# Pathophysiology of bone disease in nephropathic cystinosis
# Report-1- CYSTEABONE-2020 project

**Principal investigator**
Pr Justine Bacchetta, MD, PhD
Reference Center for Rare Renal Diseases
Hôpital Femme Mère Enfant, Bron, France
Justine.bacchetta@chu-lyon.fr

**Co-principal investigator**
Dr Irma Machuca-Gayet, PhD
INSERM Research Unit 1033 LYOS
Lyon, France
Irma.machuca-gayet@inserm.fr

## 1- Summary/Abstract

**Bone impairment has been recently described in patients with nephropathic cystinosis** [1–3], with **international recommendations for diagnosis and management published in 2019**; the concept of "**cystinosis metabolic bone disease**" is currently emerging [4]. Even though **its exact pathophysiology remains unclear**, at least five distinct but complementary entities can explain bone impairment in patient with cystinosis: long-term consequences of renal Fanconi syndrome, malnutrition and copper deficiency, hormonal disturbances, myopathy, and intrinsic/iatrogenic bone defects. This complication has a **significant impact on patients' quality of life**, because of an increased frequency of bone pains, deformations and fractures occurring in late teenage and early adulthood.

**The CYSTEABONE-2020 project** aims **to better understand the underlying mechanisms of bone impairment in cystinosis**, and **to identify putative new therapeutic approaches to improve (or prevent the onset of) bone symptoms**. Indeed we had previously reported specific cystinosis-related bone damage in patients independent of mineral and bone abnormalities secondary to chronic disease [1,5]. The first steps of the CYSTEA-BONE project have been funded by the Cystinosis Research Foundation in 2018. This first CRF funding had allowed us to better understand the underlying mechanisms of cysteamine bone toxicity, and to demonstrate intrinsic osteoclastic defect in cystinosis. We have recently achieved an overlapping 2018/2020 study on genotype /phenotype correlation in osteoclasts from NC patients [6].

**The Cysteabone 2020 project is focused on a cell based model to:**

**Aim 1: fully dissect the mechanisms of the impaired cross-talk between osteoblasts and osteoclasts observed in the murine model, with and without cysteamine.**

**Aim 2: confirm experimentally that the IL1 pathway and inflammasome- dependent- or independent IL1 production are clinically relevant for bone complications in cystinosis.**

**Aim 3: confirm the dysregulation of the osteoblast-dependent-osteoclastogenesis in human cystinosis**, mainly by implementing the CRISPR technology on cell lines carrying the mutations representative of two classes of phenotypes we have identified.

## 2- Global Context

Since September 2020 our lab has re-opened and progressively reached somehow full activity, however we as others, have experienced enormous delays in animal house facilities, deliveries in goods and services explaining that aims that were designed in 2018 as RNAseq analysis will be only completed at the end 2021 /2022.

We have been able with to set up **a one year** Post-Doc contract (recipient : Dr Candide Alioli, PhD) on ANR fundings from national French research (coordinator Dr Olivier Peyruchaud and Dr Irma Machuca-Gayet) to boost and support the Cysteabone project as we considered it a strong line of our research. Dr Candide Alioli will apply for a 2022 post-doctoral grant.
In the meantime, we have been awarded a grant from AIRG France which allowed us to start the CRISPR project.

## 3-Results : Response to Cysteamine in Osteoclasts Obtained from Patients with Nephropathic Cystinosis: A Genotype/Phenotype Correlation

cells

MDPI

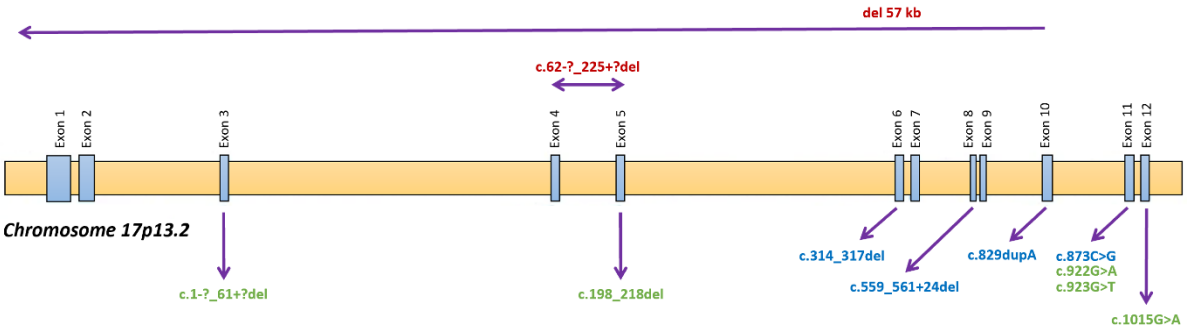
*Article*

### Response to Cysteamine in Osteoclasts Obtained from Patients with Nephropathic Cystinosis: A Genotype/Phenotype Correlation

Thomas Quinaux [1,2], Aurélia Bertholet-Thomas [1,2], Aude Servais [3], Olivia Boyer [4], Isabelle Vrillon [5], Julien Hogan [6], Sandrine Lemoine [7,8], Ségolène Gaillard [9], Candide Alioli [2], Sophie Vasseur [10], Cécile Acquaviva [10], Olivier Peyruchaud [2], Irma Machuca-Gayet [2,†] and Justine Bacchetta [1,2,8,*,†]

The main objectives of the study were to determine in vitro the impact of CTNS mutations and cysteamine therapy on human osteoclasts and to carry out a genotype-phenotype analysis related to osteoclastic differentiation. Human osteoclasts were differentiated from peripheral blood mononuclear cells (PBMCs), and were treated with increasing doses of cysteamine (0, 50, 200 µM) and then assessed for osteoclastic differentiation. A total of 17 patients (mainly pediatric) were included, at a median age of 14 (2-61) years, and an eGFR of 64 (23-149) ml/min/1.73m². Most patients (71%) were under conservative kidney management (CKM). There were no dialysis patients, the others were kidney transplant recipients. Three functional groups were distinguished for CTNS mutations : 1/ cystinosin variant with residual cystin efflux activity (RA, residual activity), 2/ inactive cystinosin variant (IP, inactive protein), 3/ absent protein (AP).

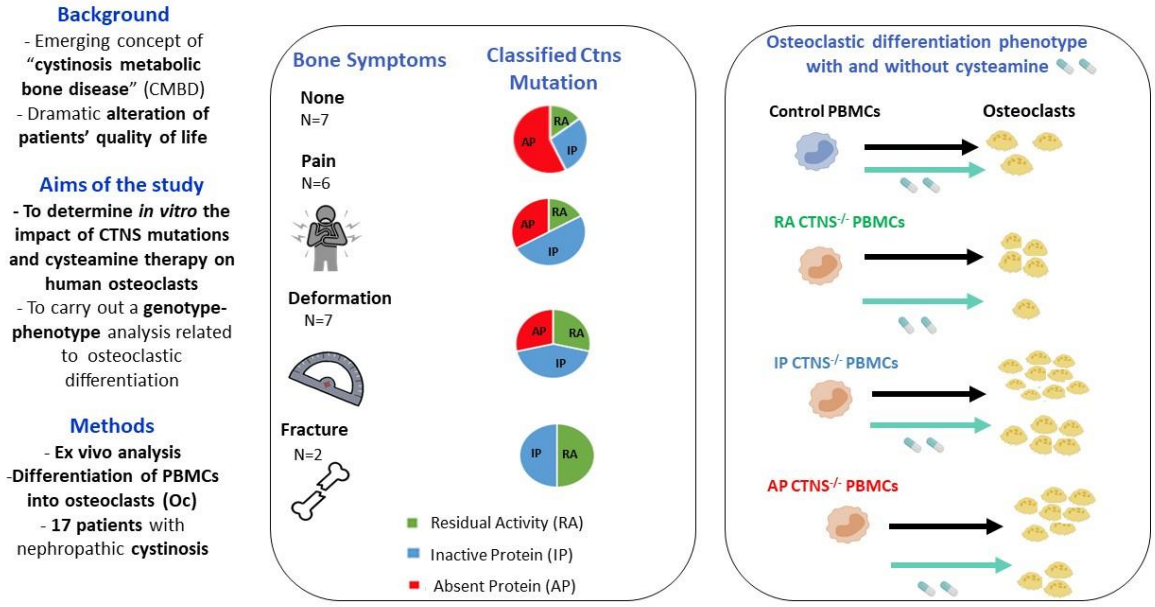
We performed a genotype/phenotype analysis, the endpoint being the osteoclastic differentiation of the patients' monocytic progenitors (PBMCs) according to the underlying genotype, as illustrated in the figure below.




***Schematic illustration of the CTNS gene with genomic localization of mutations in our cohort.***
*Exonic mutations displayed in the lower part, and large deletions in the upper area.*

*Green: Residual activity (RA); Blue: Inactive protein (IP); Red: Absent protein (AP)*

PBMCs from patients with residual cystinosin activity generate significantly less osteoclasts than those obtained from patients of the other groups. In all groups, cysteamine exerts an inhibitory effect on osteoclastic differentiation at high doses. This study highlights a link between genotype and osteoclastic differentiation, as well as a significant impact of cysteamine therapy on this process in humans, results are summarized in the graphical abstract below and published very recently [6].




***Thus, this genotype/phenotype analysis had helped to delineate the group of mutations that are prone to generate more osteoclasts and have been used to the design the human osteoblast CRISPR strategy developed in Aim 3.***

## 4- Research in progress

For reasons of confidentiality, we only present here the outline of our current work. Unpublished detailed results have been presented to the scientific committee of the Cystinosis Research Foundation in the scientific report.

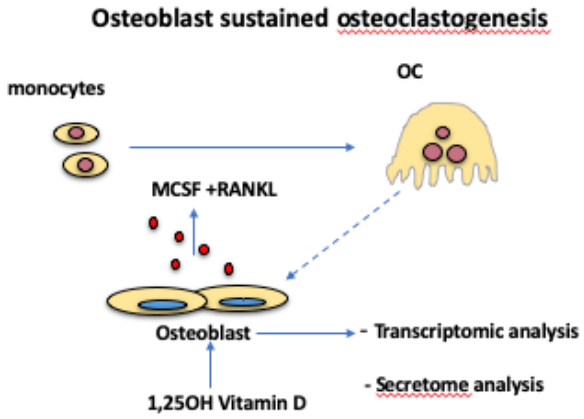
### Aim1: to fully dissect the mechanisms of the impaired cross-talk between osteoblasts and osteoclasts observed in the murine model, with and without cysteamine

Our purpose of studying the cross-talk between osteoblasts and osteoclasts was based on the ground of bone physiology knowledge that osteoblasts support osteoclastogenesis by the production of pro-osteoclastic factors induced by 1,25-OH vitamin D3. Among these factors, cytokines as M-CSF (macrophage stimulating factor) and RANKL (receptor activator of nuclear factor kappa-B ligand) that are essential to form multinucleated osteoclasts derived from the fusion monocytes. In addition OPG (Osteoprotegerin) that is RANKL decoy receptor (i.e., blocking RANKL), is down regulated by 1,25-OH Vitamin D3 treatment leading to an increased RANKL/OPG ratio therefore favoring osteoclastic formation [7]. The facts that in our

previous work we found that 1/ PBMC from NC patient form more small osteoclasts than healthy controls [8], 2/ despite cysteamine treatment, fractures, deformities, bone pain and cortical impairment were frequent in patients from the clinical Cysti-Bone study [9], and 3/ unusual localized patches of osteoclasts were found in pediatric bones from NC patients, as illustrated in the figure below [5], led us to examine Ob/Oc cross-talk in cystinosis context.

Bone biopsy at the tibia, Goldner's trichrome: bone tissue with a partly fibrotic bone marrow (white star) and unusual resorption areas, with many multinucleated osteoclasts (red arrow), from [5].

Here we did not intend to compare murine Ctns$^{-/-}$ Ob versus WT Ob, these analysis having already been performed [10]. Our interest relies on the response to 1,25-OH Vitamin D3 treament of Osteoblasts derived from Ctns-/- mice compared to WT Ob response. It appears to us much more relevant to compare these two conditions in the light of our clinical observations and in vitro work on PBMCs from NC patients under conservative management that are indeed treated with cysteamine but also with regular doses of Vitamin D3 (either native or active or both).




**Co-cultures to explore Ob/Oc cross talk : increased osteoclastogenesis supported by VD3 treated Ctns$^{-/-}$**

We evaluated in a mouse model the ability of wild-type and Ctns -/- osteoblasts to support osteoclast differentiation of bone marrow-derived monocytes from wild-type mice, as illustrated in the Figure above. To do this, as a read out, we have counted the TRAP-positive cells obtained after 5 to 7 days of co-culture in an osteogenic medium containing L-ascorbic acid and 1,25-hydroxyvitamin D3.

**Expression of Oc differentiation pivotal cytokine: RANK-L/OPG ratio**

**Aim 2: to confirm experimentally that the IL1 pathway and inflammasome- dependent- or independent IL1 production are clinically relevant for bone complications in cystinosis**

**Pro inflammatory context in both mouse models and patients**

**Proteome Profiler Array**

Target proteins identified by this method and strongly induced are growth factors, or enzymes of cytokines/chemokine proteolytic pathways showing an increase of 3 to 5 fold. Then cytokines or chemokines found in response to inflammatory process were evaluated, as well as specific cytokine as M-CSF or factor OPN (osteopontin) involved in pro-osteoclasic differentiation. These results also support our previous results.

**Ctns$^{-/-}$ secretome pro-Osteoclastic assay: Osteoblast sustained osteoclastogenesis**

To further confirm that VD3 Ctns$^{-/-}$ Ob secretome is more efficient than the WT one to generate osteoclast (similarly to what we observed in co-culture experiments), we conducted osteoclast differentiation assays in presence of osteoblast 8 x concentrated conditioned media (CM).

**Interleukin Receptor expression in Cystinosis patients**

**Transcriptome analysis of VD3 Ctns $^{-/-}$ Ob versus VD3 WT Ob**

**Aim 3: to confirm the dysregulation of the osteoblast-dependent-osteoclastogenesis in human cystinosis, mainly by implementing the CRISPR technology on cell lines carrying the mutations representative of two classes of phenotypes we have identified.**

**Human Cell based model to study the Ob/Oc cross talk: CRISPR edited CTNS Osteoblast.** For obvious ethical reasons, bone marrow mesenchymal stem cells from patients to generate osteoblasts, the bone forming cells, are not available. Therefore, we took advantage of the CRISPR technology to generate two human osteoblastic lines carrying two different mutations among the ones we identified [6]. We chose to edit Saos-2 human osteosarcoma cell line which express Vitamin D3 receptor, PTH receptor 1 and high levels of alkaline phosphatase activity. These cells showed are able to differentiate into osteoblasts producing a mineral matrix [11].

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