Int J Clin Exp Med 2016;9(4):7585-7595
www.ijcem.com /ISSN:1940-5901/IJCEM0019577

Original Article

Population-based studies of genetic variants of *FRZB* with osteoarthritis at three joint sites: a comprehensive meta-analysis

Jie Ma[1], Shanshan Wu[1], Rui Zhang[1,2], Qian Zhang[2], Lingling Zhang[2], Cuicui Liu[2], Jing An[2], Jingjing Zhao[2], Jianfeng Yao[3]

[1]Department of Biochemistry and Molecular Biology, Xi'an Jiaotong University Health Science Center, Xi'an 710061, Shaanxi, China; [2]Translational Medicine Center, Hong Hui Hospital, Xi'an Jiaotong University Health Science Center, Xi'an 710054, Shaanxi, China; [3]Department of Joint Surgery, Hong Hui Hospital, Xi'an Jiaotong University Health Science Center, Xi'an 710054, Shaanxi, China

Received November 11, 2015; Accepted January 23, 2016; Epub April 15, 2016; Published April 30, 2016

Abstract: To address the ambiguities raised by inconsistent results among molecular genetic studies between the rs288326 (R200W) and rs7775 (R324G) variants of the *FRZB* gene and osteoarthritis (OA), we synthesized standardized data on hip, knee, and hand OA according to a common meta-analysis protocol. There were nine independent case-control studies that were candidates for performing an association analysis of the SNPs rs288326 and rs7775 in the *FRZB* locus of patients with OA at three joint sites. For SNP rs288326, the case and control sample sizes were 3,803 and 5,972 for hip OA, 2,305 and 2,514 for knee OA, and 2,761 and 1,463 for hand OA, respectively, and for SNP rs7775, the case and control sample sizes were 3,849 and 8,843 for hip OA, 2,315 and 2,506 for knee OA, and 2,796 and 1,456 for hand OA, respectively. Using a fixed-effect model, the summary effect for the T-allele of rs288326 was statistically significant in females with knee OA between patients and controls (Subtotal OR=1.24, 95% CI=1.01-1.53, Z=2.02, P=0.04), and an association trend was revealed for this SNP in females with hip OA (Subtotal OR=1.12, 95% CI=0.99-1.28, Z=1.75, P=0.08). In summary, our study supports the hypothesis that FRZB may play a small but important role in the pathogenesis of OA in females, at least in females with hip OA or knee OA. Further gender-based studies are needed to elucidate the actual role of FRZB in the physiology of human bone and cartilage and in the pathogenesis of skeletal disorders.

Keywords: Osteoarthritis, FRZB, SNP, gender, meta-analysis

Introduction

Osteoarthritis (OA) is a common complex disorder affecting ~5% of the population mostly above 45 years of age [1]. OA can result in joint pain and disability, and its development and progression are associated with both genetic and environmental risk factors, including age, gender, ethnicity, behavioral influences, obesity, and occupation [2]. From the results of epidemiological studies in twins, sibling pairs, and families, genetic components of OA have an estimated heritability of 60% for the hip, 40% for the knee, and 65% for the hand [3, 4].

Though a genome-wide linkage analysis and association study, Loughlin *et al*. initially identified the single-nucleotide polymorphism (SNP) rs7775 (a R324G substitution) in the frizzled-related protein (FRZB) gene associated with hip OA in females [3]. *FRZB* is located at chromosomal position 2q32.1, spans ~33.5 kb, consists of six exons, and encodes a secreted glycoprotein that inhibits signalling for the wingless-type (Wnt) ligands through Frizzled receptor [5, 6].

The Wnt/β-catenin signalling pathway has been implicated in the pathogenesis of OA, and this pathway is of key importance during skeletal patterning in embryogenesis, and is also involved in the homeostasis of bone and cartilage in adults [7-11]. Accumulated results from human studies have demonstrated a link between the rs288326 (R200W) and rs7775 and OA [5, 7, 12-14]. However, not every study has replicated these findings [6, 15-18], and two recent meta-analyses failed to demon-

strate a consistent link between *FRZB* variants and OA [14, 16]. Since then, multiple studies on the relationship of OA and *FRZB* have been published [7, 17]. Therefore, to address the ambiguities raised by inconsistent results among molecular genetic studies, additional studies and more reliable estimates of OA with *FRZB* are warranted [19, 20].

In the current study, we synthesized standardized data on hip, knee, and hand OA according to a common meta-analysis protocol, and performed a comprehensive meta-analysis of individual-level data on the relationship of the rs288326 and rs7775 SNPs within the *FRZB* locus with OA at three different joint sites.

Methods

Identification of eligible studies and data extraction

A search for studies that examined associations between *FRZB* polymorphisms and OA was performed. The literature was searched using the PubMed (http://www.ncbi.nlm.nih.gov), SCOPUS (http://www.scopus.com), and EMBASE (http://www.elsevier.com/solutions/embase) databases to identify available studies in which *FRZB* polymorphisms were analyzed in OA patients. Combinations of keywords, such as "frizzled-related protein", "FRZB", "polymorphism", "osteoarthritis", and "OA", were entered as medical subject heading (MeSH) components and as text words. References in identified studies were also investigated to identify additional studies not indexed by the above-mentioned databases [21]. A study was included in the present meta-analysis if the following criteria were met: (1) the study was published by October 2015; (2) the study was published in a peer-reviewed English journal; (3) the study presented original data on allele and/or genotype frequencies in both case and control samples; (4) the study was independent of other studies, i.e., studies that included and/or re-analyzed a previously published data set were not regarded as independent, and in such cases, only the first published data set was included in the meta-analysis; and (5) the study contained sufficient data to calculate an effect size [20, 22]. The exclusion criteria were as follows: (1) studies in which family members were studied, such as by a transmission disequilibrium test, because their analyses were based on linkage considerations; (2) studies that presented non-original data, such as reviews, editorials, opinion papers, or letters to the editor; and (3) studies that used non-human subjects or specimens. The following information was extracted from each included study: (1) the first author; (2) the journal; (3) the year of publication; (4) the study design; (5) the ethnicity of the subjects; (6) the sample size; (7) the phenotype information; and (8) the allele and/or genotype distribution of subjects with and without OA [23].

Quality control

For genetic association studies with conflicting results on the same genetic variants, the quality of the study design should be controlled by appropriate criteria to limit the risk of introducing bias into the meta-analysis. Therefore, three phenotypes of OA, i.e., hip OA, knee OA and hand OA, were addressed separately. Population-based studies were also subdivided into females and males.

Evaluation of statistical associations

Allele and/or genotype distribution data were entered into tables. If genotypic data were available, the Hardy-Weinberg equilibrium (HWE) was assessed separately for the control group of each study. Deviation from HWE was considered nominally statistically significant if $P<0.05$. The allele frequencies in these studies were analyzed using the Epi_Info program (http://www.cdc.gov/epiinfo), and $P<0.05$ was used as the criterion for statistical significance. Before the pooling procedure, Cochran's Chi-squared-based *Q*-statistic, which is considered significant at $P<0.10$, was performed to assess the heterogeneity within the group of odds ratios (ORs) [1, 14]. The natural logarithm of the OR estimates were determined using random-effects models or fixed-effects models. Random-effects models yield wider confidence intervals (CIs) when heterogeneity exists; otherwise, random and fixed effects coincide [22]. The significance of the pooled OR was determined by a Z test. A sensitivity analysis, which determines the influence of individual studies on the pooled OR, was performed by sequentially removing each study and recalculating the pooled OR and 95% CI. Furthermore, an ancillary procedure for funnel plot asymmetry was also used to qualitatively assess evidence of publication bias. The above-described statistical analyses were performed using the soft-

Table 1. Characteristics of the included case-control studies between rs288326 of FRZB and OA

Studies	Country	Assessment of cases	HWE	Allele (T/C)								
				Mixed			Female			Male		
				Cases	Controls	*P*-value	Cases	Controls	*P*-value	Cases	Controls	*P*-value
Hip OA												
Loughlin et al, 2004	UK	Hip replacement	>0.05	145/814	189/1132	0.588	86/494	91/612	0.331	59/320	98/520	0.903
Min et al, 2005	Netherlands	K/L grade ≥2	>0.05	37/259	237/1597	0.840						
Lories et al. 2006	Belgium	Hip replacement	>0.05				26/126	66/410	0.326			
Lane et al, 2006	USA[a]	JSN grade ≥3	>0.05				157/981	307/2327	0.066			
Rodriguez-Lopez et al, 2007	Spain	Hip replacement	>0.05	71/543	89/499	0.068	37/335	26/204	0.597	34/208	63/295	0.247
Kerkhof et al, 2008	UK	K/L grade ≥2	NA				10/82	177/1373	0.872			
Evangelou et al, 2009	Iceland	NDI	>0.05	317/3135	194/2144	0.244						
Baker-LePain et al, 2012	USA[a]	Hip replacement	>0.05				116/786	151/1051	0.839			
Knee OA												
Rodriguez-Lopez et al, 2007	Spain	Knee replacement	>0.05	76/466	89/499	0.596	59/377	26/204	0.413	17/89	63/295	0.709
Valdes et al, 2007	UK	NDI	>0.05	155/1051	135/1063	0.233	79/531	57/541	0.060	76/520	78/522	0.898
Kerkhof et al, 2008	UK	K/L grade ≥2	NA				88/630	99/805	0.414			
Evangelou et al, 2009	Iceland	NDI	>0.05	201/1943	194/2144	0.204						
Hand OA												
Rodriguez-Lopez et al, 2007	Spain	ACR	>0.05	55/421	89/499	0.090	50/372	26/204	0.836	5/49	63/295	0.124
Evangelou et al, 2009	Iceland	NDI	>0.05	452/4594	194/2144	0.351						

K/L=Kellgren/Lawrence; JSN=Joint Space Narrowing; NDI=no detailed information; ACR=American College of Rheumatology; NA=Not applicable. [a]Caucasian descent.

Table 2. Characteristics of the included case-control studies between rs7775 of FRZB and OA

Studies	Country	Assessment of cases	HWE	Allele (G/C)								
				Mixed			Female			Male		
				Cases	Controls	*P*-value	Cases	Controls	*P*-value	Cases	Controls	*P*-value
Hip OA												
Loughlin et al, 2004	UK	Hip replacement	>0.05	89/918	111/1288	0.428	68/530	56/678	**0.019**[b]	21/388	55/610	0.052
Min et al, 2005	Netherlands	K/L grade ≥2	>0.05	22/272	144/1760	0.961						
Lories et al. 2006	Belgium	Hip replacement	>0.05				15/137	46/428	0.952			
Lane et al, 2006	USA	JSN grade ≥3	>0.05				100/1040	684/7588	0.564			
Rodriguez-Lopez et al, 2007	Spain	Hip replacement	>0.05	75/529	64/514	0.473	52/316	26/198	0.379	23/213	38/316	0.699
Kerkhof et al, 2008	UK	K/L grade ≥2	NA				3/87	121/1397	0.109			
Evangelou et al, 2009	Iceland	NDI	>0.05	318/3190	202/2136	0.576						
Baker-LePain et al, 2012	USA	Hip replacement	>0.05				81/821	107/1095	0.950			
Knee OA												
Rodriguez-Lopez et al, 2007	Spain	Knee replacement	>0.05	62/486	64/514	0.898	49/395	26/198	0.825	13/91	38/316	0.615
Valdes et al, 2007	UK	NDI	>0.05	105/1101	91/1107	0.320	57/553	39/559	0.070	48/548	52/548	0.702
Kerkhof et al, 2008	UK	K/L grade ≥2	NA				49/655	75/827	0.313			
Evangelou et al, 2009	Iceland	NDI	>0.05	200/1972	202/2132	0.515						
Hand OA												
Rodriguez-Lopez et al, 2007	Spain	ACR	>0.05	56/418	64/514	0.707	51/369	26/198	0.842	5/49	38/316	0.742
Evangelou et al, 2009	Iceland	NDI	>0.05	442/4676	202/2132	0.979						

K/L=Kellgren/Lawrence; JSN=Joint Space Narrowing; NDI=no detailed information; ACR=American College of Rheumatology; NA=Not applicable. [a]Caucasian descent. [b]Significant *P* values (<0.05) are in boldface.

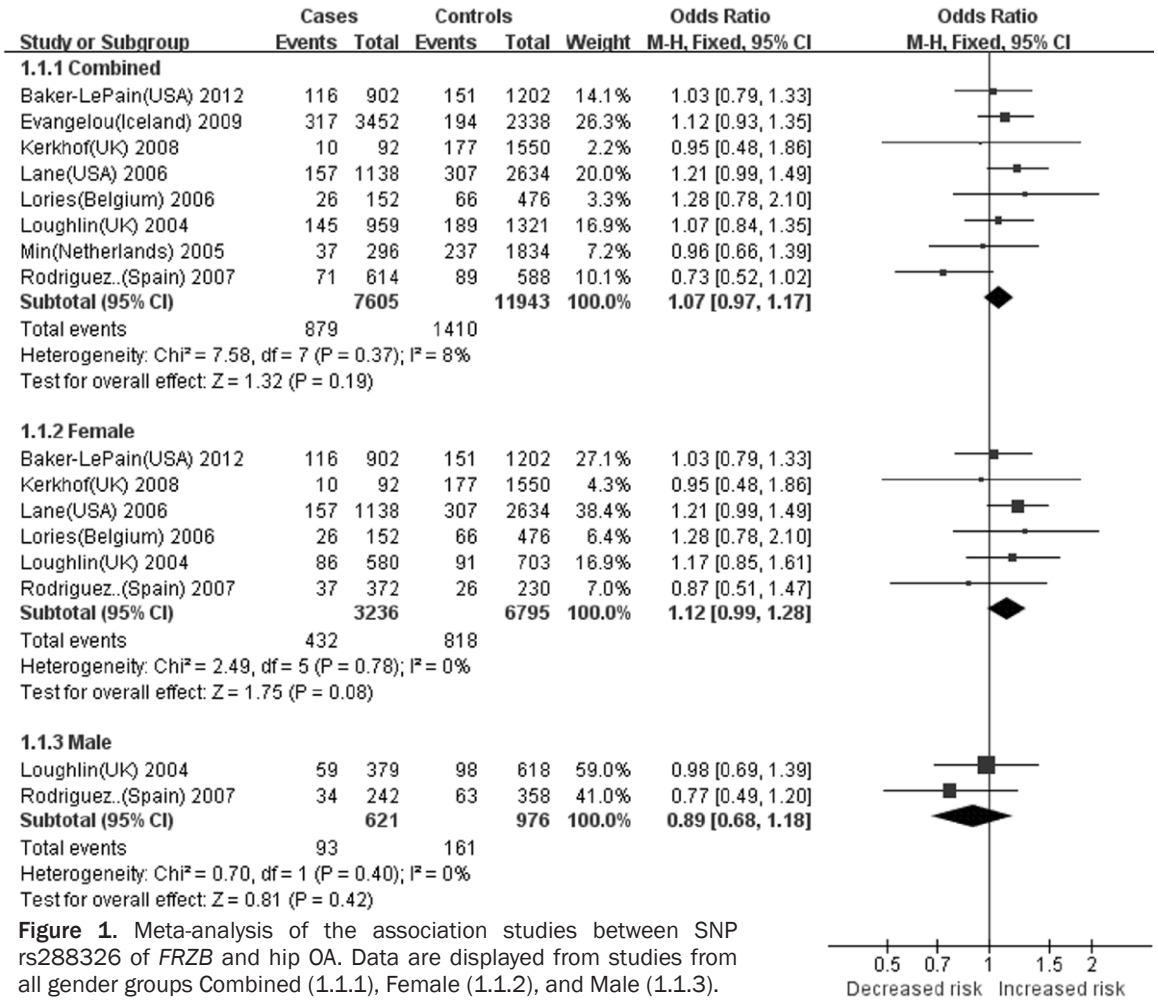


Figure 1. Meta-analysis of the association studies between SNP rs288326 of *FRZB* and hip OA. Data are displayed from studies from all gender groups Combined (1.1.1), Female (1.1.2), and Male (1.1.3).

ware program RevMan version 5.2 (http://www.cochrane.org/revman) [24].

Results

Available studies

26 references were identified by the combined search. After discarding reviews and studies that were not written in English, 23 references remained. Furthermore, 14 references were excluded, because they did not clearly meet the criteria, or were not association studies of SNPs. This resulted in nine independent case-control studies that were candidates for use in an association analysis of SNPs rs288326 and rs7775 in the *FRZB* locus with OA at three joint sites [3, 5-7, 12-16]. Among these available studies, only three studies showed genotypic data and had an applicable HWE test for their samples [6, 7, 12]. For these studies, two SNPs were in HWE (P>0.05) in both the case and control samples (data not shown). Moreover, an analysis of genetic models was not performed because of limited genotyping data.

In the end, for SNP rs288326, the case and control sample sizes were 3,803 and 5,972 for hip OA, 2,305 and 2,514 for knee OA, and 2,761 and 1,463 for hand OA, respectively, and for SNP rs7775, the case and control sample sizes were 3,849 and 8,843 for hip OA, 2,315 and 2,506 for knee OA, and 2,796 and 1,456 for hand OA, respectively. All of the subjects were of Caucasian descent (**Tables 1** and **2**).

SNP rs288326 and OA

Hip OA. No significant evidence of between-study heterogeneity was found among the group of allele-wise ORs in the combined samples (P=0.37), in females (P=0.78), and in males (P=0.40). Using a fixed-effect model, a trend of association was revealed between the patients and controls for the T-allele of rs288326 in females (Subtotal OR=1.12, 95% CI=0.99-

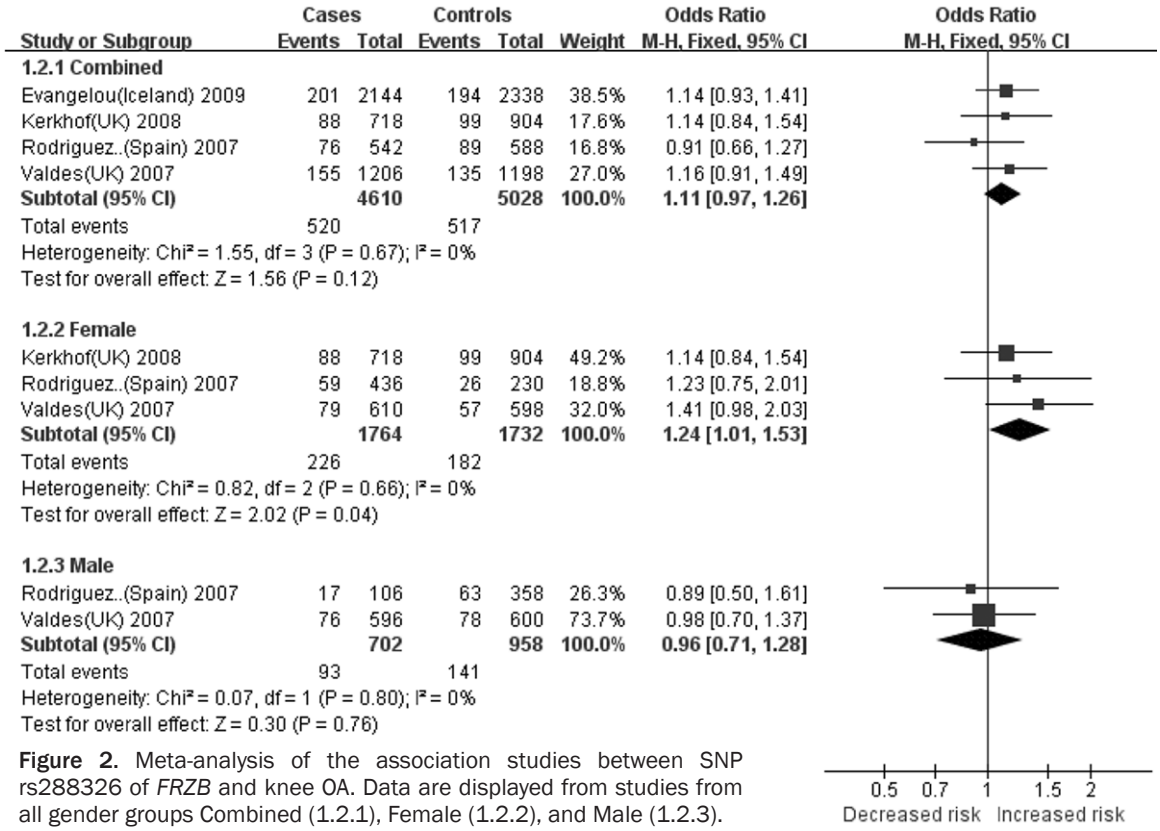


Figure 2. Meta-analysis of the association studies between SNP rs288326 of *FRZB* and knee OA. Data are displayed from studies from all gender groups Combined (1.2.1), Female (1.2.2), and Male (1.2.3).

1.28, Z=1.75, P=0.08). However, no significant difference was identified in the combined samples (Subtotal OR=1.07, 95% CI=0.97-1.17, Z=1.32, P=0.19) and in males (Subtotal OR= 0.89, 95% CI=0.68-1.18, Z=0.81, P=0.42) (**Figure 1**).

Knee OA. The fixed-effects model was utilized in the meta-analysis because no heterogeneity was observed in the three analysis groups (P=0.67, P=0.66, and P=0.80, respectively). The summary effect for SNP rs288326 was statistically significant in females (Subtotal OR=1.24, 95% CI=1.01-1.53, Z=2.02, P=0.04), but not in the combined samples (Subtotal OR=1.11, 95% CI=0.97-1.26, Z=1.56, P=0.12) and in males (Subtotal OR=0.96, 95% CI=0.71-1.28, Z=0.30, P=0.76) (**Figure 2**).

Hand OA. The result of the homogeneity test in the meta-analysis showed significant heterogeneity (X^2=3.73, *df*=1, P=0.05), and no significant difference was found between patients and controls with the random-effects model (Subtotal OR=0.92, 95% CI=0.63-1.35, Z=0.42, P=0.68) (Supplementary Figure 1A).

SNP rs7775 and OA

Hip OA. No significant heterogeneity was detected in the combined samples (P=0.86), in females (P=0.23), and in males (P=0.29). Using a fixed-effect model, a non-statistically significant summary OR was found in three samples (Combined samples: Subtotal OR=1.05, 95% CI=0.94-1.17, Z=0.91, *P*=0.36; Females: Subtotal OR=1.10, 95% CI=0.95-1.28, Z=1.32, *P*= 0.19; Males: Subtotal OR=0.72, 95% CI=0.50-1.05, Z=1.71, P=0.09) (**Figure 3**).

Knee OA. No significant evidence of between-study heterogeneity was found in three samples (P=0.55, P=0.12, and P=0.53, respectively), and no significant association was observed with a fixed-effect model in the combined samples (Subtotal OR=1.04, 95% CI=0.91-1.20, Z=0.59, P=0.55), in females (Subtotal OR=1.04, 95% CI=0.81-1.32, Z=0.29, P=0.77), and in males (Subtotal OR=0.99, 95% CI=0.69-1.40, Z=0.07, P=0.94) (**Figure 4**).

Hand OA. No significant difference was found (Subtotal OR=1.01, 95% CI=0.86-1.18, Z=0.13, P=0.89) between the patients and controls

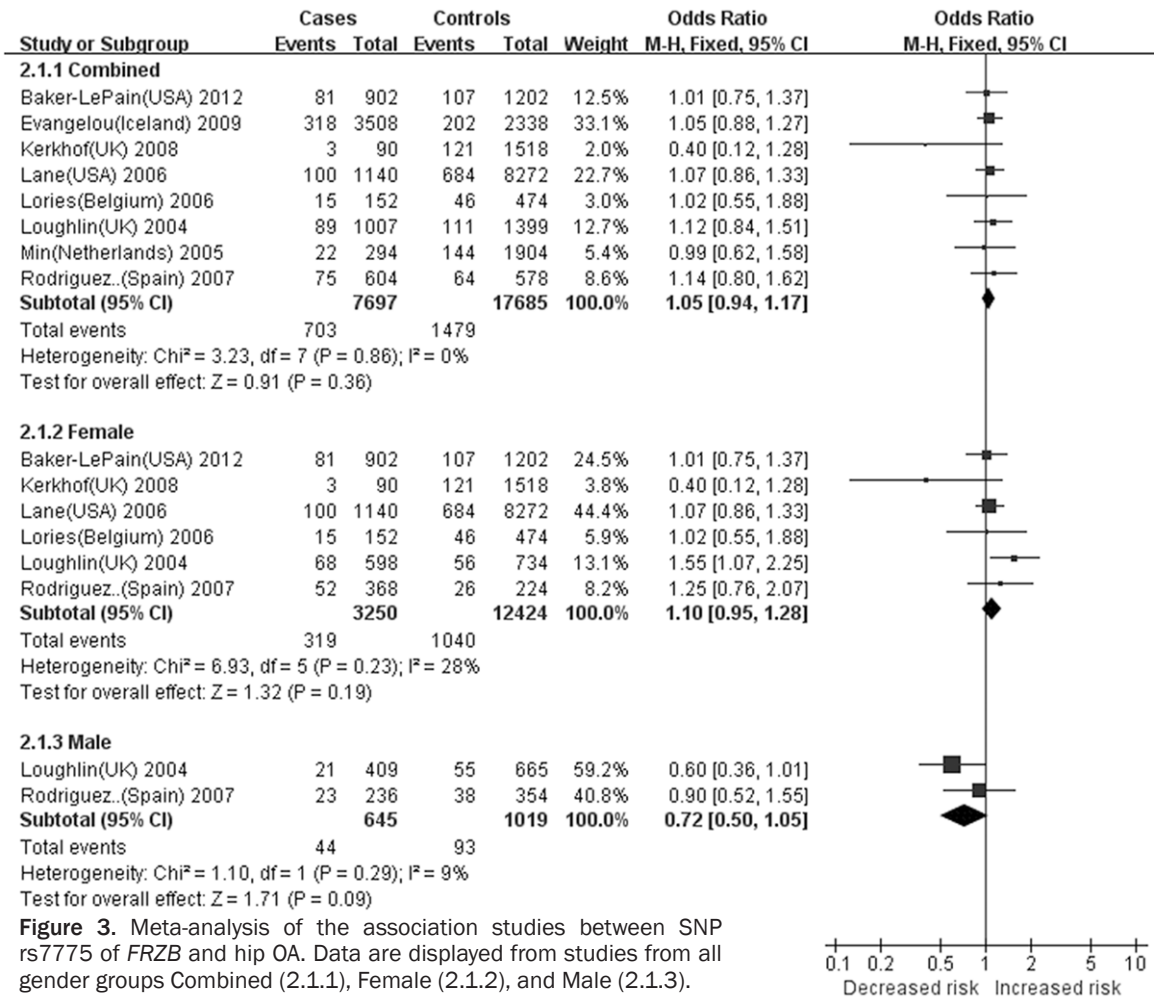


Figure 3. Meta-analysis of the association studies between SNP rs7775 of *FRZB* and hip OA. Data are displayed from studies from all gender groups Combined (2.1.1), Female (2.1.2), and Male (2.1.3).

with the fixed-effects model (Heterogeneity: P=0.72) (Supplementary Figure 1B).

For SNPs rs288326 and rs7775, the sensitivity analysis showed that the removal of any one of the studies did not significantly change the heterogeneity of the population in the above-described three analysis groups, and the *P* value of Z test was not significantly altered (data not shown). The results of publication bias tests for these SNPs are presented in Supplementary Figures 2 and 3. No significant evidence of publication bias was revealed within the analysis group.

Discussion

We performed a meta-analysis due to the concern that the individual studies may have insufficient power to detect the small effect of the genetic variants of *FRZB* on OA susceptibility in populations of Caucasian descent [1]. In the present study, we investigated the association of the SNPs rs288326 and rs7775 in the *FRZB* locus with OA at three joint sites using gender-diverse indepenent samples detailed in nine published references from six countries across two continents.

For SNP rs288326, the *P* value in females was 0.08, indicating a potential association with hip OA. A further investigation revealed that the *P* value of this SNP for the association tests was significant (0.04), which provides evidence for an association with knee OA in females. However, we failed to find an association between SNP rs288326 and hand OA in females. For SNP rs7775, no associated signal was detected between this SNP and OA at three joint sites in females, in males, or in the combined populations (P>0.05). To the best of our knowledge, these results are similar to those obtained in a meta-analysis previously performed by Evangelou *et al*. [14]. A comparison of our results with those reported by Evangelou *et al*. revealed that two SNPs were analysed in both studies.

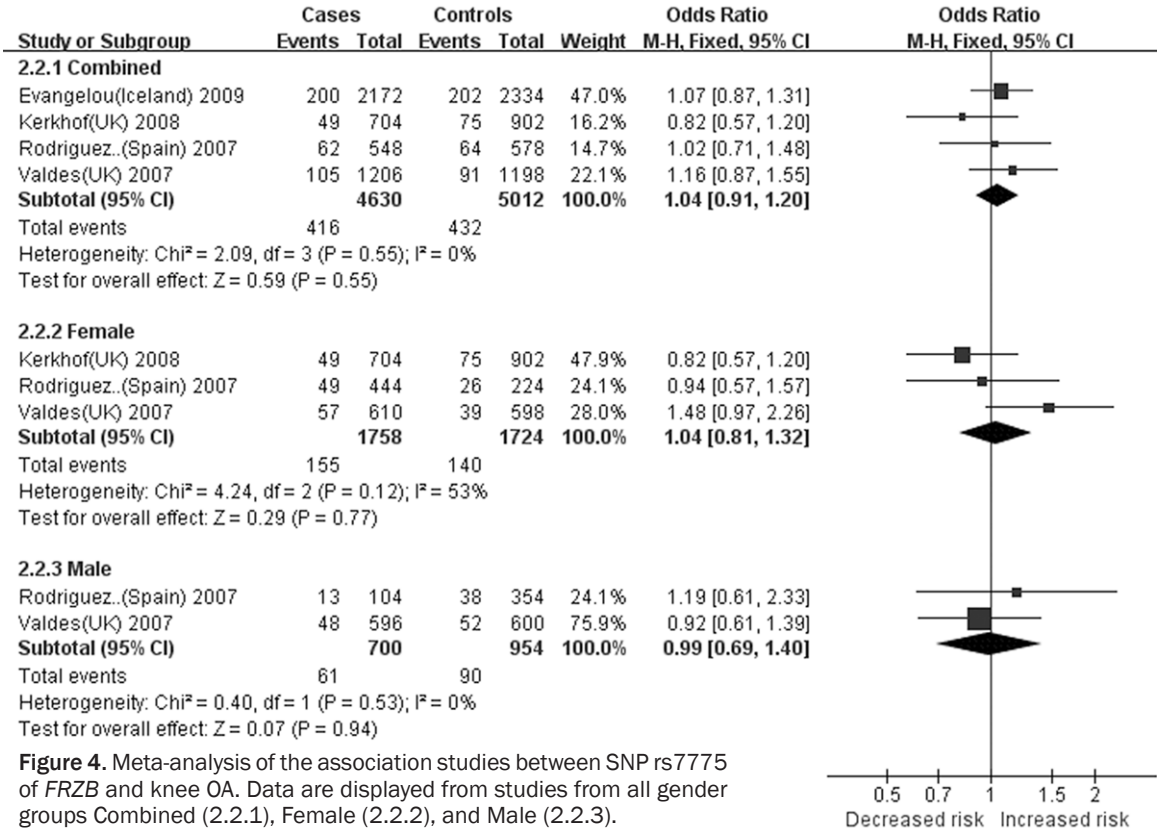


Figure 4. Meta-analysis of the association studies between SNP rs7775 of *FRZB* and knee OA. Data are displayed from studies from all gender groups Combined (2.2.1), Female (2.2.2), and Male (2.2.3).

Of these, neither the analysis performed by Evangelou *et al*. nor our analysis found an association between SNP rs7775 and OA. Interestingly, SNP rs288326 showed a significant association in female subjects with hip OA in the study conducted by Evangelou *et al*. (P=0.007), and a trend of association in our female samples with hip OA. In addition, this SNP showed a potential association in female subjects with knee OA in the study conducted by Evangelou *et al*. (P=0.06) and a significant difference in our female samples with knee OA. The slight difference for SNP rs288326 between the two studies may be due to the different sample sizes obtained based on the different inclusion criteria used [14].

Based on the above-described analysis, sex appears to play a small but important role in the pathogenesis of OA, at least in hip OA and/or knee OA. In fact, epidemiological observations provid evidence for an association between oestrogen deprivation and OA development, as demonstrated by the finding that the age-related increases in the incidence and prevalence of hip, knee, and hand OA were greater in males than in females prior to 50 years of age, but subsequently greater in females at older age [1, 25]. Spector *et al*. found that hysterectomy was significantly more prevalent among cases with OA than among controls [26, 27]. In a study of OA, hormone replacement therapy (HRT) was associated with trends toward lower prevalences of symptomatic hip and knee OA [28]. A longitudinal study in the Chingford population found that HRT offers significant protection against radiological knee OA compared with a control group [29]. Therefore, oestrogen deprivation at menopause may be associated with increases in the frequency of OA, and HRT due to menopause appears to decrease the incidence and progression of hip and knee OA [25].

The importance of Wnt/β-catenin signalling in bone and cartilage homeostasis was recently highlighted by the observation that animals deficient in *Frzb* (also called secreted Frizzled-related protein 3, SFRP3) [7, 30], present alterations in hip shape and articular cartilage properties. FRZB consists of two protein domains, a cysteine-rich domain responsible for Wnt/β-catenin interactions, and a netrin domain identified as a unique C-terminal basic domain in laminin-related modular proteins called netrins [30, 31]. The netrin domain is homologous to

the N-terminal domain of tissue inhibitors of metalloproteinases, and the interactions between FRZB and matrix metalloproteinase 2 and 3 (MMP-2, MMP-3) have been demonstrated [30, 32]. Cytosolic β-catenin protein is the principal mediator of Wnt/β-catenin signalling [33]. In the activation of Wnt/β-catenin signals, β-catenin is dephosphorylated and accumulates in the cytoplasm, which leads to the subsequent translocation of the protein into the cell nucleus. Nuclear β-catenin interacts with DNA bound T-cell factor/lymphoid enhancer factor (TCF/LEF) proteins and thereby induces the expression of Wnt-target genes [34]. The upregulation of Wnt/β-catenin signalling may be deleterious for the joint cartilage because it induces the release of metalloproteases that promote cartilage catabolism and degradation. Wnt/β-catenin signals also have a stimulatory effect on bone formation [9]. Serine/threonine protein kinase GSK3β is a key negative regulator of β-catenin in the Wnt/β-catenin signalling pathway because it phosphorylates and promotes the degradation of β-catenin in quiescent cells [35]. Interestingly, 17β-estradiol (E2) can induce the phosphorylation of Akt at serine 473 and subsequently activate the phosphorylation of GSK-3β at serine 9, leading to the inhibition of GSK-3β activity, and the activation of Wnt/β-catenin signalling [36].

In summary, our study supports the hypothesis that FRZB may play a small but important role in the pathogenesis of OA in females, at least in females with hip OA or knee OA. Together, the results suggest that the function of FRZB (Wnt signals) may influence bone anabolism in females with OA, and that oestrogen receptor signalling may be an important regulator of bone mass and bone cell differentiation. However, the underling mechanisms of FRZB in females with OA are not fully understood. Therefore, further gender-based studies are needed to elucidate the actual role of FRZB in the physiology of human bone and cartilage and in the pathogenesis of skeletal disorders.

Acknowledgements

We are indebted to all of the individuals who have participated in, or helped with, our research. This study was supported by the National Natural Science Foundation of China (No. 81272023; No. 81301151; No. 31371298); the China Postdoctoral Science Foundation (No. 2013M542337; No. 2015T81016); the Program for New Century Excellent Talents in University (No. NCET-13-0452); the Key Project of International Scientific Cooperation of Shaanxi Province (No. S2013KW25-02); and the Fundamental Research Funds for the Central Universities (No. 2012JDGZ07).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Rui Zhang and Jianfeng Yao, Translational Medicine Center, Hong Hui Hospital, Xi'an Jiaotong University Health Science Center, Xi'an 710054, Shaanxi, China; Department of Joint Surgery, Hong Hui Hospital, Xi'an Jiaotong University Health Science Center, Xi'an 710054, Shaanxi, China. E-mail: zhangruity12@163.com (RZ); yaojf58@163.com (JFY)

References

[1] Chapman K, Takahashi A, Meulenbelt I, Watson C, Rodriguez-Lopez J, Egli R, Tsezou A, Malizos KN, Kloppenburg M, Shi D, Southam L, van der Breggen R, Donn R, Qin J, Doherty M, Slagboom PE, Wallis G, Kamatani N, Jiang Q, Gonzalez A, Loughlin J and Ikegawa S. A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5' UTR of GDF5 with osteoarthritis susceptibility. Hum Mol Genet 2008; 17: 1497-1504.

[2] Valdes AM, Loughlin J, Timms KM, van Meurs JJ, Southam L, Wilson SG, Doherty S, Lories RJ, Luyten FP, Gutin A, Abkevich V, Ge D, Hofman A, Uitterlinden AG, Hart DJ, Zhang F, Zhai G, Egli RJ, Doherty M, Lanchbury J and Spector TD. Genome-wide association scan identifies a prostaglandin-endoperoxide synthase 2 variant involved in risk of knee osteoarthritis. Am J Hum Genet 2008; 82: 1231-1240.

[3] Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, Ferreira A, Ciesielski C, Carson DA and Corr M. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. Proc Natl Acad Sci U S A 2004; 101: 9757-9762.

[4] Zeggini E, Panoutsopoulou K, Southam L, Rayner NW, Day-Williams AG, Lopes MC, Boraska V, Esko T, Evangelou E, Hoffman A, Houwing-Duistermaat JJ, Ingvarsson T, Jonsdottir I, Jonnson H, Kerkhof HJ, Kloppenburg M, Bos SD, Mangino M, Metrustry S, Slagboom PE, Thorleifsson G, Raine EV, Ratnayake M, Ricketts M, Beazley C, Blackburn H, Bumpstead S, Elliott KS, Hunt SE, Potter SC, Shin SY, Yadav VK, Zhai G, Sherburn K, Dixon K, Arden E, Aslam N, Battley PK, Carluke I, Doherty S, Gor-

don A, Joseph J, Keen R, Koller NC, Mitchell S, O'Neill F, Paling E, Reed MR, Rivadeneira F, Swift D, Walker K, Watkins B, Wheeler M, Birrell F, Ioannidis JP, Meulenbelt I, Metspalu A, Rai A, Salter D, Stefansson K, Stykarsdottir U, Uitterlinden AG, van Meurs JB, Chapman K, Deloukas P, Ollier WE, Wallis GA, Arden N, Carr A, Doherty M, McCaskie A, Willkinson JM, Ralston SH, Valdes AM, Spector TD and Loughlin J. Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. Lancet 2012; 380: 815-823.

[5] Min JL, Meulenbelt I, Riyazi N, Kloppenburg M, Houwing-Duistermaat JJ, Seymour AB, Pols HA, van Duijn CM and Slagboom PE. Association of the Frizzled-related protein gene with symptomatic osteoarthritis at multiple sites. Arthritis Rheum 2005; 52: 1077-1080.

[6] Lories RJ, Boonen S, Peeters J, de Vlam K and Luyten FP. Evidence for a differential association of the Arg200Trp single-nucleotide polymorphism in FRZB with hip osteoarthritis and osteoporosis. Rheumatology (Oxford) 2006; 45: 113-114.

[7] Baker-Lepain JC, Lynch JA, Parimi N, McCulloch CE, Nevitt MC, Corr M and Lane NE. Variant alleles of the Wnt antagonist FRZB are determinants of hip shape and modify the relationship between hip shape and osteoarthritis. Arthritis Rheum 2012; 64: 1457-1465.

[8] Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, Rosier RN, O'Keefe RJ, Zuscik M and Chen D. Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. J Bone Miner Res 2009; 24: 12-21.

[9] Velasco J, Zarrabeitia MT, Prieto JR, Perez-Castrillon JL, Perez-Aguilar MD, Perez-Nunez MI, Sanudo C, Hernandez-Elena J, Calvo I, Ortiz F, Gonzalez-Macias J and Riancho JA. Wnt pathway genes in osteoporosis and osteoarthritis: differential expression and genetic association study. Osteoporos Int 2010; 21: 109-118.

[10] Thomas RS, Clarke AR, Duance VC and Blain EJ. Effects of Wnt3A and mechanical load on cartilage chondrocyte homeostasis. Arthritis Res Ther 2011; 13: R203.

[11] Garcia-Ibarbia C, Delgado-Calle J, Casafont I, Velasco J, Arozamena J, Perez-Nunez MI, Alonso MA, Berciano MT, Ortiz F, Perez-Castrillon JL, Fernandez AF, Fraga MF, Zarrabeitia MT and Riancho JA. Contribution of genetic and epigenetic mechanisms to Wnt pathway activity in prevalent skeletal disorders. Gene 2013; 532: 165-172.

[12] Lane NE, Lian K, Nevitt MC, Zmuda JM, Lui L, Li J, Wang J, Fontecha M, Umblas N, Rosenbach M, de Leon P and Corr M. Frizzled-related protein variants are risk factors for hip osteoarthritis. Arthritis Rheum 2006; 54: 1246-1254.

[13] Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, Doherty M and Spector TD. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. Arthritis Rheum 2007; 56: 137-146.

[14] Evangelou E, Chapman K, Meulenbelt I, Karassa FB, Loughlin J, Carr A, Doherty M, Doherty S, Gomez-Reino JJ, Gonzalez A, Halldorsson BV, Hauksson VB, Hofman A, Hart DJ, Ikegawa S, Ingvarsson T, Jiang Q, Jonsdottir I, Jonsson H, Kerkhof HJ, Kloppenburg M, Lane NE, Li J, Lories RJ, van Meurs JB, Nakki A, Nevitt MC, Rodriguez-Lopez J, Shi D, Slagboom PE, Stefansson K, Tsezou A, Wallis GA, Watson CM, Spector TD, Uitterlinden AG, Valdes AM and Ioannidis JP. Large-scale analysis of association between GDF5 and FRZB variants and osteoarthritis of the hip, knee, and hand. Arthritis Rheum 2009; 60: 1710-1721.

[15] Rodriguez-Lopez J, Pombo-Suarez M, Liz M, Gomez-Reino JJ and Gonzalez A. Further evidence of the role of frizzled-related protein gene polymorphisms in osteoarthritis. Ann Rheum Dis 2007; 66: 1052-1055.

[16] Kerkhof JM, Uitterlinden AG, Valdes AM, Hart DJ, Rivadeneira F, Jhamai M, Hofman A, Pols HA, Bierma-Zeinstra SM, Spector TD and van Meurs JB. Radiographic osteoarthritis at three joint sites and FRZB, LRP5, and LRP6 polymorphisms in two population-based cohorts. Osteoarthritis Cartilage 2008; 16: 1141-1149.

[17] Minafra L, Bravata V, Saporito M, Cammarata FP, Forte GI, Caldarella S, D'Arienzo M, Gilardi MC, Messa C and Boniforti F. Genetic, clinical and radiographic signs in knee osteoarthritis susceptibility. Arthritis Res Ther 2014; 16: R91.

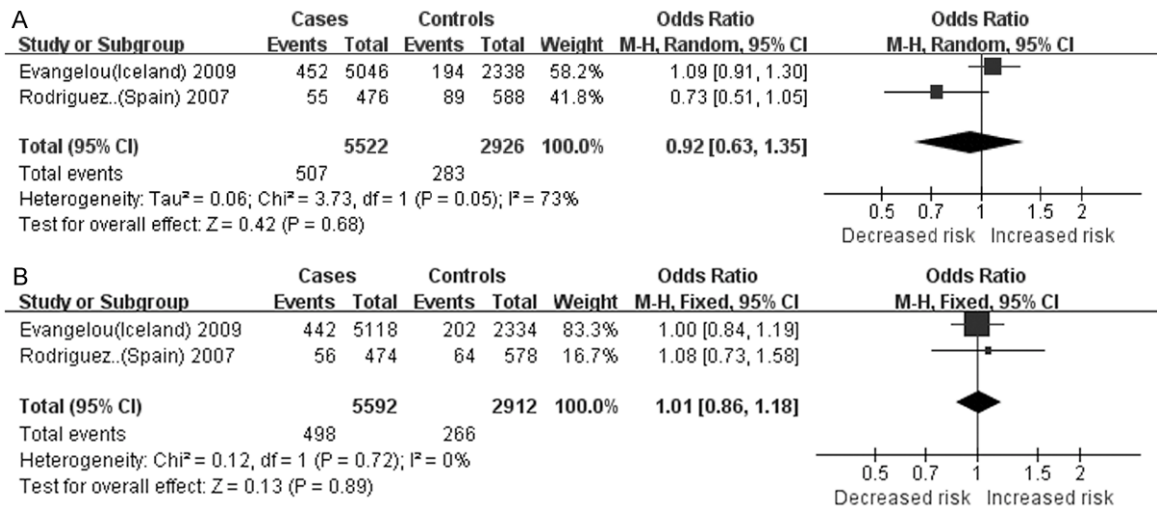
[18] Gao G, Zhang ZL, He JW, Zhang H, Yue H, Hu WW, Gu JM, Fu WZ, Hu YQ, Li M, Liu YJ and Yu JB. No association of the polymorphisms of the frizzled-related protein gene with peak bone mineral density in Chinese nuclear families. BMC Med Genet 2010; 11: 1.

[19] Liu J, Cai W, Zhang H, He C and Deng L. Rs143383 in the growth differentiation factor 5 (GDF5) gene significantly associated with osteoarthritis (OA)-a comprehensive meta-analysis. Int J Med Sci 2013; 10: 312-319.

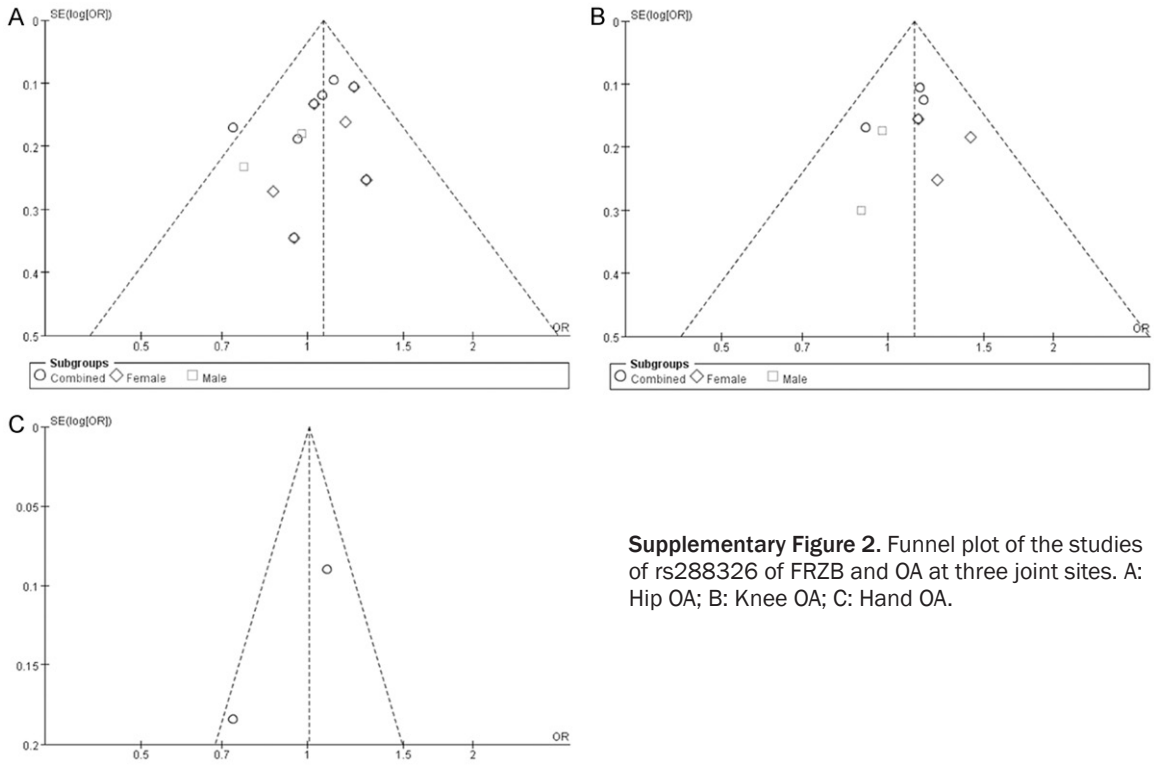
[20] Zhang R, Yao J, Xu P, Ji B, Luck JV, Chin B, Lu S, Kelsoe JR and Ma J. A comprehensive meta-analysis of association between genetic variants of GDF5 and osteoarthritis of the knee, hip and hand. Inflamm Res 2015; 64: 405-414.

[21] Song GG, Kim JH and Lee YH. A meta-analysis of the relationship between aspartic acid (D)-repeat polymorphisms in asporin and osteoarthritis susceptibility. Rheumatol Int 2014; 34: 785-792.

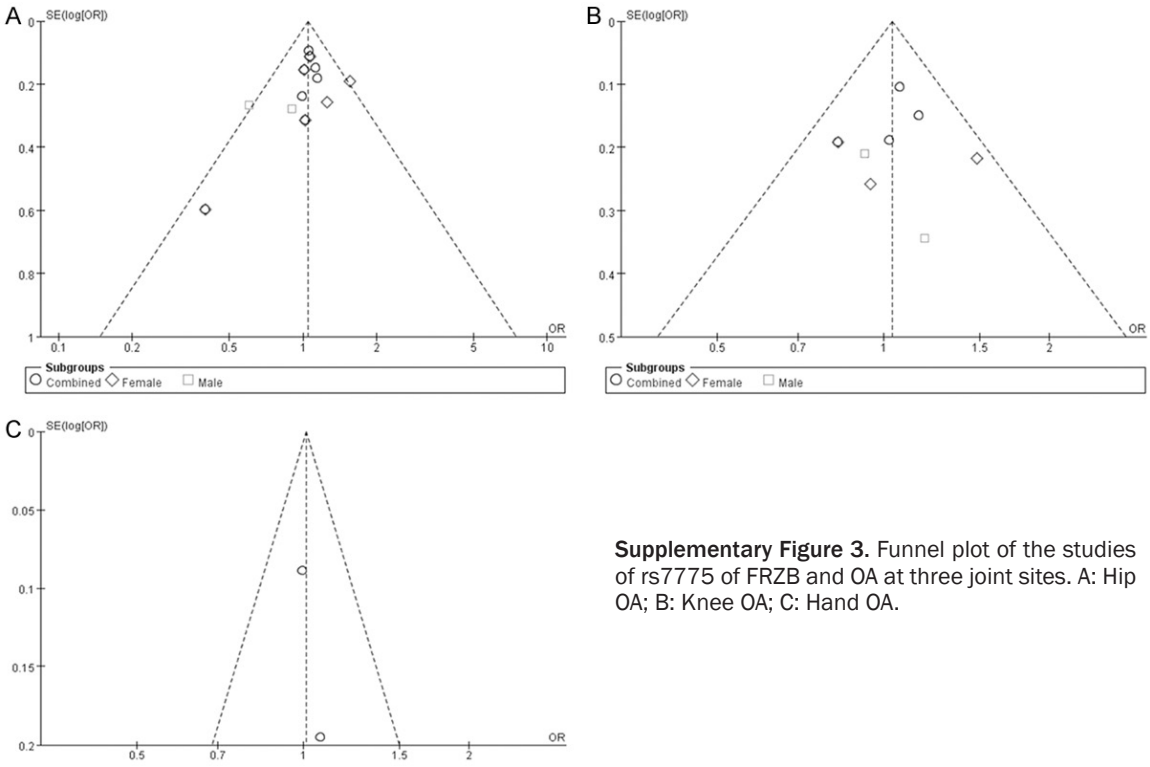
[22] Xu M, St Clair D and He L. Testing for genetic association between the ZDHHC8 gene locus and susceptibility to schizophrenia: An integrated analysis of multiple datasets. Am J Med Genet B Neuropsychiatr Genet 2010; 153B: 1266-1275.
[23] Nie F, Wang X, Zhao P, Yang H, Zhu W, Zhao Y, Chen B, Valenzuela RK, Zhang R, Gallitano AL and Ma J. Genetic analysis of SNPs in CACNA1C and ANK3 gene with schizophrenia: A comprehensive meta-analysis. Am J Med Genet B Neuropsychiatr Genet 2015; 168: 637-48.
[24] Zhang R, Yan JD, Valenzuela RK, Lu SM, Du XY, Zhong B, Ren J, Zhao SH, Gao CG, Wang L, Guo TW and Ma J. Further evidence for the association of genetic variants of ZNF804A with schizophrenia and a meta-analysis for genome-wide significance variant rs1344706. Schizophr Res 2012; 141: 40-47.
[25] Richette P, Corvol M and Bardin T. Estrogens, cartilage, and osteoarthritis. Joint Bone Spine 2003; 70: 257-262.
[26] Spector TD, Hart DJ, Brown P, Almeyda J, Dacre JE, Doyle DV and Silman AJ. Frequency of osteoarthritis in hysterectomized women. J Rheumatol 1991; 18: 1877-1883.
[27] Spector TD, Perry LA and Jubb RW. Endogenous sex steroid levels in women with generalised osteoarthritis. Clin Rheumatol 1991; 10: 316-319.
[28] Samanta A, Jones A, Regan M, Wilson S and Doherty M. Is osteoarthritis in women affected by hormonal changes or smoking? Br J Rheumatol 1993; 32: 366-370.
[29] Spector TD, Nandra D, Hart DJ and Doyle DV. Is hormone replacement therapy protective for hand and knee osteoarthritis in women?: The Chingford Study. Ann Rheum Dis 1997; 56: 432-434.
[30] Lories RJ, Peeters J, Bakker A, Tylzanowski P, Derese I, Schrooten J, Thomas JT and Luyten FP. Articular cartilage and biomechanical properties of the long bones in Frzb-knockout mice. Arthritis Rheum 2007; 56: 4095-4103.
[31] Serafini T, Kennedy TE, Galko MJ, Mirzayan C, Jessell TM and Tessier-Lavigne M. The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. Cell 1994; 78: 409-424.
[32] Zi X, Guo Y, Simoneau AR, Hope C, Xie J, Holcombe RF and Hoang BH. Expression of Frzb/secreted Frizzled-related protein 3, a secreted Wnt antagonist, in human androgen-independent prostate cancer PC-3 cells suppresses tumor growth and cellular invasiveness. Cancer Res 2005; 65: 9762-9770.
[33] Gordon MD and Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem 2006; 281: 22429-22433.
[34] Qi J, Yu Y, Akilli Ozturk O, Holland JD, Besser D, Fritzmann J, Wulf-Goldenberg A, Eckert K, Fichtner I and Birchmeier W. New Wnt/beta-catenin target genes promote experimental metastasis and migration of colorectal cancer cells through different signals. Gut 2015; [Epub ahead of print].
[35] Wu D and Pan W. GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem Sci 2010; 35: 161-168.
[36] Yin X, Wang X, Hu X, Chen Y, Zeng K and Zhang H. ERbeta induces the differentiation of cultured osteoblasts by both Wnt/beta-catenin signaling pathway and estrogen signaling pathways. Exp Cell Res 2015; 335: 107-114.



Supplementary Figure 1. Meta-analysis of the association studies between two SNPs of *FRZB* and hand OA. A: rs288326; B: rs7775.



Supplementary Figure 2. Funnel plot of the studies of rs288326 of FRZB and OA at three joint sites. A: Hip OA; B: Knee OA; C: Hand OA.



Supplementary Figure 3. Funnel plot of the studies of rs7775 of FRZB and OA at three joint sites. A: Hip OA; B: Knee OA; C: Hand OA.