

## Introduction

- **Osteoporosis** is a multifactorial disease whose interaction between genetic and environmental factors lead to a reduction of bone mineral density accompanied by changes in bone microarchitecture level, leading to a significant decrease in bone strength and an increased fracture risk.
- **Iron** is known to play a relevant role in the development of osteoporosis as it suppresses osteoblast formation and may also stimulate osteoclast resorption of bone. As so, polymorphisms in genes affecting iron homeostasis can increase the susceptibility for the development of osteoporosis.
- **HFE** is a major histocompatibility complex class I-like protein which gene is commonly mutated in Hereditary Hemochromatosis, a disorder characterized by excessive intestinal iron absorption and its deposition in several organs. It has been postulated that HFE may contribute to iron metabolism regulation by activating hepcidin synthesis in hepatocytes and regulating the expression of iron metabolism-related genes (ferroportin) in duodenum and other cells.
- The locus encoding HFE is located on the long arm of chromosome 6 (6q22.2) and contains 2 major polymorphisms. A 845G-A transition resulting in a cys282-to-tyr (C282Y) substitution and a C-to-G transversion in exon 2 resulting in a his63-to-asp substitution (H63D).
- **Haptoglobin (Hp)** is an acute phase protein that binds free hemoglobin (Hb) released from erythrocytes with high affinity and thereby inhibits its oxidative activity.
- The locus encoding haptoglobin is located on the long arm of chromosome 16 (16q22.2) and presents a copy number variation polymorphism (CNV) that results from an internal duplication of a gene segment (exons 3 and 4). This gives rise to three different genotypes (Hp1.1, Hp 2.1 and Hp2.2) that modulate the half-life of Hp-Hb complex, its plasma concentration as well as other functions (angiogenesis, immune, etc).

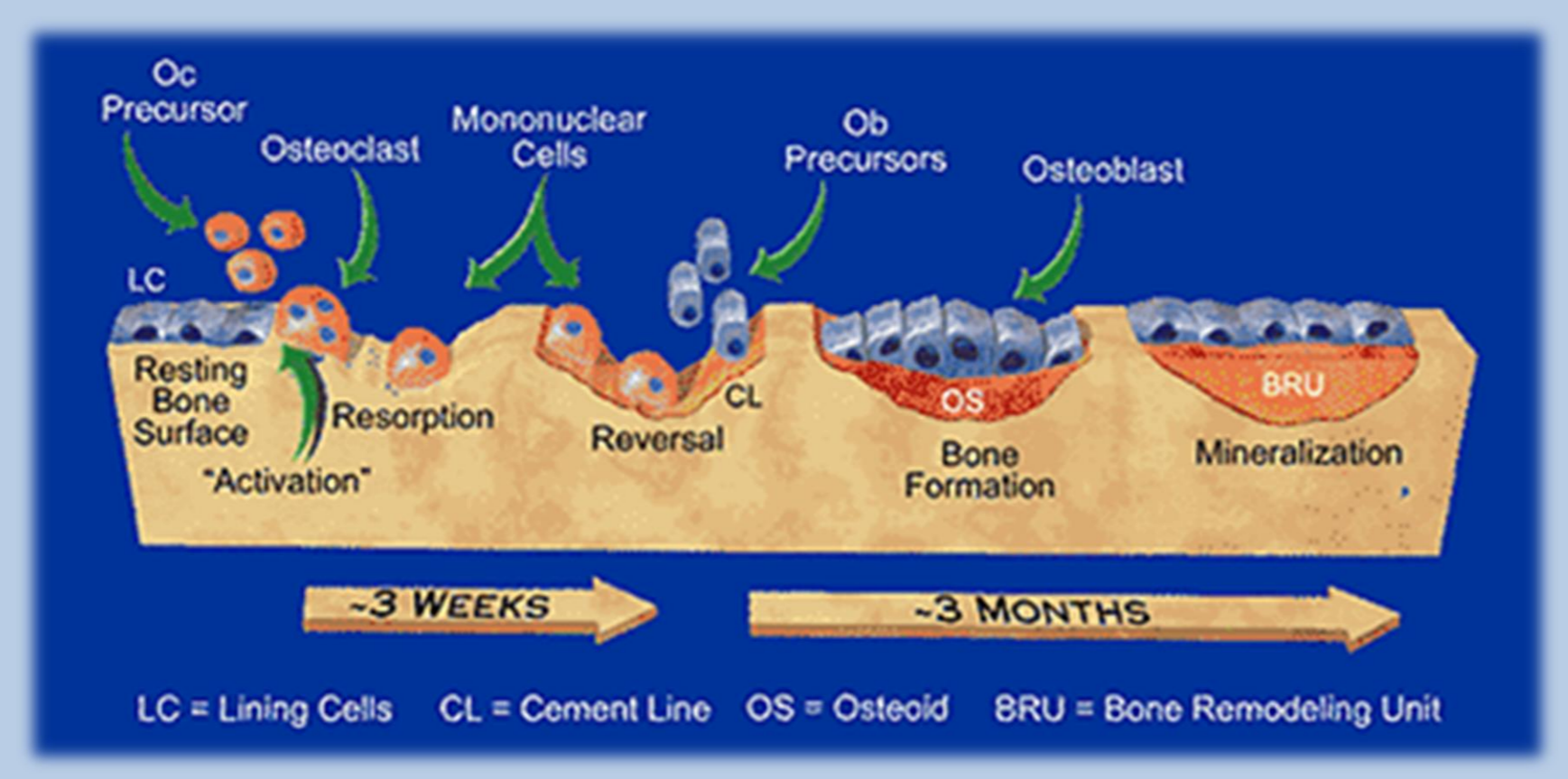


Fig. 1: Bone Remodeling Mechanism

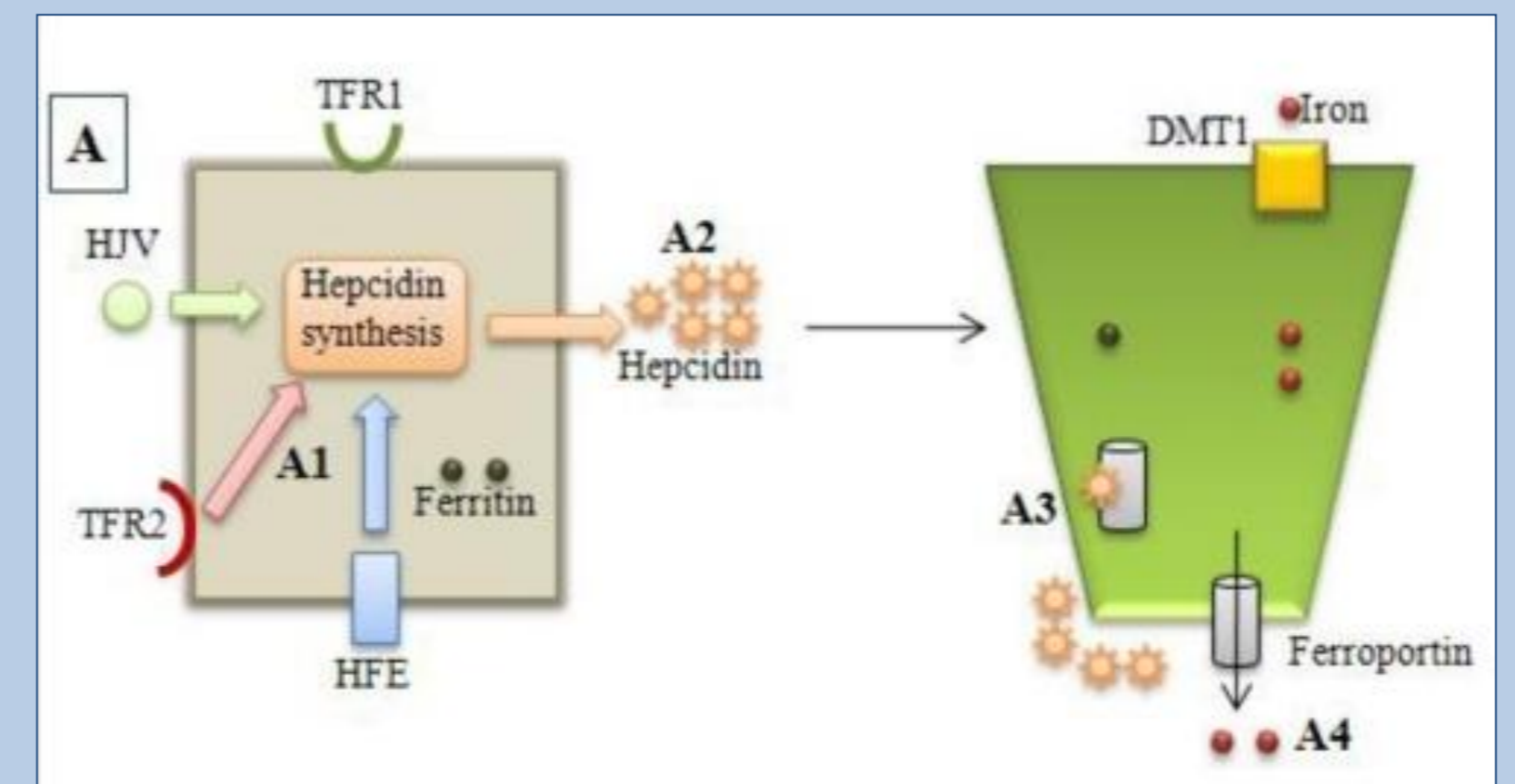


Fig. 2: HFE and Iron Metabolism Regulation

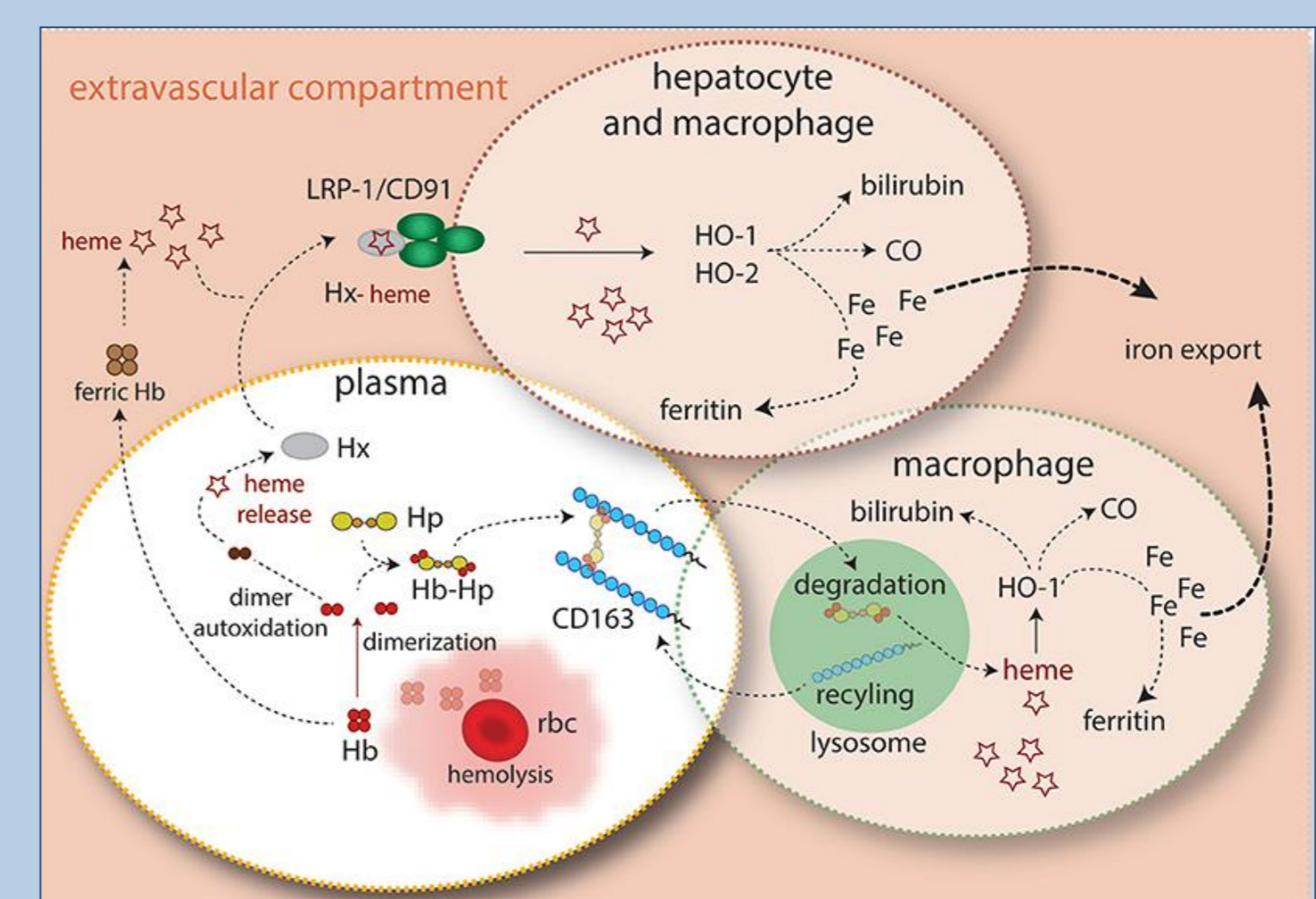


Fig. 3: Hp and Iron Metabolism Regulation

## Aims

To study the association of Hp, HFE\_C282Y and HFE\_H63D polymorphism with bone mineral density and metabolic parameters of bone remodelling.

## Population

- 202 female subjects:
- 72 normal BMD – (61,7±5.1 years; 31,6±5.1 kg/m<sup>2</sup>)
  - 130 osteoporosis (62.3±7.5 years; 27.2±4.1 kg/m<sup>2</sup>)

## Methods

- BMD (g/cm<sup>2</sup>) was measured at the lumbar spine, femoral neck and distal radius, as well as the total body soft tissue composition by DEXA.
- Metabolic and bone remodelling parameters were analyzed: LDL, HDL, total cholesterol, triglycerides, HOMA<sub>IR</sub>, alkaline phosphatase (AP), bone alkaline phosphatase (BAP), osteocalcin and calcemia.
- Hp polymorphism was determined by PAGE.
- HFE polymorphisms were evaluated by PCR-RFLP.
- Statistical analysis with SPSS 21.0 and Primer of Biostatistics were applied to the results.

## Results

1. We did not find significant differences in the distribution of H63D, C282Y and Hp genotypes among women with normal BMD and osteoporosis.
2. Female carriers of Hp2.2 and H63D\_HH, in combination, are at increased risk for developing osteoporosis (\*p value adjusted for IMC, LDL and HDL values).

| Hp/HFE_H63D   | Normal BMD |           | Osteoporosis |       | p value | OR, 95CI                    |
|---------------|------------|-----------|--------------|-------|---------|-----------------------------|
|               | n (%)      | n (%)     | n (%)        | n (%) |         |                             |
| Hp2.2/H63D_HH | 9 (18.4)   | 34 (38.2) |              |       | 0.027*  | OR = 2.747 [1.186 to 6.365] |
| OTHERS        | 40 (81.6)  | 55 (61.8) |              |       |         |                             |

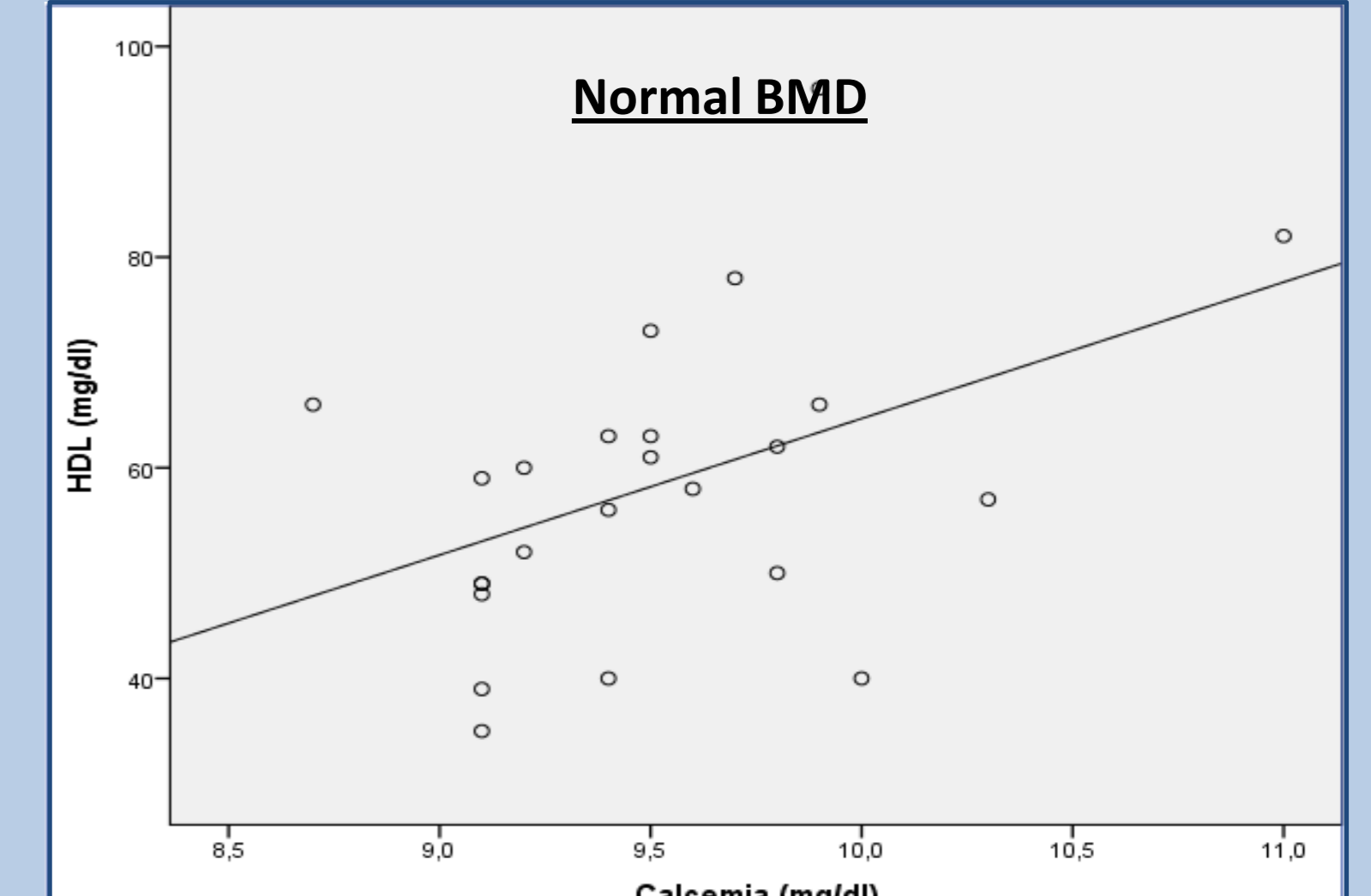
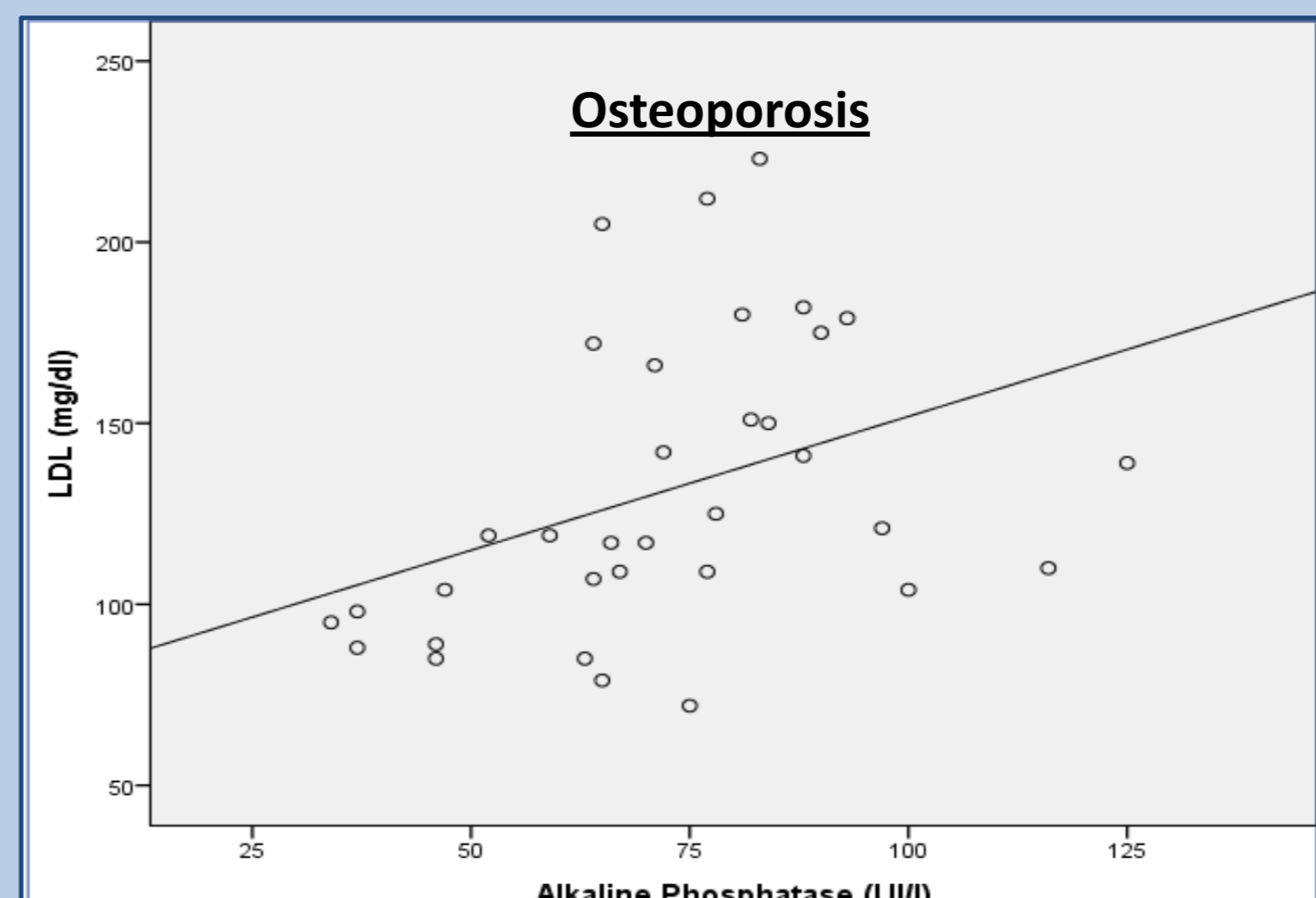
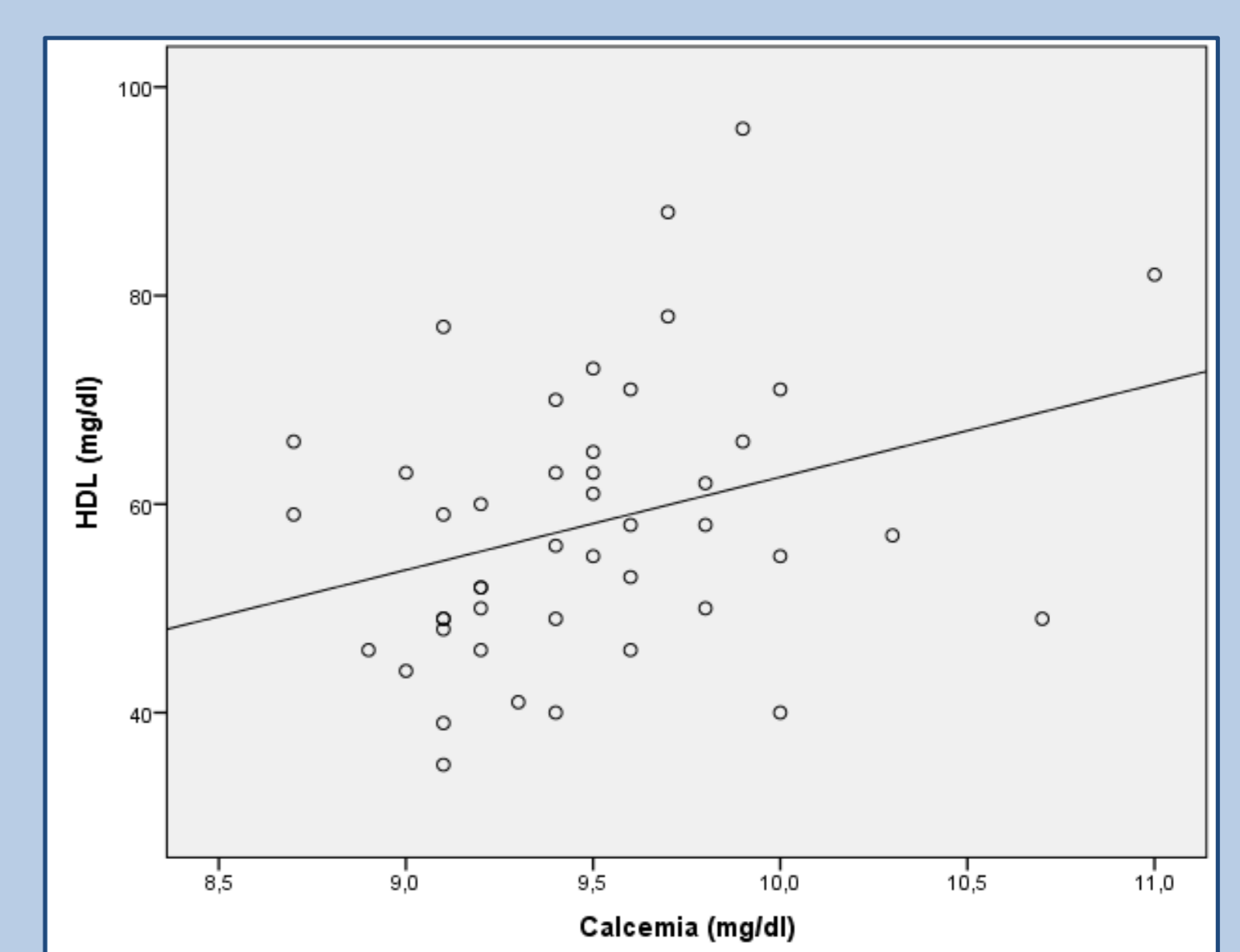
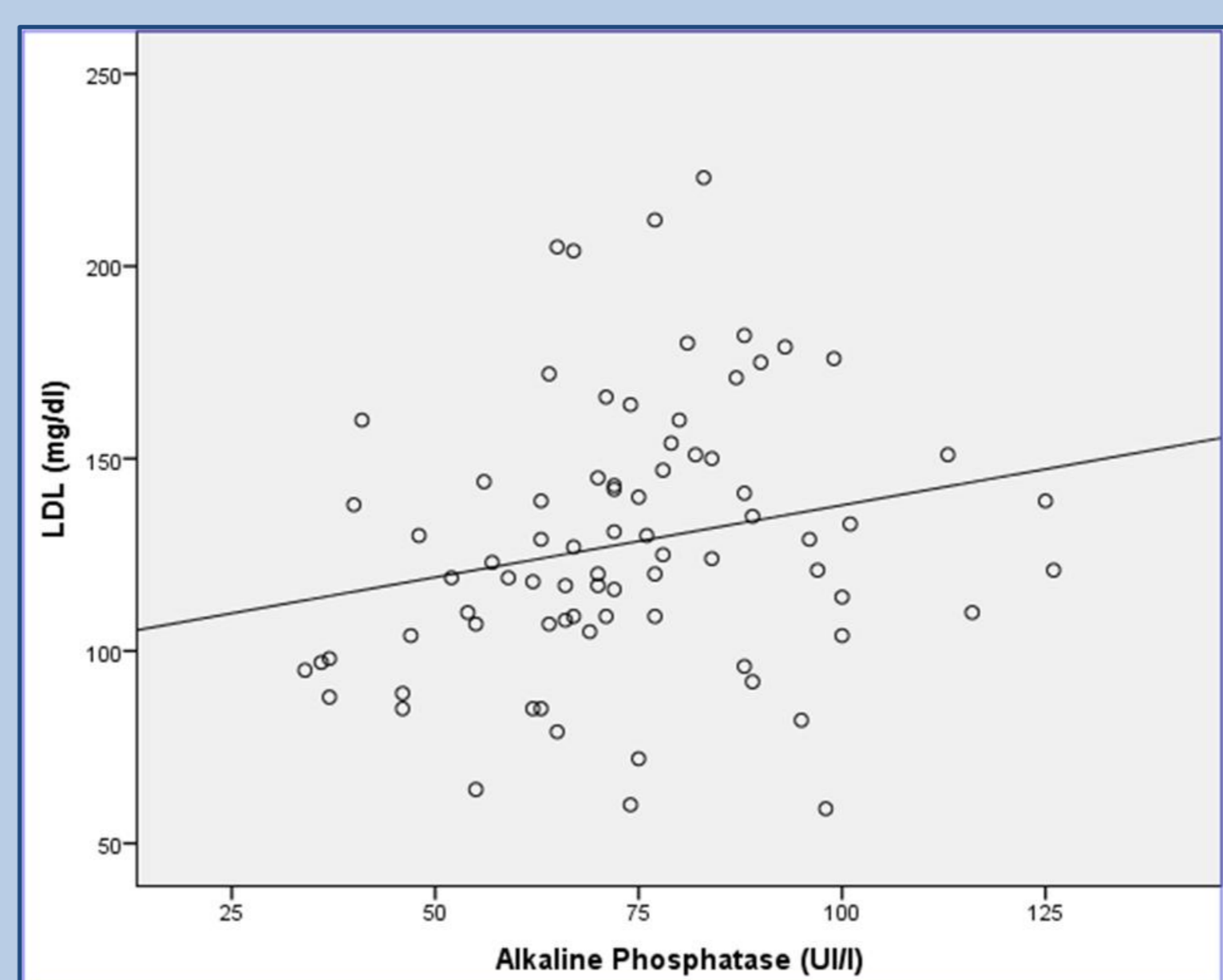
3. In osteoporosis have found increased levels of LDL.

| Parameter   | Normal BMD<br>(mean SD; n) | Osteoporosis<br>(mean SD; n) | p value |
|-------------|----------------------------|------------------------------|---------|
| LDL (mg/dl) | 131.9 36.4 (63)            | 145.1 35.1 (119)             | 0.018   |

4. In the general population, we found association between Hp polymorphism and LDL and HDL levels. Female Hp1.1 or Hp2.1 showed higher levels of LDL and lower HDL values.

| Parameter         | Hp Genotype              |                    | p value |
|-------------------|--------------------------|--------------------|---------|
|                   | Hp1.1+Hp2.1<br>(mean SD) | Hp2.2<br>(mean SD) |         |
| LDL (mg/dl), n=59 | 146.2 36.5               | 131.6 32.0         | 0.018   |
| HDL (mg/dl), n=61 | 54.6 14.2                | 60.3 13.2          | 0.019   |

5. We also observed positive correlation between LDL and AP (R=0.296, p=0.009; n = 78) and HDL and calcemia (R=0.312, p=0.037; n=45).
  6. LDL-AP correlation only remains significant in osteoporosis (R=0.525, p=0.001; n=34) while HDL-calcemia correlation only remains significant in normal BMD (R=0.432, p=0.035; n=24).
- The same results were obtained for BAP.



## Conclusion

• Hp2.2 in combination with HFE\_H63D HH genotype appear to increase the risk for developing osteoporosis. However, only the polymorphism of Hp seems to modulate some metabolic parameters either directly associated with bone loss or correlated with others indirectly associated with the remodeling of bone metabolism.