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C. L. NOBLE*, J. MCCULLOUGH*, W. HO*, C. W. LEES*, E. NIMMO*, H. DRUMMOND*, S. BEAR†, J. HANNAN‡, C. MILLAR‡, S. H. RALSTON† & J. SATSANGI*

*Gastrointestinal Unit and †Rheumatology Unit, Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh, UK; ‡Medical Physics Department, Western General Hospital, Edinburgh, UK

Correspondence to:
Dr C. Noble, Gastrointestinal Unit, Molecular Medicine Centre, Western General Hospital, Edinburgh EH4 2XU, UK.
E-mail: noblecolin@hotmail.com

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SUMMARY

Background
Osteoporosis is a recognized complication of inflammatory bowel disease (IBD).

Aim
To investigate the role of environmental factors and vitamin D receptor (VDR) variants on the prevalence of osteoporosis.

Methods
DEXA scans and case note review were performed on 440 IBD patients from 1997 to 2006. All the IBD patients and 240 healthy controls were genotyped for VDR variants *Taq*-1 and *Apa*-1 using PCR-RFLP.

Results
Osteoporosis and osteopenia rates were 15% and 18% for IBD, 16% and 18% for Crohn's disease (CD) and 13% and 19% for ulcerative colitis, respectively. On univariate analysis of the CD patients, low body mass index (BMI, <18.5) and smoking status ($P = 0.008$ and 0.005 respectively) were associated with osteoporosis and osteopenia. Low BMI was also associated with osteoporosis on multivariate analysis in CD ($P = 0.021$, OR 5.83, CI 1.31–25.94). No difference was observed between *Taq*-1 and *Apa*-1 VDR polymorphisms in IBD, CD, ulcerative colitis and healthy controls. However, CD males were more likely to carry the variant *Taq*-1 polymorphism than healthy controls males ($P = 0.0018$, OR 1.94, CI 1.28–2.92) and female CD patients ($P = 0.0061$, OR 1.60, CI 1.17–2.44).

Conclusions
In this well-phenotyped cohort of IBD patients, a relatively low prevalence of osteoporosis was observed. Low BMI was the only independent risk factor identified to be associated with osteoporosis.

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INTRODUCTION

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are immune-mediated diseases that result in chronic, relapsing inflammation of the gastrointestinal (GI) tract. These diseases occur in genetically susceptible individuals who are exposed to as yet poorly defined environmental stimuli.[1, 2]

Osteoporosis is defined by the World Health Organisation (WHO) as: 'A disease characterized by low bone mass and micro architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk.'[3] The estimated prevalence of osteoporosis in the IBD population ranges widely from 13% to greater than 50%.[4–7] This complication has important implications in terms of morbidity and population-based studies from Nottingham, UK have shown a 60% increased risk of fracture in IBD patients.[5]

The aetiology of low bone mineral density (BMD) in IBD is multifactorial. Risk factors include low body mass index (BMI).[4, 8, 9] Recent data from England are pertinent. Bartram *et al.* in Newcastle observed that CD patients with osteoporosis had a mean BMI of 22 compared with those without osteoporosis where the BMI was 25 ($P < 0.0001$).[4] A number of studies have shown that corticosteroid use is also a risk factor for the development of osteoporosis in patients with IBD[6, 7, 10, 11] and other factors that include smoking, hypogonadism, increased secretion of osteoclast stimulating cytokines, calcium and vitamin D deficiency, disease location and duration have been implicated.[4, 7–14]

Twin studies have suggested that up to 80% of a patient's BMD is genetically determined.[15] In view of its chromosomal location and function, there has been sustained interest in the contribution of germline variation of the vitamin D receptor (VDR) gene. The VDR gene is located within the IBD2 susceptibility locus on chromosome 12 and it spans at least 105 kb.[16–18] It encodes a steroid receptor that mediates the effects of $1,12(OH)_2$ vitamin D_3 by regulating the transcription of cellular genes.[19] The gene plays a well-described role in skeletal metabolism but has also been shown to have immunomodulatory effects as well as possible roles in the growth of cancer cells and in insulin secretion.[20]

The index study implicating VDR polymorphisms in the regulation of bone density published in *Nature* in 1994 suggested that the *Bsm*-1 variant of the VDR gene in intron 8 conferred 75% of the genetic variability of BMD; however, subsequently genotyping errors were identified and the results of this study were modified.[21] Two other VDR polymorphisms, *Apa*-1 and *Taq*-1 were found to be in strong linkage disequilibrium with the original *Bsm*-1 variant and the *Bsm*-1–*Apa*-1–*Taq*-1 haplotype was shown to have a moderate association with osteoporosis.[22, 23]

More recently, the contribution of the VDR gene was studied in comprehensive detail by Fang *et al.* who observed a modest association between promoter variation and 3′ untranslated haplotypes and fracture risk.[16] These data contrast with data from a large participant level meta-analysis of European patients, where no association was observed between low BMD and any of the VDR variants examined (*Cdx*-2, *Fok*-1, *Bsm*-1, *Apa*-1, *Taq*-1).[24]

In a cohort of 245 CD patients from Newcastle, UK, no association was observed between VDR variants *Fok*-1 and *Taq*-1 and low BMD[25]; however, in a further cohort of CD patients from Oxford, UK, there were more VDR, *Taq*-1 homozygotes in the CD cohort when compared with a healthy control population.[26]

The aims of the present study were to investigate the prevalence of osteoporosis in our IBD cohort, and to evaluate the contribution of specific environmental and genetic factors on the development of osteoporosis in this IBD population.

METHODS

Patients and controls

Four hundred and forty IBD patients attended the Western General Hospital, Edinburgh and had demographic data collected and DNA stored as part of the IBD database. All the patients selected had a confirmed diagnosis of IBD, using the Lennard–Jones criteria.[27] All had undergone at least one DEXA scan. Patients under the age of 20 at the time of the DEXA scan were excluded. For each patient, the following data were generated: age, gender, age at diagnoses, disease duration (in years), BMI, azathioprine therapy, infliximab therapy, smoking status, family history, surgery, the location of disease at diagnosis and behaviour of disease at 5 years follow-up using Montreal classification.[28] Two hundred and eighty-six CD patients and 154 UC patients were recruited (Table 1).

Table 1. Demographics of the Crohn's disease (CD), ulcerative colitis (UC) and control cohorts

	CD (n = 286)	UC (n = 154)	Controls (n = 240)
Gender (M:F)	107:179	74:79	118:122
Age at diagnosis (years)	31.9	39.7	40.2
Disease duration (years)	15.7	11.8	
Montreal location at diagnosis (CD)			
L1 (terminal ileum)	89		
L2 (colonic)	94		
L3 (ileo-colonic)	57		
L4 (upper GI, proximal to terminal ileum)	8		
L1 + L4	13		
L2 + L4	3		
L3 + L4	10		
Oral Only	3		
Montreal behaviour at 5 years			
B1 (inflammatory)	140		
B2 (stricturing)	43		
B3 (penetrating)	42		
B1P (inflammatory + perianal)	28		
B2P (structuring + perianal)	3		
B3P (penetrating + perianal)	7		
Montreal at diagnosis (UC)			
E1 (proctitis)		38	
E2 (distal to splenic flexure)		61	
E3 (extensive)		33	
Current or ex-smoker	170	78	
Never smoked	114	76	
Normal (BMI = 18.5–24.9)	150	66	
Underweight (BMI < 18.5)	9	1	
Overweight (BMI > 24.9)	112	73	
Surgery			
Yes	166	20	
No	117	134	
Azathioprine treated			
Yes	140	87	
No	120	61	
Infliximab treated			
Yes	44	4	
No	240	150	

Full demographic data were available on 91% of the CD patients and 85% of the UC patients.

In all 45 osteoporotic CD patients and an age- and gender-matched group of 45 non-osteoporotic CD patients, a case note review was undertaken to calculate the number of months each patient had spent on corticosteroids. For the genetic study, the healthy control population comprised 240 individuals recruited from the Blood Transfusion Donor register and healthy staff of the GI department of the WGH, all without GI pathology. Informed consent was obtained from all patients and controls – Lothian Local Research Ethics Committee LREC 2000/4/192.

Bone mineral density measurement

DEXA scans were performed between 1997 and 2006 using a Hologic QDR4500A machine (Syngene, Cambridge, UK) at the left hip, left femoral neck and the lumbar spine on an anterior projection. The T-score at the lumbar spine was selected for analysis as tight correlation was observed between T-scores from the femoral neck and lumbar spine-median T-score at the hip −0.80, median T-score at the lumbar spine −0.94, r^2 = 0.410, $P < 0.001$. Using the WHO criteria, osteoporosis

was defined as a T-score of below −2.5 and osteopenia when the T-score was between −1.0 and −2.5.[3]

Vitamin D receptor genotyping

Genomic DNA was extracted from peripheral venous blood by a modified salting-out technique, and re-suspended in 1× TE [10 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0)] at a final concentration of 100 ng/μL.[29] VDR *Taq*-1 (rs17880019) and *Apa*-1 (rs17879735) genotyping was carried out using restriction digestion polymerase chain reaction (PCR). The primers used were forward 5′-CAGAGCATGGACAGGGAGCAA-3′, back 5′-CACTTCGAGCACAAGGGGCGTTAG-3′. PCR conditions were as follows – initial denaturation at 94 °C for 4 min followed by 33 cycles of (94 °C for 50 s, 64 °C for 60 s, followed by 72 °C for 90 s) and a final extension at 72 °C for 8 min and 30 s. Ten microlitres of PCR product was then added to 10 μL of *Taq*-1 or *Apa*-1 digest mixture and incubated at 65 °C overnight. The digestion products were then separated on 1.5% agarose gel with ethidium bromide. The gel was visualized on Genegenius Bioimager (Syngene) with GENESNAP software using ultraviolet light. The images were recorded digitally.

Statistical analysis

Data were compared using the chi-squared test or when appropriate Fisher's exact test using MINITAB software (Minitab Ltd, Coventry, UK). The Mann–Whitney *U*-test was used to compare nonparametric BMD data. To assess differences between steroid dosages in the osteoporotic and non-osteoporotic groups, an unpaired *t*-test with Welch correction was used. To identify significant independent variables associated with phenotype, multivariate logistic regression analysis was carried out. Each single nucleotide polymorphism (SNP) was analysed for association with IBD overall, CD, UC and disease phenotype and allele frequencies were determined for each polymorphism. In the control group, each allele was in Hardy–Weinberg equilibrium. HAPLOVIEW software was used to estimate haplotypes (Broad Institute of MIT and Harvard, Boston, MA, USA). A $P < 0.05$ was considered significant.

RESULTS

Prevalence of osteoporosis and osteopenia

When all of the 440 IBD patients were investigated, 15% were osteoporotic, 18% had osteopenia and 67% had normal T-scores at their lumbar spine (Figure 1). In the CD patients, 16% were osteoporotic, 18% were osteopenic and 66% had normal T-scores at their lumbar spine and in the UC patients, 13% were osteoporotic, 19% were osteopenic and 68% had a normal T-score.

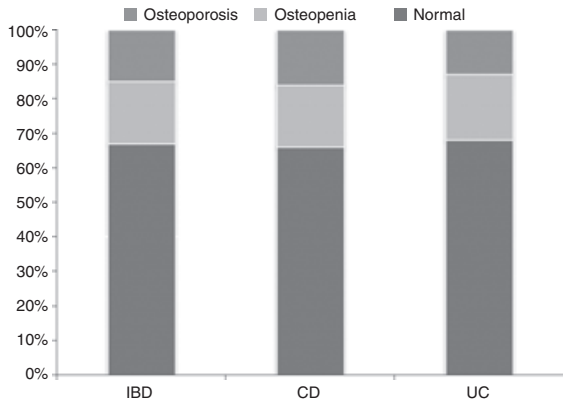

Figure 1. Low prevalence rates of osteoporosis and osteopenia were observed in patients with inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC).

Risk factors for reduced BMD

In CD patients, osteoporosis was associated with low BMI (<18.5) – 44% of the low BMI patients had osteoporosis compared with 14% of the patients with a normal BMI who had osteoporosis, $P = 0.048$, OR 4.9 CI 1.2–19.8. A linear correlation between T-score at the vertebral spine and BMI was observed in patients with CD ($r^2 = 0.034$, $P = 0.0009$) (Figure 2). When BMD was compared with BMI, again a linear correlation was observed ($P = 0.0362$).

Further analysis was carried out to include CD patients at increased risk of fracture – those with osteoporosis and osteopenia. Low BMI ($P = 0.0008$) and history of being a current or ex-smoker ($P = 0.005$) were associated with osteoporosis and osteopenia (Table 2). No association was observed between the number of months a CD patient was on corticosteroid therapy and osteoporosis. Osteoporotic CD patients had a median of 30.4 months of corticosteroid therapy and the non-osteoporotic CD patients had a median of 29.5 months of corticosteroid therapy ($P = 0.92$). No phenotypic associations were observed with osteoporosis in the UC cohort.

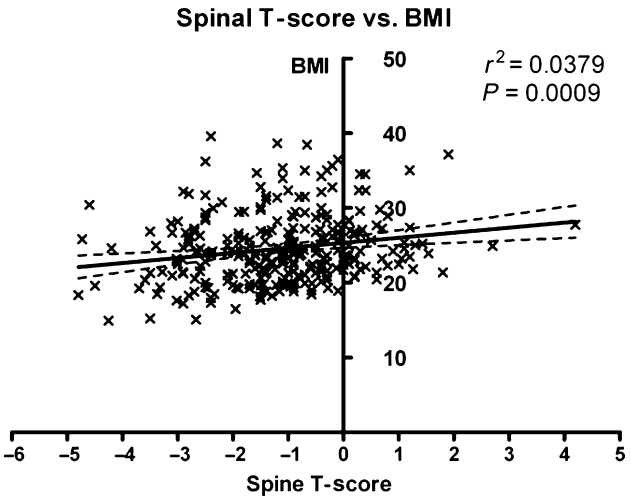

Figure 2. Body mass index (BMI) plotted against T-scores at the lumbar spine of patients with Crohn's disease. A linear correlation was observed between T-scores at the vertebral spine and BMI in patients with Crohn's disease (n = 286).

Logistic regression analysis of the CD patients was performed using a model that considered BMI groups, smoking status, Montreal location, family history, surgery, infliximab treatment, azathioprine treatment, gender and Montreal behaviour at 5 years follow-up, with the outcome being osteoporosis in patients with CD. Low BMI (<18.5) was independently associated with osteoporosis (P = 0.021, OR 5.83, CI 1.31–25.94). Using this model, the percentage variance of osteoporosis explained by BMI was 8.41%, being a current or ex-smoker was 1.63%, female gender was 0.92%, ileal disease location was 0.87%, exposure to azathioprine was 0.52% and exposure to infliximab therapy was 0.42%. When cumulative corticosteroid doses were added to the analysis, increased corticosteroid intake contributed 0.4% to the variance of BMD.

Genotype results

When allelic frequencies of the VDR *Apa*-1 variant were examined, there was no difference among patients with IBD (45.8%), CD (45.3%), UC (46.7%) and the control group (46.6%) (P > 0.7) (Table 3). When the CD patients were split into those with and without osteoporosis, there was also no difference in allelic frequencies of the *Apa*-1 variant (45.7% osteoporosis

Table 2. Analysis of the effect of phenotypic variables on the prevalence of osteoporosis and osteopenia in Crohn's disease (CD) patients

Phenotype	Total n	Osteoporosis and osteopenia % (n)	P-value	OR (CI)
BMI				
Underweight BMI < 18.5/normal	9/150	88.9 (8)/33.3 (50)	0.0008	16 (2–93)
Azathioprine treated				
Yes/no	136/119	34.6 (47)/34.5 (41)	0.99	1.0 (0.5–1.8)
Infliximab treated				
Yes/no	44/240	45.5 (20)/32.1 (77)	0.086	1.8 (0.9–3.4)
Family history*				
Yes/no	55/229	32.7 (18)/34.5 (79)	0.80	0.9 (0.5–1.7)
Surgery†				
Yes/no	166/117	37.3 (62)/29.9 (35)	0.19	1.4 (0.8–2.3)
Gender				
Male/female	107/178	39.6 (42)/30.3 (54)	0.11	1.5 (0.9–2.5)
Smoking status				
Current and ex/never	170/114	41.3 (69)/21.9(28)	0.005	2.1 (1.2–3.6)
Montreal location				
L1 and L4/ L2	100/94	33 (33)/34 (32)	0.88	1.0 (0.5–1.7)
L1/L2	89/94	29.2(26)/36.2 (34)	0.32	0.7 (0.4–1.4)
Montreal behaviour at 5 years				
B1/B2 and B3	140/85	32.1(45)/34.1 (29)	0.70	0.9 (0.5–1.6)

BMI, body mass index.
* Having one or more first degree relative with CD or UC.
† Surgery due to intra-luminal complication of CD or UC.

Table 3. Allelic frequencies of vitamin D receptor (VDR) variants *Apa*-1 (rs17879735) and *Taq*-1 (rs17880019) in the control and inflammatory bowel disease (IBD) populations

	Control	IBD	UC	CD	Osteoporotic CD
Apa-1	46.6%	45.8% (P = 0.8, CI 0.8–1.2)	46.7% (P = 1.0, CI 0.74–1.34)	45.3% (P = 0.7, CI 0.82–1.35)	42.9% (P = 0.6, CI 0.73–1.86)
Taq-1	37.6%	40.1% (P = 0.4, CI 0.7–1.1)	41.2% (P = 0.35, CI 0.6–1.16)	39.6% (P = 0.52, CI 0.7–1.18)	44% (P = 0.27, CI 0.5–1.22)
Taq-1 males	34.2%	47.8% (P = 0.003, CI 1.3–2.5)	45.6% (P = 0.029, CI 1.1–2.5)	49.4% (P = 0.0017, CI 1.3–2.8)	42.5% (P = 0.3, CI 0.7–2.8)
Taq-1 females	41%	36.8% (P = 0.27, CI 0.6–1.2)	37.0% (P = 0.4, CI 0.6–1.3)	36.7% (P = 0.3, CI 0.6–1.2) *(P = 0.006, CI 1.2–2.4)	45.5% (P = 0.6, CI 0.6–2.3)

Allelic frequencies of VDR *Apa*-1 (G → T) and *Taq*-1 (T → C) variants in IBD, ulcerative colitis (UC) and Crohn's disease (CD) populations overall and separated by gender. P-values and confidence intervals have been calculated by comparing each of the disease groups against the respective control population.
* In the CD population allelic frequency of *Taq*-1 was compared in the male and female CD patients.

compared with 42.9% non-osteoporosis, P = 0.72). When the controls and patients were analysed by gender, there was no significant difference among all of the disease groups.

There was no association among the allelic frequencies of the VDR *Taq*-1 SNP and IBD (40.1%), CD (39.6%), UC (41.2%) and control populations (37.6%) (P > 0.35), nor with osteoporosis in the CD cohort (44% osteoporotic compared with 38.7% non-osteoporotic, P = 0.40). However, when allelic frequencies of *Taq*-1 variants were examined in males and females in the CD patients, there was a higher frequency of variants in the male CD population (49.4% vs. 36.7%, P = 0.0061, OR 1.69, CI 1.2–2.4). There were also more *Taq*-1 variants in the male IBD, UC and CD population compared with the male controls 34.2% healthy controls, 47.8% IBD (P = 0.003), 45.6% UC (P = 0.029) and 49.4% CD (P = 0.0017). No genotypic or VDR *Apa*-1–*Taq*-1 haplotype associations were observed and no phenotypic associations were observed.

DISCUSSION

Our retrospective data show that in this well-phenotyped cohort of IBD patients, relatively low levels of osteoporosis and osteopenia were observed. Low BMI was a robust predictor of osteoporosis. VDR variants were not associated with osteoporosis; however, intriguing gender-specific differences were observed.

The prevalence of osteoporosis in our CD (16%) and UC (13%) population is in line with recent CD data published from Newcastle, UK, where 11.6% of CD patients were osteoporotic at either the lumbar spine or femoral neck.[4] The data are also comparable to data from London, wherein patients with CD and UC the incidence of osteoporosis of the vertebrae and the hip was between 17% and 28%.[30] There are, however, studies from Israel where 42% of the patients had osteoporosis[14] and Cardiff where 31% of IBD patients were osteoporotic.[6]

Patient selection bias may have a part to play, as access to DEXA scanning varies considerably amongst gastroenterologists. Our cohort of patients represent essentially an unselected group of IBD patients from the Edinburgh region. Our access to DEXA scans is good, allowing physicians to check bone density in the majority of IBD patients. In other centres, with more limited resources, patients need to be prioritized on the basis of clinical suspicion, and of disease

severity and this may explain why the osteoporosis levels are lower than those reported in this series.

In the same context, it is noteworthy that DEXA scans in our cohort were performed closer to the date of diagnosis compared with previous studies. Thirty-two per cent of patients had their DEXA scan within 2 years of diagnosis and the median duration to the first DEXA was 6 years. As the median age of diagnosis of IBD was 29.5 years, this cohort also represents a younger population of IBD patients compared with previous series. Our series also includes substantially more patients than previous studies and this may result in a more accurate estimation of the prevalence of osteoporosis.

Low BMI was the strongest risk factor for osteoporosis in our CD population, consistent with data from a number of previous studies.[4, 6, 8, 9] Despite only 3% of patients having a BMI of less than 18.5, this was a significant risk factor when logistic regression analysis was undertaken. Using a multivariate model, variance in BMI accounted for approximately 8.4% of the variance in BMD, a percentage lower than that suggested by previous studies.[4] However, it is worth noting that overall only 13% of the variance in BMD was explained by the environmental factors we have studied (including corticosteroid dose). These data would suggest that, in our cohort, there are either other unidentified critical environmental modifiers, or a critically important genetic component involved in determining BMD.

Increasing data are emerging both in patients with IBD and in the healthy population that low BMI is a significant independent risk factor for osteoporosis. In a recent study of postmenopausal women in the USA, Asomaning *et al.* observed a significant linear trend across BMI categories with those with the lowest BMI having the lowest BMD.[31] The authors went on to evaluate BMI as a continuous variable and observed a decrease of 12% in BMD for each point decrease in BMI. Low BMI has also been observed to predict risk of hip fracture even after adjustment for BMD. In a meta-analysis of 60 000 patients from 11 prospective studies, the relative risk of fracture rose from 1.4 in females with a BMI of 20 to 2.2 in females with a BMI of 15.[32]

In this study, being a current or ex-smoker was also associated with osteoporosis and osteopenia on univariate analysis. The mechanisms underlying smoking-associated bone loss and fracture risk remain poorly understood and previous studies have suggested that the effect of smoking appears to be dose-dependent, and may be reversible.[33] Smoking was not an independent risk factor in the multivariate analysis, however, suggesting that it plays a smaller role than low BMI in determining BMD in the present cohort.

No association was observed between corticosteroid use and osteoporosis when the osteoporotic CD patients were compared with a matched non-osteoporotic CD group. Although the role of corticosteroids in the development of osteoporosis in patients with IBD remains controversial, this result is in line with previous data published from our centre in 1994 where low bone mineralization was observed in patients with CD at diagnosis and prior to any corticosteroid therapy.[34] Collating cumulative steroid exposure retrospectively by case notes analysis is difficult. Our design has allowed us to address this question to a similar extent to previous studies, but we acknowledge this as a potential limitation of the present study and we feel strongly that further prospective studies are required. In this study, we also did not collate data on the use of bisphosphonates; however, we sought to minimize this potential confounding factor by studying a young cohort and using the index DEXA scan for analysis.

When the VDR variants *Apa*-1 and *Taq*-1 were examined in our cohort of IBD patients, no association was observed between osteoporosis and these variants. Our results are consistent with data from Newcastle where in patients with CD no association was observed between VDR variants *Taq*-1 and *Fok*-1 and osteoporosis.[25] Results from both Newcastle and our data are also consistent with a population based European meta-analysis of 26 242 patients where no association was observed between any of the five VDR variants that were examined and low BMD.[24]

Although Fang *et al.* did not observe an association between VDR variants and low BMD, they did observe an association with fracture risk and the authors went on to provide functional data showing reduced VDR expression in variant reporter assays and increased mRNA degradation.[16] They further speculated that these variants may alter fracture risk by modifying bone micro-architecture rather than influencing BMD. Fracture incidences were not measured in this study and it may be that new imaging techniques will allow investigators to assess better bone micro-architecture rather than BMD alone to determine the fracture risk better.

Interestingly in our sub-group analysis, we observed that the *Taq*-1 VDR variant was more prevalent in the male CD population compared with

the female CD population and also in the male IBD, CD and UC patients compared with the male controls. The strongest signal in the data came from the male CD population and whilst the confounding factor of multiple comparisons must be taken into consideration when undertaking genotype–phenotype analysis, our data are internally consistent across the disease groups that were examined. The *Taq*-1 polymorphism represents a synonymous T–C base substitution at codon 352 in exon 8 of the VDR gene and the index study by Morrison *et al*. suggested that *in vitro* the risk haplotype may be associated with increased gene transcription.[21] Data from Oxford showed more *Taq*-1 homozygotes in the CD population compared with controls; however, gender-specific data were not provided.[26] Previous IBD genetic studies using gender stratification have found male-specific linkage to the human leukocyte antigen (HLA) region of chromosome 6 and no association with the IBD2 locus.[35] Although the numbers in both studies are small, the trends in the data sets are the same and our own detailed studies of the IBD2 region involving 1400 SNPs and 2024 IBD patients may help resolve this issue.

In conclusion, our results show relatively low prevalence of osteoporosis in a large cohort of Scottish patients with IBD. Low BMI was the strongest risk factor associated with osteoporosis. Longitudinal follow-up studies to assess the benefits of nutritional intervention in these patients, on BMI, bone density and fracture risk will be of great interest.

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