)	0.45
	G	169 (70.4)	346 (75.5)	1.00	Reference	
	A	71 (29.6)	112 (24.5)	*1.51*	(1.02–2.25)	*0.03*

OR, odds ratio; *CI*, confidence interval; *OR** , value adjusted for age, sex, BMI, and admixture in a logistic regression model. Significant *P* values are in italic

Discussion

OA is the most common joint disease, imposing a major economic burden to health systems due to the costs associated with healthcare and disability [34]. Several studies have been performed aimed to identify potential genes of therapeutic targets [35]. It is well-known that knee OA pathogenesis is multifactorial, and its complexity is primarily due to its polygenic nature. Given this polygenic nature, it has been difficult to prove gene-gene interactions associated with knee OA; in this sense, MDR has been applied to identify gene-gene interactions conferring susceptibility to common multifactorial diseases, including hypertension, bladder cancer, type 2 diabetes, and RA [36]. To date, only two published reports have evaluated gene-gene interactions by the MDR method in knee OA, which allow the identification of predictive models for the disease development based on the analyzed pathways (TGF-β/Smad3 and ADIPOQ/PON1) [37, 38]. In the present study, we applied the MDR method to assess the epistasis of genes related to the HIF-1α signaling pathway due to its central participation in the articular cartilage homeostasis.

Our main findings reveal important gene-gene interactions between the *AKT2*, *IGF1*, *COL2A1*, and *GSK3B* genes and knee OA. HIF-1α expression is regulated through the PI3K/Akt pathway, and both kinases are important in cell survival and apoptosis; especially, it has been shown that apoptosis of chondrocytes can be regulated by this signaling pathway, which is closely related to the occurrence and development of osteoarthritis [39, 40]. In our study, we observed that the carriers of the G/G homozygous genotype and the G minor allele of the *AKT2* rs8100018 polymorphism showed a significant association with a lower risk to knee OA development. To our knowledge, data on the associations between common genetic variations in *AKT2* gene and knee OA are scarce. But in pathologies such as rectal cancer, it has been observed that the rs8100018 variant is associated with low risk in progress to cancer, suggesting that this variant might play an important role in the *AKT2* function [41].

On the other hand, the insulin-like growth factor-1 (IGF-1) is a small 70-amino acid polypeptide mediator with a potent anabolic impact on cartilage homeostasis. IGF-1 is expressed in cartilage, where it can act in a paracrine and autocrine manner to stimulate cartilage extracellular matrix (ECM) synthesis

Table 4 Results of MDR analysis

Locus number	Best model	Training Bal Acc	Testing Bal Acc	Cross-validation consistency	*P* value*
1	COL2A1_rs1793953	0.6044	0.5005	6/10	0.828
2	AKT2_rs8100018, IGF1_rs2288377	0.7678	0.7678	10/10	0.001
3	GSK3B_rs6438552, IGF1_rs35767, COL2A1_rs1793953	0.8086	0.7698	8/10	0.001

**P* values were based on 1000 permutations

MDR, multifactor dimensionality reduction; *Testing Bal Acc*, testing-balanced accuracy

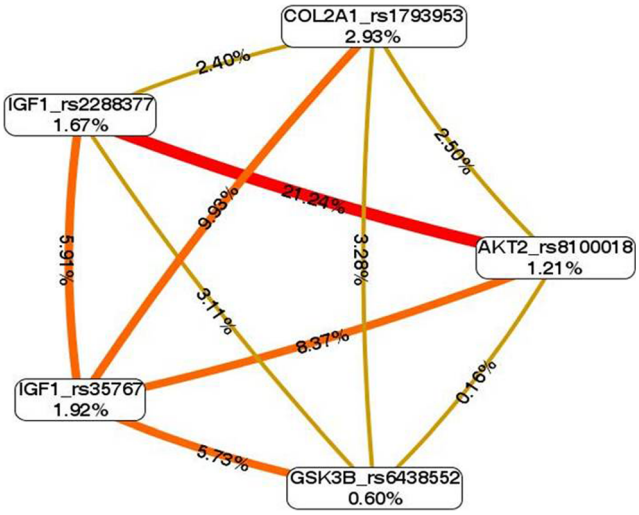

Fig. 1 Interaction map for knee OA risk. The interaction model describes the percentage of the entropy (information gain) that is explained by each factor or two-way interaction. Values inside nodes indicate information gain of individual attributes or main effects, whereas values between nodes show information gain of pairwise combinations of attributes or interaction effects. Positive entropy (plotted in red or orange) indicates interaction, which can be interpreted as a synergistic or nonadditive relationship; while negative entropy (plotted in yellow-green) indicates independence or additivity (redundancy)

as well as inhibit matrix degradation [42, 43], and it has a close relationship in the expression of HIF-1α under hypoxic conditions such as occurrence in articular cartilage [44]. In our study, we evaluated the rs35767 and rs2288377 polymorphisms of the *IGF1* gene, and we observed that the carriers of the heterozygous genotype and the minor allele in both polymorphisms have higher risk to develop OA. Today, the role of these polymorphisms in the development of OA is not clear. In other pathologies such as osteoporosis, the rs35767 polymorphism has also been associated with risk, especially with low levels of bone mineral density of the femoral neck [45]; however, in the study performed by Chen YC et al., they found that the rs2288377 polymorphism was not associated with osteoporosis risk [46]. In view of these reports, our results may help to elucidate the role that plays the rs35767 and rs2288377 polymorphisms in pathologies that affect the joint and adjacent tissues, but more studies are needed to support it.

Also, we observed that the rs1793953 polymorphism of the *COL2A1* gene was associated with protection against OA. It is known that this gene codifies for the alpha chain of type II collagen, which is the main component of the ECM of the articular cartilage. Alterations in this gene have been associated with OA and early onset family OA, among other cartilage disorders [47]. In the study performed by Gálvez-Rosas et al., they analyzed a polymorphic site in the *COL2A1* gene of primary knee OA patients and observed a significant association with KL grade 4 patients [48]. Moreover, Valdes et al. analyzed the rs1635560 polymorphism of the *COL2A1* gene in OA patients and found an association with a decrease in knee OA risk, but only among male patients (OR = 0.68, $P < 0.005$) [49]. Deng Y et al. analyzed the rs1793953 polymorphism of the *COL2A1* gene in intervertebral disc degeneration patients, and they found that the carriers of the A/A homozygous genotype and of the A minor allele showed a significant association with a lower risk of developing this disease ($P = 0.004$ and $P = 0.010$, respectively) [50]. The controversy of these results is highly interesting, suggesting for instance a dual role of the gene in the disease, or even a possible interaction with environmental or genetics factors not taken into account in the latter studies. Thus, it is necessary to explore other polymorphic variants in *COL2A1* in our population and elucidate their involvement in OA.

Finally, in the present work, we evaluated the rs6438552 polymorphism of the glycogen synthase kinase-3B (*GSK3B*) gene in knee OA patients, and we observed that the carriers of the heterozygous A/G genotype increase the risk of OA. Several studies have suggested a proinflammatory role for GSK-3 activity based on cytokine profiles during GSK-3 inhibition. GSK-3 inhibition has been demonstrated to ameliorate collagen-induced arthritis and collagen antibody-induced arthritis in mice, which is consistent with a proinflammatory role; however, its activity may have procatabolic or chondroprotective effects depending on the pathologic scenario, with important implications for the proposed use of GSK-3 inhibitors as therapeutic agents in arthritis [51].

The gene-gene interaction analysis allows us to know whether two or more polymorphisms impact OA genetic susceptibility. Our study allowed us to identify gene-gene interactions implemented by MDR with high-degree synergy between *AKT2* and *IGF1* genes (Fig. 1). Examination of these genes in the interaction model reveals a testable hypothesis for further studies; not only does the evaluation of interactions between genes increase the detection capacity, but it also helps to understand the genetics behind the underlying biological and biochemical pathways of the disease. Another important aspect is that with the MDR method, high-risk and low-risk genotypes were identifying in knee OA patients, suggesting an essential role of the polymorphisms involved in HIF-1α signaling pathway (Fig. 2). Because the MDR method allows the identification of risk predictive models in OA, it can also be used to provide support in preclinical diagnosis; in addition, knowing the mechanisms of interaction, it could help to designed specific therapeutic strategies where several molecular targets should be taken into account for OA.

Finally, the haplotypes analysis makes it possible to evaluate whether there are polymorphism blocks (groups) of a single gene that are jointly segregated and might be linked to the disease development. Our results show that the presence of CTG and AT haplotypes of the *HIF1A* and *IGF1* genes are significantly associated ($P < 0.05$) with knee OA (Supplementary Table 2). The

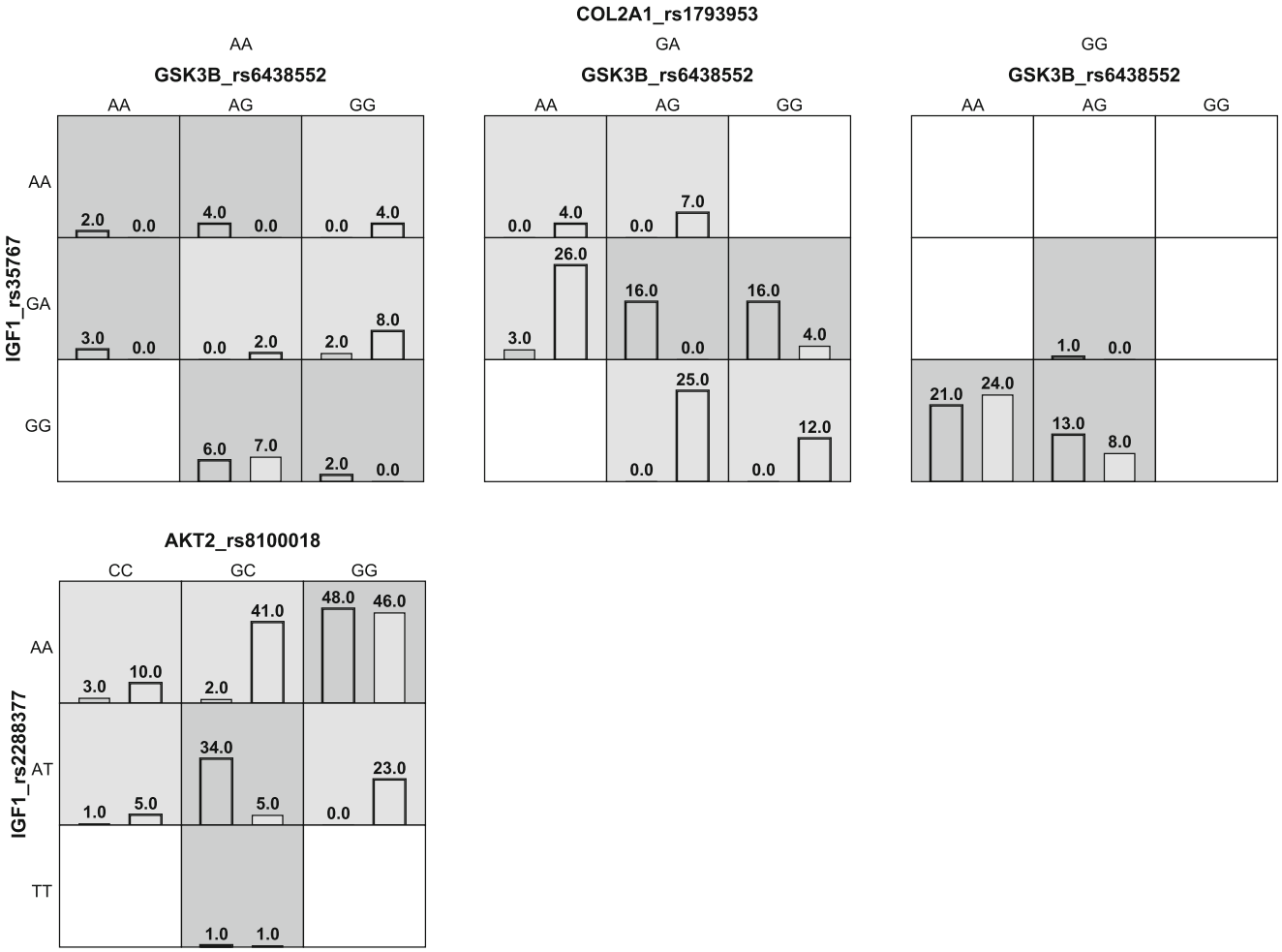

Fig. 2 Distribution of high-risk and low-risk genotypes in the best two- and three-*locus* model. The distribution shows high-risk (dark shading) and low-risk (light shading) genotypes associated with knee OA in the two- and three-*locus* interaction detected by MDR analysis. The percentage of osteoarthritic subjects (left black bar in boxes) and control subjects (right hatched bar in boxes) is shown for each two- and three-locus genotype combination. Boxes were labeled as high-risk if the ratio of the percentage of cases to controls met or exceeded the threshold of 1.0. Boxes were labeled as low-risk if the threshold was not exceeded. Based on the pattern of high-risk and low-risk genotypes, this two- and three-*locus* model is evidence of gene-gene interaction

data obtained points out the potential role that these genes play in knee OA development.

It is worth mentioning some strengths of our study. a) The population stratification was not biased, given that we included the ethnicity of each participant in the regression models assessed by AIMs; b) our study is the first that evaluated the wide number of genes related to the HIF-1α signaling pathway among Mexican patients with knee OA; and c) unlike genetic classical analysis, our main approach highlights the importance to evaluate in an integral manner the effect of genetic variants in knee OA.

Yet, it is important to highlight some aspects. We are aware of the limitations of our study; first, our sample size is limited; however, we believe that after performing a multivariate analysis and a rigorous selection of our patients and controls, the presented data reinforce the biological plausibility of the SNPs in the OA. Second, our association study was limited to two populations, so more studies in different populations are needed to support our findings, as well as to evaluate the functionality of the associated SNPs and be able to show evidence of whether they have a causal effect or not. Finally, there are more variants of the same gene that were not analyzed, as well as other genes of the HIF-1α signaling pathway that were not considered and whose impact on OA development is unknown.

Conclusions

We analyzed polymorphisms related to the HIF-1α signaling pathway in Mexican knee OA patients. Knowing the gene-gene interactions of these polymorphisms involved in HIF-1α signaling pathway could provide a new diagnostic support tool to identify individuals at high risk of developing knee

OA which can serve as a therapeutic target; additionally, a large-scale study to assess HIF-1α signaling pathway polymorphisms and mechanisms of interaction is needed to clarify the role of HIF-1α polymorphisms in the pathogenesis of knee OA.

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Compliance with ethical standards Written informed consent was obtained from all participants according to the Declaration of Helsinki and the study protocol was approved by ethics committee of the National Research Centre.

Disclosures None.

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