Pathophysiology of bone disease in nephropathic cystinosis
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Justine Bacchetta, MD, PhD, and Irma Machuca-Gayet, PhD
Reference Center for Rare Renal Diseases Hôpital Femme Mère Enfant INSERM 1033 Research Unit Lyon, France justine.bacchetta@chu-lyon.fr

SUMMARY

Cystinosis metabolic bone disease (**CMBD**) corresponds to the **specific bone impairment** observed in **patients with infantile nephropathic cystinosis**; CMBD has been officially recognized in an international consensus paper in 2019 [1]. CMBD has a significant impact on patients' quality of life, because of an increased frequency of bone pains, deformations and fractures occurring as early as late teenage and early adulthood. We have previously showed **intrinsic specific cystinosis-related bone impairment** in patients independently of mineral and bone abnormalities secondary to chronic kidney disease (CKD) [2–5].

Since local bone cell dysfunctions contributing to CMBD are still poorly understood, **the goal of the 2022 Cysteabone project is to keep dissecting the molecular mechanisms underlying the intrinsic defects in specific bone cells, namely osteoblasts, the bone forming cells** o mesenchymal origin **and osteoclasts, the bone resorbing cells** of hematopoietic origin **in cystinosis.**

Despite the fact that cystinosis is a well identified lysosomal storage disease, **clinical outcomes are very hetereogenous** as well as findings observed directly on bone tissues or cell phenotypes from INC patients. Indeed, **we have also reported very distinct cellular phenotypes related to their differentiation or activity and diverse response to cysteamine according to the embryonic cell lineage from which they are derived [4].** Therefore, we hypothesized that deep analyses of each bone cell type in cystinosis will allow **to identify the "signature" molecular pathways of CMBD** but also **to provide new target gene candidates to design new therapeutical approaches**.

Thus, **we have developed a cell-based model** (coculture of osteoblast/osteoclasts) mimicking "the *in vivo* bone remodeling unit" **to study the cross-talk of bone cells in a cystinosis context**. This model allowed us **to investigate and to identify the molecular cell response to hormonal supplementation** ($1,25(OH)_2$ Vitamin D_3, $1,25VD_3$) and **cysteamine** treatment. It is a setting appropriately designed **to evaluate osteoblast-dependent osteoclastogenesis**, a process that is representative of Osteoblast/Osteoclast crosstalk. Using this approach, **we have identified active Interleukin1β (IL1β) that is over-secreted by Ctns$^{-/-}$ osteoblasts**. To validate IL1β as a potential candidate for biotherapy, **we have provided the proof of concept** using the IL1β receptor antagonist Anakinra, that **reduces osteoclastic formation**. Furthermore, supporting our hypotheses and results, IL1β pathway was also recently identified as a potential target to attenuate cachexia in muscle cells by another team[6]. Interestingly enough, muscle cells and osteoblasts derive both fom mesenchymal cell lineage.

The aims of the 2022 CYSTEABONE project were the following: 1/ to finalize the experimental part of the interleukin 1 project, so as to obtain a strong rationale to further propose a proof-of-concept trial in patients, 2/ to analyze the 1,25VD3 treated Ctns$^{-/-}$ Osteoblastic transcriptome to explore other regulatory pathways involved in CMBD, and 3/ to identify new targets to improve bone health in cystinosis using high-throughput pharmacological screening in a cystinosin CRISPR edited human Osteoblast-dependent osteoclastogenesis.

We have also applied in Spring 2024 for a 2-year post-doc application, and we would like to express our deepest gratitude to Cystinosis Research Foundation for the continuous support for the CYSTEABONE project.

Current state of the CYSTEABONE project at one glance

Murine models	Human models
Cytokine Proteome profiler confirming and strengthening the results obtained by qRT-PCR: 2020 application, performed Transcriptomic analyses of the cross-talk in murine cells: 2020 application, performed. In view of the results, dissection of the underlying pathways: identification of a deregulated hormonal system in the bone/endocrine axis, ongoing Confirmation of the alteration of IL1β signaling in cystinosis: transcriptome and proteome of other members of IL1 family and of genes involved in RANKL independent osteoclastogenesis, performed Proof of concept with the use of anti IL1β / anti-IL1β -R in co-cultures: performed	Confirmation that IL1β- signaling is affected in cystinosis: evaluation of the expression of other members of IL1 family, according to the underlying genotype, additional ECYSCO funding for results of serum Il1 in patients: ongoing Cell based models of cystinosis: 2 human CRISPR edited osteoblasts lines baring identified mutations (absence *versus* residual activity respectively) to assess and confirm the crosstalk defect in human bone cells. Aim 1 of the 2022 application. These lines were characterized, in terms of osteoblast commitment and effect of $1.25VD_3$ + the expression (qRT-PCR) and secretion (cell lysates, medium, with and without starving, with and without $1.25VD_3$). We were not able to detect any response to $1.25VD_3$ even for the "canonical" vitamin D-induced osteoblatic genes and for Il1. Also, using these CRISPR lines, limited osteoclastogenesis was obtained, because of increased OPG in committed $1,25VD_3$ treated osteoblasts so that we will stop using this model. As such, we will now focus on a novel cell based model of cystinosis, i.e., the induced pluripotent stem cells (iPSCs), in collaboration with Pr Antignac's lab. Expression of IL1β-R in PBMCs from patients and inflammasome monitoring: 2020 application, performed, paper submitted Resorption analyses from patients depending on the underlying genotype2020 application, not performed (not enough material available to date, will be performed if we have enough material with human samples, IRB still ongoing) Histology on bone from patients: IL1ß staining: 2020 application, not performed (no material available to date)

Submitted paper submitted

Title
Cystinosis Bone Metabolic Disease: inflammatory profile in human PBMCs and derived osteoclasts
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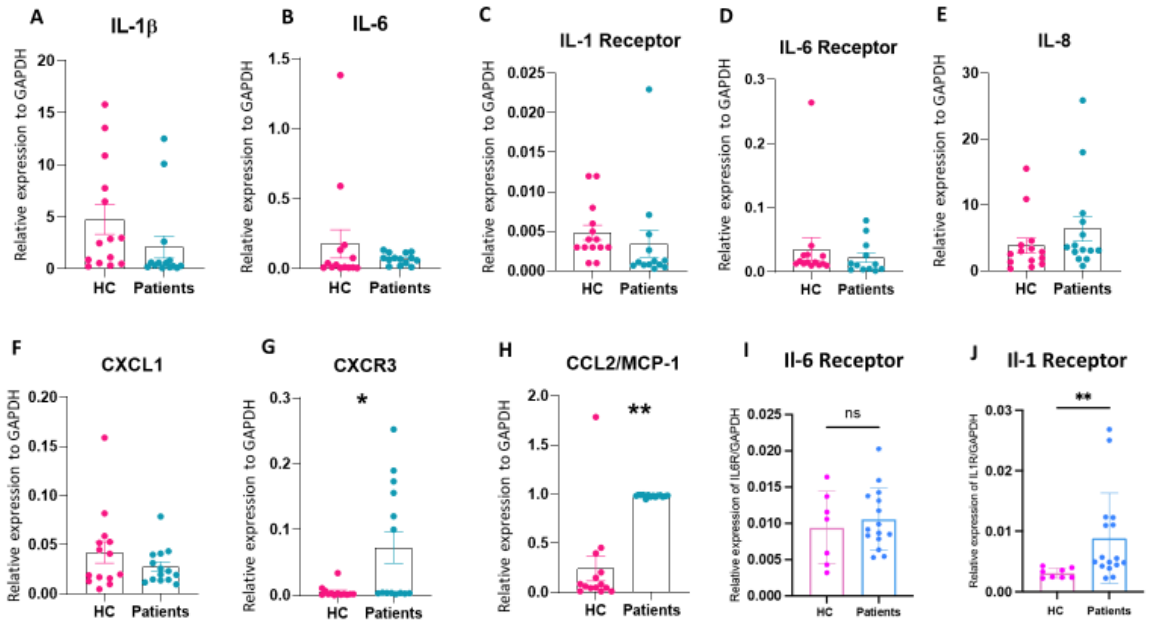
Figure: expression of the different inflammatory cytokines in PBMCs and osteoclasts in patients with cystinosis



HC: healthy controls; results of RT-qPCR for the 8 genes involved in inflammation and chemotaxis (IL1β, IL6, IL8, CXCL1, CCL2 -also named MCP1-, IL1R, IL6R, CXCR3) in PBMCs from 14 patients and 13 controls (A-H), and in fully-differentiated osteoclasts from 4 patients and 3 controls (I-J). Gene expression was quantified and normalized to hGAPDH values, then expressed as relative expression using the 2^(-ΔCq) method. *, p<0.01 and **, p<0.001.

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