Molecular Biology Reports (2019) 46:2049–2058
https://doi.org/10.1007/s11033-019-04654-5

ORIGINAL ARTICLE

Epistasis between *ADIPOQ* rs1501299 and *PON1* rs662 polymorphisms is potentially associated with the development of knee osteoarthritis

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Received: 4 December 2018 / Accepted: 24 January 2019 / Published online: 8 February 2019

Abstract

Overweight produces oxidative stress (OS) on the articular cartilage, with the subsequent risk of developing knee osteoarthritis (OA). Associations between genetic polymorphisms related to OS and OA have been reported, but it is currently unknown whether there exist interactions among them that affect OA development. To identify and evaluate interactions between multiple SNPs related to OS in Mexican knee OA patients. Ninety-two knee OA patients were included in the study, which were compared to 147 healthy controls. Nine variants of six genes (*PEPD, AGER, IL6, ADIPOQ, PON1*, and *CA6*) related to OS were genotyped in both study groups through the OpenArray system. Epistasis was analyzed with the multifactor dimensionality reduction (MDR) method. The MDR analysis revealed a significant interaction ($p = 0.0107$) between polymorphisms rs1501299 (*ADIPOQ*) and rs662 (*PON1*), with an entropy value of 9.84%; in addition, high and low risk genotypes were identified between these two polymorphisms. The effect of the interaction between rs1501299 (*ADIPOQ*) and rs662 (*PON1*) polymorphisms seems to play an important role in OA pathogenesis; so the epistasis analysis may provide an excellent tool for identifying individuals at high risk for developing OA.

Keywords Knee osteoarthritis · Oxidative stress · Multifactor dimensionality reduction · Epistasis · Single nucleotide polymorphism

Introduction

Primary osteoarthritis (OA) is a multifactorial and polygenic disorder with a complex etiology that affects the structure and composition of articular tissues. It is characterized by progressive loss of articular cartilage, presence of osteophytes and subchondral bone sclerosis, resulting in pain and disability. Overweight, age, and gender are the main factors involved in OA development [1], but more detailed studies on OA pathogenesis acknowledge genetic factors and inflammatory processes as critical elements for its development [2]. In addition to these factors, an imbalance in cartilage homeostasis is favored by pro-inflammatory molecules, such as cytokines, lipid mediators, reactive oxygen species (ROS) and reactive nitrogen species (RNS), triggering an oxidative stress (OS) state [3]. Recent studies have suggested that OS plays a critical role in the development of many pathological conditions, such as OA, due to oxidation of proteins of the articular cartilage such as type II collagen [4–6]. Current studies on proline recycling by prolidase enzyme involved in collagen synthesis, show that in this process significantly

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favors ROS production, which promote OA development [7–12].

On the other hand, production of advanced glycation end products (AGEs) is an unavoidable process in vivo, and their accumulation in different tissues is importantly related to aging and systemic OS [13]. AGEs are the result of the non-enzymatic, post-translational addition of reducing sugars to proteins or apolipoproteins [6]. Accumulation of AGEs in the joints of OA patients leads to cartilage stiffness and fragility. AGEs bind the receptor for advanced glycation end products (RAGE) to trigger the intracellular pro-inflammatory response and promote hypertrophy development in chondrocytes [14].

As well as AGEs, the pro-inflammatory mediator interleukin-6 (IL-6) plays a critical role in very early stages of OA with increasing serum levels as the disease progresses [15]. In addition to IL-6, a direct correlation between serum levels of tumor necrosis factor-α (TNF-α) and IL-1β, and the OA radiographic grade has been observed. These three cytokines are the main mediators in cartilage degradation associated with OA. A recent study showed that IL-1β decreases expression of type II collagen and aggrecan, and increases production of matrix metalloproteinases-1 and -3 (MMPs). Both IL-1β and the cartilage mechanic charge promote nitric oxide (NO) production by positively regulating nitric oxide synthase 2 (NOS2), which induces chondrocyte apoptosis [16].

Overweight is linked to adipose tissue accumulation in different parts of the body. Adipose tissue is a metabolically active endocrine organ that secretes IL-1β, TNFα, and adipokines, such as adiponectin, leptin, and resistin. These inflammatory mediators have been shown to be related to articular cartilage degeneration and OA development [17]. However, the role of adiponectin has not been fully elucidated, as there are inconsistencies regarding its protecting, or rather, damaging role in maintaining articular cartilage homeostasis [18].

On the other hand, the antioxidant system plays a critical role in the homeostasis of several tissues, such as cartilage. Paraoxonase-1 (PON-1) is a Ca^{2+}-dependent esterase with antioxidant properties that is associated with high density lipoproteins (HDL). However, PON-1 enzyme activity is reduced in high OS diseases, such as heart disease, dyslipoproteinemias, inflammatory processes, lung cancer, type 2 diabetes mellitus, and as recently shown, OA [19–21].

Another group of enzymes with antioxidant activity are carbonic anhydrases (CAs), which regulate pH inside and outside the cell, catalyzing the reversible conversion of HCO_3^- and H^+ ions into water and CO_2. Since CAs are involved in multiple body processes, their inhibition is associated with a series of disorders, such as macular edema, congestive heart failure-induced edema, glaucoma, obesity, cancer, and osteoporosis [22]. Derived from the avascularity in the articular cartilage, oxygen and glucose concentrations are low, so oxygen and nutrients are basically supplied by subchondral bone and synovial fluid diffusion [23, 24]. When chondrocytes suffer from oxygen deficit, glycolysis is the main mechanism for energy sustenance. However, lactic acid is generated as a subproduct of this process, contributing to acidification [25]. In this sense, significant changes in pH are critical for the articular cartilage and subchondral bone calcification processes through mineralization and formation of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] and precipitation of calcium carbonate ($CaCO_3$), where CAs play an essential role [26].

Changes in the expression of the aforementioned proteins may be due to the presence of genetic polymorphisms in their respective genes, thus affecting OA development. Several studies suggest a significant association of single-nucleotide polymorphisms (SNPs) in OA development [27, 28]. Nevertheless, the main limitations of association studies on complex diseases are limitation in the control of variables, especially environmental factors with potentially confusing effect; and sample size, which can significantly influence the statistic power to detect small effects. Moreover, SNP distribution is generally affected by the ethnicity of populations. On the other hand, studies related to analyzing gene–gene or SNP–SNP interactions, better known as epistasis, are usually conducted with logistic regression models, linkage disequilibrium (LD), and Hardy–Weinberg equilibrium (HWE), all of which have their own limitations. Logistic regression models require a very large "N" to detect statistical significance; also, polymorphisms that are poorly represented or of low frequency are difficult to detect; and the interactions between the polymorphisms can only be evaluated by pairs. With respect to linkage disequilibrium (LD), also evaluates by pairs and not in group. If two or more polymorphisms are very close to each other, they can generate false positives without an interaction taking place. Finally, with respect to the HWE, it can be affected when a polymorphism is not in equilibrium in complex diseases (such as OA), but this measure of disequilibrium can be given by other causes such as gene drift (when a trait is randomly fixed and inherited more), by positive selection, or selective pressure.

In this sense, the multifactor dimensionality reduction (MDR) approach is a non-parametric method that detects and characterizes non-linear interactions between discrete attributes (for instance, SNP, smoking, gender, etc.), which are predictive of a discrete result (for example, cases and controls). Furthermore, MDR combines attribute selection and construction, and classification with cross-validation to offer a powerful approach for modeling interactions [29, 30].

According to the information described above, the objective of this study was to identify and evaluate epistasis between multiple SNPs related to OS in Mexican knee OA patients.

Materials and methods

Subjects

Ninety-two unrelated patients with primary knee OA from the Outpatient Department of the "Luis Guillermo Ibarra Ibarra" National Rehabilitation Institute (INRLGII) were included in this case-control study. The knee OA was diagnosed under the guidelines of the American College of Rheumatology [31]; the clinical exam and the X-ray evaluation were performed by a rheumatologist and a radiologist, respectively. OA severity was evaluated using the Kellgren–Lawrence radiological scale (K–L) [32]. Patients with K–L ≥ 2 were included due to from this radiological grade the presence of osteophytes is confirmed and there is possible narrowing of the joint space; however, in K–L < 2 grade, the presence of osteophytes is still doubtful or null. Patients with other etiologies causing knee diseases, such as inflammatory arthritis (rheumatoid arthritis or any other autoimmune disease), post-traumatic arthritis, post-septic arthritis, poliomyelitis, or skeletal dysplasia, were excluded. Additionally, 147 healthy employees from the Departments of Human Communications and Human Resources, as well as janitorial staff of the INRLGII, with no signs, symptoms or family history of OA, were selected as the control group.

All the procedures with the participants of this study were conducted under the ethical standards of the Institutional Research and Ethics Committee of the INRLGII (CONBIOETICA-09-CEI-031-20171207) and under the Helsinki Declaration (1964). This study was approved by the ethics committee of the INRLGII and is derived from a main study with registration INR-18/13. All the participants signed an informed consent letter before the study, their minimum age is 40 years old, and they all are from the same geographic region (Mexico City and bordering states).

SNPs selection and genotyping

Nine candidate SNPs of six genes involved in OS development were selected. The search strategy was as follows: (a) information of each SNP was obtained from the public databases Hap Map (http://www.hapmap.ncbi.nlm.nih.gov/) and the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp); (b) the previously selected SNPs have been evaluated in OA or other pathologies [33–39]; and (c) the minor allele frequency (MAF) in the Mexican population must be > 10% (Table 1). Considering that the ancestral structure of the Mexican population is primarily composed of Amerindian, European, and African populations, a panel of eight ancestry-informative markers (AIMs) was included to distinguish each of these

Table 1 SNPs evaluated in this study

Closest gene	SNP rs ID	Chromosome: position	Major/minor allele (MAF in Mexican population)	Most severe consequence
PEPD	rs10425678	19: 33977396	T/C (0.52)	Intron variant
AGER	rs1800624	6: 32152387	A/T (0.26)	Upstream gene variant
AGER	rs1035798	6: 32151222	G/A (0.26)	Splice region variant
IL6	rs1474347	7: 22768124	A/C (0.17)	Non-coding transcript exon variant
ADIPOQ	rs2241766	3: 186570892	T/G (0.14)	Synonymous variant
ADIPOQ	rs1501299	3: 186571123	G/T (0.33)	Intron variant
PON1	rs662	7: 94937446	C/T (0.48)	Missense variant
PON1	rs854560	7: 94946084	A/T (0.19)	Missense variant
CA6	rs17032907	1: 9010405	C/T (0.12)	Intron variant
AIMs				
SAP30L	rs3340	5: 153831867	T/C (0.48)	Intron variant
–	rs2695	9: 82884577	C/T (0.60)	Intergenic variant
DRD2	rs1800498	11: 113291588	G/A (0.33)	Intron variant
–	rs2862	15: 35145553	T/C (0.59)	Upstream gene variant
–	rs223830	16: 57451971	T/C (0.40)	Downstream gene variant
CA10	rs203096	17: 50011769	G/T (0.50)	Intron variant
CKM	rs4884	19: 45810035	G/A (0.58)	Synonymous variant
–	rs722098	21: 16685598	G/A (0.58)	Intergenic variant

SNP single nucleotide polymorphisms, *MAF* minor allele frequency, *AIMs* ancestry informative markers

three populations with a value of $\delta > 0.44$ to adjust all the analysis [40].

Peripheral blood of all participants was obtained through venipuncture in order to isolate genomic DNA using a commercial method (QIAmp 96 DNA Blood Kit, Qiagen, Hilden, Germany). Isolated DNA was adjusted with molecular grade water at 50 ng/µl and maintained at −80 °C until it was used. Genotyping was conducted with the OpenArray technology on a QuantStudio 12K flex System equipment (Thermo Fisher Scientific). A mixture of 2.5 ng/µl of DNA and 2.5 µl of TaqMan OpenArray Genotyping Master Mix (Thermo Fisher Scientific) was prepared and loaded into 384-well trays. 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 software v1.2.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). The *P*-values were calculated with Student's *t* test (continuous variables) or Fisher's exact test (categorical variables), when appropriate. HWE was calculated using the chi-squared test and SNPs not in HWE in the control group were excluded. Gene and allele frequencies among cases and controls were calculated using the chi-squared test or Fisher's exact test, when appropriate. Associations of each SNP with OA risk were calculated with logistic regression models adjusted by age, gender, body-mass index (BMI), and ancestry. All statistical analyses were performed with the STATA v14.0 statistical package (StataCorp, Texas, USA), with a significance level of $\alpha = 0.05$. The Bonferroni's test was used to determine the significance level to correct multiple test errors, in which taking into account the nine selected SNPs, an adjusted *P*-value < 0.005 (α/number of *loci*) was considered statistically significant. The STRUCTURE software v2.3.4 (Pritchard Lab, Stanford University, USA) was used to evaluate the effect of population stratification on the associations found for each population k (k = 3) with the genotypes of the eight AIMs selected. The interactions between the nine SNPs selected in OA were evaluated with the MDR software v3.0.2, according to the algorithm proposed by Ritchie et al. [29]. Additionally, the OR-value of the high and low risk genotypes was calculated from the best model obtained, following the method described by Chung et al. [41].

Results

Characteristics of the study population

The characteristics of the study population are shown in Table 2. Cases were older than controls (47.2 ± 12.5 years old vs. 40.9 ± 12.0, respectively, $P = 0.0001$), and the group of women was more representative than the group of men, both in cases and controls ($P = 0.005$). BMI shows significant differences between both study groups, being higher in OA patients than in controls (29.0 ± 4.19 vs. 24.8 ± 4.38, $P < 0.0001$). The place of birth of the participants was similar among both study groups ($P = 0.40$), with the majority originating from Mexico City.

Gene and allele frequency of oxidative stress SNPs

Table 3 shows the genotype and allele distribution in cases and controls with OR-values adjusted by age, gender, BMI, and ancestry; there were no significant differences in their distribution in both study groups. The genotype distributions

Table 2 Characteristics of the study population

Parameter	Total (n = 239)	OA (n = 92)	Controls (n = 147)	*P*
Age (years)	43.4 ± 12.5	47.2 ± 12.4	40.9 ± 12.0	**0.0001**[#]
Gender				
Female (%)	185 (77.4)	80 (87.0)	105 (71.4)	**0.005***
Male (%)	54 (22.6)	12 (13.0)	42 (28.6)	
BMI (Kg/cm^2)	26.5 ± 4.76	29.0 ± 4.19	24.8 ± 4.38	**< 0.0001**[#]
Place of birth				
Mexico City	195 (81.5)	71 (77.2)	124 (84.3)	0.40*
Other states	44 (18.5)	21 (22.8)	23 (15.7)	

Data are expressed as mean ± SD. Normal: 18.5–24.9; overweight: 25.0–29.9; obesity: ≥ 30.0. Significant *P*-values are in bold

BMI body-mass index

[#]*P*-values were estimated using Student's *t* test, α = 0.05

**P*-values were estimated using Fisher's exact test, α = 0.05

Table 3 Genetic and allelic frequencies of SNPs studied in OA patients and controls

Gene (SNP rs ID)	OA N (%)	Controls N (%)	Adjusted OR*	(95% CI)	*P*
PEPD (rs10425678)					
TT	18 (21.2)	19 (21.6)	1.00	Reference	
TC	39 (45.9)	42 (47.7)	0.80	(0.32–2.00)	0.64
CC	28 (32.9)	27 (30.7)	1.07	(0.40–2.84)	0.88
T	75 (44.1)	80 (45.5)	1.00	Reference	
C	95 (55.9)	96 (54.5)	1.06	(0.65–1.71)	0.80
HWE	0.521	0.725			
AGER (rs1800624)					
AA	40 (44.9)	59 (47.6)	1.00	Reference	
AT	39 (43.8)	49 (39.5)	1.27	(0.67–2.39)	0.44
TT	10 (11.2)	16 (12.9)	0.78	(0.30–2.02)	0.61
A	119 (66.9)	167 (67.3)	1.00	Reference	
T	59 (33.1)	81 (32.7)	0.98	(0.63–1.52)	0.94
HWE	0.915	0.257			
AGER (rs1035798)					
GG	39 (45.3)	56 (46.3)	1.00	Reference	
GA	37 (43.0)	50 (41.3)	1.19	(0.63–2.25)	0.57
AA	10 (11.6)	15 (12.4)	0.86	(0.33–2.23)	0.75
G	115 (66.9)	162 (66.9)	1.00	Reference	
A	57 (33.1)	80 (33.1)	1.00	(0.64–1.55)	0.99
HWE	0.786	0.465			
IL6 (rs1474347)					
AA	67 (83.7)	61 (72.6)	1.00	Reference	
AC	12 (15.0)	19 (22.6)	0.67	(0.29–1.56)	0.35
CC	1 (1.25)	4 (4.76)	0.21	(0.02–2.12)	0.18
A	146 (91.2)	141 (83.9)	1.00	Reference	
C	14 (8.80)	27 (16.1)	0.55	(0.27–1.12)	0.10
HWE	0.587	0.138			
ADIPOQ (rs2241766)					
TT	56 (64.4)	59 (56.7)	1.00	Reference	
TG	28 (32.2)	42 (40.4)	0.59	(0.29–1.19)	0.14
GG	3 (3.40)	3 (2.90)	0.98	(0.13–7.09)	0.98
T	140 (80.5)	160 (76.9)	1.00	Reference	
G	34 (19.5)	48 (23.1)	0.72	(0.40–1.27)	0.26
HWE	0.826	0.160			
ADIPOQ (rs1501299)					
GG	55 (63.2)	61 (57.0)	1.00	Reference	
GT	29 (33.3)	40 (37.4)	0.53	(0.26–1.10)	0.09
TT	3 (3.50)	6 (5.60)	0.69	(0.13–3.52)	0.66
G	139 (79.9)	162 (75.7)	1.00	Reference	
T	35 (20.1)	52 (24.3)	0.66	(0.37–1.16)	0.15
HWE	0.728	0.867			
PON1 (rs662)					
CC	46 (61.3)	71 (69.6)	1.00	Reference	
CT	24 (32.0)	26 (25.5)	1.09	(0.49–2.41)	0.81
TT	5 (6.70)	5 (4.90)	1.95	(0.42–8.99)	0.39
C	116 (77.3)	168 (82.3)	1.00	Reference	
T	34 (22.7)	36 (17.7)	1.26	(0.68–2.35)	0.45
HWE	0.450	0.214			
PON1 (rs854560)					
AA	19 (22.6)	25 (23.4)	1.00	Reference	

Table 3 (continued)

Gene (SNP rs ID)	OA N (%)	Controls N (%)	Adjusted OR*	(95% CI)	*P*
AT	48 (57.1)	51 (47.6)	1.23	(0.53–2.80)	0.62
TT	17 (20.3)	31 (29.0)	0.96	(0.36–2.57)	0.94
A	86 (51.2)	101 (47.2)	1.00	Reference	
T	82 (48.8)	113 (52.8)	0.98	(0.61–1.58)	0.95
HWE	0.188	0.651			
CA6 (rs17032907)					
CC	30 (33.7)	45 (36.6)	1.00	Reference	
CT	46 (51.7)	57 (46.3)	1.41	(0.73–2.71)	0.30
TT	13 (14.6)	21 (17.1)	0.86	(0.35–2.15)	0.76
C	106 (59.6)	147 (59.8)	1.00	Reference	
T	72 (40.4)	99 (40.2)	1.01	(0.66–1.54)	0.95
HWE	0.491	0.685			

*The multi-variable model was adjusted for age (continuous data), gender (male, female), BMI (continuous data) and admixture; OA, osteoarthritis patients; OR, Odds ratio; CI, confidence interval. HWE, Hardy–Weinberg equilibrium; if $P < 0.05$, not consistent with HWE

were all in HWE ($P > 0.05$), indicating that the study subjects were representative of the study field.

Interquartile range of the three main components of the study groups

There was no significant difference in the genetic structure between the study groups ($P > 0.05$). The interquartile range of the three main ancestral components of the Mexican population was: Amerindian component, k1 = 0.34 ± 0.08, $P = 0.116$; European component, k2 = 0.3 3 ± 0.16, $P = 0.128$; African component, k3 = 0.34 ± 0.08, $P = 0.141$.

Evaluation of gene–gene interactions: MDR

Table 4 summarizes the results of the exhaustive MDR analysis, which analyzed all possible combinations of the studied polymorphisms. According to the MDR analysis, the best model includes the *ADIPOQ* (rs1501299) and *PON1* (rs662) polymorphisms. This model had a balanced accuracy test of 0.6171, a cross-validation consistency of 10/10 and an interaction *P*-value = 0.0107. Figure 1 shows a dendogram and interaction map of the studied polymorphisms, based on entropy measures among individual variables. (A) Dendrogram shows strong or weak interactions between polymorphisms of OS in knee OA. (B) A strong interaction effect was observed between *ADIPOQ* (rs1501299) and *PON1* (rs662) polymorphisms, and between *ADIPOQ* (rs1501299) and *AGER* (rs1800624) polymorphisms, with information gain values of 9.84% and 7.08%, respectively.

Moreover, our model allowed us to identify interactions between two *loci* in high risk genotypes of the *ADIPOQ* (rs1501299) and *PON1* (rs662) polymorphisms, and the most representative were [GG+CC], [GT+CC] and [TT+CT], respectively; and low risk genotypes [GT+CT] and [TT+TT], respectively (Fig. 2, left). Interestingly, when these two SNPs were merged as a single variable and the data reanalyzed it clearly came out as the best model for OA risk prediction (Fig. 2, right). According to this model, Table 5 shows the OR-values generated between *ADIPOQ* (rs1501299) and *PON1* (rs662) polymorphisms.

Table 4 Results of MDR analysis

Locus number	Best combination	Cross-validation consistency	Testing Bal Acc	*P*
1	ADIPOQ_rs1501299	8/10	0.5115	0.6230
2	ADIPOQ_rs1501299, PON1_rs662	10/10	0.6171	**0.0107**
3	ADIPOQ_rs1501299, PON1_rs662, PON1_rs854560	4/10	0.5429	0.1719
4	ADIPOQ_rs1501299, AGER_rs1800624, PON1_rs662, PON1_rs854560	7/10	0.6107	0.0547

P-values were based on 1000 permutations and were obtained from sign test

Significant *P*-values are in bold

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

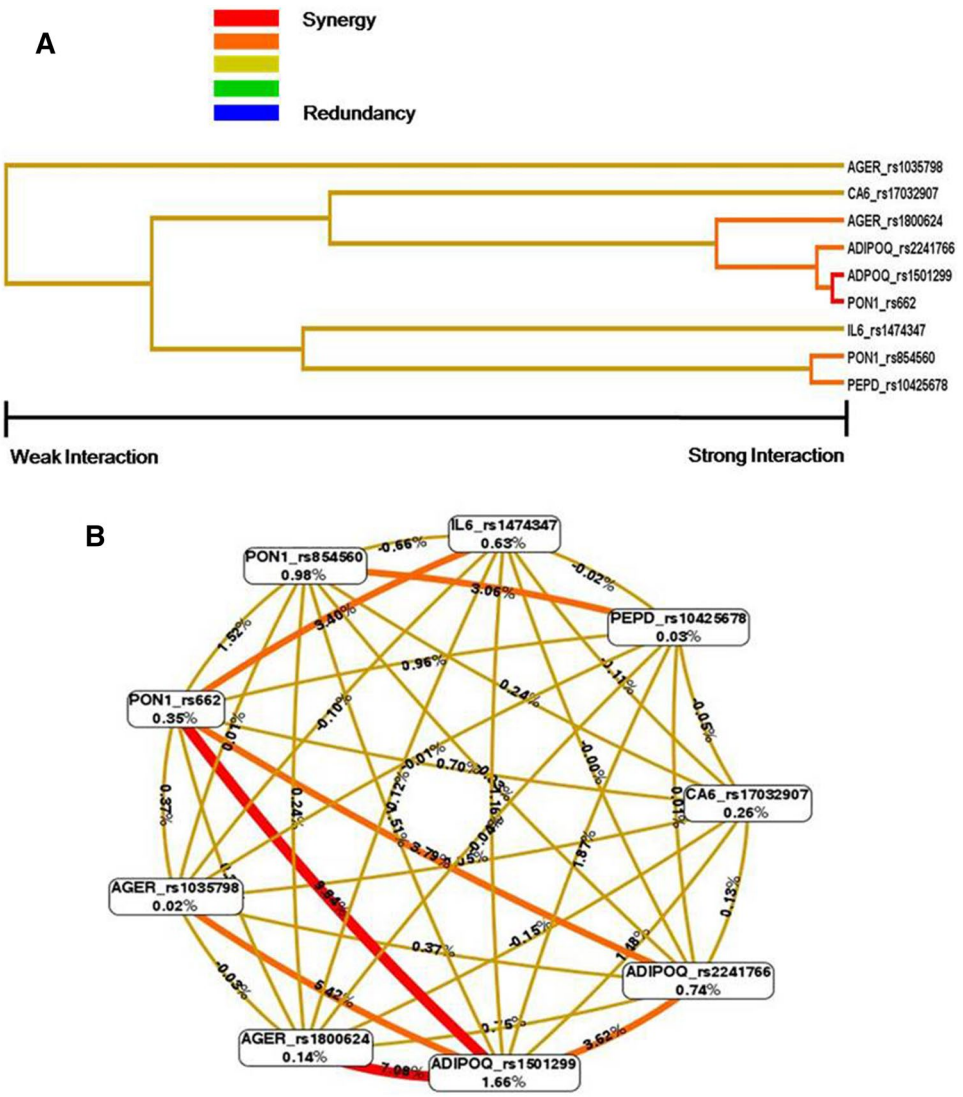

Fig. 1 **a** Dendrogram of interactions between oxidative stress polymorphisms in knee osteoarthritis patients. The colors used depict the degree of synergy, ranging from red (highest information gain) to blue (highest information redundancy). **b** Interaction map for knee osteoarthritis risk. The interaction model describes the percentage of the entropy (information gain) that is explained by each factor or 2-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 or green) indicates independence or additivity (redundancy). (Color figure online)

Discussion

In this study, we evaluated the epistasis by MDR of genetic polymorphisms related to OS in knee OA patients. By definition, OS is a perturbation in the balance of production of ROS and antioxidant defenses resulting in macromolecular damage and interruption of signaling and redox control [3]. At the articular tissue level, ROS play a major role in the inflammatory intracellular signaling mechanisms of the synovial membrane and adjacent tissues [4]. Even though the environment and lifestyle seem to be key factors for the onset and progression of metabolic diseases, some of them, such as obesity and type 2 diabetes are subject to genetic susceptibility, with the subsequent risk of developing OA [6]. Despite the fact that we could not establish a significant association of the genetic polymorphisms of OS in OA development, our findings show significant evidence of interaction between variants rs1501299 (*ADIPOQ*) and rs662 (*PON1*).

Adiponectin is a 30-kDa hormone that is synthesized in the adipose tissue and carries out several biological functions in the organism; its receptors (AdipoR1 and AdipoR2) are expressed on the surface of human chondrocytes, suggesting an important role in OA etiology [17]. Even though its dual role (protector or risk factor) in articular cartilage maintenance is speculated upon, it was recently shown to be related to adiponectin activity and expression of articular cartilage damage markers, with production of pro-inflammatory and catabolic factors in OA [42]. Recently, Zhan et al. [36] evaluated polymorphisms rs2241766 and rs1501299 of the *ADIPOPQ* gene in knee OA patients with no significant association found. Nevertheless, when they stratified patients by radiological grades 2, 3, and 4, they identified significant risk association with rs1501299 variant only. In our study, we analyzed the same variants and our results were similar.

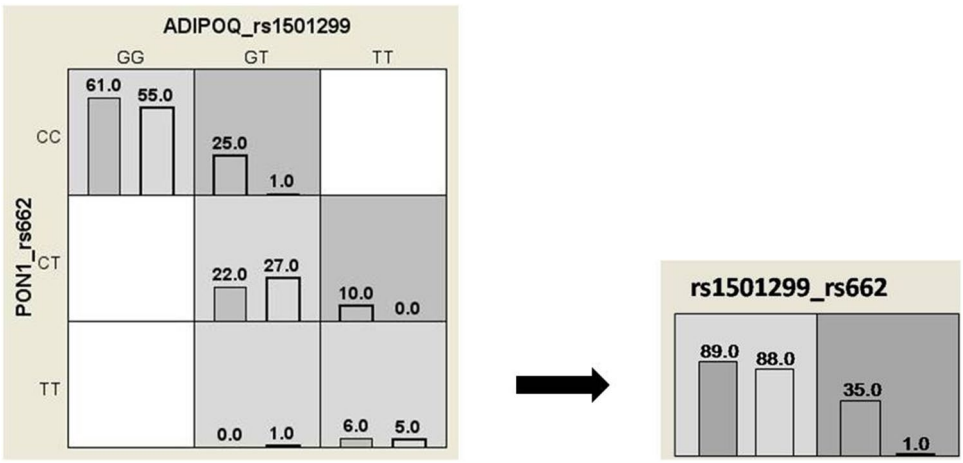

Fig. 2 Distribution of high-risk and low-risk genotypes in the best two-*locus* model. The distribution shows high-risk (dark shading) and low-risk (light shading) genotypes associated with knee osteoarthritis (KOA) in the two-*locus* interaction detected by MDR analysis. The percentage of KOA subjects (left black bar in boxes) and control subjects (right hatched bar in boxes) is shown for each two-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-*locus* model is evidence of gene–gene interaction. Summary for *ADIPOQ* (rs1501299) and *PON1* (rs662) SNPs combinations associated with risk to OA as (left) independent variables and as (right) a single merged variable

Table 5 OR-values according to the best model between *ADIPOQ*_rs1501299 and *PON1*_rs662 polymorphisms

rs1501299 genotype	rs662 genotype	Cell frequency[a]	OR
GG	CC	61:55	1.77
GT	CC	25:1	39.9
TT	CT	10:0	–
GT	CT	22:27	1.30
TT	TT	6:5	1.92

[a]The first number indicates the number of cases and the second the number of controls in each cell from Fig. 2 (left)

Jiang et al. [43] evaluated variants rs182052, rs2082940, and rs6773957 of *ADIPOQ*, and only variant rs182052 was associated with a potential risk of developing knee OA in a Chinese population. The association results might be consistent, but to our knowledge, there is not enough evidence of the contribution of the *ADIPOQ* gene variants in the serum levels of adiponectin that could directly influence OA development, as is the case with heart disease. A correlation between the presence of certain genotype or allele of variant rs1501299 and the serum levels of adiponectin has been observed in this pathology, with the subsequent risk of suffering a heart attack. In other words, higher serum levels of ADIPOQ turn out to be protective [44].

PON-1 is a 45-kDa glycoprotein primarily synthesized in the liver, with an antioxidant activity modulated by age and OS. With aging, OS increases and the enzymatic activity of PON-1 decreases, which correlates with an increase in HDL oxidation susceptibility [21]. Soran et al. [20] and Ertürk et al. [45] observed that OS favors lipid peroxidation while decreasing the enzymatic activity of PON-1 in OA patients. Moreover, several polymorphisms that might affect the risk of development of disease have been identified in the *PON1* gene; the most widely studied among them, rs854560 (L55M) and rs662 (R192Q), are located in the coding region. One study revealed that these two polymorphisms dramatically affect PON-1 activity and concentration in the serum of patients with a cardiovascular disease [46]. As far as we know, there is no scientific evidence evaluating the influence of polymorphisms rs854560 and rs662 on PON-1 activity in OA patients, but there is for other rheumatic pathologies. Tanimoto et al. [47] evaluated PON-1 serum activity subject to variant rs662 in rheumatoid arthritis patients, and they found it to be reduced in the group of patients. On the other hand, Xu et al. [48] evaluated variants rs662 and rs854560 in patients with ankylosing spondylitis, and like the previous report, they found a lower PON-1 activity in patients compared to the control group. The data presented above suggest that serum levels of adiponectin and paraoxonase-1 in OA patients are regulated by the presence of polymorphisms in their respective genes.

Regarding the MDR analysis, we found significant evidence of interaction between variants rs1501299 of *ADIPOQ* and rs662 of *PON1* in OA patients. This interaction is interesting, as both adiponectin and paraoxonase-1 are actively involved in OA development, suggesting a multi-*loci* effect of genes *ADIPOQ* and *PON1*, i.e., these two genes may need each other in order to have an effect

on OA development. This approach can be supported by the fact that the genotype analysis allowed us to identify low- and high-risk genotypes only for these two polymorphisms. Nevertheless, the calculated OR-values clearly show a risk interaction. However, we must highlight that our study was exclusively focused on one population, and the number of polymorphisms that were evaluated did not encompass the majority of those related to OS. Likewise, the design of our study does not allow us to know if there is a significant difference between the interaction of both polymorphisms and the different degrees of OA; so, the sample size should be increased to have representativeness in each of the radiological degrees. The interaction strength or intensity of any polymorphism can be lost if the genes are examined individually without considering the potential interactions with other genes, particularly those in related pathways. Therefore, further studies analyzing other pathways involved in OA development, as well as other populations, are needed.

In conclusion, we analyze polymorphisms related to OS in Mexican knee OA patients and found no significant associations, but the effect of the interaction between polymorphisms *ADIPOQ* rs1501299 and *PON1* rs662 seems to play an important role in the OA pathogenesis. When we evaluate the polymorphisms individually, we observe that the effect is relative small for it to be detected as statistically significant with our sample size; however, when the polymorphisms are analyzed in a combined manner by the MDR method, a significant interaction can be identified since the combined effect of both polymorphisms is stronger; one of the virtues of the MDR method is that it does not require large sample sizes to detect significant interactions. Finally, the epistasis analysis may provide an excellent tool for identifying individuals at high risk for developing OA (and others complex diseases, such as diabetes mellitus, cancer, and rheumatoid arthritis, among others.) which can serve as a therapeutic target.

Acknowledgements We thank the support provided by Dr. Daniela Garrido-Rodríguez in facilitating the laboratory where we genotyped the samples. The authors thank Dr. Alberto López-Reyes for supporting the purchase of the genotyping chip.

Funding The study was funded by departmental resources.

Compliance with ethical standards

Conflict of interest The authors declare that they have not conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the INRLGII-Institutional Research and Ethical Committee (CONBIOETICA-09-CEI-031-20171207) and with the Helsinki Declaration (1964). This study was approved by the ethics committee of the INRLGII.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Prieto-Alhambra D, Judge A, Javaid MK, Cooper C, Diez-Perez A, Arden NK (2014) Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. Ann Rheum Dis 73:1659–1664
2. Conway R, McCarthy GM (2015) Obesity and osteoarthritis: more than just mechanics. EMJ Rheumatol 2:75–83
3. Lepestos P, Papavassiliou AG (2016) ROS/oxidative stress signaling in osteoarthritis. Biochim Biophys Acta 1862:576–591
4. Ziskoven C, Jäger M, Kircher J, Patzer T, Bloch W, Brixius K et al (2011) Physiology and pathophysiology of nitrosative and oxidative stress in osteoarthritic joint destruction. Can J Physiol Pharmacol 89:455–466
5. Regan EA, Bowler RP, Crapo JD (2008) Joint fluid antioxidants are decreased in osteoarthritic joints compared to joints with macroscopically intact cartilage and subacute injury. Osteoarthr Cartil 16:515–521
6. Courties A, Gualillo O, Berenbaum F, Sellam J (2015) Metabolic stress-induced joint inflammation and osteoarthritis. Osteoarthr Cartil 23:1955–1965
7. Aslan M, Duzenli U, Esen R, Soyoral Y (2017) Serum prolidase enzyme activity in obese subjects and its relationship with oxidative stress markers. Clin Chim Acta 473:186–190
8. Phang JM, Pandhare J, Liu Y (2008) The metabolism of proline as microenvironmental stress substrate. J Nutr 138:2008S–2015S
9. Ercan AC, Bahceci B, Polat S, Cenker OC, Bahceci I, Koroglu A et al (2017) Oxidative status and prolidase activities in generalized anxiety disorder. Asian J Psychiatr 25:118–122
10. Clavijo-Cornejo D, Martínez-Flores K, Silva-Luna K, Martínez-Nava GA, Fernández-Torres J, Zamudio-Cuevas Y et al (2016) The overexpression of NALP3 inflammasome in knee osteoarthritis is associated with synovial membrane prolidase and NADPH oxidase 2. Oxid Med Cell Longev 2016:1472567
11. Altay MA, Ertürk C, Bilge A, Yaptı M, Levent A, Aksoy N (2015) Evaluation of prolidase activity and oxidative status in patients with knee osteoarthritis: relationships with radiographic severity and clinical parameters. Rheumatol Int 35:1725–1731
12. Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M (2007) Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. Rheumatol Int 27:339–344
13. Drinda S, Franke S, Rüster M, Petrow P, Pullig O, Stein G et al (2005) Identification of the receptor for advanced glycation end products in synovial tissue of patients with rheumatoid arthritis. Rheumatol Int 25:411–413
14. Zhao J, Yu Y, Wu Z, Wang L, Li W (2017) Memantine inhibits degradation of the articular cartilage extracellular matrix induced by advanced glycation end products (AGEs). Biomed Pharmacother 91:1193–1198
15. Azim S, Nicholson J, Rebecchi MJ, Galbavy W, Feng T, Rizwan S et al (2018) Interleukin-6 and leptin levels are associated with preoperative pain severity in patients with osteoarthritis but not with acute pain after total knee arthroplasty. Knee 25:25–33
16. Panina SB, Krolevets IV, Milyutina NP, Sagakyants AB, Kornienko IV, Ananyan AA et al (2017) Circulating levels of proinflammatory mediators as potential biomarkers of post-traumatic knee osteoarthritis development. J Orthop Traumatol 18:349–357

17. Gandhi R, Takahashi M, Smith H, Rizek R, Mahomed NN (2010) The synovial fluid adiponectin-leptin ratio predicts pain with knee osteoarthritis. Clin Rheumatol 29:1223–1228
18. de Boer TN, van Spil WE, Huisman AM, Polak AA, Bijlsma JW, Lafeber FP et al (2012) Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. Osteoarthr Cartil 20:846–853
19. Goswami B, Tayal D, Gupta N, Mallika V (2009) Paraoxonase: a multifaceted biomolecule. Clin Chim Acta 410:1–12
20. Soran N, Altindag O, Cakir H, Celik H, Demirkol A, Aksoy N (2008) Assessment of paraoxonase activities in patients with knee osteoarthritis. Redox Rep 13:194–198
21. Ertürk C, Altay MA, Selek S, Koçyiğit A (2012) Paraoxonase-1 activity and oxidative status in patients with knee osteoarthritis and their relationship with radiological and clinical parameters. Scand J Clin Lab Invest 72:433–439
22. Supuran CT (2008) Carbonic anhydrases: an overview. Curr Pharm Des 14:603–614
23. Sophia Fox AJ, Bedi A, Rodeo SA (2009) The basic science of articular cartilage: structure, composition, and function. Sports Health 1:461–468
24. Pfander D, Swoboda B, Cramer T (2006) The role of HIF-1alpha in maintaining cartilage homeostasis and during the pathogenesis of osteoarthritis. Arthritis Res Ther 8:104
25. Schultz M, Jin W, Waheed A, Moed BR, Sly W, Zhang Z (2011) Expression profile of carbonic anhydrases in articular cartilage. Histochem Cell Biol 136:145–151
26. Boskey AL (2003) Biomineralization: an overview. Connect Tissue Res 44(Suppl 1):5–9
27. Fernández-Moreno M, Rego I, Carreira-García V, Blanco FJ (2008) Genetics in osteoarthritis. Curr Genom 9:542–547
28. Wang T, Liang Y, Li H, Li H, He Q, Xue Y et al (2016) Single nucleotide polymorphisms and osteoarthritis: an overview and a meta-analysis. Medicine 95:e2811
29. Ritchie MD, Hahn LW, Moore JH (2003) Power of multifactor dimensionality reduction for detecting gene–gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. Genet Epidemiol 24:150–157
30. Hahn LW, Ritchie MD, Moore JH (2003) Multifactor dimensionality reduction software for detecting gene–gene and gene–environment interactions. Bioinformatics 19:376–382
31. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K et al (1986) Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and therapeutic criteria committee of the American Rheumatism Association. Arthritis Rheum 29:1039–1049
32. Kellgren JH, Lawrence JS (1957) Radiological assessment of osteo-arthritis. Ann Rheum Dis 16:494–502
33. Cho YS, Chen CH, Hu C, Long J, Ong RT, Sim X (2011) Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet 44:67–72
34. Han Z, Liu Q, Sun C, Li Y (2012) The interaction between obesity and RAGE polymorphisms on the risk of knee osteoarthritis in Chinese population. Cell Physiol Biochem 30:898–904
35. Li F, Xu J, Zheng J, Sokolove J, Zhu K, Zhang Y et al (2014) Association between interleukin-6 gene polymorphisms and rheumatoid arthritis in Chinese Han population: a case-control study and a meta-analysis. Sci Rep 4:5714
36. Zhan D, Thumtecho S, Tanavalee A, Yuktanandana P, Anomasiri W, Honsawek S (2017) Association of adiponectin gene polymorphisms with knee osteoarthritis. World J Orthop 8:719–725
37. Zhan D, Yuktanandana P, Anomasiri W, Tanavalee A, Honsawek S (2014) Association of adiponectin + 276G/T polymorphism with knee osteoarthritis. Biomed Rep 2:229–232
38. Gupta N, Singh S, Maturu VN, Sharma YP, Gill KD (2011) Paraoxonase 1 (PON1) polymorphisms, haplotypes and activity in predicting cad risk in North-West Indian Punjabis. PLoS ONE 6:e17805
39. Li ZQ, Hu XP, Zhou JY, Xie XD, Zhang JM (2015) Genetic polymorphisms in the carbonic anhydrase VI gene and dental caries susceptibility. Genet Mol Res 14:5986–5993
40. Fernández-Torres J, Zamudio-Cuevas Y, López-Reyes A, Garrido-Rodríguez D, Martínez-Flores K, Lozada CA et al (2018) Gene–gene interactions of the Wnt/β-catenin signaling pathway in knee osteoarthritis. Mol Biol Rep 45:1089–1098
41. Chung Y, Lee SY, Elston RC, Park T (2007) Odds ratio based multifactor-dimensionality reduction method for detecting gene–gene interactions. Bioinformatics 23:71–76
42. Koskinen A, Juslin S, Nieminen R, Moilanen T, Vuolteenaho K, Moilanen E (2011) Adiponectin associates with markers of cartilage degradation in osteoarthritis and induces production of proinflammatory and catabolic factors through mitogen-activated protein kinase pathways. Arthritis Res Ther 13:R184
43. Jiang L, Zhu X, Rong J, Xing B, Wang S, Liu A et al (2018) Obesity, osteoarthritis and genetic risk: The rs182052 polymorphism in the ADIPOQ gene is potentially associated with risk of knee osteoarthritis. Bone Joint Res 7:494–500
44. Zhang Z, Li Y, Yang X, Wang L, Xu L, Zhang Q (2018) Susceptibility of multiple polymorphisms in ADIPOQ, ADIPOR1 and ADIPOR2 genes to myocardial infarction in Han Chinese. Gene 658:10–17
45. Ertürk C, Altay MA, Bilge A, Çelik H (2017) Is there a relationship between serum ox-LDL, oxidative stress, and PON1 in knee osteoarthritis? Clin Rheumatol 36:2775–2780
46. Shunmoogam N, Naidoo P, Chilton R (2018) Paraoxonase (PON)-1: a brief overview on genetics, structure, polymorphisms and clinical relevance. Vasc Health Risk Manag 14:137–143
47. Tanimoto N, Kumon Y, Suehiro T, Ohkubo S, Ikeda Y, Nishiya K et al (2003) Serum paraoxonase activity decreases in rheumatoid arthritis. Life Sci 72:2877–2885
48. Xu H, Qu Y (2017) Correlation of PON1 polymorphisms with ankylosing spondylitis susceptibility: a case-control study in Chinese Han population. Medicine 96:e7416