

# Allelic variation in the vitamin D receptor, lifestyle factors and lumbar spinal degenerative disease

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## Abstract

**Objective**—To describe the relation between spinal degenerative disease, allelic variation in the vitamin D receptor gene, and lifestyle factors in a population-based association study.

**Methods**—Random population-based sample of 110 men and 172 women over 60 years of age participating in the Dubbo Osteoporosis Epidemiology Study who had spinal radiographs (performed according to a standardised approach), assessment of lifestyle factors, bone densitometry as well as blood taken for genotyping.

**Results**—Spinal degenerative disease of varying severity was common in this sample. Multivariate analysis of genetic and lifestyle factors simultaneously strengthened the statistical significance of each indicating the presence of additive gene environment interaction. Allelic variation in the vitamin D receptor gene was associated with severity of osteophytosis (adjusted OR "TT" v "tt" 0.41, 95% CI 0.17, 0.97), presence of disc narrowing (adjusted OR "TT" v "tt" 0.45, 95% CI 0.20, 0.99) and weakly with presence of osteophytosis (adjusted OR "TT" v "tt" 0.47, 95% CI 0.19, 1.16) but not with severity of disc narrowing (OR "TT" v "tt" 1.05, 95% CI 0.40, 2.72) or apophyseal arthritis (OR "TT" v "tt" 0.63, 95% CI 0.24, 1.59). Adjustment for femoral neck bone density did not change these findings suggesting that the association is not mediated through bone density. Presence and severity of spinal degenerative disease increased with age at all sites. Current smoking increased both the presence (adjusted OR 9.70, 95% CI 2.08, 45.1) and severity (adjusted OR 2.91, 95% CI 1.16, 9.03) of spinal osteophytosis with intermediate values for past smokers. Severity of osteophytosis was also independently associated with body mass index and quadriceps strength consistent with a contributory effect of physical loading.

**Conclusions**—In this elderly sample, both genetic and lifestyle factors were associated with the presence and severity of spinal degenerative disease. There were site specific differences in associations at the spine, which may be because of misclassification of disease status or may indicate possible environmental and genetic differences in the pathophysiology of spinal degenerative disease. Further studies are required to confirm these findings

**in different population samples and to further explore potential aetiological mechanisms particularly gene environment interaction.**

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Osteoarthritis (OA) is a leading cause of morbidity in Western society. Both environmental and genetic factors seem to be important in the aetiology of this heterogeneous condition. Studies have suggested that obesity,<sup>1</sup> trauma,<sup>2</sup> and past surgery<sup>3</sup> have been associated with site specific OA. Family and twin studies indicate that certain patterns (Heberdens disease,<sup>4</sup> knee,<sup>4</sup> and generalised OA<sup>6</sup> as well as lumbar disc degeneration<sup>7</sup>) have a strong familial component with approximately 50% of the variance in the twin model being caused by genetic factors.

A number of candidate genes have been implicated in rarer forms of OA. These include the gene for type II collagen (COL2A1) in those with generalised OA secondary to chondrodysplasia.<sup>8,9</sup> Information on gene linkage to the more common forms of OA is limited. Recently two loci on chromosome 2q were linked with nodal OA in sib pairs.<sup>10</sup> A further possibility is the vitamin D receptor allele (VDR). Polymorphisms in this gene have been previously associated with bone mass although its exact significance remains controversial.<sup>11,12</sup> Given that an inverse association between osteoporosis and OA has been proposed<sup>13</sup> it was reasonable to hypothesise that allelic variation in this gene may also be associated with OA. Recent studies have suggested that this is the case for radiographic OA of the knees particularly severity of osteophytosis and possibly joint space narrowing<sup>14,15</sup> but there are no data at other sites that would appear necessary given the site specific variations in the aetiology of OA. Furthermore, as indicated above, lifestyle factors also seem equally important in the genesis of OA. Such factors may interact with genetic factors but few studies have considered the effect of both simultaneously. Therefore, a randomly selected population based sample of elderly men and women, who had previously been assessed for the presence and severity of spinal degenerative disease, were studied to determine if VDR genotype or lifestyle factors, or both, were associated with spinal degenerative disease and whether there was gene environment interaction.

## Methods

The city of Dubbo has a population of approximately 32 000 people and is situated 400 km north west of Sydney, Australia. This

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Table 1 Demographic details of study subjects

| Study factor                         | Men<br>(n=110) | Women<br>(n=172) |
|--------------------------------------|----------------|------------------|
| Age (y)                              | 69.3 (6.4)     | 69.6 (6.4)       |
| Body mass index (kg/m <sup>2</sup> ) | 25.8 (3.8)     | 26.0 (4.2)       |
| Current smokers (%)                  | 8              | 15               |
| Former smokers (%)                   | 30             | 26               |
| <i>Spinal degenerative disease</i>   |                |                  |
| Osteophytes (%)                      | 71             | 67               |
| Disc narrowing (%)                   | 55             | 52               |
| Apophyseal osteoarthritis (%)        | 100            | 99               |
| <i>Genotype frequency (n(%))</i>     |                |                  |
| tt                                   | 17 (16)        | 20 (12)          |
| Tt                                   | 46 (42)        | 89 (52)          |
| TT                                   | 47 (43)        | 63 (36)          |

community included approximately 1600 men and 2100 women aged 60 years or over (as at 1 January 1989) who were 98.6% white. It is well suited to epidemiological research being relatively isolated with its own centralised health services. The Dubbo Osteoporosis Epidemiology Study (DOES) started in 1989, and 2136 subjects out of an initial total target Dubbo population of 3860 (55%) have participated. After allowing for an excess of deaths in the non-participants, the participation rate as at 1 January 1994 (the time of recruitment of these subjects) was 58%.

This study examined the relation between spinal degenerative disease and allelic variation in the vitamin D receptor allele in subjects who

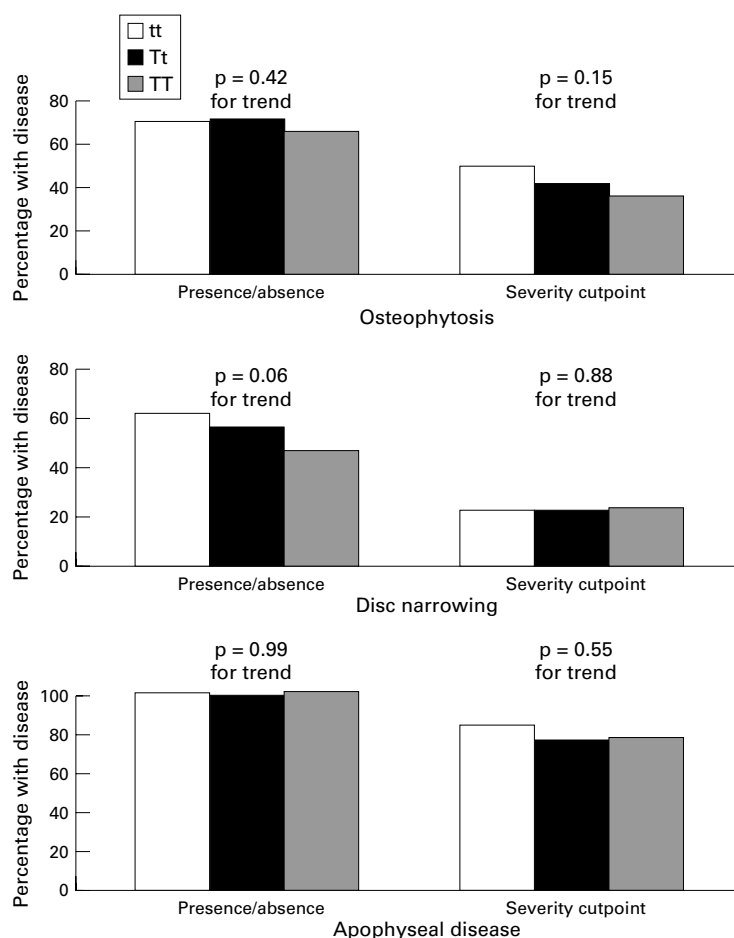


Figure 1 Allelic variation in the vitamin D receptor gene and spinal degenerative disease. For most categories there is a trend for decreasing presence and severity of spinal degenerative disease. However, this was not statistically significant at any site for the unadjusted data.

were randomly selected from the total cohort by computer generated random numbers for a spinal radiograph (n=300) and had venepuncture performed at a later date (n=282).

Lateral radiographs, performed by a standardised procedure with a target to film distance of 105 cm, were read in a blinded fashion by one of us (GJ) for the presence and severity of spinal degenerative disease. Radiographs were assessed at L1–L4 for osteophytes, disc narrowing, and posterior element changes. Osteophytes were scored using previously published criteria<sup>16</sup> as follows: 0, no osteophytes; 1, small osteophytes at one or two vertebral interspaces; 2, large osteophytes at one or two vertebral interspaces or small osteophytes at three or four vertebral interspaces and; 3, large osteophytes at three or four interspaces.

Thus a score could range from 0–3. Intraobserver variation was good ( $\kappa=0.71$ , 95%CI 0.57, 0.85). Disc and posterior element scores were assessed using our previously published criteria<sup>17</sup> as follows:

Disc (for each level of lumbar spine): 0, no narrowing; 0.5 <20% narrowing; 1 > 20% narrowing. Normal scores were obtained by comparison with disc spaces above and below. If all were clearly abnormal, they were compared visually with disc spaces from a same sex sample of unequivocally normal subjects. Total score could vary from 0–4. Intraobserver variation was acceptable ( $\kappa=0.46$ , 95%CI 0.32, 0.60).

Posterior elements (for L1–L4): 0, no involvement; 0.5, mild sclerosis; 1, moderate/severe sclerosis were assessed on the standard lateral view. Total score could vary from 0–4. Intraobserver variation was poor but better than chance alone ( $\kappa=0.29$ , 95%CI 0.13, 0.45).

Lifestyle factors were also assessed as previously published.<sup>18</sup> These included age, body mass index, smoking (current/ past/ never), medication use, dietary calcium, co-morbid illness, quadriceps strength, and postural instability. Bone density was measured using the technique of dual energy x ray absorptiometry using one Lunar DPX-L densitometer (Lunar Corporation, Madison, Wisconsin, USA). All scans were analysed using software program 3.6. Reproducibility, checked at fortnightly intervals by an aluminium spine phantom, had a longitudinal coefficient of variation of 1.6%. The coefficient of variation of bone density at our institution for normal subjects is 1.5% for the lumbar spine and 1.3% for the femoral neck.<sup>19</sup>

VDR genotype was determined by PCR amplification and Taq-1 restriction endonuclease digestion of a 740 bp fragment from genomic DNA isolated from peripheral blood leucocytes using a forward primer in intron 8 and a reverse primer in exon 9 as previously described.<sup>20</sup> The Taq-1 polymorphism is located within 1.1 kb 3' of the polymorphic Bsm-1 site and is in strong linkage disequilibrium with the previously reported Bsm-1 polymorphism such that there is up to 97% concordance of genotype in the Sydney population. Thirty six samples from the Dubbo population were analysed for the Bsm-1

Table 2 Univariate and multivariate associations between genotype, lifestyle factors, and spinal degenerative disease

|                               | Univariate             | Multivariate*          |
|-------------------------------|------------------------|------------------------|
|                               | Odds ratio<br>(95% CI) | Odds ratio<br>(95% CI) |
| <b>A Osteophytosis</b>        |                        |                        |
| <i>Presence/absence</i>       |                        |                        |
| Age (y)                       | 1.04 (1.00, 1.09)      | 1.08 (1.03, 1.14)      |
| Current smoking<br>(Y/N)      | 6.16 (1.75, 21.3)      | 9.70 (2.08, 45.1)      |
| Past smoking (Y/N)            | 1.59 (0.87, 2.89)      | 1.43 (0.74, 3.06)      |
| Genotype                      | 0.73 (0.34, 1.58)      | 0.47 (0.19, 1.16)      |
| <i>Severity cutpoint</i>      |                        |                        |
| Age (y)                       | 1.07 (1.03, 1.11)      | 1.10 (1.05, 1.16)      |
| Current smoking<br>(Y/N)      | 2.27 (1.06, 4.85)      | 2.91 (1.16, 9.03)      |
| Past smoking (Y/N)            | 1.77 (1.01, 3.10)      | 1.63 (0.85, 3.06)      |
| BMI (kg/m <sup>2</sup> )      | 1.06 (1.00, 1.13)      | 1.09 (1.01, 1.17)      |
| Quadriceps strength<br>(kg)   | 1.01 (0.98, 1.04)      | 1.03 (1.00, 1.06)      |
| Genotype                      | 0.59 (0.29, 1.13)      | 0.41 (0.17, 0.97)      |
| <b>B Disc narrowing</b>       |                        |                        |
| <i>Presence/absence</i>       |                        |                        |
| Age (y)                       | 1.07 (1.03, 1.12)      | 1.06 (1.02, 1.11)      |
| Genotype                      | 0.50 (0.25, 1.03)      | 0.45 (0.20, 0.99)      |
| <i>Severity cutpoint</i>      |                        |                        |
| Age (y)                       | 1.07 (1.03, 1.12)      | 1.06 (1.01, 1.12)      |
| Genotype                      | 1.06 (0.45, 2.48)      | 1.05 (0.40, 2.72)      |
| <b>C Apophyseal arthritis</b> |                        |                        |
| <i>Severity cutpoint</i>      |                        |                        |
| Age (y)                       | 1.07 (1.02, 1.13)      | 1.10 (1.04, 1.17)      |
| Genotype                      | 0.78 (0.33, 1.82)      | 0.63 (0.20, 1.59)      |

\*Adjusted for age, sex, body mass index, quadriceps strength, current smoking, and past smoking.

polymorphism by Southern blot and found to be in 100% concordance with the Taq-1 polymorphism. Because of this strong linkage and the presence of an internal control in the Taq-1 RFLP, only the Taq-1 RFLP was determined in the present study population. Presence (t) and absence (T) of the polymorphic Taq-1 restriction endonuclease site are in linkage with the absence (B) and presence (b) respectively of the polymorphic Bsm-I site.

#### STATISTICAL METHODS

Prevalence of the different categories of spinal degenerative disease as well as severity was analysed by genotype for two cutpoints; presence versus absence of each type and moderate/severe disease versus mild/absent disease. The cutpoints for this were as follows: osteophytes (score >1 versus <1), disc narrowing and posterior element scores (score >2 versus <2). Univariate and multivariate logistic regression analysis was used to examine the association between the different categories of spinal degenerative disease, genotype and the various lifestyle factors as well as additive interaction. Examining the raw data suggested that a linear relation was most appropriate. Therefore, ranked ordinal variables were allotted to genotype as follows "tt"-0, "Tt"-0.5, "TT"-1.0. Variables were included in the final model if the p value was less than 0.10 or they were the stated study factors. Final results are listed with 95% confidence intervals. No correction was made for multiple comparisons. All statistical calculations were carried out using SPSS version 6.1 for Windows.

#### Results

Of the original 300 subjects, 282 (94%) were available and willing to have blood drawn for genotyping (table 1 shows the demographic

details of the patients and genotype frequencies). There were 110 men and 182 women of similar mean age. More men than women had been smokers but fewer were current smokers possibly representing a survivor bias. The different categories of spinal degenerative disease had similar prevalence in both men and women. Apophyseal arthritis was present in virtually all subjects, osteophytes were present in approximately 70% with more than half showing some evidence of disc narrowing. The vitamin D receptor allele frequencies of "T" 63% and "t" 37% were comparable in men and women and similar to previously reported frequencies.

Figure 1 gives the prevalence of the various categories of spinal degenerative disease by presence, severity partition, and genotype. In general, osteophytosis was progressively more common and severe in the "tt" than in the "Tt" and "TT" subjects. There was a similar relation for the presence, but not the severity, of disc degeneration. Apophyseal involvement was present in all subjects with equal prevalence and a non-statistically significant trend to decreasing severity for "TT" versus "tt" with "Tt" in between.

Table 2 shows the univariate and multivariate associations between age, smoking, and other lifestyle factors and genotype with the various categories of spinal degenerative disease. Figures for presence of apophyseal arthritis were not included because of lack of variation. The presence of osteophytosis was predicted, in multivariate analysis, by age and smoking history while its severity was predicted by these factors as well as body mass index,

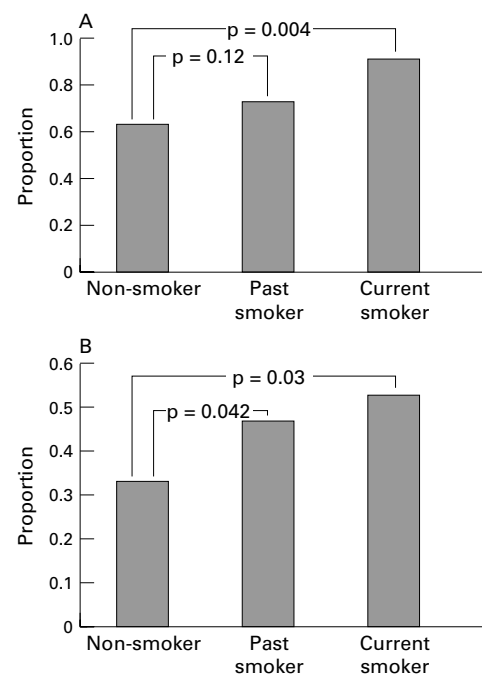


Figure 2 Smoking status and spinal osteophytosis. For both presence (A) and severity (B) of osteophytosis the proportion with osteophytosis is increased in smokers compared with non-smokers with past smokers having an intermediate value. Data are presented unadjusted for potential confounders and the p values represent the difference between each category compared with non-smokers after excluding the other category.

Table 3 Bone density (mean(SD)) at spine and hip partitioned by sex and genotype

|  | <i>tt</i>   | <i>Tt</i>   | <i>TT</i>   |
|--|-------------|-------------|-------------|
| <i>Spine (g/cm<sup>2</sup>)</i>        |             |             |             |
| Men                                    | 1.16 (0.19) | 1.17 (0.24) | 1.15 (0.28) |
| Women                                  | 1.15 (0.24) | 1.09 (0.21) | 1.10 (0.23) |
| <i>Femoral neck (g/cm<sup>2</sup>)</i> |             |             |             |
| Men                                    | 0.83 (0.13) | 0.84 (0.15) | 0.86 (0.16) |
| Women                                  | 0.81 (0.21) | 0.81 (0.15) | 0.81 (0.14) |

quadriceps strength, and VDR genotype. Figure 2 shows the unadjusted prevalence of osteophytes (both presence and severity) by smoking category. The presence of disc narrowing was predicted by age and VDR genotype, although only age was a significant predictor of its severity. Disc narrowing and osteophyte severity scores were correlated with each other (Spearman's  $r=0.31$ ,  $p<0.001$ ). Forcing the disc narrowing score into the model for osteophyte severity led to a non-significant result for genotype ( $p=0.14$ ) indicating that it is not possible, in this case, to assess whether the genotype association is mediated separately by either osteophytosis or disc narrowing. The genetic associations with spinal degenerative disease were not changed by adjustment for femoral neck bone mineral density (data not shown) suggesting that these associations are not mediated by bone density.

Table 3 shows bone density stratified by site, sex, and genotype. No association is evident between genotype and bone density at either site in this sample. This lack of association persisted after multivariate adjustment for potential confounders (data not shown).

### Discussion

This cross sectional population-based study of elderly men and women has shown, for the first time, an association between allelic variation in the vitamin D receptor and spinal degenerative disease. Taken as a whole the data suggest a linear decrease in the risk of spinal osteophytosis (both presence and severity) and presence of disc narrowing from "tt" to "TT". The magnitude of the genetic association is substantial with those in the "TT" group having a 50–60% reduction in risk compared with the "tt" group with heterozygotes in between. Increasing age and body mass index were associated with increased prevalence and severity of spinal degenerative disease as were smoking and quadriceps strength for osteophytosis.

The genetic and lifestyle associations in this sample were most evident for osteophytosis and less clear for disc narrowing and apophyseal osteoarthritis. This is consistent with the two previous studies reporting an association at the knee where strong associations were reported with osteophytes but not with joint space narrowing.<sup>14 15</sup> However, in our sample it is probable that at least some of this discrepancy is a result of definition difficulties within the various categories of spinal degenerative disease. Intra-observer variation was good for osteophytosis but was suboptimal for the other two categories particularly apophyseal osteoarthritis (where there was also lack of variation). The overall effect of this non-differential misclassification would be to weaken the

strength of the associations between the various study factors and these two categories of spinal degenerative disease. This proposition would seem consistent with our data and indicates the need for the development of more reproducible methods of assessment for these categories perhaps using morphometry or a standard atlas.

The results from this study do not allow us to separate the associations between the VDR and osteophytes or disc narrowing as the associations were not independent of each other. Biologically, it is plausible that the VDR may have a direct effect on the pathophysiology of OA through either of these categories. It may have its effect through increased bone density leading to increased local damage to articular cartilage by differential impact loading. However, this does not seem probable in this case as adjustment for bone density did not change the associations (a finding similar to the above mentioned Dutch study<sup>15</sup>). However, bone density may not reflect more subtle changes in bone quality particularly subchondral stiffness, which may be important in the pathogenesis of this disease. The VDR is expressed in both osteoblasts<sup>21</sup> and chondrocytes,<sup>22</sup> both of which are found in osteophytes<sup>23</sup> suggesting a possible role in osteophyte formation or progression, or both. Our results would suggest a greater role in osteophyte progression. In addition to the VDR being expressed in chondrocytes, vitamin D can also influence proteoglycan synthesis by articular chondrocytes *in vitro*.<sup>24</sup> These findings suggest that the VDR may be involved in the pathophysiology of OA in bone or cartilage, or both, at a molecular level.

The direction of the genetic effect on spinal degenerative disease in this study is in the same direction as would be expected for fracture given the effect seen for bone mass in other studies.<sup>11</sup> This finding was somewhat unexpected given the reported inverse association between osteoporosis and OA that we and others have previously published on including in this particular sample.<sup>16 17 25</sup> However, our finding is in the same direction and of similar magnitude to that reported recently by Uitterlinden *et al*<sup>15</sup> who studied a comparable elderly group to our own. Keen *et al*,<sup>14</sup> in a younger sample with early arthritis reported an association in the opposite direction. These variations between studies may be caused by a number of potential reasons in addition to the differing age groups. The first is that of population stratification. This seems unlikely in all three studies as cases and controls were selected in a nested fashion from within ethnically homogeneous cohorts. Secondly, there may be variations in gene environment interactions in the different communities resulting in variations in disease expression within the various genotypes. The other two studies do not report on gene environment interactions suggesting this may be a potential explanation. However, linkage disequilibrium is a more likely reason for the discrepancies between studies. The vitamin D receptor gene is located on chromosome 12q. Other potential candidate genes for OA that are also located in this region include type

2 collagen (COL2A1) and insulin-like growth factor type 1. In particular, COL2A1 maps within 920 kilobases from the VDR.<sup>26</sup> The degree of linkage disequilibrium may vary between population groups<sup>9</sup> thus, despite the biological plausibility outlined above, it remains possible that the VDR is not directly involved in the genesis of OA but is a marker of other gene(s).

The VDR was not associated with bone mass in this sample possibly because of sample size considerations where considerably larger samples are required to demonstrate an association between VDR and bone density. This lack of sample size is contributed to by the fact that 38% of the study group are male in whom association with the VDR genotype has not been reported. In the whole sample from the cohort who have been genotyped the association with BMD is weak (unpublished data) perhaps reflecting the high prevalence of spinal degenerative disease of widely varying severity in these elderly subjects with its resultant artefactual changes in bone mass particularly at the spine.<sup>17</sup>

This study has also shed new light on the relation between lifestyle factors and spinal degenerative disease. As expected, the prevalence of all types of OA increases with age and our findings are consistent with this for each of the three categories. Body mass index was associated with osteophytosis particularly severity suggesting consistent with a contributory effect of mechanical loading on the spine. Quadriceps strength was also associated with severity of osteophytosis suggesting that physical activity may also play a part in its development or progression, or both, at the spine. The associations with body mass index and quadriceps strength were independent of one another. In contrast with most other studies, smoking considerably increased the risk of osteophytosis in this sample. This was most evident for current smokers but there was also an intermediate increase in the risk for past smokers suggesting a duration effect. This association persisted and even increased after adjustment for potential confounders such as age and body mass index so it appears real. Previous studies at other sites have found conflicting results. Two American studies found a modest 20% decrease in the risk of knee OA.<sup>27-28</sup> However, a British study found an increased risk of OA at most sites although this was only statistically significant for Heberden's disease and the confidence limits for the knee estimate overlapped those from the above two studies.<sup>29</sup> Taken as a whole these findings suggest that smoking may have site specific effects on osteophytosis. Furthermore, smokers in our total cohort had lower BMD at the spine<sup>18</sup> while the smokers in this sample had higher spinal BMD ( $p=0.03$ ) consistent with an association between smoking and osteophytosis and possibly indicating that osteophytosis is more common in this random sample than in the total cohort. This evidence of selection bias was somewhat unexpected as our sample were selected by computer generated random numbers and is very similar to the whole cohort in terms of age,

body mass index, smoking, and self reported OA.<sup>30</sup> There have, however, been no other studies that have examined this question in relation to the spine so our findings need to be confirmed in other populations. Potential mechanisms for this association are not clear but our analysis suggests that it is unlikely to be mediated by changes in body weight, age or physical activity and may thus be a direct effect of smoking. The presence of additive gene environment interaction in our sample is also of clinical significance. The statistical significance of both environmental and particularly genetic factors increased after adjustment for the other. This is presumably a result of a reduction in the "noise" introduced by random variation in genetic and lifestyle factors when each is considered separately. However, the present sample did not have sufficient power to study multiplicative interaction suggesting that further studies will need to be considerably larger and consider both lifestyle and genetic factors. Furthermore, the clinical application of these results would suggest that both factors will need to be considered for more accurate assessment of risk of disease at both the individual and population level and that prevention may be better targeted to high risk groups.

In conclusion, both genetic and lifestyle factors are associated with the presence and severity of spinal degenerative disease in our elderly sample. There were site specific differences in associations at the spine that may be because of misclassification of disease status or may indicate possible environmental and genetic differences in the pathophysiology of spinal degenerative disease. Further studies are required to confirm these findings in different population samples and to further explore potential aetiological mechanisms particularly gene environment interaction.

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