

Bone structural effects of variation in the *TNFRSF1B* gene encoding the tumor necrosis factor receptor 2

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Abstract

Summary The 1p36 region of the human genome has been identified as containing a QTL for BMD in multiple studies. We analysed the *TNFRSF1B* gene from this region, which encodes the TNF receptor 2, in two large population-based cohorts. Our results suggest that variation in *TNFRSF1B* is associated with BMD.

Introduction The *TNFRSF1B* gene, encoding the TNF receptor 2, is a strong positional and functional candidate gene for impaired bone structure through the role that TNF has in bone cells. The aims of this study were to evaluate the

role of variations in the *TNFRSF1B* gene on bone structure and osteoporotic fracture risk in postmenopausal women.

Methods Six SNPs in *TNFRSF1B* were analysed in a cohort of 1,190 postmenopausal Australian women, three of which were also genotyped in an independent cohort of 811 UK postmenopausal women. Differences in phenotypic means for genotype groups were examined using one-way ANOVA and ANCOVA.

Results Significant associations were seen for IVS1 +5580A>G with BMD and QUS parameters in the Australian population ($P=0.008-0.034$) and with hip BMD parameters in the UK population ($P=0.005-0.029$). Significant associations were also observed between IVS1 +6528G>A and hip BMD parameters in the UK cohort ($P=0.0002-0.003$). We then combined the data from the two cohorts and observed significant associations between both IVS1+5580A>G and IVS1+6528G>A and hip BMD parameters ($P=0.002-0.033$).

Conclusions Genetic variation in *TNFRSF1B* plays a role in the determination of bone structure in Caucasian postmenopausal women, possibly through effects on osteoblast and osteoclast differentiation.

Keywords Bone mineral density (BMD) · Elderly women · Osteoporotic fracture · Single nucleotide polymorphisms (SNPs) · Osteoporosis

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Introduction

Postmenopausal osteoporosis is a systemic bone disease characterised by low bone mass and disturbed micro-architecture of bone tissue [1]. This results in an increased fragility and is a major risk factor for fracture [1]. Peak bone mass is achieved in early adult life but declines in

postmenopausal women due to a reduction in oestrogen production with effects on bone and intestinal and renal calcium handling [2]. According to the WHO definition of osteoporosis, which is based on the use of DXA densitometry as a non-invasive measurement of bone mass and size, 30% of Caucasian postmenopausal women are afflicted with osteoporosis [1].

In addition to the effects of oestrogen and calcium on bone structure and fracture there is a strong genetic effect on bone structure both for peak bone mass and for bone loss and fracture rates in postmenopausal women [3–6]. Twin and family studies show that 50%–90% of the variance in peak bone mass is heritable [3, 4, 6] and 27%–68% of the variance in osteoporotic fracture is heritable [5].

It is clear that the genetic effect for common variation in bone structure as assessed by DXA bone mineral density (BMD) is under polygenic control [7]. Many allelic variations in genes contributing to variation in BMD in postmenopausal women have already been identified using the candidate gene approach, some examples include the genes *TGFBI*, *APOE* and *COL1A1* [8–11]. In addition, the whole genome scanning approach often using a linkage design has identified many potential positional candidates [12–15]. Thus although the proof of principle for gene discovery for bone structure is clear, there is much work to be undertaken to identify the most important and consistent gene polymorphisms across populations.

The 1p36 region of the genome has been identified as containing a QTL for BMD in many studies, including our own [14], most reporting linkage of hip BMD to this genomic region [12–17]. The tumour necrosis factor receptor subfamily member 1B (*TNFRSF1B*) gene, which encodes the 75Kd tumour necrosis factor- α (TNF) receptor 2 (p75), is situated within this locus (1p36.2–36.3) and is a strong functional candidate for regulation of BMD. TNF is a cytokine that has a role in the pathogenesis of postmenopausal osteoporosis [18, 19]. Menopause brings about a drop in oestrogen level, which has been shown to increase the number of TNF-producing T cells in the bone marrow of ovariectomised mice and an increase in the quantity of TNF produced [20]. This increase in TNF production results in bone loss apparently through increased osteoclastogenesis [20].

On the basis of this positional and candidate gene data, the present study was undertaken in order to evaluate the influence of polymorphisms in the *TNFRSF1B* gene on bone structure and fracture frequency in a well described large cohort of normal, ambulatory Australian women. Replication of significant results in a similar cohort of postmenopausal women from the UK would be attempted should they occur.

Materials and methods

Subjects

The Australian population used in this study consists of women aged between 72 and 85 years of age, as described previously [9, 21]. Out of 1,499 women approached, 1,190 agreed to provide DNA samples, with heel quantitative ultrasound (QUS) and DXA BMD measurements obtained from 1,148 and 896 of these individuals, respectively. The subjects were recruited in 1998 as described previously [22].

The UK population was recruited in 1988 to participate in a longitudinal epidemiologic study of rheumatic diseases [23, 24]. Women between the ages of 45 and 64 were recruited from a single large general practice in Chingford, North-East London using a population-based method. DNA samples were obtained from 811 individuals, with hip and spine DXA BMD data obtained from 775 and 779 of these individuals, respectively. The studies on both cohorts were approved by the local ethics committees.

Demographic, anthropometric and lifestyle factors

Demographic and lifestyle factor data were obtained by questionnaires [25] completed in 1998 for the Australian population and in 1988 for the UK population. Height and weight were measured by standard methods at these times and weight was measured again in the UK population at the time of DXA scan.

DXA bone density and QUS measurements

In the Australian population, BMD was measured in 1999 as described previously [9, 21].

In the UK population bone density measurements were undertaken in 1998 approximately 10 years after initial recruitment as described previously [26].

Fracture status

A detailed fracture history in the Australian population was collected as described previously [9, 21].

The UK subjects were categorised as having had a previous fracture on the basis of self-report.

Biochemistry

Blood and urine samples were obtained from the women in the Australian population with serum osteocalcin and urine deoxypyridinoline analysis performed as described previously [9, 21].

Table 1 Demographics, bone density, quantitative ultrasound and biochemistry of the Australian and UK populations

| Variables | Australia | UK |
|---------------------------------------|----------------------|-----------|
| Demographics | (n=1,190) | (n=811) |
| Age (years) | 75.2±2.7 | 62.7±5.9 |
| Weight (kg) | 68.3±12.2 | 69.1±12.6 |
| Dietary calcium (mg/day) | 959±352 | – |
| Ever smoked (%) | 35.9 | 46.5 |
| Prevalent fractures (%) | 25.7 | 34.4 |
| Incident fractures (%) | 17.5 | – |
| Hip DXA BMD | (n=896) | (n=775) |
| Total hip (mg/cm ²) | 811±126 | 869±128 |
| Femoral neck (mg/cm ²) | 689±104 | 747±119 |
| Trochanter (mg/cm ²) | 637±107 | 672±107 |
| Intertrochanter (mg/cm ²) | 951±158 | 1018±154 |
| Spine DXA BMD | (n=0) | (n=779) |
| Spine L1–L4 (mg/cm ²) | – | 955±155 |
| Heel quantitative ultrasound | (n=1148) | (n=0) |
| BUA (Db/MHz) | 100.4±7.9 | – |
| SOS (m/s) | 1513±26 | – |
| Stiffness (% mean young adult) | 70.5±11.5 | – |
| Bone biochemistry | (n=215) | (n=0) |
| Osteocalcin (µg/l) ^a | 4.70 (2.70, 7.15) | – |
| U-Dpd/Creat (µmol/mol) ^a | 26.76 (21.41, 34.00) | – |

Results are given as *mean*±*SD*

^aMedian (25th, 75th percentiles)

Genotyping

All single nucleotide polymorphism (SNP) genotyping was performed in a single Australian laboratory on genomic DNA obtained from each subject using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-ToF) mass spectrometry [27]. Primer sequences for these PCR reactions were 5'-TTGCAAAGTGAAGCCTTGTG-3' and 5'-CTTCTTTCCTCCCTGCCTTT-3' for IVS1+5580A>G, 5'-CCCACACTGTAGGAAAGTCCTTA-3' and 5'-ACAGCCAAGTACCTCAAACCAC-3' for IVS1+6528G>A, 5'-CAGCAAGGAAAGAGCTTCCA-3' and 5'-CACATGCCGGCTCAGAGA-3' for c.257T>C, 5'-CACCCCAGCCACTCTGTC-3' and 5'-CCAACTGGAA GAGCGAAGTC-3' for c.676A>C and c.783G>A and 5'-CCCCACCACTAGGACTCTGA-3' and 5'-CACAGA GAGTCAGGGACTTGC-3' for c.1690T>C. The sequence

of the genotyping primers used was 5'-CCCACAGCCTTTGC CACAATAC-3', 5'-TCCTGGAAGAAGCCCTTTGTGCG-3', 5'-CTGCCCTCACCCGGCGAGCA-3', 5'-TGGACGTGCA GACTGCATCC-3', 5'-CAACACACGCAGCCAACTCCA-3' and 5'-GGAAAGCCTCTGCTGCCATGG-3' for IVS1+5580A>G, IVS1+6528G>A, c.257T>C, c.676A>C, c.783G>A and c.1690T>C, respectively.

Statistical analysis

Statistical analysis was performed using Statistica for Windows Version 5.1 (Statsoft Inc). Chi-square testing was used to confirm that the SNPs were in Hardy-Weinberg equilibrium. Differences in phenotypic means for genotype groups were examined using one-way ANOVA. BMD and QUS data were adjusted for the covariates age, weight, calcium supplementation and smoking habits by univariate Analysis of Co-Variance (ANCOVA). Genotype effects on incident and prevalent fracture rates were examined using a Chi-square test. Assessment of linkage disequilibrium (LD) within the gene was undertaken using the JLIN program [28]. QTPHASE, a program for quantitative trait analysis under UNPHASED software, was used to investigate the effect of haplotypes on quantitative traits. The program employs likelihood ratio tests in a log-linear model [29]. Throughout, two-tailed *P* values are reported, and values *P*≤0.05 are considered significant.

Results

The demographic and morphometric characteristics of the populations are detailed in Table 1. In the Australian population, genotyping was completed for six SNPs in the *TNFRSF1B* gene, all of which were in Hardy-Weinberg equilibrium. The position and allele distributions of these six SNPs are presented in Fig. 1 and Table 2. The six SNPs are IVS1+5580A>G (rs496888 or SNP 1), IVS1+6528G>A (rs976881 or SNP 2), c.257T>C (rs945439 or SNP 3), c.676A>C (rs1061622 or SNP 4), c.783G>A (rs5746026 or SNP 5) and c.1690T>C (rs3397 or SNP 6), and are located in intron 1, intron 1, exon 2, exon 6, exon 6 and the 3' UTR, respectively.

Fig. 1 Schematic drawing of SNP localisation in *TNFRSF1B*. Vertical bars represent the exons of *TNFRSF1B* with the receptor domains and the positions of the six SNPs analysed indicated

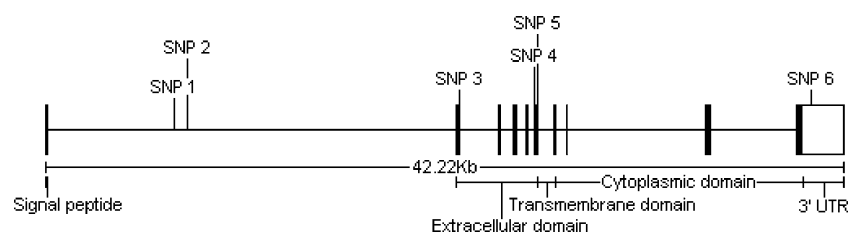


Table 2 Nomenclature, position and allele distribution of the analysed SNPs on chromosome 1 in relation to the *TNFRSF1B* gene

| SNP number | 1 | 2 | 3 | 4 | 5 | 6 |
|-------------------------|-------------------------------|-------------------------------|---|-------------------------------|-------------------------------|-------------------------------|
| Designation | IVS1+5580A>G | IVS1+6528G>A | c.257T>C | c.676A>C | c.783G>A | c.1690T>C |
| dbSNP ID | rs496888 | rs976881 | rs945439 | rs1061622 | rs5746026 | rs3397 |
| Chromosome position* | 12167072 | 12168020 | 12183208 | 12187221 | 12187328 | 12201558 |
| Function / Location* | Intron 1 | Intron 1 | Exon 2 | Exon 6 | Exon 6 | 3' UTR |
| | | | Synonymous change of aa 56 (Lys/Lys) | Change of aa 196 (Arg/Met) | Change of aa 232 (Lys/Glu) | |
| Allele distribution (%) | AA 49.5 AG 40.1 GG 10.5 | AA 10.1 AG 43.6 GG 46.3 | CC 6.6 TC 33.9 TT 59.5 | AA 61.6 AC 32.6 CC 5.7 | AA 0.2 AG 5.1 GG 94.6 | CC 13.3 TC 43.4 TT 43.4 |

*From GenBank reference sequence NM_001066, Genome Build 35.1. aa - amino acid

Locus specific analyses: effects of individual SNP genotypes on phenotypic data

The genotype effects of each SNP on hip BMD, heel QUS and bone biochemistry data in the Australian population were

investigated by one-way ANCOVA. Significant associations with BMD parameters were observed for SNP 1 ($P=0.008-0.018$) (Table 3). BMD was significantly lower in subjects homozygous at SNP 1 for the less common G allele. At the total hip, intertrochanter and femoral neck sites, the differ-

Table 3 DXA and quantitative ultrasound parameters in relation to the allele distribution of SNP 1 and SNP 2 in the Australian, UK and combined populations

| SNP 1 | Australian population | | | | UK population | | | | Combined population | | | |
|----------------------------------|-----------------------|----------------------|-----------------------|-------|-----------------------|-----------------------|----------------------|--------|-----------------------|----------------------|----------------------|-------|
| | AA | AG | GG | P | AA | AG | GG | P | AA | AG | GG | P |
| Hip DXA BMD | n=401 | n=328 | n=99 | | n=386 | n=294 | n=62 | | n=787 | n=622 | n=161 | |
| Total Hip (mg/cm ²) | 814±126 ^a | 816±125 ^a | 782±122 ^b | 0.018 | 859±124 ^a | 880±133 ^b | 857±125 ^a | 0.029 | 834±127 ^a | 845±134 ^b | 815±127 ^c | 0.005 |
| Troch (mg/cm ²) | 640±105 | 640±108 | 618±101 | 0.088 | 665±105 ^a | 680±112 ^b | 662±99 ^a | 0.08 | 650±106 ^{ab} | 658±112 ^a | 637±101 ^b | 0.033 |
| Intertroch (mg/cm ²) | 954±158 ^a | 960±160 ^a | 915±153 ^b | 0.016 | 1009±151 ^a | 1029±160 ^b | 999±155 ^a | 0.071 | 978±157 ^a | 992±165 ^b | 952±157 ^c | 0.003 |
| Fem Neck (mg/cm ²) | 692±103 ^a | 692±104 ^a | 662±98 ^b | 0.008 | 735±113 ^a | 760±124 ^b | 739±119 ^a | 0.005 | 711±110 ^a | 723±120 ^b | 696±112 ^c | 0.002 |
| Heel QUS | n=527 | n=427 | n=107 | | | | | | | | | |
| BUA (Db/MHz) | 101±8 ^a | 101±8 ^a | 98±8 ^b | 0.008 | | | | | | | | |
| SOS (m/s) | 1514±26 ^a | 1513±26 ^a | 1507±27 ^b | 0.034 | | | | | | | | |
| Stiffness (%) | 71±11 ^a | 71±12 ^a | 67±12 ^b | 0.01 | | | | | | | | |
| SNP 2 | GG | AG | AA | P | GG | AG | AA | P | GG | AG | AA | P |
| Hip DXA BMD | n=401 | n=379 | n=93 | | n=386 | n=310 | n=75 | | n=787 | n=689 | n=168 | |
| Total Hip (mg/cm ²) | 817±129 | 809±123 | 813±125 | 0.558 | 876±128 ^a | 867±126 ^a | 822±117 ^b | 0.0003 | 845±132 ^a | 836±129 ^b | 818±121 ^c | 0.009 |
| Troch (mg/cm ²) | 640±105 | 639±109 | 629±110 | 0.611 | 677±105 ^a | 671±111 ^a | 635±97 ^b | 0.003 | 657±106 ^a | 654±112 ^a | 633±104 ^b | 0.01 |
| Intertroch (mg/cm ²) | 963±165 ^a | 946±151 ^b | 955±156 ^{ab} | 0.235 | 1025±155 ^a | 1016±152 ^a | 963±142 ^b | 0.0007 | 992±163 ^a | 979±157 ^b | 959±150 ^b | 0.009 |
| Fem Neck (mg/cm ²) | 691±106 | 691±103 | 695±98 | 0.925 | 753±122 ^a | 747±115 ^a | 700±106 ^b | 0.0002 | 720±119 ^a | 717±113 ^a | 697±102 ^b | 0.026 |

Results are given as mean±SD. The Australian bone structural data are adjusted for age, weight, calcium supplementation and smoking habits. The UK bone structural data are adjusted for age, weight, and smoking habits.

The combined structural data are adjusted for age, weight, calcium supplementation and study centre.

^a significantly different from ^b and ^c in post hoc analysis ($P<0.05$)

ences in BMD were -4.1% , -4.3% and -4.5% , respectively, compared to individuals homozygous for the *A* allele, and -4.3% , -4.9% and -4.5% compared to individuals with the *AG* heterozygote genotype. Importantly, significant associations were also observed between SNP 1 and the QUS parameters BUA, SOS and Stiffness ($P=0.008-0.034$) (Table 3). Again the less common *G* allele was associated with lower quality bone. No significant associations between genotype and the covariates age, weight, calcium supplementation (calcium or placebo) or smoking (pack years) were found ($P=0.38-0.98$). Data for SNP 2 showed a trend for total hip, trochanter and intertrochanter BMD towards a reduced BMD for the less common allele but these failed to reach statistical significance (Table 3). There were no significant associations seen between any of the other four SNPs genotyped in this population and bone phenotypes ($P=0.06-0.98$).

Based on the a priori hypothesis developed during examination of the genotype effects of SNP 1 on the Australian population, the influence of this polymorphism on hip and spine BMD was examined in the UK population. SNP 2 and SNP 6 were also genotyped in the UK population, all of which were in Hardy–Weinberg equilibrium. Significant associations were observed between SNP 1 and total hip and femoral neck BMD phenotypes in the UK population ($P=0.005-0.029$) in the same direction as that seen in the Australian population, although these appear to be caused by a high mean BMD for heterozygous individuals (Table 3). However, significant associations were observed between SNP 2 and total hip, trochanter, intertrochanter and femoral neck BMD in the UK cohort ($P=0.0002-0.003$), with the less common *A* allele associated with a lower BMD (Table 3). At the total hip, trochanter, intertrochanter and femoral neck sites, the differences in BMD were -6.6% , -6.6% , -6.4% and -7.6% , respectively, compared to individuals homozygous for the *G* allele and -5.5% , -5.7% , -5.5% and -6.7% compared to individuals with the *AG* heterozygote genotype. No significant associations were seen between these SNPs and spine BMD in this cohort ($P=0.06-0.08$). No

significant associations between genotype and the covariates age, weight or smoking (ever smoked) were found for either SNP ($P=0.06-0.71$). No significant associations were seen between SNP 6 and any of the BMD phenotypes in the UK population ($P=0.13-0.47$).

After examination of the data from SNP 1 and SNP 2 in each population, it was decided that combining the genotype data from the two populations and correcting for the covariates age, weight, calcium supplementation and study centre could potentially be informative. Both SNPs were significantly associated with total hip, trochanter, intertrochanter and femoral neck BMD in the combined population ($P=0.002-0.033$; $n=1,570-1,644$), with individuals homozygous for the less common allele associated with a lower BMD at both SNP sites (Table 3). For SNP 1 at the total hip, trochanter, intertrochanter and femoral neck sites, the differences in BMD were -2.3% , -2.0% , -2.7% and -2.2% , respectively, compared to individuals homozygous for the *A* allele and -3.7% , -3.3% , -4.2% and -3.9% compared to individuals with the *AG* heterozygote genotype. For SNP 2 at the total hip, trochanter, intertrochanter and femoral neck sites, the differences in BMD were -3.3% , -3.8% , -3.4% and -3.3% , respectively, compared to individuals homozygous for the *G* allele and -2.2% , -3.3% , -2.1% and -2.9% compared to individuals with the *AG* heterozygote genotype. BMD was also found to be significantly higher in subjects from the Australian population after correction for age at the total hip ($P=0.02$), trochanter ($P=0.02$) and intertrochanter ($P=0.02$). No significant associations between genotype and the covariates weight, calcium supplementation or study centre were found for either SNP, although an association was seen between SNP 2 and age in the combined cohort ($P=0.016$) with individuals homozygous for the *G* allele associated with a slightly younger age (Table 4).

Linkage disequilibrium and haplotype analysis

Pairwise LD D' and r^2 values for the six SNPs in the Australian population are given in Fig. 2. Three separate

Table 4 Demographics of study subjects grouped by SNP 1 and SNP 2 genotype in the combined population

| | SNP 1 | | | | SNP 2 | | | |
|-----------------------------|-----------------|-------------------|-------------------|-------|--------------------------------|--------------------------------|-------------------|-------|
| | AA | AG | GG | P | GG | AG | AA | P |
| Age (years) | n=942 70±7.5 | n=754 69.9±7.7 | n=181 70.7±7.4 | 0.467 | n=939 69.6±7.7 ^a | n=828 70.6±7.5 ^b | n=203 70.6±7.2 | 0.016 |
| Weight (kg) | 68.8±12.3 | 68.6±12.6 | 68.3±12.6 | 0.868 | 68.6±12.3 | 68.8±12.5 | 68.6±11.9 | 0.925 |
| Calcium supplemented (%) | 28.7 | 30.6 | 35.4 | 0.182 | 30 | 30.1 | 34.5 | 0.431 |
| Study centre (% Australian) | 57.7 | 58.5 | 63.5 | 0.35 | 57.1 | 60.6 | 61.1 | 0.26 |

Results are given as *mean*±*SD*

^asignificantly different from ^b in post hoc analysis ($P<0.05$)

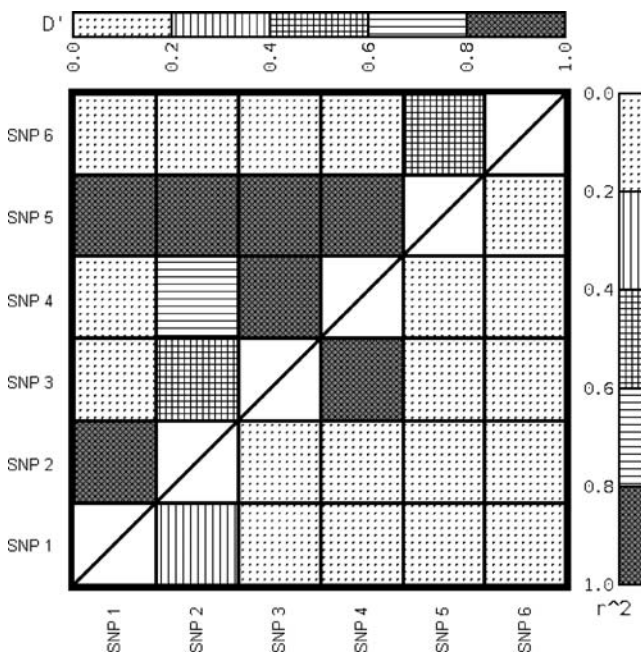


Fig. 2 Linkage disequilibrium (LD) for six SNP pairs in the *TNFRSF1B* gene in the Australian population. Gray-shaded coding represents the strength of LD according to the scale shown on the top (D') and the right (r^2)

LD blocks within the gene were identified represented by SNP 1, SNP 3 and SNP 6. Using imputed haplotypes, researchers found a 3-marker haplotype analysis involving representative SNPs with high and low LD values (SNP 1, SNP 3 and SNP 6) suggested that 100% of the population can be represented by eight haplotypes. No significant associations between the haplotypes and hip BMD or heel QUS were observed based on a likelihood ratio test in a log-linear model ($P=0.24-0.89$).

Biochemistry and fracture rates

There were no significant associations seen between any of the six SNPs examined in the Australian population and bone related biochemistry ($P=0.17-1.0$). In the Australian population 306 subjects had a fracture at baseline giving a prevalent fracture rate of 25.7%. In the 60 month follow-up period 208 subjects (17.5%) experienced an osteoporotic fracture confirmed by X-ray, no significant association between prevalent or incident fracture and any of the SNPs was observed ($P=0.06-0.87$).

In the UK population 279 subjects had suffered a fracture in the past, giving a prevalent fracture rate of 34.4%. No significant association between prevalent fracture and any of the three SNPs analysed in this population was observed ($P=0.56-0.66$).

Discussion

The data suggest that the a priori hypotheses that variation within the *TNFRSF1B* gene impairs bone structure has been supported. In particular two intronic polymorphisms in the *TNFRSF1B* gene, SNP 1 and SNP 2, are associated with impaired bone structure of the hip in postmenopausal women who are homozygous for the less common allele. The overall effect size is a 2–3% reduction in hip BMD which was not large enough to alter fracture frequency. This is a similar effect size to that reported for the genes *LRP5* [30], *COL1A1* [31] and *TGFBI* [31]. Fracture risk rises by about twofold for a reduction in hip BMD of one SD [32], which in this study was about 125mg/cm² or about 15% of the mean. Thus the increase in fracture rate for the “disease” genotype would be expected to be about 20%. Under these circumstances the power of the study to detect a fracture effect was only about 0.2. The association between SNP 1 and the heel QUS parameters in the Australian population suggests that the polymorphism affects bone structure at the heel and is therefore likely to have a generalised effect on bone structure in postmenopausal women [33]. These associations were not explained by other demographic covariates such as age, weight and smoking and persisted after adjustment for these variables and for calcium treatment, which was part of the primary study in the Australian population. The fact that the associations observed for SNP 2 were primarily related to the slightly younger UK cohort suggests that the effect of the less common *A* allele on BMD at this SNP site may be related more to attainment of peak BMD, with the effect of the allele decreasing with increased age.

Since SNP 1 and SNP 2 are located within an intron, it is unlikely that polymorphism at these sites could directly cause functional changes to the *TNFRSF1B* protein or affect its production. It is possible that these SNPs are in strong LD with another polymorphic site in the same region of the *TNFRSF1B* gene that can cause structural changes to the p75 receptor. There is one non-synonymous coding change in the *TNFRSF1B* gene that occurs at a relatively high frequency (SNP 4); however, this has been examined on multiple occasions with no significant associations with bone phenotypes seen [34–37], including in this study. Another non-synonymous coding change was analysed in this study (SNP 5), although it had a very low minor allele frequency. Again, no significant associations were seen between this SNP and bone phenotypes.

It seems more plausible that polymorphism at SNP 1 and/or SNP 2 affect the quantity of the *TNFRSF1B* protein produced. It is possible that these SNPs are in LD with a polymorphic site located at the 3' end of intron 1, where there has been reported to be several transcription factor

binding sites and a regulatory region [38]. Another possibility is that they are in LD with a polymorphism in the promoter region of *TNFRSF1B*, which in turn affects the transcription of the gene.

Other investigators have reported associations between polymorphisms in the *TNFRSF1B* gene and BMD [34–36, 39–41]. Most relate to haplotypes of three SNPs in the 3' untranslated region (UTR) of *TNFRSF1B* [34–36, 39] c.1663G>A (G593A or rs1061624), c.1668T>G (T598G or rs5030792) and c.1690T>C (T620C, rs3397 or SNP 6). However, there are discrepancies between the findings from different groups. For example, Spotila et al. [36, 39] found that the 1663A-1668G-1690T (AGT) haplotype was associated with a lower spine and femoral neck BMD, whereas Albagha et al. [34] and Tasker et al. [35] reported the 1663A-1668T-1690C (ATC) haplotype to be associated with a reduced femoral neck BMD.

Xiong et al. [41] analysed 12 SNPs in and around the *TNFRSF1B* gene including SNP 1 and SNP 3 from our study. They did not report any significant associations between these SNPs and BMD parameters. However, they did report a significant association between a haplotype window in the 5' end of *TNFRSF1B*, which included SNP 1, and spine BMD. They also reported several associations between two haplotype windows covering the central region of the gene and the qualitative trait osteoporosis at the hip, spine and ultradistal radius. However, the cohort used in their study is considerably different to that used in ours: the study by Xiong et al. [41] is a family-based cohort containing both males and females across a wide range of ages. Interestingly, they found that the associations observed with *TNFRSF1B* were primarily driven by females. It would be interesting to analyse specifically the female subsample of their cohort in relation to this gene.

The p75 TNF receptor is composed of a single membrane-spanning domain and an extracellular domain [42]. TNF receptor superfamily members contain cysteine-rich extracellular regions made up of three to six disulfide-linked domains, which are thought to be important for ligand binding [42]. Another member of the TNF receptor superfamily is the 55 Kd receptor 1 (p55), the product of the *TNFRSF1A* gene. Both the p55 and p75 receptors have been found to be highly expressed on osteoclast precursors [43]. The main ligands for the p55 and p75 receptors are soluble and membrane-residing TNF, respectively [43]. When activated by TNF, the p55 receptor has been found to stimulate osteoclastogenesis and inhibit osteoblast differentiation [44], whereas the p75 receptor suppresses osteoclastogenesis *in vitro* [43]. The external domain of the p75 receptor, which is shed, competes with the p55 receptor for soluble TNF [45], where it has a higher ligand affinity than the p55 receptor [46]. Thus impaired function or a decrease in the quantity of the p75 receptor produced could

result in a decrease in the suppression of osteoclastogenesis. Alternatively a decrease in the quantity or activity of the external domain of the p75 receptor shed into the extracellular space could result in an increase in the level of unbound circulating soluble TNF, which in turn would increase the stimulation of p55 receptors resulting in increased osteoclast recruitment and decreased osteoblast differentiation. Either mechanism would result in a reduction in bone mass and structure. Further studies of variants of the *TNFRSF1B* gene need to be carried out to determine which polymorphic site or sites are directly responsible for these effects.

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