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Association of vitamin D status with knee pain and radiographic knee osteoarthritis

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SUMMARY

Objective: The objective of the present study was to explore the association of serum vitamin D concentration and polymorphism in the vitamin D receptor (VDR), with knee pain and radiographic knee osteoarthritis (OA) among men and women in a large population-based UK cohort study.
Methods: Seven hundred and eighty-seven participants in the Hertfordshire Cohort Study (399 men, 388 women; mean age 65.6 ± 2.7 years) underwent a questionnaire on knee pain and radiographic knee examination. This study examined the association of Fok1, Cdx2 and Apa1 polymorphism in the gene for the VDR and serum 25(OH)D concentration with knee pain and radiographic knee OA by a generalized estimating equations population averaged logistic regression analysis in the Hertfordshire Cohort Study.
Results: There were no associations of Fok1, Cdx2 and Apa1 polymorphisms of the VDR with knee OA except for Aa for Apa1 compared with AA [Odds ratio (OR) 0.59, 95% confidence interval (CI) 0.36–0.95, $P = 0.031$]. While, ff for Fok1 (OR 1.60, 95% CI 1.07–2.39, $P = 0.022$) and AA for Cdx2 polymorphism (OR 2.21, 95% CI 1.07–4.56, $P = 0.032$) was significantly associated with higher prevalence of knee pain compared with FF for Fok1 and GG for Cdx2, respectively. None of these are statistically significant after adjusting for the three polymorphisms tested. 25(OH)D level was not significantly associated with radiographic knee OA, while, low tertile of 25(OH)D level tended to be associated with knee pain compared with high tertile of 25(OH)D level.
Conclusion: The present cross-sectional study using a large-scale population from the Hertfordshire Cohort study indicated that vitamin D may be associated with pain rather than radiographic change, but the evidence for an association between vitamin D genetic variation and pain in knee OA is very weak in the present study. Further replication of our results will be required to elucidate the association of vitamin D and knee OA.

Introduction

Knee osteoarthritis (OA) is a major public health issue that causes chronic pain and disability[1–3], although at present the pathogenesis of this condition remains largely unknown. Several environmental factors have been associated with OA, including obesity[4–6], previous injury[7], knee-bending occupations[8,9], and other metabolic factors[10,11]. A previous population-based UK study of twins has also demonstrated a clear genetic influence on radiologic knee OA in women, with up to 65% of the variance being explained by genetic factors[12].

Vitamin D has been shown to stimulate synthesis of proteoglycan by mature articular cartilage *in vitro*[13], and this suggests that vitamin D may directly affect articular cartilage metabolism. Vitamin D receptor (VDR) is found in many types of tissues, including chondrocytes[14,15]. A previous study showed that VDR gene polymorphism was associated with bone[16], although it is still controversial[17]. The relationship between osteoporosis and OA suggests that VDR gene polymorphisms may be associated with both diseases[18]. However, the association of VDR gene polymorphisms with knee OA is

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controversial[19–23]. This may be partly due to different races, differences in environmental factors related to vitamin D metabolism, or the presence of other genetic factors that influence VDR function.

The association of vitamin D level with knee OA is also controversial[24–28]. In previous studies, McAlindon suggested that low serum levels of vitamin D were associated with progression of knee OA[24]. A recent study has also shown that serum 25(OH)D levels were associated with decreased knee cartilage loss[28], but Hunter *et al.* found that there was no significant association between vitamin D levels and knee osteophytes after adjusting for age, body mass index (BMI) and relatedness[25]. The Framingham study also found no association of vitamin D levels with knee OA worsening[26]. This may be partly explained by VDR gene polymorphism, because vitamin D exerts its endocrine and autocrine/paracrine local effects upon binding to and activating its intracellular receptor VDR. In other words, the association of vitamin D level with knee OA may be different by VDR gene polymorphisms, but, to the best of our knowledge, there were no studies investigating the association of vitamin D level with knee OA by VDR gene polymorphisms.

The principal clinical symptom of knee OA is pain[29], but the correlation between pain and radiographic severity is inconsistent[4,30–32]. Fewer studies have addressed factors which might influence knee pain[32–35]; among these, older age, female gender, and physically demanding work, have all been proposed[30–33]. Previous studies, however, have not addressed the role of vitamin D status or fixed genetic variation in the VDR.

The objective of this study was to clarify the association of VDR gene polymorphism with knee pain and radiographic knee OA among men and women in the general population, as well as to examine the association between circulating vitamin D concentration and these indices of OA.

Subjects and methods

Subjects

The Hertfordshire Cohort Study is a population-based cohort study in the UK. Details of the study design have been published previously[36], thus, a brief summary is provided here. The selection procedure was as follows: using the National Health Service Central Registry at Southport, and Hertfordshire Family Health Service Association, we traced men and women who were born during 1931–1939 in Hertfordshire, and still lived there during the period 1998–2003. After obtaining written permission from each subject's general practitioner (GP), we approached each person by letter, asking him or her if they would be willing to be contacted by one of our research nurses. If subjects agreed, a research nurse performed a home visit and administered a structured questionnaire. This included information on socioeconomic status, medical history, drug history, cigarette smoking, alcohol consumption, and reproductive variables in women.

At a subsequent clinic, height was measured to the nearest 0.1 cm using a Harpenden pocket stadiometer (Chasmors Ltd, London, UK) and weight to the nearest 0.1 kg on a SECAfloor scale (Chasmors Ltd). Fasting venous whole-blood samples were taken at this clinic visit. Eligible subjects were then invited to book a return visit for knee radiography. Weightbearing anteroposterior and lateral semiflexed radiographs of both knees were taken at the same hospital using the same radiographic equipment; a standard tube to film distance of 100 cm was used. Radiographs were performed at a median duration of 6 months [interquartile range (IQR) 4.8–7.2] after the clinic visit. Radiographs were graded at the tibiofemoral joints using the Kellgren Lawrence (KL) grade[37]. One trained reader graded the radiographs; KL grade ≥ 2 was the threshold for a definition of knee OA. Subjects were also asked "Have you had any pain in or around your right knee on most days in the last month?" and "Have you had any pain in or around your left knee on most days in the last month?" Knee pain reported in this way was defined as having knee pain. A total of 498 men and 468 women completed a home questionnaire, attended clinic, and underwent knee radiography.

A fasting morning blood sample was obtained from all subjects at the first clinic visit, and the serum separated and stored at -70°C. 25(OH)vitamin D was assayed using a DiaSorin Liason automated chemiluminescent assay with equal specificity for both D2 and D3 (coefficient of variation for vitamin D across the assays was 10–12% for within batch and 10–15% between batch).

Genomic DNA was extracted from whole-blood samples according to standard procedures. VDR genotype was determined by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, and three VDR polymorphic sites (Fok1, Cdx2 and Apa1) were analyzed.

Ethical permission for the study was granted by the East and North Hertfordshire Ethical Committees. All participants gave written informed consent.

Statistical analysis

To assess gene polymorphism effects on radiographic knee OA and knee pain, indicator variables were created for Fok1 (FF and ff), Cdx2 (GA and AA) and Apa1 (AA and aa) polymorphism. As both knees have a pain score and a radiographic grade, a generalized estimating equations (GEE) population averaged logistic regression model was used to adjust for clustering of knees within patients. To examine 25(OH)D levels and their association with knee OA and knee pain, we classified subjects into three categories; high tertile (>51.5 nmol/l), middle tertile (35.5–51.5 nmol/l) and low tertile (<35.5 nmol/l). A GEE population averaged logistic regression analysis was used to determine the association of vitamin D level with knee OA and knee pain with and without adjustment for age, gender, BMI, season of the clinic visit and KL grade. To decide whether statically significant associations between VDR polymorphisms and knee outcomes are noteworthy, we used Wacholder's method to calculate the False Positive Report Probability (FPRP) [Wacholder in JNCI 2004]. Data analyses were performed using SAS version 9.0 (SAS Institute, Cary, NC, USA) and Stata version 11.2 (Stata, College Station, TX, USA).

Results

Of 984 subjects, 170 (17.3%) provided incomplete pain questionnaires. A further 19 (1.9%) lacked genotypic information. We also excluded eight subjects with total knee arthroplasty, leaving 787 (399 males and 388 females) participants in this analysis. Comparison between the 787 subjects with complete information and those without complete information revealed no statistically significant differences in mean age (responders 65.6 years, nonresponders 65.7 years; $P = 0.66$), sex (responders 80.3% women, nonresponders 79.8% women; $P = 0.87$), BMI (responders 27.0, nonresponders 27.4; $P = 0.19$) or prevalence of knee OA (responders 15.2% women, nonresponders 16.3% women; $P = 0.74$). The characteristics of these participants are shown in Table I. The men were slightly younger than the women, and they had a lower mean BMI; serum vitamin D concentration was also significantly higher among men than women. There were no significant differences in mean values [IQR] of 25(OH)D concentration (nmol/l) among VDR gene polymorphisms of Fok1 [FF 45.5 (30.0–56.0), Ff 47.1 (31.5–56.8), ff 51.3 (31.0–69.0)], Cdx2 [GG 45.9 (30.6–56.0), AG 47.5 (31.2–60.2), AA 54.1 (36.7–68.0)] and Apa1 [AA 48.4 (32.9–61.3), Aa 47.5 (30.0–59.1), aa 42.7 (31.0–50.9)]. There were no significant

Table I
Characteristics of participants

	Overall	Men	Women	*P*-value
Number of subjects	787	399	388	
Age, years	65.6 (2.7)	64.8 (2.6)	66.4 (2.6)	<0.001
BMI, kg/m^2	27.0 (4.3)	26.8 (3.6)	27.2 (4.9)	0.22
25(OH)D level, nmol/l* means, (IQR)	42.5 (30.8, 57.3)	44.4 (34.7, 64.2)	41.0 (28.3, 54.1)	<0.001
Radiographic knee OA, *n*, (%)	120 (15.3)	70 (17.5)	50 (12.9)	0.069
Knee pain, *n*, (%)	309 (39.3)	147 (36.8)	162 (41.8)	0.16

Except where indicated otherwise, values represent means (standard deviation). The differences in age, BMI and 25(OH)D level between men and women were examined by the non-paired Student's *t*-test. The differences in prevalence of radiographic knee OA and knee pain between men and women were examined by chi square test.

* Of 787 subjects, 25(OH)D was measured in 683 subjects.

differences in the prevalence of radiographic knee OA and knee pain between genders. Of 120 subjects with radiographic knee OA, 79 (65.8%) had knee pain, while, of 667 subjects without radiographic knee OA, 230 (34.5%) had knee pain. Knee pain was significantly associated with radiographic knee OA after adjustment for age, gender and BMI [Odds ratio (OR); 3.03, 95% confidence interval (CI); 1.98–4.68].

We examined the association of VDR gene polymorphisms and radiographic knee OA (Table II). There were no associations of Fok1, Cdx2 and Apa1 polymorphisms of the VDR with knee OA except for Aa for Apa1 compared with AA after adjustment for age, gender and BMI, and FPRP values were low for association of Apa1 (Aa) on radiographic knee OA suggesting this association may be noteworthy. We also examined the associations of the alleles with knee OA. f for Fok1 tended to associate with higher prevalence of knee OA than F ($P = 0.06$). The alleles for Cdx2 and Apa1 were not significantly associated with knee OA ($P = 0.94$ and 0.64).

We also examined the association of VDR gene polymorphisms and knee pain (Table III). Unlike radiographic knee OA, Fok1 and Cdx2 polymorphism was significantly associated with prevalence of knee pain after adjustment for age, gender BMI and KL grade, and FPRP values were low for association of Fok1 (ff) for knee pain, suggesting this association may be noteworthy. There were no associations of Apa1 polymorphisms with knee pain. When analyzed in men and women separately, Fok1 polymorphism was significantly associated with knee pain after adjustment for age, BMI and KL grade in women (Ff: OR; 1.17, 95% CI; 0.75–1.81, $P = 0.486$, ff: OR; 2.46, 95% CI; 1.38–4.39, $P = 0.002$, compared with FF), while, not in men (Ff: OR; 1.10, 95% CI; 0.71–1.73, $P = 0.649$, ff: OR; 1.01, 95% CI; 0.58–1.76, $P = 0.98$, compared with FF). We also examined the associations of the alleles with knee pain. f for Fok1 had significantly associated with higher prevalence than F ($P = 0.01$). The alleles for Cdx2 and Apa1 were not significantly associated with knee pain ($P = 0.49$ and 0.64, respectively).

We next examined the association of 25(OH)D level and knee OA (Table IV). GEE logistic regression analysis showed that 25(OH)D level was not significantly associated with radiographic knee OA. For knee pain effect of vitamin D level was non-linear, so we classified subjects into three groups; high tertile (>51.5 nmol/l, $n = 225$), middle tertile (35.5–51.5 nmol/l, $n = 229$) and low tertile (<35.5 nmol/l, $n = 229$); low tertile of 25(OH)D level tended to be associated with knee pain compared with high tertile of 25(OH)D level after adjustment for age, gender, BMI, season of the clinic visit and KL grade (Table IV).

Discussion

This is the first study to examine the association of radiographic knee OA and knee pain with vitamin D level and VDR gene polymorphism at the same time. A Fok1 polymorphism of the VDR was significantly associated with radiographic knee OA and knee pain. There were no associations between radiographic knee OA and 25(OH)D level, while 25(OH)D level tended to be associated with knee pain.

The association of VDR gene polymorphism with OA is controversial[19–23]. In previous studies, a nested case-control study in Britain showed the 'T' allele was associated with knee OA in women[19]. The Rotterdam Study showed that the 'bAT' haplotype was associated with reduced prevalence of OA[20]. While, The Framingham study found no evidence for an association of the VDR gene with knee OA[23]. In a case-control study in Japan, there was also no significant association between VDR gene polymorphism and knee OA[21], although cases were sampled from hospital attenders in the study and controls did not undergo X-rays, causing the inevitable selection bias to occur. This inconsistency may also be due to differences in the relative importance of this gene in different races, differences in environmental factors related to vitamin D metabolism, or the presence of other genetic factors that influence VDR function. Further, the association of genetic factors with knee OA is diminishing later in life due to the effects of lifestyle factors, thus it may be difficult to find out their association in the elderly. In the present study, a Fok1 polymorphism of the VDR was significantly associated with radiographic knee OA. Vitamin D has been shown to stimulate synthesis of proteoglycan by mature

Table II
Association of VDR gene polymorphisms and radiographic knee OA

	Total	Number (%) with knee OA	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)*	*P*-value	Power†	FPRP prior probability	
								0.1	0.01
Fok1									
FF	328	39 (11.9)	1.00		1.00				
Ff	333	60 (18.0)	1.50 (0.96, 2.34)	0.072	1.54 (0.96, 2.46)	0.071	0.47	0.58	0.94
ff	108	18 (16.7)	1.38 (0.75, 2.57)	0.301	1.56 (0.82, 2.94)	0.173	0.27	0.85	0.98
Cdx2									
GG	491	75 (15.3)	1.00		1.00				
AG	248	37 (14.9)	1.03 (0.66, 1.59)	0.903	0.94 (0.60, 1.48)	0.781	0.49	0.93	0.99
AA	29	5 (17.2)	1.30 (0.48, 3.57)	0.605	1.09 (0.43, 2.74)	0.858	0.11	0.99	1.00
Apa1									
AA	213	36 (16.9)	1.00		1.00				
Aa	388	51 (13.1)	0.64 (0.40, 1.03)	0.068	0.59 (0.36, 0.95)	0.031	0.39	0.42	0.89
aa	166	31 (18.7)	1.12 (0.65, 1.91)	0.687	1.04 (0.60, 1.81)	0.884	0.28	0.97	1.00

Of 787 subjects, genotyping was completed for 769, 768 and 767 with Fok1, Csk2 and Apa1 polymorphism of the VDR, respectively.

* As both knees have a radiographic grade, GEE population averaged logistic regression analysis after adjustment for age, gender and BMI was used to calculate adjusted OR.

† To detect an OR of 1.5, we are looking for a difference in proportions of 15.3% vs 21.3% for radiographic knee OA.

Table III
Association of VDR gene polymorphisms and knee pain

	Total	Number (%) with knee pain	Crude OR (95% CI)	*P*-value	Adjusted OR* (95% CI)	*P*-value	Power†	FPRP prior probability	
								0.1	0.01
Fok1									
FF	328	115 (35.1)	1.00		1.00				
Ff	333	139 (41.7)	1.19 (0.89, 1.61)	0.244	1.14 (0.84, 1.56)	0.398	0.71	0.83	0.98
ff	108	51 (47.2)	1.50 (1.00, 2.24)	0.052	1.60 (1.07, 2.39)	0.022	0.40	0.33	0.84
Cdx2									
GG	491	189 (38.5)	1.00		1.00				
AG	248	94 (37.9)	1.05 (0.78, 1.42)	0.733	0.99 (0.73, 1.34)	0.936	0.71	0.92	0.99
AA	29	15 (51.7)	2.20 (1.08, 4.47)	0.03	2.21 (1.07, 4.56)	0.032	0.14	0.67	0.96
Apa1									
AA	213	87 (40.8)	1.00		1.00				
Aa	388	151 (38.9)	0.90 (0.65, 1.23)	0.5	0.93 (0.67, 1.30)	0.678	0.62	0.91	0.99
aa	166	64 (38.6)	0.97 (0.65, 1.43)	0.864	0.92 (0.61, 1.40)	0.71	0.45	0.93	0.99

Of 787 subjects, genotyping was completed for 769, 768 and 767 with Fok1, Csk2 and Apa1 polymorphism of the VDR, respectively.
* As both knees have a pain score, GEE population averaged logistic regression analysis after adjustment for age, gender, BMI and KL grade was used to calculate adjusted OR.
† To detect an OR of 1.5, we are looking for a difference in proportions of 39.3% vs 49.3% for knee pain.

articular cartilage *in vitro*[13], and this suggests that vitamin D may directly affect articular cartilage metabolism. Further, *in vitro* experiments confirmed that loss of VDR in chondrocytes reduced osteoclastogenesis by inducing receptor activator of NF-κB ligand (RANKL) expression[38], indicating that polymorphism of the VDR may affect osteophyte formation. In addition, the VDR gene has a thymine to cytosine single nucleotide polymorphism (SNP) at the Fok1 restriction site in the first of two potential start (ATG) codons located in the 50 region, resulting in a VDR protein that is shorter by three amino acids[39]. The F allele lacks the first ATG; thus, translation starts at the second ATG, instead of the first ATG, where translation of the f allele starts[40]. Most data indicate that the F allele is more effective than the f allele in transactivation of the 1,25-dihydroxyvitamin D signal[41]. However, a meta-analysis studying the association between VDR polymorphisms and OA[42] found no associations between VDR variation and OA. The ongoing GWAS studies on OA did not also find the foci polymorphism[43,44]. In the present study, the best *P*-value is only 0.022 which would be at least 0.066 when adjusted. Given the lack of a replication cohort, the evidence for an association between vitamin D genetic variation and pain in knee OA is very weak. In addition, considering that the sample size is modest for association studies in general, and more specifically for genetic association studies, the significant association of VDR gene polymorphism with radiographic knee OA in the present study may be due to random error. Additional and larger studies will be required, and, longitudinal studies may also determine whether this locus has any influence on the progression of joint damage at the knee.

IOF Working Group suggests that 75 nmol/L is the appropriate target level of serum 25(OH)D for individuals[45]. Vitamin D insufficiency, defined as 25(OH)D levels <75 nmol/L is prevalent worldwide[46], and the present study also showed that 604/683 (88.4%) had vitamin D insufficiency defined as <75 nmol/L. While, the association of serum vitamin D level and radiographic knee OA is controversial[24–27], McAlindon suggested that subjects with low serum levels of vitamin D are approximately three times more likely to have progression of established knee OA than subjects with high serum levels[24], but the number of subjects with progressive knee OA were comparably small in the study. Hunter *et al.* found that there was evidence of decreased vitamin D levels in subjects with knee osteophytes compared to those without osteophyte, but after adjusting for age, BMI and relatedness, the significant differences disappeared[25]. While, the Framingham study also found no association of vitamin D levels with knee OA worsening, defined as joint space loss on radiography or as worsening cartilage score on magnetic resonance imaging (MRI)[26]. In the present study, contrary to VDR gene polymorphisms, there were no significant association between vitamin D level and radiographic knee OA. Further, there were no differences in association of vitamin D level with radiographic knee OA among VDR gene polymorphisms.

Like radiographic knee OA, a Fok1 polymorphism of the VDR was significantly associated with knee pain in the present study. Further, knee pain also tended to be associated with vitamin D level, although it was not associated with radiographic knee OA. The correlation with the radiographic severity of knee OA is controversial[4,30–32]. In our previous study, 10% of men and 20% of women without radiographic knee OA had knee pain, and approximately 50% of men and 40% of women with severe radiographic knee OA had no knee pain in the elderly[4]. This indicates

Table IV
Association of 25(OH)D level with radiographic knee OA and knee pain

	Radiographic knee OA					Knee pain				
	n (%)	Crude OR (95% CI)	*P*-value	Adjusted OR* (95% CI)	*P*-value	*n* (%)	Crude OR (95% CI)	*P*-value	Adjusted OR† (95% CI)	*P*-value
25(OH)D level		0.99 (0.90, 1.10)	0.889	1.03 (0.92, 1.16)	0.627		–	–	–	–
Tertile 3 (51.2–147)	30/225 (13.3)	–	–	–	–	79/225 (35.1)	1.00		1.00	
Tertile 2 (35.9–51)	41/229 (17.9)	–	–	–	–	89/229 (38.9)	1.10 (0.77, 1.58)	0.598	1.04 (0.70, 1.56)	0.832
Tertile 1 (17–35.8)	36/229 (15.7)	–	–	–	–	105/229 (45.9)	1.48 (1.04, 2.10)	0.031	1.47 (0.95, 2.25)	0.08

OR of continuous vitamin D is for a 10-unit increase. For knee pain effect of vitamin D level was non-linear, so stratified into tertiles. Of 787 subjects, 25(OH)D was measured in 683 subjects.
* As both knees have and a radiographic grade, GEE population averaged logistic regression analysis after adjustment for age, gender, BMI and season of the clinic visit was used to calculate adjusted OR.
† As both knees have a pain score, GEE population averaged logistic regression analysis after adjustment for age, gender, BMI, season of the clinic visit and KL grade was used to calculate adjusted OR.

that there may be other factors associated with knee pain rather than radiographic knee OA, but there were few studies regarding factors associated with knee pain. Previous studies have shown that age, female sex and physical demanding work were associated with knee pain[32–35], but these factors were also reported as those associated with radiographic knee OA[4,9]. In the present study, vitamin D level tended to be associated with knee pain without association with radiographic knee OA, indicating that the association of vitamin D level with knee pain may be independent of radiographic knee OA. In fact, the result was almost similar after adjustment for radiographic knee OA, although it did not reach significance. Previous study has shown that vitamin D deficiency was related to quadriceps weakness[47], which is strongly associated with knee pain and disability in the community, even when activation and psychological factors are taken into account[48]. This may partly explain the association of vitamin D level and knee pain.

There are several limitations in the present study. First, the sample size was modest for association studies in general, and more specifically for genetic association studies. Further, we did not make multiple testing adjustments in the present study. In addition, studies reporting biomarker associations and, even more so, genetic associations have suffered from the report of false positives and the best way of addressing this is by testing these associations in independent cohorts and replicating the results. Thus the association of VDR gene polymorphisms with knee pain may be due to random error. However, FPRP values were low for association of Apa1 (Aa) on radiographic knee OA, and Fok1 (ff) for knee pain, suggesting these associations may be noteworthy, thus, these may merit replication in further studies. Second, we did not analyze Bsm and Taq, although these SNP are near Apa1. Third, 25(OH)D should have different association with different feature of ROA such as joint space narrowing or osteophytosis, but we did not analyze the association of joint space narrowing or osteophytosis with 25(OH)D or VDR polymorphisms.

In conclusion, the present cross-sectional study using a large-scale population from the Hertfordshire Cohort study revealed that a Fok1 and Cdx2 polymorphism of the VDR were significantly associated with knee pain, but not with radiographic knee OA. There were no associations between radiographic knee OA and vitamin D level, but it tended to be associated with knee pain. Further replication of our results will be required to elucidate the association of vitamin D and knee OA.

Author contributions

All authors have made substantial contributions to all three of sections (1), (2) and (3) below:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data,

(2) drafting the article or revising it critically for important intellectual content,

(3) final approval of the version to be submitted.

Conflict of interest

There are no conflicts of interest.

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