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“CAROL DAVILA” UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
DOCTORAL SCHOOL
FIELD OF MEDICINE

Metabolic Syndrome, Sarcopenia, and Risk of Osteoporotic Fracture: Clinical Interferences and Genetic Substrate

SUMMARY of DOCTORAL THESIS

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INTERNATIONAL SCIENTIFIC CONTEXT, OBJECTIVES, AND WORKING HYPOTHESIS

INTERNATIONAL SCIENTIFIC CONTEXT. Osteoporosis (OP) is the most common metabolic bone disease, with a global prevalence of 18.3% (Salari et al., 2021). In Romania, the prevalence among individuals over 50 years of age has been estimated to be 20.5% in women and 6.2% in men (Grigorie et al., 2013). Regarding fragility fractures, it is estimated that one in three women and one in five men over the age of 50 will suffer at least one osteoporotic fracture (Kanis et al., 2000).

Currently, OP is diagnosed by measuring bone mineral density (BMD) or by identifying fragility fractures. For many years, research in the field of OP has focused on methods for assessing bone tissue (BMD or bone turnover markers) and, more recently, on genetic predisposition. While the genetics of OP began to be understood through the study of a few *Mendelian genetic syndromes*, recent years have seen remarkable progress in the genetic analysis of complex diseases, including OP. Over the past decade, the introduction of Genome-Wide Association Studies (GWAS) has enabled the identification of genes responsible for the development of OP or fragility fractures.

Research on OP at the "C.I. Parhon" National Institute of Endocrinology, conducted by the group led by Prof. Univ. Dr. Cătălina Poiană, was initiated within this international, competitive context, involving significant advancements in fields such as bioinformatics, statistics, and new methods for genome genotyping. The genetics of complex *non-Mendelian* diseases poses enormous challenges due to their polygenic nature and high diversity. However, the completion of the *Human Genome Project* in 2003 and the discovery of SNPs (*single nucleotide polymorphisms*), along with the development of the international HapMap program and the 1000 Genomes Project (1KGP), have opened new avenues for using genetic markers. Like other complex diseases, such as cardiovascular diseases, obesity, type 2 diabetes (T2D), and even neurodegenerative diseases, OP has benefited from these advancements.

In this international context, during this thesis, I completed a doctoral internship at the *Molecular Endocrinology Laboratory* at IURC (*Institut Universitaire de Recherche Clinique*) in Montpellier (France), under the guidance of Prof. Florin Grigorescu, a

researcher at INSERM. This allowed me to familiarize myself with new bioinformatics and genotyping methods. This internship also enabled me to formulate new working hypotheses while benefiting from the clinical expertise of the "C.I. Parhon" Institute.

We chose to focus our research on the relationship between OP and insulin resistance, particularly in connection with *obesity* and *metabolic syndrome* (MetS). The motivation for selecting this topic stemmed from the current international literature on the incidence of MetS in OP, which shows extremely varied and even contradictory results (Greere et al., 2023; Wong et al., 2016). It should be noted that the prevalence of OP and fragility fractures is also expected to increase in the next decade, given the aging population phenomenon. Numerous factors contribute to this trend, such as the rising prevalence of obesity and diabetes (especially type 2 diabetes), which may also be associated with a higher risk of fractures, independent of BMD.

Due to its prevalence, OP is considered a serious public health issue, given that more than 200 million people worldwide suffer from this condition. In the United States and Europe, 30% of postmenopausal women are diagnosed with OP, and at least 40% of these women and 15-30% of men with OP will experience one or more fragility fractures (Clynes et al., 2020).

A significant issue among postmenopausal women, particularly at advanced ages, is the onset of metabolic complications, including obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome (MetS), all characterized by *insulin resistance* (IR). This IR has a complex pathogenesis, where insulin and IGF-1 (*insulin-like growth factor-1*), known for their impact on growth, glucose homeostasis, and lifespan, play crucial roles. The mechanisms involved in IR and the role of MetS in the pathogenesis of osteoporosis (OP) are not fully understood. When exploring MetS, the literature reports contradictory results (Wong et al., 2016). Some studies suggest a high prevalence of MetS in OP (interpreted as a pathogenic effect), while others report a lower prevalence (interpreted as a protective effect). Additionally, OP and *sarcopenia* are two conditions with significant morbidity, whose frequency increases with age. Muscle system parameters positively correlate with bone mass or bone mineral density (BMD), and the explanations for these correlations are varied. The mechanostat hypothesis (introduced by Frost in 1964) suggests that the skeletal response selectively varies depending on bone deformation and amplitude through a

mechanical effect. Thus, physical effort maintains the proper functioning of the musculoskeletal system components, but many factors (*vitamin D, testosterone, growth hormone/IGF-1, and estrogens*) can influence muscle and bone growth. Notably, sarcopenia is also associated with an increased risk of falls, which negatively impacts OP by causing fragility fractures.

The general theme of this thesis concerns the relationship between metabolic MetS, obesity, and the pathogenesis of primary OP in postmenopausal women, including simple or severe OP with fragility fractures.

OBJECTIVES. Given the contradictory results in the literature regarding the relationship between MetS and OP, the general objective was to *identify clinical or genetic factors that might explain these contradictions*. Specifically, we aimed to study the complex relationship between obesity, MetS, and OP through a clinical approach on one hand, and on the other hand, by examining genetic factors that could influence the pathogenesis of obesity and OP with or without fractures. Therefore, we focused on a key obesity-related gene, the *FTO (fat mass and obesity-associated)* gene.

More specifically, during this thesis, we set the following specific objectives:

1) Recruitment of a population of postmenopausal women with and without osteoporosis at the *C.I. Parhon National Institute of Endocrinology* through a prospective protocol.

2) Study of the possible relationship between *in vivo* IR, MetS, obesity, and OP with and without fractures.

3) Investigation of the potential role of the *FTO* gene in MetS and OP with and without fractures.

WORKING HYPOTHESIS. For the clinical study, we hypothesized the establishment of a complex relationship between the degree of obesity and systemic insulin resistance (IR). IR often accompanies postmenopausal women through the onset of MetS and various cardiovascular complications, but this situation is greatly complicated by at least three fundamental aspects:

a) Recent studies from Columbia University (USA) have highlighted the existence of a *specific insulin resistance in bone* ("*bone insulin resistance*") that has a complex relationship with *systemic IR* through the secretion of regulatory hormones such as osteocalcin (OCN) or leptin (Karsenty, G. and Ferron, M., 2012).

b) Another fundamental aspect is related to the observation that *bone marrow tissue*, which produces various bone cells (*osteoblasts and osteoclasts*) from stem cells, is a very particular tissue. In bone marrow, adipocytes exhibit significant proliferation and remain highly sensitive to insulin despite peripheral IR (Tencerova et al., 2019), potentially undergoing an *aging* process with major consequences for bone metabolism.
c) Given that the *FTO* gene undeniably contributes to the pathogenesis of obesity and MetS, it warrants analysis in the context of OP. We hypothesize that SNPs of this gene (intron 1) will clarify how obesity or MetS influences OP (Zhang et al., 2019).

In the field of OP genetics, our working hypothesis was pertinent. We questioned the possible role of an obesity gene (Frayling et al., 2007) within the complex relationship between *body mass index* (BMI) variation and IR. This hypothesis proved to yield extraordinary results, capable of altering all existing working hypotheses in the field of OP. The strategy we proposed was original and differed from traditional GWAS. Instead of relying on a large number of individuals, we opted for a smaller, but well-characterized, cohort ($n \approx 200$), for which we applied original and effective fine-scale haplotype mapping strategies.

RESULTS

STUDY 1: *The Relative Contribution of Metabolic Syndrome Components Related to Obesity and Insulin Resistance in Postmenopausal Osteoporosis*

This initial study aimed to analyze the contribution of metabolic syndrome (MetS) in the pathogenesis of OP by examining the correlation between the **cumulative criteria of MetS**, IR, obesity, and BMD among our patients with OP, both with and without fractures (Greere et al., 2024a). Between May 28, 2020, and April 1, 2022, we recruited 188 postmenopausal women, with and without OP, excluding all forms of *secondary osteoporosis* or chronic diseases (except for T2D). The research protocol was submitted to the Ethics Committee, and the study was conducted in accordance with international regulations and the Declaration of Helsinki (2022). Written informed consent was obtained from each patient after they were informed via a letter. Each patient was assigned a unique code, and the DNA collection was anonymized. All patients underwent a series of laboratory tests to establish their hormonal and metabolic profiles. Particular attention was

given to DEXA (Dual X-ray Absorptiometry) analysis, with BMD and *Trabecular Bone Score* (TBS) assessments for the lumbar spine at L1-L4. OP was defined according to the guidelines of the *American Association of Clinical Endocrinologists* (AACE) and the *National Osteoporosis Foundation*, as follows: 1) T-score of -2.5 SD (standard deviations) or lower at the lumbar spine, femoral neck, total proximal femur, or 1/3 radius; 2) a vertebral or hip fracture following low-energy trauma, regardless of BMD value; 3) a T-score between -1.0 and -2.5 SD with fragility fractures of the proximal humerus, pelvis, or distal forearm; 4) a T-score between -1.0 and -2.5 SD with a high fracture risk according to FRAX®, based on country-specific thresholds (Kanis et al., 2019). The FRAX PLUS (TBS) score was used to assess the 10-year risk of fragility fractures, calculated using the country-specific website (https://www.fraxplus.org/). Severe OP was diagnosed based on WHO criteria, specifically a T-score of -2.5 SD or lower (e.g., < -3) combined with fragility fractures.

Particular attention was also given to muscle strength and physical performance tests, including the following assessments: bilateral handgrip strength (HGS), the chair stand test (CST), gait speed (GS), the Timed Up and Go (TUG) test, and the Tinetti score. To summarize the muscle test results, we developed a statistical tool (SUMstat) that considered the number of tests—ranging from 0 to 5—with values outside the normal range (scored as 0/1).

The diagnosis of Metabolic Syndrome (MetS) was based on the presence of at least three criteria from the *National Cholesterol Education Program* (NCEP) and the *Adult Treatment Panel III* (ATP-III). IR was assessed using the HOMA-IR (*Homeostasis Model Assessment of Insulin Resistance*) index or as a nominal variable defined by values exceeding the threshold of 1.92, which was calculated from fasting insulin levels in normal-weight subjects without OP, plus 2 standard error of the mean (SEM), as previously described (Haydar et al., 2019).

Staistical Analysis. The statistical power was calculated using the Raosoft program (www.Raosoft.com), and variables were tested using non-parametric methods, specifically the Mann-Whitney U test or the Kruskal-Wallis test. In the ANOVA, the interaction factor α was set at 5%. Nominal variables were analyzed using the χ^2 test. For the logistic regression analysis of MetS, we used the backward stepwise method to determine P-values,

odds ratios (OR), and 95% confidence intervals (CI). The analysis began by comparing controls with normal BMD or osteopenia (non-OP) to subjects with OP. The correlation analysis between BMD and HOMA-IR with the cumulative criteria of MetS was conducted using ANOVA. BMD was analyzed at all anatomical sites. The MetS statistics accounted for BMD variation according to the degree of obesity (BMI *percentiles*) with specific cutoff values: 21.0, 23.8, 27.2, 31.1, and 35.5 kg/m². **The inflection point** for BMD was determined by inspecting residuals, calculated as the difference between the measured BMD values and the predicted values based on the relationship between BMD and BMI in the non-OP population. The analysis was extended after **newly stratifying OP patients** as either normal weight (lean OP) or overweight/obese (OW/OB) based on **the inflection point of 27.2 kg/m²**. Statistical significance was considered at $P < 0.05$ in all cases. The statistical analysis was performed using Statview 5.0, Abacus Concepts, Berkeley, CA, as previously described (Attaoua et al., 2008; Haydar et al., 2019).

Results. Patients with osteoporosis (OP) showed a 2 times lower prevalence of obesity (based on BMI) and 6 times lower prevalence of central obesity (based on abdominal circumference). Severe OP with fragility fractures accounted for 48.3% of OP cases. No significant difference was found in the prevalence of insulin resistance (IR) or HOMA-IR values. BMD was reduced at all anatomical sites, as was the Trabecular Bone Score (TBS) at L1-L4. In OP patients, MetS was 1.7 times less prevalent compared to non-OP subjects ($p < 0.031$), consistent with a 2.3-fold reduction in obesity and the prevalence of all five ATP III criteria for MetS.

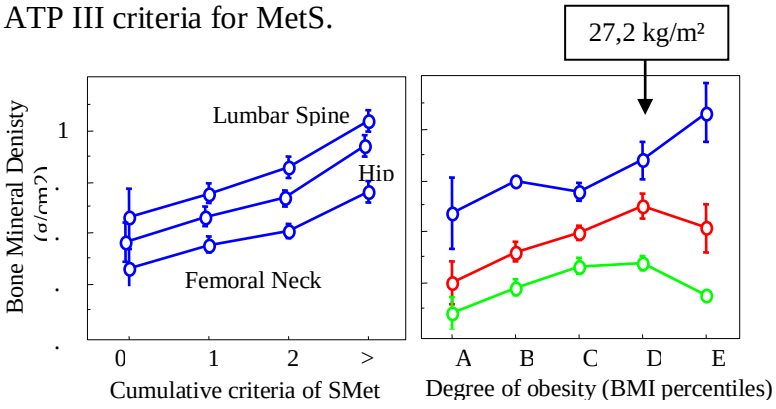

Figure 1. Correlation between BMD and Cumulative MetS Criteria or Degree of Obesity.

An important finding was the observation of a **strong correlation between cumulative MetS criteria and BMD at all anatomical sites, including the radius**

(Figure 1). BMD variation can be explained by cumulative MetS criteria ($p < 0,0001$, $\alpha = 0.99$, ANOVA), as well as the HOMA-IR index ($p < 0,0001$, $\alpha = 0.99$), with no difference between individuals with or without OP. Understanding the role of MetS components remains crucial, as an increase in cumulative MetS criteria indicates a buildup of metabolic abnormalities, while higher BMI is associated with greater BMD (and reduced OP risk).

In response to this situation, we aimed to **reassess the relative contribution of MetS to OP after reclassifying subjects into lean and overweight/obese groups based on the inflection point (BMI of 27.2 kg/m²).** We then examined the profiles of patients with OP, both with and without MetS. In logistic regression, the most influential MetS components in lean individuals were blood **glucose** ($p < 0.0031$, OR 4.14, 95% CI [1.44-11.05]), triglyceride **(TG) levels** ($p < 0.0126$, OR 6.82, 95% CI [1.4-33.12]), and **hypertension** ($p < 0.0012$, OR 11.1, 95% CI [2.14-56.55]). A completely different pattern was observed in OW/OB patients with and without MetS. The determining factors for MetS in these patients were higher levels of blood **glucose, HbA1c, and TG**, **particularly the HOMA-IR index** (up to 3.8). Thus, in logistic regression, the influential MetS components in OW/OB patients were only two factors: **HbA1c** with $p < 0.0037$, OR 9.6, 95% CI [1.64-55.6], and similarly significant, **IR** with $p < 0.0076$, OR 6.7, 95% CI [1.49-30.8]. **These data indicate that IR emerged as a significant factor primarily in more obese OP patients, stratified by BMI at the BMD inflection point.**

To visualize this situation, we **plotted the residual BMD** values against the predicted values based on the BMD-BMI correlation from control subjects. It was found that in OP, the BMD values for MetS were relatively lower than predicted, a trend observed across all anatomical sites and proportional to the cumulative MetS criteria.

In conclusion, in this first study, we characterized MetS and its components in postmenopausal women with OP, while simultaneously assessing IR measured by the HOMA-IR index through an analysis of the correlation between BMD and cumulative MetS criteria in relation to obesity levels. **My personal contribution involved observing a *nonlinear, biphasic relationship* between BMD and BMI, with a noticeable decline in BMD after a BMI of 27.2 kg/m². These findings are significant for studies on MetS in OP patients, which often focus solely on reporting the prevalence of this syndrome.**

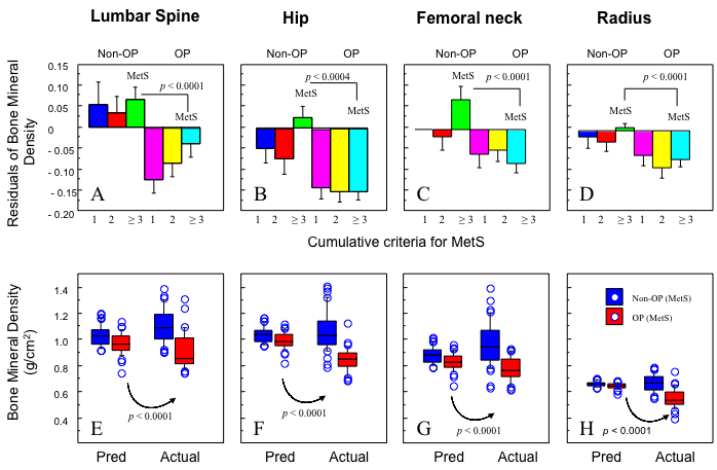

Figure 2. BMD at Different Anatomical Sites in Patients with Osteoporosis and Metabolic Syndrome Compared to Predicted Values Based on BMI. Estimated BMD values based on BMI were calculated using linear regression for subjects without osteoporosis (non-OP). These estimated values were then compared with actual values in non-OP and OP with Metabolic Syndrome together (Panels E, F, G, and H), showing residual values (Panels A, B, C, and D).

Identifying the harmful effects of MetS in the context of OP is crucial, as, by definition, MetS patients include a higher proportion of individuals with obesity and, consequently, a *relatively higher* BMD that might suggest a protective effect. However, our data actually indicate a *relatively lower* BMD in OP compared to the values predicted based on the BMI-BMD relationship observed in control subjects.

STUDY 2: *Fine-Scale Haplotype Mapping Reveals an Association of the FTO Gene with Osteoporosis and Fracture Risk in Postmenopausal Women*

The interaction between obesity and OP is complex because obese women often have higher BMD due to mechanical loading or estrogen production by adipose tissue, which is interpreted as having a protective role. However, increased BMD does not necessarily translate to a reduced fracture risk, a phenomenon known as the *"obesity paradox"* (Rinonapoli et al., 2021). Among the many factors that may contribute to an increased fracture risk, genetic predisposition plays a decisive role. A recent bivariate GWAS meta-analysis identified three loci (2p23.2, 16q12.2, and 18q21.32) with pleiotropic effects on both obesity and OP, corresponding to the genes *TRMT61B* (tRNA methyltransferase 61B), *FTO* (fat mass and obesity-associated gene), and the *MC4R* gene for the melanocortin-4 receptor (Pei et al., 2020). Since the discovery of *FTO* as a leading

obesity gene, it has garnered particular attention in other metabolic diseases, such as T2D, non-alcoholic fatty liver disease, hypertension, and more recently, OP (Frayling et al., 2007; Li et al., 2020). The *FTO* gene encodes a *Fe(II) and 2-oxoglutarate-dependent oxygenase* involved in transcriptional *epigenetic regulation*, functioning as an mRNA demethylase, particularly in the demethylation of N(6)-methyladenosine (m6A) (Chen et al., 2020). Studies on cell cultures and transgenic animal models have implicated FTO in adipogenesis, adipocyte apoptosis, and the differentiation of *bone marrow mesenchymal stem cells* into adipocytes or osteoblasts. In addition to its expression in adipose tissue, brain, muscle, and heart, the *FTO* gene is also expressed in bone marrow, making it a promising candidate for genetic predisposition to OP (Zhang et al., 2019). Research on *FTO*'s role in OP, especially in postmenopausal women, is very limited. Guo et al. (2011) identified six SNPs in intron 8 of the FTO gene associated with increased hip BMD. Other studies have reported several SNPs in intron 1 of FTO associated with hip fractures, although without affecting BMD (De Dios et al., 2024; Tran et al., 2013).

In this context, our objective was to investigate the *FTO* gene in postmenopausal OP by analyzing five SNPs in intron 1 previously associated with obesity (Greere et al., 2024b). Haplotyping using phased DNA revealed even stronger associations with OP and fragility fractures, correlated with reduced BMD. The study was conducted on the population described in Study 1, with genomic DNA extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA). Five SNPs in intron 1 of the *FTO* gene (rs8057044, rs8050136, rs9939609, rs62033406, rs9930506) were selected based on previous studies in French and Romanian populations (Haydar et al., 2019; Attaoua et al., 2008). Additionally, we genotyped the rs3736228 SNP in the LRP5 gene, identified as a candidate gene from GWAS studies. Genotyping was performed using allele discrimination tests (KASPar technique by LCG Genomics, Teddington, UK). For phased DNA, haplotypes were reconstructed in the population using the PHASE 2.1 software and visualized for the *linkage disequilibrium* (LD) map in HAPLOVIEW 3.1. Predictions of transcriptional activity were examined using HaploReg v.4.1 (http://archive.broadinstitute.org/mammals/haploreg).

Statistical Analysis. The analysis was conducted using StatView 5.0 (Abacus Concepts, Berkeley, CA), consistent with the previous study. Logistic regression was

performed using the stepwise method to assess SNP associations, and Bonferroni corrections were applied in Rv3.2.1. The LD map between SNPs was calculated using the NIH database (https://ldlink.nih.gov) and HAPLOVIEW 3.1. Genotype-phenotype correlations were analyzed in the OP population, and results for *haplotype pairs* were compared with those from independent SNPs on unphased DNA. BMD was tested at all anatomical sites, with values adjusted for BMI. Genetic association was assessed by comparing non-OP subjects (controls) with OP subjects (cases).

Results. Genetic Association. Five SNPs in the *FTO* gene (rs8057044, rs8050136, rs9939609, rs62033406, and rs9930506) and one SNP in the *LRP5* gene (rs3736228) showed a *minor allele frequency* (MAF) comparable to European populations. Preliminary results indicated that the most significant genetic model was "over-dominant." In logistic regression, SNP rs9930506 (GA) was significantly associated with OP with a strong OR, while the associations for rs8057044 (GA) and rs9939609 (TA) appeared as trends. The association of rs9930506 was confirmed after **Bonferroni correction** ($P < 0.0175$). No association was detected for the rs3736228 marker in the LRP5 gene. Notably, all five SNPs were significantly associated with severe OP with fractures, with rs9939609 showing a protective effect, while the others had a pathogenic effect. The associations with severe OP and fractures were also supported by **Bonferroni correction** for rs8057044 ($P < 0.013$) and rs9930506 ($P < 0.001$). Conditional analysis showed that rs9930506 and rs9939609 were independently associated ($P < 0.0001$ and 0.01, respectively) within the corresponding LD block. SNPs rs8057044 and rs9930506 were found to be in low LD ($r^2 = 0.70$). Therefore, for further correlation investigations, we considered three SNPs—rs8057044, rs9939609, and rs9930506—as lead SNPs.

Haplotype Mapping. To understand the biological effects of the SNPs, we performed haplotype mapping using the PHASE program. A total of nine haplotypes were reconstructed within the population and assigned to dizygotic individuals as **haplotype pairs**. Two haplotypes (H1 and H9) were more common (>40%), while the others (H2 to H8) were rare or very rare. Seventeen haplotype pairs were identified in the population, with **three pairs being frequent**: H1/H1 (24.5%), H1/H9 (35.6%), and H9/H9 (20.2%).

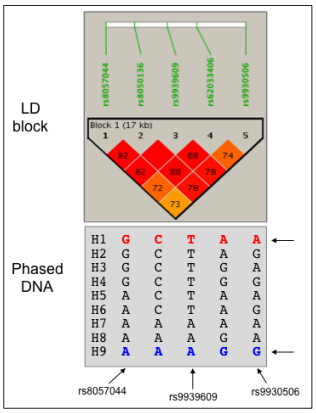

Figure 3. Linkage Disequilibrium Map of SNPs in Intron 1 of the *FTO* Gene and Haplotypes Reconstructed Using the PHASE Program.

A surprising result emerged from the **analysis of these haplotype pairs**. While the H1/H1 pair remained non-significant, the **H9/H9 pair was associated with severe OP, with a protective effect**. Conversely, the **heterozygous H1/H9 pair was associated with both OP and severe OP, showing a high OR**, particularly in insulin-resistant individuals with an OR of 3.92, 95% CI [1.48-10.367], P < 0.0029. To understand the metabolic consequences, OR variations were examined based on the presence or absence of SMet or its components. When the population was stratified into those with and without SMet, a significant association was detected for **H1/H9 in the absence of SMet** (P < 0.03, OR 1.9, 95% CI [1.043-3.626]), the absence of low HDL-C levels (P < 0.0057, OR 2.3, 95% CI [1.249-4.242]), in individuals with **hypertension** (P < 0.0093, OR 2.5, 95% CI [1.228-5.148]), and particularly **with elevated TG** levels (P < 0.0079, OR 4.58, 95% CI [1.35-15.98]). The same pattern was observed for the H1/H9 association in the OP subgroup with fractures, where the OR for the H1/H9 pair increased to 9.1 (P < 0.0067) in individuals with elevated TG levels. These findings consistently indicate that the H1/H9 haplotype pair is pathogenic for OP and fractures, especially in the context of insulin resistance, elevated TG levels, and hypertension.

Genotype-Phenotype Correlation. To analyze the metabolic consequences and bone alterations, we examined the genotype-phenotype correlation in OP. **Carriers of the H1/H9 haplotypes exhibited a leaner phenotype** (12.2% obesity) linked to the heterozygous genotypes GA, TA, or GA of rs8057044, rs9939609, and rs9930506, respectively. Despite the leaner phenotype, this combination of SNPs (H1/H9) proved to be pathogenic for bone, being associated with 53% fractures and a higher prevalence of

muscle changes (51%). In contrast, **H9/H9 homozygous carriers** with the AA, AA, and GG alleles of the three leading SNPs were more obese (30.2% obesity), with larger waist circumference (97.2 cm), more central obesity (86%), and higher systolic blood pressure values. H1/H1 homozygous carriers of the GG, TT, and AA alleles of the SNPs displayed an intermediate phenotype. We found no effects on the HOMA-IR index, IR as a nominal variable, fasting glucose, or MetS, although H1/H1 carriers showed a tendency towards a higher prevalence (39.3%) of MetS and lower HDL-C levels (46.4%, $P < 0.0003$). These latter effects may be due to the dominant effect of the rs9930506 AA genotype. In the next stage, our focus shifted to **bone metabolism**, particularly BMD, TBS score, and bone turnover markers. Carriers of the pathogenic H1/H9 pair exhibited lower BMD values at all anatomical sites, while H9/H9 carriers showed higher values.

In this paper, we presented evidence supporting the association of certain SNPs in intron 1 of the *FTO* gene with OP and severe OP with fragility fractures, **designating the *FTO* gene as a promising candidate for OP**. These findings reinforce previous studies on the *FTO* gene, and through haplotype mapping, we have provided new data in the identification of biomarkers for genetic predisposition to OP. **My personal contribution in this field lies in identifying SNPs in intron 1 of the FTO gene as being associated with primary OP and severe OP with fragility fractures, consistent with reduced BMD at various anatomical sites.**

STUDY 3: *Bioinformatics Analysis in the Genetics of Osteoporosis and Metabolic Syndrome*

It is worth mentioning that the field of genetics research has evolved significantly in recent years with the introduction of bioinformatics analysis, an essential step today, providing researchers with a range of public databases. This Study 3 presents the results obtained in the field of bioinformatics and genetic analysis of OP, conducted during a research internship in France. Before proposing the genotyping of candidate genes (*FTO* or *LRP5*), we considered it necessary to examine the data on OP through bioinformatics analysis of GWAS studies and RI, MetS, or sarcopenia. Thus, it is not a simple literature review, but rather an integration of data from public databases and the analysis of results using specific software, with access to high-capacity computing resources. The hypothesis was that certain genes involved in OP might be shared with those implicated in RI or MetS.

Results. GWAS Studies in OP. Since 2007, more than 40 GWAS studies have been conducted on OP, identifying over 518 loci associated with various osteoporotic phenotypes at genome-wide significance (P < 10^-8). These loci explain 20% of BMD variability. These studies have already been reviewed in the literature, and here we will present only the aspects where we made an original contribution.

GWAS Studies in Metabolic Syndrome (MetS). Similarly, we reviewed studies on the genetics of MetS. Over 100 loci have been associated with BMI, as shown in a GWAS meta-analysis of 340,000 predominantly European subjects, accounting for 2.7% of BMI variation. So far, there are few GWAS studies with MetS as the phenotype. One study, in which MetS was diagnosed according to the IDF definition, included 4,560 South Asians but found no significant associations. However, several influential genes were reported, such as *CETP*, *LPL*, *FADS1*, *FADS2*, and *FLJ41733*, associated with HDL-C (P < 10^-6), and the *TCF7L2* gene, associated with T2D (P < 10^-6) (Zabaneh and Balding, 2010). A meta-analysis combined 13 studies within the STAMPEED consortium (22,161 subjects) to investigate genetic associations with MetS, defined by NCEP ATP III. The results implicated the *LPL*, *CETP*, and *APOA5* gene cluster (*ZNF259*, *BUD13*, and *APOA5*). In association with bivariate combinations of MetS components, 27 SNPs were reported across 11 genes, including *GCKR*, *C2orf16*, *ZNF512*, *CCDC121*, *ABCB11*, *TFAP2B*, *TRIB1*, and *LIPC* (Kraja et al., 2011). Regarding insulin resistance (IR), more than 60 loci have been identified through GWAS, with the top 10 in statistical significance and replication annotated to the genes *IRS1*, *PPARG*, *GRB14*, *PEPD*, *PDGFC*, *MAP3K1*, *ARL15*, *FAM13A*, *RSPO3*, and *LYPLAL1*.

Common Genetics between OP, MetS, and IR. These review studies allowed us to bioinformatically identify the genes common to the three conditions by querying the HugeNavigator site. We included 174 genes for MetS and 1,514 genes for IR, which were intersected with the 76 genes associated with OP (Trajanovska et al. 2018), plus 19 genes common to OP and fragility fractures. Remarkably, very few genes were found to be shared. Among the genes associated with OP and fragility fractures, only three were common with MetS (*LRP5*, *ESR1*, and *F2*). Between the genes for OP and IR, only 16 were common: *TNFRSF11B*, *LRP5*, *TNFSF11*, *ESR1*, *SOST*, *TNFRSF11A*, *CTNNB1*, *F2*, *SOX6*, *RSPO3*, *LEKR1*, *FAM9B*, *PPP1R3B*, *SLC8A1*, *AQP1*, *ETS2*, and *MBL2*. Furthermore,

when the intersection was made with genes from the Wnt signaling pathway, only three genes were found (*CTNNB1*, *LRP5*, and *PPARD*). It is noteworthy, however, that for the first time, the *FTO* and *IRS-1* genes appeared among the common genes, even though previous GWAS studies did not implicate the *FTO* gene. There is no clear explanation as to why the *FTO* gene was ignored in OP for many years, especially considering that Pei et al. in 2020 demonstrated the pleiotropic effect of *FTO* on obesity and BMD.

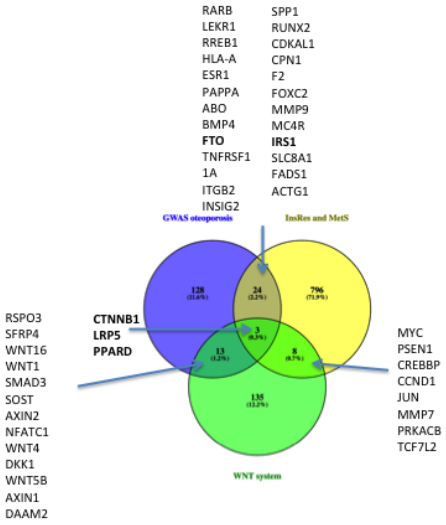

Figure 3. The Venn diagram for the intersection between the genes associated with Osteoporosis (OP), Metabolic Syndrome (MetS), Insulin Resistance (IR), and genes from the Wnt signaling pathway.

These studies, conducted to prepare more precise objectives, have allowed the development of new working hypotheses and demonstrate that bioinformatics research is becoming an indispensable step in genetics. We hypothesized that the *FTO* gene might be linked to OP through yet unknown mechanisms**, possibly acting at the bone marrow level by redirecting stem cell differentiation towards adipocytes instead of osteoblasts**. Although unpublished, during the same period, we initiated a GWAS study on MetS in a collection of patients with and without MetS from Romania, Turkey, and France, focusing on genes involved in OP. However, no gene achieved a significant P-value.

In conclusion, these studies have been crucial in formulating study hypotheses. Furthermore, these data enable us to revisit the *FTO* gene, for which we have 4,000 SNPs available, allowing for the future creation of a dense haplotype map, unmatched on an international level.

CONCLUSIONS

Primary postmenopausal osteoporosis is a complex disease with significant public health implications due to its increased risk of fractures. **In Study 1, we observed a nonlinear, biphasic relationship between BMD and BMI in patients with osteoporosis, where BMD significantly decreases after a BMI threshold of 27.2 kg/m².** This finding highlights the detrimental effect of MetS components on bone metabolism and underscores the need for more elaborate research protocols and the adoption of individualized therapeutic approaches.

Osteoporosis is strongly influenced by genetic predisposition, and the advent of new investigative strategies in molecular genetics, such as genome-wide association studies (GWAS), has been beneficial. Given its polygenic nature, applying genetic results as predictive markers in clinical settings remains challenging. The difficulties arise from multiple factors, including the polygenic nature of the disease and the pleiotropic effects of many genes.

In Study 2, we analyzed SNPs in intron 1 of the FTO gene (rs8057044, rs8050136, rs9939609, rs62033406, and rs9930506) in relation to osteoporosis and fragility fractures. These SNPs were associated with an increased risk of osteoporosis (especially rs9930506) and severe osteoporosis with fractures (rs9939609 with a protective effect, while all others had a pathogenic effect). This study is the first to report the association of SNP rs8057044 with osteoporosis and fracture risk. **Moreover, haplotype mapping using phased DNA revealed even stronger associations with fragility fractures in osteoporosis, driven by specific haplotype combinations and correlated with reduced BMD at all anatomical sites studied.**

The discovery of the FTO gene's role in osteoporosis (Study 2) will shift research in OP pathogenesis, bringing the disease into transcriptomics, involving both epigenetic and genetic mechanisms. This may lead to identifying predictive markers for clinical use. Additionally, given FTO’s role in obesity and its interaction with nutrition, this gene will open new therapy-related research directions. Considering the complex relationship between obesity, MetS, and osteoporosis (Study 1), obesity could modulate the relationship between MetS, systemic IR, and OP, making the description of predictive genetic markers crucial.

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LIST OF PUBLISHED SCIENTIFIC WORKS

Published Articles in Specialty Journals

1. **Greere, D.**, Grigorescu, F., Manda, D., Lautier, C. and Poiana, C. (2023). Insulin Resistance and Pathogenesis of Postmenopausal Osteoporosis. *Acta Endocrinologica*, 19(3) :349–363. doi:https://doi.org/10.4183/aeb.2023.349.
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3. **Greere, D.**, Haydar, S., Grigorescu, F., Manda, D., Voicu, G., Lautier, C. and Poiană, C. (2024b). Fine-Scale Haplotype Mapping Reveals an Association of the FTO Gene with Osteoporosis and Fracture Risk in Postmenopausal Women. Genes, 15(9). doi:https://doi.org/10.3390/genes15091152.
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