expression may also be altered in the respiratory epithelium of cystic fibrosis (CF) patients, which may contribute to a dysregulated immune response, causing chronic airway inflammation and progression of CF-related lung disease. We evaluated urinary clusterin levels in CF patients and healthy controls (HC) and determined if there was a relationship between urinary clusterin and pulmonary function in CF.
Methods: A single-centre cross-sectional study of children with CF and age and sex matched healthy controls (HC). Urine was assayed for clusterin using an immunobead-based assay. Lung function testing was performed in CF subjects. Results are expressed as median [IQR].
Results: 25 CF and 25 HC subjects were included (52% male, median age 9.5 years [5.9–12.3] and 9.4 years [5.2–12.8] respectively). Urinary clusterin levels were significantly lower in CF subjects compared with HC, (2.5 ng/mL [1.0–8.1] and 7.9 ng/mL [2.6–25.1] respectively, $p = 0.011$) and also when corrected for creatinine (2.8 ng/mg Cr [1.7–5.4] and 6.7 ng/mg Cr [2.7–15.6] respectively, $p = 0.019$). There was no correlation between clusterin and FEV_1% (n = 21), however, there was a strong positive correlation between clusterin (ng/mg Cr) and LCI, (n = 5, r = 0.997, p < 0.001), suggesting that higher clusterin levels are associated with worse pulmonary function.
Conclusion: Urinary clusterin may be a potential novel biomarker of lung disease in children with CF. Further investigation to explore the potential role of extracellular (secreted) clusterin potentiating lung disease in children with CF is warranted. The overall reduction in urinary clusterin in CF compared to HC also warrants further investigation.

WS04.6
Dynamic Chest Radiography (DCR) in cystic fibrosis: initial experience

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Objectives: DCR is a novel technology that uses real-time low-dose X-ray to obtain sequential chest radiographs to assess moving structures such as the chest wall and diaphragm. It is quick to perform and carries a similar radiation dose to a PA chest X-ray. It has previously demonstrated good correlation between diaphragm motion with vital capacity (VC) and FEV_1 in non-CF healthy volunteers (Hida *et al*, EJR, 2019), and with the severity of lung disease in COPD (Hida *et al*, EJR, 2019). For the first time, we report its use in assessing pulmonary physiology in people with CF (pwCF).
Methods: 11 pwCF (age range 17–56, 6 male) with stable lung function ($ppFEV_1$ range 26–101) underwent DCR as part of their scheduled CF annual review. DCR was performed during a tidal/deep breathing manoeuvre using a dynamic imaging system (Konica Minolta®) that applies real-time sequential PA X-ray in the standing position at 15fps. Software localisation of the mid-diaphragm image was performed, and excursion (mm) and speed (mm/s) over a time of up to 20 s were recorded. Spirometry was performed on the same day.
Results: DCR was well tolerated with no adverse events during image capture. Time to perform DCR was similar to that of a standard PA chest X-ray, as was image quality. Hemi-diaphragm maximum speed and excursion during deep breathing correlated with FVC (maximum speed: right r = -0.701, p = 0.001; left r = -0.638, p = 0.003; maximum excursion: right r = -0.627, p = 0.004; left r = -0.0524, p = 0.034), with a trend towards FEV_1 with right hemi-diaphragm speed (right p = 0.061, r = -0.437, left p = 0.126, r = -0.364).
Conclusion: This imaging system is promising in clinical settings, as it provides physiological and functional information, and is quick to perform. Further work to correlate larger sample sizes with more detailed pulmonary function tests is warranted.

WS05 CFTR structure and trafficking

WS05.1
AVX-770 binding site within CFTR membrane spanning domain 2 enables ATP-independent channel activation

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Objectives: Cystic Fibrosis is an autosomal recessive genetic disease caused by mutations of the *CFTR* gene.
During these last years, modulators of CFTR channel have been developed to target some defects associated with specific mutations, affecting the folding of CFTR protein (correctors; e.g. VX809) or the opening probability of the channel (potentiators; e.g. VX-770). Our aim is to understand the mechanistic basis of VX-770 potentiation`.
Methods: We combined a structural approach including molecular dynamics simulations with site directed mutagenesis of key amino acids and functional assays.
Results: The identification of a point mutation insensitive to VX-770 led us to search for a possible binding site in the second membrane spanning domain of CFTR. Structural data currently available, combined with molecular dynamics simulations, allowed us to identify a pocket able to accommodate the potentiator.
Mutation of key amino acids was realized in order to confirm their implication in VX-770 binding.
Moreover, we identified a gain of function mutation (GOF) which allow to rescue CFTR activity in a G551D background following an ATP-independent mechanism.
Finally, the sensitivity of VX-770-resistant mutations to other CFTR potentiators was evaluated.
Conclusion: Altogether, these results give new insights into our fundamental understanding of the mode of action of potentiators and open new perspectives for CFTR-specific, structure-based drug design.

WS05.2
Role of cholesterol in the function of current cystic fibrosis medications

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Objectives: Despite their importance, the mechanisms of action of clinically approved Cystic Fibrosis (CF) drugs remain unclear. VX-809 is a corrector of CFTR, while VX-770, a CFTR potentiator, works by binding to CFTR at a protein-lipid interface. CFTR resides in regions of membrane that are highly enriched in cholesterol, yet a possible integral role of cholesterol in facilitating drug-CFTR interactions has not been specifically investigated. Accordingly, we sought to explore the role of cholesterol in:
1) the interaction of VX-770 and VX-809 with the mammalian membrane;, and
2) stabilizing and folding of hairpin constructs of the CFTR transmembrane domain.
Methods: The interaction(s) of VX-770 and VX-809 with the lipid bilayer were assayed by several biophysical techniques.

i. A terbium-based liposome disruption assay was used to determine drug effects on the membrane.
ii. To explore the effect of cholesterol on secondary structure/protein folding, circular dichroism (CD) spectroscopy was performed on WT and CF-phenotypic mutant hairpin constructs consisting of the transmembrane segments 3 and 4 of CFTR in lipid bilayers, in the presence and absence of cholesterol. CD experiments were also conducted on hairpins in the presence of VX-809.
iii. Förster Resonance Energy Transfer spectroscopy was used to explore the general role of cholesterol in membrane protein folding and stabilization.