

Table 2. Associations of minimum JSW and osteophyte area with WOMAC domain scores*

	Pain				Physical function			
	Crude regression coefficient (95% CI)	P	Adjusted regression coefficient (95% CI)†	P	Crude regression coefficient (95% CI)	P	Adjusted regression coefficient (95% CI)†	P
Overall population								
Minimum JSW	-0.71 (-0.81, -0.60)	<0.0001	-0.37 (-0.48, -0.25)	<0.0001	-2.33 (-2.66, -1.99)	<0.0001	-0.97 (-1.34, -0.59)	<0.0001
Osteophyte area	0.07 (0.05, 0.08)	<0.0001	0.03 (0.02, 0.04)	<0.0001	0.25 (0.21, 0.29)	<0.0001	0.14 (0.10, 0.18)	<0.0001
Men								
Minimum JSW	-0.47 (-0.64, -0.31)	<0.0001	-0.29 (-0.47, -0.11)	0.002	-1.34 (-1.86, -0.82)	<0.0001	-0.48 (-1.04, 0.08)	0.10
Osteophyte area	0.07 (0.04, 0.11)	<0.0001	0.03 (-0.005, 0.07)	0.09	0.30 (0.19, 0.41)	<0.0001	0.20 (0.09, 0.32)	0.0005
Women								
Minimum JSW	-0.83 (-0.97, -0.69)	<0.0001	-0.41 (-0.57, -0.25)	<0.0001	-2.89 (-3.35, -2.43)	<0.0001	-1.22 (-1.72, -0.72)	<0.0001
Osteophyte area	0.06 (0.05, 0.08)	<0.0001	0.03 (0.01, 0.04)	0.0001	0.24 (0.20, 0.29)	<0.0001	0.12 (0.08, 0.17)	<0.0001

* WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; 95% CI = 95% confidence interval.

† Calculated by multiple regression analysis with age, sex, body mass index, minimum joint space width (JSW), and osteophyte area as the independent variables in the overall population and with age, body mass index, minimum JSW, and osteophyte area as the independent variables in the groups of men and women only.

were larger than those for osteophyte area (-0.15 and 0.11, respectively), while the absolute values of the beta values for minimum JSW for scores on the physical

function domains of the WOMAC were smaller than those for osteophyte area (-0.14 and 0.15, respectively).

When the analysis was restricted to the partici-

Table 3. Associations of minimum JSW and osteophyte area with WOMAC domain scores in the subjects with knee OA*

	Pain				Physical function			
	Crude regression coefficient (95% CI)	P	Adjusted regression coefficient (95% CI)†	P	Crude regression coefficient (95% CI)	P	Adjusted regression coefficient (95% CI)†	P
Overall population								
Minimum JSW	-0.81 (-0.97, -0.65)	<0.0001	-0.51 (-0.69, -0.33)	<0.0001	-2.77 (-3.32, -2.22)	<0.0001	-1.46 (-2.05, -0.87)	<0.0001
Osteophyte area	0.06 (0.04, 0.07)	<0.0001	0.03 (0.01, 0.04)	0.0007	0.22 (0.18, 0.27)	<0.0001	0.12 (0.07, 0.17)	<0.0001
Men								
Minimum JSW	-0.59 (-0.86, -0.31)	<0.0001	-0.42 (-0.72, -0.11)	0.009	-1.95 (-2.81, -1.08)	<0.0001	-0.97 (-1.97, -0.01)	0.05
Osteophyte area	0.07 (0.02, 0.11)	0.003	0.02 (-0.02, 0.07)	0.40	0.34 (0.21, 0.48)	<0.0001	0.24 (0.10, 0.39)	0.001
Women								
Minimum JSW	-0.89 (-1.09, -0.68)	<0.0001	-0.56 (-0.78, -0.34)	<0.0001	-3.00 (-3.71, -2.29)	<0.0001	-1.61 (-2.35, -0.88)	<0.0001
Osteophyte area	0.05 (0.04, 0.07)	<0.0001	0.03 (0.01, 0.04)	0.002	0.20 (0.15, 0.26)	<0.0001	0.11 (0.05, 0.16)	0.0001

* Knee osteoarthritis (OA) was defined as a Kellgren/Lawrence grade of ≥ 2 . WOMAC = Western Ontario and McMaster Universities OA Index; 95% CI = 95% confidence interval.

† Calculated by multiple regression analysis with age, sex, body mass index, minimum joint space width (JSW), and osteophyte area as the independent variables in the overall population and with age, body mass index, minimum JSW, and osteophyte area as the independent variables in the groups of men and women only.

pants with knee OA, the results were almost the same (Table 3). In men with knee OA, minimum JSW was independently associated with pain domain scores (β coefficient -0.17), but not with physical function domain scores (β coefficient 0.05). In women with knee OA, both minimum JSW and osteophyte area were independently associated with physical function domain scores, but the beta value for minimum JSW for physical function domain scores was smaller than that for osteophyte area (-0.12 and 0.20 , respectively).

DISCUSSION

This is the first study to separately examine the associations of JSN and osteophytosis with QOL, measured by a disease-specific scale such as WOMAC, using a large-scale population-based Japanese cohort. In addition, JSN and osteophytosis were estimated not by categorical grade but by continuous values such as minimum JSW and osteophyte area at the knee. In the present study, JSN as well as osteophytosis was independently associated with QOL.

The present study showed that both JSN and osteophytosis reduce QOL. Osteophytosis appears to begin with the activation of periosteal layers, with initial generation of chondrocytes and subsequent calcification to real osteophytes. The process is probably an adaptive reaction of the joint in order to cope with joint instability, and thus osteophyte area may indicate the severity of joint instability (22), which might lead to loss of QOL. When men and women were analyzed separately, minimum JSW was significantly associated with scores on the WOMAC pain domain but not the WOMAC physical function domain in men, while osteophyte area was associated with scores on the physical function domain but not the pain domain. According to the methodology of the WOMAC, pain domains estimate the severity of pain, indicating that JSN may be strongly associated with pain. In contrast, physical function domains assess difficulties in activities of daily living, indicating that osteophytosis may be mainly associated with activities of daily living, particularly in men.

Our findings also indicated differences between the sexes in the associations of JSN and osteophytosis with QOL. Minimum JSW was significantly associated with scores on the physical function domains of the WOMAC in women, but not in men. Similarly, osteophyte area was associated with scores on the pain domains of the WOMAC in women, but not in men. These differences may indicate that JSN and osteophytosis were more strongly associated with loss of QOL

in women than in men. Our previous study also showed that the odds ratio of knee pain for K/L grade 3 or 4 knee OA was approximately twice as high in women as in men (4). This may be partly explained by the lower muscle mass in women than in men. Previous reports have shown that muscle mass is also associated with QOL (23,24). In men, muscular strength may obscure the associations of JSN and osteophytosis with QOL loss; thus, these were not associated with some QOL parameters in men.

The present study has several limitations. First, this is a large-scale, population-based study, with a cross-sectional study of baseline data. Thus, causal relationships could not be determined. The ROAD study is a longitudinal survey, so further progress may help elucidate any causal relationships. Second, we did not include other weight-bearing forms of OA, such as hip OA, in the analysis, although this disorder may also affect QOL. However, the prevalence of K/L grade 3 or 4 hip OA is 1.4% and 3.5% in Japanese men and women (25), respectively, which is lower than the prevalence of K/L grade 3 or 4 knee OA (13.5% and 24.6% in Japanese men and women, respectively) (4). Thus, it is possible that including hip OA would not strongly affect the results of the present study. Third, the QOL questionnaire was completed by 74.2% of all participants age 40 years or older in the ROAD study. Participants who completed the questionnaire were younger and more likely to have knee OA than the participants who did not complete the questionnaire, and thus the participants included in this study may have had better QOL than those who did not complete the questionnaire, and our results may have overestimated QOL. Fourth, although osteophytes may be even more pronounced in the contralateral tibiofemoral compartment (26), at present the KOACAD system can only measure medial osteophytes at the tibia. We are now developing the KOACAD system to measure osteophytes at other sites; thus, we may be able to clarify the association between osteophytes at other sites and QOL in the near future.

In conclusion, the present cross-sectional study using a large-scale population from the ROAD study revealed that JSN and osteophytosis are independently associated with QOL. Further studies, along with continued longitudinal surveys in the ROAD study, will help clarify the mechanisms of JSN and osteophytosis at the knee, and their relationship with QOL.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Muraki 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.

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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 polymorphisms 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.

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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 injury⁷, 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 factors¹².

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 bone¹⁶, although it is still controversial¹⁷. The relationship between osteoporosis and OA suggests that VDR gene polymorphisms may be associated with both diseases¹⁸. 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 OA²⁴. A recent study has also shown that serum 25(OH)D levels were associated with decreased knee cartilage loss²⁸, 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 relatedness²⁵. The Framingham study also found no association of vitamin D levels with knee OA worsening²⁶. 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 pain²⁹, 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 previously³⁶, 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 SECA floor 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) grade³⁷. 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 women¹⁹. The Rotterdam Study showed that the 'bAT' haplotype was associated with reduced prevalence of OA²⁰. While, The Framingham study found no evidence for an association of the VDR gene with knee OA²³. In a case-control study in Japan, there was also no significant association between VDR gene polymorphism and knee OA²¹, 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- κ B ligand (RANKL) expression³⁸, 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 acids³⁹. 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 starts⁴⁰. Most data indicate that the F allele is more effective than the f allele in transactivation of the 1,25-dihydroxyvitamin D signal⁴¹. However, a meta-analysis studying the association between VDR polymorphisms and OA⁴² 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 individuals⁴⁵. Vitamin D

insufficiency, defined as 25(OH)D levels <75 nmol/L is prevalent worldwide⁴⁶, 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 levels²⁴, 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 disappeared²⁵. 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 elderly⁴. 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 weakness⁴⁷, which is strongly associated with knee pain and disability in the community, even when activation and psychological factors are taken into account⁴⁸. 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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Reference values for hand grip strength, muscle mass, walking time, and one-leg standing time as indices for locomotive syndrome and associated disability: the second survey of the ROAD study

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Abstract

Background We established reference values for hand grip strength, muscle mass, walking time, and one-leg standing time as indices reflecting components of locomotive syndrome and associated disability using a large-scale population-based sample from the second survey of the Research on Osteoarthritis/Osteoporosis Against Disability (ROAD) cohort.

Methods We measured the above-mentioned indices in 2,468 individuals ≥ 40 years old (826 men, 1,642 women; mean age 71.8 years) during the second visit of the ROAD study. Disability was defined as certified disability according to the long-term care insurance system through public health centres of each municipality.

Results Mean values for hand grip strength (weaker side), muscle mass of the thighs, walking time for 6 m at the

usual pace, and the fastest pace for men were 32.7 kg, 7.0 kg, 5.6 s, and 3.7 s, respectively, and those for women were 20.8 kg, 5.2 kg, 5.9 s, and 4.1 s, respectively. The median values for one-leg standing time (weaker side) were 14 s for men and 12 s for women. The prevalence of disability in men aged 65–69, 70–74, 75–79, and ≥ 80 was 0.0, 1.0, 6.3, and 8.8%, respectively, and in women was 3.4, 3.5, 9.2, and 14.7%, respectively. There were significant associations between the presence of disability and walking time for 6 m at the usual pace and at the fastest pace, and between the presence of disability and walking speed.

Conclusions We established reference values for indices reflecting components of locomotive syndrome, and identified significant associations between walking ability and disability.

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Introduction

Musculoskeletal diseases, including osteoarthritis (OA) and osteoporosis (OP), are major public health problems among the elderly that affect activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality. According to the recent National Livelihood Survey by the Ministry of Health, Labour, and Welfare in Japan, OA is ranked fourth, while falls and osteoporotic fractures are ranked fifth among diseases that cause disabilities and subsequently require support for ADL [1]. Previous studies have reported increased mortality following osteoporotic fractures at the hip and other sites [2], and have estimated that a total of 47,000,000 people (21,000,000 men and 26,000,000 women) aged ≥ 40 years will eventually be affected by either OA or OP. Considering that the population of Japan is aging very rapidly—more than 22% of the population is aged ≥ 65 years [3]—a comprehensive and evidence-based prevention strategy for musculoskeletal diseases is urgently needed.

The Japanese Orthopaedic Association has proposed the term ‘locomotive syndrome’ to designate a condition in high-risk groups with musculoskeletal diseases who are highly likely to require nursing care [4]. Locomotive syndrome is caused by weakening of musculoskeletal organs such as bone, joint, and muscle, which in turn interferes with physical performance, especially self-transportation. Loss of locomotor abilities such as walking causes disabilities requiring support. Therefore, to prevent decline into disability, it is important to maintain a healthy range of bone, joint, muscle, and physical performance.

These four components, bone, joint, muscle, and physical performance, each have objective measurements that can be used as indices to evaluate their present condition. For example, bone mineral density (BMD) is a representative index of the condition of the bone. Joint space width (JSW), joint space area (JSA), and osteophyte area (OPA) are indices reflecting the condition of the joint. Regarding muscle, although the best index remains controversial, hand grip strength can be used to reflect muscle strength [5], and muscle mass is one index of muscle volume [6]. In addition, as objective indices of physical performance, walking speed and/or one-leg standing times are candidates [7, 8]. However, at present, it is difficult to use such indices for evaluating, diagnosing, or predicting the future occurrence and progression of locomotive syndrome in Japan, because there is little information on reference values for such indices to distinguish patients at risk from normal individuals in a large population-based cohort.

In 2005–2007, we began a large-scale population-based cohort study entitled Research on Osteoarthritis/

Osteoporosis Against Disability (ROAD), consisting of 3,040 participants in three communities located in urban, mountainous, and coastal areas (baseline study). Following the baseline study, a second survey was performed in the same communities in 2008–2010, in which 2,674 inhabitants participated (second visit).

Through analysis of the baseline data of the ROAD, the age-gender distribution of BMD has been reported as an index for bone mass [3], and the medial and lateral JSW, medial and lateral JSA, OPA, and femorotibial angle of the knee have been reported as indices of the health of joints [9] in these populations. However, there is still scant information regarding the condition of the muscles and physical performance. Therefore, in the present study, we aimed to establish reference values for hand grip strength as an index of muscle power, muscle mass as an index of muscle volume, and walking time and one-leg standing time as indices of physical performance, classified by age and gender, using the data from the second visit of the ROAD study. This information is expected to be valuable for early diagnosis and prevention of locomotive syndrome. In addition, we evaluated the prevalence of disabilities in participants in the ROAD study second visit, and identified associations between hand grip strength, muscle mass, walking time, and one-leg standing time and the presence of disability.

Participants and methods

Participants

Reference values were obtained from the results of cross-sectional measurements of participants enrolled in the second visit of the ROAD study. The ROAD study, which began in 2005, is a nationwide prospective study comprising population-based cohorts established in three communities, such as urban, mountainous, and coastal regions in Japan. Recruitment methods for this study have been described in detail elsewhere [3]. To date, participants in the urban region, aged ≥ 60 years, were recruited from among those enrolled in a randomly selected cohort study from the previously established Itabashi Ward resident's registration database. The response rate was 75.6%. Participants in the mountainous and coastal regions, aged ≥ 40 years, were recruited from listings of resident registration. Residents aged ≤ 60 years in the urban area and ≤ 40 years in the mountainous and coastal areas who were interested in participating in the study were invited. We have completed development of a baseline database including clinical and genetic information for 3,040 inhabitants aged 23–95 years (1,061 men, 1,979 women).

The second visit of the ROAD study began in 2008 and was completed in 2010. All the participants in the baseline study were invited to participate in the second visit. In addition to the former participants, inhabitants aged ≥ 60 years in the urban area and those aged ≥ 40 years in the mountainous and coastal areas who were willing to participate in the ROAD survey performed in 2008–2010 were also included in the second visit. In addition, residents aged ≤ 60 years in the urban area and ≤ 40 years in the mountainous and coastal areas who were interested in participating in the study were invited to be examined as well as the baseline.

The inclusion criteria of participants were as follows: (1) ability to walk to the clinic where the survey was performed, (2) ability to provide self-reported data, and (3) ability to understand and sign an informed consent form. No other exclusion criteria were used.

Thus, a total of 2,674 residents (892 men, 1,782 women) aged 21–97 years participated in the second visit. In the present study, we analysed the data for 2,468 individuals (826 men, 1,642 women; mean age 71.8 years); this population comprised 956 individuals (318 men, 638 women) in the urban region, 726 individuals (258 men, 468 women) in the mountainous region, and 786 individuals (250 men, 536 women) in the coastal region who participated in the second visit and were ≥ 40 years old.

All the participants provided written informed consent, and the study was conducted with the approval of the ethics committees of the participating institutions.

Hand grip strength, muscle mass, walking time, and one-leg standing time

Hand grip strength was measured bilaterally using a Toei Light handgrip dynamometer (Toei Light Co., Ltd., Saitama, Japan). Both hands were tested, and the better value was used to characterise the maximum muscle strength of the subject.

Among the 2,468 participants who participated in the second visit of the ROAD study, 778 residents (248 men, 530 women) in the coastal town of Taiji were examined to determine their segmental muscle mass using the bioelectrical impedance method (BIP; Physion MD; Physion Inc., Kyoto, Japan). We obtained values for the muscle masses of the right and left forearms, upper arms, upper limbs, quadriceps, thighs, lower legs, and lower limbs. This method had previously been validated as having a close correlation to muscle volume as measured by magnetic resonance imaging [10].

Among the 2,468 participants who participated in the second visit of the ROAD study, 1,637 residents (559 men, 1,078 women) of the mountainous town of Hidakagawa and the coastal town of Taiji were examined to determine

their walking time. Walking time was measured as the time required to complete a 6-m course. All participants walked the 6-m course twice; they first walked at their usual walking speed and then repeated the course at their fastest pace.

Among the 2,468 participants who participated in the second visit of the ROAD study, one-leg standing time with eyes open was measured on both sides for 2,433 individuals (816 men, 1,617 women). The time until the raised leg was set down on the floor was measured, with a maximum time of 60 s recorded for those who could stand on one leg for at least that length of time. The shorter value of the two measurements was used as the worse side and the longer measurement as the better side for the one-leg standing time of the subject.

Mean values and standard deviations (SDs) of hand grip strength, muscle mass, and walking time were classified by gender and age group (40, 50, 60, 70, and ≥ 80 s) to establish age-gender reference values for the general population. However, reference values classified by gender and age group for one-leg standing time were established using median (50th percentile) values and 25th–75th percentile ranges. These values were recorded using a maximum time of 60 s for anyone who could exceed that time; thus, the data do not fit a normal distribution, and use of means and SDs is unsuitable for one-leg standing time reference values.

Presence of disability

Disability in the present study was defined as ‘cases requiring long-term care’ as determined by the long-term care insurance system based on the Long-Term Care Insurance Act of 1997 in Japan. The procedure for identifying cases requiring long-term care is as follows: (1) each municipality establishes a long-term care approval board consisting of clinical experts, physicians, and specialists at the Division of Health and Welfare in each municipal office; (2) the long-term care approval board investigates the insured person using an interviewer-administered questionnaire consisting of 82 items regarding mental and physical condition and makes a screening judgement based on the opinion of a regular doctor; and (3) ‘cases requiring long-term care’ are determined according to standards for long-term care certification uniformly and objectively applied nationwide [11].

During the 3 years between the baseline and the second visit of the ROAD study, we annually obtained information on the participating residents regarding deaths, changes of residence, and presence or absence of certified disability according to the long-term care insurance system from the public health centres of the participating municipalities.

Statistical analysis

All statistical analyses were performed using Stata statistical software (Stata, College Station, TX, USA). Differences in the values of the indices were tested for significance using analysis of variance for comparisons among multiple groups. Scheffé's least significant difference test was then used for pairs of age groups.

To ascertain associations between the presence of disability and hand grip strength, muscle mass, walking time, and one-leg standing time, logistic regression analyses were performed using the presence of disability (yes, 1; no, 0) as an objective factor, and values for hand grip strength, muscle mass, walking time, and one-leg standing time as the explanatory factor after adjusting for age, gender, and body mass index (BMI, kg/m²).

Results

Characteristics of participants

Summary characteristics including age, height, weight, and BMI of the participants in the present study are shown in Table 1. Two-thirds of the 2,468 subjects were women, and the mean age of the female participants was 1 year younger than that of the male participants. Height and weight were

significantly lower for women than for men, but no significant difference in BMI was noted between the genders. All anthropometric measurements other than BMI of females tended to decrease with age. BMI of women in their 80s and older was significantly lower than that in younger age groups, while there were no significant differences among age groups 40–70 years old.

Reference values for hand grip strength, muscle mass, walking time, and one-leg standing time

Table 1 also shows the age-gender distribution of hand grip strength for both the better and worse sides. Mean hand grip strength in men was significantly higher than that in women ($p < 0.001$) and decreased with age in both men and women ($p < 0.001$).

Mean muscle mass for both forearms, both upper arms, both upper limbs, both quadriceps, both thighs, both lower legs, and both lower limbs are shown in Table 2. Muscle masses for all parts of the body were significantly higher in men than in women ($p < 0.001$). Mean muscle mass in men decreased with age for all areas except the lower leg. Particularly in the quadriceps and thighs, muscle masses in men aged ≥ 70 were significantly lower than those in their 40s–50s ($p < 0.05$). By contrast, although muscle mass for women aged ≥ 80 and older tended to be lower than those of younger age groups (other than the lower legs), there

Table 1 Mean values (standard deviation) of anthropometric measurements and hand grip strength of the participants classified by sex and gender

Age strata (years)	Number of subjects	Weight (kg)	Height (cm)	Body mass index (g/cm ²)	Grip strength (better side) (kg)	Grip strength (worse side) (kg)
Men						
40–49	32	73.5 (10.2)	170.3 (7.3)	25.4 (3.6)	49.5 (8.2)	49.3 (8.4)
50–59	100	68.8 (10.6)	168.0 (5.2)	24.3 (3.3)	47.3 (7.0)	42.6 (6.9)
60–69	137	65.4 (11.1) ^a	165.2 (6.2) ^{a,b}	23.9 (3.5)	41.4 (6.6) ^{a,b}	36.9 (7.9) ^{a,b}
70–79	308	60.0 (8.1) ^{a,b,c}	161.1 (5.7) ^{a,b,c}	23.1 (2.7) ^{a,b}	35.4 (6.8) ^{a,b,c}	31.5 (7.1) ^{a,b,c}
80 and older	249	57.2 (8.9) ^{a,b,c,d}	159.7 (6.0) ^{a,b,c}	22.4 (2.9) ^{a,b,c}	29.7 (6.2) ^{a,b,c,d}	26.3 (6.3) ^{a,b,c,d}
Total	826	61.6 (10.3)	162.5 (6.7)	23.3 (3.1)	36.6 (9.1)	32.7 (9.1)
Women						
40–49	93	55.9 (9.5)	157.0 (4.4)	22.6 (3.5)	31.2 (4.3)	28.2 (4.4)
50–59	191	55.3 (8.9)	154.4 (5.8) ^a	23.2 (3.7)	28.7 (4.9) ^a	25.4 (4.9) ^a
60–69	316	54.2 (8.0)	152.0 (5.5) ^{a,b}	23.4 (3.2)	26.6 (4.3) ^{a,b}	23.77 (4.5) ^{a,b}
70–79	599	51.3 (8.5) ^{a,b,c}	148.4 (5.9) ^{a,b,c}	23.3 (3.5)	22.6 (4.6) ^{a,b,c}	19.7 (4.7) ^{a,b,c}
80 and older	443	47.4 (8.3) ^{a,b,c,d}	145.5 (5.9) ^{a,b,c,d}	22.4 (3.6) ^{c,d}	19.4 (4.4) ^{a,b,c,d}	16.6 (4.6) ^{a,b,c,d}
Total	1,642	51.6 (8.9)	149.5 (6.7)	23.0 (3.5)	23.7 (5.8)	20.8 (5.8)

^a Significantly different ($p < 0.05$) from values of the age group in their 40s

^b Significantly different ($p < 0.05$) from values of the age group in their 50s

^c Significantly different ($p < 0.05$) from values of the age group in their 60s

^d Significantly different ($p < 0.05$) from values of the age group in their 70s

Table 2 Mean values (standard deviation) of segmental muscle mass (kg) in total right and left sides classified by age and gender

Age strata (years)	Number of subjects	Forearm	Upper arm	Upper limb	Quadriceps	Thigh	Lower leg	Lower limb
Men								
40–49	25	1.20 (0.19)	1.59 (0.36)	2.79 (0.54)	3.91 (0.64)	7.76 (1.19)	3.41 (0.66)	11.16 (1.69)
50–59	60	1.18 (0.16)	1.53 (0.28)	2.71 (0.41)	3.73 (0.64)	7.45 (1.22)	3.41 (0.66)	10.86 (1.60)
60–69	67	1.15 (0.17)	1.50 (0.28)	2.65 (0.42)	3.50 (0.68)	7.02 (1.28)	3.52 (0.86)	10.54 (1.89)
70–79	66	1.17 (0.20)	1.43 (0.28)	2.60 (0.46)	3.37 (0.66) ^a	6.78 (1.26) ^a	3.51 (0.68)	10.29 (1.70)
80 and older	30	1.11 (0.17)	1.37 (0.26)	2.48 (0.38)	3.10 (0.62) ^{a,b}	6.27 (1.18) ^{a,b}	3.92 (1.11)	10.18 (2.05)
Total	248	1.16 (0.18)	1.48 (0.29)	2.65 (0.44)	3.52 (0.69)	7.04 (1.30)	3.53 (0.80)	10.57 (1.79)
Women								
40–49	67	0.77 (0.12)	0.86 (0.19)	1.63 (0.30)	2.65 (0.60)	5.37 (1.12)	2.65 (0.47)	8.02 (1.45)
50–59	124	0.76 (0.10)	0.82 (0.16)	1.58 (0.24)	2.56 (0.44)	5.20 (0.82)	2.58 (0.51)	7.78 (1.20)
60–69	161	0.78 (0.11)	0.84 (0.16)	1.62 (0.25)	2.55 (0.45)	5.18 (0.84)	2.57 (0.42)	7.74 (1.10)
70–79	130	0.80 (0.12) ^b	0.85 (0.16)	1.66 (0.27)	2.54 (0.46)	5.17 (0.85)	2.66 (0.53)	7.83 (1.24)
80 and older	48	0.79 (0.43)	0.82 (0.16)	1.61 (0.28)	2.39 (0.45)	4.90 (0.84)	2.91 (0.69) ^{b,c}	7.81 (1.38)
Total	530	0.78 (0.11)	0.84 (0.16)	1.62 (0.26)	2.55 (0.47)	5.18 (0.88)	2.63 (0.51)	7.81 (1.23)

^a Significantly different ($p < 0.05$) from values of the age group in their 40s

^b Significantly different ($p < 0.05$) from values of the age group in their 50s

^c Significantly different ($p < 0.05$) from values of the age group in their 60s

were no specific trends in muscle mass among age groups ≤ 79 years old. However, as for men, the muscle mass of the quadriceps in women tended to decline with age, although the difference was not statistically significant.

Mean 6-m walking time and the calculated walking speed (m/s) using the walking time, classified by age and gender, are shown in Table 3. Six-meter walking time was significantly lower in men than in women ($p < 0.05$), indicating that men tended to walk faster than women in this study population. Mean 6-m walking time for both men and women increased with age. In particular, 6-m walking times for men and women ≥ 70 years old were significantly higher than those in younger age groups ($p < 0.05$).

Table 4 shows median one-leg standing time classified by age and gender with 25th–75th percentile ranges. For both men and women in their 40s–50s, all median, 25th percentile, and 75th percentile values were 60 s, with no gender difference. One-leg standing times for men ≥ 60 years old tended to be higher than those for women, and median values declined with age in both men and women.

Prevalence of disability among subjects ≥ 65 years old

Among the 2,468 participants in the second visit of the ROAD study, we surveyed 1,845 subjects (625 men, 1,220 women) ≥ 65 years old and obtained information on the presence or absence of disability certified for long-term care insurance. We found a total of 149 individuals (8.1%;

36 men, 5.8%; 113 women, 9.3%) that were certified as requiring support. Figure 1 shows the prevalence of disability classified by gender and age. The prevalence of disability in men 65–69, 70–74, 75–79, and ≥ 80 years old was 0.0, 1.0, 6.3, and 8.8%, respectively, and that in women in the same age groups was 3.4, 3.5, 9.2, and 14.7%, respectively (Fig. 1). The prevalence of disability in women was significantly higher than that in men ($p < 0.05$) and increased with age in both genders ($p < 0.01$).

Associations between disability and hand grip strength, muscle mass, walking speed, and one-leg standing time

Logistic regression analysis was performed using the presence of disability (1, yes; 0, no) as an objective factor, and hand grip strength on the better side and the worse side; muscle mass of the forearms, upper arms, upper limbs, quadriceps, thighs, lower legs, and lower limbs; walking time for 6 m at the usual pace and at the fastest pace; and quartile of one-leg standing time [0: 0–25% (highest quartile), 1: 25–50% (higher quartile), 2: 50–75% (lower quartile), 3: 75–100% (the lowest quartile)] on the better and worse sides as explanatory factors, after adjusting for age, gender, and BMI. No significant associations were found between the presence of disability and hand grip strength, muscle mass, or one-leg standing time. However, there were significant associations between the presence of disability and 6-m walking time at the usual

Table 3 Mean values (standard deviation) of 6-m walking time (s) and walking speed (m/s) with usual pace and the fastest pace classified by age and gender

Age strata (years)	Number of subjects	Usual pace		Fastest pace	
		Time for 6 m (s)	Walking speed (m/s)	Time for 6 m (s)	Walking speed (m/s)
Men					
40–49	32	4.4 (0.6)	1.38 (0.19)	3.0 (0.5)	2.09 (0.43)
50–59	100	4.8 (0.9)	1.29 (0.20)	3.2 (0.6)	1.97 (0.36)
60–69	134	5.1 (0.9)	1.21 (0.20) ^a	3.4 (0.7)	1.82 (0.33) ^a
70–79	196	5.9 (1.8) ^{a,b,c}	1.09 (0.25) ^{a,b,c}	4.0 (1.4) ^{a,b,c}	1.62 (0.39) ^{a,b,c}
80 and older	97	6.8 (3.0) ^{a,b,c,d}	0.99 (0.33) ^{a,b,c,d}	4.5 (1.8) ^{a,b,c,d}	1.48 (0.44) ^{a,b,c}
Total	559	5.6 (1.9)	1.15 (0.27)	3.7 (1.3)	1.73 (0.42)
Women					
40–49	92	4.7 (1.0)	1.32 (0.24)	3.2 (0.6)	1.95 (0.31)
50–59	190	4.9 (0.9)	1.27 (0.23)	3.3 (0.7)	1.87 (0.33)
60–69	299	5.1 (1.1)	1.22 (0.23)	3.7 (0.8)	1.71 (0.32) ^{a,b}
70–79	345	6.3 (2.4) ^{a,b,c}	1.03 (0.25) ^{a,b,c}	4.4 (1.5) ^{a,b,c}	1.46 (0.36) ^{a,b,c}
80 and older	152	8.4 (3.9) ^{a,b,c,d}	0.82 (0.27) ^{a,b,c,d}	5.8 (2.7) ^{a,b,c,d}	1.17 (0.36) ^{a,b,c,d}
Total	1,078	5.9 (2.4)	1.12 (0.29)	4.1 (1.6)	1.60 (0.42)

^a Significantly different ($p < 0.05$) from values of the age group in their 40s

^b Significantly different ($p < 0.05$) from values of the age group in their 50s

^c Significantly different ($p < 0.05$) from values of the age group in their 60s

^d Significantly different ($p < 0.05$) from values of the age group in their 70s

Table 4 Values of median (25–75 percentile) of one-leg standing time (s, maximum = 60 s) in a better side and a worse side classified by age and gender

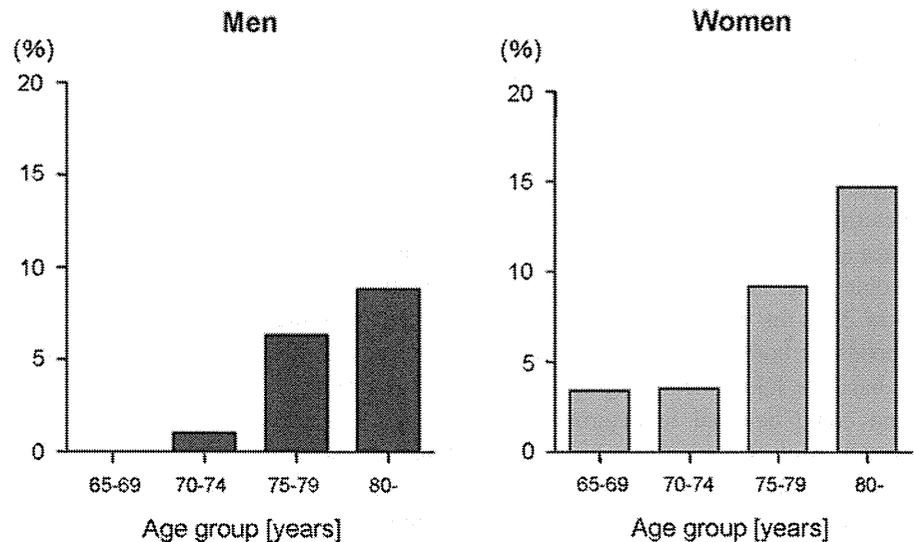
Age strata (years)	Number of subjects	One-leg standing time (better side) (s)	One-leg standing time (worse side) (s)
Men			
40–49	32	60 (60–60)	60 (60–60)
50–59	99	60 (60–60)	60 (60–60)
60–69	136	60 (34.5–60)	45 (14.25–60)
70–79	303	27 (9–60)	9 (4–35)
80 and older	246	8 (4–32)	4 (2–12)
Total	816	39.5 (8–30)	14 (4–60)
Women			
40–49	92	60 (60–60)	60 (60–60)
50–59	191	60 (60–60)	60 (43–60)
60–69	317	60 (41.5–60)	43 (13–60)
70–79	593	21 (8–57.5)	8 (3–25)
80 and older	424	7 (3–18.8)	3 (2–7)
Total	1,617	31 (8–60)	12 (4–60)

pace [+1 s, odds ratio (OR) 1.15, 95% confidential interval (CI) 1.07–1.24, $p < 0.001$] and at the fastest pace (+1 s, OR 1.22, 95% CI 1.08–1.38, $p < 0.01$). In addition, there were significant associations between the presence of disability and walking speed at the usual pace (+1 m/s, OR 0.07, 95% CI 0.02–0.27, $p < 0.001$) and at the fastest pace (+1 m/s, OR 0.16, 95% CI 0.06–0.41, $p < 0.001$).

Discussion

In this study, we established age-gender-classified mean values for hand grip strength as an index of muscle strength, muscle mass as an index of muscle volume, and walking time and median one-leg standing time as indices of physical performance, using data for a large-scale

Fig. 1 Prevalence of disability among subjects ≥ 65 years old classified by gender and age



population-based cohort. We found that mean hand grip strength, muscle mass, walking time, and median one-leg standing time were higher in men than in women, and decreased with age (with the exception of the muscle mass of the lower legs).

The Japanese Ministry of Education, Culture, Sports, Science, and Technology has reported ranges for physical strength and sporting ability in 69,745 Japanese men and women 6–79 years old. Mean hand grip strength in both men and women reaches peak values between the ages of 20–40, and decreases with age after 40 [12]. In the present study of a population aged ≥ 40 years, hand grip strength declined significantly with age, consistent with the previous report of the Japanese government.

Although computed tomography or MRI scans are the most reliable methods of measuring segmental muscle mass, these methods are not suitable for a large-scale population-based study. The BIA method is rapid, inexpensive, portable, and importantly, a noninvasive measuring method. Previous studies have shown that there is a strong correlation between BIA resistance and measurements of skeletal muscle mass in the arms [13], legs [13], and whole body [14]. For the BIA method used in the present study, Miyatani et al. [10] had previously compared values for muscle mass using a series of cross-sectional images of the forearm, upper arm, lower leg, and thigh on the right side of 22 male subjects as determined by the BIA and MRI methods. The BIA impedance index (L^2/Z) for every segment, calculated as the ratio of the segment length squared to the impedance, was significantly correlated with the muscle volume measured by MRI, with $r = 0.902$ – 0.976 ($p < 0.05$). These previous results demonstrate that the BIA method used in the present study is sufficiently reliable as an index of muscle volume.

In the present study, age-related differences were observed in the thighs and quadriceps. In addition, the age-related decreases in muscle mass were greater in the lower limbs than in the upper limbs, and in proximal sites than in distal sites. Yamada et al. [15] estimated the bioelectrical impedance (BI) index, calculated as the ratio of the square of segmental length to impedance in a Japanese population consisting of 1,006 individuals (374 men, 632 women) and reported that the BI index decreased most with age in the thighs, whereas there were no significant age-related changes in the forearms or lower limbs. Miyatani et al. [16] studied muscle thickness at nine sites, the forearm, anterior and posterior upper arm, abdomen, subscapular, anterior and posterior thigh, and anterior and posterior lower leg, using brightness-mode ultrasonography in 348 Japanese men aged 20–79 years. They found a greater decrease in muscle thickness in the trunk and anterior thigh than at other sites, consistent with our results. They speculated that site-related differences in muscle loss with aging may be attributed to age-related changes in the patterns of loading to and/or activation of individual muscles in daily life. However, because these results were obtained from a cross-sectional study, longitudinal data would be required to determine the mechanism of these differences. We have begun the third visit of the ROAD study, 6 years after the baseline and 3 years after the second visit, to measure losses of skeletal muscle mass at various sites. Losses of muscle mass in the quadriceps and/or thighs may result in a decrease in walking ability, including walking speed. Therefore, establishment of reference values for muscle mass is useful for prediction of future disability.

Walking ability is regarded as the most important activity for the elderly to maintain an independent life in the community, and walking speed is an important index of

walking ability. Reference values have been published for populations in western countries [17, 18]; however, there has been little information available for the Japanese population. Takahashi et al. [19] surveyed walking speed at 130 crosswalks and reported that at least 1.0 m/s was required to safely cross the street. In the present study, we determined that the mean 6-m walking time at the usual pace at an age of ≥ 40 years was 5.6 s for men and 5.9 s for women, and the mean 6-m walking time at the fastest pace was 3.7 s in men and 4.1 s in women. These walking speeds for both the usual pace and the fastest pace provide a baseline for clinical judgments of patient performance and could be used to determine which subjects would benefit from therapeutic intervention to improve locomotive function.

The Japanese Ministry of Education, Culture, Sports, Science and Technology published mean values for one-leg standing time with a maximum time of 120 s using 5,500 individuals (2,741 men, 2,759 women) with an age range of 65–79 years in each prefecture who participated in an examination of sporting ability, including walking ability [12]. They reported that mean one-leg standing times for men 65–69, 70–74, and 75–79 years old were 79.9, 66.5, and 50.5 s, respectively, and those for women were 80.8, 62.1, and 45.0 s, respectively. These values were measured up to 120 s, and ours were measured up to 60 s. Because the measuring method was different and their outcomes are means while our results are medians, the results cannot be compared directly. However, one-leg standing time was significantly lower with age in both studies. Again, establishment of reference values for physical performance, including walking and standing ability, would be useful for prediction of future disability.

We then evaluated associations between hand grip strength, muscle mass, walking time, and one-leg standing time, as indices reflecting components of locomotive syndrome and the presence of disability. We found that the 6-m walking time may be a useful index for detection of disability. To evaluate the independence of elderly persons in daily life, physical performance has been measured using various outcomes. Walking speed has been reported to be one important index that can predict future disability, hospitalisation, and mortality in the general geriatric population [20, 21]. In a Japanese population, Shinkai et al. [22] demonstrated that lower scores on baseline performance measures, particularly maximum walking speed, predicted an increased risk of onset of functional dependence, based on their 6-year follow-up of a cohort in a rural community consisting of 736 participants. In the present study, a 1-s slower normal walking time for 6 m was associated with a 15% increase in the presence of disability, and a 1-s slower fastest walking time for 6-m was associated with a 22% increase in the presence of disability. Our study evaluated

only walking ability and the presence of disability, not the occurrence of disability; however, we expect to follow these populations and clarify the predictive ability of walking speed for the occurrence of disability over the next few years.

On the other hand, no associations were found between indices such as hand grip strength, muscle mass, and one-leg standing time and the presence of disability. There is growing evidence that reduced hand grip strength is associated with adverse outcomes in older years, including morbidity, lower quality of life, higher fracture rates, increased length of hospital stay, and mortality [23–25]. Progressive decline in muscle mass has been defined as sarcopenia, which represents an impaired state of health associated with morbidity disorders, increased risk of falls and fractures, impaired ADL, loss of independence, and increased risk of death [6, 26–29]. Lang et al. [29] stated that loss of muscle mass and power increases the difficulties associated with procuring adequate nutrition and the effort required to undertake exercise; the combination of nutritional loss and reduced physical activity levels results in further loss of muscle mass and power. The resulting decrements in power, endurance, and physical performance lead to a loss of independence. In addition to muscle strength and mass, balance appears to be an important index of disability. Shinkai et al. [22] measured the one-leg standing time of 736 participants in a cohort established in a rural community, and the individuals in the lowest performance quartile had a significantly higher occurrence of disability.

Self-selection bias is suggested as a possible reason for the lack of associations between hand grip strength, muscle mass, and one-leg standing time and disability observed here, compared with previous reports. Self-selection bias is one type of sampling bias exhibited by subjects who voluntarily enrol in an epidemiological study. In this second visit of the ROAD study, volunteers who could walk to the clinic where the survey was performed, and could understand and sign an informed consent form, and who wanted to learn about their bone and joint conditions were welcomed. Therefore, the participants in the second survey may have been healthier than the general Japanese population. In fact, the estimated number of persons with disability in Japan using the age-gender prevalence of the second visit and the age-gender distribution of the Japanese population based on the national census in 2007 would be estimated at 1,510,000 (350,000 men, 1,160,000 women), considerably lower than the 4,940,000 reported by the government in 2010. Thus, self-selection bias likely affected the reference values; the reference values for hand grip strength, muscle mass, and one-leg standing time obtained from the present study may be higher, and walking speed faster, than the actual values. However, self-selection bias is somewhat unavoidable in such an

examination, because it is impossible to obtain measurements for individuals who cannot grasp a handgrip dynamometer or walk 6 m. This bias should be taken into consideration when reference values are used, including not only those obtained from the present study, but also from the national survey of physical strength and sporting ability published by the government.

In addition to self-selection bias, this study has several limitations. First, our results were obtained from a cross-sectional study of the second visit of the ROAD study; thus, we can not conclude causal relationships between such indices and disability, since some of the indices, such as muscle mass and one-leg standing time, were first introduced and performed during the second visit. We have begun the third visit of the ROAD study to clarify the relationships between physical performance and the occurrence of disability. Once the significance of indices reflecting components of locomotive syndrome can be determined as predictors for the occurrence of disability, appropriate thresholds can be developed as predictors of future disability. In addition, because of the lack of sufficient information, we could not determine the disorders that caused the disability. Thus, the disabled status of the participants in the present study might have been affected by various diseases such as cardiovascular diseases, dementia, or other diseases. However, regardless of the cause of disability, we found that walking ability was significantly associated with the presence of disability.

Conclusions

We have established reference values for hand grip strength, muscle mass, walking time, and one-leg standing time using data for a large-scale population-based cohort, and identified gender and age differences in the reference values. In addition, we determined that walking ability, including walking time and walking speed at the usual and maximum pace, was significantly associated with the presence of disability.

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Conflict of interest No conflict of interest has been declared by the authors.

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Incidence and Risk Factors for Radiographic Knee Osteoarthritis and Knee Pain in Japanese Men and Women

A Longitudinal Population-Based Cohort Study

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Objective. To examine the incidence and progression of radiographic knee osteoarthritis (OA) and the incidence of knee pain, and their risk factors in Japan, using the large-scale population of the nationwide cohort study ROAD (Research on Osteoarthritis/osteoporosis Against Disability).

Methods. Subjects from the ROAD study who had been recruited in 2005–2007 were followed up with knee radiography 3 years later. A total of 2,262 paired radiographs (74.4% of the original sample) were scored using the Kellgren/Lawrence (K/L) grading system, and the incidence and progression rate of knee OA was

examined. The incidence rate of knee pain was also examined. In addition, risk factors were tested for their association with incident and progressive radiographic knee OA and incident knee pain.

Results. Given the ~3.3-year followup, the rate of incident K/L grade ≥ 2 radiographic knee OA was 6.9% and 11.9% in men and women, respectively, while that of K/L grade ≥ 3 knee OA was 8.4% and 13.9% in men and women, respectively. The rate of progressive knee OA was 17.8% and 22.3% in men and women, respectively. The incident rate of knee pain was 21.2% and 27.3% in men and women, respectively. Female sex was a risk factor for incident K/L grade ≥ 2 knee OA, but was not associated with incident K/L grade ≥ 3 knee OA or progressive knee OA. Knee pain was a risk factor for incident and progressive knee OA. Previous knee injury was a risk factor for knee pain but not for radiographic knee OA.

Conclusion. The present longitudinal study revealed a high incidence of radiographic knee OA in Japan.

Knee osteoarthritis (OA), characterized by pathologic features including joint space narrowing and osteophytosis, is a major public health issue causing chronic pain and disability in the elderly in most developed countries (1). The prevalence of radiographic knee OA is high in Japan (2), with 25,300,000 subjects ages ≥ 40 years estimated to experience radiographic knee OA (3). According to the recent National Livelihood Survey of the Ministry of Health, Labor, and Welfare in Japan, OA is ranked fourth among diseases that cause

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