

Radiographic osteoarthritis at three joint sites and FRZB, LRP5, and LRP6 polymorphisms in two population-based cohorts

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Summary

Objective: To examine the association of genetic variation in key players in the Wnt signaling pathway with aspects of osteoarthritis (OA) in two population-based cohort studies: the Rotterdam Study and the Chingford Study.

Methods: Radiographic OA (ROA) was defined as a Kellgren/Lawrence score (K/L) score ≥ 2 for the knee and hip. Total hip replacement (THR) was scored. Hand OA was defined as presence of ROA (K/L ≥ 2) in two out of three hand joint groups [distal interphalangeal (DIPs), proximal interphalangeal (PIPs), first carpometacarpal (CMC1)/trapezio-scaphoid joint (TS)] of each hand. The concentration of urinary C-terminal cross-linked telopeptide of type II collagen (CTX-II) was standardized to the total urine creatinine. Genotypes for the amino acid variants, Arg200Trp and Arg324Gly of Frizzled-Related protein gene (FRZB), Ala1330Val of Low-density lipoprotein receptor-related protein 5 (LRP5) and Ile1062Val of Low-density lipoprotein receptor-related protein 6 (LRP6), were obtained using the Taqman allelic discrimination assay. A meta-analysis was performed for the FRZB Arg324Gly polymorphism and hip- and knee-OA using RevMan version 4.3.

Results: No consistent associations were observed between the FRZB, LRP5 and LRP6 amino acid variants and radiographic hip-, knee-, or hand-OA or THR, in either study population. While power was limited for most studies to date, a meta-analysis of all published studies regarding the FRZB Arg324Gly polymorphism was performed for hip- and knee-OA separately. This showed no significant associations between the Gly324 allele and risk for hip- or knee OA, although there was large heterogeneity between studies for hip OA in females.

Conclusion: No association was seen between FRZB, LRP5 and LRP6 variants with radiographic osteoarthritic outcomes in two population-based cohorts. In future studies, increased power and standardization of OA-phenotypes are highly recommended for replication studies and to allow meta-analysis.

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Key words: Polymorphism, Osteoarthritis, Wnt, FRZB, LRP5, LRP6.

Introduction

Osteoarthritis (OA) is a chronic, age-related, degenerative disease of the synovial joints. It is characterized by cartilage degradation, formation of osteophytes, subchondral sclerosis and synovitis¹. The etiology of OA is multifactorial, i.e., environmental and genetic factors play an important role in the development of OA. Primary OA has an estimated heritability of 40% for the knee, 60% for the hip and 65% for the hand². This means that a substantial proportion of variation in risk for OA can be attributed to genetic variation, i.e., polymorphisms in genes involved in the etiology of OA. The vast majority of the genes are unknown and their

identification could explain much of the pathogenesis of OA, which at the moment remains unclear.

Evidence is accumulating, showing that the Wnt signaling pathway is involved in cartilage degeneration and OA^{3–11}. Several Wnt signaling proteins were shown to play an important role in regulating many aspects of chondrogenesis and skeletal development, including limb formation and osteoblast maturation⁴. Genes encoding proteins of the Wnt signaling pathway are therefore interesting candidates to search for common genetic variants that could contribute to risk for OA.

Frizzled-Related protein gene (FRZB) is a key player in the Wnt signaling pathway with regard to cartilage metabolism and OA^{4,6,7,10–12}. Several studies have investigated the relationship between OA and two polymorphisms in the FRZB gene: the FRZB Arg200Trp and Arg324Gly variants. Loughlin *et al.* observed in female carriers of the FRZB Gly³²⁴ allele an increased risk for total hip replacement (THR)⁶. However, subsequent studies have yielded conflicting results^{7,11,13–15}. This could partially be explained by the

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Received 1 March 2007; revision accepted 8 February 2008.

variety of OA-definitions across studies. In general, many different phenotypes are used as endpoints in genetic studies of OA and those on FRZB are no exception. Such phenotypes may vary from clinical endpoints such as THR to biochemical assessments [such as with C-terminal cross-linked telopeptide of type II collagen (CTX-II)], and include radiographic definitions of OA as defined by the Kellgren/Lawrence (K/L) score at several possible sites (knee, hip or hand), and be even based on composites and/or sub-phenotypes from this score. Furthermore, individual studies might be underpowered, which could be a reason why true associations might not be observed. A meta-analysis could help to overcome such limitations and document the true effect (if any) of such genetic variants on the risk of OA.

In three papers, the relationship between the rare FRZB “Trp²⁰⁰–Gly³²⁴” haplotype (frequency of 0.6–5.0%) and OA has been studied^{6,11,13}. They found that female carriers of this haplotype have an increased risk of THR⁶, severe JSN of the hip¹¹ and clinical knee OA¹³ further supporting a role for FRZB variants in OA. In addition, Gordon *et al.*¹⁶ showed recently that the FRZB Trp²⁰⁰ allele was associated with a decreased risk of osteolysis after total hip-arthroplasty. In this study, we examined the relationship between combined genotypes (FRZB Arg200Trp and Arg324Gly variants) and hip- and knee-OA.

Low-density lipoprotein receptor-related protein 5 and 6 (LRP5 and LRP6) are two known co-receptors for Wnt proteins¹⁷. Since Wnt signaling is involved in cartilage degeneration and OA^{3–11} and LRP5 and LRP6 are key players in the Wnt signaling pathway^{17,18}, these genes are logical candidate genes to study in relation to OA. Several studies have shown that genetic variation in the LRP5 and LRP6 genes is associated with bone-related outcomes^{19–26}. With respect to OA, studies of LRP5 and LRP6 polymorphisms are scarce. One study observed a common haplotype of the LRP5 gene, to be associated with an increased risk of knee OA²⁷.

In the present study, we examined the association of the Arg200Trp and Arg324Gly variants of FRZB, the LRP5 Ala1330Val variant and the LRP6 Ile1062Val variant with the risk of radiographic hip-, knee- and hand-OA, THR, and urinary CTX-II levels. We investigated these associations in two population-based cohort studies: the Rotterdam Study and the Chingford Study. In addition, a meta-analysis was performed for the FRZB Arg324Gly polymorphism and hip- and knee-OA.

Materials and methods

THE ROTTERDAM STUDY

The Rotterdam Study is a large prospective population-based cohort study of men and women aged 55 years and older. The study design and rationale are described elsewhere in detail²⁸. In summary, the objective of the study is to investigate the determinants, incidence and progression of chronic disabling diseases in the elderly²⁸. Depending on the OA outcome studied, there were 2685–3001 subjects with data available for OA outcomes. For example, for THR we had data available for 2998 subjects. The medical ethics committee of Erasmus University Medical School approved the study and written informed consent was obtained from each participant.

Clinical examination: height and weight were measured at baseline examination with the subject in a standing position with indoor clothing without shoes. The presence of knee/hip/hand pain (“did you have joint complaints of your right/left knee/hip/hand during the last month”) was asked during the home interview at baseline^{29,30}. The definitions for coronary heart disease, diabetes and lower limb disability are described elsewhere^{31–33}.

THE CHINGFORD STUDY

The Chingford Study is a prospective population-based longitudinal cohort. The Chingford Study includes 1003 women derived from the age/sex

register of a large general practice ($n > 11,000$) in North London, who are representative of the general UK population in terms of weight, height and smoking characteristics³⁴. The study design and rationale are described elsewhere in detail^{35–37}.

Clinical examination: all anthropometrical measurements were taken in a standardized manner, measured in standing position. Knee and hip pain were defined as having pain on most days of the month in at least one episode, in one or both joints during the last year.

ASSESSMENT OF RADIOGRAPHIC OSTEOARTHRITIS (ROA)

Radiographs were scored for the presence of a THR and ROA of the hip, knee and hand according to the K/L score³⁸. Knee and hip ROA were defined as a K/L score ≥ 2 of one or both joints^{29,39}. Also, a joint space width (JSW) in one or both hips ≤ 1.5 mm was defined as ROA. Hand OA was defined as presence of a K/L score ≥ 2 in two out of three hand joint groups [distal interphalangeal (DIPs), proximal interphalangeal (PIPs), first carpometacarpal (CMC1)/trapezio-scaphoid joint (TS)] of each or both hands³⁰. Furthermore, in a subgroup of the Rotterdam Study, we used a K/L score ≥ 3 of the knee and K/L score ≥ 2 of the metacarpophalangeal (MCP) joints of the hand as additional outcomes for severe OA. Clinical OA was defined as having a K/L ≥ 2 plus joint complaints in that specific joint.

BIOCHEMICAL MEASUREMENT

Urinary CTX-II was measured as described before^{29,40}. The concentration of CTX-II (ng/L) was standardized to the total urine creatinine (mmol/L).

GENOTYPING

Genotyping of both the Rotterdam Study and the Chingford Study was done by the Genetic Laboratory of the Department of Internal Medicine in Rotterdam. Genomic DNA was extracted from peripheral venous blood samples according to standard procedures. Genotypes were determined using the Taqman allelic discrimination assay. The Assay-by-Design service (www.appliedbiosystems.com) was used to set up a Taqman allelic discrimination assay for the FRZB Arg324Gly, the FRZB Arg200Trp, the LRP5 Ala1330Val and the LRP6 Ile1062Val polymorphisms. The primers and probes used are available on request and conditions of the assay were as previously described²⁶.

STATISTICAL ANALYSIS

Allele frequencies were estimated by allele counting and Hardy–Weinberg equilibrium (HWE) was tested using a χ^2 test. For reasons of power, homozygous and heterozygous carriers of the risk alleles were pooled for estimation of the odds ratio (OR) for all SNPs studied. Differences in baseline characteristics and CTX-II levels were evaluated by analysis of co-variance (ANCOVA). In order to compare the CTX-II levels in the two cohorts, sex-specific standard deviation (SD) scores were calculated separately for each subject in each cohort as described elsewhere in detail⁴¹. ORs, crude and adjusted for age and body mass index (BMI), were estimated from logistic regression for cross-sectional analysis of all the binary OA variables. Linkage Disequilibrium (LD) between the Arg200Trp and Arg324Gly FRZB SNPs was estimated by D' and r^2 and was calculated using Haploview. SPSS version 11.0 (SPSS INC., Chicago, USA) was used to construct combined genotypes. Interaction between the different SNPs was tested using the multi-locus model implemented in FAMHAP and is described elsewhere in detail⁴². Power calculations were performed with the program PS (<http://biostat.mc.vanderbilt.edu/twiki/bin/view>). Stratification according to gender was performed. A P -value of ≤ 0.05 was considered significant. If not stated otherwise, we used SPSS version 11.0 software for all analyses.

Power considerations: for FRZB Arg324Gly, LRP5 Ala1330Val and LRP6 Ile1062Val polymorphisms, we performed power calculations for detectable effect sizes in our study at $\beta = 0.80$ and $\alpha = 0.05$.

META-ANALYSIS

We conducted a meta-analysis of data from all published studies regarding the FRZB Arg324Gly polymorphism, in order to assess whether the Gly³²⁴ allele is associated with increased risk for hip- or knee OA. ORs for the individual studies were estimated using the number of subjects and allele frequencies presented in the papers. Forest plots were created using RevMan Analyses version 1.0⁴³. By eyeballing and using the Q -statistic (Mantel–Haenszel χ^2) and I^2 -statistic, heterogeneity between studies was assessed. A p -value ≤ 0.10 was considered significant for the Q -statistic. The DerSimonian and Laird test was used in RevMan Analyses version 1.0⁴³ to estimate an OR in case of

heterogeneity (random-effects model), otherwise a Mantel–Haenszel test was performed (fixed-effect model). Analyses were performed separately for males and females, since positive studies only found an association in females. In the case more than one definition of hip- or knee-OA occurred in one study, only the definition for which most power was observed, was included in the meta-analysis.

Results

GENOTYPING

In the Rotterdam Study, all genotypes were in HWE. However, in the Chingford Study we observed a HWE deviation for LRP5 Ala1330Val and LRP6 Ile1062Val genotype distributions, but this was borderline significant (0.03 and 0.05, respectively). To exclude genotyping errors we re-genotyped all homozygote carriers of the LRP5 Val¹³³⁰ allele and the LRP6 Val¹⁰⁶² allele, but no discrepancies were detected. While all genotyping was done at the same laboratory, it is unlikely that genotyping errors are the cause of the slight imbalance in HWE in the Chingford Study. Allele frequencies for all polymorphisms were similar to the ones found in previous studies on Caucasians^{6,7,11,14,27}.

BASELINE CHARACTERISTICS

Characteristics of the Rotterdam Study and the Chingford Study are shown in Table I. In women, there were no associations between these characteristics and the two studied polymorphisms in the FRZB gene, neither in the Rotterdam Study nor in the Chingford Study (data not shown). For males in the Rotterdam Study, carriers of the FRZB Gly³²⁴ allele were on average 1 year older as the non-carriers (data not shown).

OSTEOARTHRITIC OUTCOMES

Table II shows no consistent significant associations between the FRZB Gly³²⁴ variant and hip-, knee-, or hand-OA outcome measures in the Rotterdam, nor in the Chingford Study. Adjusting for co-morbidity factors such as lower limb disability, coronary heart disease and diabetes did not change these results. There were no significant associations between the three other studied variants, FRZB Trp²⁰⁰, LRP5 Ala¹³³⁰, LRP6 Ala¹⁰⁶², and hip-, knee- or hand-OA (Supplementary Tables IV–VI) and there were no associations between the FRZB, LRP5 and LRP6 variants and other osteoarthritic outcomes such as JSW of the hip, clinical OA, OA in

the MCP joints of the hand and a K/L ≥ 3 for the knee as a proxy for severe OA. Similarly, no associations were observed between the genetic variants and THR, although power was limited.

We next examined CTX-II levels as a measure of generalized cartilage degradation. As shown in Fig. 1, in the Rotterdam Study, female carriers of the FRZB Gly³²⁴ allele had 0.32 SD lower levels of CTX-II ($P = 0.002$), while a similar non-significant trend was seen in males with 0.13 SD lower levels for Gly³²⁴ carriers. In the Chingford Study, we also observed a similar – though not significant – trend with 0.14 SD lower CTX-II levels in FRZB Gly³²⁴ carriers. When we combined males and females from the Rotterdam Study, and females from the Chingford Study according to SD scores, carriers of the FRZB Gly³²⁴ allele had 0.29 SD lower CTX-II levels ($P = 0.001$).

For alleles of the FRZB Arg200Trp and Arg324Gly polymorphisms we could not estimate haplotypes because LD was too low ($D' = 0.06$; $r^2 = 0.003$ in the Rotterdam Study). We therefore analyzed combined genotypes and compared the combination of Trp²⁰⁰ and Gly³²⁴ carriers with non-carriers of these two variants. There were no significant associations for hip- or knee-OA in the Rotterdam Study or the Chingford Study. In females of the Rotterdam Study, we observed an OR of 0.94 for hip OA [95% confidence interval (CI) 0.44–2.01] and 1.33 for knee OA (95% CI 0.79–2.22) and in the Chingford Study we observed an OR of 0.46 (95% CI 0.17–1.24) for hip OA and 1.29 for knee OA (95% CI 0.58–2.96), however, for both studies power was very limited. In the Rotterdam Study, we constructed a multiple locus association model incorporating all four polymorphisms in the three genes of the Wnt signaling pathway to test for possible interactions. No significant interactions between the polymorphisms in relation to OA endpoints were observed (data not shown).

POWER CONSIDERATIONS

We calculate power for all published studies and our own study (see Table III). For the FRZB Arg324Gly polymorphism we had 80% power to detect risks of at least 1.6 for knee OA in females of the Rotterdam Study and a risk of 1.9 in the Chingford Study. For females in the Rotterdam Study, we had 80% power to detect risks of 1.9 for hip OA and 1.6 for hand OA. For THR we had 80% power to detect a risk of 2.6, indicating very limited power in our study to detect the originally reported OR of 1.5 for THR by FRZB genotype⁶. For the LRP5 and LRP6 variants we had higher power, given the higher allele frequencies. For example, for

Table I
Characteristics of the Rotterdam Study and the Chingford Study for the FRZB Arg324Gly polymorphism

	Rotterdam Study		Chingford Study
<i>n</i> Males/ <i>n</i> Females	<i>n</i> = 1765 males	<i>n</i> = 2448 females	<i>n</i> = 780 females
Age (years)*	67.1 ± 7.3	68.0 ± 7.9	64.2 ± 6.2
Height (cm)*	175.1 ± 6.7	161.9 ± 6.5	160.6 ± 6.1
Weight (kg)*	79.1 ± 10.5	70.1 ± 11.1	69.1 ± 12.4
BMI (kg/m ²)*	25.8 ± 2.9	26.8 ± 4.1	26.9 ± 4.7
No. THR cases/total (%)	18/1276 (1.4)	62/1722 (3.6)	NA
No. Hip OA cases/total (%)	113/1276 (8.9)	168/1722 (9.8)	245/750 (32.7)
No. Knee OA cases/total (%)	164/1063 (15.4)	479/1622 (29.5)	291/773 (37.6)
No. Hand OA cases/total (%)	267/1329 (20.1)	600/1672 (35.9)	100/636 (15.7)
CTX-II levels (ng/mmol)	142.8	209.4	227.9

NA = not applicable.

*Values are averages with SDs.

Table II
Hip-, knee-, and hand-OA risk by FRZB Arg324Gly genotype in both study cohorts

OA phenotype	Rotterdam Study						Chingford Study					
	Allele frequency			OA by genotypes			Allele frequency			OA by genotypes		
	Controls	Cases	OR (95% CI)*	Arg/Arg	Arg/Gly + Gly/Gly	OR (95% CI)*	Controls	Cases	OR (95% CI)*	Arg/Arg	Arg/Gly + Gly/Gly	OR (95% CI)*
Females												
THR	0.09	0.06	0.55 (0.23–1.29)	56/1433 (3.9) [†]	6/289 (2.1)	0.55 (0.23–1.29)	NA	NA	NA	NA	NA	NA
K/L hip	0.09	0.09	0.99 (0.64–1.53)	141/1433 (9.8)	27/289 (9.3)	0.99 (0.64–1.53)	0.09	0.05	0.56 (0.34–0.91)	224/648 (34.6)	21/102 (20.6)	1.10 (0.71–1.71)
K/L knee	0.08	0.09	1.09 (0.80–1.48)	399/1363 (29.3)	80/259 (30.9)	1.09 (0.80–1.48)	0.08	0.07	1.10 (0.71–1.71)	257/689 (37.3)	42/116 (36.2)	0.82 (0.41–1.63)
Hand OA	0.09	0.08	0.94 (0.71–1.25)	503/1387 (36.3)	97/285 (34.0)	0.94 (0.71–1.25)	0.08	0.06	0.82 (0.41–1.63)	89/560 (15.9)	12/96 (12.5)	NA
Males												
THR	0.09	0.06	0.53 (0.12–2.37)	16/1044 (1.5)	2/232 (0.9)	0.53 (0.12–2.37)	NA	NA	NA	NA	NA	NA
K/L hip	0.09	0.11	1.14 (0.70–1.86)	90/1044 (8.6)	23/232 (9.9)	1.14 (0.70–1.86)	NA	NA	NA	NA	NA	NA
K/L knee	0.09	0.09	0.81 (0.50–1.30)	137/882 (15.5)	27/181 (14.9)	0.81 (0.50–1.30)	NA	NA	NA	NA	NA	NA
Hand OA	0.10	0.09	0.78 (0.54–1.14)	223/1089 (20.5)	44/240 (18.3)	0.78 (0.54–1.14)	NA	NA	NA	NA	NA	NA

NA = not applicable; THR = total hip replacement; K/L = Kellgren/Lawrence score

*ORs are adjusted for age and BMI.

[†]Number of subjects defined as case/total number of subjects with data on this OA phenotype (%).

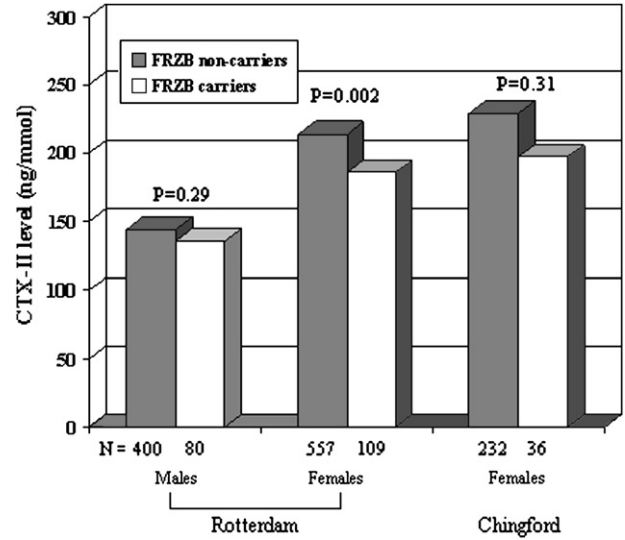


Fig. 1. CTX-II levels by FRZB Arg324Gly genotype of males and females of the Rotterdam Study and of females of the Chingford Study. Values are adjusted for age and BMI.

knee OA in females in the Rotterdam Study, we could detect (with 80% power) an OR of 1.5 for the LRP5 variant and an OR of 1.4 for the LRP6 variant (data not shown). In the Rotterdam Study and the Chingford Study we had very low power to investigate the role of the rare FRZB combined genotype and OA. In the Rotterdam Study, we had 80% power to detect a OR of 2.3 in females for hip OA, in the Chingford Study this was a OR of 2.5.

META-ANALYSIS

In Table III some characteristics of all published studies are shown that were considered for the meta-analysis, including our own data from the current study. For the FRZB Arg324Gly polymorphism, the forest plots for hip OA and knee OA are depicted in [Fig. 2(A) and (B)], respectively. Large heterogeneity was observed between the studies on hip OA in females reflected by a Q-statistic of 16.69 (df = 5), with a P-value of 0.005 and an I² of 70%. Therefore, a random-effects analysis was performed for this phenotype. This analysis showed no evidence of association between the FRZB Gly³²⁴ allele and hip OA in females (combined effect estimate OR 1.09, 95% CI 0.80–1.47, P = 0.58). For knee OA no large heterogeneity between studies was observed, and so both fixed- and random-effect analysis were performed. Both analyses showed no evidence of association between the Gly³²⁴ allele and knee OA in males and females (combined effect estimate OR 1.04, 95% CI 0.89–1.20, P = 0.63).

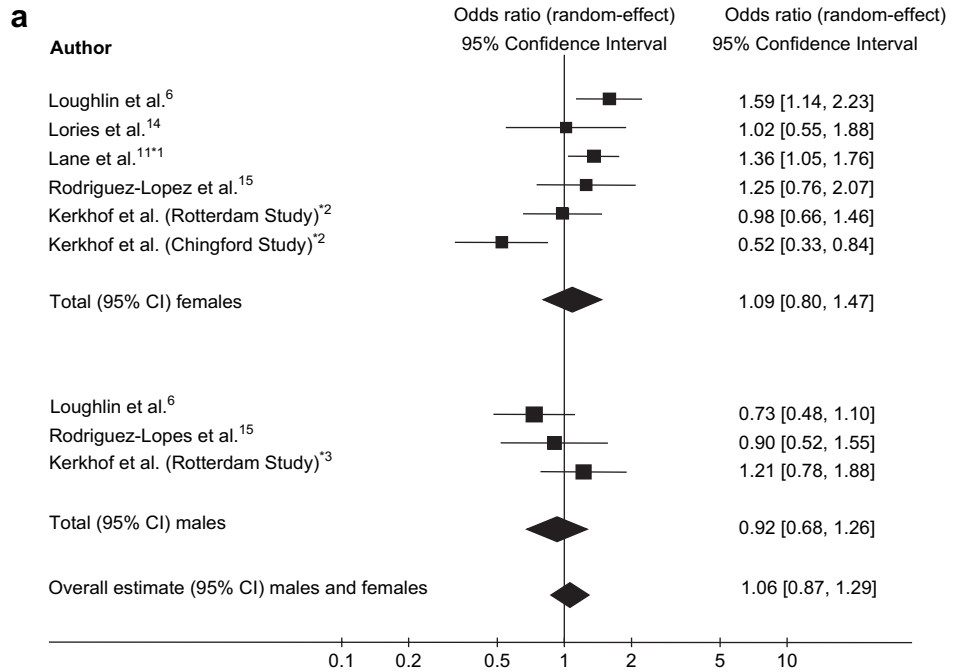
Discussion

The present study showed that in two population-based cohort studies no consistent associations were observed between selected polymorphisms in the FRZB, LRP5 and LRP6 genes and several measures of OA including, radiographic hip-, knee- and hand-OA and THR. A meta-analysis of all published studies and including our own data indicated no evidence of association between the FRZB Gly³²⁴ allele and hip- or knee-OA, although large

Table III
 Overview of published studies on the relationship of the FRZB Arg324Gly polymorphism with OA outcomes

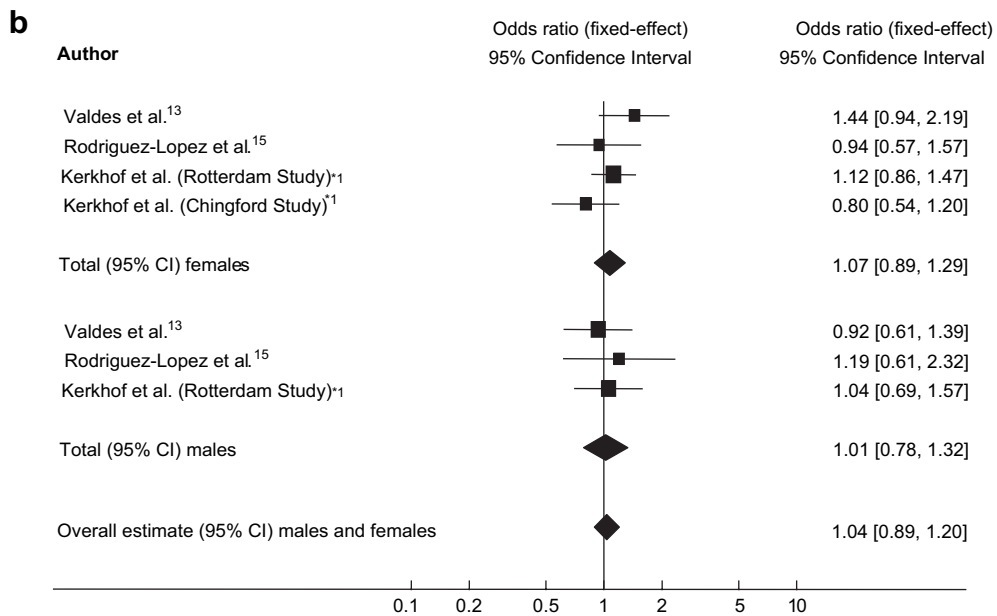
Author and study population (n)	OA Phenotypes	Hip OA						Knee OA						Hand OA				Generalized OA		CTX-II levels	
		THR		Radiographic OA		Power‡		Clinical OA		Radiographic OA		Power‡		Clinical OA		Radiographic OA		Radiographic			
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Loughlin <i>et al.</i> ⁶ Oxford (n = 1696)	Case-control	■	■			1.9	1.8					NA	NA								
Min <i>et al.</i> ⁷ Rotterdam Study (n = 1369)	Cohort			■	■	NA	NA					NA	NA					■	■		
Min <i>et al.</i> ⁷ GARP (n = 382)	Family study*					NA	NA					NA	NA					■	■		
Lories <i>et al.</i> ¹⁴ Leuven (n = 314)	Case-control		■			NA	2.8					NA	NA								
Lane <i>et al.</i> ¹¹ SOF (n = 4706)	Cohort			■		NA	1.5					NA	NA								
Valdes <i>et al.</i> ¹³ Nottingham/Oxford (n = 1202)	Case-control†					NA	NA	■	■			2.0	2.2								
Rodriguez <i>et al.</i> ¹⁵ Spanish patients (n = 1123)	Case-control	■	■			2.5	2.2	■	■			3.1	2.1	■	■						
Kerkhof <i>et al.</i> Rotterdam Study (n = 4472)	Cohort	■	■	■	■	2.2	2.0			■	■	2.0	1.6			■	■			■	■
Kerkhof <i>et al.</i> Chingford Study (n = 814)	Cohort				■	NA	1.9			■	■	NA	1.8			■	■				■

SOF = Study of Osteoporotic Fractures, M = males, F = females, TKR = total knee replacement, CTX-II = urinary CTX-II levels.
 *One thousand eighty two control subjects from the Rotterdam Study are shared between that study and the Rotterdam Study described in this paper.
 †One hundred and eighty five female control subjects from Oxford are shared between that study and the original study by Loughlin *et al.*
 ‡Power is expressed as ORs that can be detected with beta = 80% and alpha = 0.05.
 ■ No association found □ Significantly increased risk Gly³²⁴ allele ▣ Significantly decreased risk Gly³²⁴ allele □ Not studied



¹Joint space narrowing is used as definition of hip OA. Other hip OA phenotypes are excluded from the analysis.

^{2/3}A Kellgren/Lawrence score ≥ 2 is used as definition of hip OA. The phenotype total hip replacement is excluded from the analysis.



¹A Kellgren/Lawrence score ≥ 2 is used as definition of knee OA. The phenotype total knee replacement is excluded from the analysis.

Fig. 2. Forest plot of studies regarding the FRZB Arg324Gly polymorphism. (A) Forest plot of studies regarding FRZB Arg324Gly polymorphism and hip OA. (B) Forest plot of studies regarding FRZB Arg324Gly polymorphism and knee OA.

heterogeneity was observed between studies concerning hip OA in females. Recently, Loughlin *et al.* observed in 957 females the Gly³²⁴ variant of the FRZB gene to be associated with a 1.5 times higher risk of THR⁶. In our study, however, we did not observe an increased risk for THR by

genotype for the FRZB Arg324Gly or FRZB Arg200Trp polymorphisms, although power in our population was very limited for purposes of replication of this phenotype. For other hip-related OA outcome measures such as the JSW and the K/L score, we had somewhat higher power,

but we could not detect an association or a trend in the predicted direction. Within the Rotterdam Study we can only exclude risks of 2.0 for hip ROA based on power calculations, while Loughlin *et al.* found an OR of 1.5 for the FRZB Gly³²⁴ allele (and then for THR, rather than hip OA defined by radiography). Therefore, the lack of power, especially for hip OA, could be one of the reasons for our negative findings. Alternatively, the difference in severity of OA (hip replacement cases vs radiological defined milder OA in our study) could be another reason for the negative findings. Lastly, negative findings could be based on confounding due to co-morbidity factors. However, in the Rotterdam Study no differences in risk for OA were observed after adjusting our results for co-morbidity factors such as lower limb disability, coronary heart disease and diabetes.

We also explored the association with more clinical/severe definitions of OA in a subset of our subjects. We identified a subgroup with a JSW ≤ 1.5 mm of the hip, a K/L score ≥ 3 of the knee, current pain in the hip/knee/hand coinciding with ROA of that joint and K/L score ≥ 2 of the MCP joints of the hand as a proxy for severe hand OA³⁰. However, again there were no associations observed between these more severe and clinical phenotypes and the four polymorphisms. In line with our observation, also three other reports found conflicting results for the role of the FRZB Arg324Gly polymorphism in OA, although each study used a different definition of OA^{7,11,14}. This highlights a general difficulty in genetic studies of OA: the lack of consensus on OA-definitions or outcomes to use and how to compare different joint sites for replication⁴⁴. In Table III, we compared OA endpoints used in different studies on the FRZB Arg324Gly polymorphism. This table shows: (1) that different studies used different outcomes, (2) only very few studies analyzed the same phenotypic OA outcome, and (3) that there is no consistency in the results for this association. Considering that results are conflicting and power of individual studies is limited, a meta-analysis may help in determining the true effect sizes if any. The meta-analysis of all published studies and including our results showed no evidence of association between the FRZB Gly³²⁴ allele and hip- or knee-OA. In the process of the meta-analysis, we had difficulty with handling the different OA-phenotypes used in the published studies. For example, for hip OA it was necessary to combine both clinical and ROA outcomes, resulting in a large heterogeneity between studies. Therefore, the power becomes lower to observe an association. Recently, general recommendations have been published by working groups of HuGenet and NCI-NHGRI^{45–47} for replication studies in genetic epidemiology studies. One of the recommendations was to preferably investigate the same or a very similar phenotype in replication studies and for genetic studies in OA this represents a substantial challenge as is discussed above in some more detail.

Previously, Min *et al.*⁷ published a paper regarding the FRZB Arg324Gly polymorphism and OA using a random sample of 1369 subjects of the Rotterdam Study. They found a significant association of the FRZB Arg324Gly polymorphism with generalized OA. In the present study, apart from testing additional polymorphisms, we focused more on the many different joint sites of OA, including hip-, knee- and hand-OA and CTX-II measurement. Furthermore, we used more subjects of the Rotterdam Study (4472 instead of 1369, average overlap between the study of Min *et al.* and the Rotterdam Study for hip-, knee- and hand-OA is 79%), thereby increasing power.

In the Chingford Study and in the Rotterdam Study we used a cut-off point for the K/L score of the hip of grade

2, which is considered the golden standard for hip OA⁴⁸. However, the prevalence of hip OA was three times higher in the Chingford Study compared with the Rotterdam Study when using grade 2+. This is highly likely due to the fact that agreement between observers for the grading of OA is rather low, particularly for hip OA⁴⁸. This is a problem we cannot overcome at this moment and is a general problem for all OA-studies. The differences in K/L scoring between different cohorts is becoming apparent because of the population-based design of our study. It was picked up due to frequency differences between the two cohorts. These frequency differences cannot be observed in a case-control study.

Cartilage degradation is one of the main characteristics of OA and is commonly assessed by the JSW measurement. However, significant cartilage degradation has to occur in order to visualize a reduction in JSW on radiographs⁴⁹. Biochemical markers may be more sensitive than radiographs to pick up early changes in the cartilage^{50,51}. CTX-II levels are a biochemical marker of cartilage degradation²⁹. CTX-II levels are elevated in diseases that are characterized by increased cartilage turnover such as rheumatoid arthritis and OA^{49,50,52}. We observed a highly significant association with the FRZB Arg324Gly polymorphism and CTX-II levels in females of the Rotterdam Study, which suggests a protective effect for OA (assessed by CTX-II levels) for carriers of the FRZB Gly³²⁴ allele. The Chingford Study showed a non-significant trend in the same direction. This finding is counter-intuitive since previous research lead to the hypothesis that carriers of the FRZB Gly³²⁴ allele are less capable of antagonizing Wnt signaling⁶, which would lead to a higher Wnt signaling in cartilage and hence, in an increased risk of OA^{3,8–10}. Although a false positive result due to multiple testing is still possible, the partial confirmation in Chingford and overall significance of the two combined study populations makes this a less likely explanation. It therefore suggests a true effect of FRZB genotype on CTX-II levels but this observation still warrants further validation in other cohorts.

One earlier report examined the relationship between polymorphisms in the LRP5 gene and OA and found an association between a certain haplotype of the LRP5 gene and knee OA²⁷. In this study, we examined the relationship between the LRP5 Ala1330Val variant and OA. This is not a direct replication of the study of Smith *et al.*²⁷. Previous research showed that genetic variation in exon 18 of LRP5, in particular the Ala1330Val variant, modulates Wnt signaling⁵³, and therefore the Ala1330Val variant is a promising variant to study in relation to OA. In our study, we did not observe any relation between the LRP5 Ala1130Val variant with knee-, hip- and hand-OA or CTX-II levels in either cohort. Considering the earlier results of Smith *et al.* who studied other polymorphisms in this gene as we did, we cannot exclude that the LRP5 gene does play a role in the pathogenesis of OA, but if so perhaps through other variants than the Ala1330Val variant. A tagging approach of the large LRP5 gene would be most appropriate to investigate the role of this gene in the pathogenesis of OA, however, we did not use this approach in our study, which is a limitation of this study. To our knowledge, we are the first to study the relationship between the LRP6 amino acid variant and OA. de Ferrari *et al.* showed that the LRP6 Val¹⁰⁶² allele leads to a decreased beta-catenin signaling⁵⁴ and could therefore decrease the risk of OA. However, we did not observe significant associations between this variant and osteoarthritic outcomes.

Lastly, we would like to discuss power issues. For females in the Rotterdam Study, we had 80% power for

the FRZB Arg324Gly variant to detect risks of 2.0 for hip-OA and 1.6 for knee-OA as defined by the K/L score, while for THR power was 2.6. For the LRP5 and LRP6 variants and other osteoarthritic endpoints we had overall somewhat higher power, given the higher allele frequencies. Therefore, it is possible that in our study some true associations were not detected due to lack of power, especially for THR. Yet, we note that most previously published studies on this association had very limited power and so a meta-analysis of all published studies including our own results could have overcome this problem, if this was the only reason for missing the association in our study. However, the meta-analysis failed to provide convincing evidence to support an association of the FRZB Arg324Gly polymorphism with several OA endpoints, although for hip OA in females, large heterogeneity existed between studies.

In conclusion, we studied polymorphisms in several key players of the Wnt signaling pathway but did not observe any association between the FRZB, LRP5 and LRP6 amino acid variants with osteoarthritic outcomes in two population-based cohorts. A meta-analysis for the FRZB Gly³²⁴ allele showed no evidence of association with hip- or knee-OA. This study underscores that more consensus is needed to standardize phenotypes of interest in future genetic studies of OA as well as increasing sample sizes. The recently formed European Consortium to explore the genetics of OA called Treat-OA (www.TreatOA.eu) has access to up to 30,000 DNA samples and hopefully should be able to overcome some of these issues in the near future.

Conflict of interest

All authors state that there are no financial and personal relationships with other people or organizations that could inappropriately influence this work.

Acknowledgments

This project was funded by European Commission Grant QLK6-2002-02629 (GENOMOS). Arthritis Research Campaign. This article was part of Hanneke Kerkhof's training program of the Master of Science Clinical Epidemiology at the Netherlands Institute of Health Sciences. We would like to thank the Trustfonds Erasmus University Rotterdam for contributing financially to the Masters program. Furthermore, we would like to thank all participants of the Rotterdam Study and the general practitioners, pharmacists, and the many field workers at the research center in Ommoord, Rotterdam, The Netherlands. We are very grateful to Dr E. Odding, Prof. H. A. Valkenburg (deceased), Dr A. P. Bergink, Dr M. Reijman and Dr S. Dahaghin for scoring the knee, hip and hand radiographs, we thank Dr S. C. E. Schuit and F. Imani for help with collection and transfer of urine samples and Taye Hussien Hamza for help with statistical analyses. The Chingford Study is supported by a special purpose grant from the Arthritis Research Council and we are very grateful to Dr Doyle, Dr Thompson, Dr Hakim and Maxine Daniels for support of the cohort.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.joca.2008.02.007.

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