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**Epigenome-wide association study shows that smoking alters DNA methylation in blood cells triggering aggressive bone resorption of osteoclasts in vivo and in vitro**

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differentiation of BMSCs. It may hold the potential as a therapeutic target for treatment of osteoporosis.

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## COP12

### The inositol phosphatase SHIP1 regulates skeletal development

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**Background/Introduction:** Src-homology (SH) 2 domain-containing inositol-5-phosphatase 1 (SHIP1) is a lipid phosphatase expressed mainly in hematopoietic cells. SHIP1 regulates cell proliferation, differentiation, and survival via the PI3K/Akt signaling pathway. SHIP1-deficient (*Styx*) mice are osteoporotic, which is associated with an increased number of osteoclasts (OC).

**Purpose:** This study aimed to investigate the underlying mechanisms through which SHIP1 controls osteoporosis.

**Methods:** Osteoclast progenitor cells (OPC) were generated by incubating bone marrow cells with CSF-1. To develop OC, OPC from *Styx*, *Styx het* (heterozygous) and *wt* (wild type) mice were cultured with RANKL and CSF-1. Osteoclastogenesis was evaluated using an XTT cell viability assay, TRAP activity (OC marker) and qRT-PCR. Micro-computed tomography (Micro-CT) of vertebrae and femora were performed to evaluate the bone structure.

**Results:** Deficiency in SHIP1 affected several aspects of bone. Compared to *Styx het* and *wt* controls, OPC-derived *Styx* OC presented several developmental defects, including a lower TRAP/XTT ratio and a 52% decrease in *Calcr* transcripts (encoding for the Calcitonin Receptor) ( $p < 0.001$ ). *In vivo*, there was a strong reduction of BV/TV in vertebrae and femora of *Styx* versus *wt* animals (39.6% and 35%, respectively,  $p < 0.01$ ). In particular, trabeculae in *Styx* vertebrae were increased by 8% ( $p < 0.05$ ) in numbers while decreased by 37% in thickness ( $p < 0.001$ ). In contrast, in *Styx* femora both the number and thickness of the trabeculae were decreased by 16% and 14%, respectively. These different phenotypes in *Styx* femora versus vertebrae indicate different paths to osteoporosis in bones with primary or secondary spongiosa.

**Conclusion(s):** Taken together, our data indicate a central role for SHIP1-dependent PI3K/Akt signalling in bone remodeling. Further investigation will address the role of osteoblasts in the development of osteoporosis in SHIP1-deficient *Styx* mice.

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## Concurrent Oral Presentations 2: Clinical / Public Health: New Pathophysiology Insights

### COP13

#### Epigenome-wide association study shows that smoking alters DNA methylation in blood cells triggering aggressive bone resorption of osteoclasts in vivo and in vitro

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**Background/Introduction:** Smoking is a risk factor for osteoporosis, but the mechanisms remain unclear. Human osteoclasts can resorb bone in two modes, the aggressive trench-mode and the slower pit-mode. Recently, we have shown<sup>1</sup> that e.g. donors' age correlate with osteoclast trench-mode *in vitro* suggesting an epigenetic regulation through monocytes.

**Purpose:** Investigate if smoking affects bone resorption of osteoclasts *in vivo* and *in vitro* and if this may be explained by changes in DNA methylation.

**Methods:** Based on data from our publication<sup>1</sup> we applied an epigenome-wide association study (EWAS)(n=34 healthy women, 40-66 years, ethical approval S-20150059). Methylation levels on DNA from donors' peripheral blood mononuclear cells were analysed using Illumina's EPIC array (850k CpGs). Osteoclasts generated from donors were reseeded onto bone slices for 72h and evaluated for % trench surface/eroded surface (%TS/ES).

**Results:** Number of cigarettes smoked throughout life (0 to 263,000) correlated with %TS/ES *in vitro* ( $r_s = 0.40, p = 0.02$ ) and an increasing imbalance between bone resorption (CTX) and formation (PINP) *in vivo* (CTX/PINP) ( $r_s = 0.45, p = 0.008$ ). A direct correlation between %TS/ES *in vitro* and CTX/PINP *in vivo* was also found ( $r^2 = 0.20, p = 0.009$ ). EWAS for single CpGs on %TS/ES interacting with number of cigarettes smoked throughout life showed a total of 2035CpGs with both positive and negative directions of significance associations between smoking and %TS/ES (FDR < 0.05,  $p = 9.88e-06$ ). 2755CpGs were significant for CTX/PINP ratio *in vivo* interacting with the number of cigarettes smoked throughout life (FDR < 0.05,  $p = 4.703e-11$ ). 1731CpGs were found overlapping in common.

**Conclusion(s):** Results suggest that smoking directly affects osteoclasts *in vivo* and *in vitro* triggering aggressive osteoclasts. This may be mediated through epigenome-wide alterations in circulating osteoclast precursors. Our data may be of interest to predict individual risk of osteoporosis, especially in the context of smoking history.

**Reference:** [1] Møller, A.M.J. et al.; Bone Res. 8, 27 (2020).

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### COP14

#### Association between environmental air pollution and rheumatoid arthritis flares

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**Background/Introduction:** Environmental air pollution has been linked to the pathogenesis of Rheumatoid Arthritis (RA). Nevertheless, evidence linking higher concentrations of air pollutants with the risk of RA reactivations is missing.

**Purpose:** The objective of the study was to determine the association between RA flares and air pollution.

**Methods:** We designed a case-crossover study. We compared the exposure to pollutants in the 30-day and 60-day periods preceding an arthritic flare referent to the 30-day and 60-day preceding a low-