UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Genetic epidemiology of longitudinal change in bone mineral density

by Krisel De Dios

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Doctor of Philosophy

Under the supervision of

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Associate Professor Nham Tran

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Faculty of Engineering and Information Technology

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Certificate of Original Authorship

I, Krisel De Dios, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biomedical Engineering at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abstract

Osteoporosis is characterised by low bone mineral density (BMD), microarchitectural deterioration of bone tissue, and an increased risk of fracture. These hallmark features arise from disrupted bone homeostasis, where bone resorption occurs at a higher rate than bone formation, leading to progressive skeletal decline. This thesis aimed to investigate the relationship between bone loss and fracture risk and to identify both overlapping and distinct genetic variants associated with longitudinal bone change. By moving beyond static BMD measures, this work provides novel insights into bone loss trajectories and their implications for fracture prediction and clinical management.

This thesis is based on several large, prospective longitudinal cohort studies, including the Study of Osteoporotic Fractures (SOF), the Osteoporotic Fractures in Men Study (MrOS), the Dubbo Osteoporosis Epidemiology Study (DOES), and the Vietnam Osteoporosis Study (VOS). The findings demonstrate that bone loss is an independent risk factor for hip fracture in men, even after adjusting for pre-defined covariates. Furthermore, genetic analyses identified associations between specific FTO single nucleotide polymorphisms (SNPs) and hip fracture risk, with minor homozygous alleles emerging as potential clinical biomarkers of increased susceptibility. In addition, novel loci linked to longitudinal bone change were discovered, underscoring the value of capturing dynamic skeletal processes rather than relying solely on cross-sectional BMD measures.

In conclusion, this thesis highlights bone loss as a central element contributing to low BMD, microarchitectural deterioration, and fracture risk. It also establishes that overlapping and distinct genetic determinants underlie longitudinal bone change, pointing to new skeletal pathways for further investigation. Collectively, these findings advance the understanding of osteoporosis pathophysiology and provide a framework for incorporating bone loss trajectories into future diagnostic, predictive, and therapeutic approaches.

Research Outputs

The findings presented in this dissertation represent the outcomes of a study undertaken from September 2021 to September 2025 at the School of Biomedical Engineering, Faculty of Engineering and Information Technology, University of Technology Sydney, under the supervision of Prof. T.V Nguyen and A/Prof. N Tran. Chapters 3, 4 and 5 have been composed in the form of independent manuscripts and therefore a certain degree of redundancy and repetition may exist between these chapters and chapters 1 (Introduction), 2 (Study design and Analytical methods) and 6 (Conclusions and Future Directions). Results of this study have been reported as follows:

Published papers:

1. **Dios, K**., Huynh, N., Tran, T. S., Center, J. R. & Nguyen, T. V. Association between Fat Mass and Obesity-Related Transcript Polymorphisms and Osteoporosis Phenotypes. *J Bone Metab* **31**, 48-55 (2024). https://doi.org:10.11005/jbm.2024.31.1.48
2. Huynh, N., **De Dios, K**., Tran, T. S., Center, J. R. & Nguyen, T. V. Association between the Sp1-binding-site polymorphism in the collagen type I alpha 1 (COLIA1) gene and bone phenotypes: the Dubbo Osteoporosis Epidemiology Study. J Bone Miner Metab, doi:10.1007/s00774-024-01558-8 (2024).

Manuscripts submitted or In Preparation

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Oral presentations

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2. Rate of bone loss is associated with fracture risk: The Study of Osteoporotic Fractures. International Congress of Osteoporosis by the Korean Society of Osteoporosis in Seoul, Korea: 3 – 5 November 2023
3. Rate of bone loss is associated with fracture risk: The Study of Osteoporotic Fractures. Annual Scientific Meeting of Australian and New Zealand Bone Mineral Society in Newcastle, Australia: 22 – 25 October 2023

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3. Rate of bone loss is associated with fracture risk: The Study of Osteoporotic Fractures. Annual Meeting of American Society for Bone and Mineral Research in Vancouver, Canada: 13 – 16 October 2023

4. Contribution of fat mass and obesity-associated (FTO) gene to osteoporosis phenotypes. Annual Meeting of American Society for Bone and Mineral Research in Austin, Texas: 9 – 12 September 2022

5. Contribution of fat mass and obesity-associated (FTO) gene to osteoporosis phenotypes. Australian and New Zealand Bone and Mineral Society (ANZBMS), Molecular & Experimental Pathology Society of Australasia (MEPSA) and the Australian & New Zealand Orthopaedic Research Society (ANZORS) Joint Scientific Meeting in Gold Coast, Australia: 1 – 4 August, 2022

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5. Winner of 3MT Wildcard competition (2023), UTS Library, Australia
6. People's Choice award of 3MT Faculty competition (2023), The Faculty of Engineering and Information Technology, UTS, Australia
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BMD	Bone Mineral Density
BMI	Body Mass Index
DOES	Dubbo Osteoporosis Epidemiology Study
DXA	Dual-energy X-ray Absorptiometry
FN	Femoral Neck
GCTA	Genome-wide Complex Trait Analysis
GWAS	Genome-Wide Association Study
HR	Hazard ratio
IBD	Identity by Descent
LD	Linkage Disequilibrium
LS	Lumbar Spine
LSC	Least Significant Change
MAF	Minor Allele Frequency
MrOS	Osteoporotic Fractures in Men Study
MR	Mendelian Randomisation
PCA	Principal Component Analysis
QC	Quality Control
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SOF	Study of Osteoporotic Fractures
VOS	Vietnam Osteoporosis Study

Chapter 1. Introduction

1.1 Osteoporosis

Osteoporosis is a common musculoskeletal disease which is the result of an unbalanced bone homeostasis. Osteoporosis is characterised by low bone mineral density (BMD), microarchitectural deterioration of bone tissue and an increased risk of fracture[1]. Osteoporosis is often referred to as a "silent disease', as bone loss accumulates over a long period of time and a lack of symptoms to frequently monitor during this time. It remains unmonitored until a fracture event occurs, consequently followed with secondary health issues and possibly death[2].

Bone strength describes both the quality and density of bone. The density is commonly referred to as the "bone mineral density" (BMD). BMD is calculated as the amount of bone mineral in a defined area of bone tissue, and it is measured in grams by area (g/cm^2). BMD is usually measured using a 2-dimensional scan with a dual energy X-ray absorptiometry (DXA) in clinical practice. The World Health Organisation and National Osteoporosis Foundation define osteoporosis based on a T-score categorisation. A T-score between +1 to -1 is deemed a normal bone density. Those with a lower bone mass, referred to as osteopenia, will exhibit values between -1 to -2.5. Individuals with a score of -2.5 or lower indicates osteoporosis[3]. However, most individuals who sustain a fracture do not meet this T-score standardised model that defines osteoporosis (-2.5 or below). The acquisition of lower bone mass is due to the loss of bone over time, and it is very important to monitor this bone change over time[4].

The quality aspect of bone strength describes the bone turnover, mineralisation, and micro-architecture. Most studies on osteoporosis are related to areal bone density (g/cm^2), however, true bone density (g/cm^3) can also be measured. Quantitative computed tomography (QCT) can measure volumetric bone mass, such as cortical and trabecular BMD at the hip and lumbar spine[5]. QCT is much more effective in detecting lower bone mass than DXA which can only attain the bone mineral density and is unable to differentiate trabecular bone from cortical. The QCT technique utilises a specialised 3D volume technology that examines the soft bone, an area that is more susceptible to bone loss changes earlier than hard bone[6,7]. However, QCT is an expensive alternative to DXA and exposes patients to a higher dosage of radiation[5].

DXA and volumetric QCT is typically used to determine the bone macrostructure but not the bone microstructure. Instead, bone microstructure is quantified using preclinical imaging techniques such as microCT, microMR, high-resolution CT, and high-resolution MR[8]. Although from lumbar spine DXA images, the trabecular bone score (TBS) is a novel tool that can indirectly measure the bone microarchitecture. In cross-sectional and longitudinal studies, TBS has also reported to be able to predict fractures[9]. Therefore, TBS can be a highly useful tool to monitor for low bone mass and predict fracture risk.

A compromised bone architecture with low bone mass leads to reduced bone strength. This reduced bone strength ultimately results to one of the severe outcomes of osteoporosis, fragility fractures. When the relative lifetime risk of fracture and other diseases were assessed, it was observed that the lifetime risk of hip (16%), Colles' (15%), and vertebral (32%) fracture was higher than the risk of developing endometrial (3%) or breast (9%) cancer[10]. BMD is used as a fundamental

predictor of fracture risk. For every 1 standard deviation reduction of the normal mean BMD, it correlates to between 1-2 times increase in fracture risk, with a 2.5-fold increase for subsequent hip fracture[11-13]. In terms of residual lifetime risk of fracture, it was reported to be 44% and 25% for women and men, respectively. But when BMD T-scores are -2.5 or below, the risks escalated to 65% in women and 42% in men[14].

For several decades, osteoporosis wasn't classified as a primary disorder of the skeleton. Instead, when inspected on plain film, it would be classified as a syndrome when a combination of vertebral fractures, osteopenia and back pain was reported[4]. Now, it is considered as the most highly common disease among the elderly population. The prevalence of osteoporosis around the world is estimated to be more than 200 million people. Osteoporosis is more common in women than men, with approximately 1 in 3 women and 1 in 5 men aged over 50 years estimated to develop osteoporosis[2]. Additionally, as women and men age, the prevalence of osteoporosis will progressively increase. A study showed the prevalence of osteoporosis in women in their 50s was 15.2% and exponentially increased to 85.8% in their 80s. A similar trend was also seen for men, which shows the severity of advancing age on osteoporosis prevalence[15].

Several studies have investigated the prevalence of osteoporosis in different populations around the world. Although the prevalence and risk of osteoporosis can also differ between ethnicities. This can be due to several different reasons. A huge portion of studies related to osteoporosis have been focused on Caucasian populations or those of European descent. A study with multiple ethnicities, including African, Asian, Hispanic, Native American, and predominantly of European ancestry participants depicted BMD and fracture risk differences between the various

demographics. Amongst the various ethnicities, individuals of African ancestry exhibited the highest BMD, whereas Asians had the lowest. In terms of fracture risk, Caucasians and Hispanics had the highest risk of fracture, followed by Native Americans, blacks, and Asian Americans in descending order[16]. Despite the strong relationship between BMD and fracture, it is intriguing to note that the ethnic population that had the highest BMD did not have the lowest risk of fracture. Typically, Caucasian and Asian populations have been reported to exhibit a higher risk of fracture compared to other ethnicities[17].

Fracture is associated with increased morbidity and mortality. The incidence of a fracture dramatically increases the risk of morbidity and mortality, compared to those without a fracture incidence. After sustaining a fracture at the hip, vertebral and other fracture sites, patients aged 50 years and older have a 1.5 to 4-fold greater mortality rate within the first year of the fracture incident[18-20]. Particularly for hip fracture incidences, only 30-40% of patients will fully recover their prior functionality[21,22]. Hip fracture results to informal care after hospitalisation of patients. Patients with vertebral fractures will also experience a great health burden[23].

Fractures are associated with increased economic burden. This economic burden is partitioned into direct (hospitalisation, surgery, medication, rehabilitation, outpatient services) and indirect costs (loss of functional status and working days, disability, home equipment, home care costs). Indirect costs were estimated to be higher than direct costs. The increase in fracture risk is reflected in all skeletal sites. Hip and vertebral fractures are predominantly the classic osteoporotic fractures considered[12]. However, the incidence of other fractures can be more frequent than hip and vertebral fracture; and collectively have a greater economic cost. The cost of hip or vertebral fractures is a larger financial burden at 60 years and older, however,

other fractures contribute to higher costs prior the age of 60 years[24,25]. The direct annual cost of fractures is estimated to be between $5000 to $6500 billion. In addition, indirect costs incur further financial burdens on the health system. When the costs of hip, vertebral and other fractures are calculated in combination, it is a huge economic burden, especially with the rapid increase of an ageing population globally[26].

1.2 Bone loss

BMD is a dynamic trait of bone that varies with age and gender (Figure 1.1). During the early years of life, BMD slowly accumulates until adolescence. During this critical developmental period, BMD increases rapidly with approximately 90% of bone mass acquired by age 20. This adolescent period represents a unique opportunity to maximise bone strength and mass, as the skeleton is highly responsive to mechanical loading, nutrition such as calcium and vitamin D, and hormonal influences. Gender differences emerge prominently during sexual maturation, as on average, men attain higher peak BMD than women due to larger skeletal dimensions, greater muscle mass, and androgenic hormonal effects[27]. Women reach peak bone mass earlier, generally at the ages 15-20 years compared to men who will reach it on average around their early to mid-20s[28].

Following peak bone mass, BMD stabilises for approximately 10 years between the ages of 25-40, during which bone remodelling maintains structural integrity without drastic net gain or loss. This plateau period ends around age 40, when BMD begins declining due to disrupted balance between bone resorption and formation[29,30]. This imbalance stems from multiple factors: hormonal changes such as estrogen deficiency in postmenopausal women, reduced mechanical loading from

decreased physical activity, impaired osteoblast function, and cellular aging processes. Women experience substantially greater bone loss than men, particularly during the first 5-10 years after menopause, while men can show a more gradual decline beginning around ages 40-50[31-34]. Notably, the rate and extent of bone loss vary considerably between individuals with similar baseline BMD (illustrated by diverging trajectories in Figure 1.1 after age 40), influenced by genetic factors, nutritional status, physical activity levels, and comorbidities[28,35].

Bone loss refers to the reduction of bone mass over time in an individual and occurs at both cellular and tissue levels. At the cellular level, increased bone turnover leads to more remodelling units, with osteoclast-mediated resorption exceeding osteoblast-mediated formation so that remodelling cycle removes slightly more bone than it replaces. Age-related changes such as oxidative stress, altered osteoblast and osteoclast responses, and accumulation of genetic and molecular damage further disrupt this balance and contribute to progressive skeletal fragility[36]. At the tissue level, these processes manifest as thinning and perforation of trabeculae and increased porosity of cortical bone, so that the volume of newly formed bone is greatly outnumbered by bone that has been resorbed[28,37]. Even individuals who achieve similar peak BMD in early adulthood can experience very different rates of age-related bone loss, driven by differences in lifestyle factors including diet, physical activity, and smoking; comorbidities; medications, and inherited susceptibility[28,35]. Genetics appears to account for a substantial proportion of variability in both peak bone mass and subsequent bone loss among individuals with comparable baseline BMD.

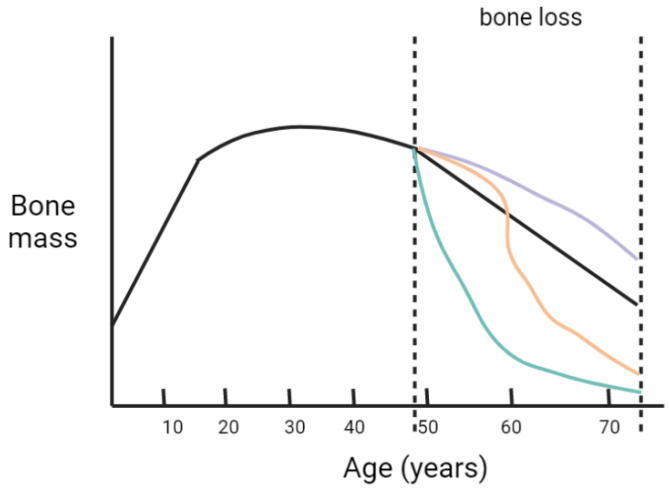

Figure 1.1 BMD progress over an individual's lifespan.

The black curve illustrates rapid bone mass accumulation during adolescence until peak bone mass is attained. The subsequent plateau in early adulthood reflects a period of relative skeletal stability, followed by the onset of age-related bone loss from about 40-50 years onwards. The three coloured lines after midlife represent slow, mild, and accelerated bone loss, emphasising how bone loss varies between individuals – despite acquiring the same baseline BMD.

1.3 Significance of bone loss

Bone loss is a major contributor of osteoporosis. Bone loss is a consequence of disruption of normal bone remodelling, the tightly regulated process that maintains skeletal strength. Under healthy and normal conditions, bone is continually renewed in 'basic multicellular units' in which osteoclasts resorb small packets of old or micro-damaged bone and osteoblasts subsequently replace this tissue with new bone. This sequence is coordinated by osteocytes, which sense mechanical loading and microdamage and regulate the coupling of resorption and formation through signalling pathways (e.g., RANKL/OPG and Wnt-related signals). In youth, resorption and formation are generally balanced, so bone mass remains stable. With age – and under the influence of hormonal changes (particularly reduced estrogen), oxidative stress, inflammation, inactivity, and other risk factors – this coupling becomes less efficient: osteoclast-mediated resorption is prolonged or increased, while osteoblast-mediated formation and mineralization decline[36,38]. As a result, each remodelling

cycle can end with a small deficit, so that slightly more bone is removed than replaced. Over time, this chronic negative balance leads to a gradual reduction in bone mineral density and deterioration of bone microarchitecture (e.g., trabecular thinning/perforation and cortical thinning/porosity), ultimately increasing susceptibility to fragility fractures from minimal trauma, such as a fall from standing height[39].

Bone loss is an endpoint for osteoporosis treatment. Once the detrimental outcomes of bone loss occur; treatment is needed. Typically, the treatments are hormone-replacement therapy or the prescription of drugs such as bisphosphonates, denosumab, and bone-building medications. Often, these treatments and therapy are used to alleviate symptoms of osteoporosis but can't clear osteoporosis in patients[40]. Instead of managing and minimising symptoms of osteoporosis, an effective alternative would be to provide earlier interventions with a focus on bone loss. The appropriate prescription of lifestyle behaviours, with an individual's genes taken into consideration, that promote bone growth and health can be an effective alternative.

Bone loss is a major risk factor for fracture. The gradual and continuous loss of bone decreases the quantity and quality of bone, to the point where the bones become very weak and brittle. The other risk factors of fracture, such as age, smoking, alcohol consumption, nutritional deficiency, sedentary lifestyle, and co-morbidities, are closely linked to bone loss. The changes of bone are influenced by these risk factors of fracture, which can influence if bone resorption occurs more frequently than bone formation and remodel the bone to be thinner and weaker[41,42]

As part of the pathophysiological domino effect of bone loss – leading to the development of osteoporosis and increased risk of fracture, it is also ultimately linked

to a higher rate of mortality and morbidity. Following a fracture incident, a drastic change to an individual's lifestyle occurs with a lower health status from pain and reduced mobility. The worst outcome is fracture events resulting to death[43]. The risk of mortality is higher for those with fracture incidences at the hip and vertebral. Particularly, men exhibit a higher rate of mortality than women[44].

1.4 Epidemiology of bone loss

Determinants of peak bone mass and subsequent bone loss are better viewed on a continuum rather than as strictly 'modifiable' or 'unmodifiable'. Some factors are largely fixed (e.g., age, sex, ancestry and inherited genetic variation), whereas others are biologically regulated and partly heritable but may still be altered in certain settings (e.g., hormonal milieu, body composition, and nutrient metabolism including vitamin D/calcium absorption). Behavioural and environmental exposures such as smoking, alcohol intake, diet, and physical activity are conventionally treated as modifiable, although susceptibility to these behaviours is also partially heritable, reflecting gene–environment correlation and interaction. Epidemiological methods (twin/family designs, within-family analyses, polygenic score adjustment, Mendelian randomisation where appropriate, and formal gene–environment interaction models) can help disentangle genetic liability from environmental effects and identify modifiable pathways through which bone loss and fracture risk may be reduced[45,46].

1.4.1 Age and sex

As we age, bone loss occurs at a faster rate and the biological mechanisms for bone formation is slower. From the ages of 25 to 40, our bone mass stays relatively stable with an equal quantity of bone formation and resorption. However, after the age of 40 and beyond, the breakdown of bone begins to outpace bone

formation. This imbalance causes an acceleration of bone loss. Ultimately, resulting to the weakening of our bones and increased risk of osteoporosis[39].

Women are at a greater risk of bone loss than men. Women begin with a lower peak bone mass, smaller bones, and reduced bone strength than men. Once menopause begins, the loss of bone is further exacerbated. Due to this, women tend to have a higher prevalence of osteoporosis and risk of fracture than men. Despite this, men still have a high risk as well. Women tend to have more fracture incidences but when men sustain a fracture, they often have worse outcomes. It is important to keep in mind the disparity with the number of studies of osteoporosis in older men compared to postmenopausal women. There is extensive information on the impact and extent of bone loss and fracture risk in women, but the studies on men does not match that. Therefore, the impact and effect in men could be much worse than previously reported[47].

1.4.2 Smoking and alcohol

Heavy drinking and smoking have detrimental effects on bones. Tobacco contains many harmful compounds, most notably nicotine which is the most abundant and toxic component in cigarettes. Nicotine affects the permeability of blood vessel walls, in turn, adversely affects absorption and activity of nutrients in and out of blood vessels, such as protein and calcium. The other toxic compounds in tobacco will additionally stimulate higher rates of bone resorption, decrease the production of osteoblasts, impair the protective effects of estrogen replacement therapy, and introduce excessive acidity in the blood. In addition, smoking can promote early menopause, thereby accelerates the timeline of metabolic degradation of estrogen. The combination of these detrimental effects results to the accumulation of increased bone loss and susceptibility to develop osteoporosis[48].

Excessive alcohol consumption during the adolescence and early adult years compromises peak bone mass and overall bone health. It is challenging to elucidate the exact mechanisms of action of alcohol intake on bone, as the impact of alcohol can vary based by the timeline and level of alcohol exposure. The direct effects of alcohol on bone metabolism is inhibiting the differentiation of mesenchymal stem cells to osteoblasts[49]. Consequently, the damaging effects of chronic alcohol consumption on bone can't be reversed, even if alcohol consumption has ceased.

1.4.3 Body composition

There is an extensive collection of epidemiology studies that have shown the association between bone mass and body weight, most distinctly, observations of high BMI correlates to high bone mass in individuals. Based on these observations, it has been implied that individuals with a higher body weight will be protective against bone loss and fracture[49,50]. In contrast, older women with smaller body sizes, therefore reduced BMD at their hip were at higher risk of hip fractures[51]. However, this is not applicable to everyone as another study comparing the BMD and fracture risk differences among older women did not find the same trend[16]. Asians, who were noted as showing the lowest BMD did not subsequently show the highest risk of fracture. Rather, the opposite was observed compared to the other ethnicities in the study. Therefore, the effect of body composition on bone mass is conflicting.

Body weight is separated into two main components – lean mass (LM) and fat mass (FM). Several studies show LM as a stronger predictor of BMD across ages, sexes, and skeletal sites including femoral neck, spine, and hip, primarily due to muscle contractions generating mechanical loading on bones[52-54]. FM shows a more complex relationship including varying effects at different skeletal sites, an initial increase of BMD by FM plateaus; and impairment of bone strength index or fracture

risk. In contrast, loss of LM shows a relatively higher increased fracture risk than FM gain[55-58]. To preserve BMD, promoting LM over total weight gain is key, especially when FM effects can weaken at higher BMI[54,59].

1.4.4 Physical activity

Physical activity is a source for changes in mechanical loading and muscular activity which is why the skeleton is designed as a dynamic tissue. As previously discussed, during the early and adolescence years is the crucial period when peak bone mass is acquired, therefore, it is also an important time period to strengthen bones. A regular exercise routine is one of the main non-pharmacological interventions used to enhance bone mass and integrity and neuromuscular aptitude. During puberty, regular physical activity helps to strengthen bone architecture and expand the bone surface. Subsequently improving the skeleton's resistance against falls and fractures in the future[60].

To acquire an ideal peak BMD and geometry, health professionals can prescribe an exercise regime that includes the optimal duration, type, frequency and intensity tailored for a given individual. Additionally, a long duration for each exercise sessions isn't required when the appropriate number of cycles has reached the threshold. It is highly recommended that exercise styles that follow novel, short-duration and high-intensity are applied. Cross-sectional studies of high-impact sports such as squash, gymnastics, and volleyball have proven to be effective in applying multi-directional stress to the bone to accumulate significantly larger BMD. These dynamic sports were shown to enhance BMD at the femoral neck and lumbar spine by 20-30%, compared to running and swimming which mainly move in single directions[61,62]. Furthermore, the implementation of regular physical activity has

shown indirect benefits, including a higher exposure of vitamin D and absorption of calcium.

1.4.5 Nutrition

Our bones require the appropriate nutrients for healthy growth and development, such as calcium, vitamin D, protein, magnesium, and potassium. Calcium and vitamin D are the primary nutrients that are crucial for a healthy peak bone mass and maintenance of bone health. Our bones contain approximately 99.5% of the calcium in our body, which emphasises how integral it is for building and maintaining the architecture of bone[63]. When there is an insufficient amount of calcium in our diet, calcium is removed from the bone and resourced to other areas of the body that require calcium. The removal of calcium is orchestrated by the parathyroid hormone (PTH), a key contributor of bone resorption. PTH targets osteoblasts which indirectly increases differentiation and activity of osteoclasts[64].

The importance of vitamin D is intimately linked with calcium. Vitamin D's major active form is 1,25-dihydroxyvitamin D, and insufficient levels of vitamin D leads to reduced calcium absorption. This causes a downstream effect of calcium imbalance, thereby an imbalance of bone homeostasis that favours bone resorption. A common intervention to alleviate the symptoms of osteoporosis is to maintain an adequate level of calcium and vitamin D[65].

1.4.6 Medication

A primary cause of secondary osteoporosis is drug-induced, typically glucocorticoids are the common source of drug-induced osteoporosis. Glucocorticoids is a drug commonly used to fight inflammation and treat health conditions with overactive immune systems. However, glucocorticoids are not

beneficial for bone health. Glucocorticoids promote bone loss by reducing the number and activity of osteoblasts, in turn, enhancing osteoclast activity. Glucocorticoids can directly increase the expression of RANKL and macrophage colony stimulating factor to produce more osteoclasts. Furthermore, the other pathways that glucocorticoids can contribute to bone resorption is by reducing the expression of sex hormones, calcium absorption and vitamin D activity[66].

1.4.7 Co-morbidity

The presence of co-morbidities can exacerbate an individual's current poor bone health or contribute to developing a lower bone mass. Diseases such as cancer, chronic inflammatory conditions, endocrine disorders, and gastrointestinal conditions are associated with low bone density and can directly contribute to bone loss. The mechanisms of each disease are complex and mediated through the drastic fluctuations of bone remodelling imposed by these diseases.

Chronic inflammatory diseases are commonly related to systemic bone loss. Examples of chronic inflammatory disorders include rheumatoid arthritis, Crohn's disease, coeliac disease, chronic obstructive pulmonary disease, asthma, renal diseases, and conditions affecting muscle and nerve. The systemic inflammation associated with these diseases directly affects the bone remodelling dynamic, often contributing to bone loss than bone gain. To treat inflammatory diseases, glucocorticoids is a common option, however, as mentioned previously, glucocorticoids have a negative effect on bone. Those who suffer from an inflammatory disease undergo lifestyle changes to their nutritional and active state. These diseases can induce the loss of lean mass, exercise and mobility which reduces the mechanical stimulation and strengthening of bone[67].

Cancer patients have a higher risk for bone loss and fractures from the effects of cancer and side effects of cancer therapies on the skeleton. Cancer-associated bone loss has primarily been studied in breast and prostate cancers; however, almost all cancers have a negative effect on a patient's bone health. The deleterious effects of cancer on bone loss are mediated through the changes in sex hormones, particularly estrogen and testosterone, during the development and treatment of cancer[68,69]. Breast and prostate cancer are characterised by estrogen and testosterone deficiencies which result to excessive breakdown of bone, subsequently followed by degradation of the bone microarchitecture and bone loss[68].

Inflammatory bowel disease (IBD) and other gastrointestinal diseases is associated with developing low bone mass, thereby placing patients at a higher risk of bone loss. IBD is a type of intestinal disorder characterised by inflammation in the digestive tract. The effect of gastrointestinal diseases on bone loss is driven by nutritional deficiencies from the defective absorption of calcium, vitamin D and other minerals, chronic inflammation, and long-term use of corticosteroids. The nutritional deficiencies will directly affect attaining the optimal peak bone mass and maintaining a relatively high and stable BMD before the age of 40[70,71].

There are many other conditions that contribute to the pathophysiology of bone loss, such as endocrine disorders, dementia, chronic kidney disease, hypogonadism, diabetes and several others. The following papers discuss the effect of co-morbidities on the severity of osteoporosis in greater detail[72-75].

1.4.8 Genetics

Previous twin and family studies have so far identified genetic factors that account for 25-35% of variance in bone loss[76,77]. In contrast, there has been a

greater number of genetic studies that have identified hundreds of susceptibility loci for osteoporosis, BMD, and fractures at a single time point. To the extent where the heritability of BMD has been determined with a variance of 50 to 90%[78-80]. The majority of the susceptibility loci fall within the non-coding regions of the genome[81-83]. Based on this, it is highly likely that the bone loss susceptibility loci will also mostly be located within the non-coding regions.

The extrinsic, modifiable factors can only contribute a small percentage of the variance in bone loss. A genome-wide association analysis of bone loss is required to explain the variation and genes of bone loss. Most of the genome-wide association studies (GWAS) conducted have been focused on BMD, fractures, and other related-phenotypes. These GWAS have identified hundreds of important susceptibility loci[83]. The current genetic studies on longitudinal bone change have been very limited, with the current GWAS investigations on heel BMD[84], and distal radius and tibia[85]; and candidate gene studies on FTO and COLIA1[86,87]. To date, GWAS and related genetic studies on bone loss – a key contributor of osteoporosis – remains very limited. This limitation makes it hard to understand several aspects of bone loss and how it heavily influences osteoporosis in varying ways in each individual. Such as, what is the genetic basis of low bone mass, inter-individual differences in bone loss rates despite the same bone mass, why low bone mass doesn't always correlate to a higher fracture risk?

Despite the lack of GWAS conducted on bone loss, the genes and pathways associated with BMD and other osteoporosis-related phenotypes are likely attractive candidates for bone loss. Genes involved in skeletal development, maintenance of bone turnover, and estrogen endocrine pathway have been identified as candidates to influence BMD. However, most of these potential bone loss-related genes were

identified in GWAS or genetic studies in cross-sectional BMD studies[83,88]. To gain a better understanding of the genetics of age-related bone loss, longitudinal BMD studies are required. Serial BMD measurements over time can better elucidate the dynamic changes of bone loss in each individual and between individuals. This can show the inter- and intra-individual variance of bone loss-related genes.

1.5 Mechanism of bone loss

Bone is a dynamic tissue which is the structural unit of the skeleton, an organ that is constantly adapting its structure. It undergoes changes either through bone modelling or remodelling[89]. The shape and size of bone can be malleable and receptive to changes from different mechanical loadings. Shaping and reshaping bones is accomplished in the process known as bone modelling. During bone modelling, either bone formation or resorption occurs for a given bone surface. Whereas remodelling regulates bone homeostasis by maintaining the skeleton's mechanical integrity when it needs to repair and replace damaged bone. During this maintenance, bone resorption followed by bone formation will occur at the same spatial location. Through the remodelling process, bone undergoes this continual self-regeneration process until we hit an older age when bone resorption surpasses bone formation[8,90]. Bone loss occurs when the normally coordinated activities of osteoclasts and osteoblasts become uncoupled, such that osteoclast-driven resorption persistently exceeds osteoblast-driven formation. Even small deficits in each remodelling cycle accumulate over time, resulting in progressive reductions in bone mass and deterioration of bone microarchitecture[38].

The influence of muscle forces is a key determinant of the growth, development and changes in bone structure. In particular, the relationship between

body mass and bone mass underlines the effect of muscle forces[91]. For example, the concept of greater body mass imposing a larger mechanical loading on bone, thereby stimulating the bone to increase in mass to accommodate the greater load. When the mechanical load is greater than their bone strength, it results to skeletal fractures[92]. Therefore, additional attention on muscle growth and its positive effect on bone structure should be kept in mind.

Systemic hormones play a key role in the muscle-bone relationship, referred to as the “bone-muscle unit”. Specifically, estrogen and testosterone are crucial for skeletal development and the maintenance of bone health. Deficiencies in sex hormones can be seen both in the earlier and later years of women and men[93,94].

Estrogen is a key hormone that plays a significant role in bone maintenance in both women and men. It is a key promoter of osteoblasts and inhibits bone resorption. At the cellular level, estrogen helps maintain remodelling balance by limiting osteoclast formation and activity while promoting osteoblast survival and function. When estrogen declines, this balance is disrupted and bone resorption increasingly outweighs bone formation, so each remodelling cycle ends with a slight net loss. Over time, these repeated losses contribute to lower BMD and weakening of bone structure[95]. The effect of estrogen is commonly studied in the context of women’s bone health; however, estrogen also has a direct effect on age-related bone loss in men. In women, the reduction in ovarian estrogens during menopause is followed by major declines in BMD. After 10 years, an estimated loss of 9.1% and 10.6% is observed at the femoral neck and lumbar spine. As men age, the level of their sex steroids drops which also makes them vulnerable to declines in BMD[96].

Although estrogen is the primary hormone in the bone health of women and men, testosterone still plays a role on the bone. Along with estrogen, age-related testosterone deficiency contributes to bone loss in elderly men. Low levels of testosterone are associated with a decreased BMD and increased fracture risk in men[97]. The effects of testosterone have primarily been studied in men, however, there are evidence of potential effects on the bone mass of women. Higher levels of testosterone were associated with higher BMD at the femoral neck and lumbar spine. Additionally, testosterone was linked to greater lean mass which is a protective factor for bone health[98].

1.6 Conclusion and Future research

There have been many extensive genetic studies, including GWAS, conducted on BMD and other osteoporosis phenotypes, such as fracture risk, femoral neck geometry, bone turnover markers, and ultrasound bone properties[99,100]. However, there has been minimal genetic studies and no GWAS conducted on bone loss. Despite the importance of bone loss on peak bone mass and low BMD, little is known about the genes or genetic variants associated with age-related bone loss.

Due to this, a GWAS on bone loss is required to understand the complexities of low bone mass. For example, to better understand why two individuals may have the same baseline BMD but one of them will develop a lower BMD and higher risk of a fracture event. Environmental factors aren't sufficient to explain the variance in bone loss, as the pathophysiology lies in the genetic profile of an individual. Individuals with low BMD are often at a greater risk of fracture. To unpack the genetic architecture of bone loss, I propose to pursue the following objectives:

To ascertain the association between bone loss and fracture risk in elderly men and women.

To analyse the association between bone loss and the single nucleotide polymorphisms (SNPs) of a candidate gene.

To undertake a genome-wide association study (GWAS) to identify SNPs that are related to bone loss in elderly men and women.

Chapter 2. Study design and Analytical methods

2.1 Overview

Four prospective, longitudinal studies were used to study the genetic epidemiology of bone loss (Chapters 3 – 5). The first study was based on two prospective US cohorts – The Study of Osteoporotic Fractures (SOF) and The Osteoporotic Fractures in Men Study (MrOS). These two cohorts were used to investigate the association between bone loss and fragility fractures, one of the most severe outcomes of osteoporosis (Chapter 3). Next, the second study involved the Australian prospective, population-based cohort – the Dubbo Osteoporosis Epidemiology Study (DOES). In this Australian cohort, the association between polymorphisms at the fat mass and obesity-related transcript (*FTO*) gene and osteoporosis phenotypes, including bone loss, was analysed (Chapter 4). The final study was extended to search across the entire genome for genetic variants associated with bone loss. The final study was based on the Vietnam Osteoporosis Study (VOS).

This methods chapter presents an overview of the main contributing cohorts, their phenotypes and outlines the study design and methodology constructed for the genetic and epidemiological analyses in this thesis. Please note: - I was not involved in the subject recruitment or the construction of the study design of each cohort. A more comprehensive description of the study design, measurements, outcome assessments of each cohort is provided in each results chapter. The study designs of each cohort are also provided below.

2.2 Study population

2.2.1 Study of Osteoporotic Fractures (SOF)

SOF is a large prospective cohort study that has been an invaluable resource for studies on osteoporosis and aging[11,101]. The study recruited 9704 community-dwelling white women aged 65 years and older from four areas of the United States: Baltimore County, MD; Minneapolis, MN; Portland, OR; and the Monongahela Valley near Pittsburgh, PA. African American women were excluded from the initial SOF study because of their lower rate of hip fracture.

2.2.2 Osteoporotic Fractures in Men Study (MrOS)

MrOS is a multicentre prospective study of risk factors for osteoporosis and fractures[102,103]. The study recruited 5995 community-dwelling predominantly white men aged 65 years and older at six clinical sites in the United States: Birmingham, AL; Palo Alto, CA; Minneapolis, MN; San Diego, CA; Portland, OR; Pittsburgh, PA. Of the 5995, 633 (10.6%) were non-white or Hispanic. Women and men with bilateral hip replacements and those unable to walk without assistance were not eligible to participate. Consent forms and conduct for SOF and MrOS were approved by the Institutional Review Boards at all participating institutions.

2.2.3 Dubbo Osteoporosis Epidemiology Study (DOES)

The protocols and study structure of DOES has been described elsewhere[104]. In short, DOES was designed as a prospective, population-based investigation that was operational from 1989 to 2020. Since 1989, all men and women aged 60 years and older living in Dubbo, a city of ~32,000 people 400 km northwest of Sydney, Australia were invited to participate in the study. Approximately 4000 men and women aged 60 years and older have been recruited and followed up for almost 30

years. The study was approved by the St Vincent's Campus Research Ethics Committee and written informed consent was acquired from each participant.

2.2.4 Vietnam Osteoporosis Study (VOS)

The rationale, study design, and operating procedures for the VOS have been reported elsewhere[105]. In brief, VOS is a population-based family cohort established in 2015 in Ho Chi Minh City and nearby rural regions of Vietnam. It was designed to provide a comprehensive assessment of skeletal health, quantify the burden of osteoporosis, fractures, osteoarthritis, multimorbidity, and related conditions (e.g., diabetes, hypertension, sarcopenia), and to explore genetic determinants and gene–environment effects on these outcomes. The primary objectives were to: (1) estimate the prevalence of osteoporosis and osteoarthritis and the incidence of fractures; (2) determine the extent to which genetic factors contribute to inter-individual variation in these traits; and (3) evaluate gene–environment interactions.

Ethical approval was obtained from the research and ethics committee of People's Hospital 115 (Ho Chi Minh City), and all procedures adhered to the Declaration of Helsinki. The cohort comprises 4,167 participants (65% women; mean age ~52 years; ~45% aged ≥50) from 817 families, recruited through random community sampling and media outreach. The sample is predominantly urban, with low obesity prevalence (~3%), diabetes prevalence of ~11% among participants aged ≥40, and osteoporosis prevalence of ~14% in women and ~5% in men aged ≥50 (femoral neck BMD T-score ≤ −2.5). Detailed phenotyping included areal BMD, body composition, trabecular and cortical bone parameters, lifestyle assessments, fasting blood collection for plasma glucose, HbA1c, and bone turnover markers, and whole blood for genomic DNA extraction. Written informed consent was obtained from all participants, who were free to withdraw at any time.

2.3 Phenotypic and covariates data

2.3.1 Dual X-ray Absorptiometry

VOS

BMD at the femoral neck (FN) and lumbar spine (LS) was measured using the Hologic densitometer (Hologic Corp., Bedford, MA, USA). As a quality control, before every measurement, the densitometer was calibrated using a standard phantom scan. The co-efficient of variation was 1.8% and 2% for femoral neck BMD (FNBMD) and lumbar spine BMD (LSBMD), respectively.

DOES

BMD (g/cm^2) at the femoral neck (FNBMD) and lumbar spine (LSBMD) was measured at baseline and biennially by a DXA instrument using a LUNAR DPX densitometer (GE-Lunar, Madison, WI, USA). The coefficient of variations of BMD measurement of the femoral neck and lumbar spine was 1.5% and 1.3%, respectively[106]. All measurements were conducted by the same technicians, following the same standard operating procedure.

SOF and MrOS

BMD at the proximal femur and lumbar spine were measured using dual-energy X-ray (DXA) absorptiometry with the QDR-1000, QDR-200 (SOF: visit 2; 1988-1990), or QDR-4500 (MrOS: visit 1; 2000-2002) scanners (Hologic, Waltham, MA). For both studies, the right proximal femur was scanned unless the participant reported a right hip replacement, other metal object(s) or severe degenerative change, in which case the left hip was scanned. Standardised procedures for participant positioning and analysis of scans were conducted for all scans. All DXA operators at the clinical centres were trained and certified centrally in their scanning and analysis techniques. As a quality control, including for longitudinal changes in

machine performance, DXA operators at the coordinating centre (University of California, San Francisco) reviewed a random sample of all scans, including regular scans of Hologic spine, exceptionally high or low BMD, problematic scans, and whole-body phantom scans at each site.

2.3.2 Anthropometric and lifestyle measurements

VOS

Participants were given a standardised questionnaire to collect demographic and clinical data. The standardised questionnaire included anthropometric and demographic characteristics, lifestyle factors, nutrition and dietary calcium intakes, reproductive history (women), clinical history (e.g., diseases), medication history, fracture history, fall history, and biochemical test results. Weight (kg) and height (cm), without shoes and indoor clothing, was measured using an electronic balance and by a handheld stadiometer. Body mass index (BMI, kg/m^2) was calculated as the weight (kg) divided by the square of height (m^2).

DOES

A nurse co-ordinator used a structured questionnaire to obtain baseline measurements (1989) and subsequent visits at approximately 2-3 yearly intervals. The structured questionnaire included age, weight, height, smoking and alcohol intake, physical activity, calcium intake, medication, number of falls in the last year, and fracture history. Weight (kg) and height (cm), without shoes and in light clothing, was measured on an electronic scale (to the nearest 0.1 kg) and by a wall-mounted stadiometer (to the nearest 0.1 cm). BMI (kg/m^2) was calculated as the ratio of weight (kg) and squared height (m^2).

SOF and MrOS

In both the SOF and MrOS cohorts, demographic, lifestyle, anthropometric, and medical history data were obtained through a questionnaire, interview, and physical examination. The variables obtained included demographics (e.g., age, height, weight), smoking status, alcohol intake, physical activity, fracture history (since age 50 years), and falls history. Weight (kg) and height (cm) were measured on a standard balance beam or digital scales and by a Harpenden stadiometer, respectively. Body mass index (BMI, kg/m^2) was calculated as the ratio of weight (kg) and squared height (m^2).

2.3.3 Outcome assessment

VOS

Anteroposterior and oblique digital X-rays (FCR Capsula XLII, Fujifilm Corp., Tokyo, Japan) were obtained from each participant. Two radiologists assessed the same radiograph independently, with X-ray readings reread if there was any discrepancy between their readings. A final decision on the X-ray results would be decided by the corresponding radiologist. Any discrepancies were solved by a joint consensual reading. The kappa coefficient among readers was 0.67.

DOES

All fractures were ascertained from the two or three radiology centres servicing the Dubbo area, and circumstances surrounding fracture were ascertained by personal interview following the fracture event. Only low-trauma fractures caused by a fall from a standing height or less were included in the study. Fractures caused by high trauma, such as motor vehicle accident, pathological fractures from bone diseases other than osteoporosis (e.g., hyperparathyroidism, Paget's disease), or fractures of the skull and digits were excluded. Vertebral fractures were determined

either through clinical diagnosis or detected incidentally on X-ray. Deaths were ascertained from funeral lists, obituary reports, and Dubbo media reports, and verified from the New South Wales Bureau of Births, Deaths, and Marriages.

SOF and MrOS

In both cohorts, participants were contacted by postcard every four months, with telephone follow-up for non-responders, to ascertain whether they had sustained a fracture. Additionally, they were also asked to notify the local clinical centre as soon as possible after any fracture. Follow-up contacts were >95% complete. Physicians assessed the radiological reports to confirm all fracture incidents and denote the degree of trauma. Fractures caused by high trauma, such as motor vehicle accidents, falls from greater than a standing height (e.g., ladder), physical assaults, or fractures of the skull and digits were excluded.

2.4 Genetic data

2.4.1 Array based genotyping

For the VOS cohort, genotyping was performed on 4056 subjects on an Illumina Infinium assay platform using a Global Screening Array microchip by the Australian Genome Research Facility at the Peter MacCallum Cancer Centre, Melbourne, Australia. Genome Studio (Illumina Inc.) was used for data pre-processing.

2.4.2 Quality control of genotyping data

VOS genotyping data were cleaned to identify and remove potential sources of confounding and bias, to avoid producing spurious associations[107]. All quality control was performed using a combination of PLINK[108] and R language[109], with the

tutorial developed by Marees *et al*. as a reference guide[110] (see Chapter 5 for condensed cohort summary and Figure 5.1 for exclusion criteria overview).

Autosomal heterozygosity

Participants with extremely high or low heterozygosity rates outside of ~4 standard deviations from the mean were excluded as this can suggest poor sample quality or little genetic variability (e.g., inbreeding).

Gender discrepancies

The observed (based on the genotyped X chromosome homozygosity) and self-reported gender of each participant was compared. Discrepancies between the observed and self-reported genders were excluded as they can indicate sample handling errors, incorrect gender allocation, or X chromosome anomalies.

Missing genotyping rates

Individuals exhibiting high levels of missing genotyping rates >3% were excluded, as this represents poor DNA quality of technical issues.

Hidden (cryptic) relatedness

To characterise potential cryptic relatedness within this family-based cohort, we calculated KING-robust kinship coefficients. Although we considered applying a 3–5% cut-off (approximately corresponding to third-degree cousin relationships), no participants were excluded; the full kinship distribution is provided in Supplemental Figure 1. Genome-wide association analyses were conducted using fastGWA-GLMM in the Genome-wide Complex Trait Analysis (GCTA) software package[111], which accounts for relatedness by incorporating a genetic relationship matrix[112,113].

Aligning to 1000G reference data

The genetic variants of the sample population were aligned to the 1000 Genomes (1000G) Phase 3 (hg38), to identify shared SNPs between the sample

population and 1000G reference data. SNPs from sample population that failed to align due to the presence of multiallelic variants (e.g., 3+ alleles present) were flipped and aligned again to the 1000G reference data. Genetic variants that still exhibited multi-allelic issues or had at least one non-A/C/G/T allele name were excluded.

Linkage disequilibrium-based SNP pruning

Pairwise linkage disequilibrium (LD) between SNPs was calculated using a 50-SNP sliding window (5-SNP shift, r^2 threshold = 0.5). SNPs in high LD were pruned, yielding a subset of genetic variants in approximate linkage equilibrium (pairwise $r^2 < 0.5$).

Population stratification

To control for populational stratification, a genetic relationships matrix of the SNP data was estimated among individuals using the GCTA software package[111]. A principal component analysis (PCA) was performed with the component scores plotted against a known ethnic structure (1000G data) to identify individuals who deviated from the samples target population – Asian ethnicity. Ethnic outliers were removed.

2.5 Genome-Wide Association Analysis

Following application of the quality control process, a genetic relationship matrix (GRM) was generated from our genotype data to account for relatedness within the sample. A sparse GRM was subsequently constructed to improve computational efficiency while retaining the essential structure of genetic relationships. Using the sparse GRM, quantitative trait analyses were performed through a linear model association framework, with appropriate covariates included

to minimise confounding effects. All genome-wide association analyses were conducted using the GCTA software package, which enabled efficient estimation of SNP effects in the presence of related individuals. For a comprehensive description of the statistical models applied, including the specific covariates used, please refer to the Methods section of Chapter 5 – Genome-wide association analysis of longitudinal change in bone mineral density: the Vietnam Osteoporosis Study (Section 5.2.4).

Chapter 3. Bone loss is associated with fracture risk

Preface: Bridge between Chapters 1-2 and Chapter 3

The first study aimed to establish the importance of bone loss as a risk factor for the development of osteoporosis. Low BMD is one of the key characteristics of osteoporosis, and to attain low BMD, a significant level of bone must be loss. Subsequently, leading to sustaining a fracture, another key characteristic of osteoporosis and a severe outcome.

The results from the Study of Osteoporotic Fractures (SOF) of this chapter were orally presented in the 33rd Australian and New Zealand Bone Mineral Society annual scientific meeting, Newcastle, Australia, 2023

Abstract

Background and Aim. Low bone mineral density (BMD) is a recognised predictor of osteoporotic fractures, but whether longitudinal bone loss independently contributes to fracture risk remains uncertain. This study aimed to test whether femoral neck bone loss predicts any fragility and hip fractures in elderly men and women.

Design and Methods. We analysed 5581 women from the Study of Osteoporotic Fractures (SOF) and 4623 men from the Osteoporotic Fractures in Men Study (MrOS), each with at least three femoral neck BMD measurements by dual-energy X-ray absorptiometry (Hologic). Bone loss rates were estimated using linear mixed-effects regression and classified as unchanged (<–1%/year), mild (–1% to –2%/year), or rapid (≤–2%/year). Incident fractures at 13 sites, including hip, were radiographically confirmed. Cox proportional hazards regression adjusted for baseline BMD, age, BMI, lifestyle factors, and prior fractures assessed the associations.

Results. During a median follow-up of 9 years, 1220 women and 513 men sustained fragility fractures, of which 522 and 120 were hip fractures, respectively. In women, bone loss was associated with fracture risk in age-adjusted models, but this was attenuated after full adjustment. In men, mild bone loss remained independently associated with hip fracture risk (hazard ratio ≈1.7).

Conclusion. Femoral neck bone loss is an independent predictor of hip fracture in men, but not women, after adjustment for baseline BMD and covariates. Monitoring longitudinal bone change may enhance fracture risk prediction beyond static BMD measures.

3.1 Introduction

The dynamic change of bone mineral density (BMD) is due to the development of peak bone mass and subsequent age-related loss of bone[27]. A combination of lifestyle factors and genetics can influence the rate of bone loss after attaining peak bone mass[41]. Some elderly individuals can gradually lose bone each year; while others experience no change or even a gain in bone[114,115].

Multiple clinical risk factors that contribute to osteoporotic fracture risk (e.g., age, BMD at the femoral neck, glucocorticoid use) have been studied, but the association between BMD change and fracture risk remains controversial. There have been contradicting reports where a change in BMD does predict the risk of fragility fractures[116-122] while others dispute this[123] or report repeat BMD measurements provide little additional value for fracture risk discrimination[124-126]. Previous reports have employed the use of two repeat BMD measurements, different statistical models, sample sizes, and follow-up time – likely establishing this uncertainty of BMD change as an independent risk factor for fractures.

Longitudinal biomarkers like bone loss are important phenotypes that reflect a dynamic view of BMD over time, revealing whether the bone density is stable, elevating, or declining – key information critical to assessing future fracture risk. Longitudinal phenotypes as clinical biomarkers have revealed a better approach to capturing an individual's risk trajectory and disease progression in COVID-19, cardiometabolic conditions and other diseases[127-130]. With the increasing digitalisation of health data, there is an unprecedented opportunity to leverage longitudinal biomarkers for improved clinical decision making[131-133]. The present study sought to determine the status of the trajectory of bone over time as an

independent risk factor for fracture in women and men by utilising a robust study design and longitudinal analysis methods.

3.2 Methods

3.2.1 Study design

Women were participants in the Study of Osteoporotic Fractures (SOF), a large prospective cohort study of risk factors for hip and other osteoporotic fractures[11,101]. The study recruited 9704 community-dwelling white women aged 65 years and older from four areas of the United States: Baltimore County, MD; Minneapolis, MN; Portland, OR; and the Monongahela Valley near Pittsburgh, PA. A total of 662 African American women were enrolled at the sixth visit and followed up till the ninth visit alongside the original cohort. This African American group was excluded from the analysis due to the limited BMD measurements available and fracture ascertainment after the third BMD measurement of the African American cohort.

Men were participants in the United States Osteoporotic Fractures in Men Study (MrOS), a multicentre prospective study of risk factors for fracture[102,103]. The study recruited 5995 community-dwelling predominantly white men aged 65 years and older at six clinical sites in the United States: Birmingham, AL; Palo Alto, CA; Minneapolis, MN; San Diego, CA; Portland, OR; Pittsburgh, PA. Women and men with bilateral hip replacements and those unable to walk without assistance were not eligible to participate. Consent forms and conduct for SOF and MrOS were approved by the Institutional Review Boards at all participating institutions.

Our analysis consisted of 5581 women and 4623 men with at least three BMD measurements and had not sustained a fracture during their first 3 BMD

measurements. We primarily included major osteoporotic fractures at 13 sites, including hip, upper and lower leg, pelvis, humerus, rib, clavicle, elbow, wrist, knee, ankle, hand, and foot. Vertebral fractures were not included in our analysis as only a time interval (i.e., visit 1-8; visit 3-8) was recorded in the SOF cohort, making the accurate estimation of the follow-up time impossible. Two fracture outcomes were analysed: 1) any fragility fracture including all 13 sites, and 2) hip. Any fragility fracture was reported as missing if at least one fracture site out of the 13 sites was recorded as missing. Due to this, we have varying sample sizes for each fracture outcome analysed (see **Figure 3.1**).

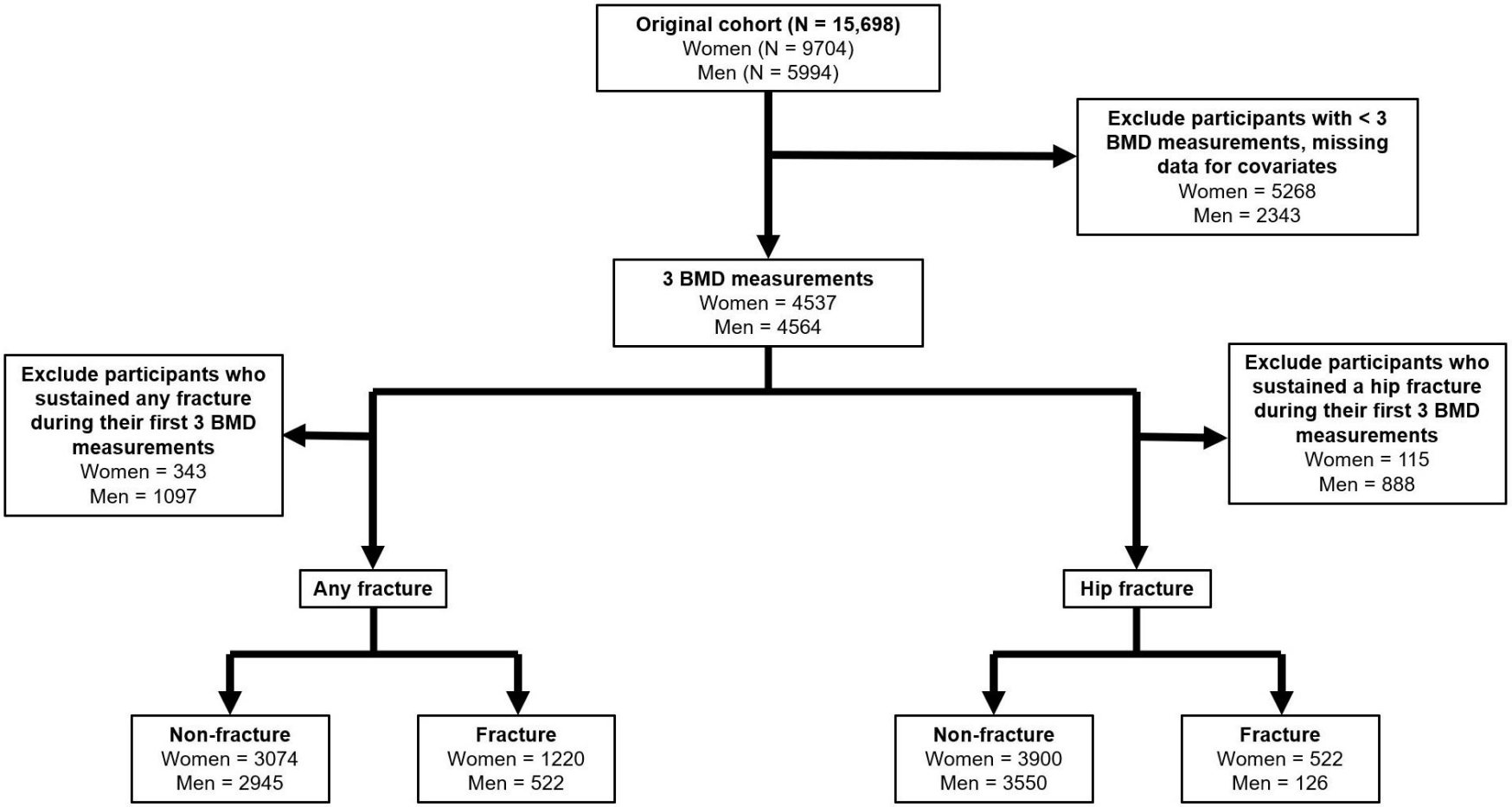

Figure 3.1 Flowchart of recruitment and follow-up. Abbreviations: BMD, bone mineral density.

Abbreviations: BMD, bone mineral density.

3.2.2 Bone mineral density measurements

BMD at the proximal femur and lumbar spine were measured using dual-energy X-ray (DXA) absorptiometry with the QDR-1000, QDR-200 (SOF: visit 2; 1988-1990), or QDR-4500 (MrOS: visit 1; 2000-2002) scanners (Hologic, Waltham, MA). For both studies, the right proximal femur was scanned unless the participant reported a right hip replacement, other metal object(s) or severe degenerative change, in which case the left hip was scanned. Standardised procedures for participant positioning and analysis of scans were conducted for all scans. All DXA operators at the clinical centres were trained and certified centrally in their scanning and analysis techniques. As a quality control, including for longitudinal changes in machine performance, DXA operators at the coordinating centre (University of California, San Francisco) reviewed a random sample of all scans, including regular scans of Hologic spine, exceptionally high or low BMD, problematic scans, and whole-body phantom scans at each site.

Our study primarily focused on femoral neck BMD, because it is less likely to be affected by degenerative changes compared to lumbar spine BMD measurement. In addition, we used femoral neck BMD to represent bone change due to the strong relationship of femoral neck BMD and fracture risk[134]. The coefficients of variations for proximal femur were 1.2% and 0.3-0.6% for women and men, respectively[103,135].

3.2.3 Covariate measurements

Demographic, lifestyle, anthropometric, and medical history data were obtained through a questionnaire, interview, and physical examination. The variables obtained included demographics (e.g., age, height, weight), smoking status, alcohol intake, physical activity, fracture history (since age 50 years), and falls history. Weight (kg) and height (cm) were measured on a standard balance beam or digital

scales and by a Harpenden stadiometer, respectively. Body mass index (BMI, kg/m^2) was calculated as the ratio of weight (kg) and squared height (m^2). To account for variations between groups, ethnicity was utilised as a covariate in our analyses for men. As the African American participants in SOF were not included, ethnicity was not included as a covariate for women.

3.2.4 Fracture ascertainment

Participants were contacted by postcard every four months, with telephone follow-up for non-responders, to ascertain whether they had sustained a fracture. Additionally, they were also asked to notify the local clinical centre as soon as possible after any fracture. Follow-up contacts were >95% complete. Physicians assessed radiographs or radiological reports to confirm all fracture incidents. Fractures caused by high trauma, such as motor vehicle accidents, falls from greater than a standing height (e.g., ladder), physical assaults, or fractures of the skull and digits were excluded. For this analysis, the first fracture incident sustained after the third BMD measurement, either any fragility or hip fracture, was our outcome.

3.2.6 Mortality ascertainment

Follow-up for vital status among women was 99.2% complete as of February 1997. Copies of original death certificates for all deceased participants were sent to the coordinating centre, where a study investigator, blinded to bone loss measurements and other predictor variables determined the cause of death using International Classification of Diseases, Ninth Revision (ICD-9) codes and, when available, hospital discharge records. Causes of death were classified as coronary heart disease (CHD; codes 410–414), stroke (codes 430–438), atherosclerosis (codes 401–404, 410–414, 425, 428, 429.2, 430–438, 440–444, and 798), cancer (codes 140–239), or other non-cancer, non-atherosclerotic causes.

Men were contacted via mailed questionnaires every four months following their initial visit, and clinic staff were typically informed of a participant's death during follow-up for missed contacts. Deaths were adjudicated centrally through physician review of death certificates and, when available, hospital discharge summaries, with causes classified using ICD-9 codes as cardiovascular disease (CVD; codes 396.9–442, 996.71, 785.51), cancer (codes 141.9–208.0), or other non-cancer, non-CVD causes.

3.2.6 Data analysis

A linear mixed-effects regression was employed to estimate the absolute rate of BMD change over time (g/cm^2/year) for each participant using the first three BMD measurements. A linear mixed-effects regression was shown to be more statistically robust to a conventional linear regression for analysis of repeated measurement as not only is it able to account for within- and between-subject variations, but also adjust for a regression-toward-the-mean phenomenon[136,137]. The relative rate of BMD change (%/year) was calculated by dividing absolute rate of BMD change by baseline BMD[116].

The rate of BMD change as a continuous variable was not normally distributed and unable to meet the assumption of co-linearity. Due to this, we categorised the rate of BMD change into distinct groups – unchanged, mild, and rapid bone loss. Given, the documented average loss of BMD being between 0.25 to 0.75% per year[138-142] – we denoted a BMD loss of less than -1% as the reference category that included individuals whose BMD remained unchanged, minimally lost (0 to 1% per year) or slightly gained. Additionally, we denoted individuals with -1 to -2% and less than -2% BMD loss per year as mild and rapid BMD loss, respectively.

For our primary analysis, a Cox's proportional hazards regression was used to quantify the association between categories of BMD change (%/year) and fracture risk, adjusted for predefined covariates of baseline BMD, age, BMI, smoking, drinking, physical activity, prior history of fractures, and ethnicity. The follow-up time for participants who had sustained an incident fracture was calculated between after their third BMD measurement and the date of fracture. For those without a fracture, the follow-up time was computed as a time interval between after their third BMD measurement and study end (2008), or date of death, whichever came first. The assumption of proportional hazards was graphically checked using the scaled Schoenfeld residuals[143].

We conducted a sensitivity analysis to account for the competing risk of death in our investigation. This analysis included the i) cause-specific hazard model, and ii) Fine-Gray sub-distribution hazard model. The cause-specific hazard model estimates the risk of a particular event – such as fracture – by modelling the cause-specific hazards for both the event of interest and competing events (e.g., death without fracture) separately. It is particularly suitable for aetiological research, as it assesses the effect of independent variables on the hazard of the primary outcome among individuals who are still at risk, allowing for a valid estimation of exposure-outcome associations. The Fine-Gray sub-distribution model estimates the absolute risk of an outcome over time by modelling the effects of independent variables on the cumulative incidence of the event, making it particularly suitable for predictive research. Unlike the cause-specific model, the Fine-Gray approach retains individuals who experience a competing event (e.g., death without fracture) in the risk set with diminishing weights, allowing for a more accurate prediction of real-

world event probabilities. The analyses were performed using R language[144] with a P-value of less than 0.05 considered statistically significant.

3.3 Results

3.3.1 Characteristics of subjects

This study was based on 4436 women and 3651 men whose baseline characteristics are shown in Table 3.1. Women with major osteoporotic fractures were significantly older, had a lower weight, BMI, and BMD at the femoral neck (FNBMD) than those without an incident fracture. In addition, women who sustained any fragility fractures were significantly older and were more likely to have a history of any fragility fracture after the age of 50 years. A similar trend was seen in men, with those who suffered any fragility and hip fracture incidents being significantly older and having a lower weight, BMI, and FNBMD. Furthermore, alcohol consumption was a significant lifestyle factor for hip fractures in men.

Intriguingly, women who sustained any fragility and hip fractures exhibited a significantly higher average annual rate of femoral bone loss at -1.00% and -1.11%, per year respectively, compared to those without fractures. Comparably, men fracture patients had 0.13-0.4% higher loss per year than their non-fracture counterparts. This significant difference is further displayed in the bone loss groups – unchanged, mild and rapid loss (**Table 3.1**).

Table 3.1 Baseline characteristics of participants classified by any and hip fracture status.

Women				
Variable	Non-fracture (N=3074)	Any fracture (N=1220)	Non-hip fracture (N=3900)	Hip fracture (N=522)
Age (years)	78 ± 4	**79 ± 4**	78 ± 4	**80 ± 4**
Weight (kg)	67 ± 13	**65 ± 12**	67 ± 13	**63 ± 11**
Height (cm)	158 ± 6	158 ± 6	158 ± 6	158 ± 6
BMI (kg/m^2)	27 ± 5	**26 ± 4**	27 ± 5	**25 ± 4**
Smoking: Current	249 (8.10%)	93 (7.62%)	315 (8.08%)	36 (6.90%)
Past	912 (29.7%)	373 (30.6%)	1177 (30.2%)	162 (31.0%)
Never	1913 (62.2%)	754 (61.8%)	2408 (61.7%)	324 (62.1%)
Alcohol consumers	1386 (45%)	547 (45%)	1772 (45%)	225 (43%)
Physical activity	2221 (72%)	873 (72%)	2819 (72 %)	368 (71%)
Prior fracture	911 (30%)	**527 (43%)**	32 (0.82%)	9 (1.72%)
FNBMD (g/cm^2)	0.65 ± 0.12	**0.60 ± 0.11**	0.64 ± 0.12	**0.57 ± 0.09**
Rate of BMD change (%/year)	-0.81 (0.92)	**-1.00 (0.95)**	-0.82 (0.92)	**-1.11 (0.97)**
Bone loss (%/year):				
Unchanged (loss <-1%/year)	1938 (63.0%)	**672 (55.1%)**	2434 (62.4%)	**257 (49.2%)**
Mild (loss from 1 to 2%/year)	894 (29.1%)	**402 (33.0%)**	1156 (29.6%)	**182 (34.9%)**
Rapid (loss > -2%/year)	242 (7.87%)	**146 (12.0%)**	310 (7.95%)	**83 (15.9%)**
Men				
Variable	Non-fracture (N=2945)	Any fracture (N=525)	Non-hip fracture (N=3550)	Hip fracture (N=126)
Age (years)	79 ± 5	**80 ± 5**	79 ± 5	**81 ± 5**
Weight (kg)	82 ± 13	**80 ± 13**	82 ± 13	**79 ± 12**
Height (cm)	173 ± 7	174 ± 7	173 ± 7	174 ± 7
BMI (kg/m^2)	27 ± 4	**26 ± 4**	27 ± 4	**26 ± 4**
Smoking: Current	51 (2%)	15 (3%)	64 (2%)	5 (4%)
Past	1718 (58%)	310 (59%)	2075 (58%)	74 (59%)
Never	1176 (40%)	200 (38%)	1411 (40%)	47 (37%)
Alcohol consumers	1867 (64%)	315 (60%)	2256 (64%)	**63 (50%)**
Physical activity	2116 (72%)	376 (72%)	2544 (72%)	84 (67%)
Prior fracture	442 (15 %)	**112 (21%)**	588 (17%)	21 (17%)
Ethnicity: Caucasian	2633 (89%)	**496 (95%)**	3200 (90%)	120 (94%)
African American	111 (4%)	**6 (1%)**	121 (3%)	2 (2%)
Asian	110 (4%)	**13 (2 %)**	127 (4%)	2 (2%)
Other	91 (3%)	**10 (2%)**	102 (3%)	2 (2%)
FNBMD (g/cm^2)	0.78 ± 0.13	**0.72 ± 0.12**	0.77 ± 0.13	**0.67 ± 0.12**
Rate of BMD change (%/year)	-0.41 (0.62)	**-0.54 (0.78)**	-0.42 (0.64)	**-0.82 (0.98)**
Bone loss (%/year):				
Unchanged (loss <-1%/year)	2555 (87%)	**425 (81%)**	3058 (86%)	**86 (68%)**
Mild (loss from 1 to 2%/year)	327 (11%)	**85 (16%)**	413 (12%)	**34 (27%)**
Rapid (loss > -2%/year)	63 (2%)	**15 (3%)**	79 (2%)	**6 (5%)**

Values are mean ± SD, or frequencies (percentages). FNBMD, femoral neck bone mineral density. Bold values indicate P values less than 0.05. P-values were derived from t-test for continuous variables and from chi-squared test for binary variables.

3.3.2 Bone loss and fracture risk

During the median follow-up of 9 years (IQR:5-15), 1220 women sustained at least one incident fracture, yielding an incidence of 32 fractures/1000 person-years (95% CI: 30-34). We found 522 women sustained a hip fracture with an incident rate

of 12/1000 person-years (95% CI: 11-13). After accounting for age differences, mild and rapid loss were significantly associated with 30-80% increased risk of any fracture, and 40% to 2.6-fold increased risk in hip fracture. Nevertheless, these associations were no longer significant when adjusted for additional confounding effects of baseline BMD, BMI, lifestyle factors and prior history of fractures.

During the median follow-up of 9 years (IQR:5-12), 513 men sustained at least one incident fracture, yielding an incidence of 19/1000 person-years (95% CI: 17-21). We found 120 men sustained a hip fracture with an incident rate of 3/1000 person-years (95% CI: 3-4). After adjusting for age, we found mild and rapid bone loss were associated with a 3-fold and 4-fold increased risk of hip fracture, respectively. Notably, the association for mild bone loss remained statistically after full adjustment for confounding factors, with the risk reduced to 68%. Although the association for rapid bone loss weakened after further covariate adjustments, it still indicated a 31% increased risk of hip fracture, though this was not statistically significant. A similar trend was observed for any fragility fracture in our age-adjusted analysis with mild bone loss linked to a 69% increased risk. When there is a rapid annual bone loss of over 2%, the risk for any fragility fracture increased further to 2-fold. After adjusting for confounding effects of our predefined covariates, the association was reduced for mild and rapid bone loss to a 20% and 31% increased risk of any fracture, respectively.

As part of our prespecified sensitivity analyses, we accounted for the competing risk of death using both the cause-specific and Fine-Gray models. The cause-specific model, which is recommended for identifying exposure-outcome associations in aetiological research, yielded results consistent with the primary Cox’s proportional hazards model, reinforcing our findings. Similarly, the Fine-Gray

model reflected the same risk trends, though with attenuated effect sizes, confirming the consistency of associations observed in our main analysis.

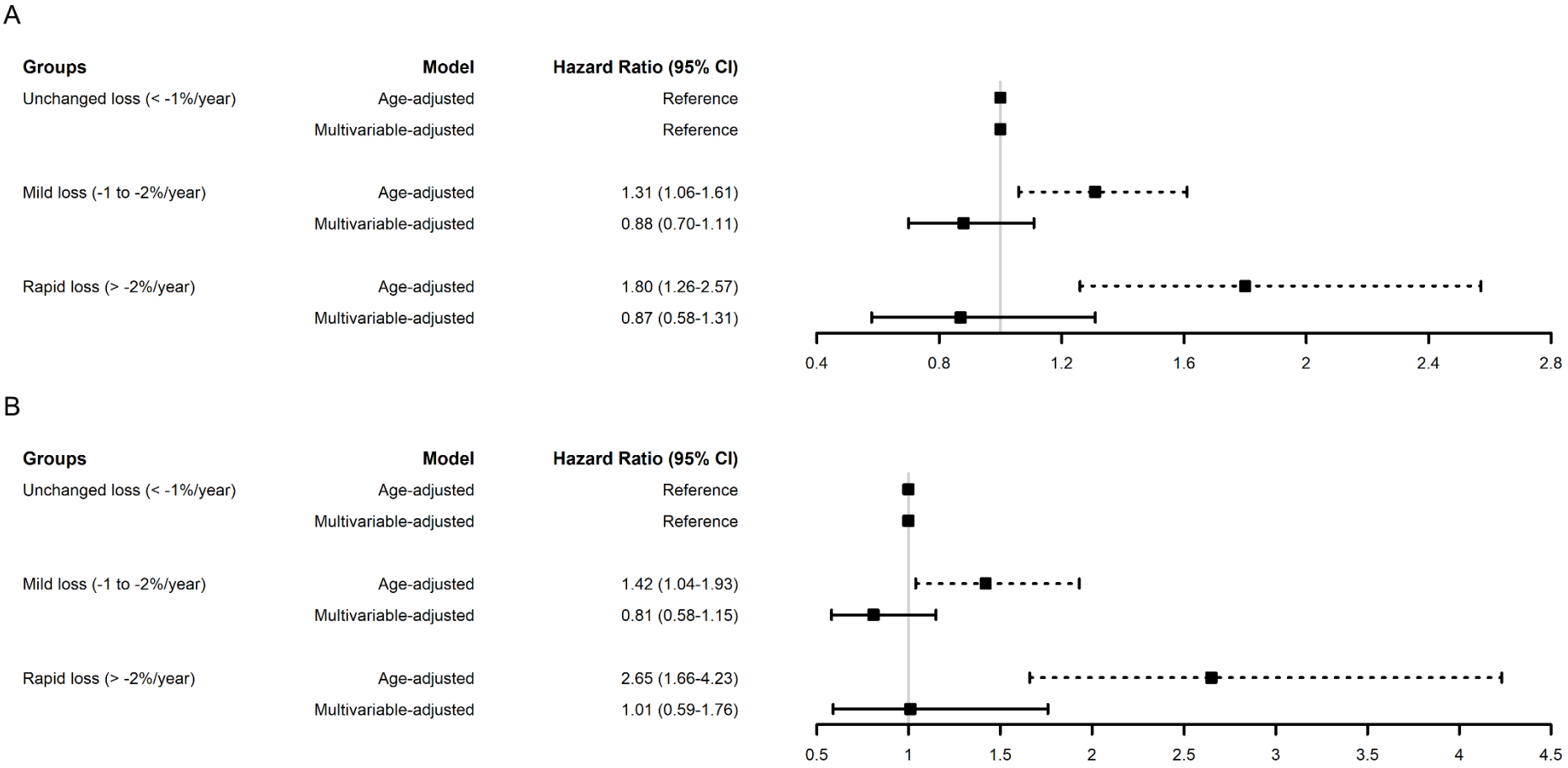

Figure 3.2. Association between bone loss and (A) any fragility fracture or (B) hip fracture in women.

Rate of BMD change (%/year) separated into three groups: mild (loss from -1 to -2%/year) (*N* = 1079; 1223) and rapid (loss >-2%/year) (*N* = 283; 334) bone loss with unchanged (loss <-1%/year) (*N* = 2301; 2556) denoted as the reference. For each group, the two *N* values correspond to participants included in the analyses of any fragility fracture and hip fracture, respectively. Multivariable-adjusted model: adjusted for age, femoral neck BMD, BMI, smoking, drinking, physical activity, prior history of fractures.

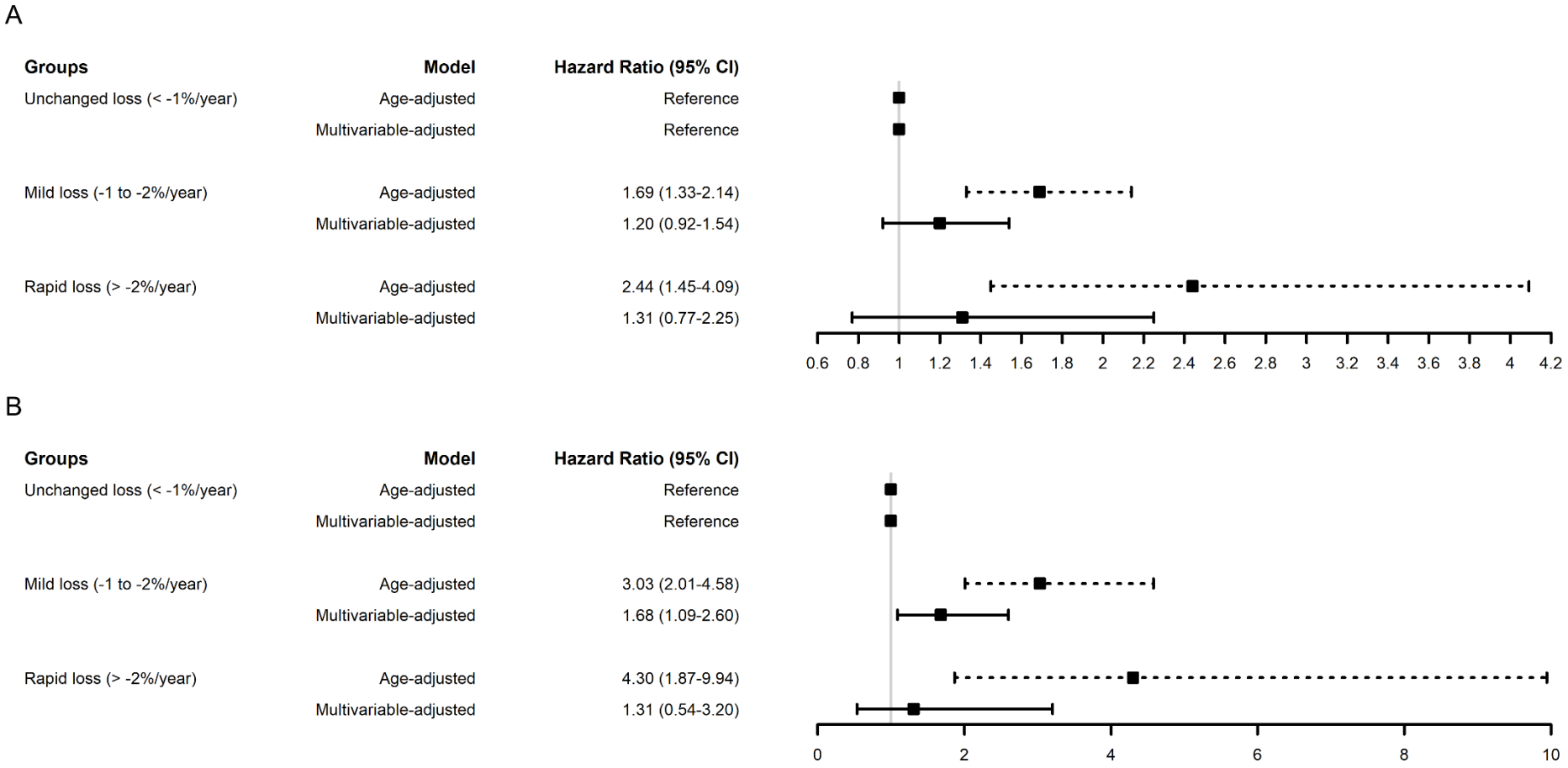

Figure 3.3. Association between bone loss and (A) any fragility fracture or (B) hip fracture in men.

Rate of BMD change (%/year) separated into three groups: mild (loss from -1 to -2%/year) (*N* = 411; 446) and rapid (loss >-2%/year) (*N* = 77; 84) bone loss with unchanged (loss <-1%/year) (*N* = 2952; 3116) denoted as the reference. For each group, the two *N* values correspond to participants included in the analyses of any fragility fracture and hip fracture, respectively. Multivariable-adjusted model: adjusted for age, femoral neck BMD, BMI, smoking, drinking, physical activity, prior history of fractures.

3.4 Discussion

Although low BMD is a well-known risk factor for osteoporotic fractures, the association with bone loss remains unclear due to inconsistent findings across studies. Using two prospective cohorts with at least three BMD measurements, we found that a bone loss rate of -1% to -2% per year at the femoral neck significantly predicted hip fracture risk in men, independent of other risk factors. In women, the association was weaker and only significant in age-adjusted models. Mild bone loss appeared more consistently linked to fracture risk than rapid loss, possibly due to limited fracture cases in the latter group. These findings highlight the clinical value of monitoring BMD change over time, offering a more dynamic and informative assessment than single-point measurements.

Unlike previous studies[116,121], our investigation incorporated three BMD measurements per participant and excluded individuals who experienced fractures during this measurement period. This design choice was crucial, as earlier studies that included overlapping fracture events likely reported inflated associations between bone loss and fracture risk – some estimating a 2- to 7-fold increased risk in men. Prior evidence[145] reported fractures can accelerate bone loss and increase the likelihood of subsequent fractures, potentially biasing the observed relationship as seen in other studies. By removing individuals with early fracture events and focusing on bone change before any incident fracture, our study provides a clearer and more accurate estimate of the association between bone loss and fracture risk. These findings, particularly the identification of BMD loss as an independent risk factor for hip fracture in men, are strongly supported by our robust longitudinal design and application of mixed-effects regression modelling, which accounts for within- and between-subject variability over time.

In women, we observed that femoral neck BMD loss was associated with increased risk of fragility and hip fractures in age-adjusted models; however, these associations were no longer statistically significant after adjusting for baseline BMD, BMI, lifestyle factors, and prior fractures. Our findings contrast with several earlier studies that reported positive associations between bone loss and fracture risk but varied considerably in design. For example, the Australian study (n=996)[122] found a significant association independent of age and baseline BMD but did not adjust for key covariates such as BMI and lifestyle factors, which were accounted for in our larger sample (n=5,581). Other studies differed in imaging modalities (e.g., single-photon absorptiometry), sites measured (forearm, radius, lumbar spine), frequency and duration of BMD measurements, and analytic methods—many using linear regression[117,119,121] with only two measurements and overlapping fracture events, which may inflate associations. In contrast, our study used three BMD measurements, excluded overlapping fracture cases, and applied mixed-effects models for a more accurate and unbiased estimation of bone loss over time. Collectively, while prior studies suggest a relationship between bone loss and fracture risk in women, our more robust design highlights the importance of accounting for multiple confounders and supports a multifactorial approach to fracture risk assessment.

The strengths of the present study distinguish it from many previous investigations and lend greater confidence to our findings. First, despite applying strict inclusion criteria requiring three repeated BMD measurements, we retained a large sample size, enabling statistically reliable analyses. Second, the long duration of follow-up allowed for the accumulation of a sufficient number of fracture events, enhancing the power to detect meaningful associations. Third, our approach to

estimating bone loss—using three BMD measurements per participant in combination with linear mixed-effects regression—provides one of the most accurate and robust methods available for capturing individual changes over time, as it accounts for both fixed and random effects. Lastly, fracture outcomes were confirmed through radiological reports, ensuring a high level of accuracy and minimizing potential misclassification. Together, these methodological strengths set our study apart from earlier research and support the validity of our conclusions.

A key limitation of this study is the potential for healthy selection bias, as participants who remained in the study and completed all required BMD measurements were likely healthier and more health-conscious than those who were unable to participate or dropped out. This type of bias is common and often unavoidable in observational studies. While we did not find direct evidence that this bias was more pronounced in women than in men, it may partly explain the weaker associations observed in women, potentially leading to an underestimation of the true effect of BMD change on fracture risk. As such, the generalisability of our findings to populations with poorer health status or multiple comorbidities may be limited. Additionally, the modest year-to-year changes in BMD (~1–2% per year) may be close to – or below – the least significant change (LSC) for hip and spine DXA (approximately 2–5.6% at the 95% confidence level). However, this is the average change observed at the population level, not an individual level where LSC is relevant. Still, observed short-term changes within an individual may partly reflect measurement imprecision, positioning variability, or normal fluctuation rather than true biological bone loss[146]. Associations involving annual BMD change should therefore be interpreted cautiously, as such variability could have diluted the fracture-risk estimates observed in our study.

In conclusion, BMD loss at the femoral neck was identified as an independent risk factor for hip fracture in elderly men, even after accounting for baseline BMD and other clinical variables. These findings highlight the potential value of repeated BMD measurements over time to monitor bone health and better identify individuals at elevated fracture risk. In women, further research is needed to more precisely quantify the association between BMD change and fracture risk, as the current sample may not fully capture this relationship. Future studies should also focus on uncovering the underlying biological mechanisms linking bone loss to fracture risk in men. This could involve in vitro and in vivo experiments, such as gene expression profiling, proteomics, and pathway analysis, to explore how genetic and molecular pathways contribute to skeletal deterioration and increased fracture susceptibility via bone loss.

Chapter 4. Association between *FTO* polymorphisms and osteoporosis phenotypes

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Preface: Bridge between Chapters 4 and Chapter 5

The second study of this thesis aimed to examine the association between genetic variants of a single gene and several osteoporosis phenotypes, in particular, bone loss. The fat mass and obesity-related transcript (*FTO*) gene is strongly correlated with obesity, another phenotype that is closely linked with osteoporosis. Due to this, genetic variants at *FTO* were selected to ascertain the association between genetic factors and bone loss.

The main content of this chapter was published in Journal of Bone Metabolism 2024:

Dios, K., Huynh, N., Tran, T. S., Center, J. R. & Nguyen, T. V. Association between Fat Mass and Obesity-Related Transcript Polymorphisms and Osteoporosis Phenotypes. *J Bone Metab* **31**, 48-55 (2024). https://doi.org:10.11005/jbm.2024.31.1.48

Summary

Common variants in the fat mass and obesity-related transcript (*FTO*) gene are associated with obesity, which is related to bone mineral density (BMD) and fracture risk. Our study results showed that *FTO* variants were associated with hip fracture risk, but the association is unlikely mediated through BMD or bone loss.

Abstract

Background and Aim. Common variants in the fat mass and obesity-related transcript (*FTO*) gene are related to body mass index (BMI) and obesity, suggesting its potential association with bone mineral density (BMD) and fracture risk. This study sought to define the association between FTO gene variants and the following phenotypes: (i) BMD, (ii) bone loss, and (iii) fracture risk.

Design and methods. This analysis was based on the Dubbo Osteoporosis Epidemiology Study that included 1277 postmenopausal women aged 60 years and above living in Dubbo, Australia. BMD at the femoral neck and lumbar spine was measured by dual-energy X-ray absorptiometry (GE-LUNAR Corp, Madison, WI) biennially. Fractures were radiologically ascertained. Six single nucleotide polymorphisms (SNPs) (rs1421085, rs1558902, rs1121980, rs17817449, rs9939609 and rs9930506) of the *FTO* gene were genotyped using TaqMan assay.

Results. Women homozygous for the minor allele (GG) of rs9930506 had significantly higher risk of hip fracture (adjusted hazard ratio, 1.93; 95% CI: 1.15-3.23) than women homozygous for the major allele (AA) after adjusting for potential confounding effects. Similar associations were also observed for the minor allele of rs1121980. However, there was no significant association between the FTO SNPs and BMD or the rate of bone loss.

Conclusion. Common variations in the *FTO* gene are associated with hip fracture risk in women, and the association is not mediated through BMD or bone loss.

4.1 Introduction

The fat mass and obesity-related transcript (*FTO*) gene encodes the nuclear protein, Fe(II)/2-oxoglutarate dependent methylase, a type of RNA demethylase[147]. In 2007, a type 2 diabetes genome-wide association study (GWAS) identified *FTO* single nucleotide polymorphisms (SNPs) were associated with body mass index (BMI) and obesity[148]. Other studies conducted independently on Asian[149,150], African[151,152] and European[153,154] populations have also shown the correlations between SNPs at intron 1 of *FTO* with waist to hip ratio, obesity, type 2 diabetes, body weight and other BMI-related phenotypes. Among hundreds to thousands of *FTO* SNPs studied in these populations, the *FTO* variants rs9939609, rs1421085, rs17817449 and rs1121980 have been consistently shown to be significantly associated with body weight and related phenotypes in various ethnicities[149,150,152-154].

BMI and obesity are strongly associated with several osteoporosis phenotypes, including BMD and fracture risk[155,156]. Each unit increase in BMI (kg/cm^2) has been found to be associated with approximately 0.0082 g/cm^2 increase in BMD amongst American adults[157]. Greater BMI has been found to be associated with lower risk of fracture, independently of BMD[158,159]. The relationship between BMI and fracture risk is complex, and dynamic due to the association between BMI and BMD[158].

Although several GWAS on BMD and other osteoporosis phenotypes have been conducted[83], there was no signal for the *FTO* gene. We hypothesise *FTO* genotypes are associated with different osteoporosis phenotypes. Specifically, the present study sought to test that hypothesis by pursuing the following specific aims: to quantify the association between *FTO* polymorphisms with (i) BMD, (ii) bone loss and (iii) fracture in post-menopausal women. By determining the association of *FTO* polymorphisms with osteoporosis phenotypes, we can utilise *FTO* as a genetic factor to predict the development of osteoporosis, including fracture prediction.

4.2 Study design and methods

4.2.1 Study design

This study was based on the Dubbo Osteoporosis Epidemiology Study (DOES) with the protocols and study structure described elsewhere[104]. Briefly, DOES was designed as a prospective, population-based investigation that was operational from 1989 to 2020. Approximately 4000 men and women aged 60 years and older have been recruited and followed up for almost 30 years. The study was approved by the St Vincent's Campus Research Ethics Committee and written informed consent was acquired from each participant. As this study was primarily focused on post-menopausal women, we excluded men and any participants without *FTO* genotype data (**Figure 4.1**).

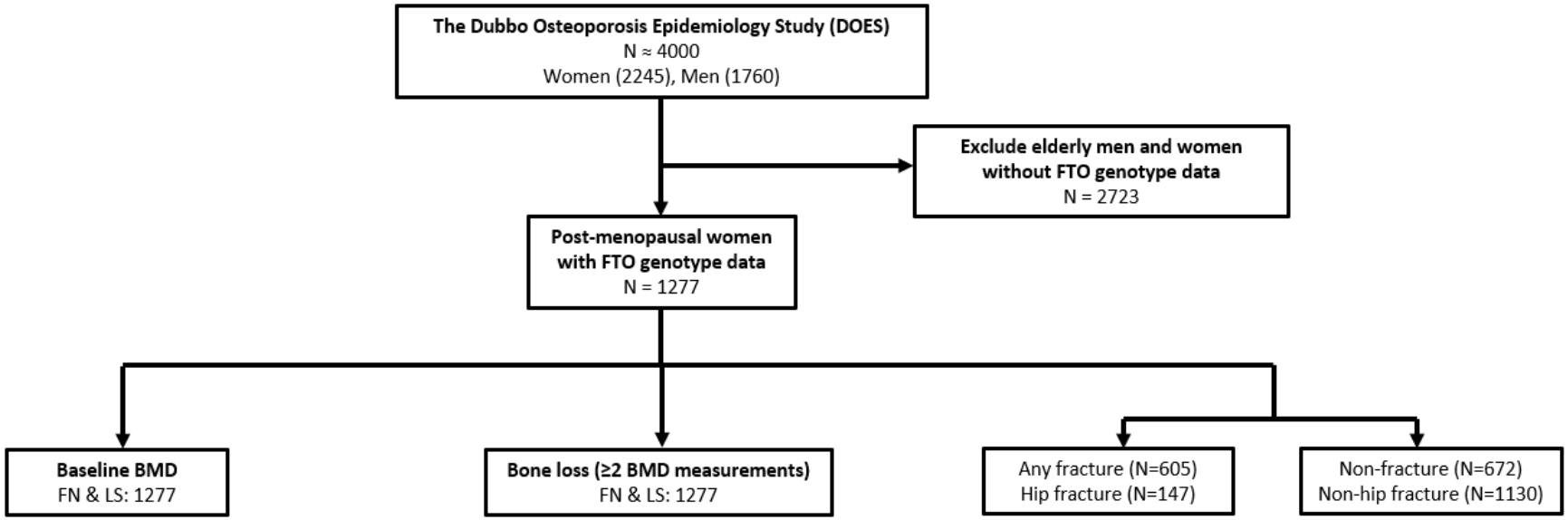

Figure 4.1 Flow chart of recruitment and follow up.

Abbreviations: BMD, bone mineral density; FN, femoral neck; LS, lumbar spine.

4.2.2 Measurements

A nurse co-ordinator used a structured questionnaire to obtain baseline measurements at 1989 and subsequent visits at approximately 2-3 yearly intervals. The structured questionnaire included age, weight, height, smoking and alcohol intake, physical activity, calcium intake, medication, number of falls in the last year, and fracture history. Weight (kg) and height (cm), without shoes and in light clothing, was measured on an electronic scale (to the nearest 0.1 kg) and by a wall-mounted stadiometer (to the nearest 0.1 cm). Body mass index (BMI, kg/m^2) was calculated as the ratio of weight (kg) and squared height (m^2).

BMD (g/cm^2) at the femoral neck (FNBMD) and lumbar spine (LSBMD) was measured at baseline and biennially by a DXA instrument using a LUNAR DPX densitometer (GE-Lunar, Madison, WI, USA). The coefficient of variations of BMD measurement of the femoral neck and lumbar spine was 1.5% and 1.3%, respectively[106]. All measurements were conducted by the same technicians, following the same standard operating procedure.

4.2.3 Assessment of outcomes

All fractures were ascertained from the two or three radiology centres servicing the Dubbo area, and circumstances surrounding fracture were ascertained by personal interview following the fracture event. Only low-trauma fractures caused by a fall from a standing height or less were included in the study. Fractures caused by high trauma, such as motor vehicle accident, pathological fractures from bone diseases other than osteoporosis (e.g., hyperparathyroidism, Paget's disease), or fractures of the skull and digits were excluded. Vertebral fractures were determined either through clinical diagnosis or detected incidentally on X-ray. Deaths were

ascertained from funeral lists, obituary reports, and Dubbo media reports, and verified from the New South Wales Bureau of Births, Deaths, and Marriages.

4.2.4 Genotyping

The genotype data for this study was drawn from a previous investigation, which describes the genotyping protocol used[160]. Briefly, blood samples were collected and stored at -80°C. Extraction of DNA was done either by using QIAamp DNA Mini Blood Kit (Qiagen Pty Ltd., Valencia, CA, USA) according to the manufacturer's instructions, or phenol/chloroform. Six single nucleotide polymorphisms (rs1421085, rs1558902, rs1121980, rs17817449, rs9939609 and rs9930506) in the first intron of the *FTO* gene were genotyped using a predesign of Taqman SNP Genotyping Assay (Applied Biosystem, Foster City, CA, USA). The ABI 7900 Sequence Detection System (SDS) software was used to compute the allelic discrimination. From the participants of DOES, the DNA of 1277 women and 758 men were able to be genotyped. The number of fractures was too small to conduct a sensitive analysis for elderly men, therefore, we primarily focused on post-menopausal women with *FTO* genotype data.

4.2.5 Data analysis

The first analysis included participants with BMD measured at baseline. We performed a multiple-adjusted linear regression to quantify the association between *FTO* genotypes and baseline BMD at femoral neck using the AA genotype as the reference group. The model was adjusted for predefined covariates of age and BMI. A predefined sensitivity analysis was also considered for lumbar spine BMD. To control for potential confounding effects of metabolic diseases, an exploratory analysis was conducted to further adjust for the presence of diabetes mellitus.

For the second aim, the rate of change in BMD was calculated for each participant with at least two BMD measurements using a linear mixed-effects regression. We also conducted a linear mixed-effects regression, adjusted for the predefined covariates to examine the association between *FTO* genotypes and bone loss. A linear mixed-effects regression has been shown to be statistically superior to a conventional linear regression in analysis of repeated measurement[136,137]. A linear mixed-effects regression is capable of accounting for not only a regression-toward-the-mean phenomenon, but also missing data which are very common in any analysis with repeated measures[136]. Moreover, a linear mixed-effects regression is also able to account for variation between and within subjects[137].

For our third aim, the Cox's proportional hazards regression was used to quantify the association between *FTO* genotypes and fracture risk, adjusted for age, BMD, and BMI. The follow-up time for participants with a fracture was calculated between the study entry and the date of fracture. For participants without a fracture, their follow-time was computed as a time interval between the study entry and date of death or the study end (5 March 2018), whichever came first. The assumption of proportional hazards was graphically checked using the scaled Schoenfeld residuals[143].

We computed the E-value[161] as a predefined sensitivity analysis to assess the robustness of our findings in the potential of residual confounding effects. Technically, the E-value quantifies possibilities that an uncontrolled confounder would make the reported association statistically non-significant. No adjustments were made for multiple comparisons. The analyses were performed using R language[109] with a P-value of less than 0.05 considered statistically significant.

4.3 Results

4.3.1 FTO genotypes and baseline BMD

The current study involved 1277 women with an average age of 69 years (±6.6) whose *FTO* genotype data were available (**Figure 4.1**). The distribution of *FTO* genotypes is consistent with the Hardy-Weinberg principle as the proportion of the major (32-38%), heterozygous (44-49%) and minor (16-20%) allele are similar in the *FTO* SNP rs9930506 (**Table 4.1**) and other *FTO* SNPs - rs1421085, rs1558902, rs1121980, rs17817449 and rs9939609 (**Supplemental Table 1**). Given the great similarities between the *FTO* SNPs and their close proximity to each other at intron 1 of the *FTO* gene, we present the results of rs9930506 in the main paper and leave those of other *FTO* genotypes in the appendix.

Table 4.1 Baseline characteristics by FTO genotypes (rs9930506) in women.

	AA (N=349)	GA (N=546)	GG (N=209)
Age (years)	69 (7)	69 (7)	70 (6)
Height (cm)	160 (6)	160 (6)	160 (6)
Weight (kg)	67 (13)	67 (13)	66 (12)
Body mass index (kg/m^2)	26 (5)	26 (5)	26 (4)

Values presented as mean (SD). The differences between the genotypes were examined using ANOVA test.

Table 4.2 illustrates the association between *FTO* genotypes rs9930506 and baseline BMD. We found *FTO* genotypes were not associated with BMD after accounting for potential confounding effects of age and BMI (Table 2). Similarly, there was no association between BMD and the other *FTO* SNPs – rs1421085, rs1558902, rs1121980, rs17817449 and rs9939609 (Table S2-S6).

Table 4.2 Association of *FTO* genotypes (rs9930506) and BMD in women.

	AA* (N=349)	GA (N=546)	GG (N=209)	GA vs AA	GG vs AA	GA vs GG
Femoral neck BMD (g/cm^2)	0.79 (0.13)	0.81 (0.14)	0.80 (0.14)	0.017 (0.002, 0.033)	0.013 (-0.006, 0.033)	-0.004 (-0.022, 0.014)
Lumbar spine BMD (g/cm^2)	1.05 (0.19)	1.05 (0.20)	1.05 (0.21)	-0.001 (-0.025, 0.024)	0.005 (-0.026, 0.037)	0.006 (-0.024, 0.035)

*reference genotype: AA. BMD values are provided as mean (SD). The association between *FTO* genotype and BMD is presented as mean difference (95% CI) derived from a multivariable-adjusted linear regression, adjusted for age and BMI.

4.3.2 FTO genotypes and longitudinal bone mineral density change

In our study population of post-menopausal women, the overall rates of BMD change were -0.004 g/cm^2 per year at the femoral neck and 0.002 g/cm^2 per year at the lumbar spine. In the *FTO* SNP rs9930506, the annual rate of bone change was similar across the *FTO* genotypes at the femoral neck (-0.004 g/cm^2) and lumbar spine (0.002 g/cm^2) (**Table 4.3**). The analysis did not find a significant difference in the rate of BMD change over time between the *FTO* genotypes after taking the potential confounding effect of age and BMI into account (**Table 4.3, Supplemental Tables 2-6**).

Table 4.3 Mean annual BMD change rates (95% CI) by FTO rs9930506 genotype in women.

	AA*	GA	GG	GA vs AA	GG vs AA	GA vs GG
Femoral neck BMD (g/cm^2)	-0.004 (0.004)	-0.004 (0.003)	-0.004 (0.003)	< -0.001 (-0.001, 0.001)	< -0.001 (-0.001, 0.001)	< 0.001 (-0.001, 0.001)
Lumbar spine BMD (g/cm^2)	0.002 (0.006)	0.002 (0.007)	0.002 (0.006)	< 0.001 (-0.001, 0.001)	0.001 (-0.001, 0.003)	0.001 (-0.001, 0.003)

*reference genotype: AA. Bone change rates are provided as mean (SD). The association between *FTO* genotype and bone loss is presented as mean difference (95% confidence interval) derived from a multivariable-adjusted linear mixed-effects regression, adjusted for age and BMI.

4.3.3 FTO genotypes and fracture risk

During a median follow-up of 14 years (IQR: 10-22 years), 605 women sustained at least one incident fracture, yielding a fracture rate of 40/1000 person-years (95% CI: 37-44). We also found 147 women with a hip fracture rate of 8/1000 person-years (95% CI: 6-9). We found *FTO* genotypes were not independently associated with the risk of any fracture (**Table 4.4; Supplemental Tables 4-5**). However, women with the minor allele (GG) were associated with a significantly greater risk of hip fracture (HR: 1.82, 95% CI: 1.10-3.03). The association remained significant after adjusting for potential confounding effects of age, BMI and BMD. Interestingly, we found the E value for the FTO-hip fracture association was as high as 3.25 (95% CI: 1.57-5.85), indicating that the finding would have become non-significant only if there was a very strong residual confounding effect associated with almost a three-fold risk of hip fracture existing in the study population. Similarly, the minor allele of *FTO* SNPs rs1121980 was also associated with a greater risk of hip fracture (**Supplemental Table 4**). The association between the minor allele of rs1421085 (P=0.09), rs1558902 (P=0.06) and rs17817449 (P=0.05) with hip fracture

only achieved marginally statistically significant results (**Supplemental Tables 2, 3 & 5**). The exploratory analysis further adjusted for the presence of metabolic diseases provided consistent results (**Supplemental Table 7**), confirming the robustness of our primary findings.

Table 4.4 Association between *FTO* genotypes (rs9930506) and fracture risk in women.

Any fracture					
		Age-adjusted		Multivariable adjusted**	
Genotype	Number*	HR (CI)	P-value	HR (CI)	P-value
AA (N=357)	165 (46.2%)	Reference	-	Reference	-
GA (N=556)	266 (47.8%)	1.08 (0.88-1.31)	0.47	1.12 (0.92-1.37)	0.26
GG (N=213)	96 (45.1%)	1.01 (0.78-1.30)	0.96	1.04 (0.81-1.34)	0.76
Hip fracture					
		Age-adjusted		Multivariable adjusted**	
Genotype	Number*	HR (CI)	P-value	HR (CI)	P-value
AA (N=357)	30 (8.4%)	Reference	-	Reference	-
GA (N=556)	65 (11.7%)	1.38 (0.89-2.13)	0.15	1.40 (0.91- 2.17)	0.10
GG (N=213)	30 (14.1%)	**1.82 (1.10-3.03)**	**0.02**	**1.92 (1.15- 3.20)**	**0.01**

*data presented as number of patients with a fracture (%). HR = Hazard ratio; CI = 95% confidence interval. **adjusted for age, femoral neck BMD and BMI. **Bold-faced number indicates statistical significance.**

4.4 Discussion

Common variants in the fat mass and obesity-related transcript (*FTO*) gene are related to BMI and obesity, suggesting its potential contribution to the key osteoporosis phenotypes, such as BMD and fracture risk. Using data from a well-established, prospective cohort study, we have presented polymorphic variation at the *FTO* gene was associated with hip fracture risk, independent of age, BMD, and BMI. However, our analysis did not find a significant association between the *FTO*

gene and either BMD or bone loss, suggesting that the association between the *FTO* gene and hip fracture is unlikely mediated via BMD or bone loss.

Our finding is consistent with a previous study that could not find an association between the *FTO* gene and BMD in Caucasians[156]. They however found the *FTO* gene was significantly associated with BMD in Chinese individuals. Studies have shown weight-related health risks were greater in Asians than Caucasians, Africans, and Hispanics[162,163] with Asians usually exhibiting a higher body fat percentage than Caucasians[164]. Given the close correlation of *FTO* with body weight and obesity, it is possible that *FTO* polymorphisms exhibit a greater effect on populations strongly affected by weight-related health risks, such as Asians.

Our finding of the relationship between *FTO* and fracture risk supports previous findings[160] that polymorphic variation at the *FTO* gene was associated with hip fracture risk in post-menopausal women, even after accounting for potential confounding effects. Although the findings suggest an increased risk of hip fracture virtually associated with all *FTO* SNPs, only rs1121980 and rs9930506 were found to be significantly associated with hip fracture after accounting for potential confounding effects. Indeed, the *FTO* SNPs rs1421085, rs1558902 and rs17817449 and were associated with 1.5-fold increased risk of hip fracture, though the associations only achieved marginally statistically significant (P≈0.10) results. Taken together, our finding suggests that neither BMD nor bone loss mediates the association between *FTO* and an increased risk of hip fracture among post-menopausal women.

The biological mechanism underlying the association between *FTO* polymorphic variation and increased hip fracture is unknown. Lean mass is known to be associated with reduced bone fracture. A stronger influence is observed with lean

mass opposed to fat mass which has a positive association with hip strength[165]. Taken together, these findings suggest that the association between *FTO* variants and hip fractures maybe mediated through lean mass. However, this hypothesis needs to be verified in future studies. Notably, these SNPs localise to an FTO intronic enhancer where rs1421085 disrupts ARID5B repression of IRX3/IRX5, driving obesity via adipocyte dysfunction[166]. Their lack of BMD and bone loss association in our study supports BMD-independent fracture effects.

The present study's findings must be interpreted within the context of potential strengths and weaknesses. To our knowledge, this is the first study that examines the association between *FTO* polymorphic variations and bone loss. The study was based on a well-characterised cohort, a reasonably large sample size with long duration of follow-up, which allows accurate assessment of bone loss at the individual level and sufficient statistical power for analysing fracture risk. Additionally, BMD was measured using the DXA, considered the robust and standard method, whereas fractures were ascertained radiologically, minimizing the risk of measurement biases. The linear mixed-effects regression we used in this study is considered much more robust than the conventional linear regression.

However, the study is an observational investigation, and it is not possible to make any causal inference concerning the association between FTO genotypes and bone phenotypes. The finding might have been affected by residual confounding effects that were not measured in this study. However, given the E-value of 3.25, it is unlikely that the association between *FTO* gene and hip fracture was only confounded by unmeasured confounders associated with at least three-fold risk of fracture. Such a very strong residual confounding effect is considered uncommon in reality, indicating our findings are statistically robust. Finally, the study population

was limited to Caucasians, and extrapolation to non-Caucasian populations require careful consideration.

In conclusion, the present study has demonstrated that polymorphic variation at the *FTO* gene is associated with hip fracture risk, but this association is unlikely mediated through low bone mineral density of bone loss. Further research is needed to examine the candidate genes of associated causal variants with osteoporosis phenotypes, as GWAS alone cannot comprehensively explain the biological mechanism of the identified genetic variants[167].

Chapter 5. Genome wide association analysis of longitudinal change in bone mineral density: the Vietnam Osteoporosis Study

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Preface: Bridge between Chapters 4 and Chapter 5

Building on the findings of the previous chapter, where no single-gene variants were identified as being associated with bone loss, the final study of this thesis broadened the investigation to the entire genome. In this genome-wide approach, more than 700,000 genetic variants were scanned to uncover potential associations with bone loss. The work presented in this chapter represents the culmination of approximately 18 months – spanning nearly half the duration of this dissertation – I have devoted to the optimisation and troubleshooting of quality control procedures and the analysis of genotyped data. Importantly, this chapter lays the groundwork for a forthcoming meta-analysis I shall conduct, which will be undertaken as part of the future directions of this research following the completion of the thesis.

5.1 Introduction

Low bone mineral density (BMD) is a well-established and robust risk factor for osteoporotic fracture. Prospective cohort studies have consistently shown that each standard deviation decrease in BMD is associated with a 1.5- to 3-fold increase in fracture risk, particularly at the hip, spine, and wrist — the most common sites of osteoporotic fracture[12,115,168]. Osteoporotic fractures, especially hip fractures, are a major contributor to morbidity, reduced quality of life, and increased mortality in the aging population.

The regulation of BMD is strongly influenced by genetic factors, with heritability estimates ranging from 50% to 85% based on family, twin, and population-based studies[169,170]. Studies in monozygotic and dizygotic twins have shown particularly high heritability estimates for both trabecular and cortical bone density, suggesting a substantial genetic contribution to BMD variation. Over the past decade, genome-wide association studies (GWAS) have successfully identified hundreds of loci associated with BMD, revealing important insights into the genetic architecture of skeletal traits. Large-scale meta-analyses conducted by the GEFOS consortium and UK Biobank, among others, have reported over 500 independent signals at hundreds of loci linked to areal and volumetric BMD, as well as fracture risk[171-173]. These loci highlight genes involved in bone metabolism, *WNT* signalling, osteoblast differentiation, and skeletal development.

However, BMD is not a static trait because it changes over time, particularly with aging, and this change itself is a critical determinant of fracture risk. Emerging evidence suggests that the rate of bone loss is also influenced by genetic factors[100,174], independent of baseline BMD. Despite its clinical relevance, the

genetic basis of longitudinal BMD change remains largely unexplored, and to date, no GWAS has systematically investigated genetic variants associated with bone loss.

In this study, we performed a GWAS to identify genetic variants associated with BMD change over time. By leveraging longitudinal data from the Vietnam Osteoporosis Study, our goal is to uncover novel genetic determinants of bone loss and improve understanding of the molecular mechanisms contributing to skeletal aging and fracture risk.

5.2 Methods

5.2.1 Study participants and phenotypes

The protocols and study structure of the Vietnam Osteoporosis Study (VOS) have been described elsewhere[105]. Briefly, VOS was designed as a population-based family investigation that was operational from 2015 to 2020. Since 2015, the study randomly recruited 4157 men and women aged 20 years and older (the average age being 51) from 817 families in the general community of Ho Chi Minh City (formerly Saigon) and rural areas. The study was approved by the research and ethics committee of the People's Hospital 115, Ho Chi Minh City. The study was conducted according to the ethical principles of the Declaration of Helsinki, and all participants gave written informed consent.

From the VOS cohort, our genome-wide analysis consisted of 2343 women and men with a minimum of two BMD measurements at the femoral neck, total hip, and lumbar spine. Of 2343 participants, 324 (14%) were <40 years at baseline (mean age 51±12 years); all analyses adjusted for age and sex as covariates. In addition, participants with their reported baseline age were included. Participants

with ≥2 BMD measurements were older with lower baseline BMD and differed in sex distribution compared to those with only one measurement (Appendix A: Supplemental Table 7).

5.2.2 Outcome and covariate measurements

BMD at the lumbar spine (L2–L4), femoral neck, and total hip was measured using a single Hologic Horizon densitometer (Hologic Corp., Bedford, MA, USA). The device was daily quality-controlled and calibrated throughout the study using the same standard phantom; a phantom scan was performed before each participant measurement. Scans were acquired by qualified DXA technologists, with measurements performed consistently by the same radiographer across the study period. The coefficients of variation were 1.7–1.8% for hip BMD and 1.5–2.0% for lumbar spine BMD.

A standardised questionnaire was used to collect demographic and clinical data. The standardised questionnaire included anthropometric and demographic characteristics, lifestyle factors, nutrition and dietary calcium intakes, reproductive history (women), clinical history (e.g., diseases), medication history, fracture history, fall history, and biochemical test results. Weight (kg) and height (cm), indoor clothing and without shoes, was measured using an electronic balance and wall-mounted stadiometer. Body mass index (BMI, kg/m^2) was calculated as the weight (kg) divided by the square of height (m^2).

5.2.3 Genotyping and quality control

Genotyping was performed on 4056 samples from the VOS cohort on an Illumina Infinium assay platform using a Global Screening Array microchip by the Australian Genome Research Facility (AGRF). Genome Studio (Illumina Inc.) was

used for data pre-processing. Of the 700 604 SNPs genotyped, we retained 387 323 SNPs for analysis. 203 414 SNPs were excluded because they violated the Hardy-Weinberg equilibrium (1×10^{-4}); 5649 SNPs were excluded because they had genotype call rates of 95% or less; and 104 218 SNPs were excluded because they had a minor allele frequency of less than 0.005. Furthermore, individuals with excessively high or low rates of autosomal heterozygosity; high missing genotyping rates; discordance of observed gender and estimated gender; and non-East Asian ancestry clusters based on the 1000G reference populations were excluded. The VOS cohort retained 3855 individuals that passed the standard quality control (see **Figure 5.1**; see Chapter 2 for additional details).

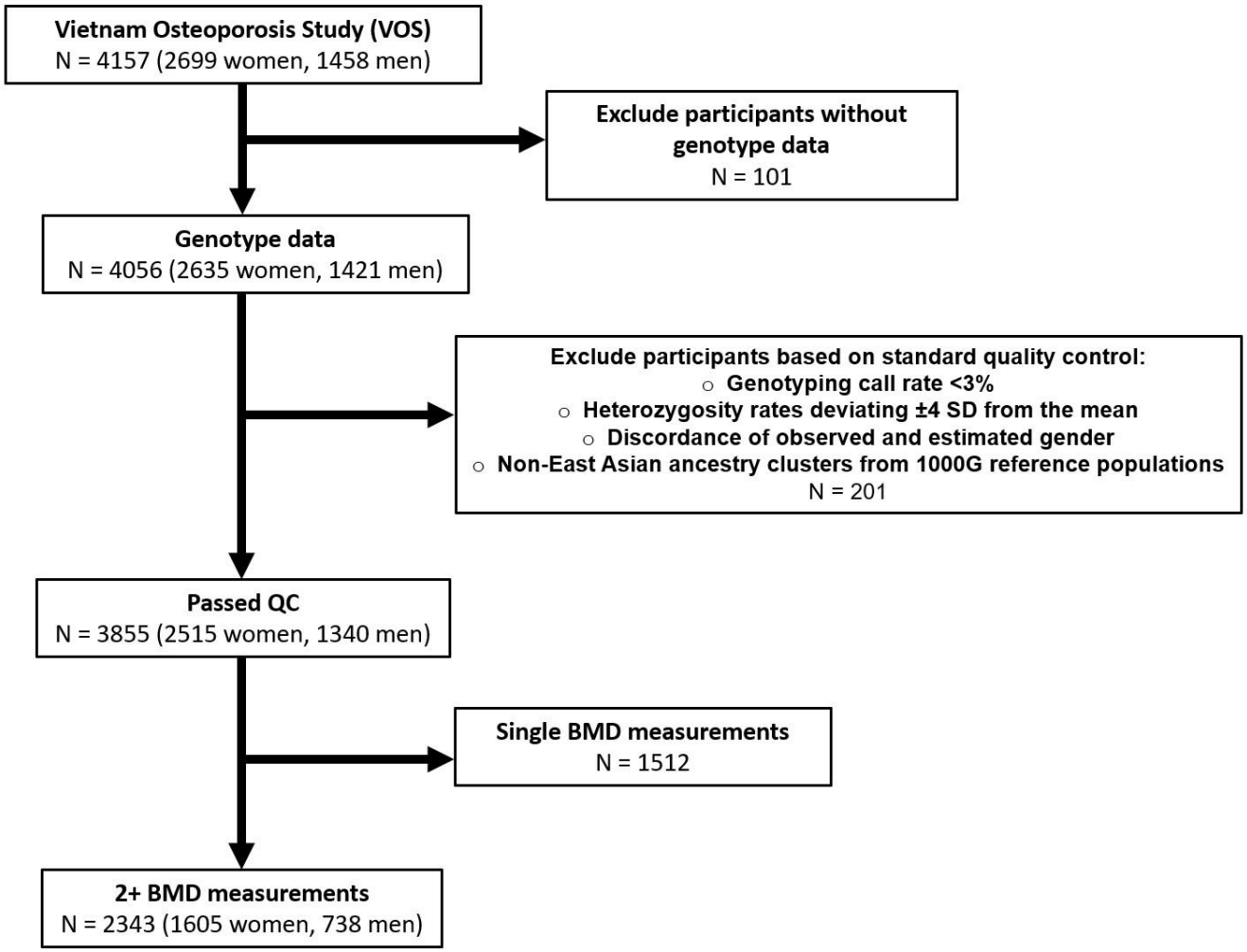


Figure 5.1 Flowchart for genome-wide association analysis of longitudinal bone change.

Abbreviations: BMD, bone mineral density.

5.2.4 Statistical analysis

A linear mixed-effects regression was employed to estimate the absolute rate of BMD change over time (g/cm^2/year) for each participant with two or more BMD measurements. A linear mixed-effects regression was shown to be more statistically robust to a conventional linear regression for analysis of repeated measurements as not only is it able to account for within- and between-subject variations, but also adjust for a regression-toward-the-mean phenomenon[136,137]. The relative rate of BMD change (%/year) was calculated by dividing absolute rate of BMD change by baseline BMD[175].

We conducted a genome-wide association analysis of BMD change at the femoral neck, total hip, and lumbar spine using the Genome-wide Complex Trait Analysis (GCTA)[111] software package with age and sex as covariates. Our GWAS analysis combined women (N=1604) and men (N=738) with sex included as a covariate to maximise power compared to sex-stratified analyses. For each phenotype, we performed genome-wide association analysis using the fastGWA linear mixed model[112]. We used a p-value threshold of 5×10^{-8} (Bonferroni correction, based on 0.05 multiplied by 1 million independent SNPs) for genetic variants reaching genome-wide significance. Furthermore, we used 5×10^{-6} to indicate variants with suggestive associations[176,177]. Post-hoc calculations (N=2343, $\alpha=5\times10^{-8}$) indicate ≥80% power to detect common variants (MAF≥0.20) explaining ≥1.5% phenotypic variance in BMD change (Supplemental Table 8).

5.3 Results

5.3.1 Characteristics of subjects

Of the 3855 participants who passed quality control, we used 2343 individuals with multiple BMD measurements, complete genotype, and covariate data in this study. The basic characteristics of the study population are shown in **Table 5.1**. The mean age was 55 years for men, and 56 years for women. As expected, men exhibited a higher amount of BMD at the total hip, femoral neck, and lumbar spine. Although, the average rate of bone change showed women to lose more bone per year than men at the total hip, femoral neck, and lumbar spine.

Table 5.1. Basic characteristics of study participants.

Characteristic	Women (N=1604)	Men (N=738)
Age (years)	56 ± 12	55 ± 13
Weight (kg)	54 ± 8	63 ± 10
Height (cm)	153 ± 5	163 ± 6
BMI (kg/cm^2)	23 ± 3	24 ± 3
Total hip (g/cm^2) Rate of bone change (%/year)	0.80 ± 0.13 -0.56 ± 0.55	0.88 ± 0.14 -0.38 ± 0.46
FNBMD (g/cm^2) Rate of bone change (%/year)	0.68 ± 0.13 -0.21 ± 0.57	0.76 ± 0.15 -0.09 ± 0.47
LSBMD (g/cm^2) Rate of bone change (%/year)	0.87 ± 0.15 -0.02 ± 0.68	0.94 ± 0.14 0.07 ± 0.54

Values are described as mean ± SD. SD, standard deviation; BMI, body mass index; FNBMD, femoral neck bone mineral density; LSBMD, lumbar spine bone mineral density.

5.3.2 Identification of longitudinal BMD change loci

We performed a genome-wide association analysis on the longitudinal BMD change at total hip, femoral neck, and lumbar spine. In our Vietnamese sample population, we identified five novel genome-wide significant (GWS, $P<5\times10^{-8}$) variants associated with longitudinal BMD change at the femoral neck (**Figure 5.2A**) and total hip (**Figure 5.2B**) in our GWAS after adjusting for sex and age. We also identified 16 genetic variants with suggestive association (SA, $P<5\times10^{-6}$) (**Table 5.2**). Among the GWS and SA genetic variants, only one (rs1332099, $P = 3.7\times10^{-06}$) has

been reported on the GWAS catalog database[178]. GWAS Catalog annotation displayed two studies have previously reported the rs1332099 SNP to be associated with Crohn's disease, rheumatoid arthritis, and paediatric autoimmune diseases.

From our GWS SNPs associated with femoral neck and total hip BMD change, we identified *ARHGAP6* and *ENSG00000267175* as candidate genes. The *ARHGAP6* gene is a member of the rhoGAP family of proteins and plays a role in actin cytoskeleton remodelling and functions as a GTPase-activating protein. Functional studies have observed the *ARHGAP6* gene to be linked with various human diseases, such as neurodevelopmental disorders, lung and breast cancer. *ENSG00000267175* is a novel transcript and RNA gene related to the long non-coding RNA class. Intriguingly, there are no previous studies on the *ENSG00000267175* gene. Furthermore, in our current study, the rs117047862 SNP at *ENSG00000267175* displays a different direction of effect (β = -0.242) direction to most of our reported SNPs with positive effect directions.

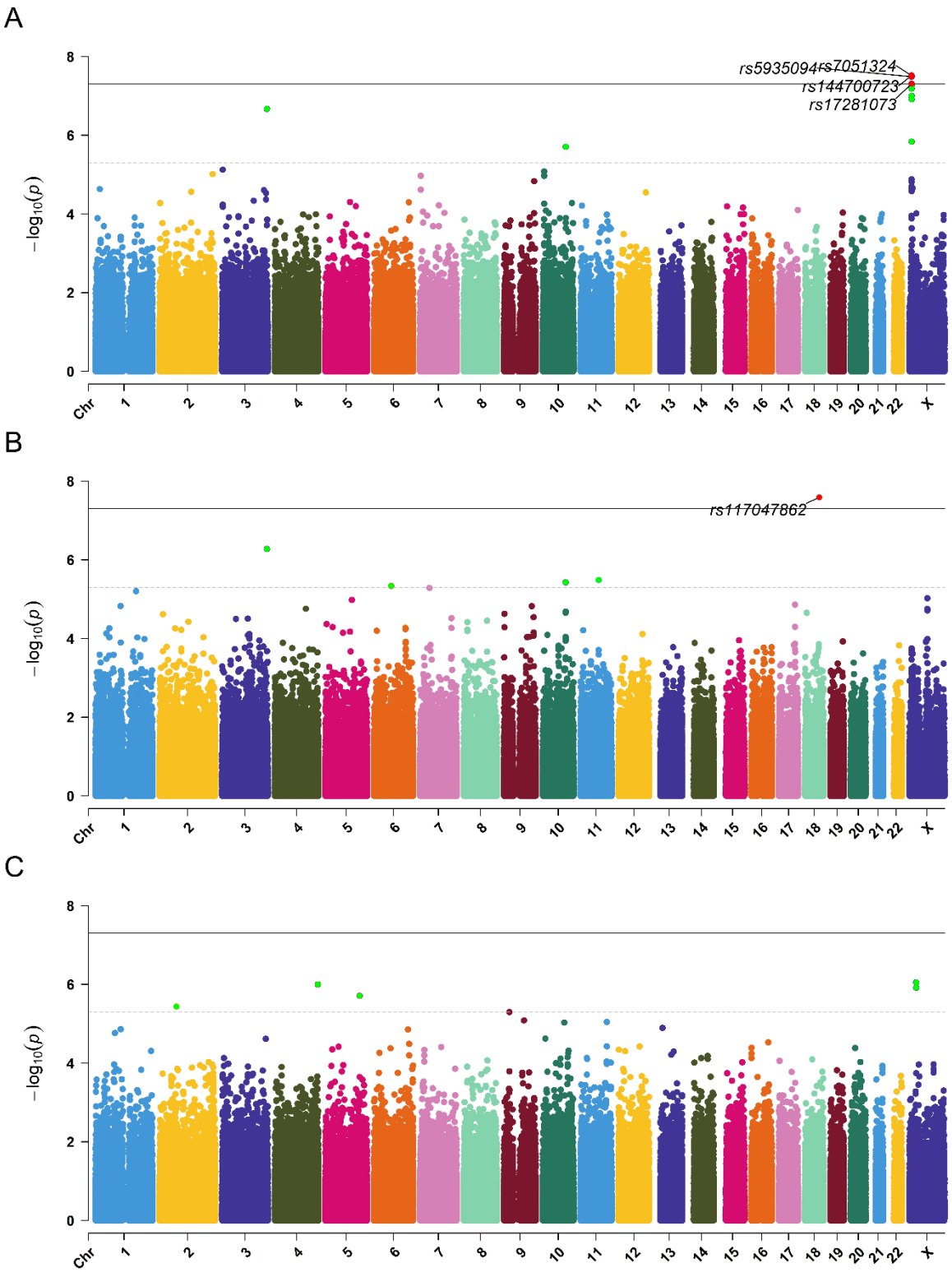


Figure 5.2 Manhattan plot of -log10 association P values for GWAS of longitudinal bone change at the (A) femoral neck, (B) total hip, and (C) lumbar spine.

Black line: Genome-wide significant signals (P<5×10-8); Grey dotted line: Suggestive significant signals (P<5×10-6).

Table 5.2. Summary of loci associated with longitudinal bone change at the femoral neck, total hip, and lumbar spine.

Nearest gene	SNP	Position	A1/A2	Annotation	GWAS Catalog	Beta	p-value
FNBMD							
ARHGAP6	rs144700723	X:11417249	A/G	Intron Variant	Novel	0.105	**3.0×10^{-08}**
	rs17281073	X:11438214	G/A	Intron Variant	Novel	0.102	**4.9×10^{-08}**
	rs5935094	X:11514281	C/A	Intron Variant	Novel	0.103	**3.3×10^{-08}**
	rs7051324	X:11532402	C/A	Intron Variant	Novel	0.103	**3.1×10^{-08}**
	rs5935064	X:11441800	G/A	Intron Variant	Novel	0.102	5.5×10^{-08}
	rs200979100	X:11444743	G/A	Intron Variant	Novel	0.101	6.6×10^{-08}
	rs59199930	X:11453192	A/C	Intron Variant	Novel	0.080	1.5×10^{-06}
	rs17281094	X:11481592	A/G	Intron Variant	Novel	0.100	1.2×10^{-07}
	rs202233144	X:11504058	A/C	Intron Variant	Novel	0.098	10.0×10^{-08}
TMEM44	rs12631612	3:194611560	G/A	Intron Variant	Novel	0.232	2.1×10^{-07}
DNMBP	rs4919399	10:99919106	A/G	Intron Variant	Novel	0.082	2.0×10^{-06}
Total hip BMD							
ENSG00000267175	rs117047862	18:61624110	G/A	Intron Variant	Novel	-0.242	**2.6×10^{-08}**
TMEM44	rs12631612	3:194611560	G/A	Intron Variant	Novel	0.214	5.3×10^{-07}
COL12A1	rs17186509	6:75117312	G/A	Intron Variant	Novel	0.240	4.6×10^{-06}
NKX2-3	rs1332099	10:99538694	A/G	Intergenic Variant	Reported	-0.072	3.7×10^{-06}
TENM4	rs649931	11:78987242	A/G	Intron Variant	Novel	-0.080	3.3×10^{-06}

LSBMD							
EMX1	rs12470805	2:72935412	A/G	Intergenic Variant	Novel	0.141	3.6×10^{-06}
LINC02492	rs78286952	4:187660806	G/A	Intron Variant	Novel	-0.104	1.0×10^{-06}
SH3TC2-DT	rs11168086	5:149104624	A/G	Intron Variant	Novel	-0.091	1.9×10^{-06}
DMD	rs4521910	X:31917836	A/G	Intron Variant	Novel	0.137	8.9×10^{-07}
	rs12008181	X:31919466	A/G	Intron Variant	Novel	0.138	1.2×10^{-06}

SNPs with $P < 5 \times 10^{-6}$ reported, with those at the genome-wide significant threshold ($P < 5 \times 10^{-8}$) in boldface. FNBMD, femoral neck bone mineral density; total hip BMD, total hip bone mineral density; LSBMD, lumbar spine bone mineral density.

5.4 Discussion

Using longitudinal BMD measurements from the VOS cohort, we identified five genome-wide significant and sixteen suggestively associated genetic variants linked to bone loss at the femoral neck and total hip. These associations were derived after adjusting for key covariates including age and sex; and consistent with an adequate statistical power (≥80% for MAF≥0.2) variants explaining ≥1.5% BMD change variance (Supplemental Table 8). Among the genome-wide significant findings, two candidate genes – *ARHGAP6*, a member of the Rho GTPase-activating protein family, and *ENSG00000267175*, a long non-coding RNA gene – were highlighted for their potential involvement in the regulation of bone change. Interestingly, *ENSG00000267175* showed a negative direction of effect compared to the predominantly positive associations observed in other variants. The genetic architecture captured in this cohort suggests novel pathways influencing BMD change, offering valuable insights into skeletal ageing beyond baseline BMD alone.

Our study shares a conceptual focus with previous investigations[84,85] that have aimed to elucidate the biological basis of skeletal aging by investigating longitudinal changes in bone. However, our study focuses explicitly on BMD change at key skeletal sites (total hip, femoral neck, lumbar spine), while the investigation using the Hertfordshire Cohort Study[85] emphasises microarchitectural parameters such as trabecular number and cortical thickness. Additionally, the investigation based on the UK Biobank[84] examines both the mean BMD trajectory and within-subject variability using large-scale, multi-timepoint data. Our study's sample size (n ≈ 2300) is considerably smaller than the UK Biobank cohort (n ≈ 141 000), limiting its statistical power but providing valuable insights into an underrepresented Southeast Asian population. Notably, our study cohort used DXA-based site-specific BMD

measurements, while UK Biobank employed ultrasound-based heel BMD as a proxy trait, which may introduce differences in trait sensitivity and fracture relevance.

In terms of genetic analysis, all three studies employed genome-wide approaches, but with different scopes and methodologies. Our analyses utilized the fastGWA linear mixed model with age and sex as covariates to assess SNP associations with the rate of BMD change, while He et al.[84] leveraged TrajGWAS, a specialized longitudinal tool that models both mean BMD change and intra-individual variability. Fuggle et al.[85] in contrast, did not perform a full GWAS but instead evaluated established BMD loci from prior GWAS for their associations with microarchitectural traits and fracture risk, offering a more targeted candidate gene approach. Our investigation identified novel loci such as *ARHGAP6* and *ENSG00000267175*, which had not been previously linked to BMD change or trajectory, suggesting distinct mechanisms may underpin longitudinal bone change. Meanwhile, the UK Biobank study replicated known loci (e.g., *WNT16, CPED1, FAM3C*) and identified new candidates related to variability. This contrast highlights that while the genetic determinants of bone status and structure have significant overlap, BMD change as a dynamic phenotype may involve a distinct, partially unexplored genetic architecture, as supported by our findings, and He and colleagues. Furthermore, the ethnic specificity of the VOS cohort underscores the importance of genetic studies in non-European populations to capture the full spectrum of osteoporosis risk.

Although we identified several novel genome-wide significant SNPs associated with longitudinal BMD change – particularly those within *ARHGAP6*, *TMEM44*, and *LINC02492* – were not reported in prior large-scale GWAS of BMD, fracture, or related osteoporosis traits such as those by the GEFOS consortium or

UK Biobank[83]. Nevertheless, intriguing functional and structural parallels exist between the genes highlighted in our investigation and previously established loci. For instance, *ARHGAP6* belongs to the same Rho GTPase-activating protein family as *ARHGAP1*, a gene previously implicated in skeletal phenotypes and cytoskeletal remodelling[179]. Similarly, *TMEM44* is part of the broader transmembrane (TMEM) gene family, where *TMEM135* has been linked to mitochondrial function and osteogenic differentiation[180]. Additionally, LINC02492 is a long intergenic non-coding RNA (lincRNA), a class of RNA genes that includes LINC00326[181], which was identified in earlier studies exploring non-protein-coding regulation of BMD. These parallels suggest that while the specific loci may be novel, they reside within biologically relevant gene families and functional categories, potentially pointing toward shared regulatory pathways or converging mechanisms in skeletal biology.

Of particular note is the SNP rs1332099 located near *NKX2-3*, which was the only variant in our analyses to have prior documentation in the GWAS Catalog. However, its previous associations were not related to bone traits. Instead, *NKX2-3* has been implicated in autoimmune[182] and psychiatric conditions[183]. The first of these associations emerged from a meta-analysis of ten paediatric-onset autoimmune diseases, where inverse χ^2 methods revealed consistent effects across multiple immune-mediated traits. The second association was reported in a cross-case genome-wide association study (CC-GWAS) investigating psychiatric disorders, further highlighting the gene's pleiotropic nature. While its relevance to bone biology remains speculative, the identification of *NKX2-3* in the VOS cohort raises compelling questions about potential immuno-skeletal interactions or pleiotropic pathways that warrant further exploration. Collectively, these findings from VOS underscore the value of examining underrepresented populations and dynamic

skeletal phenotypes, as they may uncover new biological insights beyond those observed in traditional BMD-focused GWAS.

Our study offers several strengths. Notably, the use of longitudinal BMD measurements provides a unique perspective on skeletal aging that cross-sectional studies cannot capture. This design allows for the detection of genetic variants associated with the rate of bone loss rather than just baseline BMD. Additionally, the phenotype of interest – bone change over time – more closely reflects the biological processes that lead to osteoporosis and fracture. However, the study also has limitations. The modest sample size may have limited the power to detect associations with smaller effect sizes (<1% variance); however, post-hoc calculations (Supplementary Table 8) confirm adequate power (≥80%) for moderate effects observed in our genome-wide significant loci. Furthermore, the use of a genotyping array focused on common variants may have missed associations with rare but potentially impactful genetic changes. Selection bias may also be present, as participants with only one BMD measurement were younger with higher baseline BMD and different sex distribution than the analytic cohort with ≥2 BMD measurements (Appendix A: Supplemental Table 7). Furthermore, because the study was conducted exclusively in an Asian population, the applicability of our findings to other ethnic groups remains uncertain. Finally, functional validation of the implicated loci is required to understand the biological mechanisms through which these variants influence bone change.

In conclusion, our study contributes to the growing body of literature on the genetic determinants of age-related bone loss by identifying loci associated with longitudinal BMD change in an Asian cohort. These findings highlight the importance of dynamic bone phenotypes in genetic studies and support the need for further

research in diverse populations, with larger sample sizes and functional follow-up, to improve our understanding of the biological underpinnings of osteoporosis.

Chapter 6. Conclusions and Future Directions

Preface

In this chapter, I summarise the findings of the three primary studies that make up this thesis. Furthermore, I discuss the implications and future aims to follow from the collective findings of this thesis.

6.1 Summary

Modern advances in medicine have significantly improved human health and extended life expectancy, but this success has contributed to an exponentially growing ageing population both in Australia (AIHW, 2024)[184] and globally (WHO, 2020)[185]. With age comes an increased prevalence of chronic degenerative diseases, such as osteoporosis, which pose serious clinical and public health challenges. This demographic shift brings about substantial burdens to healthcare systems, individuals, and communities due to the rising demand for medical care, loss of independence, and increased healthcare costs.

The purpose of this thesis was to build upon the current knowledge of bone and mineral research using methods from genetic epidemiology, statistical genetics, and bioinformatics to investigate an understudied phenotype in osteoporosis – bone loss. Briefly, the primary findings of this thesis are as follows.

6.1.1 Bone loss is an independent risk factor

A longitudinal analysis of BMD revealed that annual femoral neck bone loss is significantly associated with an increased risk of hip fracture in elderly men. Participants were grouped based on their annual BMD loss into three categories: unchanged (<1% loss/year), mild loss (1–2% loss/year), and rapid loss (>2% loss/year). In men, mild and rapid bone loss were associated with a 3-fold and 4-fold increase in hip fracture risk, respectively, after adjusting for age. Further adjustment for baseline BMD, BMI, lifestyle factors, and fracture history attenuated these associations, but mild bone loss remained a statistically significant predictor, with a 68% increased risk of hip fracture. This suggests that mild bone loss may be a

clinically relevant indicator for future fracture risk in elderly men, even when accounting for traditional risk factors.

In women, the association between femoral neck bone loss and fracture risk was less pronounced. Although women with fractures had significantly greater average annual bone loss (-1.00% to -1.11% per year) compared to non-fracture women, the predictive value of bone loss was largely diminished after full adjustment for confounders. These findings indicate that while bone loss is a marker of fracture risk in women, its independent contribution is less robust than in men. Notably, the exclusion of early fracture events and the use of three BMD measurements per participant provided a more accurate assessment of longitudinal bone change, avoiding the overestimation of risk commonly seen in studies with only two measurements and overlapping fracture events.

These results highlight the importance of incorporating longitudinal BMD measurements into clinical assessment models, particularly for elderly men. The dynamic view of bone loss offers valuable insight beyond static BMD measures and suggests that monitoring bone loss over time could help identify individuals at elevated risk of fracture. This study underscores the potential for using bone loss trajectories as a predictive biomarker and supports further exploration into the biological mechanisms linking bone loss to fracture susceptibility.

6.1.2 FTO SNPs associated with hip fracture risk

A prospective study from the Dubbo Osteoporosis Epidemiology Study (DOES) investigated the association between common *FTO* gene variants and osteoporosis phenotypes, including BMD, longitudinal bone loss, and fracture risk in post-menopausal women. Six SNPs in intron 1 of *FTO* were genotyped, with

rs9930506 selected as the representative variant due to its similarity with the others. Analysis showed that *FTO* polymorphisms were not associated with baseline BMD at the femoral neck or lumbar spine, nor with longitudinal rates of bone loss. However, when fracture risk was examined over a median 14-year follow-up, the minor homozygous allele (GG) of rs9930506 was significantly associated with hip fracture risk (HR: 1.82, 95% CI: 1.10–3.03), independent of age, BMI, and BMD. This association was further supported by a robust E-value of 3.25, suggesting it would require an unusually strong unmeasured confounder to fully explain the result.

These findings highlight that *FTO* genetic variation exerts a modest but independent effect on hip fracture risk, without mediation through BMD or bone loss. While the association between *FTO* and obesity-related phenotypes is well established across multiple ethnic groups, this study extends its relevance to skeletal health, particularly in fracture susceptibility. Importantly, the absence of association with BMD and bone loss suggests that *FTO*'s influence on fracture may be mediated through alternative biological pathways, potentially involving body composition or adiposity-related mechanisms. The findings were largely consistent across other *FTO* SNPs (rs1121980, rs1421085, rs1558902, rs17817449, and rs9939609), though only rs1121980 showed a similarly strong association with hip fracture.

Taken together, this study provides novel evidence that common *FTO* polymorphisms may act as genetic biomarkers for hip fracture risk in elderly women. While their predictive value in isolation is limited, these variants may hold promise when incorporated into polygenic risk scores to improve fracture risk models. Future studies are needed to replicate these findings in non-Caucasian populations, given the stronger weight-related health risks in Asian groups, and to explore the underlying mechanisms linking *FTO* variation to skeletal fragility.

6.1.3 Novel genetic variants associated with longitudinal bone change

A GWAS of longitudinal BMD change was performed in the VOS cohort to investigate the genetic determinants of age-related bone loss. Using data from 2343 men and women with multiple BMD measurements at the femoral neck, total hip, and lumbar spine, a linear mixed-effects regression model was applied to calculate annual rates of bone change. Genome-wide analysis identified five novel genome-wide significant variants ($P<5\times10^{-8}$) associated with BMD change at the femoral neck and total hip, along with 16 suggestive variants. Among these, *ARHGAP6* and *ENSG00000267175* were highlighted as candidate genes, implicating pathways linked to actin cytoskeleton remodelling and long non-coding RNA regulation. Notably, most loci identified have not been previously reported in the context of bone loss, suggesting that longitudinal phenotypes may reveal novel biological insights not captured by cross-sectional studies.

Our findings extend the current understanding of skeletal genetics by demonstrating that longitudinal BMD change has a distinct genetic architecture from baseline BMD. While cross-sectional GWAS have identified over 500 loci for BMD[82], few studies have examined genetic factors underlying bone loss trajectories. The identification of *ARHGAP6*, a gene with known roles in disease processes such as cancer and neurodevelopmental disorders, underscores the importance of exploring shared pathways between skeletal health and other biological systems. Likewise, the discovery of *ENSG00000267175*, a novel RNA gene, opens avenues for further investigation into regulatory elements contributing to skeletal aging. Although our sample size was modest compared to larger meta-analyses, the study provides valuable insight into genetic factors influencing longitudinal bone loss in an Asian population, an underrepresented group in osteoporosis research. Replication in

diverse cohorts and functional validation of implicated loci will be crucial to establish their biological relevance and potential as clinical biomarkers of fracture risk.

Osteoporosis is a common skeletal disorder characterized by reduced bone mass and structural deterioration, predisposing individuals to an increased risk of fracture. BMD, a key diagnostic metric for osteoporosis, changes dynamically across the lifespan and is influenced by both peak bone mass acquisition and age-related bone loss. While GWAS have identified numerous loci associated with cross-sectional BMD, few have investigated the genetic determinants of longitudinal BMD change, particularly bone loss. In this study, we performed a GWAS on longitudinal BMD change at the femoral neck, total hip, and lumbar spine using data from 2343 individuals with multiple BMD measurements and complete genotype and covariate data. Several genome-wide significant loci were identified, including *ARHGAP6* (associated with femoral neck BMD change) and the novel transcript *ENSG00000267175* (associated with total hip BMD change). Suggestive associations for lumbar spine BMD change were detected at *EMX1*, *LINC02492*, *SH3TC2-DT*, and *DMD* loci. Notably, most of these loci have not been previously implicated in cross-sectional BMD GWAS, suggesting they may contribute specifically to age-related bone loss rather than peak bone mass. These findings provide new insight into the genetic architecture underlying skeletal aging and highlight potential biological pathways distinct from those influencing static BMD measurements. Further functional validation of these loci may facilitate the development of personalized diagnostic tools and therapeutic strategies for osteoporosis prevention and treatment.

6.2 Future direction and perspectives

As global life expectancy rises, degenerative age-related diseases like osteoporosis are becoming more widespread, leading to a growing burden on healthcare systems. Fractures caused by osteoporosis remain a critical public health issue. To address this challenge, there is a pressing need to enhance current fracture prediction models by identifying individuals at heightened risk earlier in life. Traditional models typically rely on a single snapshot of BMD, which fails to capture the ongoing remodelling process that bone undergoes throughout the lifespan. A more informative strategy would involve monitoring BMD changes over time, offering a longitudinal perspective on bone health. Heritability estimates from twin and family studies suggest that genetic factors contribute significantly to both BMD (up to 80%) and fracture susceptibility (approximately 50%). However, the known genetic variants from genome-wide studies account for only a limited portion of this variance, suggesting a large proportion of unexplained genetic influence. Focusing research on genetic determinants of bone loss, rather than static BMD, could uncover novel biological mechanisms and targets for drug development. Although the present genome-wide association study in this thesis was limited to a single cohort – potentially contributing to the lower observed heritability – the bone loss and fracture risk association highlights the importance of integrating genetic data and longitudinal phenotypes in future osteoporosis research. Expanding these efforts across diverse populations and incorporating routinely collected digital health data may lead to more accurate, personalized fracture risk assessments and improved patient outcomes.

Following the work completed in this thesis, future research for osteoporosis can focus on these key areas:

6.2.1 GWAS Meta-analysis and Mendelian Randomisation

GWAS meta-analysis presents a powerful opportunity to deepen our understanding of the genetic determinants of bone loss. Previous GWAS meta-analyses have advanced the discovery of genetic loci underlying BMD and fracture susceptibility. For instance, large-scale efforts involving over 80,000 individuals uncovered 56 BMD-associated loci, with 14 loci also linked to fracture risk, including well-known genes such as *LRP5, MEPE,* and *DKK1*[171]. These studies underscore how combining datasets improves detection of robust associations spanning diverse skeletal traits.

Beyond enhancing statistical power, GWAS meta-analysis enables cross cohort validation and exploration of heterogeneity. By pooling data from different populations, researchers reduce random errors and assess whether loci replicate across ancestries and environments[186,187]. Moreover, gene based meta-analyses confirm established loci and suggest novel candidate genes by integrating signals from multiple variants per gene[83,171,188,189].

While GWAS meta-analyses are powerful for discovering associated loci, converting these signals into causal biological insight remains difficult. Association peaks often span multiple correlated variants, so pinpointing the true causal allele typically requires statistical fine-mapping alongside functional annotation (e.g., transcriptomic, epigenomic, and proteomic data) to prioritise the most plausible candidates[190]. Despite ongoing improvements in fine-mapping, separating correlation from causation remains a core limitation of GWAS, motivating continued development of approaches that integrate multi-omics evidence for osteoporosis-related traits[187,189,191-193].

After robust loci have been identified, Mendelian randomisation (MR) can provide a complementary strategy to strengthen causal inference[194,195]. MR leverages genetic variants as instrumental variables to test whether an exposure (e.g., BMD, bone turnover markers, or hormonal/metabolic factors) has a causal effect on outcomes such as bone loss or fracture risk. Because alleles are randomly assigned at conception, MR is generally less prone to confounding and reverse causation than conventional observational analyses[196]. Well-powered GWAS meta-analyses are particularly useful for MR because they can yield strong instruments and the summary statistics needed for reliable causal estimation[197-199].

Recent MR studies in musculoskeletal research have supported causal links between BMD, body composition, endocrine traits, and fracture risk[193,200-203], highlighting the clinical value of genetically informed causal analyses. However, MR validity depends on key assumptions – especially that instruments influence the outcome only through the exposure (i.e., no horizontal pleiotropy) – and therefore requires careful sensitivity analyses and interpretation[196]. For these reasons, MR is best positioned as a downstream analysis following adequately powered GWAS or GWAS meta-analyses, rather than being applied to exploratory association scans.

Looking ahead, integrating GWAS meta-analysis with Mendelian randomisation offers a coherent analytical pipeline for osteoporosis research. Expanded, ethnically diverse GWAS meta-analyses of bone loss will enable the discovery of robust genetic instruments, which can then be leveraged in MR analyses to prioritise causal pathways and therapeutic targets. Together with fine-mapping and multi-omics integration, this combined approach has the potential to translate genetic associations into biological mechanisms, improve personalised

fracture risk prediction, and inform the development of targeted interventions for osteoporosis.

6.2.2 Rare variants and the missing heritability

Despite numerous successes in identifying common variants associated with BMD and fracture risk, a substantial portion of osteoporosis heritability remains unexplained[204,205]. Rare genetic variants (MAF <1%) are now recognised as important contributors to “missing heritability”. These variants, often found in coding or regulatory regions, tend to have larger individual effect sizes compared to common variants and may influence disease through mechanisms not detectable by GWAS alone[206].

Because of their low frequency, rare variants are often missed in traditional GWAS, which are designed to detect common variants. However, technologies like whole-exome and whole-genome sequencing now enable researchers to comprehensively assess rare variant contributions[207]. Including rare variants in risk models has already been shown to improve the prediction of osteoporotic fractures, especially for individuals at the extremes of risk distribution[206,208]. This is crucial for clinical decision-marking, as it can help identify individuals who may benefit most from early intervention or targeted therapy.

From an evolutionary perspective, rare deleterious alleles are expected to persist at low frequencies in populations, especially if they exhibit substantial negative effects on survival fitness[206,209]. Many Mendelian disorders and familial cases of severe osteoporosis have been linked to rare variants of large effect[204]. Thus, studying rare variants not only aids in understanding osteoporosis risk at the

population level but also provides mechanistic insights that can inform therapeutic development.

Going forward, integrating rare variant analyses with studies of common variants offers a more complete picture of genetic risk. Combining these data streams can enhance the performance of PRS and uncover gene-gene interactions. Moreover, family-based studies and population isolates may be particularly useful for identifying rare variants with strong effects[210,211]. As large sequencing cohorts continue to grow, the potential to harness rare variant data for osteoporosis research will expand, offering more precise and personalised approaches to prevention and care.

6.2.3 Multi-omics data integration

While genetic data provide a foundation for understanding bone loss, a more complete picture of osteoporosis biology requires integrating multiple layers of molecular information. Multi-omics approaches – combining genomics with transcriptomics, proteomics, metabolomics, and epigenomics – enable researchers to understand how genetic risk translates into phenotypic outcomes through dynamic biological processes[212]. This integrative strategy offers significant potential for identifying molecular pathways involved in bone homeostasis, uncovering novel biomarkers, and refining disease subtypes[213].

For example, transcriptomic data can reveal how risk variants identified through GWAS influence gene expression in bone-related tissues, while proteomics can identify changes in protein abundance or modification that directly impact bone metabolism. Metabolomics can provide insights into systemic metabolic changes that contribute to bone degradation, and epigenomic data can identify regulatory changes

that modify gene activity in response to environmental factors like diet or exercise[213,214]. Together, these layers offer a comprehensive view of the pathophysiological mechanisms underlying osteoporosis.

One of the most promising applications of multi-omics is in the advancement of personalised medicine. By integrating omics data, researchers can predict individual responses to therapies, identify optimal drug targets, and develop personalised treatment plans. Spatial and single-cell multi-omics technologies further enhance this potential by mapping the molecular landscape of bone at cellular resolution, capturing heterogeneity within the bone microenvironment, and pinpointing the cell types most affected in osteoporosis[215].

Ultimately, the integration of multi-omics data can overcome the limitations of single-modality studies and reveal the full complexity of osteoporosis. As analytical tools and computational methods for multi-omics integration continue to improve, future research will be better equipped to link genetic risk with functional outcomes[216], enhancing both our understanding and management of bone loss. This systems-level approach holds the key to developing more effective, targeted, and personalised strategies for preventing and treating osteoporosis.

6.3 Conclusion

The research presented in this thesis underscores the critical role of bone loss as a key contributor to osteoporosis, particularly fracture risk in men. By validating the association between bone loss and fracture risk, this work sets a new direction for osteoporosis research – one that moves beyond static bone measurements and toward understanding bone as a dynamic tissue undergoing continual change. Preliminary findings from a single cohort have laid the groundwork for future large-

scale genome-wide meta-analyses by identifying genetic variants associated with bone loss or the dynamic change of bone. Notably, the analysis of a single candidate gene revealed that while certain biological pathways may be relevant to osteoporosis or its related traits, they may not be implicated in bone loss specifically suggesting that bone loss may follow its own distinct mechanisms. This highlights the complexity of studying bone remodelling and the need to investigate its underlying biological architecture in greater detail. Overall, the work documented here offers meaningful insight into the genetic and mechanistic underpinnings of bone loss and serves as an important stepping stone for advancing different fields of osteoporosis research.

Appendix A: Supplemental Tables

Supplemental Table 1. Baseline characteristics stratified by FTO genotype (rs1421085, rs1558902, rs1121980, rs17817449 and rs9939609).

rs1421085	**CC (N=202)**	**CT (N=549)**	**TT (N=392)**
Age (years)	69 (6)	70 (7)	70 (7)
Height (cm)	160 (6)	160 (6)	160 (6)
Weight (kg)	66 (12)	66 (12)	68 (13)
BMI (kg/m^2)	26 (5)	26 (5)	26 (5)
rs1558902	**AA (N=201)**	**AT (N=593)**	**TT (N=417)**
Age (years)	69 (6)	69 (7)	70 (7)
Height (cm)	160 (6)	160 (6)	160 (6)
Weight (kg)	65 (12)	67 (12)	66 (12)
BMI (kg/m^2)	26 (4)	26 (5)	26 (5)
rs1121980	**AA (N=214)**	**AG (N=535)**	**GG (N=374)**
Age (years)	70 (7)	69 (7)	70 (7)
Height (cm)	160 (6)	160 (6)	160 (6)
Weight (kg)	66 (13)	68 (13)	68 (13)
BMI (kg/m^2)	26 (5)	26 (5)	26 (4)
rs17817449	**GG (N=193)**	**GT (N=571)**	**TT (N=415)**
Age (years)	69 (6)	69 (7)	69 (7)
Height (cm)	160 (6)	160 (6)	160 (6)
Weight (kg)	66 (12)	67 (13)	68 (13)
BMI (kg/m^2)	26 (4)	26 (5)	26 (5)
rs9939609	**AA (N=183)**	**AT (N=480)**	**TT (N=373)**
Age (years)	69 (6)	69 (7)	69 (7)
Height (cm)	160 (6)	161 (6)	160 (6)
Weight (kg)	65 (11)	67 (13)	68 (12)
BMI (kg/m^2)	26 (4)	26 (5)	26 (5)

Supplemental Table 2. Association between FTO genotypes of rs1421085 and BMD, bone loss and fracture.

(I) BMD						
	CC (N=202)	TC (N=549)	TT* (N=392)	TC vs TT	CC vs TT	TC vs CC
FNBMD (g/cm^2)	0.80 (0.14)	0.80 (0.14)	0.80 (0.13)	0.007 (-0.007, 0.022)	0.001 (-0.018, 0.021)	-0.006 (-0.025, 0.012)
LSBMD (g/cm^2)	1.05 (0.21)	1.04 (0.20)	1.05 (0.19)	-0.006 (-0.030, 0.018)	0.002 (-0.030, 0.034)	0.008 (-0.022, 0.038)
(II) Rate of BMD change						
	CC	TC	TT*	TC vs TT	CC vs TT	TC vs CC
FNBMD (g/cm^2/year)	-0.004 (0.003)	-0.004 (0.004)	-0.004 (0.004)	< 0.001 (< -0.001, 0.001)	< -0.001 (-0.001, 0.001)	< -0.001 (-0.001, 0.001)
LSBMD (g/cm^2/year)	0.002 (0.007)	0.002 (0.007)	0.002 (0.007)	< 0.001 (-0.001, 0.001)	< 0.001 (-0.001, 0.002)	< 0.001 (-0.002, 0.002)
(III) Fracture						
Any fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	CC (N=206)	98 (47.6%)	1.00 (0.78-1.28)	0.97	1.01 (0.79-1.29)	0.94
	TC (N=559)	268 (47.9%)	1.03 (0.85-1.23)	0.78	1.04 (0.86-1.25)	0.70
	TT* (N=399)	190 (47.6%)	Reference	-	Reference	-
Hip fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	CC (N=206)	32 (15.5%)	1.46 (0.92-2.30)	0.10	1.47 (0.93-2.31)	0.09
	TC (N=559)	58 (10.4%)	0.87 (0.59-1.28)	0.47	0.87 (0.59-1.29)	0.48
	TT* (N=399)	46 (11.5%)	Reference	-	Reference	-

*reference genotype: TT. (I) BMD and (II) Rate of BMD change: values for BMD and rate of bone change are provided as mean (SD). The association between FTO genotype and BMD and bone loss are presented as mean difference (95% CI) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. Bone loss), adjusted for age and BMI. (III) Fracture: † data presented as number of patients with a fracture (%). **adjusted for age, femoral neck BMD and BMI. **Bold font indicates statistical significance.**

Supplemental Table 3. Association between FTO genotypes of rs1558902 and BMD, bone loss and fracture.

(I) BMD						
	AA (N=201)	TA (n=593)	TT* (N=417)	TA vs TT	AA vs TT	TA vs AA
FNBMD (g/cm^2)	0.79 (0.13)	0.81 (0.13)	0.79 (0.13)	0.014 (-0.001, 0.028)	-0.002 (-0.022, 0.017)	-0.016 (-0.034, 0.002)
LSBMD (g/cm^2)	1.04 (0.21)	1.04 (0.19)	1.05 (0.19)	-0.007 (-0.030, 0.016)	- 0.005 (-0.036, 0.026)	0.002 (-0.027, 0.032)
(II) Rate of BMD change						
	AA	TA	TT*	TA vs TT	AA vs TT	TA vs AA
FNBMD (g/cm^2/year)	-0.004 (0.007)	-0.004 (0.004)	-0.004 (0.004)	< 0.001 (-0.001, 0.001)	< -0.001 (-0.001, 0.001)	< -0.001 (-0.001, 0.001)
LSBMD (g/cm^2/year)	0.002 (7.26)	0.002 (0.007)	0.002 (0.006)	< 0.001 (-0.001, 0.002)	< 0.001 (-0.001, 0.002)	< -0.001 (-0.002, 0.001)
(III) Fracture						
Any fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	AA (N=204)	98 (47.6%)	1.02 (0.80-1.31)	0.85	1.03 (0.81-1.32)	0.80
	TA (N=603)	268 (47.9%)	1.03 (0.86-1.23)	0.78	1.07 (0.89-1.28)	0.48
	TT (N=426)	201 (47.2%)	Reference	-	Reference	-
Hip fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	AA (N=204)	32 (15.7%)	1.47 (0.94-2.31)	0.09	1.54 (0.98-2.42)	0.06
	TA (N=603)	65 (10.8%)	0.93 (0.64-1.36)	0.72	1.00 (0.69-1.46)	1.00
	TT (N=426)	48 (11.3%)	Reference	-	Reference	-

*reference genotype: TT. (I) BMD and (II) Rate of BMD change: values for BMD and rate of bone change are provided as mean (SD). The association between FTO genotype and BMD and bone loss are presented as mean difference (95% CI) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. Bone loss), adjusted for age and BMI. (III) Fracture: † data presented as number of patients with a fracture (%). **adjusted for age, femoral neck BMD and BMI. **Bold font indicates statistical significance.**

Supplemental Table 4. Association between FTO genotypes of rs1121980 and BMD, bone loss and fracture.

(I) BMD						
	AA (N=214)	GA (N=535)	GG* (N=374)	GA vs GG	AA vs GG	GA vs AA
FNBMD (g/cm^2)	0.79 (0.14)	0.80 (0.14)	0.79 (0.13)	0.012 (-0.003, 0.027)	0.006 (-0.013, 0.026)	- 0.005 (-0.024, 0.013)
LSBMD (g/cm^2)	1.05 (0.22)	1.04 (0.19)	1.06 (0.19)	- 0.015 (-0.040, 0.010)	< 0.001 (-0.031, 0.032)	0.015 (-0.014, 0.045)
(II) Rate of BMD change						
	AA	GA	GG*	GA vs GG	AA vs GG	GA vs AA
FNBMD (g/cm^2/year)	-0.004 (0.003)	-0.004 (0.004)	-0.004 (0.004)	< -0.001 (-0.001, 0.001)	< -0.001 (-0.002, 0.001)	< -0.001 (-0.001, 0.001)
LSBMD (g/cm^2/year)	0.002 (0.007)	0.002 (0.007)	0.002 (0.006)	< 0.001 (-0.001, 0.002)	< 0.001 (-0.001, 0.002)	< -0.001 (-0.002, 0.002)
(III) Fracture						
Any fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	AA (N=217)	102 (47.0%)	0.97 (0.76-1.24)	0.82	1.00 (0.78-1.27)	0.97
	GA (N=545)	262 (48.1%)	1.03 (0.85-1.24)	0.78	1.06 (0.87-1.28)	0.58
	GG (N=381)	181 (47.5%)	Reference	-	Reference	-
Hip fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	AA (N=217)	35 (16.1%)	**1.68 (1.06-2.65)**	**0.02**	**1.76 (1.11-2.79)**	**0.01**
	GA (N=545)	57 (10.5%)	1.03 (0.68-1.56)	0.89	1.03 (0.68-1.56)	0.88
	GG (N=381)	38 (10.0%)	Reference	-	Reference	-

*reference genotype: GG. (I) BMD and (II) Rate of BMD change: values for BMD and rate of bone change are provided as mean (SD). The association between FTO genotype and BMD and bone loss are presented as mean difference (95% CI) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. Bone loss), adjusted for age and BMI. (III) Fracture: † data presented as number of patients with a fracture (%). **adjusted for age, femoral neck BMD and BMI. **Bold font indicates statistical significance.**

Supplemental Table 5. Association between FTO genotypes of rs17817449 and BMD, bone loss and fracture.

(I) BMD						
	GG (N=193)	TG (N=571)	TT* (N=415)	TG vs TT	GG vs TT	TG vs GG
FNBMD (g/cm^2)	0.79 (0.13)	0.81 (0.14)	0.79 (0.13)	0.015 (< -0.001, 0.029)	< 0.001 (-0.020, 0.019)	-0.015 (-0.034, 0.004)
LSBMD (g/cm^2)	1.04 (0.20)	1.05 (0.20)	1.05 (0.19)	-0.005 (-0.028, 0.018)	-0.007 (-0.039, 0.024)	-0.002 (-0.032, 0.028)
(II) Rate of BMD change						
	GG	TG	TT*	TG vs TT	GG vs TT	TG vs GG
FNBMD (g/cm^2/year)	-0.004 (0.003)	-0.004 (0.003)	-0.004 (3.69)	< 0.001 (-0.001, 0.001)	< -0.001 (-0.001, 0.001)	< -0.001 (-0.001, 0.001)
LSBMD (g/cm^2/year)	0.002 (0.007)	0.002 (0.007)	0.002 (0.006)	< 0.001 (-0.001, 0.001)	< 0.001 (-0.001, 0.002)	< 0.001 (-0.001, 0.002)
(III) Fracture						
Any fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	GG (N=196)	91 (46.4%)	0.99 (0.77-1.27)	0.92	0.99 (0.77-1.28)	0.96
	TG (N=581)	272 (46.8%)	1.02 (0.85-1.23)	0.80	1.07 (0.89-1.28)	0.49
	TT (N=424)	196 (46.2%)	Reference	-	Reference	-
Hip fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	GG (N=196)	29 (14.8%)	1.59 (0.95-2.45)	0.08	1.59 (0.99-2.55)	0.05
	TG (N=581)	56 (9.6%)	0.92 (0.62-1.37)	0.69	0.98 (0.66-1.47)	0.93
	TT (N=424)	43 (10.1%)	Reference	-	Reference	-

*reference genotype: TT. (I) BMD and (II) Rate of BMD change: values for BMD and rate of bone change are provided as mean (SD). The association between FTO genotype and BMD and bone loss are presented as mean difference (95% CI) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. Bone loss), adjusted for age and BMI. (III) Fracture: † data presented as number of patients with a fracture (%). **adjusted for age, femoral neck BMD and BMI. **Bold font indicates statistical significance.**

Supplemental Table 6. Association between FTO genotypes of rs9939609 and BMD, bone loss and fracture.

(I) BMD						
	AA (N=183)	TA (N=480)	TT* (N=373)	TA vs TT	AA vs TT	TA vs AA
FNBMD (mg/cm^2)	0.79 (0.13)	0.81 (0.13)	0.80 (0.13)	9.76 (-5.70, 25.23)	-6.71 (-26.94, 13.53)	-16.47 (-35.91, 2.96)
LSBMD (mg/cm^2)	1.05 (0.20)	1.05 (0.20)	1.06 (0.19)	-14.74 (-39.67, 10.19)	-6.73 (-39.35, 25.89)	8.01 (-23.33, 39.35)
(II) Rate of BMD change						
	AA	TA	TT*	TA vs TT	AA vs TT	TA vs AA
FNBMD (mg/year)	-0.004 (0.003)	-0.004 (0.003)	-0.004 (0.004)	< -0.001 (-0.001, 0.001)	< 0.001 (-0.001, 0.001)	< 0.001 (-0.001, 0.001)
LSBMD (mg/year)	0.002 (0.008)	0.002 (0.007)	0.002 (0.006)	< 0.001 (-0.001, 0.001)	< 0.001 (-0.001, 0.002)	< 0.001 (-0.001, 0.002)
(III) Fracture						
Any fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	AA (N=187)	88 (47.1%)	1.02 (0.79-1.33)	0.83	1.01 (0.78-1.31)	0.94
	TA (N=487)	232 (47.6%)	1.10 (0.91-1.35)	0.33	1.13 (0.92-1.38)	0.24
	TT (N=381)	171 (44.9%)	Reference	-	Reference	-
Hip fracture			Age adjusted		Multivariable adjusted**	
	Variables	Number†	HR (CI)	P-value	HR (CI)	P-value
	AA (N=187)	23 (12.3%)	1.19 (0.71-1.99)	0.51	1.20 (0.71-2.00)	0.50
	TA (N=487)	51 (10.5%)	1.02 (0.68-1.55)	0.91	1.03 (0.68-1.57)	0.88
	TT (N=381)	40 (10.5%)	Reference	-	Reference	-

*reference genotype: TT. (I) BMD and (II) Rate of BMD change: values for BMD and rate of bone change are provided as mean (SD). The association between FTO genotype and BMD and bone loss are presented as mean difference (95% CI) derived from a multivariable linear regression (I. BMD) or mixed-effects regression (II. Bone loss), adjusted for age and BMI. (III) Fracture: † data presented as number of patients with a fracture (%). **adjusted for age, femoral neck BMD and BMI. **Bold font indicates statistical significance.**

Supplemental Table 7. Basic characteristics of VOS participants by number of BMD measurements.

Characteristic	Multiple (N=2430)	Single (1727)
Sex: Female Male	1664 (68.5%) 766 (31.5%)	**1035 (59.9%)** **692 (40.1%)**
Age (years)	51.5 (12.0)	**41.1 (16.0)**
Weight (kg)	56.6 (9.72)	56.6 (10.5)
Height (cm)	156 (7.43)	**158 (8.18)**
BMI (kg/cm^2)	23.2 (3.24)	**22.6 (3.41)**
Smokers	346 (14.2%)	279 (16.2%)
Alcohol consumers	398 (16.4%)	**338 (19.6%)**
Total hip (g/cm^2)	0.82 (0.14)	**0.85 (0.14)**
FNBMD (g/cm^2)	0.70 (0.14)	**0.74 (0.15)**
LSBMD (g/cm^2)	0.89 (0.15)	**0.92 (0.14)**

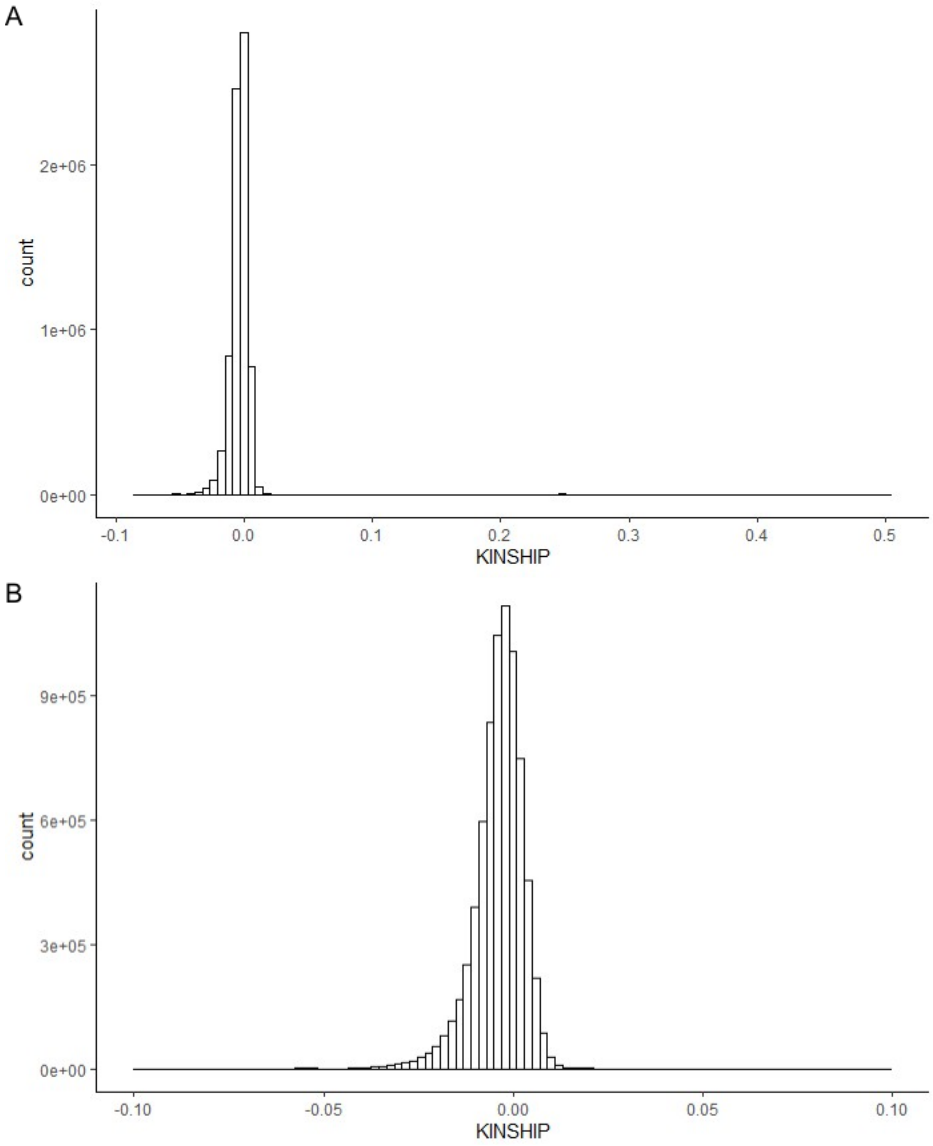
Values are mean ± SD, or frequencies (percentages). FNBMD, femoral neck bone mineral density. Bold values indicate P values less than 0.05. P-values were derived from t-test for continuous variables and from chi-squared test for binary variables.

Supplemental Table 8. Post-hoc statistical power for BMD change GWAS (N=2343, $\alpha=5\times10^{-8}$).

MAF	0.5% Var	1.0% Var	1.5% Var	2.0% Var
0.1	60%	**99%**	**100%**	**100%**
0.2	12%	73%	**98%**	**100%**
0.3	4%	44%	**85%**	**98%**
0.4	3%	31%	**74%**	**94%**

Var, variance explained by SNP; MAF, minor allele frequency. Power values ≥80% (bold) indicate effects reliably detectable.

Appendix B: Supplemental Figures



Supplemental Figure 1. Distribution of KING-robust kinship coefficients in the VOS cohort (n=3855 after quality control). (A) Full range of kinship coefficients. (B) Zoomed view limited to -0.1 to 0.1.

A 3-5% threshold, accounting for third cousins, was reviewed for characterisation but no exclusions applied. Analyses used fastGWA-GLMM to account for relatedness.

References

1 Al Anouti, F. *et al.* An insight into the paradigms of osteoporosis: From genetics to biomechanics. *Bone Rep* **11**, 100216, doi:10.1016/j.bonr.2019.100216 (2019).

2 Sözen, T., Özışık, L. & Başaran, N. An overview and management of osteoporosis. *Eur J Rheumatol* **4**, 46-56, doi:10.5152/eurjrheum.2016.048 (2017).

3 World Health, O. *WHO technical report series ; 843* (World Health Organization, Geneva, 1994).

4 Rosen, C. J. in *Endotext* (eds K. R. Feingold *et al.*) (MDText.com, Inc., 2000).

5 Alswat, K. A. Gender Disparities in Osteoporosis. *J Clin Med Res* **9**, 382-387, doi:10.14740/jocmr2970w (2017).

6 Zhang, P. *et al.* The study of bone mineral density measured by quantitative computed tomography in middle-aged and elderly men with abnormal glucose metabolism. *BMC Endocr Disord* **22**, 172, doi:10.1186/s12902-022-01076-6 (2022).

7 Grampp, S. *et al.* Comparisons of noninvasive bone mineral measurements in assessing age-related loss, fracture discrimination, and diagnostic classification. *J Bone Miner Res* **12**, 697-711, doi:10.1359/jbmr.1997.12.5.697 (1997).

8 Brandi, M. L. Microarchitecture, the key to bone quality. *Rheumatology (Oxford)* **48 Suppl 4**, iv3-8, doi:10.1093/rheumatology/kep273 (2009).

9 Rajan, R., Cherian, K. E., Kapoor, N. & Paul, T. V. Trabecular Bone Score-An Emerging Tool in the Management of Osteoporosis. *Indian J Endocrinol Metab* **24**, 237-243, doi:10.4103/ijem.IJEM_147_20 (2020).

10 Cummings, S. R., Black, D. M. & Rubin, S. M. Lifetime risks of hip, Colles', or vertebral fracture and coronary heart disease among white postmenopausal women. *Arch Intern Med* **149**, 2445-2448 (1989).

11 Cummings, S. R. *et al.* Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med* **332**, 767-773, doi:10.1056/nejm199503233321202 (1995).

12 Marshall, D., Johnell, O. & Wedel, H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *Bmj* **312**, 1254-1259, doi:10.1136/bmj.312.7041.1254 (1996).

13 Johnell, O. *et al.* Predictive value of BMD for hip and other fractures. *J Bone Miner Res* **20**, 1185-1194, doi:10.1359/jbmr.050304 (2005).

14 Nguyen, N. D., Ahlborg, H. G., Center, J. R., Eisman, J. A. & Nguyen, T. V. Residual lifetime risk of fractures in women and men. *J Bone Miner Res* **22**, 781-788, doi:10.1359/jbmr.070315 (2007).

15 Lee, J., Lee, S., Jang, S. & Ryu, O. H. Age-Related Changes in the Prevalence of Osteoporosis according to Gender and Skeletal Site: The Korea National Health and Nutrition Examination Survey 2008-2010. *Endocrinol Metab (Seoul)* **28**, 180-191, doi:10.3803/EnM.2013.28.3.180 (2013).

16 Barrett-Connor, E. *et al.* Osteoporosis and fracture risk in women of different ethnic groups. *J Bone Miner Res* **20**, 185-194, doi:10.1359/jbmr.041007 (2005).

17 Cauley, J. A. Defining ethnic and racial differences in osteoporosis and fragility fractures. *Clin Orthop Relat Res* **469**, 1891-1899, doi:10.1007/s11999-011-1863-5 (2011).
18 Guzon-Illescas, O. *et al.* Mortality after osteoporotic hip fracture: incidence, trends, and associated factors. *J Orthop Surg Res* **14**, 203, doi:10.1186/s13018-019-1226-6 (2019).
19 Tran, T. *et al.* Population-Wide Impact of Non-Hip Non-Vertebral Fractures on Mortality. *J Bone Miner Res* **32**, 1802-1810, doi:10.1002/jbmr.3118 (2017).
20 Ioannidis, G. *et al.* Relation between fractures and mortality: results from the Canadian Multicentre Osteoporosis Study. *Cmaj* **181**, 265-271, doi:10.1503/cmaj.081720 (2009).
21 O'Neill, T. W. & Roy, D. K. How many people develop fractures with what outcome? *Best Pract Res Clin Rheumatol* **19**, 879-895, doi:10.1016/j.berh.2005.06.003 (2005).
22 Cree, M. *et al.* Mortality and institutionalization following hip fracture. *J Am Geriatr Soc* **48**, 283-288, doi:10.1111/j.1532-5415.2000.tb02647.x (2000).
23 Nazrun, A. S., Tzar, M. N., Mokhtar, S. A. & Mohamed, I. N. A systematic review of the outcomes of osteoporotic fracture patients after hospital discharge: morbidity, subsequent fractures, and mortality. *Ther Clin Risk Manag* **10**, 937-948, doi:10.2147/tcrm.S72456 (2014).
24 Shi, N., Foley, K., Lenhart, G. & Badamgarav, E. Direct healthcare costs of hip, vertebral, and non-hip, non-vertebral fractures. *Bone* **45**, 1084-1090, doi:10.1016/j.bone.2009.07.086 (2009).
25 Leslie, W. D. *et al.* Direct costs of fractures in Canada and trends 1996-2006: a population-based cost-of-illness analysis. *J Bone Miner Res* **26**, 2419-2429, doi:10.1002/jbmr.457 (2011).
26 Rashki Kemmak, A. *et al.* Economic burden of osteoporosis in the world: A systematic review. *Med J Islam Repub Iran* **34**, 154-154, doi:10.34171/mjiri.34.154 (2020).
27 Henry, Y. M., Fatayerji, D. & Eastell, R. Attainment of peak bone mass at the lumbar spine, femoral neck and radius in men and women: relative contributions of bone size and volumetric bone mineral density. *Osteoporos Int* **15**, 263-273, doi:10.1007/s00198-003-1542-9 (2004).
28 Raisz, L. G. & Seeman, E. Causes of age-related bone loss and bone fragility: an alternative view. *J Bone Miner Res* **16**, 1948-1952, doi:10.1359/jbmr.2001.16.11.1948 (2001).
29 Baxter-Jones, A. D., Faulkner, R. A., Forwood, M. R., Mirwald, R. L. & Bailey, D. A. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *J Bone Miner Res* **26**, 1729-1739, doi:10.1002/jbmr.412 (2011).
30 Cosman, F. *et al.* Clinician's Guide to Prevention and Treatment of Osteoporosis. *Osteoporos Int* **25**, 2359-2381, doi:10.1007/s00198-014-2794-2 (2014).
31 Santos, L., Elliott-Sale, K. J. & Sale, C. Exercise and bone health across the lifespan. *Biogerontology* **18**, 931-946, doi:10.1007/s10522-017-9732-6 (2017).
32 Kanis, J. A., Cooper, C., Rizzoli, R. & Reginster, J. Y. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int* **30**, 3-44, doi:10.1007/s00198-018-4704-5 (2019).
33 Johnell, O. & Kanis, J. A. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int* **17**, 1726-1733, doi:10.1007/s00198-006-0172-4 (2006).

34 Management of osteoporosis in postmenopausal women: the 2021 position statement of The North American Menopause Society. *Menopause* **28**, 973-997, doi:10.1097/gme.0000000000001831 (2021).
35 Gullberg, B., Johnell, O. & Kanis, J. A. World-wide projections for hip fracture. *Osteoporos Int* **7**, 407-413, doi:10.1007/pl00004148 (1997).
36 Manolagas, S. C. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. *Endocr Rev* **31**, 266-300, doi:10.1210/er.2009-0024 (2010).
37 Seeman, E. & Delmas, P. D. Bone quality--the material and structural basis of bone strength and fragility. *N Engl J Med* **354**, 2250-2261, doi:10.1056/NEJMra053077 (2006).
38 Compston, J. E., McClung, M. R. & Leslie, W. D. Osteoporosis. *Lancet* **393**, 364-376, doi:10.1016/s0140-6736(18)32112-3 (2019).
39 Demontiero, O., Vidal, C. & Duque, G. Aging and bone loss: new insights for the clinician. *Ther Adv Musculoskelet Dis* **4**, 61-76, doi:10.1177/1759720x11430858 (2012).
40 Tu, K. N. *et al.* Osteoporosis: A Review of Treatment Options. *P t* **43**, 92-104 (2018).
41 Pouresmaeili, F., Kamalidehghan, B., Kamarehei, M. & Goh, Y. M. A comprehensive overview on osteoporosis and its risk factors. *Ther Clin Risk Manag* **14**, 2029-2049, doi:10.2147/tcrm.S138000 (2018).
42 (US), O. o. t. S. G. Assessing the Risk of Bone Disease and Fracture. (2004).
43 Hoang-Kim, A., Miclau, T., Goldhahn, J., Nijman, T. H. & Poolman, R. W. Patient-important outcome for the assessment of fracture repair. *Injury* **45 Suppl 2**, S44-48, doi:10.1016/j.injury.2014.04.008 (2014).
44 Dyer, S. M. *et al.* A critical review of the long-term disability outcomes following hip fracture. *BMC Geriatrics* **16**, 158, doi:10.1186/s12877-016-0332-0 (2016).
45 Hannan, M. T. *et al.* Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. *J Bone Miner Res* **15**, 710-720, doi:10.1359/jbmr.2000.15.4.710 (2000).
46 Zhu, X. & Zheng, H. Factors influencing peak bone mass gain. *Front Med* **15**, 53-69, doi:10.1007/s11684-020-0748-y (2021).
47 Cawthon, P. M. Gender differences in osteoporosis and fractures. *Clin Orthop Relat Res* **469**, 1900-1905, doi:10.1007/s11999-011-1780-7 (2011).
48 Al-Bashaireh, A. M. *et al.* The Effect of Tobacco Smoking on Bone Mass: An Overview of Pathophysiologic Mechanisms. *J Osteoporos* **2018**, 1206235, doi:10.1155/2018/1206235 (2018).
49 Suh, K. T., Kim, S. W., Roh, H. L., Youn, M. S. & Jung, J. S. Decreased osteogenic differentiation of mesenchymal stem cells in alcohol-induced osteonecrosis. *Clin Orthop Relat Res*, 220-225, doi:10.1097/01.blo.0000150568.16133.3c (2005).
50 Guney, E. *et al.* Effect of weight loss on bone metabolism: comparison of vertical banded gastroplasty and medical intervention. *Obes Surg* **13**, 383-388, doi:10.1381/096089203765887705 (2003).
51 Ensrud, K. E. *et al.* Body size and hip fracture risk in older women: a prospective study. Study of Osteoporotic Fractures Research Group. *Am J Med* **103**, 274-280, doi:10.1016/s0002-9343(97)00025-9 (1997).

52 Nguyen, H. G., Pham, M. T., Ho-Pham, L. T. & Nguyen, T. V. Lean mass and peak bone mineral density. *Osteoporos Sarcopenia* **6**, 212-216, doi:10.1016/j.afos.2020.10.001 (2020).
53 Leslie, W. D. *et al.* Loss in DXA-estimated total body lean mass but not fat mass predicts incident major osteoporotic fracture and hip fracture independently from FRAX: a registry-based cohort study. *Arch Osteoporos* **15**, 96, doi:10.1007/s11657-020-00773-w (2020).
54 Ho-Pham, L. T., Nguyen, U. D. T. & Nguyen, T. V. Association Between Lean Mass, Fat Mass, and Bone Mineral Density: A Meta-analysis. *The Journal of Clinical Endocrinology & Metabolism* **99**, 30-38, doi:10.1210/jc.2013-3190 (2014).
55 Chen, Z., Lohman, T. G., Stini, W. A., Ritenbaugh, C. & Aickin, M. Fat or Lean Tissue Mass: Which One Is the Major Determinant of Bone Mineral Mass in Healthy Postmenopausal Women? *Journal of Bone and Mineral Research* **12**, 144-151, doi:10.1359/jbmr.1997.12.1.144 (1997).
56 Leslie, W. D. *et al.* Estimated Lean Mass and Fat Mass Differentially Affect Femoral Bone Density and Strength Index but Are Not FRAX Independent Risk Factors for Fracture. *Journal of Bone and Mineral Research* **29**, 2511-2519, doi:10.1002/jbmr.2280 (2014).
57 Taaffe, D. R. *et al.* Race and Sex Effects on the Association Between Muscle Strength, Soft Tissue, and Bone Mineral Density in Healthy Elders: The Health, Aging, and Body Composition Study. *Journal of Bone and Mineral Research* **16**, 1343-1352, doi:10.1359/jbmr.2001.16.7.1343 (2001).
58 Shieh, A., Karlamangla, A. S., Karvonen-Guttierez, C. A. & Greendale, G. A. Menopause-Related Changes in Body Composition Are Associated With Subsequent Bone Mineral Density and Fractures: Study of Women's Health Across the Nation. *Journal of Bone and Mineral Research* **38**, 395-402, doi:10.1002/jbmr.4759 (2023).
59 Zhu, K., Hunter, M., James, A., Lim, E. M. & Walsh, J. P. Associations between body mass index, lean and fat body mass and bone mineral density in middle-aged Australians: The Busselton Healthy Ageing Study. *Bone* **74**, 146-152, doi:https://doi.org/10.1016/j.bone.2015.01.015 (2015).
60 Bailey, C. A. & Brooke-Wavell, K. Exercise for optimising peak bone mass in women. *Proc Nutr Soc* **67**, 9-18, doi:10.1017/s0029665108005971 (2008).
61 Robinson, T. L. *et al.* Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. *J Bone Miner Res* **10**, 26-35, doi:10.1002/jbmr.5650100107 (1995).
62 Pettersson, U., Nordström, P., Alfredson, H., Henriksson-Larsén, K. & Lorentzon, R. Effect of high impact activity on bone mass and size in adolescent females: A comparative study between two different types of sports. *Calcif Tissue Int* **67**, 207-214, doi:10.1007/s002230001131 (2000).
63 Bouillon, R., Van Cromphaut, S. & Carmeliet, G. Intestinal calcium absorption: Molecular vitamin D mediated mechanisms. *J Cell Biochem* **88**, 332-339, doi:10.1002/jcb.10360 (2003).
64 Silva, B. C. & Bilezikian, J. P. Parathyroid hormone: anabolic and catabolic actions on the skeleton. *Curr Opin Pharmacol* **22**, 41-50, doi:10.1016/j.coph.2015.03.005 (2015).
65 Chapuy, M. C. *et al.* Vitamin D3 and calcium to prevent hip fractures in elderly women. *N Engl J Med* **327**, 1637-1642, doi:10.1056/nejm199212033272305 (1992).

66 Canalis, E., Mazziotti, G., Giustina, A. & Bilezikian, J. P. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. *Osteoporos Int* **18**, 1319-1328, doi:10.1007/s00198-007-0394-0 (2007).
67 Hardy, R. & Cooper, M. S. Bone loss in inflammatory disorders. *J Endocrinol* **201**, 309-320, doi:10.1677/joe-08-0568 (2009).
68 Hu, M. I., Gagel, R. F. & Jimenez, C. Bone loss in patients with breast or prostate cancer. *Curr Osteoporos Rep* **5**, 170-178, doi:10.1007/s11914-007-0013-1 (2007).
69 Drake, M. T. Osteoporosis and cancer. *Curr Osteoporos Rep* **11**, 163-170, doi:10.1007/s11914-013-0154-3 (2013).
70 Oh, H. J., Ryu, K. H., Park, B. J. & Yoon, B. H. Osteoporosis and Osteoporotic Fractures in Gastrointestinal Disease. *J Bone Metab* **25**, 213-217, doi:10.11005/jbm.2018.25.4.213 (2018).
71 Katz, S. & Weinerman, S. Osteoporosis and gastrointestinal disease. *Gastroenterol Hepatol (N Y)* **6**, 506-517 (2010).
72 David, C. *et al.* Severity of osteoporosis: what is the impact of co-morbidities? *Joint Bone Spine* **77 Suppl 2**, S103-106, doi:10.1016/s1297-319x(10)70003-8 (2010).
73 Bliuc, D. *et al.* Cognitive decline is associated with an accelerated rate of bone loss and increased fracture risk in women: a prospective study from the Canadian Multicentre Osteoporosis Study. *J Bone Miner Res* **36**, 2106-2115, doi:10.1002/jbmr.4402 (2021).
74 Nickolas, T. L. *et al.* Rapid cortical bone loss in patients with chronic kidney disease. *J Bone Miner Res* **28**, 1811-1820, doi:10.1002/jbmr.1916 (2013).
75 Golds, G., Houdek, D. & Arnason, T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. *Int J Endocrinol* **2017**, 4602129, doi:10.1155/2017/4602129 (2017).
76 Deng, H. W. *et al.* Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. *J Bone Miner Res* **15**, 1243-1252, doi:10.1359/jbmr.2000.15.7.1243 (2000).
77 MacGregor, A., Snieder, H. & Spector, T. D. Genetic factors and osteoporotic fractures in elderly people. Twin data support genetic contribution to risk of fracture. *Bmj* **320**, 1669-1670; author reply 1670-1661 (2000).
78 Deng, H. W. *et al.* Determination of bone mineral density of the hip and spine in human pedigrees by genetic and life-style factors. *Genet Epidemiol* **19**, 160-177, doi:10.1002/1098-2272(200009)19:2<160::Aid-gepi4>3.0.Co;2-h (2000).
79 Dequeker, J., Nijs, J., Verstraeten, A., Geusens, P. & Gevers, G. Genetic determinants of bone mineral content at the spine and radius: a twin study. *Bone* **8**, 207-209, doi:10.1016/8756-3282(87)90166-9 (1987).
80 Guéguen, R. *et al.* Segregation analysis and variance components analysis of bone mineral density in healthy families. *J Bone Miner Res* **10**, 2017-2022, doi:10.1002/jbmr.5650101223 (1995).
81 Zheng, H. F., Spector, T. D. & Richards, J. B. Insights into the genetics of osteoporosis from recent genome-wide association studies. *Expert Rev Mol Med* **13**, e28, doi:10.1017/s1462399411001980 (2011).
82 Trajanoska, K. & Rivadeneira, F. The genetic architecture of osteoporosis and fracture risk. *Bone* **126**, 2-10, doi:10.1016/j.bone.2019.04.005 (2019).

83 Zhu, X., Bai, W. & Zheng, H. Twelve years of GWAS discoveries for osteoporosis and related traits: advances, challenges and applications. *Bone Research* **9**, doi:10.1038/s41413-021-00143-3 (2021).
84 He, D. *et al.* A longitudinal genome-wide association study of bone mineral density mean and variability in the UK Biobank. *Osteoporos Int* **34**, 1907-1916, doi:10.1007/s00198-023-06852-1 (2023).
85 Fuggle, N. R. *et al.* Level and change in bone microarchitectural parameters and their relationship with previous fracture and established bone mineral density loci. *Bone* **147**, 115937, doi:10.1016/j.bone.2021.115937 (2021).
86 Dios, K., Huynh, N., Tran, T. S., Center, J. R. & Nguyen, T. V. Association between Fat Mass and Obesity-Related Transcript Polymorphisms and Osteoporosis Phenotypes. *J Bone Metab* **31**, 48-55, doi:10.11005/jbm.2024.31.1.48 (2024).
87 Huynh, N., De Dios, K., Tran, T. S., Center, J. R. & Nguyen, T. V. Association between the Sp1-binding-site polymorphism in the collagen type I alpha 1 (COLIA1) gene and bone phenotypes: the Dubbo Osteoporosis Epidemiology Study. *J Bone Miner Metab*, doi:10.1007/s00774-024-01558-8 (2024).
88 Mitchell, B. D. & Yerges-Armstrong, L. M. The genetics of bone loss: challenges and prospects. *J Clin Endocrinol Metab* **96**, 1258-1268, doi:10.1210/jc.2010-2865 (2011).
89 Raggatt, L. J. & Partridge, N. C. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem* **285**, 25103-25108, doi:10.1074/jbc.R109.041087 (2010).
90 Allen, M. R. & Burr, D. B. in *Basic and Applied Bone Biology* (eds David B. Burr & Matthew R. Allen) 75-90 (Academic Press, 2014).
91 Frost, H. M. Muscle, bone, and the Utah paradigm: a 1999 overview. *Medicine & Science in Sports & Exercise* **32** (2000).
92 Kameda, T. *et al.* Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. *J Exp Med* **186**, 489-495, doi:10.1084/jem.186.4.489 (1997).
93 Xu, L. *et al.* The Role of Sex Hormones on Bone Mineral Density, Marrow Adiposity, and Muscle Adiposity in Middle-Aged and Older Men. *Frontiers in Endocrinology* **13** (2022).
94 Carson, J. A. & Manolagas, S. C. Effects of sex steroids on bones and muscles: Similarities, parallels, and putative interactions in health and disease. *Bone* **80**, 67-78, doi:10.1016/j.bone.2015.04.015 (2015).
95 Khosla, S., Oursler, M. J. & Monroe, D. G. Estrogen and the skeleton. *Trends Endocrinol Metab* **23**, 576-581, doi:10.1016/j.tem.2012.03.008 (2012).
96 Cauley, J. A. Estrogen and bone health in men and women. *Steroids* **99**, 11-15, doi:10.1016/j.steroids.2014.12.010 (2015).
97 Mohamad, N. V., Soelaiman, I. N. & Chin, K. Y. A concise review of testosterone and bone health. *Clin Interv Aging* **11**, 1317-1324, doi:10.2147/cia.S115472 (2016).
98 Rariy, C. M. *et al.* Higher serum free testosterone concentration in older women is associated with greater bone mineral density, lean body mass, and total fat mass: the cardiovascular health study. *J Clin Endocrinol Metab* **96**, 989-996, doi:10.1210/jc.2010-0926 (2011).
99 Arden, N. K., Baker, J., Hogg, C., Baan, K. & Spector, T. D. The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study

of postmenopausal twins. *J Bone Miner Res* **11**, 530-534, doi:10.1002/jbmr.5650110414 (1996).
100 Garnero, P., Arden, N. K., Griffiths, G., Delmas, P. D. & Spector, T. D. Genetic influence on bone turnover in postmenopausal twins. *J Clin Endocrinol Metab* **81**, 140-146, doi:10.1210/jcem.81.1.8550741 (1996).
101 Cummings, S. R. *et al.* Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group. *Jama* **263**, 665-668 (1990).
102 Blank, J. *et al.* Overview of recruitment for the Osteoporotic Fractures in Men Study (MrOS). *Contemporary clinical trials* **26**, 557-568, doi:10.1016/j.cct.2005.05.005 (2005).
103 Orwoll, E. *et al.* Design and baseline characteristics of the osteoporotic fractures in men (MrOS) study--a large observational study of the determinants of fracture in older men. *Contemp Clin Trials* **26**, 569-585, doi:10.1016/j.cct.2005.05.006 (2005).
104 Simons, L. A. *et al.* The Dubbo study: an Australian prospective community study of the health of elderly. *Aust N Z J Med* **20**, 783-789, doi:10.1111/j.1445-5994.1990.tb00423.x (1990).
105 Ho-Pham, L. T. & Nguyen, T. V. The Vietnam Osteoporosis Study: Rationale and design. *Osteoporos Sarcopenia* **3**, 90-97, doi:10.1016/j.afos.2017.06.001 (2017).
106 Nguyen, T. V., Sambrook, P. N. & Eisman, J. A. Sources of variability in bone mineral density measurements: implications for study design and analysis of bone loss. *J Bone Miner Res* **12**, 124-135, doi:10.1359/jbmr.1997.12.1.124 (1997).
107 Uffelmann, E. *et al.* Genome-wide association studies. *Nature Reviews Methods Primers* **1**, 59, doi:10.1038/s43586-021-00056-9 (2021).
108 Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-575, doi:10.1086/519795 (2007).
109 R Core Team. *R: A Language and Environment for Statistical Computing*, <https://www.R-project.org> (2021).
110 Marees, A. T. *et al.* A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. *Int J Methods Psychiatr Res* **27**, e1608, doi:10.1002/mpr.1608 (2018).
111 Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* **88**, 76-82, doi:10.1016/j.ajhg.2010.11.011 (2011).
112 Jiang, L. *et al.* A resource-efficient tool for mixed model association analysis of large-scale data. *Nat Genet* **51**, 1749-1755, doi:10.1038/s41588-019-0530-8 (2019).
113 Jiang, L., Zheng, Z., Fang, H. & Yang, J. A generalized linear mixed model association tool for biobank-scale data. *Nat Genet* **53**, 1616-1621, doi:10.1038/s41588-021-00954-4 (2021).
114 Riggs, B. L. *et al.* Changes in bone mineral density of the proximal femur and spine with aging. Differences between the postmenopausal and senile osteoporosis syndromes. *J Clin Invest* **70**, 716-723, doi:10.1172/jci110667 (1982).
115 Nguyen, T. V., Sambrook, P. N. & Eisman, J. A. Bone loss, physical activity, and weight change in elderly women: the Dubbo Osteoporosis Epidemiology

Study. *J Bone Miner Res* **13**, 1458-1467, doi:10.1359/jbmr.1998.13.9.1458 (1998).
116 Cawthon, P. M. *et al.* Change in hip bone mineral density and risk of subsequent fractures in older men. *J Bone Miner Res* **27**, 2179-2188, doi:10.1002/jbmr.1671 (2012).
117 Riis, B. J., Hansen, M. A., Jensen, A. M., Overgaard, K. & Christiansen, C. Low bone mass and fast rate of bone loss at menopause: equal risk factors for future fracture: a 15-year follow-up study. *Bone* **19**, 9-12, doi:10.1016/8756-3282(96)00102-0 (1996).
118 Gnudi, S., Malavolta, N., Lisi, L. & Ripamonti, C. Bone mineral density and bone loss measured at the radius to predict the risk of nonspinal osteoporotic fracture. *J Bone Miner Res* **16**, 1130-1135, doi:10.1359/jbmr.2001.16.6.1130 (2001).
119 Sornay-Rendu, E., Munoz, F., Duboeuf, F. & Delmas, P. D. Rate of forearm bone loss is associated with an increased risk of fracture independently of bone mass in postmenopausal women: the OFELY study. *J Bone Miner Res* **20**, 1929-1935, doi:10.1359/jbmr.050704 (2005).
120 Bruyere, O. *et al.* Loss of hip bone mineral density over time is associated with spine and hip fracture incidence in osteoporotic postmenopausal women. *European Journal of Epidemiology* **24**, 707-712, doi:10.1007/s10654-009-9381-4 (2009).
121 Berger, C. *et al.* Association between change in BMD and fragility fracture in women and men. *J Bone Miner Res* **24**, 361-370, doi:10.1359/jbmr.081004 (2009).
122 Nguyen, T. V., Center, J. R. & Eisman, J. A. Femoral neck bone loss predicts fracture risk independent of baseline BMD. *J Bone Miner Res* **20**, 1195-1201, doi:10.1359/jbmr.050215 (2005).
123 Leslie, W. D., Morin, S. N. & Lix, L. M. Rate of bone density change does not enhance fracture prediction in routine clinical practice. *J Clin Endocrinol Metab* **97**, 1211-1218, doi:10.1210/jc.2011-2871 (2012).
124 Berry, S. D. *et al.* Repeat bone mineral density screening and prediction of hip and major osteoporotic fracture. *Jama* **310**, 1256-1262, doi:10.1001/jama.2013.277817 (2013).
125 Hillier, T. A. *et al.* Evaluating the Value of Repeat Bone Mineral Density Measurement and Prediction of Fractures in Older Women: The Study of Osteoporotic Fractures. *Archives of Internal Medicine* **167**, 155-160, doi:10.1001/archinte.167.2.155 (2007).
126 Crandall, C. J. *et al.* Serial Bone Density Measurement and Incident Fracture Risk Discrimination in Postmenopausal Women. *JAMA Internal Medicine* **180**, 1232-1240, doi:10.1001/jamainternmed.2020.2986 (2020).
127 Tang, J. *et al.* Longitudinal serum proteome mapping reveals biomarkers for healthy ageing and related cardiometabolic diseases. *Nature Metabolism* **7**, 166-181, doi:10.1038/s42255-024-01185-7 (2025).
128 Scahill, R. I. *et al.* Somatic CAG repeat expansion in blood associates with biomarkers of neurodegeneration in Huntington's disease decades before clinical motor diagnosis. *Nature Medicine* **31**, 807-818, doi:10.1038/s41591-024-03424-6 (2025).
129 Li, Y. *et al.* Longitudinal plasma proteome profiling reveals the diversity of biomarkers for diagnosis and cetuximab therapy response of colorectal

cancer. *Nature Communications* **15**, 980, doi:10.1038/s41467-024-44911-1 (2024).
130 Duijvelaar, E., Gisby, J., Peters, J. E., Bogaard, H. J. & Aman, J. Longitudinal plasma proteomics reveals biomarkers of alveolar-capillary barrier disruption in critically ill COVID-19 patients. *Nature Communications* **15**, 744, doi:10.1038/s41467-024-44986-w (2024).
131 Yang, X. *et al.* Predicting interval from diagnosis to delivery in preeclampsia using electronic health records. *Nature Communications* **16**, 3496, doi:10.1038/s41467-025-58437-7 (2025).
132 Zhang, B. *et al.* Cardiovascular post-acute sequelae of SARS-CoV-2 in children and adolescents: cohort study using electronic health records. *Nature Communications* **16**, 3445, doi:10.1038/s41467-025-56284-0 (2025).
133 Mendez, K. M. *et al.* A roadmap to precision medicine through post-genomic electronic medical records. *Nature Communications* **16**, 1700, doi:10.1038/s41467-025-56442-4 (2025).
134 Watts, N. B. *et al.* Osteoporosis in Men: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism* **97**, 1802-1822, doi:10.1210/jc.2011-3045 (2012).
135 Steiger, P., Cummings, S. R., Black, D. M., Spencer, N. E. & Genant, H. K. Age-related decrements in bone mineral density in women over 65. *J Bone Miner Res* **7**, 625-632, doi:10.1002/jbmr.5650070606 (1992).
136 Garcia, T. P. & Marder, K. Statistical Approaches to Longitudinal Data Analysis in Neurodegenerative Diseases: Huntington's Disease as a Model. *Curr Neurol Neurosci Rep* **17**, 14, doi:10.1007/s11910-017-0723-4 (2017).
137 Singer, J. D. & Willett, J. B. in *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence* (eds Judith D. Singer & John B. Willett) 0 (Oxford University Press, 2003).
138 Kado, D. M., Browner, W. S., Blackwell, T., Gore, R. & Cummings, S. R. Rate of bone loss is associated with mortality in older women: a prospective study. *J Bone Miner Res* **15**, 1974-1980, doi:10.1359/jbmr.2000.15.10.1974 (2000).
139 Lim, Y. *et al.* The prevalence of osteoporosis and the rate of bone loss in Korean adults: the Chungju metabolic disease cohort (CMC) study. *Osteoporos Int* **28**, 1453-1459, doi:10.1007/s00198-016-3893-z (2017).
140 Diem, S. J. *et al.* Rates of bone loss among women initiating antidepressant medication use in midlife. *J Clin Endocrinol Metab* **98**, 4355-4363, doi:10.1210/jc.2013-1971 (2013).
141 Fink, H. A. *et al.* Association of Increased Urinary Albumin With Risk of Incident Clinical Fracture and Rate of Hip Bone Loss: the Osteoporotic Fractures in Men Study. *J Bone Miner Res* **32**, 1090-1099, doi:10.1002/jbmr.3065 (2017).
142 Diem, S. J. *et al.* Depressive symptoms and rates of bone loss at the hip in older men. *Osteoporos Int* **24**, 111-119, doi:10.1007/s00198-012-1975-0 (2013).
143 Grambsch, P. M. & Therneau, T. M. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* **81**, 515-526, doi:10.1093/biomet/81.3.515 (1994).
144 Team, R. C. R: A language and environment for statistical computing. . *R Foundation for Statistical Computing* (2022).
145 Christiansen, B. A., Harrison, S. L., Fink, H. A., Lane, N. E. & for the Study of Osteoporotic Fractures Research, G. Incident fracture is associated with a

period of accelerated loss of hip BMD: the Study of Osteoporotic Fractures. *Osteoporosis International* **29**, 2201-2209, doi:10.1007/s00198-018-4606-6 (2018).
146 Lenora, J., Åkesson, K. & Gerdhem, P. Effect of Precision on Longitudinal Follow-Up of Bone Mineral Density Measurements in Elderly Women and Men. *Journal of Clinical Densitometry* **13**, 407-412, doi:https://doi.org/10.1016/j.jocd.2010.04.004 (2010).
147 Lan, N. *et al.* FTO - A Common Genetic Basis for Obesity and Cancer. *Front Genet* **11**, 559138, doi:10.3389/fgene.2020.559138 (2020).
148 Frayling, T. M. *et al.* A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**, 889-894, doi:10.1126/science.1141634 (2007).
149 Cha, S. W. *et al.* Replication of genetic effects of FTO polymorphisms on BMI in a Korean population. *Obesity (Silver Spring)* **16**, 2187-2189, doi:10.1038/oby.2008.314 (2008).
150 Chang, Y. C. *et al.* Common variation in the fat mass and obesity-associated (FTO) gene confers risk of obesity and modulates BMI in the Chinese population. *Diabetes* **57**, 2245-2252, doi:10.2337/db08-0377 (2008).
151 Grant, S. F. *et al.* Association analysis of the FTO gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. *PLoS One* **3**, e1746, doi:10.1371/journal.pone.0001746 (2008).
152 Adeyemo, A. *et al.* FTO genetic variation and association with obesity in West Africans and African Americans. *Diabetes* **59**, 1549-1554, doi:10.2337/db09-1252 (2010).
153 Dina, C. *et al.* Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* **39**, 724-726, doi:10.1038/ng2048 (2007).
154 Scuteri, A. *et al.* Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* **3**, e115, doi:10.1371/journal.pgen.0030115 (2007).
155 Nielson, C. M., Srikanth, P. & Orwoll, E. S. Obesity and fracture in men and women: an epidemiologic perspective. *J Bone Miner Res* **27**, 1-10, doi:10.1002/jbmr.1486 (2012).
156 Guo, Y. *et al.* The fat mass and obesity associated gene, FTO, is also associated with osteoporosis phenotypes. *PLoS One* **6**, e27312, doi:10.1371/journal.pone.0027312 (2011).
157 Lloyd, J. T. *et al.* Body mass index is positively associated with bone mineral density in US older adults. *Arch Osteoporos* **9**, 175, doi:10.1007/s11657-014-0175-2 (2014).
158 Johansson, H. *et al.* A Meta-Analysis of the Association of Fracture Risk and Body Mass Index in Women. *Journal of Bone and Mineral Research* **29**, 223-233, doi:https://doi.org/10.1002/jbmr.2017 (2014).
159 Chan, M. Y., Frost, S. A., Center, J. R., Eisman, J. A. & Nguyen, T. V. Relationship between body mass index and fracture risk is mediated by bone mineral density. *J Bone Miner Res* **29**, 2327-2335, doi:10.1002/jbmr.2288 (2014).
160 Tran, B., Nguyen, N. D., Center, J. R., Eisman, J. A. & Nguyen, T. V. Association between fat-mass-and-obesity-associated (FTO) gene and hip fracture susceptibility. *Clin Endocrinol (Oxf)* **81**, 210-217, doi:10.1111/cen.12335 (2014).

161 Mathur, M. B., Ding, P., Riddell, C. A. & VanderWeele, T. J. Web Site and R Package for Computing E-values. *Epidemiology* **29**, e45-e47, doi:10.1097/ede.0000000000000864 (2018).
162 Pan, W. H. *et al.* Body mass index and obesity-related metabolic disorders in Taiwanese and US whites and blacks: implications for definitions of overweight and obesity for Asians. *Am J Clin Nutr* **79**, 31-39, doi:10.1093/ajcn/79.1.31 (2004).
163 Wen, C. P. *et al.* Are Asians at greater mortality risks for being overweight than Caucasians? Redefining obesity for Asians. *Public Health Nutr* **12**, 497-506, doi:10.1017/s1368980008002802 (2009).
164 Wulan, S. N., Westerterp, K. R. & Plasqui, G. Ethnic differences in body composition and the associated metabolic profile: a comparative study between Asians and Caucasians. *Maturitas* **65**, 315-319, doi:10.1016/j.maturitas.2009.12.012 (2010).
165 Travison, T. G., Araujo, A. B., Esche, G. R., Beck, T. J. & McKinlay, J. B. Lean mass and not fat mass is associated with male proximal femur strength. *J Bone Miner Res* **23**, 189-198, doi:10.1359/jbmr.071016 (2008).
166 Claussnitzer, M. *et al.* FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. *New England Journal of Medicine* **373**, 895-907, doi:10.1056/NEJMoa1502214.
167 Tam, V. *et al.* Benefits and limitations of genome-wide association studies. *Nat Rev Genet* **20**, 467-484, doi:10.1038/s41576-019-0127-1 (2019).
168 Kanis, J. A. *et al.* Ten year probabilities of osteoporotic fractures according to BMD and diagnostic thresholds. *Osteoporos Int* **12**, 989-995, doi:10.1007/s001980170006 (2001).
169 Nguyen, T. V., Howard, G. M., Kelly, P. J. & Eisman, J. A. Bone mass, lean mass, and fat mass: same genes or same environments? *Am J Epidemiol* **147**, 3-16, doi:10.1093/oxfordjournals.aje.a009362 (1998).
170 Pocock, N. A. *et al.* Genetic determinants of bone mass in adults. A twin study. *J Clin Invest* **80**, 706-710, doi:10.1172/jci113125 (1987).
171 Estrada, K. *et al.* Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet* **44**, 491-501, doi:10.1038/ng.2249 (2012).
172 Kemp, J. P. *et al.* Identification of 153 new loci associated with heel bone mineral density and functional involvement of GPC6 in osteoporosis. *Nat Genet* **49**, 1468-1475, doi:10.1038/ng.3949 (2017).
173 Morris, J. A. *et al.* An atlas of genetic influences on osteoporosis in humans and mice. *Nat Genet* **51**, 258-266, doi:10.1038/s41588-018-0302-x (2019).
174 Kelly, P. J. *et al.* Genetic factors in bone turnover. *J Clin Endocrinol Metab* **72**, 808-813, doi:10.1210/jcem-72-4-808 (1991).
175 Yang, S., Center, J. R., Eisman, J. A. & Nguyen, T. V. Association between fat mass, lean mass, and bone loss: the Dubbo Osteoporosis Epidemiology Study. *Osteoporos Int* **26**, 1381-1386, doi:10.1007/s00198-014-3009-6 (2015).
176 Moore, A. *et al.* Evaluating 17 methods incorporating biological function with GWAS summary statistics to accelerate discovery demonstrates a tradeoff between high sensitivity and high positive predictive value. *Commun Biol* **6**, 1199, doi:10.1038/s42003-023-05413-w (2023).

177 Hammond, R. K. *et al.* Biological constraints on GWAS SNPs at suggestive significance thresholds reveal additional BMI loci. *Elife* **10**, doi:10.7554/eLife.62206 (2021).
178 Sollis, E. *et al.* The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. *Nucleic Acids Res* **51**, D977-d985, doi:10.1093/nar/gkac1010 (2023).
179 Rivadeneira, F. *et al.* Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat Genet* **41**, 1199-1206, doi:10.1038/ng.446 (2009).
180 Moayyeri, A. *et al.* Genetic determinants of heel bone properties: genome-wide association meta-analysis and replication in the GEFOS/GENOMOS consortium. *Hum Mol Genet* **23**, 3054-3068, doi:10.1093/hmg/ddt675 (2014).
181 Paternoster, L. *et al.* Genome-wide association meta-analysis of cortical bone mineral density unravels allelic heterogeneity at the RANKL locus and potential pleiotropic effects on bone. *PLoS Genet* **6**, e1001217, doi:10.1371/journal.pgen.1001217 (2010).
182 Li, Y. R. *et al.* Meta-analysis of shared genetic architecture across ten pediatric autoimmune diseases. *Nat Med* **21**, 1018-1027, doi:10.1038/nm.3933 (2015).
183 Peyrot, W. J. & Price, A. L. Identifying loci with different allele frequencies among cases of eight psychiatric disorders using CC-GWAS. *Nat Genet* **53**, 445-454, doi:10.1038/s41588-021-00787-1 (2021).
184 Health, A. I. o. & Welfare. Older Australians. (AIHW, Canberra, 2024).
185 Rudnicka, E. *et al.* The World Health Organization (WHO) approach to healthy ageing. *Maturitas* **139**, 6-11, doi:10.1016/j.maturitas.2020.05.018 (2020).
186 Gampawar, P., Hofer, E. & Alonso, N. in *Bone Research Protocols* (ed Aymen I. Idris) 695-715 (Springer US, 2025).
187 Spain, S. L. & Barrett, J. C. Strategies for fine-mapping complex traits. *Human Molecular Genetics* **24**, R111-R119, doi:10.1093/hmg/ddv260 (2015).
188 Cheung, C. L., Sham, P. C., Xiao, S. M., Bow, C. H. & Kung, A. W. Meta-analysis of gene-based genome-wide association studies of bone mineral density in Chinese and European subjects. *Osteoporos Int* **23**, 131-142, doi:10.1007/s00198-011-1779-7 (2012).
189 Benner, C. *et al.* Prospects of Fine-Mapping Trait-Associated Genomic Regions by Using Summary Statistics from Genome-wide Association Studies. *The American Journal of Human Genetics* **101**, 539-551, doi:https://doi.org/10.1016/j.ajhg.2017.08.012 (2017).
190 Liu, L. *et al.* Twelve New Genomic Loci Associated With Bone Mineral Density. *Front Endocrinol (Lausanne)* **11**, 243, doi:10.3389/fendo.2020.00243 (2020).
191 Kanai, M. *et al.* Meta-analysis fine-mapping is often miscalibrated at single-variant resolution. *Cell Genomics* **2**, doi:10.1016/j.xgen.2022.100210 (2022).
192 Greenbaum, J. & Deng, H. W. A Statistical Approach to Fine Mapping for the Identification of Potential Causal Variants Related to Bone Mineral Density. *Journal of Bone and Mineral Research* **32**, 1651-1658, doi:10.1002/jbmr.3154 (2017).
193 Trajanoska, K. *et al.* Assessment of the genetic and clinical determinants of fracture risk: genome wide association and mendelian randomisation study. *BMJ* **362**, k3225, doi:10.1136/bmj.k3225 (2018).

194 Burgess, S., Butterworth, A. & Thompson, S. G. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* **37**, 658-665, doi:10.1002/gepi.21758 (2013).
195 Sanderson, E. *et al.* Mendelian randomization. *Nature Reviews Methods Primers* **2**, 6, doi:10.1038/s43586-021-00092-5 (2022).
196 Trajanoska, K. & Rivadeneira, F. Using Mendelian Randomization to Decipher Mechanisms of Bone Disease. *Curr Osteoporos Rep* **16**, 531-540, doi:10.1007/s11914-018-0467-3 (2018).
197 Larsson, S. C., Michaëlsson, K. & Burgess, S. Mendelian randomization in the bone field. *Bone* **126**, 51-58, doi:10.1016/j.bone.2018.10.011 (2019).
198 Zhu, K. *et al.* A causal association between osteoporosis and postural instability: a Mendelian randomization study. *Scientific Reports* **15**, 10234, doi:10.1038/s41598-025-93793-w (2025).
199 Zhao, G. *et al.* Potential drug targets for osteoporosis identified: A Mendelian randomization study. *Heliyon* **10**, e36566, doi:https://doi.org/10.1016/j.heliyon.2024.e36566 (2024).
200 Zhang, X. *et al.* Association of Bone Mineral Density and Bone Turnover Markers with the Risk of Diabetes: Hong Kong Osteoporosis Study and Mendelian Randomization. *Journal of Bone and Mineral Research* **38**, 1782-1790, doi:10.1002/jbmr.4924 (2023).
201 van Vliet, N. A. *et al.* Thyroid Stimulating Hormone and Bone Mineral Density: Evidence From a Two-Sample Mendelian Randomization Study and a Candidate Gene Association Study. *J Bone Miner Res* **33**, 1318-1325, doi:10.1002/jbmr.3426 (2018).
202 Kemp, J. P., Sayers, A., Smith, G. D., Tobias, J. H. & Evans, D. M. Using Mendelian randomization to investigate a possible causal relationship between adiposity and increased bone mineral density at different skeletal sites in children. *Int J Epidemiol* **45**, 1560-1572, doi:10.1093/ije/dyw079 (2016).
203 Oei, L. *et al.* Dissecting the relationship between high-sensitivity serum C-reactive protein and increased fracture risk: the Rotterdam Study. *Osteoporos Int* **25**, 1247-1254, doi:10.1007/s00198-013-2578-0 (2014).
204 Boudin, E., Fijalkowski, I., Hendrickx, G. & Van Hul, W. Genetic control of bone mass. *Mol Cell Endocrinol* **432**, 3-13, doi:10.1016/j.mce.2015.12.021 (2016).
205 Ralston, S. H. Genetic control of susceptibility to osteoporosis. *J Clin Endocrinol Metab* **87**, 2460-2466, doi:10.1210/jcem.87.6.8621 (2002).
206 Pallares, L. F. Searching for solutions to the missing heritability problem. *Elife* **8**, doi:10.7554/eLife.53018 (2019).
207 Duncan, E. L. & Brown, M. A. Mapping genes for osteoporosis--old dogs and new tricks. *Bone* **46**, 1219-1225, doi:10.1016/j.bone.2009.12.035 (2010).
208 Lu, T., Forgetta, V., Zhou, S., Richards, J. B. & Greenwood, C. M. T. Identifying Rare Genetic Determinants for Improved Polygenic Risk Prediction of Bone Mineral Density and Fracture Risk. *Journal of Bone and Mineral Research* **38**, 1771-1781, doi:10.1002/jbmr.4920 (2023).
209 Jang, S.-K. *et al.* Rare genetic variants explain missing heritability in smoking. *Nature Human Behaviour* **6**, 1577-1586, doi:10.1038/s41562-022-01408-5 (2022).

210 Kazma, R. & Bailey, J. N. Population-based and family-based designs to analyze rare variants in complex diseases. *Genet Epidemiol* **35 Suppl 1**, S41-47, doi:10.1002/gepi.20648 (2011).
211 Wang, L. *et al.* Family-Based Rare Variant Association Analysis: A Fast and Efficient Method of Multivariate Phenotype Association Analysis. *Genet Epidemiol* **40**, 502-511, doi:10.1002/gepi.21985 (2016).
212 Chen, C. *et al.* Applications of multi-omics analysis in human diseases. *MedComm (2020)* **4**, e315, doi:10.1002/mco2.315 (2023).
213 Yang, T. L. *et al.* A road map for understanding molecular and genetic determinants of osteoporosis. *Nat Rev Endocrinol* **16**, 91-103, doi:10.1038/s41574-019-0282-7 (2020).
214 Mullin, B. H., Ribet, A. B. P. & Pavlos, N. J. Bone Trans-omics: Integrating Omics to Unveil Mechanistic Molecular Networks Regulating Bone Biology and Disease. *Curr Osteoporos Rep* **21**, 493-502, doi:10.1007/s11914-023-00812-8 (2023).
215 Mani, S., Lalani, S. R. & Pammi, M. Genomics and multiomics in the age of precision medicine. *Pediatric Research* **97**, 1399-1410, doi:10.1038/s41390-025-04021-0 (2025).
216 Manzoni, C. *et al.* Genome, transcriptome and proteome: the rise of omics data and their integration in biomedical sciences. *Briefings in Bioinformatics* **19**, 286-302, doi:10.1093/bib/bbw114 (2018).