non-mitochondrial respiration is similar in the two subject groups. ECAR is not significantly different between the two groups.
Conclusion: Purified PBMCs in CF show sub-optimal stimulated oxygen consumption compared with controls. This has implications for cell energetics in organ systems as well as for the inflammatory response.

37

Trypsin-like protease activity predicts disease severity and patient mortality in adults with cystic fibrosis

J. Reihill[1], K.L. Moffitt[1], A. Jones[2], J.S. Elborn[3], L. Martin[1]. [1]*Queen's University Belfast, School of Pharmacy, Belfast, United Kingdom;* [2]*Manchester Adult Cystic Fibrosis Centre, Manchester, United Kingdom;* [3]*Queen's University Belfast, Centre for Experimental Medicine, Belfast, United Kingdom*

Background: Serine trypsin-like (TL) proteases, which are excessively active in CF airways, promote activation of the epithelial sodium channel (ENaC) and airways dehydration; a key initiating factor for CF lung disease pathogenesis. Furthermore TL- proteases enhance mucin gene expression and mucus hypersecretion, yet whether there is any relationship between the activity of these enzymes and CF pulmonary disease is unknown.
Objective: To determine whether TL-protease activity, measured in adult CF sputum sol, correlates with lung disease and patient outcome (survival).
Methods: In this cross-sectional, retrospective study we analysed CF sputum sol collected from 30 clinically stable adult CF patients. Protease activity was measured by monitoring the hydrolysis of a peptide-based fluorogenic substrate (QAR-AMC). Biomarkers of inflammation (IL-8 and TNF-α) were measured by ELISA. Lung function was assessed by spirometry (FEV_1). Mortality data was retrospectively obtained and time in months until death or transplantation used for subsequent survival analysis.
Results: TL-protease activity inversely correlated with lung function (FEV_1) ($r = -0.4$, $p = 0.031$) however, no relationship with IL-8 and TNF-α was observed. Kaplan-Meier analysis demonstrated significantly reduced survival for those individuals with above median TL-protease activity. Using a multivariate Cox regression analysis (adjusted for age and BMI) a significantly increased mortality hazard (HR 1.028, 95% CI 1.007–1.049; $p = 0.009$) was also identified.
Conclusions: TL-protease activity inversely correlates with lung function and patient survival. As such tryptic activity may warrant consideration when modelling CF survivorship and should be investigated further as a biomarker of CF lung disease and as a potential therapeutic target.

38

Relationship between planar cell polarity protein network signaling and airway remodeling in CF

S. Noel[1], N. Panin[1], M. Beka[1], A. Collin[2], S. Gohy[2], M.A. Mall[3], T. Leal[1]. [1]*Université Catholique de Louvain, Louvain Centre for Toxicology and Applied Pharmacology, Brussels, Belgium;* [2]*Université Catholique de Louvain, Pulmonology, ENT and Dermatology, Brussels, Belgium;* [3]*Translational Lung Research Center (TLRC), German Center for Lung Research (DZL), University of Heidelberg, Translational Pulmonology, Heidelberg, Germany*

Objectives: To understand the link between CF airway remodeling and planar cell polarity (PCP), an intracellular protein network controlling the orientation of epithelial cells in the plane of the tissue, ciliogenesis and cilia motion.
Methods: Expression of genes encoding components of PCP network, in particular the core protein CELSR3, and their regulation by TGBβ were studied *in vivo* and in mouse nasal epithelial cells in 3-D primary cultures (MNEC) from F508del-CF and *Scnn1b-tg/+* (βENaC-overexpressing; Tg/+) mice.
Results: In naïve conditions, no difference in PCP gene expression was found between CF and wild-type mouse lungs or MNECs. In bleomycin-induced lung remodeling (Huaux, Noel et al, PLoS One 2013), CELSR3 expression was more markedly decreased in CF than in wild-type cells. The effect correlated with levels of inflammatory and fibrosis markers (IL-6, TGFβ, collagen contents and TIMP-1). In Tg/+-MNECs, expression of CELSR3 and other PCP genes was significantly deregulated in comparison with wild-type cells. Cell exposure to TGFβ (15ng/ml, 6 days) decreased the expression of CELSR3 and induced epithelial to mesenchymal transition, characterized by down-regulation of epithelial markers (zonula occludens-1) and upregulation of mesenchymal markers (α-smooth muscle actin, fibronectin and vimentin). In Tg/+ cells, the effect was more marked and not fully reversed by the TGFβ-receptor type I and II inhibitor GW-788388. The inhibitor alone promoted apicobasal polarity and mucociliary differentiation (increased transepithelial electrical resistance and expression of epithelial markers, and decreased expression of mesenchymal markers) in both MNEC genotypes, with more pronounced effects in Tg/+ cells.
Conclusion: Our data support the view that PCP is deregulated in airways of CF mouse models and contributes to the pulmonary phenotype. Intrinsic and extrinsic factors impair PCP signaling, rendering CF airway epithelial cells more susceptible to tissue remodeling.

39

Is sphingosine 1-phosphate pathway involved in bone disease development in cystic fibrosis?

M.-L. Jourdain[1], D.M. Abdallah[1], C. Guillaume[1], F. Le Pimpec-Barthes[2], S.C. Gangloff[1], I. Sermet-Gaudelus[3], J. Jacquot[1], F. Velard[1]. [1]*Université de Reims Champagne-Ardenne, EA 4691 BIOS, Reims, France;* [2]*Hôpital Européen Georges Pompidou, Département de Chirurgie Thoracique, Paris, France;* [3]*Hôpital Necker, Unité de Pneumo-Pédiatrie Allergologie, Inserm U1551, Paris, France*

Objectives: The increasing life expectancy of patients with cystic fibrosis (CF) has been associated with the emergence of co-morbidities such as CF-related bone disease (CFBD). F508del mutation in Cftr gene induced a deficit of osteoblastic maturation in F508del osteoblasts (Velard et al, 2014). Bone homeostasis involves cytokines and lipid mediators such as the prostaglandin E2 (PGE2) and the sphingosine 1-phosphate (S1P). We have showed that a CFTR corrector and potentiator, the C18 (Vertex) is able to restore deficient expression of COX-2/PGE2 and reduce the RANKL production in CF cells (Velard et al, 2015; Delion et al, 2016). These results have opened new domains to explore actors of bone metabolism in CF patients including the S1P/S1P1-5/COX-2/PGE2/RANKL pathway (Jacquot et al, 2016).
Methods: To investigate the role of the S1P pathway in CFBD, we evaluated the involvement of defective CFTR on the mRNA expression level of SphK1, 2 and S1P1-5 receptors in primary F508del osteoblasts patients ($n = 5$) compared to primary normal osteoblasts ($n = 6$). The effect of the addition of the CFTR corrector C18 in F508del osteoblasts culture was also evaluated.
Results: Our results showed that F508del mutation in osteoblasts significantly reduced SphK2, but not SphK1 mRNA expression. Normal and F508del osteoblasts expressed the S1P1, 2, 3, 4 but rarely the S1P5 receptor (2 of 11 samples). The S1P4 receptor mRNA expression was slightly upregulated in F508del osteoblasts compared to normal osteoblasts. Addition of C18 in F508del osteoblasts restored the SphK2 mRNA expression, increased S1P2 and S1P3 mRNA expression and reduced the S1P4 mRNA expression.
Conclusion: These preliminary data encourage us to now explore the production level of SphK1&2, S1P and receptors at protein level in F508del osteoblasts compared to normal osteoblasts in the context of CFBD.
Acknowledgement: Vaincre la Mucoviscidose provided funding support (RF2016501518) and Vertex provided financial and material support.

40

QR-010 via inhalation is safe, well-tolerated, and achieves systemic concentrations in a single ascending dose study in subjects with cystic fibrosis homozygous for the F508del CFTR mutation

P. Drevinek[1], N. Pready[2], N. Lamontagne[3], S. Montgomery[3], N. Henig[3]. [1]*Charles University, Motol University Hospital, Department of Medical Microbiology, 2nd Faculty of Medicine, Prague, Czech Republic;* [2]*Cytel, London, United Kingdom;* [3]*ProQR Therapeutics, Leiden, Netherlands*

Background: QR-010 is an antisense oligonucleotide investigational product. It is designed to hybridize to CFTR mRNA at the F508del encoding site, and preclinical studies show improved CFTR activity in F508del models.
Methods: A multi-centre, randomized, double-blind, placebo-controlled, single dose escalation study evaluating the safety, tolerability, and pharmacokinetics of inhaled QR-010. Subjects were male or female 18 years or older with a diagnosis of CF measured by sweat chloride, confirmation of the CFTR gene homozygous for F508del mutation, and