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02/09/2015 **Dr Mattia Capulli**

Mouse model of Autosomal Dominant Osteoporosis due to the single allele mutation of the Clcn7 gene,

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encoding for the aminoacidic change G213R in the ClC-7 Protein.

Reason for private status: Data partially published in:

Generation of the first autosomal dominant osteopetrosis type II (ADO2)

disease models

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Qualitative descrition

By a knock-in strategy we inserted a missense mutation in the Cloride channel type 7 (Clcn7) gene,and generated mouse models of Autosomal Dominat Osteopetrosis Type 2 (ADO2). The mutation selected was the mouse homolog of the most frequent heterozygous mutation (p.G213R) in the Clcn7 gene found in ADO2 humans. Our results demonstrate that the heterozygous model holds true presenting with higher bone mass, increased numbers of poorly resorbing osteoclasts and a lethal phenotype in the homozygous state. Considerable variability is observed in the heterozygous mice according with the mouse background, suggesting that modifier gene could influence the penetrance of the disease gene.

Adult (3 months old) heterozygous C57Bl6/J mice had a significantly higher whole body areal Bone Mineral Density (aBMD) than wild type mice. They presented with greater distal femur trabecular bone volume, trabecular number and separation, and no changes in trabecular thickness. A similar phenotype was also observed in the tibiae and vertebrae. Increased bone mass was persistent with time as shown by analysis of bone structural parameters in aging mice. Results were similar in male and female mice, although females had a lower bone mass and more pronounced BV/TV elevation than males (**Figure 1 and Table 1**)

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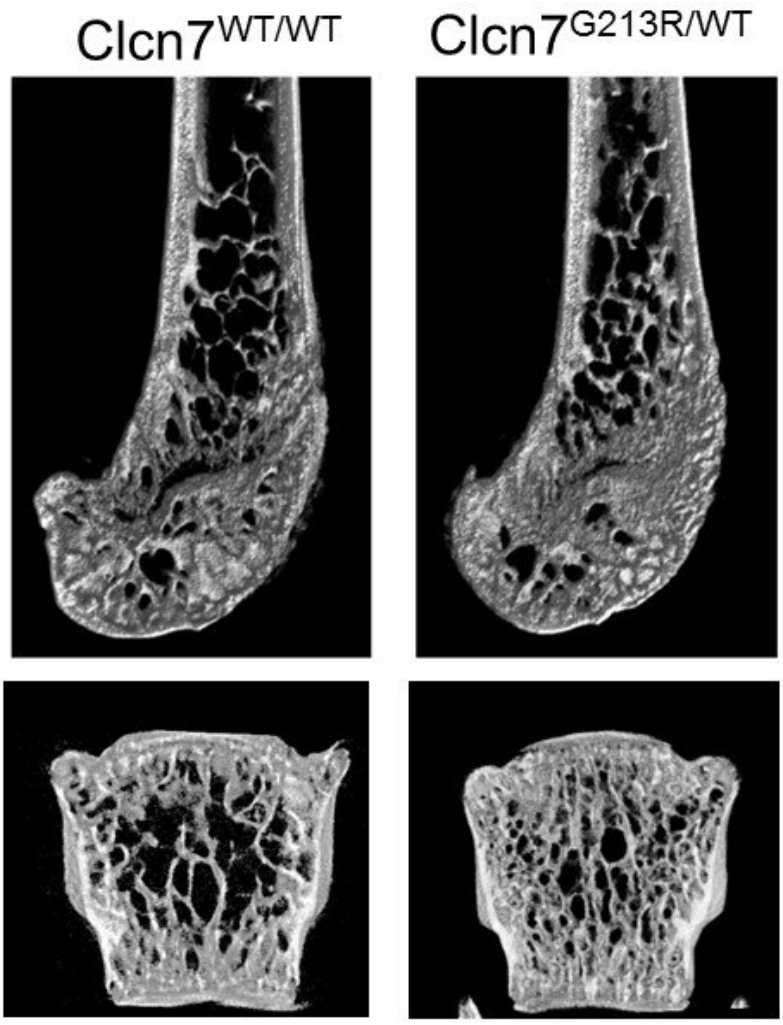

Figure (1).JPG

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Histomorphometry analysis of ADO2 bones

Histochemical examination of 3 months old heterozygous mice demonstrated a marked increase of calvarial bone whole mount staining for the osteoclast marker TRAcP (Table 2), and higher osteoclast number (Table 2) and osteoclast surface/bone surface (Table 2) in the proximal tibia compared to wild type mice. Nevertheless, a reduction of bone resorption was predicted by a *'trend of decrease'* of the serum level of the bone resorption marker CTX, normalized for the serum TRAcP (Table 2). In contrast, serum levels of a bone formation marker, osteocalcin, in addition to osteoblast, bone formation, osteoid and growth plate histomorphometric parameters were similar to wild type, suggesting no involvement of osteoblasts or chondrocytes, nor an osteopetrorickets phenotype.

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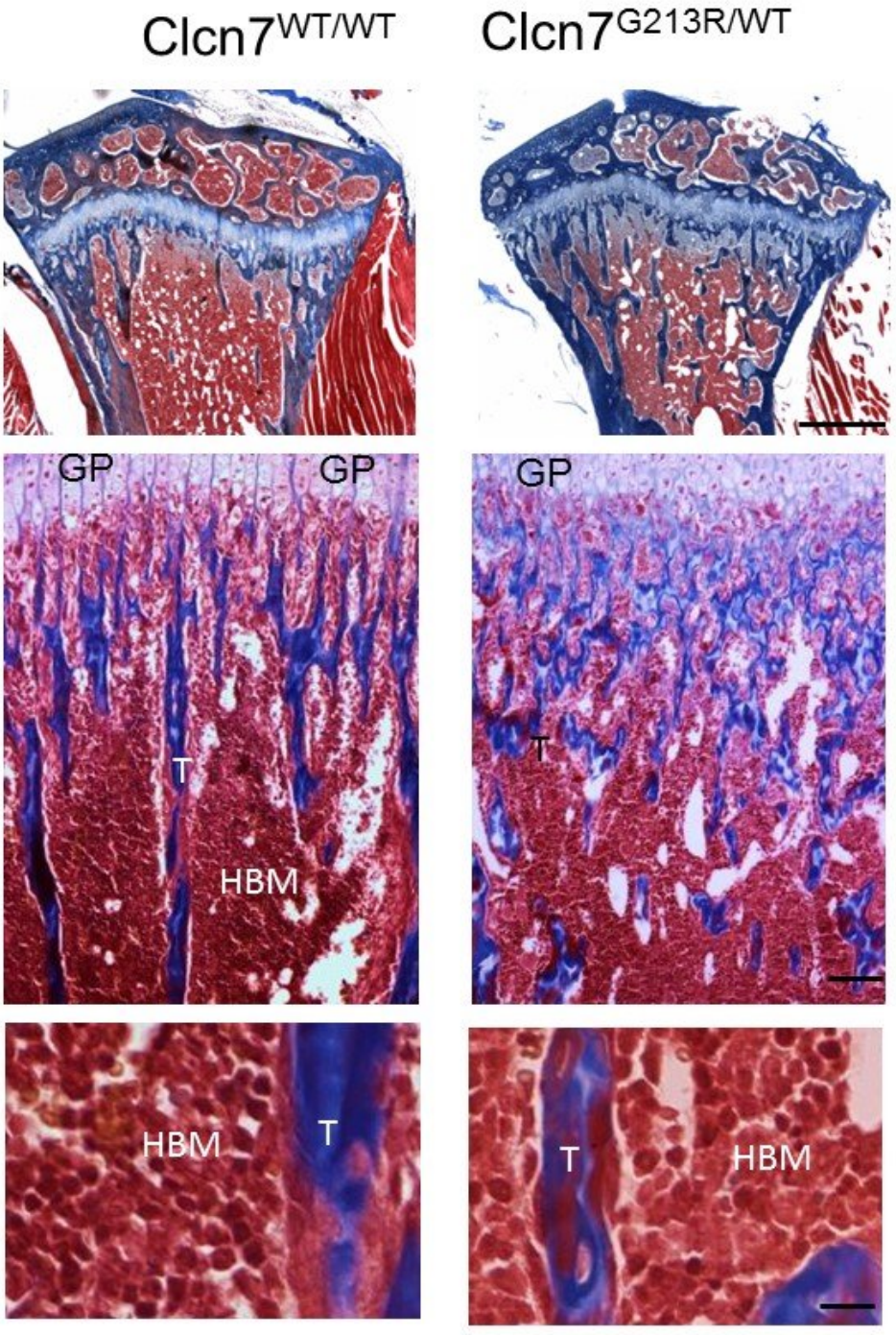

Figure (2).JPG

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Gross anatomical analysis

Heterozygous C57BL6/j were similar sized than WT mice (Figure 3, upper panel), with normal tooth eruption (Figure 3 middle panel) and normal survival (Figure 3 lower panel) and showed a mild osteopetrotic phenotype. Homozygous C57BL6/j mice were smaller (Figure 3, upper panel), lacked tooth eruption (Figure 3 middle panel) and died within 30 days of age (Figure 3 lower panel). Feeding them with soft chow did not improve survival. Homozygous C57BL6/j mice showed a severe osteopetrotic phenotype,

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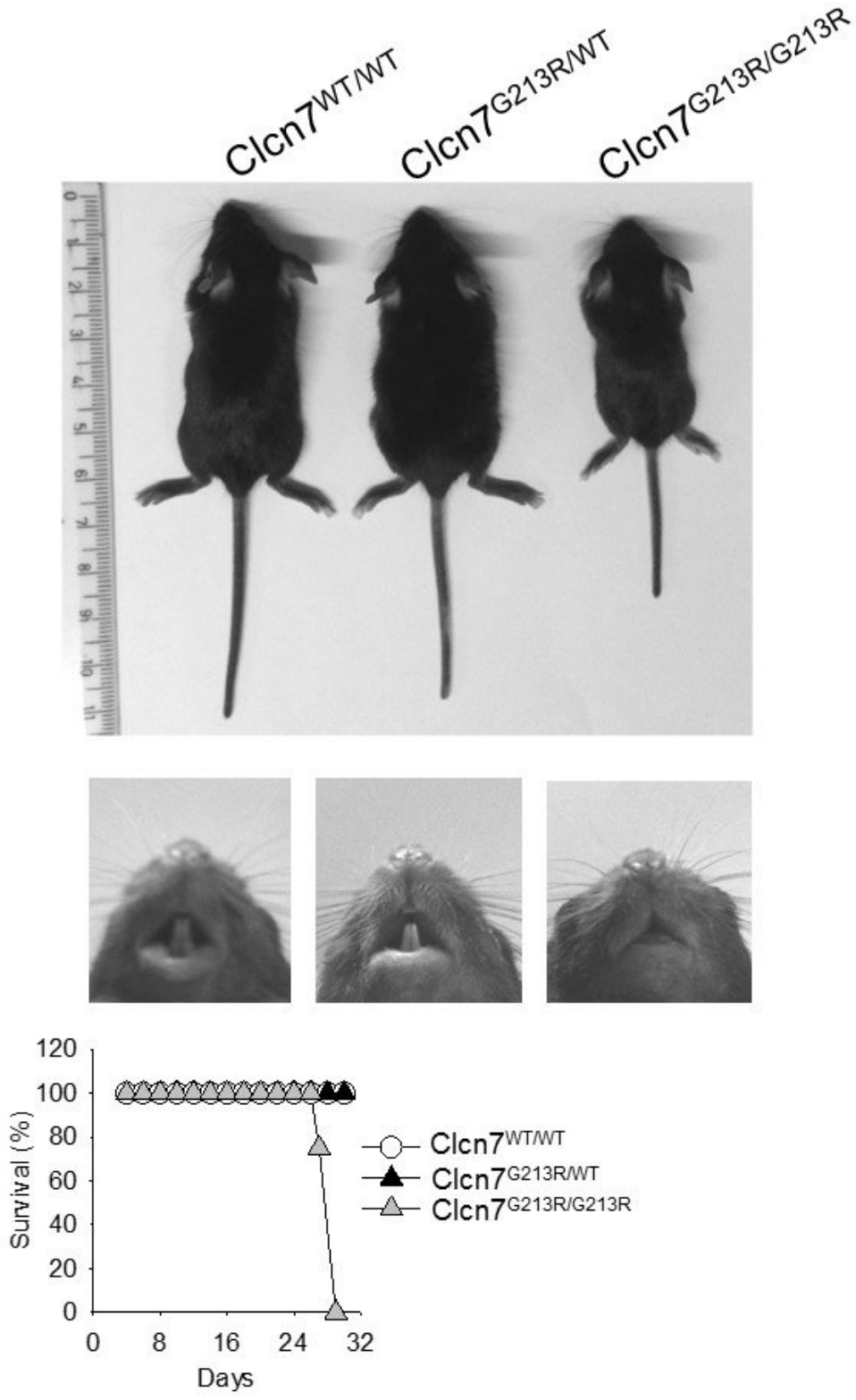

Figure (3).JPG

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Gross anatomical analysis, CD1 ADO2 mice

Heterozygous CD1 were similar sized than WT mice (Figure 4, upper panel), with normal tooth eruption (Figure 4 lower panel) and normal survival, and showed a no osteopetrotic phenotype. Homozygous CD1 mice were smaller (Figure 4, upper panel), lacked tooth eruption (Figure 4 lower panel) and died within 30 days of age. Feeding them with soft chow did not improve survival. Homozygous C57BL6/j mice showed a severe osteopetrotic phenotype,

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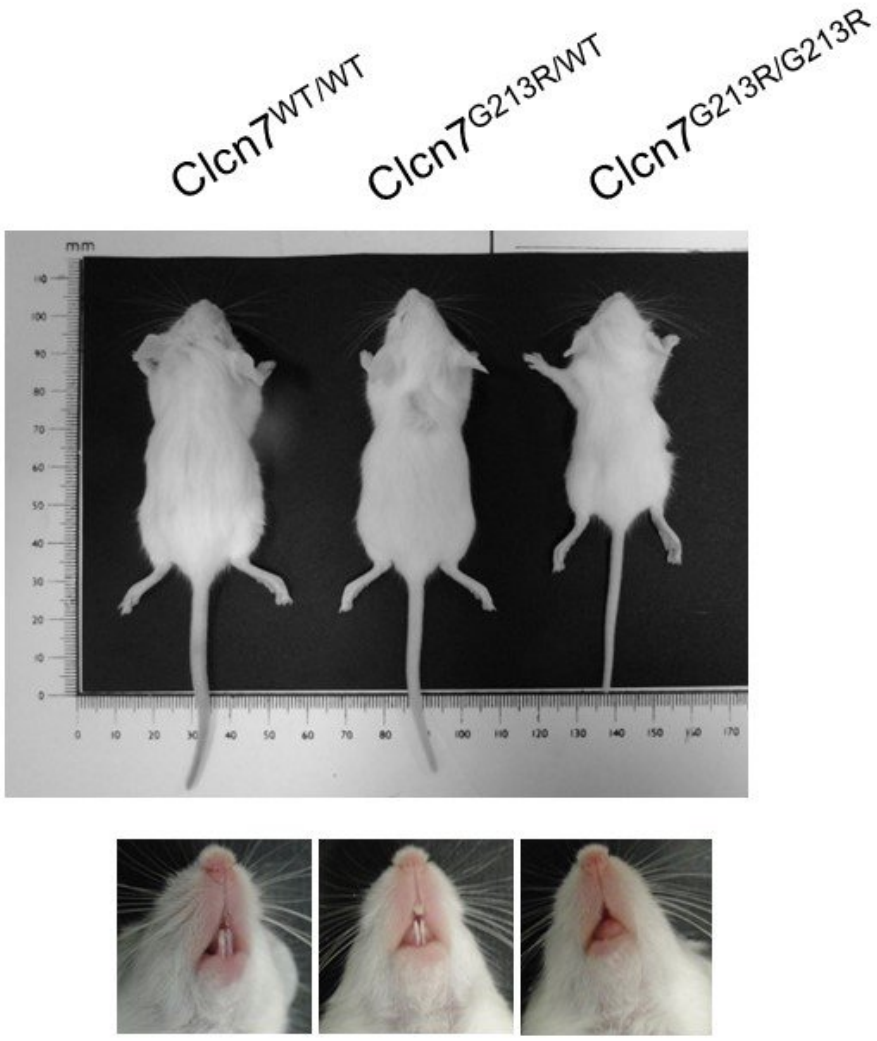

Figure (4).JPG

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MicroCT CD1 ADO2 mice

Adult (3 months old) heterozygous CD1 mice were present with a bone phenotype non statistical different than WT mice, as assessed by the similar distal femur trabecular bone volume, trabecular number, separation, and trabecular thickness. Results were similar in male and female mice, although females had a lower bone mass. (**Figure 5 and Table 1**)

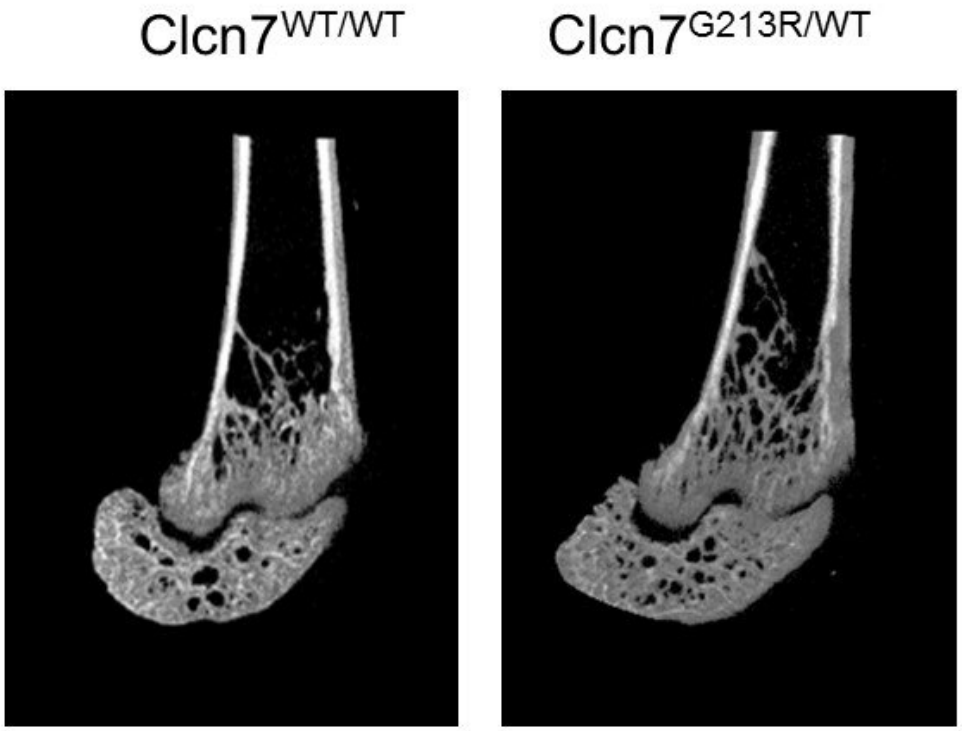

Figure (5) JPG

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MicroCT analysis protocol

Femurs and vertebrae of C57BL6/J and CD1 mice were fixed in buffered formalin, dehidrated in ethanol 70% overnight and mounted in a plastic tube wrapped in moistened paper tissue to prevent drying during scanning. The bones were imaged in the SkyScan 1174 with a voxel size of 6 µm3. The applied X-ray voltage was 50 kV. The scans were over 180°with a 0.3 degree rotation step and 2 image per rotation step. Reconstruction was carried out employing a modified Feldkamp algorithm using the Skyscan Nrecon software. Beam hardening correction and

Fourier transform-based ring artifact reduction were applied to the reconstruction. 3D and 2D morphometric parameters were calculated for the trabecular bone of selected regions of interest, 70 slices (0.6 mm) from the growth plate extending for 350 slices (2.1 mm) below. Threshold values were applied for segmenting trabecular bone

corresponding to BMD values of 0.6/cm3 CaHA. 3D parameters were based on analysis of aMarching Cubes typemodel with a rendered surface.

Calculation of all 2D areas and perimeters was based on the Pratt algorithm. Morphometric parameters were measured by CT-analyzer. Structure thickness in 3D was calculated using the local thickness or “sphere-fitting” method. Model construction was performed by the “Double time cubes” method, a modification of the Marching cubes method. Nomenclature, symbols and units of structural bone variables are those suggested by Bouxsein at al. 2010, JBMR.

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