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From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations

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ABSTRACT More than 2000 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) have been described that confer a range of molecular cell biological and functional phenotypes. Most of these mutations lead to compromised anion conductance at the apical plasma membrane of secretory epithelia and cause cystic fibrosis (CF) with variable disease severity. Based on the molecular phenotypic complexity of CFTR mutants and their susceptibility to pharmacotherapy, it has been recognized that mutations may impose combinatorial defects in CFTR channel biology. This notion led to the conclusion that the combination of pharmacotherapies addressing single defects (e.g., transcription, translation, folding, and/or gating) may show improved clinical benefit over available low-efficacy monotherapies. Indeed, recent phase 3 clinical trials combining ivacaftor (a gating potentiator) and lumacaftor (a folding corrector) have proven efficacious in CF patients harboring the most common mutation (deletion of residue F508, Δ F508, or Phe508del). This drug combination was recently approved by the U.S. Food and Drug Administration for patients homozygous for Δ F508. Emerging studies of the structural, cell biological, and functional defects caused by rare mutations provide a new framework that reveals a mixture of deficiencies in different CFTR alleles. Establishment of a set of combinatorial categories of the previously defined basic defects in CF alleles will aid the design of even more efficacious therapeutic interventions for CF patients.

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Abbreviations used: ABC, ATP-binding cassette; CF, cystic fibrosis; CFFT, Cystic Fibrosis Foundation Therapeutics, Inc.; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; PM, plasma membrane; PTC, premature termination codon.

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INTRODUCTION

Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), is characterized by a multiorgan pathology affecting the upper and lower airway, gastrointestinal and reproductive tracts, and endocrine system (Riordan *et al.*, 1989; Collins, 1992; Rowe *et al.*, 2005; Cutting, 2015). CF is one of the most common lethal autosomal-recessive diseases, with a prevalence of one in 3500 in the United States and one in 2500 in the European Union (Farrell, 2008; Pettit and Fellner, 2014). Lack of functional CFTR expression at the apical membrane of secretory epithelia results in defective Cl^- and bicarbonate secretion, coupled to enhanced Na^+ absorption and mucus secretion, which in airway epithelia leads to dehydration and acidification of the airway surface liquid (Tarran *et al.*, 2001; Chen *et al.*, 2010; Derichs *et al.*, 2011; Pezzulo *et al.*, 2012). As a consequence, impaired mucociliary clearance provokes recurrent infection and uncontrolled inflammation culminating in lung damage, which is the primary cause of morbidity and mortality in CF (Ratjen and Doring, 2003; Boucher, 2007; Stoltz *et al.*, 2015). CFTR is member of the ATP-binding cassette (ABC) subfamily C (ABCC7) (Kerr, 2002). It consists of two homologous halves, each containing a hexa-helical membrane-spanning domain (MSD1 and MSD2) and a nucleotide-binding domain (NBD1 and NBD2) that are connected by an unstructured regulatory domain (Riordan, 1993; Riordan *et al.*, 1989).

BIOLOGY OF CFTR MUTATION: TRADITIONAL CLASSIFICATION

CF is caused by ~2000 mutations in the *CFTR* gene with a wide range of disease severity (www.genet.sickkids.on.ca/home.html; www.cfr2.org; Sosnay *et al.*, 2013), which is further influenced by modifier genes (Collaco and Cutting, 2008; Cutting, 2010) and by the environmental and socioeconomic status of patients (Schechter *et al.*, 2001; Barr *et al.*, 2011; Taylor-Robinson *et al.*, 2014; Kopp *et al.*, 2015). The first classification of CF mutations into four classes according to their primary biological defect was proposed by Welsh and Smith in a landmark paper (Welsh and Smith, 1993). Currently, six major classes are distinguished (Rowe *et al.*, 2005; Zielenski and Tsui, 1995) (Figure 1).

Class I encompasses frameshift, splicing, or nonsense mutations that introduce premature termination codons (PTC), resulting in severely reduced or absent CFTR expression.

Class II mutations lead to misfolding, premature degradation by the endoplasmic reticulum (ER) quality-control system, and impaired protein biogenesis, severely reducing the number of CFTR molecules that reach the cell surface.

Class III mutations impair the regulation of the CFTR channel, resulting in abnormal gating characterized by a reduced open probability.

Class IV mutations alter the channel conductance by impeding the ion conduction pore, leading to a reduced unitary conductance (Sheppard *et al.*, 1993; Hammerle *et al.*, 2001).

Class V mutations do not change the conformation of the protein but alter its abundance by introducing promoter or splicing abnormalities (Highsmith *et al.*, 1994, 1997; Zielenski and Tsui, 1995).

Class VI mutations destabilize the channel in post-ER compartments and/or at the plasma membrane (PM), by reducing its conformational stability (Haardt *et al.*, 1999) and/or generating additional internalization signals (Silvis *et al.*, 2003). This results in accelerated PM turnover and reduced apical PM expression (Haardt *et al.*, 1999; Silvis *et al.*, 2003).

For many of the identified mutations, the disease liability is unknown, but efforts are under way to assess their functional consequence and clinical severity (www.cfr2.org; Sosnay *et al.*, 2013).

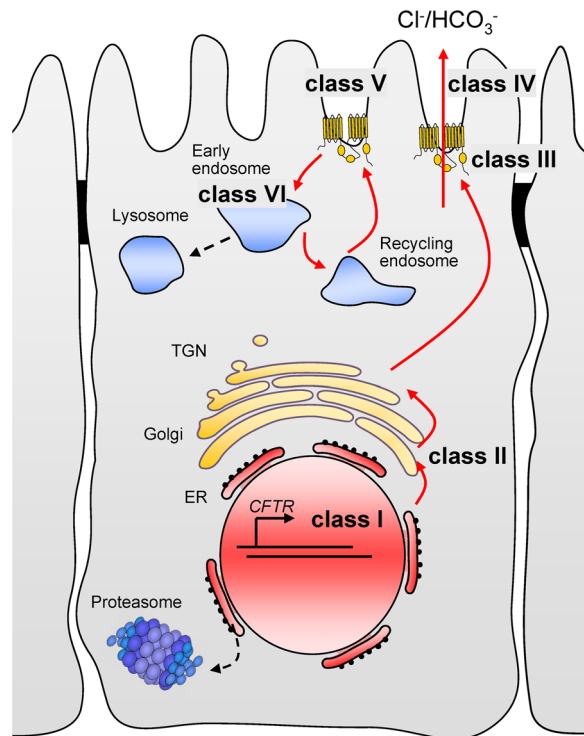


FIGURE 1: Traditional classification of CF mutations based on their cellular phenotype. Class I: protein synthesis defect; class II: maturation defect; class III: gating defect; class IV: conductance defect; class V: reduced quantity; and class VI: reduced stability. ER, endoplasmic reticulum; TGN, trans-Golgi network.

MUTATION CLASS-SPECIFIC PHARMACOTHERAPY

Defining the cellular and molecular pathology of CFTR mutations proved to be invaluable for development of small-molecule compounds targeting the underlying defect(s) in CF. The fact that some CFTR variants carrying class III or IV mutations can be expressed at the apical membrane of secretory epithelia at a density similar to that of the wild-type protein, although they are functionally impaired (e.g., G551D), led to the development of gating potentiators that increase the open probability and thereby the PM chloride conductance (Yang *et al.*, 2003). VX-770 (ivacaftor) is the first potentiator drug to be U.S. Food and Drug Administration approved for CF treatment; it directly targets the gating defect of the class III mutation G551D-CFTR (Van Goor *et al.*, 2009). This compound was developed by Vertex Pharmaceuticals in conjunction with Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT), and shows remarkable clinical benefit in patients carrying the mutation in either one or two alleles (Van Goor *et al.*, 2009; Accurso *et al.*, 2010; Ramsey *et al.*, 2011). The approval of VX-770 was extended to eight additional class III mutations (G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P, and G1349D) (Yu *et al.*, 2012; Vertex, 2014a) and recently to the class IV mutation R117H (Vertex, 2014b).

The prototypical class II mutation, ΔF508 -CFTR (Phe508del), elicits a complex folding defect that compromises both NBD1 stability and the channel's cooperative domain assembly (Du and Lukacs, 2009; Du *et al.*, 2005; Mendoza *et al.*, 2012; Rabeh *et al.*, 2012). For many years, large-scale efforts have been under way to isolate correctors that act as pharmacological chaperones by directly binding to and promoting the biogenesis of class II CFTR mutations. The most promising corrector compound at present, VX-809 (lumacaftor), partially reverts the ΔF508 -CFTR functional expression

defect by stabilizing the NBD1-MSD1/2 interface (Farinha *et al.*, 2013; Loo *et al.*, 2013; Okiyoneda *et al.*, 2013; Ren *et al.*, 2013), leading to a marked correction from 3 to 15% of wild-type channel activity in vitro (Van Goor *et al.*, 2011). A clinical trial, however, failed to observe significant clinical benefit in homozygous $\Delta F508$ -CFTR patients (Clancy *et al.*, 2012). Acute addition of VX-770 to VX-809-corrected $\Delta F508$ -CFTR doubled the PM activity in vitro (Van Goor *et al.*, 2011), and the combination therapy showed modest but significant clinical improvement (Boyle *et al.*, 2014; Wainwright *et al.*, 2015). Based on these results, the combination treatment has been approved for CF patients 12 years and older with two copies of the $\Delta F508$ mutation (Vertex, 2015). Other class II mutations that can be corrected by VX-809 in vitro include E56K, P67L, E92K, R170G, L206W, V232D, F508G, and A561E (Caldwell *et al.*, 2011; Okiyoneda *et al.*, 2013; Ren *et al.*, 2013; Veit *et al.*, 2014; Awatade *et al.*, 2015).

Ribosomal read-through allows synthesis of full-length CFTR carrying class I mutations. To this end, ataluren (PTC124) was developed as a drug that promotes near-cognate aminoacyl-tRNA incorporation at PTCs (Lentini *et al.*, 2014; Welch *et al.*, 2007). Ataluren partially restores G542X-CFTR (class I) expression in a mouse model and modestly corrects CFTR function in nasal epithelia in patients with class I mutations (Du *et al.*, 2008; Sermet-Gaudelus *et al.*, 2010; Wilschanski *et al.*, 2011). In a recent phase 3 clinical trial, however, ataluren treatment failed to produce significant clinical benefit, perhaps due to an adverse drug–drug interaction with tobramycin, which is a commonly administered, inhaled antibiotic used to treat lung infections in CF patients (Kerem *et al.*, 2014).

LIMITATIONS OF CF MUTATION CLASSIFICATION

The efficacy of available monotherapies for some mutant alleles, which have been designated as class I, class II, or class III/IV mutations, is currently limited. This could be partly explained by the pleiotropic molecular defects caused by single mutations. Thus comprehensive mapping of the multiple molecular defects caused by a single or combination of mutant alleles could offer considerable advantage for improving therapeutic interventions and for future development of drug combinations. In the following list, we present a subset of mutations that display combinatorial molecular defects.

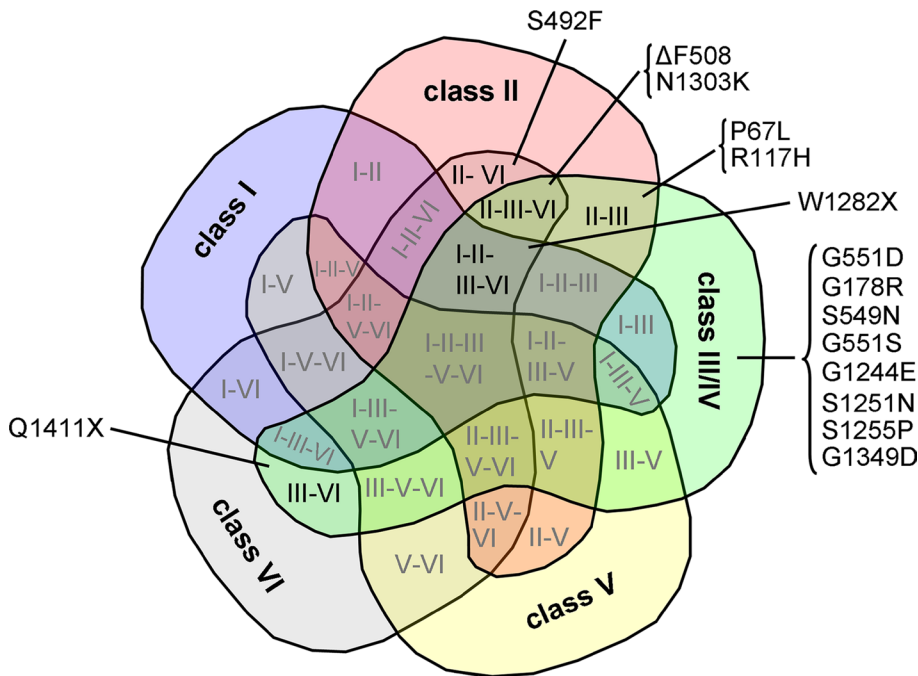
- **$\Delta F508$:** The most prevalent class II mutation impairs CFTR conformational maturation and leads to its targeting for premature ER-associated degradation (Cheng *et al.*, 1990; Cyr, 2005; Kim and Skach, 2012; Lukacs *et al.*, 1994). However, $\Delta F508$ -CFTR molecules that either constitutively or following rescue procedures escape the ER quality control and accumulate at the PM of airway epithelia exhibit a channel-gating defect, which is a hallmark of class III mutations (Dalemans *et al.*, 1991), as well as accelerated turnover in post ER compartments and at the PM, a class VI mutation characteristic (Lukacs *et al.*, 1993). Unless the folding and conformational dynamics of the rescued $\Delta F508$ -CFTR are fully restored to that of the wild-type protein by pharmacological treatment, this mutation remains partially defective and requires correction of its gating and/or peripheral stability defect. Rescue of the gating defect can be achieved with potentiators (e.g., VX-770) (Van Goor *et al.*, 2009). Peripheral stabilization of the $\Delta F508$ -CFTR could be attained by 1) the peptide inhibitor iCAL36 (Cushing *et al.*, 2010), 2) preventing post-Golgi ubiquitination (Fu *et al.*, 2015; Okiyoneda *et al.*, 2010), 3) restoring autophagosome formation (Luciani *et al.*, 2012), or 4) modulating cellular protein homeostasis (Hutt *et al.*, 2010). Thus the

most common mutant has multiple defects that extend beyond the features of a class II mutation.

- **W1282X:** This PTC represents a class I mutation, though recent studies suggest a more complex phenotype. First, the level of the W1282X transcript is reduced by nonsense-mediated RNA decay (Hamosh *et al.*, 1992; Linde *et al.*, 2007). Second, the PTC deletes part of the NBD2, which likely compromises NBD1-NBD2 dimerization and W1282X-CFTR folding and activity. Moreover, if the primary defect is corrected either with spontaneous or drug-induced read-through, some of the fully translated channel will contain nonconservative amino acid substitutions. These missense mutations may cause structural defects (class II characteristic), as suggested by the phenotype of CF patients with a missense mutation at the W1282 residue (Faucz *et al.*, 2007; Ivaschenko *et al.*, 1993; Visca *et al.*, 2008), as well as a gating defect (class III characteristic), which can be inferred based on W1282X-CFTR channel activation after exposure to VX-770 (Xue *et al.*, 2014).
- **P67L:** P67L is a mild class II mutation that results in attenuated CFTR biogenesis, as indicated by the reduced ratio between post-ER complex-glycosylated (band C) and ER-resident core-glycosylated protein (band B) (Ren *et al.*, 2013; Sosnay *et al.*, 2013; Van Goor *et al.*, 2014). Treatment with the corrector VX-809 increases the abundance of the complex-glycosylated form and PM density to nearly the level of WT-CFTR (Ren *et al.*, 2013; Veit *et al.*, 2014). However, the mutant channel is also sensitive in vitro to potentiator treatment (a class III characteristic), both in the presence and absence of corrector (Van Goor *et al.*, 2014; Veit *et al.*, 2014). Accordingly, treatment with VX-770 ameliorated the CF lung disease in a heterozygous P67L/ $\Delta F508$ patient (Yousef *et al.*, 2015).
- **R117H:** This mutation in conjunction with the 5T variant in the polythymidine tract in intron 8 was originally categorized as a class IV mutation, but it also exhibits a gating defect (class III trait) that, at least in part, can be rectified by VX-770 treatment (Sheppard *et al.*, 1993; Van Goor *et al.*, 2014). The R117H mutation also results in reduced complex-glycosylated CFTR expression, which is a class II characteristic (Fanen *et al.*, 1997; Sheppard *et al.*, 1993). This potentially explains the limited success of VX-770 treatment in patients carrying this mutation (Char *et al.*, 2014; Moss *et al.*, 2015).

AN EXPANDED CLASSIFICATION OF MUTANT CFTR BIOLOGY

We propose a modification of the current classification scheme, which would entail permutations of the traditional class I–VI CF mutations. This expanded classification of the major mechanistic categories (Welsh and Smith, 1993; Zielenski, 2000; Rowe *et al.*, 2005) accommodates the unusually complex, combinatorial molecular/cellular phenotypes of CF alleles. It consists of 31 possible classes of mutations, including the original classes I, II, III/IV, V, and VI, as well as their 26 combinations, as depicted in the Venn diagram shown in Figure 2. For the sake of simplicity, class III and IV mutations, representing functional (gating and conductance, respectively) defects, are combined. For example, according to the expanded classification, G551D will be designated as a class III mutation as before (Welsh and Smith, 1993), while $\Delta F508$ will be classified as class II–III–VI, W1282X as class I–II–III–VI, P67L as class II–III, and R117H as class II–III/IV, reflecting the composite defects in mutant CFTR biology (Figure 2 and Table 1).



A recent study by Vertex Pharmaceuticals successfully demonstrated that 24 of 54 tested missense mutations display both a processing (class II) and gating (class III) defect in the Fischer rat thyroid epithelial expression system (Van Goor et al., 2014). Characterization of several rare CF mutations is ongoing in laboratories of the CFTR2 Consortium, the CFTR Folding Consortium, CFFT, Vertex Pharmaceuticals, and many others (Caldwell et al., 2011; Yu et al., 2012; Sosnay et al., 2013; Harness-Brumley et al., 2014; Hong et al., 2014; Van Goor et al., 2014; Wang et al., 2014; Awatade et al., 2015). This work will likely provide further examples of combinatorial mechanistic defects exhibited by CF mutants.

THERAPEUTIC SUSCEPTIBILITY OF CF MUTATIONS WITH COMPLEX BIOLOGICAL DEFECTS

In-depth analysis of the biology of CF mutants distinguishes them according to their complex molecular pathology and suggests different drug combinations for treatment of different patient populations. This process, called

FIGURE 2: Refined classification of CF mutations accounting for complex phenotypes of major CFTR cellular defects. The Venn diagram indicates all combinations of mutation classes with selected examples. Possible combinations without identified mutation are indicated in gray.

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
I-II-III-VI	W1282X	X ^{1,2,3}	X ^{2,5}	X ^{2,4,5}		X ⁵	¹ HNE ² HBE ³ CFBE ⁴ CFBE ⁵ CFBE	¹ Hamosh et al., 1992 ² Cyr lab, unpublished ^a ³ Frizzell lab, unpublished ^b ⁴ Xue et al., 2014 ⁵ Lukacs lab, unpublished ^c
II-III	M1V		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor et al., 2014
II-III	E56K		X ^{5,6}	X ⁶			⁵ CFBE ^d ⁶ FRT	⁵ Lukacs lab, unpublished ⁶ Van Goor et al., 2014
II-III	P67L		X ^{3,6,7,8,9,10}	X ^{6,7,10}			³ CFBE ⁶ FRT ⁷ CFBE ⁸ Hek293 ⁹ HeLa ¹⁰ FRT	³ Frizzell lab, unpublished ⁶ Van Goor et al., 2014 ⁷ Veit et al., 2014 ⁸ Ren et al., 2013 ⁹ Sosnay et al., 2013 ¹⁰ Sorscher lab, unpublished ^e
II-III	R74W		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor et al., 2014
II-III	E92K		X ^{3,5,6,8,16}	X ⁵			³ CFBE ⁵ CFBE ⁶ FRT ⁸ HEK293 ¹⁶ HEK293	³ Frizzell lab, unpublished ⁵ Lukacs lab, unpublished ⁶ Van Goor et al., 2014 ⁸ Ren et al., 2013 ¹⁶ Brodsky lab, unpublished
II-III	P99L		X ¹¹	X ¹¹			¹¹ HeLa	¹¹ Sheppard et al., 1996
II-III	D110H		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor et al., 2014
II-III	R117C		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor et al., 2014
II-III	R117H		X ^{2,3,12,13}	X ^{2,6,12}			² HBE ³ CFBE ⁶ FRT ¹² FRT, HeLa ¹³ HeLa	² Cyr lab, unpublished ³ Frizzell lab, unpublished ⁶ Van Goor et al., 2014 ¹² Sheppard et al., 1993 ¹³ Fanen et al., 1997

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes.

Continues

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
II-III	R170G		X ^{7,14}	X ⁷			⁷ CFBE ¹⁴ BHK	⁷ Veit <i>et al.</i> , 2014 ¹⁴ Okiyoneda <i>et al.</i> , 2013
II-III	E193K		X ⁵	X ^{5,6}			⁵ CFBE ⁶ FRT ^f	⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II-III	P205S		X ¹¹	X ¹¹			¹¹ HeLa	¹¹ Sheppard <i>et al.</i> , 1996
II-III	L206W		X ^{5,6,8}	X ^{5,6}			⁵ CFBE ⁶ FRT ⁸ HEK293	⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ⁸ Ren <i>et al.</i> , 2013
II-III	V232D		X ¹⁵	X ¹⁵			¹⁵ HEK293	¹⁵ Caldwell <i>et al.</i> , 2011
II-III	R334W		X ^{2,3,5}	X ^{2,5,6,12}			² COS-7 ³ CFBE ⁵ CFBE ⁶ FRT ^f ¹² HeLa	² Cyr lab, unpublished ³ Frizzell lab, unpublished ⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹² Sheppard <i>et al.</i> , 1993
II-III	I336K		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	T338I		X ^{5,6}	X ^{5,6}			⁵ CFBE ⁶ FRT	⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II-III	S341P		X ^{5,6}	X ^{5,6}			⁵ CFBE ⁶ FRT	⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II-III	A455E		X ^{3,6,16,17}	X ⁶			³ CFBE ⁶ FRT ¹⁶ HEK293 ¹⁷ FRT, HeLa ^d	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹⁶ Brodsky lab, unpublished ⁹ ¹⁷ Sheppard <i>et al.</i> , 1995
II-III	S549R		X ^{3,5,18}	X ^{5,18}			³ CFBE ⁵ CFBE ¹⁵ FRT	³ Frizzell lab, unpublished ⁵ Lukacs lab, unpublished ¹⁸ Yu <i>et al.</i> , 2012
II-III	D579G		X ^{5,6}	X ^{5,6}			⁵ CFBE ⁶ FRT	⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II-III	R668C		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	L927P		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	S945L		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	S977F		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	L997F		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	H1054D		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	R1066H		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	A1067T		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	R1070Q		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	R1070W		X ^{6,14}	X ⁶			⁶ FRT ¹⁴ BHK	⁶ Van Goor <i>et al.</i> , 2014 ¹⁴ Okiyoneda <i>et al.</i> , 2013
II-III	F1074L		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-III	D1270N		X ⁶	X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II-VI	S492F		X ^{3,5,6}			X ⁵	³ CFBE ⁵ CFBE ⁶ FRT	³ Frizzell lab, unpublished ⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II-III-VI	R347P		X ^{3,5,6}	X ^{5,6,12}		X ⁵	³ CFBE ⁵ CFBE ⁶ FRT ¹² HeLa	³ Frizzell lab, unpublished ⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹² Sheppard <i>et al.</i> , 1993
II-III-VI	ΔF508		X ¹⁹	X ²⁰		X ²¹	¹⁹ COS ²⁰ Vero ²¹ CHO	¹⁹ Cheng <i>et al.</i> , 1990 ²⁰ Dalemans <i>et al.</i> , 1991 ²¹ Lukacs <i>et al.</i> , 1993

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes.

Continues

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
II-III-VI	A561E		X ^{6,22,23}	X ²³		X ²³	⁶ FRT ^d ²² HBE ^d ²³ BHK	⁶ Van Goor <i>et al.</i> , 2014 ²² Awatade <i>et al.</i> , 2015 ²³ Wang <i>et al.</i> , 2014
II-III-VI	L1077P		X ^{3,6,16,24}	X ²⁴		X ²⁴	³ CFBE ⁶ FRT ¹⁶ HEK293 ²⁴ CHO	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹⁶ Brodsky lab, unpublished ²⁴ Sheppard lab, unpublished ^h
II-III-VI	N1303K		X ^{2,3,5,6,16,22,24}	X ²⁴		X ⁵	² HBE ³ CFBE ⁵ CFBE ⁶ FRT ¹⁶ HEK293 ²² HBE ²⁴ CHO ⁱ	² Cyr lab, unpublished ³ Frizzell lab, unpublished ⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹⁶ Brodsky lab, unpublished ²² Awatade <i>et al.</i> , 2015 ²⁴ Sheppard lab, unpublished
III-VI	Q1411X			X ²⁵		X ²⁶	²⁵ BHK ²⁶ Cos, BHK	²⁵ Gentsch <i>et al.</i> , 2002 ²⁶ Haardt <i>et al.</i> , 1999
II	A46D		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	G85E		X ^{3,6,16,24}				³ CFBE ⁶ FRT ¹⁶ HEK293 ²⁴ CHO	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹⁶ Brodsky lab, unpublished ²⁴ Sheppard lab, unpublished
III	R352Q			X ^{5,6}			⁵ CFBE ⁶ FRT ^f	⁵ Lukacs lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II	L467P		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	V520F		X ^{3,6,16}				³ CFBE ⁶ FRT ¹⁶ HEK293	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹⁶ Brodsky lab, unpublished
II	A559T		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	R560S		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	R560T		X ^{3,6,16}				³ CFBE ⁶ FRT ¹⁶ HEK293	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014 ¹⁶ Brodsky lab, unpublished
II	R560K		X ³				³ CFBE	³ Frizzell lab, unpublished
II	Y569D		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	D614G		X ³				³ CFBE	³ Frizzell lab, unpublished
II	L1065P		X ^{3,6}				³ CFBE ⁶ FRT	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II	R1066C		X ^{3,6}				³ CFBE ⁶ FRT	³ Frizzell lab, unpublished ⁶ Van Goor <i>et al.</i> , 2014
II	R1066M		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	H1085R		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
II	M1101K		X ⁶				⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
III	D110E			X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
III	G178R			X ¹⁸			¹⁸ FRT	¹⁸ Yu <i>et al.</i> , 2012
III	R347H			X ^{6,7}			⁶ FRT ⁷ CFBE	⁶ Van Goor <i>et al.</i> , 2014 ⁷ Veit <i>et al.</i> , 2014
III	S549N			X ¹⁸			¹⁸ FRT	¹⁸ Yu <i>et al.</i> , 2012
III	G551D			X ^{27, 28}			²⁷ CHO ²⁸ L	²⁷ Bompadre <i>et al.</i> , 2007 ²⁸ Yang <i>et al.</i> , 1993
III	G551S			X ¹⁸			¹⁸ FRT	¹⁸ Yu <i>et al.</i> , 2012

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes.

Continues

Refined classification	Mutation	I	II	III/IV	V	VI	Model	Reference
III	F1052V			X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
III	K1060T			X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
III	D1152H			X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
III	S1235R			X ⁶			⁶ FRT	⁶ Van Goor <i>et al.</i> , 2014
III	G1244E			X ¹⁸			¹⁸ FRT	¹⁸ Yu <i>et al.</i> , 2012
III	S1251N			X ¹⁸			¹⁸ FRT	¹⁸ Yu <i>et al.</i> , 2012
III	S1255P			X ¹⁸			¹⁸ FRT	¹⁸ Yu <i>et al.</i> , 2012
III	G1349D			X ^{18,27}			¹⁸ FRT ²⁷ CHO	¹⁸ Yu <i>et al.</i> , 2012 ²⁷ Bompadre <i>et al.</i> , 2007

Superscript numbers refer to references in far-right column.

^aS.A.H. and D.M.C., unpublished observations

^bK.W.P. and R.A.F., unpublished observations.

^cR.G.A., H.Xu, and G.L.L., unpublished observations.

^dDoes not exhibit a gating or conductance defect in this cell model.

^eJ.S.H. and E.J.S., unpublished observations.

^fDoes not exhibit a biogenesis defect in this cell model.

^gA.N.C. and J.L.B., unpublished observations.

^hZ.C. and D.N.S., unpublished observations.

ⁱDoes not exhibit a peripheral stability defect in this cell model.

TABLE 1: Examples for CF mutations with complex or classical cellular phenotypes. Continued

“theratyping” (Cutting, 2015), will pave the way to personalized medicine in CF. However, reliable prediction of the responsiveness of a mutant phenotype to pharmacotherapy could be challenging and is dependent on the cellular model system (Pedemonte *et al.*, 2010).

Emerging evidence also suggests that the efficacy of approved and preclinical drugs may vary with different mutations within the same class. For example, while nearly complete processing correction of P67L- and R170G-CFTR (class II) was achieved with VX-809 treatment (Okiyoneda *et al.*, 2013; Ren *et al.*, 2013; Veit *et al.*, 2014), VX-809 only partially reversed the folding defect of some other class II mutants; for example, N1303K and Δ F508 (Okiyoneda *et al.*, 2013; Awatade *et al.*, 2015). This differential susceptibility to correction is attributed to the nature of the primary folding/structural defect. According to one hypothesis, robust folding correction of Δ F508-CFTR requires corrector combinations to avert its NBD1-MSD1/2 interface and NBD1 stability defects (Mendoza *et al.*, 2012; Rabeh *et al.*, 2012; He *et al.*, 2013; Okiyoneda *et al.*, 2013). The N1303K mutation in NBD2 was not rescued by VX-809, and only modest processing was observed by targeting both the NBD1/MSDs and NBD2 interfaces with C4 and C18 (a VX-809 analogue) (Okiyoneda *et al.*, 2013; Rapino *et al.*, 2015).

Some of the class III mutations also respond differently to the gating potentiator VX-770. Although R347H- and T338I-CFTR cause severe functional defects with no or modest loss of protein expression, only R347H-CFTR is potentiated by VX-770 to near wild type-like conductance (Van Goor *et al.*, 2014). Likewise, the P5 potentiator activates Δ F508-CFTR, but it has no effect on G551D-CFTR chloride permeation (Yang *et al.*, 2003). Thus identification of mutation-specific novel potentiators or their combinations may further optimize channel rescue for specific class III/IV mutations. Additive enhancement of G551D-CFTR activity by the combination of the potentiators genistein and curcumin supports the feasibility of combining potentiators (Yu *et al.*, 2011). Likewise, we envision that mutation-specific read-through drugs will ultimately need to be combined with other correctors and potentiators, based on the pleiotropic

defects associated with this class of mutations (as illustrated for W1282X above).

CONCLUDING REMARKS

The ultimate goal of theratyping is to achieve optimal correction of a specific mutant defect by selecting the most efficacious CFTR modulator(s), including correctors(s), potentiator(s), and/or read-through drugs, or a combination of these drugs. Based on accumulating observations, however, mechanistic subdivisions of some of the major classes of mutations (classes I, II, and III) may be necessary to further improve the success of drug-selection strategies. This will facilitate the theratyping of CF alleles and their combinations and expedite the identification and approval process for combination therapies. Theratyping has already proven successful in identifying class III mutations that are responsive to VX-770 (Yu *et al.*, 2012), leading to the approval of this drug for eight rare mutations besides G551D (Vertex, 2014a). In fact, the results of large-scale theratyping could be overlaid as a third dimension on the Venn diagram presented in Figure 2.

Thus, during the 22 years following the initial classification of CF mutations (Welsh and Smith, 1993), our understanding of the molecular complexity of CF alleles has evolved remarkably, establishing the need for an advanced mutation classification scheme in conjunction with personalized CF therapy.

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