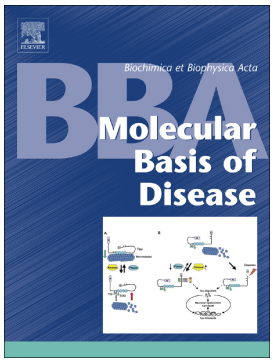
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Marie-Laure Jourdain, Johan Sergheraert, Julien Braux, Christine Guillaume, Sophie C. Gangloff, Dominique Hubert, Frédéric Velard, Jacky Jacquot

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Osteoclastogenesis and sphingosine-1-phosphate secretion from human osteoclast precursor monocytes are modulated by the cystic fibrosis transmembrane conductance regulator

Running title: Defective differentiation of osteoclasts in cystic fibrosis patients

Marie-Laure Jourdain[a,b,1], Johan Sergheraert[a,b,1], Julien Braux[a,b], Christine Guillaume[a], Sophie C. Gangloff[a], Dominique Hubert[c], Frédéric Velard[a,*], Jacky Jacquot[a,*]

[a] *Université de Reims Champagne* Ardenne, *BIOS EA 4691 «Biomatériaux et Inflammation en site osseux», 51097 Reims, France*

[b] *CHU de Reims, Pôle de Médecine Bucco-Dentaire, 51100 Reims, France*

[c] *Service de Pneumologie, Adult Cystic Fibrosis Centre, Hôpital Cochin, 75012, Paris, France*

*Corresponding authors at: Université de Reims Champagne Ardenne, EA 4691, Biomatériaux et Inflammation en site osseux (BIOS), SFR CAP-Santé (FED 4231),
1, Avenue du Maréchal Juin, 51095, Reims, France.
E-mail address: jacky.jacquot@inserm.fr or frederic.velard@univ-reims.fr
Phone: (33) 326918291

[1]Both authors contributed equally to this study.

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ABSTRACT

Osteopenia and increased fracture rates are well-recognized in patients with cystic fibrosis (CF) disease. In CF pathology, F508del is the most common CFTR mutation, with more than 85% of patients carrying it on at least one allele. The underlying molecular defect in CFTR caused by the F508del-CFTR mutation in osteoclastogenesis, i.e, on the generation and bone-resorption activity of osteoclasts (OCs) from peripheral blood-derived monocytes (PBMCs) remained unexplored. We therefore investigated whether the F508del mutation could affect the osteoclastogenic capacity of PBMCs collected from 15 adult patients bearing the F508del-CFTR mutation, to modulate their bone-resorptive abilities and the level of sphingosine-1-phosphate (S1P) produced by OCs, a key factor in the bone mineral density and formation. In the present study, a severe, defective differentiation of CF-F508del PBMCs to CF-F508del OCs without any significant difference in nuclei number per OC was found compared to non-CF healthy PBMCs from 13 subjects after 7-14-days culture periods. We observed a reduced number of formed non-CF healthy OCs in the presence of a selective inhibitor of CFTR chloride conductance (CFTR-Inh_{172}). Our data regarding OCs resorptive capabilites revealed that a loss of CFTR chloride activity in OCs led to a marked reduction in their trench-resorption mode. A 7-fold increase of the S1P release by CF-F508del OCs was found compared to non-CF healthy OCs after a 21-days culture period. We hypothesize that defective maturation of F508del-OCs precursor monocytes associated with high S1P production in the bone environment might contribute to low bone mineral density observed in the CF population.

1. Introduction

The F508del mutation within the gene encoding the chloride ion channel CFTR (Cystic Fibrosis Transmembrane conductance Regulator) results in cystic fibrosis (CF), the most common lethal autosomal recessive genetic disease that causes a number of long-term health problems [1, 2]. With the increasing life expectancy due to improvements in the treatment of pulmonary and gastro-intestinal disorders of the disease, other chronic non-pulmonary complications and comorbidities of CF have become more prevalent, such as CF-related bone disease (CFBD) with 55-65% of patients older than 40 years [3]. CFBD is a serious complication that has been correlated with severity of lung disease in the CF population [4-6]. Individuals with CF have been documented to have low bone mineral density and increased fracture rate as early as adolescence, leading to excessive kyphosis which compromises the thoracic skeletal architecture, with the consequence of an accelerated decline in lung function, ineffective cough and airway clearance [7, 8]. This bone damage is due to the combination of a low peak bone mass in prepubertal children and adolescents, and a subsequent premature bone loss during adulthood [9]. A recent study reported significant deficits in bone structure and strength that persist in young adults with CF, despite advances in care that permit them to attain relatively normal height and weight [10]. CFBD is characterized by low bone density and mass, particularly in ribs and vertebrae [11] which might be linked to an uncoupling between the bone

formation and resorption processes. The cause of this uncoupling of bone turnover has not been fully elucidated and raises the intriguing possibility that CFBD development and progression may be in part due to basic CFTR dysfunction itself [9, 12-14]. There are currently no available therapeutics to restore the bone density and mass in patients with CF. Therefore novel sources of investigation on precursors of bone-resorptive cells are becoming crucial to understand the alterations at the root of CFBD initiation.

Under physiological conditions, bone remodelling process, beginning with bone resorption by mature osteoclasts (OCs, cells derived from hematopoietic stem cells of monocytes/macrophage lineage) is directly followed by bone formation by osteoblasts (cells originated from multipotent stromal stem cells) – a phenomenon known as coupling, which ensures that the bone structure is preserved [15]. In this process, OCs control osteoblast recruitment and differentiation, through the secretion of an array of bone resorption-derived cytokines and growth factors, to start forming new bone, constituting the basis of the bone remodelling sequence [16]. Among cytokines and chemokines known as critical bioactive mediators of bone metabolism, recent insights highlight the decisive and multifaceted roles of sphingosine-1-phosphate (S1P) produced by OCs, a bioactive lysophospholipid mediator in stimulating osteo-anabolic activity [17-19] through the OCs precursor monocyte mobilization between blood and bone, and OCs-osteoblast crosstalk [15, 20-22].

However, very little data are available on the relationship between human bone health, disease and S1P; a study in postmenopausal Korean women has found a negative association between circulating S1P and bone mineral density values at some femur sites [23]. Recently, clinical data has reported that bone stiffness was inversely related to serum S1P level, and that high S1P level served as a counter-regulatory measure to increase bone formation in response to decreasing bone quality [19]. Human OCs precursor monocytes have detectable levels of functional CFTR [24, 25], and CFTR is expressed in OCs on human bone surface [26]. Interestingly, reports have demonstrated that CFTR channel is involved in cellular uptake of S1P in a mouse model of heart failure [27, 28]. Secondary to resorption, bone formation is initiated, which could indicate that some aspects of OCs bone-resorption function are important for the initiation of bone formation [29]. Compelling evidence for this is the fact that, in the normal adult skeleton, bone formation is almost exclusively initiated in areas having undergone resorption [15]. Consequently, the generation and bone-resorption activity of OCs may be crucial in determining outcomes during bone growth and remodelling.

In CF pathology, F508del is the most common CFTR mutation, with more than 85% of patients carrying it on at least one allele [30]. Several studies, including our own, have showed that F508del mutation in *Cftr* impacts bone formation in humans [14] and mice [31], and leads to elevated RANKL production and membranous RANKL expression in human osteoblasts [32]. However, the underlying molecular defect in CFTR caused by the F508del-CFTR mutation in osteoclastogenesis, i.e, on the generation and bone-resorption activity of OCs from monocytes is yet unknown. We therefore investigated whether the F508del mutation could affect the osteoclastogenic capacity of peripheral blood mononuclear cells (PBMCs) collected from patients bearing the F508del-CFTR mutation, to modulate their bone-resorptive abilities and the level of S1P secretion by OCs.

2. Patients

2.1 Patient characteristics

Fifteen CF-F508del patients with one or two F508del alleles [mean age, 42.6 (29 to 58)], followed by the Adult Cystic Fibrosis Centre at the Cochin Hospital, Paris, France, were invited to participate in this study. They exhibited neither infection nor inflammatory exacerbation in the past three months before the blood collection and their serum concentration of pro-inflammatory cytokines (i.e. IL-8 and TNF-α) analyzed by ELISA were not significantly different to that of non-CF donors (data not shown). Thirteen healthy volunteer donors [mean age 53.2 years (32 to 69)] of the "Etablissement Français du Sang Grand Est" (Authorization ALC/PIL/DIR/AJR/FO/606 Reims, France) were included as a non-CF donor control group. This study was approved by the Local Ethics Committee on Human Research (ClinicalTrials.gov ID: NCT03492567). All participants gave written informed consent in accordance with the Declaration of Helsinki before entering the study.

3. Materials and Methods

3.1. Reagents and chemicals

RPMI Medium 1640 Glutamax™, Penicillin/Streptomycin 10000 U/mL (P/S), Phosphate buffer Saline without Ca/Mg (PBS), PhalloidinAlexaFluor488® (Invitrogen), 4',6-diamidino-2-phénylindole (DAPI), Accutase™ were purchased from Life Technologies (Courtaboeuf, France). Fetal Bovine Serum Charcoal Stripped, paraformaldehyde (PFA), Bovine serum albumin (BSA), Triton X 100, ethylenediaminetetraacetic acid (EDTA) were from (Sigma Aldrich, Saint-Quentin Fallavier, France). Polymorphprep™ was bought at Proteogenix (Schiltigheim, France). Recombinant human RANK-L and M-CSF were from Merck Millipore (Molsheim, France). Glass cover slips (Deckglaser, 12 mm of diameter) were purchased from Dominique Dutscher (Brumath, France). Dentine matrix comes from ImmunoDiagnostic Systems (Pouilly en auxois, France). Fluorescent mounting medium was purchased from Dako (Les Ullis, France). Plastics were from BD Biosciences (Le Pont de Claix, France).

3.2. Isolation of PBMCs and generation of OCs

Venous whole blood (12 mL) was drawn from each CF patient and healthy volunteers by venipuncture and stored in EDTA tubes (K2E, Becton Dickinson, Le Pont de Claix, France) for further processing. PBMCs were purified using Polymorphprep™ density gradient according to our optimized differentiation protocol recently published [33]. Cells were then frozen in a mechanical -152°C freezer, in fetal bovine charcoal-stripped serum containing 5% DMSO until they were used.

PBMCs were thawed, then seeded on glass cover slips (12 mm of diameter) into 24-well plates. A low seeding density of 1.5×10^6 cells per well was used for PBMCs. They were cultured in RPMI medium

1640 Glutamax™, supplemented with 10% of fetal bovine charcoal-stripped serum (v/v), 25 ng.ml^{-1} of recombinant human M-CSF and 50 ng. ml^{-1} of recombinant human RANK-L for 7-14 and 21 days at 37°C, in humidified atmosphere containing 5% CO_2 over time. The medium was removed and replaced with fresh medium each 48-72 hours. At the end of 7, 14 and 21 days-periods, supernatants were removed and cells were fixed with 4% paraformaldehyde (v/v in PBS without Ca^{2+} and Mg^{2+}) for 10 minutes at 37°C, then permeabilized with Triton X100 0.5% (v/v in PBS without Ca^{2+} and Mg^{2+}) for 15 minutes at room temperature. After two washes of 5 minutes in PBS (without Ca^{2+} and Mg^{2+}), actin cytoskeleton was stained with AlexaFluor®488-conjugated Phalloidin (1/100 v:v) diluted into BSA 0.5% (v:v in PBS without Ca^{2+} and Mg^{2+}) for 30 minutes at room temperature protected from light. Cells were then washed with PBS without Ca^{2+} and Mg^{2+} two times for 5 min. The nuclei were labeled with DAPI 1/3000 (v:v in distilled water) for 5 minutes at room temperature protected from light, then washed two times with distilled water. Cells were visualized by fluorescent microscopy. Each analysis was performed on 5 randomly chosen microscope fields (x20 magnification, Zeiss Axiovert 200M, Axiovision Software, Zeiss, Marly-le-Roi, France). Images were processed with ImageJ software (v1.50i, NIH, USA). For the counting of multinucleated OCs and their nuclei, cell counter plugin was used. Actin ring positive cells bearing 3 or more nuclei were considered to be OCs. For the area measurement, we used freehand selection tool. For nuclei count and area measurements, at least 7 OCs were analyzed per condition.

3.3. OCs resorption assay

In order to evidence OCs resorption activity, we transferred the cells after 14 days of differentiation onto dentin matrix. The latter was previously incubated in serum-free RPMI medium 1640 Glutamax™ one hour at room temperature for rehydration purpose. Culture supernatants were discarded and cells were rinsed with PBS (without Ca^{2+} and Mg^{2+}) before being detached using Accutase™ for 30 minutes at 37°C. Detached cells were collected and rinsed in PBS (without Ca^{2+} and Mg^{2+}), centrifuged (600 g, 10 min, brake on) and then counted. Fifty thousand cells were deposited on dentin matrix for a 7-days period in differentiation medium as described above. After culture time, dentin matrix was washed with PBS two times and incubated with NaOH (1M) at room temperature for 20 minutes with 30 seconds vortex every 5 min and 10 additional minutes under ultrasonic activation. Matrix dentin was dried and kept at 37°C.

Before SEM observation, samples were sputtered with thin gold–palladium film under a JEOL ion sputter JFC 1100. The pit-and-trench resorption abilities of OCs on dentin matrix were assessed using a Schottky Field Emission Scanning Electron Microscope (JEOL JSM-7900F, JEOL, Croissy, France) to observe the formation of round holes (pits) and trenches by OCs as explained in Merrild *et al* [34]. The distinction between the trench- and pit- resorption mode was previously defined as OCs being able to resorb while migrating, thereby generating trenches which reflect long periods of resorption parallel to the bone surface.

3.4. S1P assays

The level of S1P was assessed in the 7-14-21 days-OCs culture supernatants using commercial ELISA kit (MyBiosource, San Diego, Californie, Etats-Unis).

3.5. Statistics

The significance of the biological results was assessed with a non-parametric approach owing to a lack of normal distribution of the assessed variables. Exact and stratified (when appropriate)-Wilcoxon Mann–Whitney test was used (StatXact 7.0, Cytel Inc., Cambridge, MD, USA). A value of $*p < 0.05$ was accepted as statistically significant. In all figures, red bar represents the median value, limits of the boxes represent the first and third quartile and bars represent the first and ninth decile. At least five different donors were used for each experiment.

4. Results

4.1. The F508del mutation in CFTR reduces the formed OCs' number

We first analyzed the percentage of OCs and the nuclei number/OC cell formed from CF-F508del PBMCs compared to non-CF healthy PBMCs during three 7-14 and 21 days-culture periods. Up to 20% non-CF healthy multinucleated OCs was differentiated from non-CF healthy PBMCs after the 14 days-culture period (Fig.1A). Surprisingly, a significant, defective generation of CF-F508del OCs (2% median value only) from CF-F508del PBMCs without any significant difference in nuclei number per OC was found in the 14 days-culture period compared to non-CF healthy PBMCs (Fig. 1A and B). We also observed that treatment of non-CF healthy PBMCs by a selective inhibitor of CFTR chloride conductance (CFTR-Inh_{172}, 10 µM) (35) slightly reduced the number of formed non-CF healthy OCs compared to untreated-non-CF healthy OCs in three analyzed periods (Fig.1A).

4.2. The F508del mutation in CFTR increases the OCs' size and reduces their bone-resorbing capacity

Figures 2A and B illustrate that CF-F508del OCs exhibited drastic morphological modifications with a larger surface compared to non-CF healthy OCs. Interestingly, at the 21 days-culture period (Fig. 2B), a significantly larger size was observed in both CF-F508del OCs and non-CF healthy OCs when generated in the presence of CFTR-Inh172 compared to non-CF healthy OCs. The compiled data for the measurement of OCs' surface from CF-F508del OCs and non-CF healthy OCs, and following an inhibition by CFTR-Inh_{172} in the 7-14 and 21 days culture periods are reported in the Figure 2B.

When OCs are seeded on dentin matrix for a 7 days-period, they either drill round pits and/or dig long trenches due to their resorptive function (Fig. 2C). Using OCs prepared from CF-F508del monocytes, we found that they achieved low bone resorption mainly through a pit formation, whereas non-CF healthy OCs did so through both trenches and pits formation (Fig. 2D). To obtain evidence for

a role of CFTR chloride activity in the trench-resorption mode, we incubated non-CF healthy OCs with 10μM CFTR-Inh$_{172}$. In that condition, inhibition of CFTR activity also led to a significant decrease of trench-resorption mode by non-CF healthy OCs, but not for the formation of pits. The compiled data for the quantification of trench and pit formation by mature CF-F508del OCs and non-CF healthy OCs, and after inhibition by CFTR-Inh$_{172}$ are reported in the Figure 2D. Our data thus show that defective CFTR chloride activity in OCs (either CF-F508del OCs or CFTR-Inh$_{172}$-treated non-CF healthy OCs) led to a marked reduction in their trench-resorption mode.

4.3. The F508del mutation in CFTR increases the level of S1P secretion of OCs

In humans, robust clinical data have shown that higher serum S1P levels were associated with lower bone mineral density [23]. We therefore evaluated the level of S1P secreted by CF-F508del OCs, non-CF healthy OCs and after inhibition by CFTR-Inh$_{172}$ in supernatants of 7-14-21-days culture periods (Fig. 3). A constant level of median value of S1P secretion (less of 20 ng/ml/10^4 cells) from non-CF healthy OCs was noted in all three analyzed periods. Interestingly, a strong and significant increase (by a 7-fold factor) of the S1P release by CF-F508del OCs was observed compared to non-CF healthy OCs at the 21 days-culture period. There was also a trend for the level of S1P release to be increased in the CFTR-Inh$_{172}$-treated non-CF healthy OCs in all three analyzed periods (Fig. 3).

5. Discussion

Osteopenia and increased fracture rates are becoming important clinical features as the absolute numbers of adults with CF increase [3, 13, 36]. We provide here the first evidence that PBMCs from patients with the F508del mutation in CFTR presented a lower capacity to generate *in vitro* multinucleated OCs and a high S1P release by CF-F508del OCs than did PBMCs from healthy controls. Furthermore, our data indicated that reduced osteoclastogenesis of CF-F508del PBMCs was associated with a larger size and lower bone-resorptive capabilities compared to non-CF healthy OCs. Of interest, despite a trend toward decreasing number of nuclei per CFTR-F508del OCs as compared to non-CF healthy OCs, we highlighted a similar nuclei number in the CF-F508del OCs, non-CF healthy OCs and CFTR-Inh$_{172}$ treated OCs, indicating that defective osteoclastogenesis and larger size may not be imputed to an altered OCs fusion process. The observed impact of the F508del mutation in CFTR on the reduced osteoclastogenesis is in agreement with previous reports, including from our laboratory, showing that F508del mouse model develops a severe osteopenic phenotype early on with a reduced multinucleated OCs number lining the femoral trabecular bone compared to wild-type mouse [31, 37]. Interestingly, clinical data have reported that a low bone turnover with reduced bone formation and no evidence for increased bone resorption is detectable in young adults with CF [5, 14, 38].

To gain more insights into the role of CFTR on the bone resorption, we then undertook experiments on the trench- and pit- resorption modes of OCs with or without a defective CFTR function. Our data

regarding OCs resorptive activity revealed that F508del mutation in CFTR led to a marked reduction in their trench-resorption mode. In CFTR-Inh_{172}-treated non-CF healthy OCs, we also noted a significant decrease of this trench resorption mode which suggest that this bone-resorptive activity is clearly dependent of the CFTR channel chloride activity. Deciphering if this is indicative of a defective cathepsin K production, leading to impaired collagenolysis during bone remodeling process [34, 39], a reduced motility abilities of OCs precursor monocytes exhibiting defective CFTR function [24], or both the processes remain to be investigated.

Thus, we can hypothesize that an intrinsic defect in CF-F508del OCs precursor monocytes might result in low bone-resorptive capabilities and therefore, an altered communication with osteoblasts, the cells that form bone, thereby leading to reduced bone formation in F508del-CFTR bearing patients with cystic fibrosis. It is a likely possibility that low peak bone mass often described in prepubertal children adolescents with F508del mutation [7, 9] may be caused by a defective differentiation of OCs precursor monocytes and low bone-resorptive activity, leading to a delayed initiation of bone formation process.

Increasing evidence also highlights the local actions of lipids in bone physiology [22, 40]. Studies have reported that CFTR dysfunction causes abnormalities in sphingolipid metabolism [41, 42]. In the present study, we demonstrated a marked increase of S1P level in supernatants of CF-F508del OCs compared to healthy control OCs. S1P, produced by phosphorylation of sphingosine catalysed by two sphingosine kinases (SPHK1 and SPHK2) is a well-known bioactive lipid mediator, playing important roles in many tissue repair processes, including bone regeneration and osseous tissue growth *in vivo* [19, 43]. The dysfunction of CFTR alters immune cell responses, and CFTR channel has been shown to be involved in S1P uptake in a mouse model of heart failure [27, 28, 44]. A reduced CFTR functional activity might lead to a reduced cell uptake of S1P in the CFBD context. It is tempting to speculate that CFTR could be involved in the S1P uptake in bone cells (may be deficient in F508del-OCs), which would explain some of the aberrant activities observed in CFTR-deficient OCs. In the present study, we observed a marked variability in the level of OCs differentiation and size, dentin resorption capacities and S1P release of CF-F508del OCs according to the analyzed CF patient. The causes of these variability remain to be defined, but several factors may be involved, including the presence of modifier genes [45], the level of residual CFTR function in CF cells as well as environmental factors.

That defective maturation of F508del-OCs precursor monocytes associated with elevated extracellular S1P level in the bone environment might contribute to lower bone mineral density observed in F508del-CFTR bearing patients with CF and to CFBD, later in life. In humans, clinical data have shown that higher serum S1P levels were associated with lower bone mineral density [23]. Further analysis of circulating S1P level, function of F508del-OCs precursor monocytes in patients with CF, and the association between reduced osteoclastogenesis and CFBD is needed to investigate the critical regulator role of CFTR/S1P axis in OCs-osteoblast crosstalk, and may lead to new therapeutics design in the CFBD.

New treatments that target the F508del mutation through the use of correctors and potentiators of chloride channels (see www. cff.org/Trials/Pipeline) are being developed in the care of cystic fibrosis-

related lung pathology [46-48]. We have recently reported that the CFTR potentiator ivacaftor also improved bone mineral density in G551D-bearing CF patients and supported the link between CFTR and osteoblasts and bone-resorbing osteoclasts [49]. The effect of CFTR correctors/potentiators on both the CFTR chloride channel function and maturation-related genes of blood monocytes collected from CF-F508del patients and their S1P level, and consequently their effects on the new bone formation in patients with cystic fibrosis needs to be investigated for treatment of CFBD.

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CRediT authorship contribution statement

Study concept: DH, FV, and JJ; study design: M-LJ, DH, SCG, FV, and JJ; data acquisition: M-LJ, JS, CG, SA, DH, FV, and JJ; analysis and data interpretation: M-LJ, JS, JB, FB, SA, DH, FV, and JJ; manuscript writing: ML-J, FV, and JJ.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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Figure Legends

Fig. 1. The F508del mutation in *CFTR* reduces the OCs' number but not the nuclei number/OC cell. (A) Percentage of multinucleated OCs per field and (B) the nuclei number/OC cell formed from CF-508del PBMCs, non-CF healthy PBMCs and after inhibition by CFTR-Inh_{172} in the 7, 14 and 21 days culture periods. All OCs present on five randomly chosen microscope fields were measured (1 to 95 cells per donor depending on condition, n = 5 to 8 non-CF healthy donors and 11 to 15 independent CF-F508del patients). Red bar shows median value, lower and upper limits of each box show first and third quartile, respectively and lower and upper black bars represent first and ninth decile, respectively. * means $p < 0.05$.

Fig. 2. The F508del mutation in *CFTR* increases the OCs' surface and reduces their bone-resorbing capacity. (A) Representative fluorescence images of multinucleated mature OCs differentiated from CF-F508del PBMCs, non-CF healthy PBMCs and CFTR-Inh_{172} treated non-CF healthy OCs. Actin cytoskeleton was stained with AlexaFluor®488-conjugated Phalloidin (green) and nuclei with DAPI (blue). (B) Measurement of OCs' surface of CF-F508del OCs, non-CF healthy OCs, and after inhibition by CFTR-Inh_{172} in the 7, 14 and 21 days culture periods. All OCs present on 5 randomly chosen microscope fields were measured (4 to 300 cells per donor depending on condition – objective x20, Zeiss Axiovert 200M, Axiovision Software, Zeiss, Marly-le-Roi, France, scale bar = 50 µm). n = 5 to 8 non-CF healthy donors and 11 to 15 independent CF-F508del patients. (C) Representative scanning electron microscopy images of round pits (i.e. empty arrow) and/or dug long trenches (i.e. white arrow) on dentin matrix (a bone-like substrat) by mature CF-F508del OCs, non-CF healthy OCs, and after inhibition by CFTR-Inh_{172}. Scale bar = 100 µm. (D) Measurements of pit and trench surfaces formed by CF-F508del OCs, non-CF healthy OCs, and after inhibition by CFTR-Inh_{172} (n = 9 non-CF healthy and 6 independent CF-F508del donors). In all graphs, red bar shows median value, lower and upper limits of each box show first and third quartile, respectively and lower and upper black bars represent first and ninth decile, respectively. * means $p < 0.05$.

Fig. 3. The F508del mutation in *CFTR* increases the S1P release. S1P amounts released in 7-14-21-days culture supernatants of CF-F508del OCs, non-CF healthy OCs and after inhibition by CFTR-Inh_{172} (n = 6 to 8 for non-CF healthy donors and n = 11 for CF-F508del patients). Red bar shows median value, lower and upper limits of each box show first and third quartile, respectively and lower and upper black bars represent first and ninth decile, respectively. * means $p < 0.05$.

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Highlights

1) A severe, defective differentiation of CF-F508del PBMCs to CF-F508del (OCs) osteoclasts

2) A loss of CFTR chloride activity in OCs led to a marked reduction in their trench-resorption mode

3) A 7-fold increase of the S1P release by CF-F508del OCs was found compared to non-CF healthy OCs

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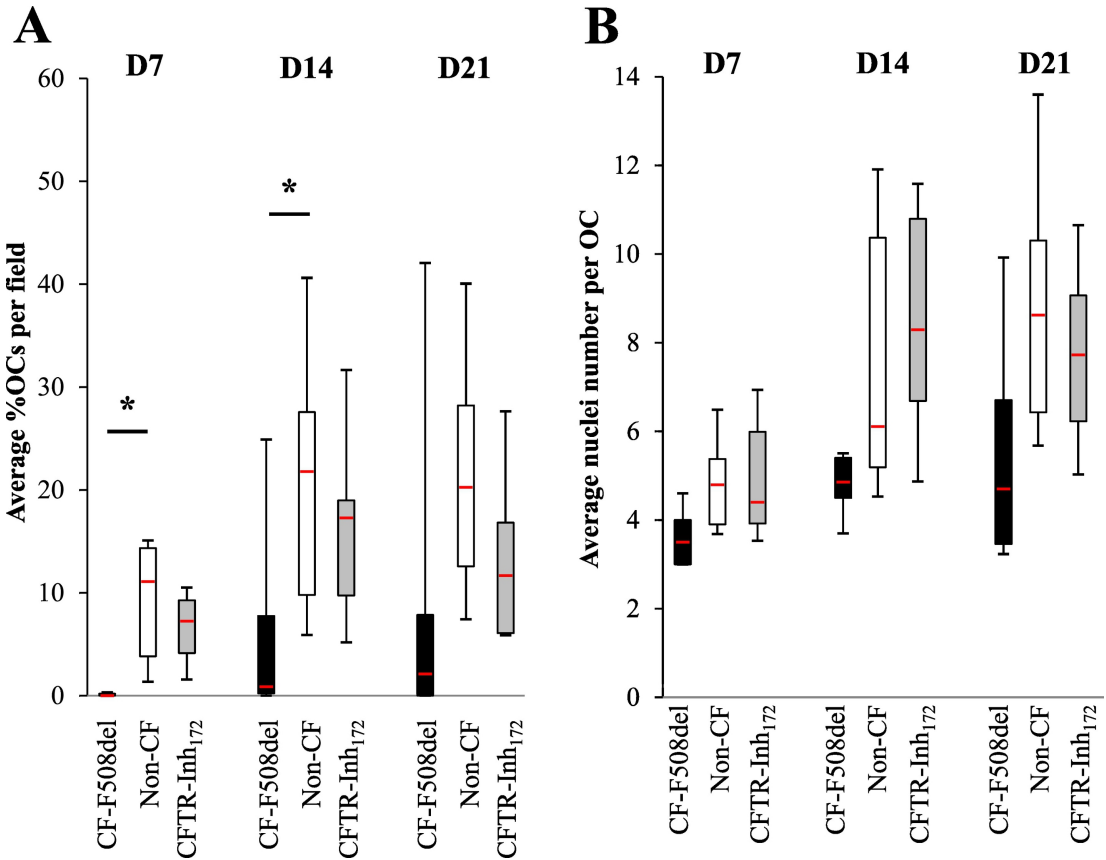

A
D7
D14
D21
Average %OCs per field
60
50
40
30
20
10
0
*
*
CF-F508del
Non-CF
CFTR-Inh172
B
D7
D14
D21
Average nuclei number per OC
14
12
10
8
6
4
2
0
CF-F508del
Non-CF
CFTR-Inh172

Figure 1

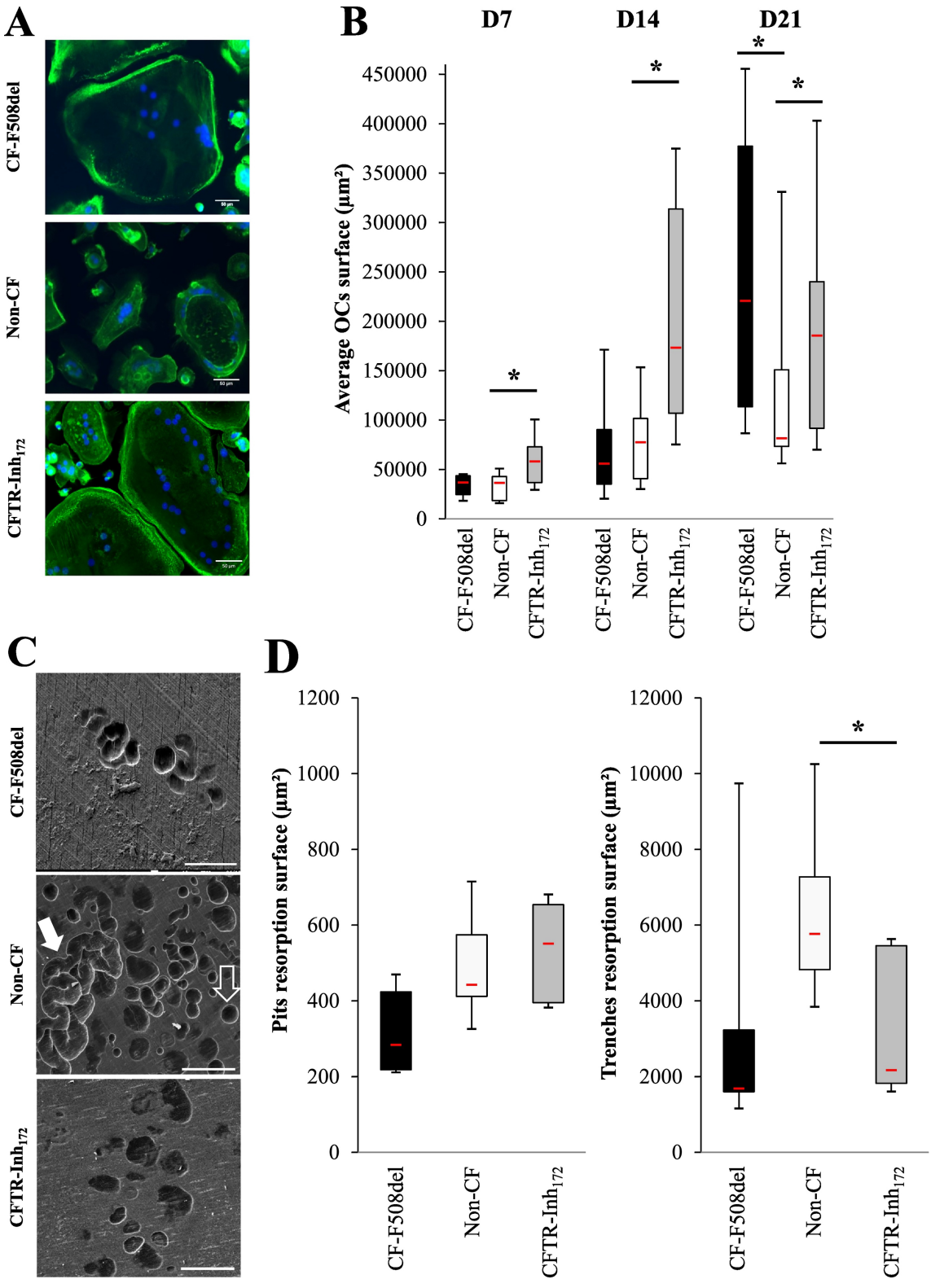

A
CF-F508del
Non-CF
CFTR-Inh172
B
D7
D14
D21
Average OCs surface (µm²)
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
*
CF-F508del
Non-CF
CFTR-Inh172
C
CF-F508del
Non-CF
CFTR-Inh172
D
Pits resorption surface (µm²)
1200
1000
800
600
400
200
0
Trenches resorption surface (µm²)
12000
10000
8000
6000
4000
2000
0
CF-F508del
Non-CF
CFTR-Inh172

Figure 2

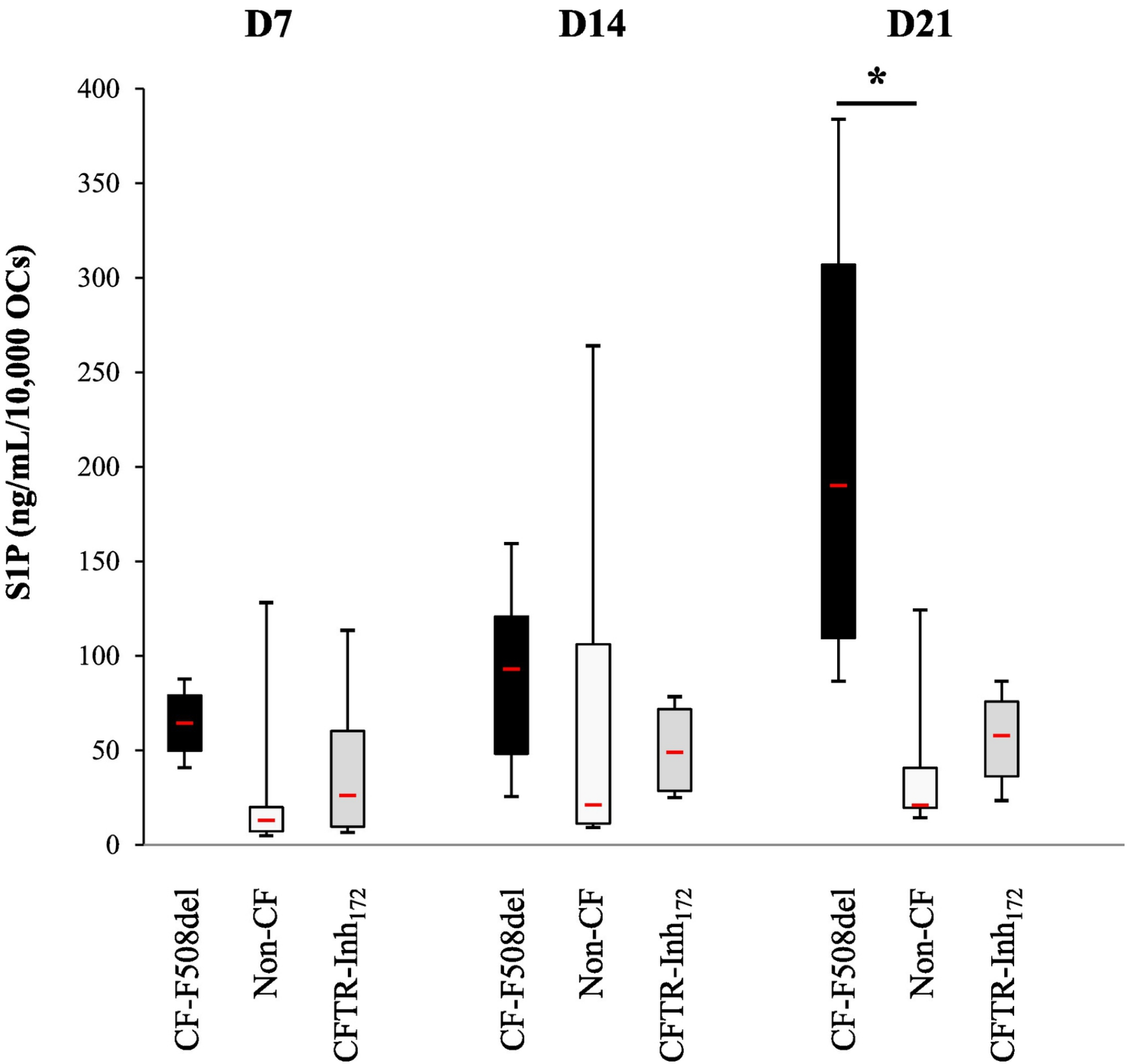

D7
D14
D21
*
S1P (ng/mL/10,000 OCs)
400
350
300
250
200
150
100
50
0
CF-F508del
Non-CF
CFTR-Inh172
CF-F508del
Non-CF
CFTR-Inh172
CF-F508del
Non-CF
CFTR-Inh172

Figure 3