Published in final edited form as:

Pharmacogenet Genomics. 2015 March ; 25(3): 149–156. doi:10.1097/FPC.0000000000000112.

PharmGKB summary: very important pharmacogene information for CFTR

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Keywords

CFTR; cystic fibrosis; pharmacogenetics; pharmacodynamics; ivacaftor; potentiator; corrector; modulator

Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR, ATP-binding cassette sub-family C, member 7, ABCC7) protein is 1480 amino acids in length. It is encoded by a single large gene with 27 exons spanning around 250kbp on chromosome 7q31.2, identified in the search to find the gene underlying cystic fibrosis (CF) disease [1–4]. The protein structure is made up of two units, each with six transmembrane helices and an intracellular nucleotide-binding domain (NBD) that can interact with adenosine triphosphate (ATP). A regulatory “R” domain connects the two units and contains sites for protein kinase phosphorylation [1]. The structure creates a channel in the plasma membrane through which anions can flow, and the gate is thought to be opened and closed by ATP binding and hydrolysis (NBDs) and phosphorylation mechanisms (R domain) which alter the protein's conformation [1, 5, 6].

CFTR is expressed predominantly in epithelial tissues, but is also found in other cell types such as smooth muscle, cardiac myocytes, macrophages, and erythrocytes [1]. CFTR is multi-functional. It is an anion channel that transports chloride (Cl⁻) and bicarbonate. It is also involved in the regulation of a range of transporters including the epithelial sodium channel (ENaC encoded by *SCNN1A*, *SCNN1B*, *SCNN1D* and *SCNN1G*) and outwardly rectifying chloride channels (ORCC) [1, 7–10]. In addition, CFTR has been proposed to be a hub for signaling pathways and may regulate a variety of other physiological processes including exocytosis and endocytosis, ATP export, proinflammatory cytokine expression

and intracellular pH [1, 10]. Defective CFTR therefore results in widespread cellular homeostasis dysfunction [10].

CF is an autosomal recessive disease resulting from a defect-causing variant on each *CFTR* allele. More than 1800 variants in the *CFTR* gene have been reported [11]. Despite a large collection of variants, there is a gap in our knowledge regarding which cause CF disease. To address this, the Clinical and Functional Translation of *CFTR* project was established to collect information regarding the functional consequences and resulting phenotypes associated with *CFTR* variants [12, 13]. Data for 39,696 subjects from 25 CF patient registries or specialty clinics were collected for the database, and an initial set of 159 *CFTR* variants (those with a frequency of $\geq 0.01\%$ in the CFTR2 database) was evaluated for whether they cause CF disease by both clinical phenotype and functional analysis [12]. A variant was defined clinically as causing CF if mean sweat chloride concentration was $\geq 60\text{mM}$ for at least three individuals with the variant or $\geq 90\text{mM}$ if only 2 individuals with the variant were available; 140 variants met the clinical criteria to be CF-causing. The variants were sorted by their predicted functional effect, and 77 were investigated further using *in vitro* assays appropriate to the genetic variant ($<10\%$ of wild-type CFTR function was considered disease-causing); 133 variants were deemed CF-causing by functional criteria. In total, 127 variants met both the clinical and functional criteria, and were defined as CF-causing. Penetrance analysis in fathers with CF children was carried out on the variants that did not meet both/either criteria and 12 variants were deemed non-CF causing, with the remaining 20 variants indeterminate [12, 13].

CF is a disease that predominantly affects the lungs but has a diverse array of phenotypes due to the expression of CFTR in different tissues, its wide-ranging physiological role, and its involvement in many signaling pathways [1, 7, 10]. Progressive lung disease, pancreatic dysfunction, infertility in males and elevated sweat electrolytes characterize a “classical” CF diagnosis [14]. CF is also associated with a reduced life expectancy (early adulthood) and an increased risk of cancer [1, 10]. There is, however, wide variability in clinical presentation, severity and the rate of disease progression between patients, which can be influenced by the underlying *CFTR* genotype as well as other genetic modifiers and environmental factors [1, 7, 10, 14–17]. The incidence of CF is thought to be around 70,000 cases worldwide [18], though it may be largely under-diagnosed in parts of Asia, Africa and Latin America [14, 19]. Genetic testing is now a routine part of CF diagnosis in many countries. A recommended panel for genetic screening for determining prenatal and preconception carrier status of CF in the US includes 23 *CFTR* variants, designed to cover variants with a frequency of at least 0.1% in CF patients that are associated with classical CF disease, for a pan-ethnic US population [20–22]. The WHO recommends sequencing of the complete *CFTR* gene in CF patients from populations where CF is likely under-diagnosed in order to establish panels of population-specific variants known to cause disease [14].

Pharmacogenetics (PGx)

Traditionally, drugs used in the treatment of CF have focused on ameliorating symptoms, fighting infection, thinning mucus and dampening inflammation, rather than directly targeting the cause: variants in the *CFTR* gene. Gene therapy techniques aimed at replacing

defective *CFTR* with a functional version of the gene have been extensively researched and remain a hope for curing CF after the discovery of the underlying disease cause. Unfortunately, gene therapy has encountered several barriers that have kept it from becoming a treatment option for CF [23], though the results of an ongoing clinical trial are eagerly awaited [24, 25]. Drugs that are designed to correct specific defects of the *CFTR* protein are being developed as novel therapies for CF; these are termed “modulators” of *CFTR*. Repurposing of drugs for CF treatment due to their mechanism of action as a *CFTR* modulator is also a potential therapeutic option (see [1]). This summary focuses on pharmacogenetics, and thus therapies that directly target defects resulting from variants in the *CFTR* gene. An interactive version of this summary, with links to further study information, is available online at <https://www.pharmgkb.org/vip/PA109>.

CFTR variants can be grouped into 6 classes depending on the resulting effect on the protein, and each could potentially be targeted by a treatment strategy aimed at the underlying defect: see Table 1. Modulator molecules also have the benefit of being administered orally, thus potentially targeting multiple organs and cell types affected by a defect in *CFTR* [26]. Included in the spectrum of modulators are “correctors” and “potentiators”. Correctors are molecules that ‘correct’ the misfolding/trafficking of defective *CFTR* protein to increase expression at the cell surface, whereas potentiators enhance the channel opening of the defective protein within the cell membrane [27].

Currently, the most commonly accepted efficacy endpoints for late phase clinical trials in CF include lung function (forced expiratory volume in one second), pulmonary exacerbation rates, growth/body mass index, and patient reported outcomes [28]. Additional outcome measures are in development and may serve to accelerate *CFTR* modulator development in CF, including multiple breath washout (lung clearance index), pulmonary imaging (including computed tomography and mucociliary clearance), cardiopulmonary exercise testing, gastrointestinal pH, a variety of sputum biomarkers and changes in microbiome [29–32]. Change in mean sweat chloride concentration is also currently used as a biomarker of CF; however, there is controversy regarding sweat chloride as a predictive biomarker for improvement in lung function [33–35]. Guidelines recommending particular biomarkers for CF therapy trials have been published [36]. Modulators may have different effects in different tissues/cells which should be taken into account when personalizing CF treatment for an individual patient [26].

Important PGx variants

G551D-*CFTR*

Variant mapping information: c. 1652G>A (NM_000492.3), Gly551Asp (NP_000483.3), rs75527207.

This variant is a single nucleotide polymorphism (SNP) at position c.1652G>A that causes a Gly to Asp change at amino acid position 551. The resulting G551D-*CFTR* protein belongs to the class III group of *CFTR* variants: it is expressed at the plasma membrane but is defective in ATP hydrolysis and channel gating (Table 1) [1, 5]. In homozygotes, or when in combination with another nonfunctional disease-causing allele, it is associated with causing

CF characterized by a pancreatic insufficiency phenotype [13]. It is one of the variants in a recommended panel for newborn screening of CF by the American College of Medical Genetics [21]. Global allele frequencies are calculated at 0.02 in Caucasian, 0.025 in African, 0.004 in Mexican, 0.003 in South American, 0.002 in Mediterranean and 0.001 in Middle Eastern CF patients (see CPIC *CFTR*-ivacaftor guideline supplement for individual references) [37].

Pharmacogenetics

Ivacaftor (VX-770, kalydeco) is a potentiator and is the first FDA-approved therapeutic developed to target a specific *CFTR* defect. It was originally indicated in CF patients 6 years and older who have at least one *G551D* variant (rs75527207 genotype AA or GA) [37]. Ivacaftor targets the gating defect of *G551D*-*CFTR* to enhance activity; *in vitro* studies show it enhances open channel probability and increases chloride transport of *G551D*-*CFTR* expressing cells [38–43]. It has also been shown to enhance cilia beating and decrease sodium absorption in bronchial epithelial cells from a patient with the *G551D/F508del* genotype [39]. It may also have anti-bacterial properties [44]. *In vivo*, clinical trials or case reports of CF patients with at least one copy of the *G551D* variant demonstrate improved lung function with ivacaftor treatment [28–30, 41, 45–47]. The response in patients with severe CF with at least one copy of the *G551D* variant seems variable, and a case report suggests that the drug may be more effective in patients homozygous for *G551D*-*CFTR* [46–49]. Ivacaftor may delay or prevent the development of diabetes or even resolve diabetes in CF patients, though more investigation into these effects is needed [50, 51]. As ivacaftor is such a new therapeutic, it is not currently possible to assess its long-term efficacy or harm-benefit balance, discussed further in [52], though a recent study provides results up to 144 weeks of treatment [53].

The indication section of the FDA-approved ivacaftor drug label was amended in February 2014 to include a further eight *CFTR* variants (corresponds to a total of ten genetic variants, listed in Table 2) [54]. All variants show defects in gating *in vitro*, as measured by decreased open channel probability and chloride transport, compared to wild-type *CFTR* [38]. The FDA-approved drug label refers to a clinical trial carried out in 39 patients who had at least one of these variants. Clinical trials of ivacaftor in patients with one of a number of non-*G551D* *CFTR* gating variants are currently underway or have been completed [55, 56], and results from the phase 3 KONNECTION study were recently announced in press releases and at the 37th European CF Society conference (June 2014) [57]. However, to our knowledge, no published peer-reviewed clinical data regarding these trials are available to date.

Ivacaftor alone or in combination with other drugs may also be effective in patients with *CFTR* variants other than the gating defects listed in Table 2, as has been shown *in vitro* [43]. A clinical trial in patients with the *R117H* (c.350G>A, rs78655421) variant has been completed (KONDUCT study), results from this trial and a rollover study of patients who completed this trial have been announced in press releases and at the 37th European CF Society conference (June 2014) but to our knowledge are not currently published in peer-reviewed literature [58]. According to press releases, Vertex plans to submit a supplemental

New Drug Application in the USA and a marketing authorization application variation in Europe for patients 18 years and older who have the *R117H* variant.

F508del-CFTR

Variant mapping information: c.1521_1523delCTT and c. 1520_1522delTCT (NM_000492.3), Phe508del (NP_000483.3), rs113993960 and rs199826652, respectively. Also referred to as Δ F508, F508del.

This variant was originally identified in 1989 after comparison of cDNA sequences from patients with CF and unaffected individuals [59]. It currently has two dbSNP reference sequence IDs (rsIDs) that represent the same variant, likely due to differences in how the DNA sequence can be read. Rs113993960 refers to a CTT deletion at cDNA position c. 1521_1523delCTT (NM_000492.3). This is the cDNA sequence referred to by the Cystic Fibrosis Mutation Database [11] and is flagged by the ClinVar database as having a pathogenic allele [60]. Rs199826652 refers to a TCT deletion (cDNA sequence 1520_1522) and is more likely to be called in sequencing analysis due to the left justification of indels - a minor allele count of 0.006 is provided from 1000 genomes [61].

Whichever way the DNA sequence is read, the 3 basepair deletion ultimately results in a loss of a phenylalanine amino acid at position 508 in the NBD of the protein [59]. It is a class II variant: F508del-CFTR gets trapped in the endoplasmic reticulum where it is prematurely degraded and largely fails to traffic to the plasma membrane (Table 1) [1, 10, 62]. It is associated with causing CF (in homozygotes or when in combination with another disease-causing allele) and is also associated with pancreatic insufficiency [13]. In most populations, this is the most frequent CF-causing *CFTR* variant, although allele frequencies vary in different population groups of CF patients, from 100% in an isolated Danish population to around 20% in Turkey [14]. In Ashkenazis from Israel the *W1282X-CFTR* (c. 3846G>A, rs77010898) variant is more common than *F508del-CFTR* [14]. Global frequencies are estimated at 0.66 in Caucasian, 0.48 in Mediterranean, 0.44 in Mexican, 0.42 in African, 0.39 in South American, and 0.21 in Middle Eastern CF patients (see CPIC *CFTR*-ivacaftor guideline supplement for individual references) [37].

Pharmacogenetics

Numerous different correctors targeting F508del-CFTR defective function are being identified, developed and investigated (Table 1) [1, 63–66]. Lumacaftor (VX-809) is an investigational drug currently undergoing clinical trials that acts as a corrector [67–69]. *In vitro* it is thought to improve F508del-CFTR maturation and chloride transport by suppressing the folding defect and increasing exit from the ER, though F508del-CFTR remains thermodynamically unstable [70–72]. Human bronchial epithelial cells from *F508del-CFTR* homozygous patients treated with lumacaftor were reported to have enhanced CFTR maturation and chloride secretion *in vitro* [70]. In a clinical trial to assess safety in CF patients homozygous for *F508del-CFTR*, sweat chloride levels were significantly decreased in patients given 100mg/day or 200mg/day lumacaftor over 28 days compared to placebo-treated patients. No improvements in other clinical parameters were observed; however, the study was not powered to determine differences in these

measurements [73]. Clinical trials of other correctors have also failed to show clinical efficacy in CF patients homozygous for *F508del-CFTR* (Table 1 footnote) [74]. Due to the complex nature of the *F508del-CFTR* defect, it is likely that combinations of correctors, and/or correctors in combination with a potentiator will be necessary to achieve clinical efficacy in these patients [74, 75]. Compounds with dual corrector and potentiator activities have been reported *in vitro* and may have therapeutic potential [26, 76].

Currently, ivacaftor monotherapy is not recommended in CF patients homozygous for the *F508del-CFTR* variant (rs113993960 or rs199826652 genotype del/del) [37, 54]. This may be due to ivacaftor's mechanism of action as a potentiator (Table 1). Since *F508del-CFTR* is a class II variant that results in minimal cell surface protein expression, ivacaftor would likely be ineffective in these patients. Indeed, a study examining the safety of ivacaftor in CF patients homozygous for the *F508del-CFTR* variant saw no differences in efficacy compared to placebo; however, was not powered to examine efficacy [77]. *In vitro* studies suggest that if expression to the cell surface is restored (by temperature treatment, a correcting mutation or a cell-free system), ivacaftor can potentiate *F508del-CFTR* activity [38–40, 43, 78–80]. Therefore, the hypothesis is that combination therapy with a potentiator such as ivacaftor and a corrector may be effective in these patients. *In vitro*, ivacaftor addition potentiates lumacaftor-corrected *F508del-CFTR* [70, 80]. Results from a double-blind, placebo-controlled phase 2 study in patients either homozygous or heterozygous for *F508del-CFTR* were recently published (NCT01225211) [81]; results for the clinical efficacy of the combination compared to placebo were less than the effect of ivacaftor monotherapy that has been demonstrated in patients with *G551D-CFTR*. The one treatment arm that showed significant differences in absolute change in percent predicted FEV₁ compared to placebo at treatment completion, was the group of patients homozygous for *F508del-CFTR* treated with a monotherapy of 600mg lumacaftor once per day for 28 days, followed by combination with 250mg ivacaftor twice a day for an additional 28 days. In this treatment group, the occurrence of dyspnoea and chest tightness during both monotherapy and combination therapy periods, compared to no occurrences in the placebo group, is of concern (at least one patient in each treatment arm withdrew due to an adverse event starting on day 1 of lumacaftor monotherapy). In this cohort, the placebo group was a mix of *F508del-CFTR* homozygotes and heterozygotes. This is therefore not reflective of the wholly homozygous or heterozygous patient treatment arms within this cohort that were compared against this mixed placebo group. Comparing treatment efficacy in patients with varying genotypes can introduce confounders that could influence study outcomes. Another issue was the lack of correlation between FEV₁ responses and sweat chloride responses observed, limiting the use of sweat chloride to predict *F508del-CFTR* restoration.

Other registered clinical trials in *F508del* homozygous patients investigating a combinational treatment of ivacaftor with lumacaftor are currently ongoing [67–69]. Results at 24 weeks of the TRAFFIC and TRANSPORT Phase 3 studies were recently announced by press release, though to our knowledge are not yet published in peer-reviewed literature. Chronic treatment of primary epithelial cells homozygous for *F508del-CFTR* or *F508del-CFTR*-expressing cell lines with ivacaftor or several other potentiators has recently been

reported to reduce stability and increase turnover of lumacaftor-corrected F508del-CFTR, and thus may have implications for long-term treatment [26, 82, 83].

Conclusion

Cystic fibrosis (CF) is a life-shortening autosomal recessive disease, caused by variants in the *CFTR* gene, with considerable treatment burden and morbidity. Strategies to modify defects in CFTR are being developed in a potential new wave of CF therapies. The first to be approved by the FDA, a potentiator named ivacaftor, targets CFTR protein variants defective in gating and is indicated in patients who carry certain underlying *CFTR* genetic variants. Correctors and combinations of modulators are currently in clinical trials in patients with the commonly found *F508del-CFTR* variant. Future hopes are a panel of therapies that can be tailored for a patient's underlying genetic variants for a more effective treatment strategy to minimize symptoms and extend longevity.

Acknowledgements

This work is supported by the NIH/NIGMS (R24 GM61374).

Conflicts of Interest

RBA and TEK are stockholders in Personalis Inc. JPC has received grant support from Vertex Pharmaceuticals to conduct clinical trials with CFTR modulators in CF patients. He has received honoraria from Vertex Pharmaceuticals for educational presentations regarding the appropriate use of ivacaftor in CF patients.

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Table 1

CFTR variants and potential treatment strategy^d

Class	Description	Associated CF phenotype	Example variants ^b	Potential treatment strategy that may target this class	Potential examples of Possible drugs/compounds ^c
I	Cause splicing defects, frameshift mutations or a premature stop codon resulting in a lack of CFTR expression and impaired biosynthesis.	Severe.	W1282X (c.3846G>A, rs77010898), G542X (c.1624G>T rs113993959), R553X (c.1657C>T rs74597325).	A suppressor which prevents premature termination by reading through premature termination codons. This allows for complete translation.	Gentamicin (repurposed from use as an anti-biotic). Synthetic aminoglycoside NB124 [84]. Ataluren (PTC-124); in a Phase 3 clinical trial it did not improve lung function in the overall CF patient population, but may be beneficial in patients not receiving chronic inhaled tobramycin [85]. There is debate over whether ataluren has suppressor function [86-88], and whether it may resurrect dormant retroelements [89].
II	Result in an immature protein that is consequently mostly degraded.	Severe.	F508del (c.1521_1523delCTT rs113993960 or rs199826652), N1303K (c.3909C>G rs80034486).	A corrector, which restores folding and increases trafficking to the membrane and/or a potentiator which increases CFTR open channel probability/gating.	See lists ^{d,e} .
Class III	Result in proteins that are present at the plasma membrane but have disrupted activation or regulation, resulting in defective CFTR channel gating.	Severe.	G551D (c.1652G>A rs75527207).	A potentiator, which increases CFTR open probability/gating.	Ivacaftor for variants detailed in Table 2, for other class III variants see list ^e .
Class IV	Result in CFTR present at the plasma membrane but with reduced conductance of chloride.	Mild.	R347P (c.1040G>C rs77932196), R334W (c.1000C>T rs121909011).	A potentiator which increases gating may be able to overcome reduced channel conductance.	See list ^e .
Class V	Result in partly defective processing or synthesis of CFTR.	Mild.	3272-26 A>G (c.3140-26A>G), 3849 +10kb C>T (c.3717+12191C>T, rs75039782).	A potentiator, which increases gating may be able to overcome reduced CFTR availability.	See list ^e .
Class VI	Result in CFTR present at the	Severe.	1811 + 1.6kb A>G	Drugs that stabilize	

Class	Description	Associated CF phenotype	Example variant ^b	Potential treatment strategy that may target this class	Potential examples of Possible drugs/compounds ^c
	plasma membrane but with reduced conductance of ions (not including chloride) or reduced membrane stability.		(c.1679+1.6kbA>G), corrected F508del.	CFTR at the plasma membrane.	

^aTable based on [1, 6, 7, 27, 90] with additional references as indicated below.

^ball examples of variants are CF disease-causing variants.

^cFor most of the compounds listed, toxicity studies and clinical trials in CF patients have not been carried out to date.

^dExamples of potential drugs/compounds that may function as CFTR correctors: Lumacaftor (VX-809)*, 4-phenylbutyrate*, miglustat, sildenafil, vardenafil, taladafil, suberoylanilide hydroxamic acid, VRT-325, CF-106951, VX-661, KM11060, Corr 2a, 3a, 4a, 4b, benzoquinolizinium, curcumin*, glatiramer, RDRI [1, 6, 7, 27, 64, 70, 71, 74, 91, 92].

^eExamples of potential drugs/compounds that may function as CFTR potentiators: ivacaftor (VX-770) (indicated for variants in Table 2), phloxine B, genistein, GPact-11a, NS004, resveratrol, phenylglycine PG-01, curcumin [1, 6, 7, 26, 27, 74, 93].

* Compounds that lacked efficacy in clinical trials with F508del-CFTR homozygous patients (reviewed in [74]).

Table 2

CFTR variants included in the indication for ivacaftor^a

	Legacy name ^{a,b}	rsID ^b	cDNA reference ^{b,c}	Protein reference ^{b,d}	Exon ^b	CF causing? ^e	Allele frequency ^e	Published evidence
1.	G551D	rs75527207	1652G>A	Gly551Asp	12	Yes	0.0202	<i>In vitro</i> and clinical data [28, 38–43, 45–48].
2.	S549N	rs121908755	1646G>A	Ser549Asn	12	Yes	0.0013	<i>In vitro</i> studies [38, 94] and a case study of a 12 year old girl with this variant who showed improved lung function after ivacaftor treatment [95].
3.	G1244E	rs267606723	3731G>A	Gly1244Glu	23	Yes	0.0007	<i>In vitro</i> study with CFTR variant-expressing Fisher Rat Thyroid cells showing significantly enhanced channel open probability [38].
4.	G1349D	rs193922525	4046G>A	Gly1349Asp	25	NA	NA	
5.	G178R	rs80282562	532G>A	Gly178Arg	5	Yes	0.0007	
6.	G551S	rs121909013	1651G>A	Gly551Ser	12	NA	NA	
7.	S1251N	rs74503330	3752G>A	Ser1251Asn	23	Yes	0.0012	
8.	S1255P	rs121909041	3763T>C	Ser1255Pro	23	NA	NA	
9.	S549R	rs121908757	1645A>C	Ser549Arg	12	Yes	0.0007	
10.		rs121909005	1647T>G					

^a according to the FDA-approved drug label for ivacaftor, amended 21st Feb 2014 [54].

^b Information from the CFTR mutation database [11].

^c cDNA sequence: NM_000492.3.

^d Protein sequence: NP_000483.3.

^e As reported in [12]. Allele frequency in CFTR2 = alleles/70,777. NA indicates that it was not included in supplemental table S2 of reference [12].