Genetic variants in obesity-related genes and the risk of osteoporotic fracture. The Hortega Follow-up Study

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Abstract

Background: Osteoporosis and obesity are major public health problems that are closely correlated, as they share various features, including a genetic predisposition. A genetic correlation between obesity and osteoporosis due to the biological common pathways of bone and fat metabolism, which implies pleiotropic genes regulating has been described. The objective of our study was to analyse whether polymorphisms in obesity-related genes modify the risk of osteoporotic bone fracture. Methods: We studied 575 subjects from the Hortega Study. The subjects were followed-up for 12–14 years. 202 subjects were overweight, 143 obese and 221 had bone fractures. The distribution of 39 genetic variants in 22 obesity-related genes were studied. Results: The results showed a relationship between polymorphisms in the FTO and NEGR1 genes and the susceptibility to osteoporotic fracture. The variant genotype of the rs2568958 NEGR1 polymorphism and the rs6499649, rs3751812, and rs8044769 genetic variants in FTO were associated with susceptibility to bone fracture. In the best of our knowledge, this is the first time that these variants in NEGR1 and FTO genes have been associated with the susceptibility to osteoporotic bone fracture, supporting the hypothesis that the NEGR1 and FTO genes might be candidates for osteoporosis and bone fracture. Conclusions: In conclusion, this study associates obesity-related polymorphisms in the NEGR1 and FTO genes with osteoporotic bone fracture, reinforcing the hypothesis that obesity and bone metabolism are closely correlated genetically.

Keywords: Osteoporosis; Bone fracture; Obesity; Polymorphism; FTO and NEGR1

1. Introduction

Osteoporosis (OMIM: 166710) is a chronic, progressive, silent, and systemic bone metabolism disease caused by reduced bone mineral density (BMD) and alterations in the tissue microarchitecture, increasing the risk of fragility fractures [1,2]. Osteoporosis is the most prevalent chronic bone metabolism disease worldwide. The main clinical complication of osteoporosis is bone fracture, and it is associated with worse quality of life and with higher disability, morbidity and mortality [3–5]. Bone is a dynamic tissue, with a balance between bone formation osteoblast-mediated and osteoclast-mediated bone resorption. The underlying pathological mechanism in osteoporosis is an unbalance between bone resorption and bone formation, which leads to increased bone fragility and higher risk of osteoporotic fracture [6].

The aetiology of osteoporosis is closely associated with genetic factors. Family studies have shown that 50–80% of the osteoporotic risk is explained by genetic variants, and multiple genes/loci have been associated with BMD phenotypes and fractures [7,8]. In addition, the risk of fragility bone fracture is higher in patients with a family history of osteoporosis [9–11]. Environmental factors are also important in the aetiology of osteoporosis, and the most accepted hypothesis is the combined action of environmental and genetic determinants [2–4].

Obesity, another major public health problem, is closely related to osteoporosis, with which it shares several features, including a genetic predisposition and a common progenitor cell [12–14]. Adipocytes and osteoblasts arise from bone marrow stem cells (BMSC) and both transdifferentiate into each other [15]. Age is an independent risk fac-
tor for osteoporosis: it has been reported that, with aging, the differentiation of BMSC shifts to favour adipocytes, decreasing osteoblast functions and leading to the risk of osteoporosis and fracture [16,17]. In addition, adipocytes have an important role in bone metabolism through the secretion of factors such as the oestrogen synthesis enzyme, aromatase, leptin and proinflammatory cytokines [15,18]. There is a common central regulation of bone metabolism and metabolic fate through the hypothalamus and sympathetic nervous system [13,19,20]. A possible protective effect of fat on the skeleton with respect to the risk of bone loss and fragility fracture has been described [13,21,22]. Genetic factors play a crucial role in osteoporosis and obesity, with a genetic correlation between them due to the biological common pathways of bone and fat metabolism, which implies pleiotropic genes regulating both processes [23–25]. Therefore, the principal objective of our study was to analyse if polymorphisms in obesity-related genes could modify the susceptibility of suffering osteoporotic bone fracture.

2. Subjects and methods

2.1 Subjects

The Hortega Follow-up Study is a cross-sectional prospective study of factors associated with chronic diseases. The Hortega Study began in 2001–2003 with the baseline screening and biological samples collection, and added information on the evolution of subjects in 2015. The participants were 1504 adults from the West Valladolid Health Area (Rio Hortega University Hospital, Spain) [26–30].

To analyse the influence of polymorphisms in obesity-related genes on the susceptibility to osteoporotic bone fracture, individuals were chosen from the Hortega Study, as previously reported [30]. In summary, of the 1504 baseline individuals, 137 subjects were excluded because lack of anthropometric, demographic or clinical data. After analysis of individuals aged over 50 years old (N = 702), we excluded 49 subjects due to lack of clinical follow-up information, 15 subjects with a history of bone fracture at baseline and 63 individuals without bone X-ray, resulting in a final study sample of 575 individuals. Cases were considered patients with bone fracture (N = 221) and individuals without fracture were (N = 354) classified as control group (Supplementary Fig. 1). Subjects aged <50 years were excluded as the risk of osteoporotic bone fracture has been reported to increase dramatically after the age of 50 years [31]. All subjects included were Caucasian.

Anthropometric, clinical and demographic characteristics such as sex, height, age, weight, body mass index (BMI), overweight and obesity status, tobacco, alcohol, menopause, corticosteroid consumption, family history of fragility bone fracture and calcaneal bone densitometry were obtained from each subject. Type 2 diabetes mellitus, hypertension, overweight and obesity were diagnosed based on World Health Organization (WHO) guidelines (http://www.who.int). Peripheral instantaneous X-ray imaging (PIXI) DXA system (General Electric Lunar Pixi, Boston, MA, USA) was used to determine the calcaneal bone densitometry of the right calcaneus. Bone fractures were collected after a clinical follow up of 12–14 years. The Genant classification was used to determine vertebral fractures [32] and non-vertebral fractures were collected with the review of the medical records.

The University Hospital of Valladolid Research Ethics Committee approved the experimental protocol. Our study was in line with the Declaration of Helsinki (2008) of the World Medical Association and it also complied with Spanish data protection laws (LO 15/1999) and specifications (RD 1720/2007). All individuals who participated signed consent form.

Table 1. Anthropometric, clinical and demographic characteristics of study individuals.

| Characteristics                     | Control group | Osteoporotic fracture group |
|-------------------------------------|---------------|-----------------------------|
|                                    | (n = 354)     | (n = 221)                   |
| Age (years)                         | 61.9 (16.3)   | 61.4 (17.8)                 |
| Sex (female)                        | 189 (53.4%)   | 107 (48.4%)                 |
| Height (cm)                         | 164.5 (9.1)   | 164.1 (9.5)                 |
| Weight (kg)                         | 71.4 (13.5)   | 71.0 (12.9)                 |
| Body mass index (kg/m²)             | 26.3 (4.3)    | 26.3 (4.1)                  |
| Obesity                             | 84 (25.0%)    | 59 (28.2%)                  |
| Overweight                          | 123 (36.5%)   | 79 (37.8%)                  |
| Central obesity                     | 82 (24.0%)    | 59 (27.4%)                  |
| High blood pressure                 | 143 (40.4%)   | 83 (37.6%)                  |
| Type 2 diabetes mellitus            | 27 (7.6%)     | 14 (6.3%)                   |
| Smoker                              | 81 (23.2%)    | 58 (26.5%)                  |
| Alcohol intake                      | 67 (19.0%)    | 54 (24.4%)                  |
| Postmenopausal women                | 165 (46.6%)   | 89 (40.3%)                  |
| Corticosteroids use                 | 38 (10.8%)    | 29 (13.1%)                  |
| Family history of bone fracture     | 1 (0.3%)      | 10 (4.5%) *                 |
| Bone mineral density (g/cm²)        | 0.55 (0.11)   | 0.53 (0.11)                 |
| Bone mineral density (t-score)      | −0.17 (1.10)  | −0.19 (1.38)                |

* p < 0.05 between control group and osteoporotic fracture group.

Quantitative variables are presented as mean (standard deviation) and qualitative variables are presented as n (%).

2.2 DNA isolation and polymorphism genotyping

Genomic DNA was extracted from nucleated peripheral blood cells of patients and healthy subjects by standard commercial procedures. The obtained genomic DNA was quantified and stored to a final concentration of 100 ng/μL. We studied 39 polymorphisms in 22 obesity-related genes (Supplementary Table 1). The selection of genetic variants was made through SYSNP [33,34] and computer-based searches in Web of Science, PubMed, Embase and Scopus databases with the following terms: “bone metabolism”, “obesity”, “polymorphisms”, “genetic variants”, “SNPs”, “obesity-related genes”, “osteoporosis”, “osteoporotic bone fracture”, “polymorphism genotyping”.


Table 2. Genetic variants in obesity-related genes significantly associated with body mass index.

| Gene   | SNP          | Genotype | N | BMI (kg/m²), mean ± SD | p-value |
|--------|--------------|----------|---|------------------------|---------|
| NEGR1  | rs2568958    | AA       | 239 | 26.71 ± 4.30            | 0.042   |
|        |              | AG       | 233 | 26.12 ± 4.02            |         |
|        |              | GG       | 74  | 25.57 ± 4.47            |         |
|        |              | AA       | 239 | 26.71 ± 4.30            | 0.047   |
|        |              | AG+GG    | 307 | 25.99 ± 4.14            |         |
|        | rs6499640    | AA       | 209 | 25.80 ± 4.04            |         |
|        |              | AG       | 258 | 26.75 ± 4.50            | 0.048   |
|        |              | GG       | 76  | 26.30 ± 3.61            |         |
|        | FTO          | AA       | 209 | 25.80 ± 4.04            | 0.022   |
|        |              | GG       | 195 | 25.82 ± 3.97            |         |
|        |              | GT       | 255 | 26.73 ± 4.59            | 0.045   |
|        |              | TT       | 95  | 26.21 ± 3.56            |         |
|        | rs3751812    | AA       | 209 | 25.80 ± 4.04            |         |
|        |              | GG       | 195 | 25.82 ± 3.97            | 0.041   |
|        |              | GT+TT    | 359 | 26.59 ± 4.34            |         |
|        | rs4788102    | AA       | 69  | 26.57 ± 4.25            | 0.038   |
|        |              | GA       | 225 | 26.79 ± 4.32            |         |
|        |              | GG       | 250 | 25.82 ± 4.09            |         |
|        |              | GA+AA    | 294 | 26.74 ± 4.30            | 0.012   |
|        | SH2B1        | TT       | 240 | 25.80 ± 3.94            |         |
|        |              | TC       | 218 | 26.70 ± 4.29            | 0.049   |
|        |              | CC       | 74  | 26.61 ± 4.17            |         |
|        | rs8049439    | TT       | 240 | 25.80 ± 3.94            |         |
|        |              | TC+CC    | 292 | 26.68 ± 4.26            | 0.015   |
|        | HTR2C        | CC       | 400 | 26.65 ± 4.26            | 0.003   |
|        | rs3813929    | CT       | 92  | 25.05 ± 4.54            |         |
|        |              | TT       | 57  | 25.94 ± 2.79            |         |
|        |              | CT+TT    | 149 | 25.39 ± 3.98            | 0.002   |

BMI, Body mass index.

“fat metabolism” and/or “bone”. Single nucleotide polymorphisms (SNPs) were selected in accordance with the following criteria: known functional or potentially functional consequence, place in promoter regions, minor allele frequency (MAF) >0.5 in Caucasian subjects (polymorphisms with allele frequencies ≤.5% were excluded), distribution and localization in the gene (upstream and downstream regions included) and low linkage disequilibrium (LD) between genetic variants (recombination frequency ≤30%). We used HapMap and NCBI databases to collect information about polymorphisms and to establish the gene and pathway involved in each SNPs. SNPlex oligonucleotide ligation assay (Applied Biosystems, Foster City, CA, USA) was used to genotyping according to the manufacturer’s guidelines.

2.3 Statistical analysis

Qualitative variables were expressed as absolute (n) and relative (%) frequencies and quantitative variables as mean (standard deviation(SD)). To analyze differences between quantitative variables was used the T-test, applying the Bonferroni adjustment. The chi-square test was used to study qualitative variables. Hardy-Weinberg equilibrium, using the chi-square test, was used to test the control group for each SNPs. To evaluate the relationship between the SNPs with overweight, obesity and osteoporotic bone fracture; odd ratios (ORs) with 95% confidence intervals (CI) were calculated for each variant applying unconditional logistic regression models. p-values were adjusted by age, sex, hypertension, menopause, BMD, and family history of osteoporotic bone fracture. The first step was a screening phase in which the influence of the SNPs in obesity-related genes on BMI levels, overweight and obesity status were analysed. Analysis of the relationship between SNPs in obesity-related genes and the susceptibility to suffer osteoporotic bone fracture only included the genetic variants which yielded significant associations with BMI, overweight or obesity.

A statistical power analysis to study the relationship between fracture and SNPs was carried out. With effect size moderate: 0.5; degree freedom: 2; sample size: 500 and level of significance: 0.05; the results showed a power = 1. The results showed a power = 0.99947 with effect size small: 0.23; degree freedom: 2; sample size: 500 and level of significance: 0.05. An OR = 1.88 (power: 0.80), OR = 2.43 (power: 0.97) and OR = 3.1 (power: 0.99) was showed by the analysis to detect the power of the statistical significance of the OR with a genotypic basal probability: 0.20, event probability: 0.40, level of significance: 0.05, and sample size: 500. The statistical analyses were made using SPSS software (version 21.0, IBM Corp., Chicago, IL, USA). A p-value < 0.05 was regarded as significant.

3. Results

The study sample included 575 subjects after a 12–14 year follow up with a mean age of 61.62 ± 17.1 years and 50.9% (n = 296) were female. The mean BMI was 26.30 (4.2). There were 202 subjects who were overweight (37.15%) and 143 who were obese (26.55%). 221 subjects with osteoporotic bone fracture and 354 individuals without bone fracture were included. Table 1 summarizes the anthropometric, demographic and clinical features between subjects with bone fractures and 354 individuals without bone fracture.

Table 2 shows the significant BMI variations according to polymorphisms in obesity-related genes. The
Table 3. Genotypic frequencies of genetic variants in obesity-related genes significantly associated with overweight.

| Gene  | SNP          | Genotype | Subjects without overweight (n (%)) | Subjects with overweight (n (%)) | p-value | OR (95% CI) |
|-------|--------------|----------|------------------------------------|---------------------------------|---------|-------------|
|       |              | CC       | 200 (61.2%)                        | 120 (62%)                       |         |             |
|       |              | CT       | 117 (35.8%)                        | 57 (29.7%)                      | 0.294   | 0.81 (0.55–1.19) |
|       |              | TT       | 10 (3.1%)                          | 15 (7.8%)                       | 0.031   | 2.50 (1.18–5.74) |
| ETV5  | rs7647305    | CC+CT    | 317 (96.9%)                        | 177 (92.2%)                     |         |             |
|       |              | CC       | 200 (61.2%)                        | 120 (62%)                       | 0.762   | -           |
|       |              | CT+TT    | 127 (38.8%)                        | 72 (37.5%)                      | 0.018   | 2.68 (1.18–6.11) |
| FTO   | rs8044769    | GG       | 83 (25.2%)                         | 69 (34.8%)                      |         |             |
|       |              | GA       | 142 (42.4%)                        | 87 (43.3%)                      | 0.065   | -           |
|       |              | AA       | 44 (13.1%)                         | 40 (19.9%)                      |         |             |
| ADRB2 | rs12654778   | GG+GA    | 291 (86.9%)                        | 161 (80.1%)                     |         |             |
|       |              | AA       | 44 (13.1%)                         | 40 (19.9%)                      | 0.038   | 1.64 (1.21–2.62) |
|       |              | GG       | 149 (44.5%)                        | 74 (36.8%)                      |         |             |
|       |              | GA+AA    | 186 (55.5%)                        | 127 (63.2%)                     | 0.086   | -           |
| KCTD15| rs11084753   | GG+GA    | 282 (84.9%)                        | 182 (91.0%)                     |         |             |
|       |              | AA       | 50 (15.1%)                         | 18 (9.0%)                       | 0.045   | 0.55 (0.31–0.78) |
|       |              | GG       | 146 (44.0%)                        | 91 (45.5%)                      | 0.732   | -           |
|       |              | GA+AA    | 186 (56.0%)                        | 109 (54.5%)                     |         |             |

OR, odds ratio; CI, confidence interval.

Variant genotypes of the rs2568958 NEGR1 and rs3813929 HTR2C polymorphisms were associated with a lower BMI (p = 0.042 and p = 0.002) and subjects with the variant genotypes of the rs8049439 SH2B1, rs47881102 SH2B1, rs6499640 FTO and rs3751812 FTO polymorphisms had a higher BMI (p = 0.015, p = 0.012, p = 0.022 and p = 0.041, respectively) (Table 2). Carrying the AG+GG genotype of the rs2568958 NEGR1 polymorphism and the CT+TT genotype of the HTR2C SNP was associated with a lower BMI (p < 0.001). The combined effect of the genotypes was observed in 80 subjects with a BMI of 24.32 ± 3.38 kg/m². Subjects carrying the variant alleles of the rs6499640 FTO, rs3751812 FTO, rs47881102 SH2B1 and rs8049439 SH2B1 polymorphisms were associated with a higher BMI (p = 0.002). This effect was observed in 109 subjects with a BMI of 27.75 ± 4.43 kg/m². The genotypic frequencies of the polymorphisms were also analysed between overweight and non-overweight subjects (Table 3) and between obese and non-obese subjects (Table 4). Polymorphisms in the ETV5, ADRB2, FTO and TMEM18 genes were associated with overweight or obesity. The variant genotype of the rs7647305 ETV5 and rs12654778 ADRB2 SNPs were associated with an increased risk of overweight (p = 0.018 and p = 0.038). The variant genotype of the rs8044769 FTO and rs11084753 KCTD15 SNPs were associated with a decreased risk of overweight (p = 0.019 and p = 0.045) (Table 3). The rs8044769 FTO SNPs was also related with a decreased risk of obesity (p = 0.039) (Table 4). The rs2867125, rs6548238 and rs4854344 polymorphisms in the TMEM18 gene were associated with obesity (p = 0.045, p = 0.034 and p = 0.041) (Table 4). Analysis of the relationship between polymorphisms in obesity-related genes and the risk of osteoporotic bone fracture only included polymorphisms associated with BMI, overweight and obesity. The genotype distribution of these polymorphisms in subjects without bone fracture was in Hardy-Weinberg equilibrium. Genetic variants in the NEGR1 and FTO genes were associated with bone fracture. The GG genotype and G allele of the rs2568958 NEGR1 polymorphism were associated with a decreased risk of osteoporotic fracture (p = 0.009 and p = 0.010) (Table 5). In addition, polymorphisms in the FTO gene were related with the susceptibility to osteoporotic fracture. The TT genotype of the rs8044769, the GG genotype of rs6499649 and the TT genotype of rs3751812 FTO gene variants were related with a decreased risk of osteo-
Table 4. Genotypic frequencies of genetic variants in obesity-related genes significantly associated with obesity.

| Gene | SNP       | Genotype | Subjects without obesity (n (%)) | Subjects with obesity (n (%)) | p-value | OR (95% CI) |
|------|-----------|----------|---------------------------------|------------------------------|---------|-------------|
|      |           |          |                                 |                              |         |             |
|      |           | GG       | 270 (69.1%)                     | 110 (78.0%)                  | 0.072   | -           |
|      |           | GA       | 109 (27.9%)                     | 30 (21.6%)                   |         |             |
|      |           | AA       | 12 (3.1%)                       | 1 (0.7%)                     |         |             |
| rs2867125 |            | GG+GA    | 379 (96.9%)                     | 140 (99.3%)                  | 0.120   | -           |
|      |           | AA       | 12 (3.1%)                       | 1 (0.7%)                     |         |             |
|      |           | GG       | 270 (69.1%)                     | 110 (78.0%)                  |         | 1.00        |
|      |           | GA+TT    | 121 (30.9%)                     | 31 (22.0%)                   | 0.045   | 0.62 (0.40–0.83) |
| TMEM18 | rs6548238 | CC       | 269 (67.9%)                     | 110 (77.5%)                  | 0.052   | -           |
|      |           | CT       | 114 (28.8%)                     | 31 (21.8%)                   |         |             |
|      |           | TT       | 13 (3.3%)                       | 1 (0.7%)                     |         |             |
|      |           | CC+CT    | 383 (96.7%)                     | 141 (99.3%)                  | 0.098   | -           |
|      |           | TT       | 13 (3.3%)                       | 1 (0.7%)                     |         |             |
|      |           | CC       | 269 (67.9%)                     | 110 (77.5%)                  |         | -           |
|      |           | CT+TT    | 127 (32.1%)                     | 32 (22.5%)                   | 0.034   | 0.61 (0.39–0.86) |
|      |           | TT       | 269 (67.8%)                     | 110 (76.9%)                  |         |             |
|      |           | TG       | 115 (29.0%)                     | 32 (22.4%)                   | 0.057   | -           |
|      |           | GG       | 13 (3.3%)                       | 1 (0.7%)                     |         |             |
| rs4854344 |            | TT+TG    | 384 (96.7%)                     | 142 (99.3%)                  | 0.097   | -           |
|      |           | TT       | 13 (3.3%)                       | 1 (0.7%)                     |         |             |
|      |           | CC       | 269 (67.8%)                     | 110 (76.9%)                  |         | -           |
|      |           | TG+GG    | 128 (32.2%)                     | 33 (23.1%)                   | 0.041   | 0.63 (0.40–0.89) |
|      |           | CC       | 111 (28.5%)                     | 52 (38.0%)                   |         | 1.00        |
|      |           | CT       | 196 (50.3%)                     | 51 (37.2%)                   | 0.011   | 0.55 (0.34–0.87) |
|      |           | TT       | 83 (21.3%)                      | 34 (24.8%)                   | 0.611   | 0.87 (0.52–1.46) |
| FTO  | rs8044769 | CC+CT    | 307 (78.7%)                     | 103 (72.2%)                  | 0.392   | -           |
|      |           | TT       | 83 (21.3%)                      | 34 (24.8%)                   |         |             |
|      |           | CC       | 111 (28.5%)                     | 52 (38.0%)                   |         | -           |
|      |           | CT+TT    | 279 (71.5%)                     | 85 (62.0%)                   | 0.039   | 0.65 (0.43–0.89) |

OR, odds ratio; CI, confidence interval.

porotic bone fracture (p = 0.001, p = 0.005 and p = 0.002) (Table 5). These SNPs were also associated with BMI, overweight and obesity (Tables 2, 3, 4). The risk of osteoporotic fracture was also analysed in subjects with overweight or obesity (Table 6). The variant genotype and variant allele of the rs2568958 NEGR1 and rs8044769 FTO SNPs were associated with a decreased risk of osteoporotic bone fracture (for subjects with overweight: p = 0.032 and p = 0.013; for subjects with obesity: p = 0.035 and p = 0.005).

4. Discussion

Obesity and osteoporosis are closely correlated genetically due to the common biological pathways of fat and bone metabolism, implying that genes may be crucial in both processes [23–25]. Multiple pleiotropic loci and candidate genes involved in obesity and osteoporosis have been studied [23,24,35], although more common genes underlying both pathologies have not been identified. Therefore, we studied whether SNPs in obesity-related genes modify the susceptibility to osteoporotic bone fracture. The results showed that polymorphisms in the NEGR1 and FTO genes were related to this risk. Analysis of the relationship between SNPs in obesity-related genes and the risk of osteoporotic fracture only included the genetic variants which yielded significant associations with BMI, overweight and obesity.

Susceptibility to obesity is determined by environmental factors, with an important genetic component. Familial and twin studies have shown that genetic factors are crucial in around 40–70% of cases [33–35]. The Src-homology (SH2)-B protein is a crucial regulator of leptin and insulin sensitivity, glucose homeostasis, and body weight [36]. It has also been reported that SH2B1 knockout mice developed obesity and diabetes [37]. We found an association between the rs4788102 and rs8049439 SH2B polymorphisms and BMI, in line with previous studies which correlated multiple genetic variants in the SH2B gene with body weight regulation [38–40]. We also found a relationship between the variant allele of the rs3813929 HTR2C polymorphism and BMI. The HTR2C gene encodes to the serotonin 5-HT-2C receptor, which is involved in appetite control, feeding behaviour and the risk of obesity [41,42]. The rs3813929 polymorphism is found in the promoter region of the HTR2C gene and has been associated with alterations in body weight, as it modified HTR2C gene expression [43,44]. Transcription factor E-
Table 5. Genotypic frequencies of genetic variants in obesity-related genes significantly associated with the risk of osteoporotic bone fracture.

| Gene   | SNP      | Genotype | Control subjects (n (%)) | Patients with OF (n (%)) | p-value | OR (95% CI) |
|--------|----------|----------|--------------------------|--------------------------|---------|-------------|
| NEGR1  | rs2568958| AA       | 137 (39.5%)              | 111 (50.5%)              | -       | 1.00        |
|        |          | AG       | 159 (45.8%)              | 90 (40.9%)               | 0.051   | 0.69 (0.48–1.10) |
|        |          | GG       | 51 (14.7%)               | 19 (8.6%)                | 0.009   | 0.46 (0.25–0.82) |
|        |          | AA+AG    | 296 (85.3%)              | 201 (91.4%)              | -       | 1.00        |
|        | rs8044769| CC       | 139 (41.1%)              | 112 (51.4%)              | -       | 1.00        |
|        |          | CT       | 103 (30.5%)              | 69 (31.7%)               | 0.358   | 0.83 (0.56–1.23) |
|        |          | TT       | 96 (28.4%)               | 37 (17.0%)               | 0.001   | 0.47 (0.30–0.75) |
|        |          | CC+CT    | 242 (71.6%)              | 181 (83.0%)              | -       | 1.00        |
|        | rs6499649| AA       | 119 (34.6%)              | 89 (40.6%)               | -       | 1.00        |
|        |          | AG       | 158 (45.9%)              | 107 (48.9%)              | 0.597   | 0.90 (0.62–1.30) |
|        |          | GG       | 67 (19.5%)               | 23 (10.5%)               | 0.005   | 0.45 (0.26–0.79) |
|        |          | AA+AG    | 277 (80.5%)              | 196 (89.5%)              | -       | 1.00        |
|        |          | TT       | 67 (19.5%)               | 23 (10.5%)               | 0.005   | 0.48 (0.29–0.80) |
|        | rs3751812| GG       | 123 (35.5%)              | 96 (43.6%)               | -       | 1.00        |
|        |          | GT       | 161 (46.5%)              | 104 (47.3%)              | 0.308   | 0.82 (0.57–1.19) |
|        |          | TT       | 62 (17.9%)               | 20 (9.1%)                | 0.002   | 0.41 (0.23–0.73) |
|        |          | GG+GT    | 284 (82.1%)              | 200 (90.9%)              | -       | 1.00        |
|        |          | TT       | 62 (17.9%)               | 20 (9.1%)                | 0.004   | 0.45 (0.25–0.78) |
|        |          | GG       | 123 (35.5%)              | 96 (43.6%)               | 0.555   | -           |
|        |          | GT+TT    | 223 (64.5%)              | 124 (56.4%)              | -       | -           |

OF, osteoporotic fracture; OR, odds ratio; CI, confidence interval.

twenty-six version 5 (Etv5) has been linked with obesity as it is involved in adipogenesis and it has been reported that \(ETV5\) knockout mice have reduced fat mass and are resistant to obesity [45,46]. The results reported a relationship between the rs7647305 \(ETV5\) polymorphism and overweight subjects, in line with other reports [47,48]. The \(ADRB2\) gene encodes to adrenergic-receptor beta 2, which plays a key role in fat metabolism by increasing lipolysis and thermogenesis [49,50]. We found the variant genotype of the rs12654778 \(ADRB2\) SNPs was associated with overweight and that the rs11084753 polymorphism in the \(KCTD15\) gene was related to overweight, similarly to the study by Willet et al. [38]. The \(KCTD15\) gene encodes to the potassium channel tetramerization protein, which is involved in various biological processes related to obesity, such as glucose metabolism, fat metabolism and adipogenesis [51]. The results also reported an association between the rs2867125, rs6548238 and rs4854344 genetic variants in the \(TMEN18\) gene and obesity, in line with previous studies [33,38,40,47,48]. Transmembrane protein 18 (Tmen18) plays a key role in the central control of appetite and body weight regulation [52] and therefore genetic variants in the \(TMEN18\) gene could modify gene expression and the susceptibility to obesity. Various studies have summarized that genetic variants in \(NEGR1\) are related to human body weight changes [38,53], and we found an association between the variant allele of the rs2568958 \(NEGR1\) SNPs and BMI. Neuronal growth regulator 1 (Negr1) is crucial in neural cell communication and, more recently, it has also been associated with intercellular cholesterol trafficking, suggesting a relationship with the predisposition to obesity [54]. We also found an association between the rs6499640, rs3751812 and rs8044769 genetic variants in the \(FTO\) gene with BMI and obesity. The \(FTO\) gene is one of the genes most commonly associated with susceptibility to obesity, due to its role in fat cell lipolysis. It has been reported that, in subjects with increased BMI, there is overexpression of the \(FTO\) gene in adipose tissue and that \(FTO\)-knockout mice have reduced fat mass. Overexpression of the \(FTO\) gene has been associated with increased food intake and obesity [55–57]. Many polymorphisms in the \(FTO\) gene have been associated with obesity [33,38,47,48]. The rs3813929 \(HTR2C\), rs8049439 \(ATXNL2\), rs6499640 \(FTO\) and rs7647305 \(ETV5\) polymorphisms were also associated with obesity in a previous study by our group that analysed the impact of obesity-related genes in Spanish subjects [58].
Genetic factors are important in the individual susceptibility of osteoporotic bone fracture [59]. Our results confirmed the association between family history of osteoporotic fracture and the risk of bone fracture. To analyse the influence of polymorphisms in obesity-related genes on the susceptibility to osteoporotic bone fracture, we studied 13 SNPs in eight obesity-related genes associated with body weight variations. The results showed that SNPs in the \textit{NEGR1} and \textit{FTO} genes were associated with the risk of osteoporotic bone fracture. Bone fragility and the increased risk of fracture observed in osteoporosis is the consequence of the imbalance between bone resorption mediated by osteoclasts and osteoblast-mediated bone formation [6]. \textit{NEGR1} could be important in bone metabolism alterations, as its role in regulating osteoblast differentiation and the cell adhesion pathway has been reported [60]. We showed that the rs2568958 genetic variant in the \textit{NEGR1} obese-related gene was associated with the susceptibility to osteoporotic bone fracture. In our knowledge, this is the first report that summarizes the association between SNPs in the \textit{NEGR1} gene and the risk of osteoporotic fracture. Polymorphisms in the \textit{NEGR1} gene could modify its expression and, therefore, osteoblast differentiation, increasing the risk of osteoporosis and bone fracture. We also described an association between the rs8044769, rs6499649 and rs3751812 SNPs in the \textit{FTO} gene and the risk of fracture. The loss of BMSC function may lead to enhanced pathological changes in the bone metabolism, including osteoporosis and bone fracture, as the differentiation of BMSC is crucial in osteogenesis [61–63]. The \textit{FTO} gene is expressed in osteoblasts and is required for maintenance of bone mass due to its key role in osteoblast differentiation from BMSC [64,65]. It is reported that \textit{FTO}-knockout mice showed growth retardation and low BMD [66]. Other genetic variants in the \textit{FTO} gene have been associated with BMD variations and susceptibility to hip fracture [67,68]. However, to our knowledge, this is the first time that the rs8044769, rs6499649 and rs3751812 \textit{FTO} polymorphisms have been associated with osteoporotic bone fracture, supporting the hypothesis that the \textit{FTO} gene might be a candidate for osteoporosis and bone fracture. Analysis of the risk of bone fracture only in subjects with overweight or obesity showed that the rs2568958 \textit{NEGR1} and rs8044669 \textit{FTO} SNPs were related with bone fracture, confirming the importance of these genetic variants in the susceptibility to bone fracture.

### Table 6. Genotypic frequencies of genetic variants in obesity-related genes significantly associated with the risk of osteoporotic bone fracture in subjects with overweight and obesity.

| Gene   | SNP    | Genotype | Control subjects (n (%)) | Patients with OF (n (%)) | p-value | OR (95% CI) |
|--------|--------|----------|--------------------------|--------------------------|---------|-------------|
|        |        |          |                          |                          |         |             |
|        |        | AA       | 51 (41.5%)               | 45 (57.0%)               | -       | 1.00        |
|        |        | AG       | 53 (43.1%)               | 30 (38.0%)               | 0.148   | 0.64 (0.35–1.17) |
|        |        | GG       | 19 (15.4%)               | 4 (5.1%)                 | 0.015   | 0.23 (0.076–0.75) |
| \textit{NEGR1} | rs2568958 | AA+AG    | 104 (84.6%)              | 75 (94.9%)               | -       | 1.00        |
|        |        | GG       | 19 (15.4%)               | 4 (5.1%)                 | 0.031   | 0.29 (0.11–0.89) |
|        |        | AA       | 51 (41.5%)               | 45 (57.0%)               | -       | 1.00        |
|        |        | AG+GG    | 72 (58.5%)               | 34 (43.0%)               | 0.032   | 0.53 (0.30–0.91) |
| Subjects with overweight |        | CC       | 46 (38.3%)               | 44 (56.4%)               | -       | 1.00        |
|        |        | CT       | 38 (31.7%)               | 23 (29.5%)               | 0.176   | 0.63 (0.32–1.22) |
|        |        | TT       | 36 (30.0%)               | 11 (14.1%)               | 0.005   | 0.31 (0.14–0.70) |
| \textit{FTO} | rs8044769 | CC+CT    | 84 (70.0%)               | 67 (85.9%)               | -       | 1.00        |
|        |        | TT       | 36 (30.0%)               | 11 (14.1%)               | 0.012   | 0.38 (0.18–0.80) |
|        |        | CC       | 46 (38.3%)               | 44 (56.4%)               | -       | 1.00        |
|        |        | CT+TT    | 74 (61.7%)               | 34 (43.6%)               | 0.013   | 0.48 (0.26–0.85) |
| Subjects with obesity |        | AA       | 32 (39.5%)               | 34 (57.6%)               | -       |             |
|        |        | AG       | 39 (48.1%)               | 22 (37.3%)               | 0.073   | -           |
|        |        | GG       | 10 (12.3%)               | 3 (5.1%)                 | -       |             |
| \textit{NEGR1} | rs2568958 | AA+AG    | 71 (87.7%)               | 56 (94.9%)               | 0.156   | -           |
|        |        | GG       | 10 (12.3%)               | 3 (5.1%)                 | -       |             |
|        |        | AA       | 32 (39.5%)               | 34 (57.6%)               | -       | 1.00        |
|        |        | AG+GG    | 49 (60.5%)               | 25 (42.4%)               | 0.035   | 0.48 (0.24–0.95) |
|        |        | CC       | 32 (41.0%)               | 38 (65.5%)               | -       | 1.00        |
|        |        | CT       | 24 (30.8%)               | 15 (25.9%)               | 0.115   | 0.52 (0.23–1.16) |
|        |        | TT       | 22 (28.2%)               | 5 (8.6%)                 | 0.003   | 0.19 (0.06–0.56) |
| \textit{FTO} | rs8044769 | CC+CT    | 56 (71.8%)               | 53 (91.4%)               | -       | 1.00        |
|        |        | TT       | 22 (28.2%)               | 5 (8.6%)                 | 0.007   | 0.24 (0.08–0.68) |
|        |        | CC       | 32 (41.0%)               | 38 (65.5%)               | -       | 1.00        |
|        |        | CT+TT    | 46 (57.4%)               | 20 (35.5%)               | 0.005   | 0.36 (0.18–0.74) |

OF, osteoporotic fracture; OR, odds ratio; CI, confidence interval.
The principal limitation of the study is that it would have been attractive to determine the relationship of these SNPs in obese-related genes with central BMD; however, we only measured calcaneal BMD. The number of the individuals included was limited although the statistical power analysis revealed that the sample size was appropriate for our objectives. Another limitation was the bias in selecting obesity-related SNPs using available databases instead of genome-wide analysis of obesity-associated genes by running a GWAS on the Hortega follow-up cohort. The principal strength of the study is that the analysis is based on a cohort of individuals from a study with a follow-up of 12–14 years. Another strength is the similarity of subjects with fracture and control group, which is crucial for the determination of the influence of genetic factors.

5. Conclusions

In conclusion, we describe associations between variants in the FTO and NEGR1 genes and the susceptibility to osteoporotic bone fracture, reinforcing the hypothesis that obesity and bone metabolism are closely correlated genetically. However, studies are needed to clarify the role of these genetic variants in the susceptibility to osteoporotic bone fracture. Developing in-vitro and in-vivo functional studies to determine the role of the FTO and NEGR1 genes and their genetic variants on the susceptibility of suffering bone fracture could be very interesting; for example, studying the importance of these genetic variants in bone remodelling.

Abbreviations

BMD, Bone mineral density; BMSC, bone marrow stem cells; BMI, body mass index; WHO, World Health Organization; PIXI, peripheral instantaneous X-ray imaging; 95% CI, 95% confidence intervals; ORs, Odd ratios; SH2, Src-homology-B; Etv5, E-twenty-six version 5; Negr1, neuronal growth regulator 1.

Author contributions

RUM and JLPC performed the statistical analysis, analysed and interpreted results and wrote the manuscript. JMV performed the statistical analysis. FLH, ABGG and FJC obtained genetic data, contributed to analysis and interpretation and reviewed the manuscript. LBF, JAO, JCME obtained population data, made critical revisions of the manuscript and contributed to the discussion. RUM, JLPC, FJC and JCME designed the study.

Ethics approval and consent to participate

The experimental protocol was in accordance with the Declaration of Helsinki (2008) of the World Medical Association and was approved by the University Hospital of Valladolid Ethics Committee. It was also in compliance with Spanish data protection laws (LO 15/1999) and specifications (RD 1720/2007). All subjects who agreed to participate gave written informed consent.

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Conflict of interest

The authors declare no conflict of interest. RUM and JLPC are serving as guest editors in this journal. We declare that RUM and JLPC had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to AG.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBL/27/1/10.31083/j.fbl2701032.

12. References

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