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Wnt Signaling Antagonists Are Potential Prognostic Biomarkers for the Progression of Radiographic Hip Osteoarthritis in Elderly Caucasian Women

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Objective. **To determine whether serum levels of 2 Wnt signaling antagonists, Frizzled-related protein (FRP) and Dkk-1, are associated with the development and progression of radiographic hip osteoarthritis (RHOA).**

Methods. **Pelvic radiographs were obtained a mean of 8.3 years apart in 5,928 Caucasian women ≥65 years of age who were enrolled in the Study of Osteoporotic Fractures. Random sampling of this cohort was performed, with ~180 subjects per group assigned to 2 nested case–control studies on RHOA incidence and progression. Baseline serum levels of FRP and Dkk-1 were measured by capture enzyme-linked immunosorbent assay. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using logistic regression analyses with adjustment for potential covariates.**

Results. **There were no differences in serum levels of FRP and Dkk-1 between case subjects with incidence or progression of RHOA and their respective control subjects. There was a trend for higher baseline serum levels of FRP to be associated with a reduced risk of incident RHOA (age-adjusted OR 0.59 [95% CI 0.32–1.09], $P = 0.09$ for women in the highest quartile versus women in the lowest quartile). There was no association of serum levels of FRP with progression of RHOA. Serum levels of Dkk-1 did not correlate with incident RHOA. However, higher serum levels of Dkk-1 were associated with diminished risk of RHOA progression (age-adjusted OR 0.43 [95% CI 0.23–0.79], $P = 0.007$ for women in the highest quartile compared with women in the lowest quartile).**

Conclusion. **Elevated circulating levels of Dkk-1 appeared to be associated with reduced progression of RHOA in elderly women, whereas the highest quartile of serum FRP levels tended to be associated with a modest reduction in risk of incident RHOA.**

Osteoarthritis (OA) is a disease that results from degradation of the articular cartilage and changes in the bone surrounding the joint. The factors that initiate OA are not well understood, and the course of joint degeneration in OA is variable (1,2). Risk factors for progression of OA in weight-bearing joints include age, increased biomechanical loading of joints through obesity, bone density, and previous injury. However, these factors explain only a very small portion of the effect (1). Definitive serologic markers for the risk, diagnosis, and prognosis of OA have yet to be determined.

One approach to investigating potential markers is to target pathways and elements known to be involved in the development or homeostasis of the joint. Recently, the Wnt/β-catenin signaling cascade, which is involved in skeletal and joint development, has received attention in OA (3,4). The potential role of the Wnt signaling pathway in the development of OA has been suggested by the increase in susceptibility for hip OA in women that is associated with 2 functional genetic variants in FRZB, which encodes Frizzled-related protein (FRP) (5–8). Secreted FRP (sFRP) is a naturally

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occurring soluble antagonist that competitively binds Wnt to antagonize its ability to signal through Frizzled receptors (9,10). FRP is expressed in adult human cartilage, and its messenger RNA expression is altered in response to cartilage injury in vitro and in vivo (8,11), suggesting that FRP might protect against the development or progression of OA.

OA is also a disease of altered bone metabolism, and subchondral sclerosis, one of the classic features of radiographic OA, might play an integral role in the progression of OA. This local increase in bone density implies a local dynamic process in which markers of bone remodeling could be systemically released with subchondral bone thickening adjacent to the OA joint (1). Natural antagonists of the Wnt pathway might influence this process, since they have significant impact on adult bone mass (12). Dkk family members are soluble antagonists of the canonical Wnt signaling pathway that bind to the Frizzled coreceptors low-density lipoprotein receptor–related protein 5 (LRP-5) and LRP-6 (13,14). Gain and loss of function mutations in LRP-5 result in high bone mass and severe osteoporosis, respectively (15–17). Serum levels of Dkk-1 in patients with multiple myeloma correlate with the number of lytic bone lesions and might correlate with the level of depression in osteoblast activity (18,19). Similarly, in OA, active bone remodeling might occur in a localized process, as seen in subchondral sclerosis, and not globally in the entire skeleton.

Based on the reported studies, we hypothesized that Wnt signaling proteins are involved in cartilage and bone metabolism and may reflect the course of OA. To test this hypothesis, we measured serum levels of 2 Wnt signaling antagonists, FRP and Dkk-1, to determine whether they were associated with the development and progression of radiographic hip OA (RHOA) in a prospective analysis of a cohort of elderly women.

PATIENTS AND METHODS

Population. Participants were in the Study of Osteoporotic Fractures (SOF), a multicenter cohort study initiated in 1986 to determine the risk factors for osteoporotic fractures in 9,704 Caucasian women. Participants were all ≥65 years of age at baseline and were recruited between September 1986 and October 1988 from population-based listings in 4 areas of the US: Baltimore, MD, Minneapolis, MN, Portland, OR, and the Monongahela Valley near Pittsburgh, PA. Nonwhite women were excluded from the original cohort because of their low incidence of hip fracture, as were women who were nonambulatory or who had undergone bilateral hip replacement (20). For this OA study, women with radiographically confirmed rheumatoid arthritis (RA), Paget's disease, or hip fracture at baseline were excluded from this analysis (21).

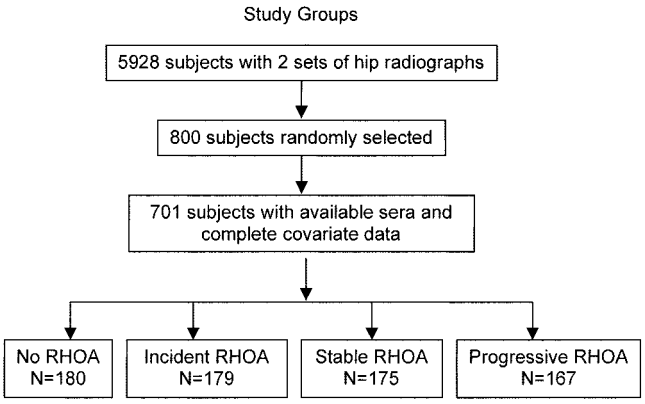

Figure 1. Study group selection. Of the 5,928 subjects with 2 sets of available hip radiographs, 800 subjects were chosen at random. Among them, 701 subjects for whom there were adequate sera and complete covariate data were divided into 4 groups based on radiographic criteria (see Patients and Methods). RHOA = radiographic hip osteoarthritis.

Radiography and interpretation. At the baseline and followup visits (average of 8.3 years followup time), supine anteroposterior radiographs of the pelvis were obtained using a standard protocol (21). Radiographs were assessed for 5 individual radiographic features of hip OA (joint space narrowing [JSN], osteophyte formation, subchondral sclerosis, cysts, and deformity) using atlas photographs to improve the reliability of the readings (22). Minimum joint space was measured using reported methods (22). A modified summary grade of 0–4 was assigned to each hip based on the individual radiographic features present. RHOA required a summary grade of ≥2 (21–23).

Hips were considered to have baseline radiographic OA if any of the following 3 findings were present: 1) a summary grade ≥2, 2) JSN superolaterally grade ≥2 or superomedially grade ≥3, or 3) definite osteophytes grade ≥2 in any location and definite JSN grade ≥2 in any location (21,22). Women were divided into those with and those without baseline findings of RHOA. Those without baseline RHOA were eligible to develop RHOA at the followup visit. Women with baseline RHOA were eligible for progression at the followup visit. A hip was defined as having developed OA (incident disease) if any of the above 3 findings were present on the 8.3-year followup radiograph in a hip that was free of all of these findings at baseline. A hip was defined as having progressed radiographically if any of the following occurred between baseline and followup for women who had baseline RHOA: a decrease in minimum joint space of ≥0.5 mm, an increase of ≥1 in the summary grade, an increase of ≥2 in total osteophyte score, or total hip replacement for OA between baseline and followup which was assessed by radiography and review of the medical records (21).

Study subject selection. We performed 2 nested case–control studies, one for incident RHOA and the other for progression of RHOA. From a cohort of 5,928 subjects who

Table 1. Baseline characteristics of subjects for the incidence and progression studies of RHOA*

	Incidence study		Progression study	
	No RHOA (n = 180)	Incident RHOA (n = 179)	Stable RHOA (n = 175)	Progressive RHOA (n = 167)
Age, years	69.8 ± 3.9	71.0 ± 4.7†	71.7 ± 5.6	72.4 ± 5.6
Weight, kg	67.8 ± 11.8	67.7 ± 12.9	67.8 ± 12.0	68.9 ± 11.9
Height, cm	159.0 ± 5.5	159.9 ± 6.0	158.0 ± 6.2	160.1 ± 6.2‡
Hip pain, no. (%)	61 (34)	60 (34)	66 (38)	78 (47)
Estrogen use, no. (%)	23 (13)	23 (13)	16 (9)	28 (17)§
Vitamin D use, no. (%)	89 (49)	80 (45)	89 (51)	81 (49)
Hip BMD, gm/cm^2	0.77 ± 0.11	0.78 ± 0.14	0.76 ± 0.12	0.79 ± 0.14
Serum Dkk-1, ng/ml	35.0 ± 20.2	34.5 ± 13.7	40.0 ± 42.1	33.9 ± 19.8
Serum FRP, ng/ml	46.1 ± 48.3	43.3 ± 58.0	41.6 ± 52.9	39.2 ± 32.4

* Except where indicated otherwise, values are the mean ± SD. RHOA = radiographic hip osteoarthritis; BMD = bone mineral density; FRP = Frizzled-related protein. See Patients and Methods for description of groups.
† $P = 0.007$ versus subjects with no RHOA.
‡ $P = 0.002$ versus subjects with stable RHOA.
§ $P = 0.03$ versus subjects with stable RHOA.

had both baseline and followup pelvic radiographs, we randomly selected 200 study subjects from each of the following 4 groups: those with no RHOA at either visit, those with incident RHOA at the followup visit, those with RHOA at baseline without progression at followup, and those with RHOA at baseline with progression at followup (Figure 1). Among them, 701 subjects were included for whom there were available sera and covariate data. For the incidence study, case subjects had no RHOA in either hip at baseline and later developed RHOA in at least 1 hip at followup, while control subjects had no RHOA at both baseline and followup. For the progression study, case subjects had RHOA at baseline that progressed in at least 1 hip, while control subjects had RHOA at baseline but their disease did not progress.

Assessment of potential confounders. All participants completed a self-administered questionnaire at baseline and followup visits that assessed a number of subject characteristics including, but not limited to, age, self-reported health status, education level, and current medication use, which included assessment of estrogen use and vitamin D supplement use. Height was measured with a wall-mounted Harpenden stadiometer (Holtain, Dyfed, UK), and weight was measured with a balance-beam scale (20,21,23). Hip pain was assessed by questionnaire at every visit and defined as hip pain "on most days for at least one month in the past year." Bone mineral density (BMD) in the hip was measured at the year 2 examination and at the year 8 followup visit using dual x-ray absorptiometry (Hologic 1000; Hologic, Waltham, MA). The protocol for the BMD measurements in the SOF has been previously reported (21,22,24).

Biochemical measurements. Serum samples were obtained at baseline. The serum was thawed and aliquoted into 0.5-ml volumes and then stored at −80°C until analysis. Serum measurements of FRP were performed by capture enzyme-linked immunosorbent assay (ELISA), in duplicate, as previously described (5). Goat anti-human FRP (R&D Systems, Minneapolis, MN) was used as the capture antibody, and the presence of bound FRP was detected with biotinylated goat anti-mouse/human FRP and streptavidin–horseradish peroxidase (HRP) (Zymed, South San Francisco, CA). Commercially prepared FRP (R&D Systems) was used as a standard, and concentrations were determined using SoftMax Pro software (Molecular Devices, Sunnyvale, CA). The lower limit of detection was reliably 1 ng/ml. Dkk-1 was assayed by capture ELISA, with goat anti-human Dkk-1 (R&D Systems) used as the capture antibody. The presence of bound Dkk-1 was detected with biotinylated goat anti-human Dkk-1 and streptavidin–HRP (Zymed). The lower limit of detection was reliably 5 ng/ml. The intraassay and interassay coefficients of variation for Dkk-1 were 4.47% and 9.6%, respectively. All samples were run in duplicate.

Statistical analysis. We compared baseline characteristics of the study subjects between the study groups (case–control incidence study and case–control progression study) using the chi-square test for dichotomous variables and the *t*-test for continuous variables. Logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for the dichotomous outcomes of incidence and progression of RHOA. The FRP and Dkk-1 levels were entered into the models as both continuous variables and quartiles, with the lowest quartile as the reference group. Exploratory classification and regression tree (CART) analyses were also performed. We considered variables for inclusion in multivariate models if they were different among cases and controls in either the incident OA case–control study group or the OA progression case–control study group with a *P* value of less than 0.10. Statistical analysis was performed using the statistical software program SAS version 8.2 (SAS Institute, Cary, NC). *P* values less than 0.05 were considered significant.

RESULTS

Subject characteristics. In the incidence study, baseline characteristics of the study subjects were similar between the group with incident RHOA and the group with no RHOA, with the exception of age (Table 1). For

the progression study, baseline characteristics of the study subjects were also similar between case and control subjects, with the exceptions of height and estrogen use (Table 1). There were no significant differences in baseline serum levels of FRP or Dkk-1 between those who developed RHOA and those who did not. Also, there was no significant increase in the risk of incident hip OA for a 1-SD increase in either serum FRP (age-adjusted OR of 0.92 per SD increase [95% CI 0.73–1.15]) or serum Dkk-1 (age-adjusted OR of 0.96 per SD increase [95% CI 0.78–1.19]). Similarly, there were no significant differences in baseline serum levels of FRP or Dkk-1 between those whose RHOA progressed and those whose RHOA did not. There was also no increased risk of RHOA progression for a 1-SD increase in either serum FRP (age-adjusted OR of 0.95 per SD increase [95% CI 0.75–1.19]) or serum Dkk-1 (age-adjusted OR of 0.75 per SD increase [95% CI 0.52–1.10]). There was also no increased risk of RHOA worsening (both incident RHOA and progression of RHOA) per SD increase in serum levels of either FRP or Dkk-1.

Stratified analysis. The association of quartiles of serum levels of FRP with the incidence and progression of RHOA is shown in Table 2. The risk of incident RHOA tended to diminish with increasing serum levels of FRP in the second, third, and fourth quartiles (by 20%, by 39%, and by 41%, respectively) compared with the lowest quartile in the age-adjusted model ($P = 0.06$ for trend). After adjustment for covariates, there was a 45% decrease in the risk of incident RHOA among women in the highest quartile of serum levels of FRP, compared with those in the lowest quartile (multivariate-adjusted OR 0.55 [95% CI 0.29–1.04], $P = 0.07$). Additional adjustments for body mass index (BMI) did not alter the results. An exploratory CART analysis found that a serum level of FRP >10.62 ng/ml reduced the risk of incident RHOA by nearly 60% (multivariate-adjusted OR 0.42 [95% CI 0.22–0.80], $P = 0.009$). The reduced risk was associated with protection against JSN. Interestingly, the risk of progressive RHOA was increased by 30% among women in the highest quartile of serum levels of FRP compared with women in the lowest quartile. However, this association was not significant (multivariate-adjusted OR 1.30 [95% CI 0.67–2.51]).

Table 2. Association between serum levels of FRP and incidence and progression of RHOA*

	Age-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)†
Incident RHOA		
Quartile (range)		
First (1.29–17.52)	Referent	Referent
Second (17.53–34.46)	0.80 (0.43–1.48)	0.71 (0.38–1.34)
Third (34.47–58.98)	0.61 (0.33–1.12)	0.58 (0.31–1.09)
Fourth (58.99–626.1)	0.59 (0.32–1.09)	0.55 (0.29–1.04)
Progressive RHOA		
Quartile (range)		
First (1.2–15.34)	Referent	Referent
Second (15.35–31.3)	1.02 (0.54–1.92)	0.93 (0.48–1.83)
Third (31.31–53.0)	0.84 (0.45–1.57)	0.76 (0.39–1.46)
Fourth (53.1–584.7)	1.16 (0.62–2.17)	1.30 (0.67–2.51)

* OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).
† Adjusted for age, hip pain at baseline, height in centimeters, and use of estrogen replacement therapy.

Table 3. Association between serum levels of Dkk-1 and incidence and progression of RHOA*

	Age-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)†
Incident RHOA		
Quartile (range)		
First (9.70–25.44)	Referent	Referent
Second (25.45–31.09)	1.06 (0.58–1.91)	1.03 (0.56–1.88)
Third (31.10–39.00)	1.50 (0.83–2.72)	1.54 (0.84–2.84)
Fourth (39.01–172.4)	1.15 (0.64–2.09)	1.25 (0.68–2.29)
Progressive RHOA‡		
Quartile (range)		
First (9.86–25.78)	Referent	Referent
Second (25.79–31.48)	0.41 (0.22–0.77)§	0.40 (0.21–0.77)¶
Third (31.49–40.50)	0.66 (0.36–1.21)	0.72 (0.38–1.36)
Fourth (40.51–500.9)	0.43 (0.23–0.79)#	0.40 (0.21–0.76)§

* RHOA = radiographic hip osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval.
† Adjusted for age, hip pain at baseline, height in centimeters, and use of estrogen replacement therapy.
‡ The risk of progressive RHOA was significantly reduced with increasing serum levels of Dkk-1 in the second and fourth quartiles ($P = 0.03$ for trend) in both age-adjusted and multivariate-adjusted models.
§ $P = 0.005$ versus first quartile.
¶ $P = 0.006$ versus first quartile.
# $P = 0.007$ versus first quartile.

Table 3 shows the association of quartiles of serum levels of Dkk-1 with the incidence and progression of RHOA. The risk of incident RHOA increased in the third and fourth quartiles compared with the lowest quartile of serum levels of Dkk-1, but the difference was not significant (fourth quartile age-adjusted OR 1.15 [95% CI 0.64–2.09]). However, the risk of progressive RHOA was significantly reduced with increasing serum levels of Dkk-1 in the second and fourth quartiles (by 59% and by 57%, respectively) compared with the first quartile (fourth quartile age-adjusted OR 0.43 [95% CI

0.23–0.79], $P = 0.007$). The association remained strong with adjustment for multiple covariates. Adjusting for BMD of the femoral neck and total hip did not change the results. In addition, the reduction in the risk of RHOA progression with increasing serum levels of Dkk-1 was associated with loss of joint space but not with osteophyte progression. After adjustment for covariates, there were 64% (OR 0.36 [95% CI 0.21–0.64], $P = 0.0004$) and 47% (OR 0.53 [95% CI 0.28–1.00], $P = 0.06$) reductions in the risks of joint space loss and osteophyte progression, respectively, among women in the highest quartiles of serum levels of Dkk-1 compared with those in the lowest quartile. Additional adjustments for BMI did not alter the results. Similarly, an exploratory CART analysis found that a serum level of Dkk-1 >24.9 ng/ml was associated with a nearly 56% reduction in progression of RHOA (multivariate-adjusted OR 0.44 [95% CI 0.24–0.80], $P = 0.007$).

We also evaluated all worsening RHOA (both incident and progressive RHOA compared with no RHOA) by quartile. Elevated levels of FRP (fourth quartile versus first quartile) were associated with a nearly 34% reduced risk of RHOA worsening (age-adjusted OR 0.56 [95% CI 0.32–0.96], $P = 0.03$), and this association remained after adjusting for estrogen replacement, hip pain, and height (OR 0.53 [95% CI 0.31–0.93], $P = 0.03$). However, there was no association of RHOA worsening with levels of Dkk-1.

DISCUSSION

In this study, high serum levels of Dkk-1, a soluble Wnt antagonist, in the sera of women with RHOA was associated with a significantly reduced risk of RHOA progression. In addition, individuals with high serum levels of FRP at baseline were somewhat less likely to develop RHOA during the next 8 years. Both of these proteins are extracellular modulators of Wnt signaling, yet their roles may be distinct in the natural progression of RHOA. The sFRP family members bind to Wnt proteins as competitive antagonists, potentially disrupting both canonical and noncanonical signaling pathways, whereas Dkk-1 predominantly disrupts the canonical β-catenin pathway (25).

The predominant effect of the elevated serum levels of Dkk-1 was seen with the reduction in the risk of JSN, which is a surrogate for cartilage loss. Increased levels of β-catenin have been reported in chondrocytes within areas of degenerative cartilage (26,27). During in vivo ectopic bone formation, bone morphogenetic protein 2 induces β-catenin–mediated signaling through Wnt ligands, and β-catenin is required for both osteogenesis and chondrogenesis (28). In contrast, studies of fetal development suggest that β-catenin is required for osteogenesis, but not for chondrogenesis. Hence, embryonic development pathways are not always recapitulated during regenerative processes later in life.

Activation of β-catenin in mature cartilage cells stimulates hypertrophy, matrix mineralization, and expression of matrix metalloproteinase 13 and vascular endothelial growth factor (29,30). Similarly, β-catenin overexpression in chondrocytes strongly stimulates expression of matrix degradation enzymes (30). Thus, Wnt/β-catenin signals may activate cartilage matrix catabolism and may have roles in cartilage destruction under pathologic conditions. The modest association that we found of increased levels of the Wnt antagonist, FRP, with a reduced risk of incident OA may occur potentially by the prevention of catabolic enzyme production or of the sequelae of catabolic stimuli.

A role for Dkk-1 in the preservation of cartilage has not been established. However, Dkk-1 has been shown to retard new bone formation and is a potent negative regulator of osteoblast differentiation in vitro and in vivo (19,31). Thus, the reduced risk of RHOA progression with elevated serum levels of Dkk-1 may reflect the ability of Dkk-1 to inhibit bone remodeling around the diseased joint. Dkk-1 is produced by synovial-like fibroblasts in patients with RA, and synovitis is often present in patients with advanced OA (32,33). Recently, Burr reviewed alterations in the subchondral bone in OA and reported that subchondral bone could affect the mechanical loading of articular cartilage and influence joint degeneration (34). It is intriguing to speculate that Dkk-1, by inhibiting bone formation and subchondral bone remodeling, may be able to retard the articular cartilage loss indirectly. Indeed, in our study, subjects with less articular cartilage loss appeared to be the subgroup with the highest serum levels of Dkk-1.

This study has a number of strengths, including a well-defined cohort of elderly Caucasian women who have been studied for an average of >8.3 years and a well-validated radiographic hip scoring method. However, there are a number of technical weaknesses. For example, the serum used to measure these Wnt signaling proteins had been thawed and refrozen numerous times, which may have influenced the results of the protein analysis. In addition, we only measured the baseline serum values, and the change over time in levels of these proteins may be a better prognostic predictor. Finally, we have not compared these proteins with other markers of bone metabolism. Perhaps a number of proteins

together might be a better determinant of progression of hip OA.

In summary, elevated levels of Dkk-1 appear to be associated with a reduction in the risk of progression of RHOA, and elevated levels of another Wnt signaling antagonist, FRP, also corresponded to a reduction in the risk of incident RHOA, although this association was more modest. Additional studies of other joints with OA, other populations, and in vivo animal models are now needed to confirm or refute these findings and to investigate the mechanism.

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AUTHOR CONTRIBUTIONS

Dr. Lane had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Lane, Nevitt, Corr.
Acquisition of data. Nevitt, de Leon, Corr.
Analysis and interpretation of data. Lane, Nevitt, Lui, Corr.
Manuscript preparation. Lane, Nevitt, Lui, Corr.
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