COMMON VARIATIONS IN ESTROGEN-RELATED GENES ARE ASSOCIATED WITH SEVERE LARGE JOINT OSTEOARTHRITIS: A MULTICENTER GENETIC AND FUNCTIONAL STUDY

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ABSTRACT

Objective.- Several lines of evidence suggest that estrogens influence the development of osteoarthritis (OA). The aim of this study was to explore the association of two common polymorphisms within the aromatase (*CYP19A1*) and estrogen receptor alpha (*ESR1*) genes with severe OA of the lower limbs.

Methods.- The rs1062033 (*CYP19A1*) and rs2234693 (*ESR1*) single nucleotide polymorphisms were genotyped in 5,528 individuals (3,098 patients with severe hip or knee OA, and 2,381 controls) from 4 centres in Spain and the United Kingdom. Gene expression was measured in femoral bone RNA samples from a group of patients.

Results.- In the global analysis, both polymorphisms were associated with OA, but there was a significant sex interaction. The GG genotype at rs1062033 was associated with an increased risk of knee OA in women (OR 1.23; p=0.04). The CC genotype at rs2234693 tended to be associated with reduced OA risk in women (OR 0.76, p=0.028, for knee OA; OR=0.84, p=0.076 for hip OA), but with increased risk of hip OA in men (OR 1.28; p=0.029). Women with unfavourable genotypes at both loci had an OR of 1.61 for knee OA (p=0.006). The rs1062033 genotype associated with higher OA risk was also associated with reduced expression of the aromatase gene *CYP19A1* in bone.

Conclusions.- Common genetic variations of the aromatase and estrogen receptor genes are associated with the risk of severe OA of the large joints of the lower limb in a sex-specific manner. These results are consistent with the hypothesis that estrogen activity may influence the development of large-joint OA.

INTRODUCTION

Osteoarthritis (OA) is a prevalent disorder that frequently impairs the quality of life in middle aged and elderly individuals and represents a considerable economical burden to health-care systems. Among individuals over 45 years of age, several studies have reported a prevalence of hip OA of between 7 and 27%, and of approximately 16% for knee OA (1). Joint replacement surgery, mostly at the hip and the knee, represents a major component of the activity of orthopaedic surgery departments, with the number of operations expected to increase 6 fold by the year 2030 (2). Therefore, a better understanding of the pathophysiology of OA is important to establish effective preventive strategies. OA is currently seen as a complex disorder resulting from the interaction between acquired and genetic factors. The heritability of OA at different sites has been estimated to be approximately 75% at the spine, 65% at the hand, 60% at the hip, and 40% at the knee, with some suggestions for a more important contribution of heredity in females than in males (3;4). Although the damage to the cartilage is frequently emphasized in OA, there is evidence suggesting that changes to the synovium and the subchondral bone are also involved in the OA disease process (5).

Estrogens are critical modulators of bone homeostasis, in females and in males (6). They have also been shown to modulate chondrocyte activity and the synthesis of a variety of factors, including metalloproteinases, nitric oxide and reactive oxygen species, involved in the anabolism and catabolism of the cartilage matrix (7-10). Estrogens act through the binding to two types of specific estrogen receptors (ER), encoded by different genes: α (or *ESR1*) and β (or *ESR2*). *ESR1* seems to have a more consistent role in both sexes (6). ERs are expressed by a variety of cells in the skeleton, including stromal cells, osteoblasts and chondrocytes (11). ER genes have been considered as appealing candidates potentially contributing to the genetic component of OA, but conflicting results have so been reported [recently reviewed by Ryder et al. (12)]. However, various studies suggest that estrogens may indeed influence the development of OA(13); low levels of estrogens have been associated with an increased risk of OA in some studies (14), whilst estrogen replacement therapy may have a protective role on the development of OA, both in experimental animals and in humans (15-18). In fact, increased osteophytosis has been reported in ER knock out mice (19). However, the protective effect of estrogens has not been demonstrated in all studies, and conflicting results have been reported in primate models of OA (20-22).

In postmenopausal women and in men, the aromatization of androgenic precursors in peripheral tissues is the main source of estrogens. The reaction is catalyzed by aromatase, the product of the *CYP19A1* gene, located on chromosome 15 (15q21.1). The synthesis of estrogens by aromatase-expressing bone cells may have important effects at the skeletal tissue level, independent of the circulating levels of estrogens. In fact, we have recently shown that aromatase expression is reduced in the bone tissue of patients with severe hip OA, in comparison to patients with hip fractures (23). Therefore, we hypothesized that allelic variants of genes influencing estrogen synthesis and/or response could influence the risk of OA. The objective of this study was to explore the association of two common polymorphisms of the aromatase and ERα genes with primary OA of the lower limb.

METHODS

Subjects.- We studied 5628 individuals aged 55 years and older (2177 patients with hip OA, 971 patients with knee OA and 2382 controls) recruited at three centres in Spain (Santander, Santiago and Coruña) and one centre in the United Kingdom, Oxford (table 1). The Spanish cohorts included Caucasian individuals of Spanish ancestry living in two regions in Northern Spain (Cantabria, Santander cohort; or Galicia, Santiago and Coruña cohorts), The Oxford cohort comprised individuals of Caucasian ethnicity from Oxford and from other parts of the UK. Patients had severe, radiographically confirmed, primary OA requiring hip or knee joint replacement surgery. Exclusion criteria included individuals with secondary OA (due to systemic diseases, rheumatoid arthritis, infections, trauma, ischemic necrosis, neurological diseases, etc.), who were excluded by clinical, laboratory and radiographic studies. The control group included volunteers recruited by voice and written announcements, patient's spouses, and individuals taking part in a cohort study of osteoporosis risk factors. They had no clinically evident OA at the lower extremities, but X-rays were not obtained routinely in the absence of a clinical justification. The study was approved by local ethics committees and all individuals gave informed written consent.

Genotyping.- DNA was extracted from the peripheral blood or buccal swabs using standard procedures. Two single nucleotide polymorphisms (SNPs) were genotyped; the rs1062033 SNP located in the 5' untranslated region (5'UTR) of the aromatase gene *CYP19A1* and the rs2234693 SNP located in intron 1 of the ERα gene *ESR1*. rs1062033 has been shown to be associated with differences in gene transcription at *CYP19A1* and with BMD in

postmenopausal women (24;25), whilst rs2234693 has been suggested to influence OA risk in some studies (26-28). In the Santander and Oxford cohorts the SNPs were genotyped by TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA, USA), as previously described (24). In the Santiago and Coruña cohorts they were genotyped by a single-base extension procedure, involving a multiplex PCR reaction (Qiagen Multiplex PCR, Valencia, CA, USA; oligonucleotide primer sequences and PCR conditions are available upon request) followed by single-base extension reactions performed with the SNaPshot Multiplex Kit (Applied Biosystems). In each laboratory, 5% samples were analyzed at least twice in different days to confirm the genotyping reproducibility. Twelve samples were genotyped in different laboratories to check the consistency of results with both genotyping methods. Alleles were designated according to the human reference sequences (minus strand for the *CYP19A1* gene, plus strand for the *ESR1* gene; Genome build 36.3).

Gene expression.- *CYP19A1* and *ESR1* expression was studied by real-time quantitative PCR, as previously described (23). Biopsy samples were obtained from the femoral head removed during joint replacement for hip OA. Small fragments of trabecular bone were extensively washed with PBS, snap-frozen in liquid nitrogen and stored at –70ºC. Unthawed fragments were ground to a powder using a tissue homogenizer into Trizol (Invitrogen) to extract RNA, whose integrity was then monitored by gel electrophoresis and whose concentration was measured by absorptiometry. Aliquots of RNA (approximately 1 μg) were reverse-transcribed with the Superscript III kit (Invitrogen), using random hexamers as primers. In negative control reactions reverse transcriptase was omitted. After RT-PCR, the expression of *CYP19A1* and *ESR1* were determined by real-time PCR in an ABI7300 apparatus (Applied Biosystems). The reactions were performed in triplicate in 96-well plates containing aliquots of cDNA obtained by reverse transcription, 5 μl of universal PCR master mix, and specific primers and probes for *CYP19A1* and *ESR1* (TaqMan Gene Expression Assays, Applied Biosystems).

The cycle threshold (Ct) was determined. This represents the cycle at which a significant increase in fluorescence is first detected and is inversely related to the amount of target cDNA in the starting material. The results were normalized to *TBP* (TATA box binding protein) expression analyzed in the same reaction plate. Control samples of reverse transcribed reference human RNA (Stratagene, La Jolla, CA) were also included. The relative RNA levels were calculated using the formula $10 \times 2^{\Delta Ct1 - \Delta Ct2}$, where ΔCt1 is the difference between the

control cDNA Ct and the sample cDNA Ct when the target gene (*CYP19A1*, *ESR1*) is amplified, and ΔCt2 is the difference between control cDNA Ct and the sample cDNA Ct when the control gene (*TBP*) is amplified.

Data analysis.- Hardy-Weinberg equilibrium in controls was tested with HWSIM software (http://krunch.med.yale.edu/hwsim/hwsim.doc). Differences in genotype distribution in each cohort and in the combined study population were analyzed with the χ^2 test and by unconditional logistic regression analysis after genotype grouping. As measures of association, the odds ratios (OR) and their 95% confidence intervals were calculated for each cohort. The global OR combining all cohorts and the adjusted Mantel-Haenszel OR (weighted by each stratatum variance) were estimated as summary estimates of effect. Assuming a minor allele frequency of 0.4, the study power to detect an effect size associated with an OR of at least 1.4 was higher than 90%, in the global and the sex-stratified analysis, under a log-additive model. The non-parametric Jonckheere-Terpstra test for ordered categories was used to analyze the differences in gene expression between subjects with different genotypes. SPSS (SPSS Inc, Chicago, IL, USA) and EPIDAT software (http://dxsp.sergas.es/ApliEdatos/ Epidat/ cas/default.asp) were used in the statistical analyses. Study power calculations were done with QUANTO (http://hydra.usc.edu/gxe/)

RESULTS

Genetic variations and osteoarthritis

In the whole study group, the genotype frequencies were similar to those reported in other Caucasian populations. The *CYP19A1* SNP rs1062033 genotypes were: CC 20%, CG 48%, and GG 32%. The *ESR1* SNP rs2234693 genotype frequencies were: TT 30%, TC 50%, CC 20%. There was no evidence for departure from the Hardy-Weinberg equilibrium in the control groups ($p>0.1$). The analyses of the unstratified data did not show significant differences in genotype distributions between cases and controls for either SNP. However, in sex-adjusted logistic regression models the genotypes at both SNPs were associated with OA

(rs1062033, p=0.023; rs2234693, p=0.03), and there was a significant interaction between sex and the genotypes (p=0.04 for *CYP19A1* and 0.006 for *ESR1*). Therefore, all subsequent analyses were stratified by sex. A dominant model for rs1062033 and a recessive model for rs2234693 best fitted the data. The genotype frequency distributions in the study cohorts are shown in table 2.

In women there was a consistent trend towards an increased risk of knee OA in those bearing the rs1062033 GG genotype. The global OR was 1.28 (95% confidence interval 1.04-1.57, p=0.020) and the combined Mantel-Haenszel OR was 1.32 (95% CI 1.06-1.63; p=0.011), without evidence of heterogeneity between the three study populations (p=0.41). On the other hand, women with the CC genotype at rs2234693 had a lower risk of knee OA, with a global OR of 0.76 (0.59-0.97; p=0.028) and combined Mantel-Haenszel OR of 0.80 (0.62-1.02; p=0.08), without evidence of heterogeneity (p=0.9) (figure 1). Both SNPs were independently associated with the risk of knee OA, and there was no significant interaction between them. In the male subgroup, there were no significant associations between knee OA and either SNP (not shown).

Similarly, the GG genotype at rs1062033 was associated with a nonsignificant trend for increased risk of hip OA in women (OR 1.12, 95% CI 0.95-1.33; p=0.18; Mantel-Haenszel OR 1.18, 95% CI 0.98-1.41, p=0.075), with relatively wide between-study differences (heterogeity p-value=0.08; proportion of between-study variance to total variance I^2=0.5) (figure 2). As for knee OA, there was a consistent trend for lower risk of hip OA in women bearing the CC genotype at rs2234693, with a global OR of 0.84, almost reaching the conventional threshold for statistical significance (95% CI 0.69-1.02; p=0.07; Mantel-Haenszel OR 0.86, 95% CI 0.70-1.06, p=0.16; heterogeneity p-value 0.8). In the male subgroup rs1062033 was not associated with hip OA. However, unlike women, men with the CC genotype at rs2234693 had a higher risk of hip OA, with a global OR of 1.28 (1.02-1.59; p=0.029; Mantel-Haenszel OR 1.25; 95% CI 1.00-1.57, p=0.048; heterogeneity p-value 0.9) (figure 3).

As shown in table 3, when the genotypes at both loci were combined, in women the gradient risk was somewhat higher than with any individual genotype, particularly for knee OA, with ORs between 1.24 and 1.61 for knee OA. In men the combined analysis did not generate more significant data than when the SNPs were analysed individually (not shown).

CYP19A1 and *ESR1* expression

Gene transcription of *CYP19A1* and *ESR1* was studied in bone samples of 49 patients undergoing hip arthroplasty (29 men, 20 women) and the results were analyzed according to the donor's genotype. rs1062033 was associated with differences in the abundance of *CYP19A1* transcript with more abundant transcript in samples from individuals bearing C alleles than in those bearing G alleles (upper panel, figure 4). Intriguingly, significant differences in *CYP19A1* transcript abundance between individuals with different genotypes of rs2234693 were also observed (lower panel, figure 4). However, neither the rs2234693 genotypes nor the rs1062033 genotypes were associated with differences in the amount of *ESR1* transcripts in the bone samples (figure 5).

DISCUSSION

Estrogens are critical in the acquisition and maintenance of bone mass, in women as well as in men (29;30), and estrogen deficiency plays a major role in involutional osteoporosis. The possible role of estrogens in OA is less well established. However, several experimental and epidemiological studies suggest that estrogen deficiency may favour the development of this disease (31). For instance, Sowers et al found an inverse relationship between serum levels of estradiol and the incidence of knee OA, even after adjustment for age and BMI (32), but others did not confirm these results (33). Likewise, conflicting results have been published regarding the possible preventive effect of hormone replacement therapy on OA (34;35).

Such conflicting results are not totally unexpected, as the local synthesis of estrogens in bone may have important effects in nearby tissue that are not captured by measuring circulating serum estradiol, whilst a single point measurement may not reflect adequately the overall exposure of skeletal cells to estrogens during the lifetime period. In this regard, it has been suggested that studies of genetic variants modulating the synthesis or activity of biological factors may be more informative than single measurements or even short interventional studies, as they originate the so-called "Mendelian randomization"(36;37). In this multicenter study we found that two common polymorphisms related to the estrogen pathway are associated with the risk of OA. Thus, women bearing a particular genotype at the aromatase-coding gene *CYP19A1* are at an increased risk for knee OA. The effect size was relatively

small and not significant in individual cohorts, but it was consistent and statistically significant in the global analysis, with an odds ratio greater than 1.2. The risk genotype was common, being present in about one third of the individuals genotyped. Therefore, it may have important consequences not only at the individual level, but also at the population level. This risk genotype was associated with reduced *CYP19A1* expression in bone samples from patients with OA, thus confirming previous results obtained in patients with fractures (38;39). We do not have data on estrogen levels, but we speculate that individuals with genotypes associated with reduced *CYP19A1* expression may have lower levels of aromatase and have therefore had a lower cumulative exposure to estrogens during their life. This would be consistent with the hypothesis that a relative estrogen deficiency, either local or systemic, may favour the development of OA (40;41), and with a recent report of reduced aromatase expression in OA (23).

Aromatase is responsible for the synthesis of estrogens in postmenopausal women and in men (42). Its important role is emphasized by studies showing a marked decline in bone mass following therapy with aromatase inhibitors (43). On the other hand, type 1 estrogen receptors (ERα) are regarded as the main receptors transducing the effect of estrogens on target cells. Based on previous results (24;38;44), we hypothesized that genetic variations of ER could also modulate the risk of OA. In fact, although to our knowledge the association of aromatase gene polymorphisms with OA has not been reported previously, several investigators have explored the relationship between *ESR1* genetic variants and OA (45-49). In common with several of these studies, we found that the *ESR1* SNP rs2234693 was also associated with the risk of OA in women, particularly at the knee (results for hip OA did not reach the conventional 0.05 statistical significance threshold) and with hip OA in men. The polymorphism is located in intron 1 and we speculated that it might influence gene transcription. However, we found no significant differences in the abundance of *ESR1* transcripts across the three *ESR1* genotypes. Interestingly, *ESR1* genotypes were associated with statistically significant differences in *CYP19A1* transcript abundance. There is no clear explanation for this result and it obviously merits further genetic and functional investigation; a relevant observation from other studies is that ER may have ligand-independent and ligand-dependent effects on the expression of genes including *CYP19A1* (50), suggesting a trans-interaction between these two genes located in different chromosomes. Whatever the explanation might be, our results suggest that the alleles associated with lower aromatase levels are associated with an increased risk of OA. They do not prove a direct causal link

between the genotypes and OA, but suggest that genotype-dependent differences in estrogen levels or action influence OA risk.

Although aromatase is critical for estrogen synthesis in both postmenopausal women and men, we observed an interaction between sex and the genotypes. Thus, whereas both rs1062033 and rs2234693 genotypes were associated with knee OA in women, in men rs1062033 genotypes were not associated with either hip or knee OA, and rs2234693 genotypes were associated with hip OA, but in an opposite direction to that observed in women. The exact mechanisms explaining the sex-related differences are unclear. They may be the consequence of differences in the relative importance of acquired factors (such as physical activity) in men and women, or reflect some sex-specific responses to changes in estrogen levels, similar to what is observed in aromatase knock-out mice and in experimental models of OA (51;52).

This multicenter study included a large number of individuals, which allowed us to increase the precision of the estimates of the genetic effects. However, it had several limitations. First, we did not systematically obtain X-rays of the control subjects. Therefore, some of them may have mild asymptomatic OA, which could bias the results towards the null effect. On the other hand, although we excluded cases with secondary OA, we had limited information about anthropometric, nutritional and other environmental factors that may influence the risk of OA. Therefore, we could not explore possible interactions between genetic and acquired factors that can be important in OA.

In conclusion, in this multicenter study we have shown that common genetic variation of the aromatase gene *CYP19A1* and the ER gene *ESR1*, which are associated with gene expression, are also associated with the risk of severe OA of the large joints of the lower limb. The sex-stratified analysis suggested that the influence of the *CYP19A1* SNP is more important in women than in men, and in knee OA than in hip OA. On the other hand, the *ESR1* SNP may have a sex-specific influence on OA risk. Overall, these results are consistent with the hypothesis that estrogen activity may influence the development of large-joint OA.

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CONFLICTS OF INTEREST

Authors do not have conflicts of interest relevant to this paper.

AUTHOR CONTRIBUTIONS

Conception and design: JAR, JL
Provision of patients and samples: CMG, JJGR, AC, AP, IRP
Clinical and laboratory data acquisition: CGI, AG, EAR, CRF, FJB, IRP AD, JJGR, CMG, AC,
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Final approval of the article: all coauthors
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Study coordination and responsibility for the integrity of the work: JAR

TABLE 1. Characteristics of study subjects

	Santander	**Coruña**	**Santiago**	**Oxford**
Individuals (n)	1433	496	1014	2585
Controls (n)	802	244	473	862
Hip OA (n)	359	252	287	1278
Knee OA (n)	272	-	254	445
Age (mean, SD)				
Controls	71±10	65 ±13	68±9	69±7
Hip OA	71±7	67±14	68±5	65±6
Knee OA	72±7	-	68±6	64±5
Sex (% women)	61	62	55	56

Table 2. Genotype and allele frequencies in males and females (percentages in parentheses)

MALES									
		rs1062033				**rs2234693**			
Cohort	**Group**	**CC/CG**	**GG**	**C**	**G**	**TT/CT**	**CC**	**T**	**C**
Santander	Control	182 (64)	103 (36)	0.36	0.64	229 (80)	56 (20)	0.55	0.45
	Knee OA	58 (61)	37 (39)	0.36	0.64	79 (83)	16 (17)	0.58	0.42
	Hip OA	109 (61)	71 (39)	0.35	0.65	133 (74)	47 (26)	0.50	0.50
Santiago	Control	190 (64)	105 (36)	0.34	0.66	250 (85)	45 (15)	0.62	0.38
	Knee OA	22 (47)	25 (53)	0.37	0.63	34 (72)	13 (28)	0.48	0.52
	Hip OA	76 (69)	34 (31)	0.31	0.69	91 (83)	19 (17)	0.59	0.41
Coruña	Control	67 (69)	30 (31)	0.46	0.54	79 (81)	18 (19)	0.56	0.44
	Knee OA	-	-			-	-		
	Hip OA	63 (70)	27 (30)	0.46	0.54	72 (80)	18 (20)	0.59	0.41
Oxford	Control	330 (70)	141 (30)	0.46	0.54	385 (82)	86 (18)	0.55	0.45
	Knee OA	125 (71)	51 (29)	0.49	0.51	144 (82)	32 (18)	0.54	0.46
	Hip OA	363 (72)	140 (28)	0.47	0.53	395 (79)	108 (21)	0.56	0.44
FEMALES									
		rs1062033				**rs2234693**			
Cohort	**Group**	**CC/CG**	**GG**	**C**	**G**	**TT/CT**	**CC**	**T**	**C**
Santander	Control	358 (69)	159 (31)	0.43	0.57	394 (76)	123 (24)	0.52	0.48
	Knee OA	107 (61)	70 (39)	0.40	0.60	140 (79)	37 (21)	0.53	0.47
	Hip OA	114 (64)	65 (36)	0.40	0.60	146 (82)	33 (14)	0.57	0.43
Santiago	Control	129 (73)	49 (27)	0.44	0.56	143 (80)	35 (20)	0.57	0.43
	Knee OA	132 (64)	75 (36)	0.43	0.57	174 (84)	33 (16)	0.55	0.45
	Hip OA	108 (61)	69 (39)	0.40	0.60	145 (82)	32 (18)	0.58	0.42
Coruña	Control	95 (65)	52 (35)	0.40	0.60	116 (79)	31 (21)	0.55	0.45
	Knee OA	-	-			-	-		
	Hip OA	116 (72)	46 (28)	0.48	0.52	130 (80)	32 (20)	0.57	0.43
Oxford	Control	281 (72)	110 (28)	0.47	0.53	310 (79)	81 (21)	0.55	0.45
	Knee OA	188 (70)	81 (30)	0.46	0.54	224 (83)	45 (17)	0.56	0.44
	Hip OA	535 (69)	240 (31)	0.44	0.56	626 (81)	149 (19)	0.57	0.43

Table 3. Combined analysis of rs1062033 and rs2234693 genotypes in women.

rs1062033 **rs2234693**	**No-GG** **CC**	**No-GG** **No-CC**	**GG** **CC**	**GG** **No-CC**	**p-value**	**p-value for trend**
Controls (n)	193	670	77	293		
Knee OA (n)	77	350	38	188		
OR	1	1.31 (0.97-1.75)	1.24 (0.77-1.98)	1.61 (1.16-2.22)	0.031	0.006
Hip OA (n)	156	717	90	330		
OR	1	1.32 (1.05-1.68)	1.45 (1.00-2.09)	1.39 (1.07-1.81)	0.063	0.051

Table 4. *CYP19A1* gene expression according to *CYP19A1* and *ESR1* genotypes.
Values are means±SEM (arbitrary units) and 95% confidence intervals in parentheses.

CYP19A1 genotype			
CC (n=8)	**CG (n=21)**	**GG (n=21)**	**p-value**
23.6±8.3 (7.3-39.9)	15.5±3.4 (8.7-22.3)	11.4±2.9 (5.8-17.0)	0.036
ESR1 genotype			
TT (n=14)	**TC (n=28)**	**GG (n=7)**	**p-value**
8.4±2.4 (3.7-13.1)	16.7±3.1 (10.6-22.8)	23.6±9.2 (5.6-41.6)	0.011

Table 5. *ESR1* gene expression according to *CYP19A1* and *ESR1* genotypes (data on 37 subjects).Values are means±SEM (arbitrary units) and 95% confidence intervals in parentheses.

CYP19A1 genotype			
CC (n=4)	**CG (n=16)**	**GG (n=17)**	**p-value**
4.5±1.5 (1.6-7.4)	13.2±6.2 (1.1-25.3)	5.1±1.4 (2.3-7.9)	0.22
ESR1 genotype			
TT (n=13)	**TC (n=18)**	**GG (n=6)**	**p-value**
5.9±1.7 (2.6-9.2)	11.8±5.6 (0.9-22.7)	4.4±2.1 (2.7-6.1)	0.90

FIGURE LEGENDS

Figure 1. Association of *CYP19A1* SNP rs1062033 GG genotype and *ESR1* SNP rs2234693 CC genotype with knee osteoarthritis in women. The individual ORs, the global combined OR and the 95% confidence intervals are shown.

Figure 2. Association of *CYP19A1* SNP rs1062033 GG genotype and *ESR1* SNP rs2234693 CC genotype with hip osteoarthritis in women. The individual ORs, the global combined OR and the 95% confidence intervals are shown.

Figure 3. Association of *CYP19A1* SNP rs1062033 GG genotype and *ESR1* SNP rs2234693 CC genotype with hip osteoarthritis in men. The individual ORs, the global combined OR and the 95% confidence intervals are shown.

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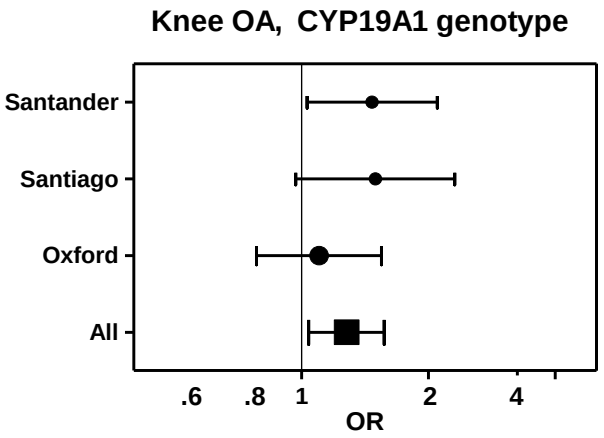
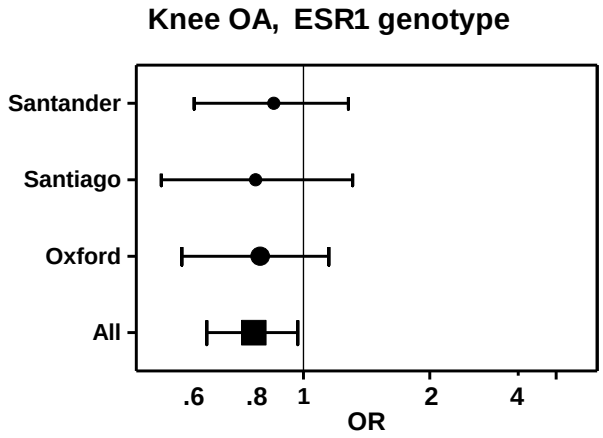
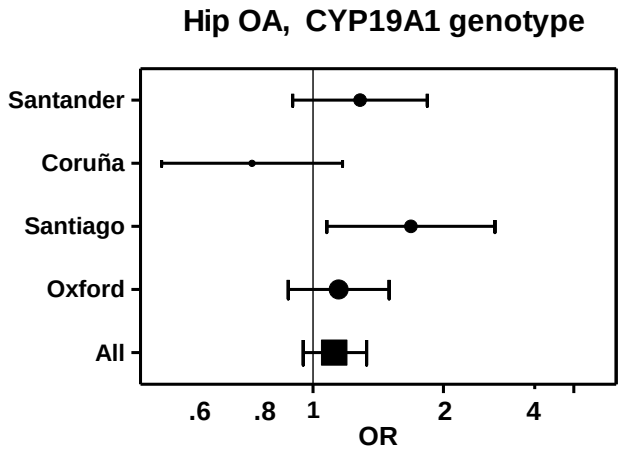
Figure

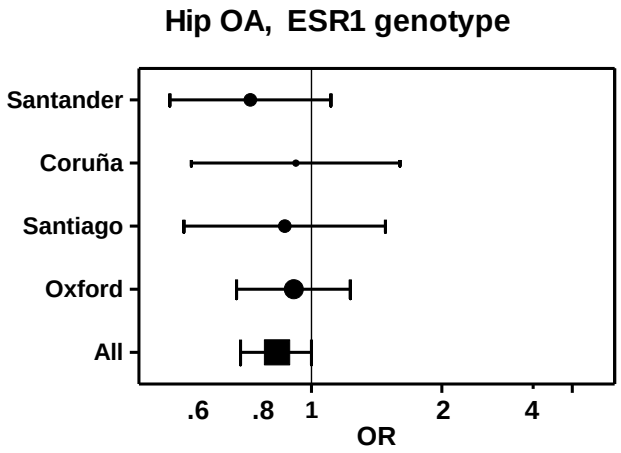
Riancho et al. Fig 1





Riancho et al. Fig 2





Riancho et al. Fig 3