MANAGEMENT OF ENDOCRINE DISEASE

Cystic fibrosis-related diabetes: novel pathogenic insights opening new therapeutic avenues

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Abstract

Cystic fibrosis (CF) is a recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*). *CFTR* is primarily present in epithelial cells of the airways, intestine and in cells with exocrine and endocrine functions. Mutations in the gene encoding the channel protein complex (*CFTR*) cause alterations in the ionic composition of secretions from the lung, gastrointestinal tract, liver, and also the pancreas. CF-related diabetes (CFRD), the most common complication of CF, has a major detrimental impact on pulmonary function, nutrition and survival. Glucose derangements in CF seem to start from early infancy and, even when the pathophysiology is multifactorial, insulin insufficiency is clearly a major component. Consistently, recent evidence has confirmed that *CFTR* is an important regulator of insulin secretion by islet β -cells. In addition, several other mechanisms were also recognized from cellular and animals models also contributing to either β -cell mass reduction or β -cell malfunction. Understanding such mechanisms is crucial for the development of the so-called 'transformational' therapies in CF, including the preservation of insulin secretion. Innovative therapeutic approaches aim to modify specific *CFTR* mutant proteins or positively modulate their function. *CFTR* modulators have recently shown *in vitro* capacity to enhance insulin secretion and thereby potential clinical utility in CFRD, including synergistic effects between corrector and potentiator drugs. The introduction of incretins and the optimization of exocrine pancreatic replacement complete the number of therapeutic options of CFRD besides early diagnosis and implementation of insulin therapy. This review focuses on the recently identified pathogenic mechanisms leading to CFRD relevant for the development of novel pharmacological avenues in CFRD therapy.

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Introduction

Cystic fibrosis (CF) is a well-established inflammatory disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene (1). *CFTR* protein is a

chloride/bicarbonate channel essential for the correct composition of epithelial cell secretion. *CFTR* dysfunction results in an ionic imbalance of secretions from several

Invited Author's profile

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organ systems, such as the lung, gastrointestinal tract, liver, and also the pancreas. The most severe feature and the main cause of premature death is the respiratory failure. Diabetes has emerged as a common comorbidity in CF (CF-related diabetes (CFRD)). The relevance of this diagnosis is related not only with the imposition of additional medical burden but also from its association with worse health outcomes in CF patients.

CF is the most common autosomal recessive disorder in Caucasians, in accordance with the high prevalence of *CFTR* mutations in the general population. The carrier frequencies observed in the North American population is 1/36 (2) and a wide mutational heterogeneity has been encountered throughout the world. Currently, over 70 000 individuals live with CF worldwide (3).

Mutations in *CFTR* may impact different physiological mechanism of the CFTR function: the synthesis of the protein, its transport to the apical membrane, the gating and the conductance of ions through the CFTR channel. Even though the basic pathophysiology of CF is caused by a defect in the CFTR protein, the reported wide spectrum of disease severity can be explained by a complex crosstalk between the CFTR malfunction, additional modifier gene variations and environmental factors (4, 5). In particular, the existence of pleiotropic modifier genes may represent the bases of complementary therapeutics that extends beyond causal gene dysfunction.

CFTR gene mutations: genotype–phenotype correlations

To date, over 2000 different sequence variations in the *CFTR* gene have been reported (CF Mutation Database (CFMDB). <http://www.genet.sickkids.on.ca/cftr/Statistics-Page.html> (accessed Feb 14, 2014)), although a detailed understanding of how *CFTR* mutations impact channel dysfunction is limited to only a few of these (6). The F508del mutation is present in 90% of CF patients worldwide (7) and only four other mutations (*G551D*, *W1282X*, *G542X*, and *N1303K*) have a minor but substantial prevalence (1–3% each) worldwide (8).

CFTR mutations are classified into six categories (classes I–VI) according to their specific consequences on CFTR protein function, namely the complete lack of synthesis (I), altered processing (II), altered gating (III), altered conductance (IV), reduced synthesis (V), and defective stability (VI) of the channel within the cell membrane (9) (Fig. 1). Class II mutations, including the most prevalent F508del, disrupt normal folding and trafficking of CFTR through the cell, which results in

Figure 1

Type of mutations in the *CFTR* gene, including those that increase the risk for diabetes (CFRD) development. Prevalence of the different mutational classes in the CF population. Most common mutations in each group are in bold. Potential therapies for class-specific correction/modulation of underlying CFTR defects are indicated. *HGF, hepatocyte growth factor enhances CFTR retention/anchoring at the cell surface (modified from Fanen *et al.* (10), Bell *et al.* (2014) (64), and Boyle *et al.* (2013) (66)).

decreased or no expression of CFTR at the epithelial surface (10).

Genotype–phenotype correlations in CF have been mainly studied with respect to clinical severity. It is established that type I to III mutations result in no CFTR function at the cell membrane and are associated with more severe phenotypes, while in patients with classes IV to VI mutations, CFTR protein may retain residual activity, resulting in less severe disease. When mutations from two different classes are present in the same patient, disease severity is unpredictable (11).

With respect to the pancreatic exocrine involvement, specific *CFTR* mutations are associated with increased risk of exocrine enzymatic insufficiency. Such risk is especially high for mutations of classes I to III, whereas harboring milder mutations (usually of class IV or V) associates with maintained exocrine secretions. Despite the above, the degree of exocrine damage at birth and the age of onset of clinically established exocrine insufficiency are variable among patients with the same *CFTR* genotypes. It is well known that pancreatic insufficiency is a risk factor for the development of CFRD (12). Such susceptibility is in part influenced by genetic variants at the SLC26A9 protein

(another epithelial chloride/bicarbonate channel that interacts with CFTR), and single-nucleotide polymorphisms (SNPs) for type 2 diabetes at or near *CDKAL1*, *CDKN2A/B*, and *IGF2BP2* were found to be associated with CFRD, as well (13), and Derbel *et al.* (14) presented the first evidence for an involvement of a polymorphism in the *CAPN10* gene in the pathogenesis of CFRD. A further candidate gene approach revealed the modifying effects of a variant in the *TCF7L2* gene that associates with both type 2 diabetes and CFRD, supporting the concept that diabetes develops in patients who may have underlying susceptibility to β -cell dysfunction (15).

Therefore, development of exocrine and endocrine pancreatic dysfunctions (diabetes) in CF is not completely independent, because most patients developing diabetes were previously exocrine insufficient (16). Thus, it is not surprising that the same types of mutations increasing the risk of pancreatic insufficiency have been found in CF patients with CFRD (classes I, II, and III shown in Fig. 1).

Natural history and importance of early diagnosis of CFRD

Diabetes is the most common complication of CF and, although classically revealed in the second decade of life, pre-diabetic states frequently exist before and are amenable to early diagnosis. The clinical symptoms of diabetes in CF are generally insidious, thereby routine screens for glucose abnormalities are performed at specialized CF units on a yearly basis, using the oral glucose tolerance test (OGTT). Glucose abnormalities that precede overt diabetes are very common, even in children younger than 10 years (17, 18, 19, 20).

After puberty, there is an age-related increase in CFRD prevalence of 5% per year, with diabetes occurring in about 15% of adolescents and 50% of adults with CF (21). Worryingly, CFRD is associated with significantly increased morbidity and mortality in both children and adults. It has a major detrimental impact on pulmonary function, nutritional status and survival (22, 23, 24). These deleterious effects of hyperglycemia frequently occur before the diagnosis of overt CFRD is made (25). Given the fact that insulin has potent anabolic actions, it was shown that early administration of insulin in CF pre-diabetic patients ameliorates lung function, nutrition, and survival (21, 26).

Early risk markers for CFRD would be clinically useful. In the future, we might begin insulin therapy as soon as screening shows β -cell insufficiency, rather than waiting until overt diabetes is established. In any case, the

importance of early screening for glucose abnormalities in CF should be highlighted to ensure prompt diagnosis and minimization of the effects of 'pre-diabetic' hyperglycemia on the overall outcome of the disease.

Circulating immunoreactive trypsinogen (IRT), a biomarker of exocrine pancreatic activity, has been shown to be present in most CF newborns. However, in those babies with severe *CFTR* genotypes, IRT declines rapidly in the first years of life, reflecting progressive pancreatic damage (27). Consistent with this progression, a less elevated newborn IRT level may reflect more severe pancreatic disease, including compromised islet compartments, and potentially increased risk of CFRD. Therefore, the levels of IRT may be of value to predict CFRD risk in early life, because maintained ductal secretion flow in the exocrine pancreas could delay the onset of CFRD (27).

Novel mechanistic views on CFRD development

The primary cause of CFRD is insulin deficiency, especially the failure in the first-phase of insulin secretion. Insulin sensitivity in CFRD patients is generally normal or only slightly decreased, except in the settings of acute infectious pulmonary illness or the use of glucocorticoids, when severe insulin resistance is present (28). Furthermore, insulin clearance may also be increased in CF individuals, whether diabetic or not (29).

Patients with CF, even those with normal glucose tolerance, tend to have decreasing insulin secretion over time that leads to a progressive alteration in carbohydrate metabolism, resulting finally in diabetes (30). The conventional assessment of glucose abnormalities (HbA1c, fasting, and OGTT 2-h glucose levels) is insensitive to early dysfunction; thus, other tools are needed for the diagnoses of early glycemic alterations. The 'disposition index' (DI), reflecting the use of glucose by tissues, is reduced in CF patients even when their glucose tolerance is normal, and the index is further decreased in those CF individuals with diabetes. In a small cohort, Merjaneh *et al.* (31) found that the measurement of DI could be clinically useful in CF to detect subtle defects in β -cell function and to identify progression of the disease.

Two major pathogenic mechanisms mediate the development of CFRD: islet cell mass reduction (especially β - and α -cells) and β -cell-specific dysfunction (Fig. 2):

Decreased β -cell mass

In the pancreas, accumulation of thick, highly concentrated secretions in the pancreatic–biliary ducts leads to

Figure 2

Pathogenic mechanisms involved in the development of CFRD. Insulin secretion leading to CFRD involves two major mechanisms: reduction in β -cell mass and β -cell dysfunction. The number of β -cells is decreased by pancreatic fibrosis derived from auto-digestion of the tissue by the enzymes retained within the pancreas (black pathway). There is also an immune imbalance against the β -cells linked with hyperglycemia and hypovitaminosis D, both resulting in β -cell dysfunction (dark blue pathway). A third route (purple pathways) impacts

on both the number of β -cells (through apoptosis) and β -cell malfunction. *CFRT* mutants in β -cells may negatively impact insulin secretion (grey pathways, see Fig. 3 for detailed explanation). Low incretin levels due to alteration in the entero-insular axis (light blue pathway) also impact β -cell function. Finally, insulin resistance and increased insulin clearance have a role in the final derangements responsible for CFRD development.

ductal obstruction, progressive fibrosis, fatty infiltration, and amyloid deposition in the islets that are associated with both decreased β -cell mass and β -cell dysfunction. β -cell mass loss in CFRD can be multifactorial (Fig. 2):

Auto-digestion of the pancreatic tissue ► Exocrine pancreatic insufficiency, present in 90% of CF patients, causes the retention of digestive pro-enzymes, premature activation of such trapped enzymes, and finally the tissue destruction and fibrosis. The concentrations of islet hormones other than insulin, such as glucagon, are also abnormal in adults with CFRD, suggesting that the total islets are destroyed by fibrosis. However, strikingly, although all patients with CF seem to have a substantial

β -cell loss by this mechanism, fibrosis cannot be the unique cause of CFRD because patients with CFRD were shown not to necessarily have more pancreatic fibrosis at autopsy than do CF patients without CFRD (32). Therefore, additional factors should be involved in CFRD pathogenesis.

Oxidative stress at the β -cell ► More recently, a study has shown that a combination of increased oxidative stress (33) and an accumulation of misfolded *CFTR* proteins in the endoplasmic reticulum (ER) of the β -cell may lead to ER stress and eventual apoptosis of this cell lineage. This effect can be further potentiated by the well-known malabsorption of dietary antioxidant in CF patients.

β-cell-specific dysfunction

Misbalance of the immune system ► CFRD is preceded by a long pre-diabetic phase associated with unexplained accelerated loss of lung function. The parallel development of CFRD and the decline in lung function before the onset of CFRD are not fully understood, but experimental evidence suggests that chronic inflammation might be involved in both processes. Patients with CF have increased glucose fluctuations and hyperglycemia and this may lead to lymphocyte dysfunction (34). A balance between pro-inflammatory T-helper 17 lymphocytes (Th17) that secrete the pro-inflammatory cytokines IL17 and Tc17, and anti-inflammatory regulatory T cells lymphocytes (Tregs) is essential to maintain immunological tolerance and prevent the onset of several autoimmune diseases (35). The Th17 pathway is involved in CF lung inflammation and, interestingly, also in β-cell destruction occurring in type 1 and type 2 diabetes (36). Also, Tregs have been shown to be dysfunctional and produce IL17 in type 1 diabetes (37). There is some evidence that Th17 cells and dysfunctional Treg lymphocytes producing IL17 could be involved in lung damage, perpetuating the excessive pulmonary inflammatory process, and also in β-cell dysfunction, providing a potential link between these two crucial and frequent abnormalities in CF (38).

In addition, non-optimal levels of vitamin D are known to aggravate the exposed immune misbalance. Hypovitaminosis D is frequently observed in CF patients, mainly due to intestinal malabsorption of dietary vitamin D precursors. Vitamin D is known to have a role in CF-related inflammation, diabetes, and the differentiation of lymphocytes (39). The lymphocytes function is susceptible not only to hyperglycemia (40) but also to non-optimal levels of vitamin D which could both trigger the abnormal immune response observed in CF (41, 42) and might promote inappropriate immune responses of Th17 and Treg cells and, in turn, possibly contribute to the deterioration of lung function, as well as the development of CFRD (43). However, human data supporting the decrease in β-cell mass by hypovitaminosis D are currently lacking.

Impaired insulin secretion ► CFTR protein is expressed in α-cells and β-cells in the rat pancreas, although its role in these cells is not well known (44). Evidence reveals a role of CFTR in glucose-induced electrical activities and insulin secretion in β-cells (45). CFTR is important for rapid exocytosis of primed granules

important for first-phase insulin release (46) (Fig. 3). Indeed, many CF patients suffer from postprandial hyperglycemia, although they have normal fasting plasma glucose levels indicating that the β-cells fail to respond upon increased insulin demand. For these patients, impaired insulin secretion is mostly apparent during the first phase, strongly indicating a defect at the level of the pancreatic β-cell. Studies in animal models for CF with modification of CFTR develop multi-system disease, including pancreas and lung disease that have very similar

Figure 3

Model describing the possible involvement of CFTR in β-cell granular priming and exocytosis. Insulin secretion in β-cells is triggered by rising blood glucose levels. Starting with the uptake of glucose by the GLUT2 transporter, the glycolytic phosphorylation of glucose causes a rise in the ATP/ADP ratio. This rise inactivates the potassium channel (formed by Kir6.2 + SUR1 proteins) that depolarizes the membrane, causing the calcium channel to open up allowing calcium ions to flow inward. The ensuing rise in the levels of calcium leads to the exocytotic release of insulin from their storage granule (with blue lumen). However, storage granules need to be 'primed' before they are ready for calcium-induced exocytosis ('pre-primed' granules with brown lumen). Such priming involves in part acidification of the granular lumen, which is achieved by the inward flow of chloride ions by the chloride transporter CLC3. The necessary Cl⁻ ions in the cytoplasm are supplied from the outside of the β-cell by the chloride transporter anosmin 1 (ANO1), which is experimentally shown to be activated or cooperate with CFTR. CFTR is stimulated by the intracellular rise in cAMP derived from the activation of incretin receptors by incretins (GLP1 or GIP). (Adapted from Edlund *et al.* 2014 (46)).

pathology to that in patients (47, 48, 49). Endocrine pancreas function in the *CFTR*-null ferret is abnormal from birth, which suggests that it is an intrinsic defect in β -cell function in CF (47). In the same way as in humans, the glucose abnormalities progress with age in the *Cftr*-null animals as pancreatic fibrosis worsens. In contrast to the current belief that insulin insufficiency in CFRD is mainly due to the destruction of the pancreatic islets, the study of Guo *et al.* (45) revealed no significant difference in pancreatic islet morphology between *CFTR* WT and F508del mice, indicating that the observed defect in insulin secretion in CF may not be caused by structural alteration in the islets. Of note, CFRD may start in CF patients at ages as children or juveniles with the absence of islet destruction. Thus, a loss of islet cells could be at most a long-term effect in CFRD patients. Ode & Moran (19) postulate that in patients a mild intrinsic defect in insulin secretion related to *CFTR* exists, which then becomes of greater clinical importance as islets are lost through scarring and fibrosis of the pancreas.

Altered entero-insular axis ► Alterations in hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP1), and glucose-dependent insulintropic peptide (GIP), which have been observed in CF patients, may also play a role in CFRD, because each of these hormones participate in insulin regulation (50).

Current and emerging therapeutic options in CFRD

The optimal management of pre-diabetic glycemic derangements and overt diabetes is an active field of research. Novel therapeutics approaches have been recently used in connection with a better understanding of *CFTR* pathophysiology, including the development of 'transformational' or 'modulatory' drugs aiming to restore *CFTR* functions (51). Despite the recent arrival of these promising novel drugs to the clinical arena, its use is so far limited. Beside transformational drugs, the early support to deteriorating β -cell function was shown capable to attain better prognosis for pre-diabetes and CFRD (52).

The current array of therapeutic tools available for CFRD is based on:

1. Early supplementation of failing β -cell function.
 - a. *Insulin therapy.* Symptomatic therapies treating end-organ manifestations of CF including CFRD have increased life expectancy of CF patients toward a mean age of 40 years. Insulin is currently

the only recommended treatment for CFRD by consensus from international diabetes and CF scientific societies. In addition to its role in controlling hyperglycemia, insulin is a potent anabolic hormone, beneficially impacting on pulmonary and nutritional states of typically catabolic CF individuals. Even though insulin therapy is a demanding treatment for patients who yet require other complex therapeutic regimens, benefits of its use clearly exceed inconveniences such as the risk of hypoglycemia. Classically, only CFRD cases showing fasting hyperglycemia were treated, usually with basal-bolus regimens (19); however, recently also CFRD with fasting normoglycemia has been treated with preprandial insulin boluses (26). Due to the need for multiple daily injections, insulin pumps could represent an ideal mode of administration for many CFRD patients. The use of insulin therapy in CF pre-diabetes is still controversial given the lack of international agreement guidelines (19). In a randomized trial, Minicucci *et al.* (53) found benefits using once-daily long-acting insulin in CF patients with glucose intolerance. Early indications that insulin treatment of non-diabetic CF patients improves nutritional and pulmonary status, together with evidence that even healthy CF children are often insulinopenic, seem to support that active treatment of subtle insulin deficiencies in CF children should be considered (54).

- b. *Oral antidiabetic drugs.* The use of oral antidiabetic drugs (OAD) in patients with CFRD is not officially approved. The latest Cochrane Database Report regarding 'Insulin and oral agents for managing CFRD' states that further studies are needed to establish whether there is clear benefit for hypoglycemic agents in this type of diabetes (55).
- c. *Islet/pancreas transplantation.* In patients with CFRD and severe alteration in pulmonary function, Spijker *et al.* (56) propose islet-transplantation after-lung intervention for those patients who could not benefit from simultaneous islet-lung or pancreas-lung allo-transplantation.
2. Supplementation of pancreatic enzymes and gastrointestinal hormones.

The correct maintenance of exocrine functions in CF indirectly but strongly impacts an adequate control of hyperglycemia. Besides, incretin hormones are involved in the regulation of adequate insulin secretion by β -cells.

a. *Pancreatic enzymes supplementation.* In CF, exocrine pancreatic insufficiency generates fat maldigestion, which still remains non-optimal despite pancreatic enzyme replacement therapy (57). In the gastrointestinal lumen, lipolysis of fat is required to slow down gastric emptying. Therefore, fat maldigestion associates with abnormally rapid gastric emptying of high-fat meals, which in turn accelerates absorption of carbohydrates, resulting in postprandial hyperglycemia (58). Such postprandial hyperglycemia is the predominant abnormality of carbohydrate metabolism in CF and it manifests early in the course toward CFRD. Thus, it is crucial to optimize pancreatic enzyme replacement with regard to doses and timing of administration, including administration before meal starts and ensuring adequate mixing with ingested nutrients. Furthermore, a recent finding that neonatal determination of serum IRT represents a reasonable biomarker risk for pancreatic exocrine deficiency later in life has allowed the close control and early replacement of enzymes in those children classified with high risk for exocrine (and indirectly endocrine) dysfunction.

b. *Incretin hormones.* Glucose homeostasis is dependent on a complex interplay of multiple hormones: insulin and amylin, produced by pancreatic β -cells; glucagon, produced by pancreatic α -cells; and gastrointestinal peptides (incretin hormones), including GLP1 and glucose-dependent insulinotropic polypeptide (GIP; gastric inhibitory polypeptide). Incretins are peptide hormones secreted by specialized cells along the gastrointestinal tract including oesophagus, stomach, small and large bowels, and the pancreas (59). Incretins are rapidly inactivated by the enzyme dipeptidyl-peptidase 4 (DPP4), resulting in a very short half-life. DPP4 inhibitors are a relatively new class of oral anti-hyperglycemic agents that enhance insulin secretion by reducing degradation of endogenous GLP1. GLP1-based therapies affect glucose control through several mechanisms, including enhancement of glucose-dependent insulin secretion, slowed gastric emptying, regulation of postprandial glucagon, and reduction in food intake (60). Because of such functions, incretin hormones are central to postprandial glycemic control. In the small intestine, lipolytic products, in addition to stimulating release of the incretins, induce neurohumoral feedback to slow gastric emptying that involves, among

other mechanisms, secretion of peptide YY and CCK (50). As previously mentioned, young people with CF have markedly increased glycemic excursions after a high-fat/high-carbohydrate meal compared with healthy controls, even in the context of CF patients with normal fasting blood glucose and normal OGTT (61). These CF subjects have abnormally low postprandial stimulation of incretin hormones which normalizes after pancreatic enzyme replacement, which further attenuates postprandial hyperglycemia. Therefore, improvement in postprandial hyperglycemia is attainable through prandial administration of GLP1 agonists that slows down gastric emptying or by augmenting the action of incretins, or through administration of DPP4 inhibitors (61). Therapies that specifically target postprandial glucose excursions are, therefore, likely to play a significant role in the improvement of clinical outcomes of CFRD patients.

3. *Functional modulation of CFRT mutants in the β -cell.* The recent development of CFTR-targeted drugs able to correct the basic protein defects promises to transform the therapeutic landscape from a trial-and-error prescription to personalized type of mutation-based medicine (62, 63). This approach would need to achieve at least 30% of normal CFTR function to have a clinical effect. Options within these protein-modulating therapies relate to the class of mutation and corresponding effects on CFTR function (Fig. 1). Class I and II mutations which reduce protein quantity need corrector drugs, while class III, IV, and V (gating, conductance, and decreased-protein defect) retaining residual function needs potentiators. This era of clinically relevant CFTR correction offers the opportunity to ascertain whether restoration of CFTR function in B-cell also ameliorates or prevents development of CFRD.

a. *CFTR correctors.* CFTR correctors improve abnormal CFTR protein folding and trafficking (class II mutations) (64), allowing a better arrival to the apical cell membrane. Lumacaftor (VX-809) is an investigational, orally bioavailable CFTR corrector, potentially promoting folding of type II CFTR mutant proteins (including the most prevalent phe508del), allowing them to escape degradation at the ER and reach the cell surface, thus increasing the number of channels present at the plasma membrane. In addition, Lumacaftor improves CFTR-mediated chloride transport *in vitro* (65). Interestingly, VX-809 also demonstrated a clear capacity to correct glucose-induced electrical

abnormalities and insulin secretion in pancreatic islet β -cells with the F508del mutation. This opens the possibility of a clinical use of Lumacaftor in CFRD. However, it remains uncertain how effectively misprocessing of F508del can be corrected *in vivo*, and how much correction is required to yield clinical benefits in patients. Recently, a combined therapy with correctors (Lumacaftor) and potentiators (Ivacaftor) has been tried in patients homozygotes for F508del, on pulmonary end-points (FEV1), with promising results based on hypothesized synergistic effects between the two types of CFTR modulators (66). Other ongoing clinical trials currently examine the safety and efficacy of such first-generation combination of corrector/potentiator drugs for F508del and results are eagerly awaited. Ivacaftor alone does not seem to provide significant clinical benefit for patients with F508del (67). Yet another CFTR corrector in development is currently known as VX-661. Preliminary results from a phase II study of its use in combination with ivacaftor have shown promising results in patients homozygous for the F508del mutation (68). It is likely that a phase III program will be developed shortly. Ibuprofen has been also identified as a CFTR corrector. Hence, ibuprofen may be suitable to be part of a future CF combination therapy (69).

- b. *CFTR potentiators*. These drugs increase the probability of CFTR mutant channels to open when they have gating (Class III) or conductance (Class IV) defects (8). In addition, evidence from *in vitro* studies suggests that CFTR potentiators may also enhance the open probability of CFTR channels with Class II mutations, such as F508del (70). Nevertheless, the recombinant-expressed channel must already be located on the cell membrane for potentiation to exist. Only one potentiator drug-targeting CFTR mutants (Ivacaftor (Kalydeco; VX-770)) is available to a small number of CF patients (71). However, there is optimism that transformational drugs will shortly become available for patients' use (72). Ivacaftor (VX-770) activates CFTR and increases the open probability of the channel by regulating its gating and possibly also its conductance (73, 74). It is the first of a group of potentiator molecules currently under development. Ivacaftor was FDA approved in December 2012 for CF patients with the G551D mutation; in February 2014, the FDA extended approval for

ivacaftor use to another eight CFTR-gating mutations. Interestingly, in a small study of patients with CF with the G551D (75) mutation and aged 6–52 years receiving ivacaftor for 1 month, significant improvement of their insulin response was observed. This pilot study further substantiates the direct role of CFTR in human insulin secretion. Interestingly, these authors suggest the need for long-term longitudinal studies to determine whether early initiation of CFTR modulation, particularly in young CF children who have not yet lost considerable β -cell mass, will delay or prevent development of diabetes in this high-risk population.

Following this rationale, a complete resolution of CFRD with ivacaftor therapy has been very recently reported in a 25-year-old CF male with the genotype F508del/G551D (76). This patient was diagnosed with CFRD 6 years earlier and was treated with 20 units glargine insulin with adequate control. After 9 months of ivacaftor, insulin requirement reduced to half and 4 months later insulinotherapy was completely withdrawn. Euglycemia with normal OGTT remains after 1 year follow-up. After 25 months under ivacaftor, his sweat chloride decreased, his BMI increased, and his spirometry also improved (76).

4. Gene therapy.

Gene therapy represents a potentially curative approach to correct the mutated CFTR gene or to deliver the WT gene to the target tissues of the disease. Correction of CFTR mutations focused on the 'read-through' of premature termination codons present in the gene using aminoglycoside antibiotics (Ataluren) has been made. Although the primary end-point of the study (FEV1% predicted) did not show improvement against placebo, a better maintenance of lung function was shown in the subset of patients not treated with inhaled antibiotics known to decrease Ataluren efficacy (77). Since cloning of the *CFTR* gene 20 years ago, a large number of pre-clinical and clinical CF gene therapy studies have been performed. However, research into gene therapy has not yet provided clear evidence of how these findings might relate with tangible benefits for clinical practice and, currently, no gene-delivery therapies have been approved for use (78). Notwithstanding the above, there are novel promising gene-correcting therapies for CF, particularly assessing the efficiency and safety of lentiviral

vectors delivery vs formerly used delivery options such as airway gene transfer agents mainly targeting lung disease (79).

Conclusion

CF is a very severe disease that seriously compromises life expectancy of patients. However, recent decades have seen notable improvement in the medical handling of the many facets of the disease, resulting in notable decrease in morbidity and progressive increase of survival time. This longer life expectancy and better diagnostic methods have made diabetes (CFRD) the most prevalent co-morbidity of CF, beside typical pulmonary and nutritional features. It is relevant to underline that early recognition and treatment of not only CFRD but also the pre-diabetic states can be of substantial benefit to compensate excessive catabolism and the overall outcome of CF. Tailored insulin regimens and the use of continuous perfusion pumps are to become more frequently used to optimize insulin therapy in CF. Defective insulin secretion derived from *CFTR* mutations can yet be aided by the use of incretins.

We currently live in exciting times for the development of novel drugs capable to modulate (correct or potentiate residual mutant channel activity) the damaging effects of *CFTR* mutants at the protein level. As for diabetes, some of them have demonstrated their capacity to ameliorate insulin secretion by pancreatic β -cells. Within the next decade, it is envisaged that it will be routine to deliver individualized therapies designed to overcome specific mutational abnormalities in the CF population.

Declaration of interest

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