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Editorial

Exploring the basic mechanisms in Cystic Fibrosis: Promoting data presentation and discussion at the 16th ECFS Basic Science Conference☆

1. Background

Thirty years ago, the identification and sequencing of the *CFTR* gene enabled the unraveling of the pathogenesis of cystic fibrosis and has paved the way for targeting the root cause of the disease [1–3]. This has stimulated tremendous interdisciplinary research efforts, which has led in recent years to significant progress in CF precision medicine. These efforts have improved our basic understanding in several branches of biology, going far beyond CFTR, with results from this community paving the way in other pathologies. In the CF research community, various disciplines come together, including genetics, physiology, biochemistry, molecular biology, biophysics, immunology, microbiology and pharmacology. The ECFS Basic Science Conference (BSC), which has been held every year since 2004, constitutes a key event for the CF research community, allowing deep discussions around topical subjects and fostering basic CF-related research in Europe and beyond. Despite enormous progress, important challenges remain, and the BSC is a perfect venue to identify critical issues and facilitate collaborations, especially through informal discussions between senior investigators and young scientists.

Two years ago, the 14th ECFS BSC was held in Albufeira, Portugal, 19 March-A1 April 2017, and a first Special Issue of the Journal of Cystic Fibrosis Basic Science Supplement, made CF research discussed there accessible to the broader CF community and beyond. This issue gathered articles authored by renowned experts in the field, providing summaries on the core content of the conference and on recent highlights and breakthroughs in basic and translational CF research. Given the interest raised by this initiative, a second special issue has been prepared for disseminating topical issues presented at the 16th ECFS Basic Science Conference, held in Dubrovnik, Croatia, 27–30 March 2019. The program of this conference, in line with the previous ones, was based on single session symposia, covering multiple aspects of CF research and two keynote lectures opening and closing the event. A pre-conference seminar, organized by European CF patient organizations and chaired by Bertrand Kleizen and David N. Sheppard, was devoted to a discussion around the new insights gained on the CFTR structure and function and implications for the development of modulators. The articles included in this special issue are intended to give a succinct summary of the core content of the conference and to identify potential avenues on key issues and challenges to further develop transformative therapies for all patients with CF.

2. CFTR: from genetics to structure, function, regulation and modulation

The development of CFTR modulators, culminating with the latest promising data of triple combination therapies (TCTs) [4–6] (with the approval of Vertex Trikafta by the FDA and the submission of a Marketing Approval Application of the same TCT to the EMA), has significantly changed the landscape for CF treatment. It became clear from data gathered from recent studies that most of the pathogenic CFTR variants confer numerous and complex abnormalities, highlighting the need to view CF precision therapeutics from an updated perspective [7,8]. In return, it has also been observed that modulators may have an effect on a broad range of variants and disease subcategories. Hence, it is now obvious that the vast majority of patients with CF should benefit from modulator therapy, combining drugs with different activities and targeting different sites, probably acting via allosteric interaction [9].

The article by Cutting and Sharma [10] traces the rich history of genetics and genomics applied to CF since the discovery of the *CFTR* gene thirty years ago and describes the efforts made via extensive studies to link the consequences of the numerous variants (missense, non-sense, frame shift, canonical splice site) on disease susceptibility including evaluation of CFTR function and response to modulators. Among others, it highlights that even minor improvements in CFTR function for patients with severe CF disease may lead to significant clinical changes. This review also reports on new perspectives, generated using genomic approaches, to evaluate the effect of genetic and environmental modifiers as determinants of phenotype variability. In line with this review, two other articles by Paranjapye and colleagues [11] and by Chevalier and Hinzpeter [12] illustrate how genetic variations within and outside the *CFTR* locus, revealed by the use of cutting-edge techniques of functional genomics, contribute to the variability of the disease severity. These involve *cis*-regulatory elements acting on the gene transcription level, various modifier genes, as well as complex alleles, defined by the presence of at least two variations on the same CFTR allele. These studies have the potential to result in the development of specific diagnostic tools and personalized therapies, as well as to aid the identification of novel therapeutic targets.

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Challenges remain in the therapeutic area, especially as *i)* CFTR modulators are not available for all CFTR mutations; and *ii)* modulators with greater efficacy are required to prevent disease progression. The success of the most recently evaluated Vertex drug, Trikafta, may go some way towards the goal of disease prevention, at least when people with CF are treated from a young age, but data about the long-term benefits and consequences are not yet available. Understanding the molecular basis of the impact of mutations and modulators on CFTR folding, structure and function will provide the foundation on which more efficient or alternative therapeutic approaches may be built.

A first article by Kleizen and colleagues [13] features the discussions held at the Pre-Conference Seminar, which was organized by the national patients organizations. It describes how combined efforts from structural biology and electrophysiology may allow better understanding of CFTR's structure-function relationships and the impact of disease-causing mutations and drugs. This field of research has unquestionably benefited from the formidable advances offered by cryo-electron microscopy, which recently suggested a potential binding site for ivacaftor [14]. However, work is still needed to cover the full, dynamical conformational landscape of the CFTR protein at the atomic resolution level, particularly regarding the different conformations of the active WT CFTR protein and of mutated proteins as well as addressing the comprehensive mapping of modulator-binding sites and modulator mechanisms of action.

A second article by Bose and colleagues [15] highlights the potential of single-molecule studies in understanding the effect of mutations and modulators on NBD1 co-translational folding as well as on the folding of transmembrane helical hairpins. These fundamental investigations of the folding mechanisms (also see [16,17] for recent articles published in the field) and of CFTR thermodynamics stability (also see [18] for a recent advance in the field) might lead to important mechanism-based therapeutic avenues targeting CFTR and other folding disorders. This review also clearly indicates how high-resolution single-channel recording on CFTR from different species provides insightful information to help understand critical sequence-structure-function relationships, with practical outcomes on the suitability of CF animal models for pharmacological studies using CFTR modulators. Finally, the article also sheds light on the emergence of co-potentiators, acting synergistically and restoring therapeutically relevant levels of activity to rare CF mutations.

CFTR belongs to a wide, dynamic network of interactions, called the CFTR functional landscape or CFTR social network, intervening on the synthesis, folding, stability, trafficking and function of the protein. The article by Amaral and colleagues [19], describes how genetic variations impact on the ability of CFTR to interact with this network and how this network can be modulated to rescue the defects associated with the most frequent CFTR variant (F508del). Focus on this CFTR "regulome" will generate novel important insights which will facilitate identification of novel therapeutic targets. This will be of particular relevance to patients carrying CFTR genotypes that are not responsive to CFTR-directed approaches [20], and deepen our understanding of how these interactions shape CFTR fate in the cell [21,22].

3. Beyond CFTR: the epithelial channelome

In addition to CFTR, focus has been given to other ion channels–including the epithelial Na^+channel (ENaC), Cl^-channels TMEM16A and SLC26A9 as well as the H^+/K^+-ATPase ATP12A–as they can be considered as alternative targets. Such channels may be the targets of CFTR mutation-agnostic therapeutic strategies, and potentially compensate for CFTR dysfunction by improving surface hydration and pH regulation in the airways. Numerous abstracts related to the study of these actors of the epithelial channelome were presented at the conference, either at the level of a dedicated symposium, in the poster sessions or within the ECFS Basic Science Working group chaired by Margarida Amaral and Jeff Beekman. The article by Quesada and Dutzler [23] summarizes the current knowledge acquired on the structure-function relationships of the chloride channels TMEM16A and SLC26A9, as well as synthetic anionophores, which can form lipid-soluble complexes allowing chloride and bicarbonate shuttling across membranes. The interest in such molecules as part of a therapeutic strategy in CF was recently supported by the demonstration that amphotericin, an antifungal medication that forms non-selective ion-passing pores, restores ion transport and antibacterial defenses when tested *in vitro* and in an *in vivo* animal model of the disease [24,25].

4. The airway epithelium and CF model systems

Lung pathology progression and disease severity in CF is linked to pathological remodeling of the airway epithelium. The article by Barbry and colleagues [26] gives an overview of the current knowledge on the mechanisms regulating airway epithelial cell regeneration and repair, which is a multi-step process leading to the redifferentiation of progenitor cells into all cell types contributing to the function of the normal epithelia. The article especially focuses on pulmonary ionocytes and deuterosomal cells, two recently described airway cell types identified by powerful single-cell RNA-sequencing techniques and suggested to have great relevance for CF [27–29]. The authors list an ensemble of future directions that need to be explored in order to specify the role of these cells and others in the homeostasis of normal and CF airway epithelium. They highlight the importance of the "omics" approach in understanding the mechanisms of airway epithelial repair in an integrative way.

Many efforts have been undertaken in the CF field to generate animal models lacking functional CFTR, yielding information on the mechanisms of pathogenesis, with organ-specific differences to the human CF phenotype [30]. Representative libraries of patient-specific epithelial cell models are developed for disease modeling, preclinical testing of drug response, and biobanking for future drug discovery. Cell models can be derived from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and tissue-resident stem cells ASCs (Adult Stem Cells). While the different models, detailed in several recent reviews (*e.g.* [31,32]) have been discussed at the conference, the article by de Poel and colleagues [33] focuses on the interest of ASC-based intestinal organoids, used in the Dutch Rainbow and European HIT-CF projects, enabling CF disease classification, drug development and personalized treatment optimization, considering the clinical heterogeneity among CF patients.

5. Genetic therapies for CF

While genetic therapies have faced challenges associated with gene delivery and expression persistence of CFTR over time, related to mucociliary barriers in the CF airways, novel technologies have generated new ways to address these issues [34,35]. There are numerous approaches in development, which were widely debated at the conference, in particular related to CFTR mRNA therapy and CRISPR gene editing, with the possibility of editing epithelial progenitor cells. The article by Boyd and colleagues [36] more specifically focuses on the potential of ENaC siRNA targeting and antisense oligonucleotide approaches to CF therapy, as well as those offered by plasmid-mediated therapies using viral or non-viral liposomal vectors, with initial data highlighting a favorable effect of gene therapy on the modulation of lung function. Questions remain relative to the challenges of delivery and to the cell-types which have to be targeted in the epithelium for such genetic therapies, given that the airway epithelium contains high and low-CFTR expressing cells, each playing distinct roles in the physiology and protection from infection [26,37].

6. Host-microbe and microbe-microbe interactions in CF

In 2018, the ECFS Basic Science pre-conference symposium focused on the lung and gut microbiome and the clinical implications for CF from data generated by new and innovative technologies, and the question was raised how to standardize operating procedures and guidelines for microbiome analysis [38]. In this issue, the article by Armbruster and colleagues [39] – summarizing the dedicated symposium of the 2019 BSC–brings an interesting perspective on how the host nutritional environment influences microbial pathogenesis and feeds back into host-microbe and microbe-microbe interactions, how these intervene on antimicrobial susceptibility of bacterial biofilms, and how the immune response may drive changes in microbiota composition.

7. Concluding remarks

Recent advances in basic and translational CF research have been described in the concise review articles presented in this special issue related to the 16th ECFS Basic Science Conference. Other topics also warrant mention including mucociliary transport (MCT) and mucociliary clearance (MCC) and the key role that mucus and mucins play in airways defense [40]. This topic was addressed at the Conference through the presentation by Michael Welsh, who described the *in vivo* imaging assay of MCT in newborn pigs [41]. Another topic related to mucus obstruction is that of the chronic, non-resolving inflammation, which remains a key issue for lung disease severity and progression in CF patients [42,43]. Finally, the reviews presented here essentially focus on novel results, directions and challenges remaining in the CF field. However, remarkable improvements in health outcomes for people with CF, especially the recent development of CFTR modulator triple drug combinations, have been made possible by a highly committed and interdisciplinary CF research community.

Declaration of Competing Interest

IC, CMF, and MM have no relevant conflicts of interest related to this work.

CRediT authorship contribution statement

Isabelle Callebaut: Writing - original draft, Writing - review & editing. **Martin Mense:** Writing - review & editing. **Carlos M. Farinha:** Writing - review & editing.

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References

[1] Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox T, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science 1989;245:1073–80.
[2] Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989;245:1066–73.
[3] Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989;245:1059–65.
[4] Davies J, Moskowitz SM, Brown C, Horsley A, Mall MA, McKone EF, et al. VX-659-Tezacaftor-Ivacaftor in patients with Cystic Fibrosis and one or two Phe508del alleles. N Engl J Med 2018;379:1599–611.
[5] Keating D, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, et al. VX-445-Tezacaftor-Ivacaftor in patients with Cystic Fibrosis and one or two Phe508del alleles. N Engl J Med 2018;379:1612–20.
[6] Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polinemi D, et al. Elexacaftor–Tezacaftor–Ivacaftor for Cystic Fibrosis with a single Phe508del allele. N Engl J Med 2019;381:1809–19.
[7] Veit G, Avramescu RG, Chiang AN, Houck SA, Cai Z, Peters KW, et al. From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. Mol Biol Cell 2016;27:424–33.
[8] Joshi D, Ehrhardt A, Hiong JS, Sorscher E. Cystic fibrosis precision therapeutics: emerging considerations. Pediatr. Pulmonol. 2019;54:S13–SS7.
[9] Veit G, Xu H, Dreano E, Avramescu RG, Bagdany M, Beitel LK, et al. Structure-guided combination therapy to potently improve the function of mutant CFTRs. Nat Med 2018;24:1732–42.
[10] Cutting G, Sharma N. The genetics and genomics of Cystic Fibrosis. J Cyst Fibr 2020;19S1:S5–9.
[11] Paranjapye A, Ruffin M, Harris A, Corvol H. Genetic variation in CFTR and modifier loci may modulate cystic fibrosis disease severity. J Cyst Fibr 2020;19S1:S10–14.
[12] Chevalier B, Hinzpeter A. The influence of CFTR complex alleles on precision therapy of cystic fibrosis. J Cyst Fibr 2020;19S1:S15–18.
[13] Kleizen B, Hunt JF, Callebaut I, Hwang T-C, Sermet-Gaudelus I, Hafkemeyer S, et al. CFTR: new insights into structure and function and implications for modulation by small molecules. J Cyst Fibr 2020;19S1:S19–24.
[14] Liu F, Zhang Z, Levit A, Levring J, Touhara KK, Shoichet BK, et al. Structural identification of a hotspot on CFTR for potentiation. Science 2019;364:1184–1188.
[15] Bose SJ, Krainer G, Ng DR, Schenkel M, Shishido H, Yoon JS, et al. Towards next generation therapies for cystic fibrosis: folding, function and pharmacology of CFTR. J Cyst Fibros 2020;19S1:S25–32.
[16] Oliver KE, Rauscher R, Mijnders M, Wang W, Wolpert MJ, Maya J, et al. Slowing ribosome velocity restores folding and function of mutant CFTR. J Clin Invest 2019;129(12):5236–53 in press.
[17] van Willigen M, Vonk AM, Yeoh HY, Kruisselbrink E, Kleizen B, van der Ent CK, et al. Folding-function relationship of the most common cystic fibrosis-causing CFTR conductance mutants. Life Sci Alliance 2019;2:e201800172.
[18] Sigoillot M, Overtus M, Grodecka M, Scholl D, Garcia-Pino A, Laeremans T, et al. Domain-interface dynamics of CFTR revealed by stabilizing nanobodies. Nat Commun 2019;10:2636.
[19] Amaral MD, Hutt DM, Tomati V, Botelho HM, Pedemonte N. CFTR processing, trafficking and interactions. J Cyst Fibr 2020;19S1:S33–6.
[20] Loureiro CA, Santos JD, Matos AM, Jordan P, Matos P, Farinha CM, et al. Network biology identifies novel regulators of CFTR trafficking and membrane stability. Front Pharmacol 2019;10:619.
[21] Santos JD, Canato S, Carvalho AS, Botelho HM, Aloria K, Amaral MD, et al. Folding status is determinant over traffic-competence in defining CFTR interactors in the endoplasmic reticulum. Cells 2019;8 pii: E353.
[22] Canato S, Santos JD, Carvalho AS, Aloria K, Amaral MD, R M, et al. Proteomic interaction profiling reveals KIFC1 as a factor involved in early targeting of F508del-CFTR to degradation. Cell Mol Life Sci 2018;75:4495–509.
[23] Quesada R, Dutzler R. Alternative chloride transport pathways as pharmacological targets for the treatment of cystic fibrosis. J Cyst Fibr 2020;19S1:S37–41.
[24] Sheppard DN, Davis AP. Fighting cystic fibrosis with small molecules. Nature 2019;567:315–17.

[25] Muraglia KA, Chorghade RS, Kim BR, Tang X, Shah V, Grillo A, et al. Small–molecule ion channels increase host defences in cystic fibrosis airway epithelia. Nature 2019;567:405–8.
[26] Barbry P, Cavard A, Chanson M, Jaffe AB, Plasschaert LW. Regeneration of airway epithelial cells to study rare cell states in cystic fibrosis. J Cyst Fibr 2020;19S1:S42–6.
[27] Montoro DT, Haber AL, Biton M, Vinarsky V, Lin B, Birket SE, et al. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. Nature 2018;560:319–24.
[28] Plasschaert LW, Žilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature 2018;560:377–81.
[29] Revinski DR, Zaragosi LE, Boutin C, Ruiz-Garcia S, Deprez M, Thomé V, et al. CDC20B is required for deuterosome-mediated centriole production in multiciliated cells. Nat Commun 2018;9:4668.
[30] Rosen BH, Chanson M, Gawenis LR, Liu J, Sofoluwe A, Zoso A, et al. Animal and model systems for studying cystic fibrosis. J Cyst Fibr 2018;17:S28–34.
[31] Awatade NT, Wong SL, Hewson CK, Fawcett LK, Kicic A, Jaffe A, et al. Human primary epithelial cell models: promising tools in the era of Cystic Fibrosis personalized medicine. Front Pharmacol 2019;9:149.
[32] Clancy JP, Cotton CU, Donaldson SH, Solomon GM, VanDevanter DR, Boyle MP, et al. CFTR modulator theratyping: current status, gaps and future directions. J Cyst Fibros 2019;18:22–34.
[33] de Poel E, Lefferts JW, Beekman JM. Intestinal organoids for Cystic Fibrosis research. J Cyst Fibr 2020;19S1:S60–4.
[34] Hart SL, Harrison P. Genetic therapies for cystic fibrosis lung disease. Curr Opin Pharmacol 2017;34:119–24.
[35] Yan Z, McCray PJ, Engelhardt JF. Advances in gene therapy for cystic fibrosis lung disease. Hum Mol Genet 2019;28(R1):R88–94.
[36] Boyd C, Guo S, Huang L, Kerem B, Oren YS, Walker AJ, et al. New approaches to genetic therapies for Cystic Fibrosis. J Cyst Fibr 2020;19S1:S54–9.
[37] Engelhardt JF, Yankaskas JR, Ernst SA, Yang Y, Marino CR, Boucher RC, et al. Submucosal glands are the predominant site of CFTR expression in the human bronchus. Nat Genet 1992;2:240–8.
[38] Héry-Arnaud G, Boutin S, Cuthbertson L, Elborn SJ, Tunney MM. The lung and gut microbiome: what has to be taken into consideration for cystic fibrosis? J Cyst Fibr. 2019;18:13–21.
[39] Armbruster CR, Coeyne T, Touqui L, Bomberger JM. Interplay between host-microbe and microbe-microbe interactions in cystic fibrosis. J Cyst Fibr 2020;19S1:S47–53.
[40] Morrison CB, Markovetz MR, Ehre C. Mucus, mucins, and cystic fibrosis. Pediatr Pulmonol 2019;54(Suppl 3):S84–96.
[41] Fischer AJ, Pino-Argumedo MI, Hilkin BM, Shanrock CR, Gansemer ND, Chaly AL, et al. Mucus strands from submucosal glands initiate mucociliary transport of large particles. JCI Insight 2019;10:124863.
[42] McElvaney OJ, Wade P, Murphy M, Reeves EP, McElvaney NG. Targeting airway inflammation in cystic fibrosis. Expert Rev Respir Med 2019;13:1041–55.
[43] Mall MA, Danahay H, Boucher RC. Emerging concepts and therapies for Mucoobstructive lung disease. Ann Am Thorac Soc 2018;15(Suppl 3):S216–SS26.