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CFTR modulator therapy for cystic fibrosis caused by the rare c.3700A>G mutation

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Abstract

BACKGROUND: The c.3700A>G mutation, a rare cystic fibrosis (CF)-causing CFTR mutation found mainly in the Middle East, produces full-length transcript encoding a missense mutation (I1234V-CFTR), and a cryptic splice site that deletes 6 amino acids in nucleotide binding domain 2 (I1234del-CFTR).

METHODS: FRT cell models expressing I1234V-CFTR and I1234del-CFTR were generated. We also studied an I1234del-CFTR-expressing gene-edited human bronchial (16HBE14o-) cell model, and primary cultures of nasal epithelial cells from a c.3700A>G homozygous subject. To identify improved mutation-specific CFTR modulators, high-throughput screening was done using I1234del-CFTR-expressing FRT cells. Motivated by the in vitro findings, Trikafta was tested in two c.3700A>G homozygous CF subjects.

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AUTHOR CONTRIBUTIONS

Puay-Wah Phuan: Performed Experiments, Data Analysis, Writing, **Peter M. Haggie:** Conceptualization, Methodology, Writing, Writing – Review & Editing, Funding Acquisition, **Joseph A. Tan:** Performed Experiments, Data analysis, **Amber A. Rivera:** Performed Experiments, **Walter E. Finkbeiner:** Methodology, **Dennis W. Nielson:** Investigation, Methodology, Writing, Writing – Review & Editing **Merlin M. Thomas:** Investigation, **Ibrahim A. Janahi:** Conceptualization, Investigation, Resources, Writing – Review & Editing, **Alan S. Verkman:** Conceptualization, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition

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CONFLICT OF INTEREST STATEMENT

Drs. Phuan, Haggie and Verkman are named co-inventors on a patent filing on co-potentiators, whose rights are owned by the University of California.

RESULTS: FRT cells expressing full-length I1234V-CFTR had similar function to that of wildtype CFTR. I1234del-CFTR showed reduced activity, with modest activation seen with potentiators VX-770 and GLPG1837, correctors VX-809, VX-661 and VX-445, and low-temperature incubation. Screening identified novel arylsulfonyl-piperazine and spiropiperidine-quinazolinone correctors, which when used in combination with VX-445 increased current ~2-fold compared with the VX-661/VX-445 combination. The combination of VX-770 with arylsulfonamide-pyrrolopyridine, piperidine-pyridoindole or pyrazolo-quinoline potentiators gave 2–4-fold greater current than VX-770 alone. Combination potentiator (co-potentiator) efficacy was also seen in gene-edited I1234del-CFTR-expressing human bronchial epithelial cells. In two CF subjects homozygous for the c.3700A>G mutation, one subject had a 27 mmol/L decrease in sweat chloride and symptomatic improvement on Trikafta, and a second subject showed a small improvement in lung function.

CONCLUSIONS: These results support the potential benefit of CFTR modulators, including co-potentiators, for CF caused by the c.3700A>G mutation.

Keywords

Cystic fibrosis; I1234V; c.3700A>G; CFTR modulators; co-potentiators; N-of-1 clinical trials

INTRODUCTION

Cystic fibrosis (CF) is caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a cAMP-activated chloride channel expressed in the lungs, pancreas and other tissues (1, 2). More than 2000 CFTR gene variants have been identified, with >300 associated with CF of varying severity (1, 3). Although F508del is the most prevalent mutation in Europe and the US, there is considerable regional variability in many CFTR mutations (4). The c.3700A>G mutation is a relatively common CF-causing mutation in the Middle East (5–8), present in nearly all CF subjects in Qatar and ~11 % of CF subjects in Saudi Arabia. Clinically, the c.3700A>G mutation is associated with lung disease, lung infections, low bone mineral density, and late-onset pancreatic insufficiency, though disease severity is variable (6, 8–10). As originally reported by Molinski, Bear and colleagues (11), c.3700A>G mutation results in the generation of a full-length protein with a missense mutation (I1234V-CFTR), and also creates a cryptic splice site that results in deletion of six amino acids (p.Ile1234_Arg1239del) in nucleotide binding domain 2 (NBD2) (herein referred to as I1234del-CFTR).

CFTR modulators are currently approved for ~90% of CF subjects (12–14). The potentiator Kalydeco (ivacaftor/VX-770) was first approved for G551D-CFTR, and, currently, for any mutation that is responsive by clinical and/or in vitro assay data (14, 15). Trikafta contains VX-770 plus the two correctors VX-661 (tezacaftor) and VX-445 (elexacaftor), and is approved for CF subjects with one F508del allele in combination with any CFTR mutation (16–18). However, there are no approved CFTR modulators for many rare CFTR mutations, including c.3700A>G (12–14).

We previously investigated the utility of small molecule modulators for CFTR mutants not benefitted by approved CFTR modulators, including the N1303K mutation and the truncated

CFTR polypeptide ($CFTR_{1281}$) generated by the W1282X mutation (19–21). Two concepts emerged from these studies: *(i)* potentiators used in combination ('co-potentiators') may greatly improve channel gating of certain rare mutants; and *(ii)* mutation-specific correctors may have greater efficacy than F508del correctors. Here, we investigated the utility of approved and investigational CFTR modulators for the c.3700A>G CFTR mutation, and report preliminary data for Trikafta efficacy in two homozygous c.3700A>G subjects.

MATERIALS AND METHODS

Chemicals

VX-809, VX-770, GLPG1837 and $CFTR_{inh}$-172 were purchased from Selleck Chemicals (Boston, MA). VX-445 and PTI-428 were purchased from MedChemExpress (Monmouth Junction, NJ). Potentiators P2, P3 and P5 are contained in an in-house repository (19). For screening, a collection of ~50,000 compounds containing diverse drug-like synthetic small molecules (ChemDiv Inc., San Diego, CA) and an in-house repository of mutant CFTR modulators was tested. Other chemicals were purchased from MilliporeSigma (Burlington, MA).

Complementary DNA constructs

Complementary DNAs (cDNAs) for the I1234V-, I1234del- mutants CFTRs were generated using standard techniques. In brief, gBLOCK gene fragments (IDT, Coralville, IA) were introduced into full-length wild type CFTR cDNA in the vector pIRESpuro3 (Takara Bio, Mountain View, CA). gBLOCKs contained CFTR sequence from a HindIII site (nucleotides 3171-3176) to the stop codon, encoding the I1234V mutation or deletion of residues 1234–1239 and containing a silent mutation in a HindIII site (nucleotides 4000-4005).

Cell culture

Fischer rat thyroid (FRT) cells were cultured as described (19–21). FRT cells stably expressing I1234V and I1234del-CFTR were generated as described (19, 20). FRT cell lines expressing F508del-CFTR (22) and gene-edited 16HBE14o- cells expressing I1234del-CFTR (23) were cultured as described. Human nasal epithelial cells were acquired by nasal brushing as approval by the UCSF Committee on Human Research. Nasal epithelial cells from a homozygous c.3700A>G subject (subject 1) and from two heterozygous c.3700A>G / W1282X subjects were cultured and expanded by conditional reprogramming as described (19, 20) and used at passages 1 and 2.

High-throughput screening

High-throughput screening to identify I1234del-CFTR correctors used FRT-YFP-I1234del cells and was performed essentially as described for $CFTR_{1281}$ (19, 20).

Short-circuit current measurements

Short-circuit measurements were performed as previously described in FRT cells (19, 20), 16HBE14o- gene-edited cells (23), and primary human airway epithelial cell cultures (19, 20).

RESULTS

Characterization of I1234V and I1234del-CFTR in transfected FRT cells

To investigate the activity of the two CFTR polypeptides resulting from the c.3700A>G mutation, FRT cell lines were generated that stably express a halide-sensitive YFP (H148Q/I152L/F46L) together with human CFTR containing the I1234V mutation (FRT-YFP-I1234V) or CFTR with 6 ammo acid (I1234 to R1239) deleted (FRT-YFP-I1234del). Figure 1A shows the location of I1234 in nucleotide binding domain 2 (NBD2), and the cDNA constructs used to generate the cell models. Figure 1B shows the short-circuit current responses of wildtype (*left*) and I1234V-CFTR (*middle*) to increasing concentrations of forskolin. Similar EC_{50} were found for forskolin activation of wildtype and I1234V-CFTR (~130 nM) (Fig. 1B, *right*), suggesting that the I1234V mutation does not interfere with CFTR channel processing or function.

In contrast, FRT cells expressing I1234del-CFTR showed little chloride current even with maximal forskolin, and limited current with a high concentration of VX-770 (Fig. 1C, *top*). As anticipated from prior studies on other NBD2 mutants (20, 21), the co-potentiator ASP-11 added together with VX-770 increased I1234del-CFTR activity by ~4-fold, with current inhibited by $CFTR_{inh}$-172. To investigate whether I1234del-CFTR is subject to folding defects as found for F508del-CFTR, cells were incubated at reduced temperature (27 °C, 24 hours) (24). Low-temperature incubation increased channel activity by >2-fold in response to VX-770 alone or in combination with ASP-11 (Fig. 1C, *bottom*). These results suggest the utility of CFTR modulators to rescue I1234del-CFTR activity, as originally reported by Molinski, Bear and colleagues (23).

Concentration-dependence studies were done to further investigate potentiator and co-potentiator action. The EC_{50} values for activation of I1234del-CFTR and G551D-CFTR by VX-770 were similar at ~0.1 μM (Fig. 1D). Measurement of ASP-11 concentration-dependence in I1234del-CFTR-expressing cells following addition of forskolin and VX-770 gave an EC_{50} of ~3 μM (Fig. 1E), similar to that found for N1303K-CFTR (20).

Functional rescue of I1234del-CFTR by correctors

The approved correctors VX-809, VX-661 and VX-445 were initially tested. Correctors were incubated with FRT cells expressing I1234del-CFTR (24 hours, 37 °C) prior to plate reader assays in which forskolin+VX-770+ASP-11 was added and channel activity determined by YFP quenching in response to an iodide gradient (Fig. 2A). Similar comparative measurements were made in FRT cells expressing F508del-CFTR treated with correctors and stimulated with forskolin+VX-770. Figure 2B (*top*) shows data for the correctors in F508del-CFTR expressing cells, with EC_{50} values of 0.20, 0.20 and 0.07 μM for VX-809, VX-661 and VX-445 respectively. Figure 2B (*bottom*) shows data for I1234del-CFTR, with EC_{50} values of 0.12, 0.17 and 0.04 μM, respectively.

Short-circuit current measurements showed that individual correctors increased I1234del-CFTR activity by ~2-fold (compared to no correctors) with forskolin+VX-770 (Fig. 2C and 2D, *open bars*). The combination of VX-661 and VX-445, the correctors in Trikafta, increased forskolin+VX-770-stimulated I1234del-CFTR current by ~4-fold (Fig. 2C and 2D,

open bars), similar to the approximately additive effect of VX-661/VX-445 on F508del-CFTR (17). When cells were treated with correctors individually, or with VX-661/VX-445, the forskolin+VX-770+ASP-11-stimulated response was consistently ~1.5–2-fold greater than that in the absence of corrector (Fig. 2C and 2D, *grey bars*). In agreement with these observations, the fold increase of VX-770 to VX-770+ASP-11 stimulated CFTR current decreased from ~2–2.5-fold with a single corrector to ~1.5-fold in the presence of VX-445/VX-661 (Fig. 2D).

Motivated by these findings, screening was done to identify I1234del-CFTR correctors with improved efficacy. Screening of ~50,000 synthetic small molecules identified two active compound classes, with the most active compounds being the 1-arylpyrazole-4-arylsulfonyl-piperazine WX_{corr}-A23 and the spiro-piperidine-quinazolinone WX_{corr}-B09 (Fig. 3A), both previously identified in a screen for $CFTR_{1281}$ correctors (19). Individually, in plate reader assays the EC_{50} of WX_{corr}-A23 and WX_{corr}-B09 were 1.6 μM and 3.2 μM respectively, similar to the values found for $CFTR_{1281}$ (19). When used individually, WX_{corr}-A23 and WX_{corr}-B09 increased the VX-770-potentiated I1234del-CFTR current by half of that with the VX-661/VX-445 corrector combination (Fig. 3B). When WX_{corr}-A23 or WX_{corr}-B09 was used together with VX-445, the VX-770-potentiated current was approximately twice that produced by VX-661/VX-445. In WX_{corr}-A23 or WX_{corr}-B09 -corrected cells, VX-770+ASP-11 increased I1234del-CFTR current ~5-fold over that with VX-770 alone. Similarly, VX-770+ASP-11-stimulated I1234del-CFTR activity was ~2-fold greater in cells corrected with VX-445/WX_{corr}-A23 or VX-445/WX_{corr}-B09 as compared to the VX-445/VX-661 corrector combination.

Co-potentiators increase I1234del-CFTR function

We previously reported evidence for two distinct classes of potentiators acting at different sites – class I (classical potentiators) such as VX-770, and class II potentiators ('co-potentiators') such as ASP-11 (21). Following VX-770, addition of the potentiators P2, P3 and P5 did not increase current (Fig. 4A, *left*), suggesting that these class I potentiators bind at or near the same site on I1234del-CFTR. Subsequent addition of ASP-11 increased I1234del-CFTR current. Activation of I1234del-CFTR was also seen for the combination of the class I potentiator GLPG1837 and ASP-11 (Fig. 4A, *center*), as reported for N1303K-CFTR (21). In each experiment, current was inhibited by $CFTR_{inh}$-172. In further studies summarized in Fig. 4A (*right*), activation of I1234del-CFTR was seen for combinations of class I and class II compounds, but not for combinations of class I-class I or class II-class II compounds.

Prior high-throughput screening identified four novel class II potentiators (21). The most potent compounds from each class, piperidinepyridoindole CP-A61, phenylazepine CP-B01, tetrahydroquinoline CP-C01 and pyrazoloquinoline CP-D123, were tested together with VX-770 on VX-661-corrected FRT-YFP-I1234del cells. CP-D123 was the most potent co-potentiator (EC_{50} ~700 nM) followed by CP-A61 (EC_{50} ~2 μM) and then CP-B01 and CP-C01 (EC_{50} >3 μM) (Fig. 4B). Measurements were done with VX445/VX-661 correction (Fig. 4C). The class I potentiator GLPG1837 increased current in VX445/VX-661-treated cells by ~1.5-fold more than VX-770, with CP-D123 producing further increases.

CFTR modulator activity in gene-edited I1234del-CFTR-expressing human bronchial epithelial cells

Short-circuit current measurements were next done in a gene-edited 16HBE14o- human airway epithelial cell model in which the endogenous CFTR gene was edited to express I1234del-CFTR (16HBEge-I1234del) (23). Limited CFTR current was seen in 16HBEge-I1234del cells with forskolin and VX-770 in the absence of corrector (Fig. 5A, Fig. 5B). The VX-661/VX-445 combination produced ~5-fold increased current. VX-445 in combination with WX_{corr}-A23 or WX_{corr}-B09 also increased current albeit to a lesser degree. Remarkably, addition of co-potentiator ASP-11 after VX-770 increased current by ~3–fold compared to VX-770 alone.

CFTR modulator testing in c.3700A>G homozygous human airway epithelial cells

Short-circuit current measurements were also done in primary cultures of human airway epithelial cells from nasal brushings on a homozygous c.3700A>G subject (subject 1, see below). Little CFTR current was seen without or with multiple correctors and potentiators/co-potentiators (Fig. 5C and 5D), including the combination VX-661/VX-445. Further, little current was seen with the CFTR amplifier PTI-428 added together with VX-661 and VX-445, or VX-445 with WX_{corr}-A23 or WX_{corr}-B09. As controls, bronchial epithelial cells from a non-CF subject cultured in an identical manner showed a robust response to forskolin, and cells from a F508del homozygous subject showed robust responses to VX-661/VX-445 and forskolin/VX-770 (Suppl. Fig. 1A and 1B, Fig. 5D). As another control, response to VX-770/ASP-11 was shown in bronchial epithelial cells from a homozygous N1303K subject (Suppl. Fig. 1C, Fig. 5D). Though nasal brushings could be obtained on only one homozygous c.3700A>G subject, brushings were done on two c.3700A>G/W1282X siblings and cultured in an identical manner to the c.3700A>G cells. Cells cultured from one c.3700A>G/W1282X sibling demonstrated a substantial VX-770/ASP-11 response of similar magnitude to the response in N1303K homozygous cells (Suppl. Fig. 1D).

Efficacy of Trikafta in two CF subjects homozygous for the c.3700A>G CFTR mutation

In an attempt to clarify the different response to CFTR modulators in cell lines vs. primary nasal epithelial cell cultures, two limited N-of-1 studies were done to test the efficacy of Trikafta in homozygous c.3700A>G subjects.

Subject 1 is a 34 year old male of Middle Eastern origin. His main complaints were abdominal bloating with alternating diarrhea and constipation, which improved but did not resolve on pancreatic enzyme replacement therapy (PERT). He never developed distal intestinal obstruction syndrome. He has had only mild respiratory symptoms, with normal lung function (FEV_1 3.33 L, 95% predicted; FVC 4.32 L, 103% predicted) prior to Trikafta. For 5 years before beginning Trikafta therapy, he had not received intravenous antibiotics, nor experienced pulmonary exacerbations requiring hospitalization, although he did receive courses of oral antibiotics for mild increases in sputum production and sinus disease. Induced sputum cultures showed light growth of two morphologic strains of *Pseudomonas aeruginosa*, both of which were broadly antibiotic susceptible. There was a history of recurrent rhinosinusitis but not within a year of starting Trikafta. There was no history of

nasal polyps or sinus surgery. He was treated with mometasone nasal spray on most days of the week.

On Trikafta, gastrointestinal symptoms subjectively resolved, PERT was self-discontinued, and weight gain occurred (body weight increased from 86 to 93 kg, body mass index from 30.5 to 32.9). Fecal elastase at two months after discontinuation of PERT was 183μg/gm stool (normal > 200, mild insufficiency 100–200). Sweat chloride was 70±6 mmol/L (mean ±S.D., n=6 over 2 years) prior to Trikafta and 43 mmol/L after two months on Trikafta. He remained free of respiratory symptoms and had normal complete blood count, liver function and renal function.

Subject 2 is the 21 year old sister of subject 1. Her main complaint has been mild chronic cough productive of thin yellow-green phlegm with occasional chest pain triggered by cough. In the year before Trikafta, she was treated twice as an outpatient with oral antibiotics and once as an inpatient with intravenous antibiotics. She also has chronic rhinosinusitis with occasional nose bleeds and complaints of right ear congestion. She has not undergone sinus surgery. She has no gastrointestinal complaints and has not been treated with PERT. Her medications include nebulized dornase and aztreonam, salbutamol, desloratadine and budesonide.

On Trikafta she did not note change in respiratory symptoms, having continued minimal daily cough with yellow sputum production and occasional nasal congestion and ear symptoms. FEV_1 was 62±1 % predicted (mean±S.D., n=3) in the year before Trikafta and 68% predicted on Trikafta, FVC values did not change. Sweat chloride was 66±1 mmol/L (n=2) on no CFTR modulator therapy, 44 mmol/L on Symdeko, and 53 mmol/L on Trikafta. Body weight (75 kg) and body mass index (28.2) did not change.

CONCLUSIONS

Remarkable advances have been made in CFTR modulator drugs (12–14), though ~10 % of CF subjects, including those with rare missense mutations and premature termination codons, have no available therapy. The objective of this study was to investigate the utility of CFTR modulators for the c.3700A>G mutation. In transfected FRT cell models, in agreement with earlier studies by Molinski *et al.* (11), we found that the I1234V missense mutation produce no apparent impairment in CFTR channel activity, though deletion of six residues in NBD2 produced a nonfunctional CFTR. In the FRT cell model, minimal I1234del-CFTR chloride current was seen with forskolin and VX-770, though approved and investigational correctors and corrector combinations significantly increased channel activity. Since cell model background can influence corrector responses (25), studies were also done in gene-edited 16HBE14o- cells expressing I1234del-CFTR, with robust responses seen similar to those in the FRT cell model. These in vitro results suggested the potential utility of the approved CFTR therapy Trikafta for CF subjects with the c.3700A>G mutation.

We previously reported that certain NBD2 mutants, including N1303K-CFTR, are responsive to a second potentiator (class II potentiator) that likely binds at a distinct location

from that of VX-770 (21, 26, 27). As anticipated based on the location of the deleted residues, the I1234del-CFTR mutant was activated by co-potentiators. To identify improved correctors for I1234del-CFTR, high-throughput screening produced the 1-arylpyrazole-4-arylsulfonyl-piperazine WX_{corr}-A23 and the spiro-piperidine-quinazolinone WX_{corr}-B09 that, in the FRT cell model, when used with VX-445 were ~2-fold more effective than VX-661. In 16HBEge-I1234del cells the VX-445/VX-661 combination was more effective than VX-445 with WX_{corr}-A23 or WX_{corr}-B09. Notwithstanding the quantitative differences in results for the FRT vs. 16HBE14o- cell models, the data support the potential utility of tailored CFTR modulator combinations for the c.3700A>G mutation.

A few prior studies addressed the biology of the c.3700A>G CFTR mutation. In agreement with results herein, the I1234V missense mutation appears to have little effect on CFTR processing or channel gating, whereas deletion of 6 amino acids in I1234del impairs both processes (11, 28). Immunoblot analysis and functional studies suggest that I1234del-CFTR does not undergo complex glycosylation and has impaired channel function in cell models (11, 23, 28). A panel of correctors studied by Molinski et al. in a HEK293 expression system, including VX-809, VX-661 and Corr-4a, increased I1234del-CFTR band C and channel activity by ~2-fold (in the absence of potentiators) (23). In 16HBEge-I1234del cells (generously provided for the current study), VX-809-mediated I1234del-CFTR band B stabilization was reported, as well as increased channel activity with VX-770 (23). In cultured primary nasal epithelial cells from two c.3700A>G homozygous siblings, one subject showed ~2-fold increased band B with VX-661 or VX-809 and ~2-fold increased channel activity, while cells from a second subject did not respond to CFTR modulators (23). Our data herein extend these prior findings, and suggest that Trikafta, or various tailored next generation modulator therapies, including co-potentiators and novel corrector combinations, may have utility for the c.3700A>G mutation.

Interestingly, we found that nasal epithelial cells cultured from a c.3700A>G homozygous CF subject did not show CFTR chloride current in response to a panel of CFTR modulator combinations. This absence of response contrasts with results in the heterologous FRT expression system and in the gene-edited 16HBE14o- cell model. To investigate these different responses, we studied nasal epithelial cell cultures generated from two c.3700A>G/W1282X siblings; cultures from one sibling showed significant CFTR chloride current in response to VX-661 and VX-770/ASP-11. Based on studies using nasal epithelial cells from a W1282X homozygous subject showing no CFTR chloride current (19), the responses in the c.3700A>G/W1282X culture probably arise from the c.3700A>G allele. We note that others have also reported disparate responses from cultured nasal epithelial cell models, including cells from c.3700A>G homozygous siblings as reported by Molinski, Bear and colleagues (23). Various factors might contribute to the different results obtained in cell culture models and primary nasal epithelial cells, such as differential drug penetration, culture method, phenotype heterogeneity, subject disease status at the time of nasal brushing, and others. Measurements in identically cultured CF cells with the F508del and N1303K mutations suggest that culture conditions and drug penetration are not likely to account for the absence of response in the c.3700A>G cells. In any case, notwithstanding the incomplete explanation for differences in cell models vs. primary cell data, our data highlight the importance of n-of-1 clinical data used together with cell models data, as well as the need

for caution in drawing conclusions about drug efficacy using data obtained from a limited patient population.

Motivated by the studies conducted in cell models, Trikafta was evaluated in two c.3700A>G homozygous subjects. In one subject, Trikafta produced a possible reduction in sweat chloride, subjective resolution of gastrointestinal symptoms and weight gain. A second subject did not experience symptomatic changes on Trikafta, but had possible mild improvement in FEV1 and sweat chloride. Further follow-up studies on these subjects, as well as studies in additional subjects, is indicted to clarify the potential clinical benefit of Trikafta for the c.3700A>G mutation.

In summary, the data herein support potential utility of CFTR modulators for the c.3700A>G mutation. Notably, the substantial increases in CFTR current conferred by co-potentiators in vitro suggest their utility as add-on agents to approved potentiator-corrector drugs for this and possibly other CFTR mutations, including N1303K, that are not benefitted by available CFTR modulators such as Trikafta. However, unresolved issues that remain to be clarified include explanation for differences in the in vitro data in transfected and gene-edited cells vs. primary human airway cultures, and the apparent heterogeneity in the human disease phenotype and response to CFTR modulator therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- The c.3700A>G mutation in the CFTR gene produces two protein products, one with single missense mutation (I1234V-CFTR) and a second with six amino acids deleted from nucleotide binding domain 2 (p.Ile1234_Arg1239del-CFTR, I1234del-CFTR)
- I1234V-CFTR function is similar to that of wild type CFTR, whereas I1234del-CFTR is impaired in its cellular processing and channel gating
- I1234del-CFTR is responsive to approved CFTR modulator drugs, including VX-445, VX-661, VX-809 and VX-770
- I1234del-CFTR can be activated by investigational CFTR modulators, including co-potentiators and mutation-specific correctors
- Trikafta therapy in two c.3700A>G homozygous cystic fibrosis subjects showed modest clinical benefit

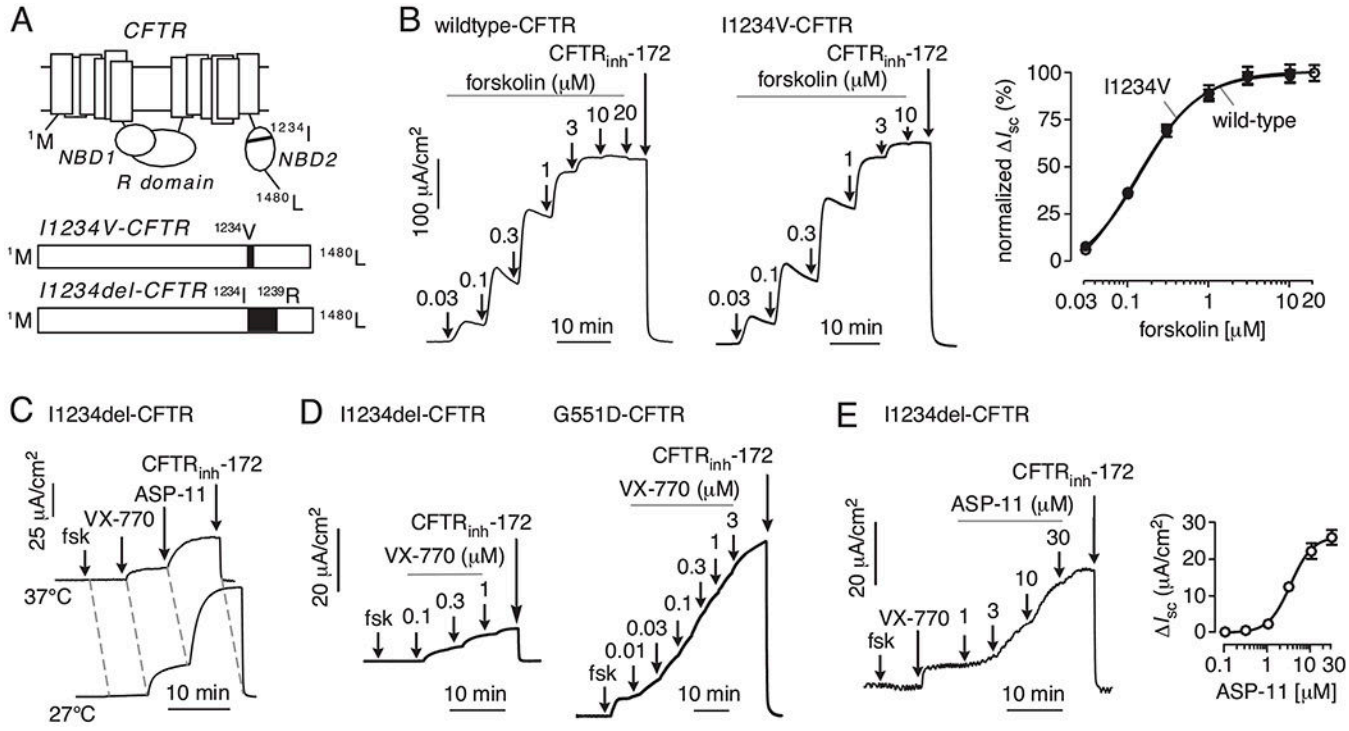

Figure 1. Characterization of I1234V-CFTR and I1234del-CFTR in transfected FRT cells.

A. (*top*) Schematic of CFTR structure showing the location of the II234V mutation and CFTR domain structure. (*bottom*) Complementary DNA expression cassettes used in this study: CFTR with point mutation (I1234V-CFTR), and CFTR containing a 6-amino acid deletion (I1234-R1239del). **B.** Short-circuit current in FRT cells expressing wildtype (*left*) and I1234V-CFTR (*middle*) in response to indicated concentration of forskolin and 10 μM $CFTR_{inh}$-172. (*right*) Summary of concentration-dependence data (n=4, mean ± S.E.M.). **C.** Short-circuit current in I1234del-CFTR expressing FRT cells showing responses to 20 μM forkolin (fsk), 5 μM VX-770, 20 μM ASP-11 and 10 μM $CFTR_{inh}$-172. Cells were for incubated for 24 h at 37 °C (*top*) or 27 °C (*bottom*) prior to measurement. **D.** VX-770 concentration-dependence in FRT cells expressing I1234del- (*left*) and G551D-CFTR (*right*) with 20 μM forskolin and 10 μM $CFTR_{inh}$-172. I1234del-CFTR cells were incubated for 24 h at 27 °C prior to measurement. Data in panels **C** and **D** is representative of n=4 experiments. **E.** (*left*) ASP-11 concentration-dependence in I1234del-CFTR cells with 20 μM forskolin, 5 μM VX-770 and 10 μM $CFTR_{inh}$-172. (*right*) Summary of concentration-dependence data (n=4, mean ± S.E.M.).

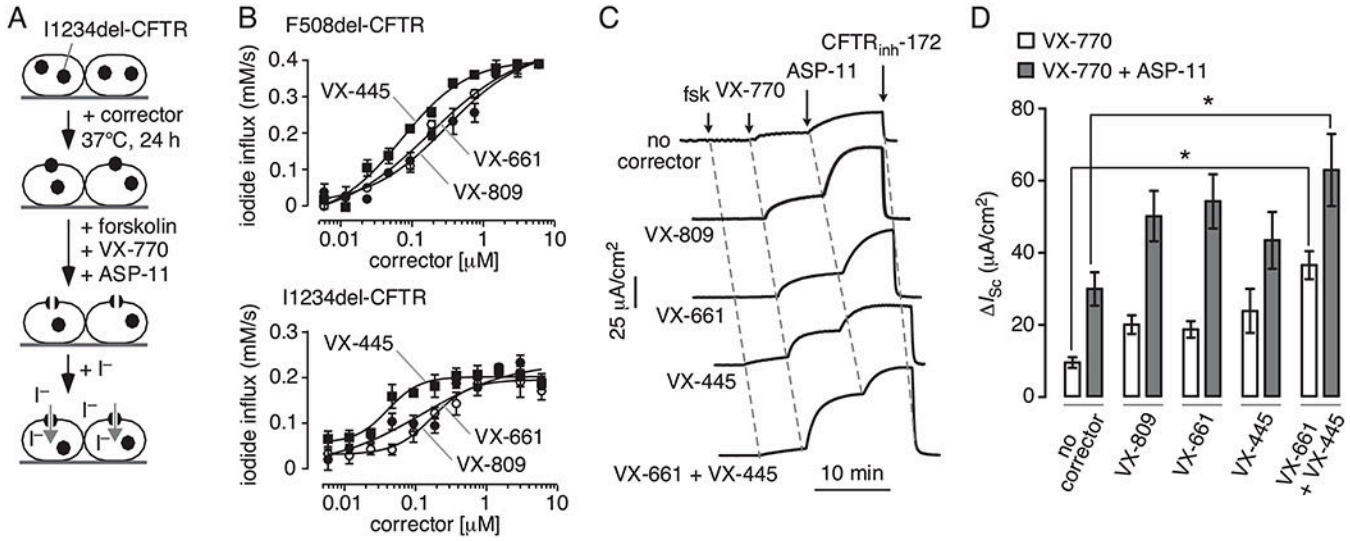

Figure 2. Approved correctors increase I1234del-CFTR function in FRT cells.

A. Plate-reader assay used to test correctors. **B.** Concentration-dependence of indicated correctors in FRT cells expressing F508del-CFTR (*top*) and I1234del-CFTR (*bottom*) (n=3–4, mean ± S.E.M.). **C.** Short-circuit current measured in FRT cells expressing I1234del-CFTR with 20 μM forskolin, 5 μM VX-770 and 20 μM ASP-11. As indicated, cells were treated with 3 μM VX-809, 3 μM VX-661, 3 μM VX-445, or 18 μM VX-661 plus 3 μM VX-445 for 24 h prior to measurements. **D.** Summary of data from C. (n=3, mean ± S.E.M., * $P < 0.01$).

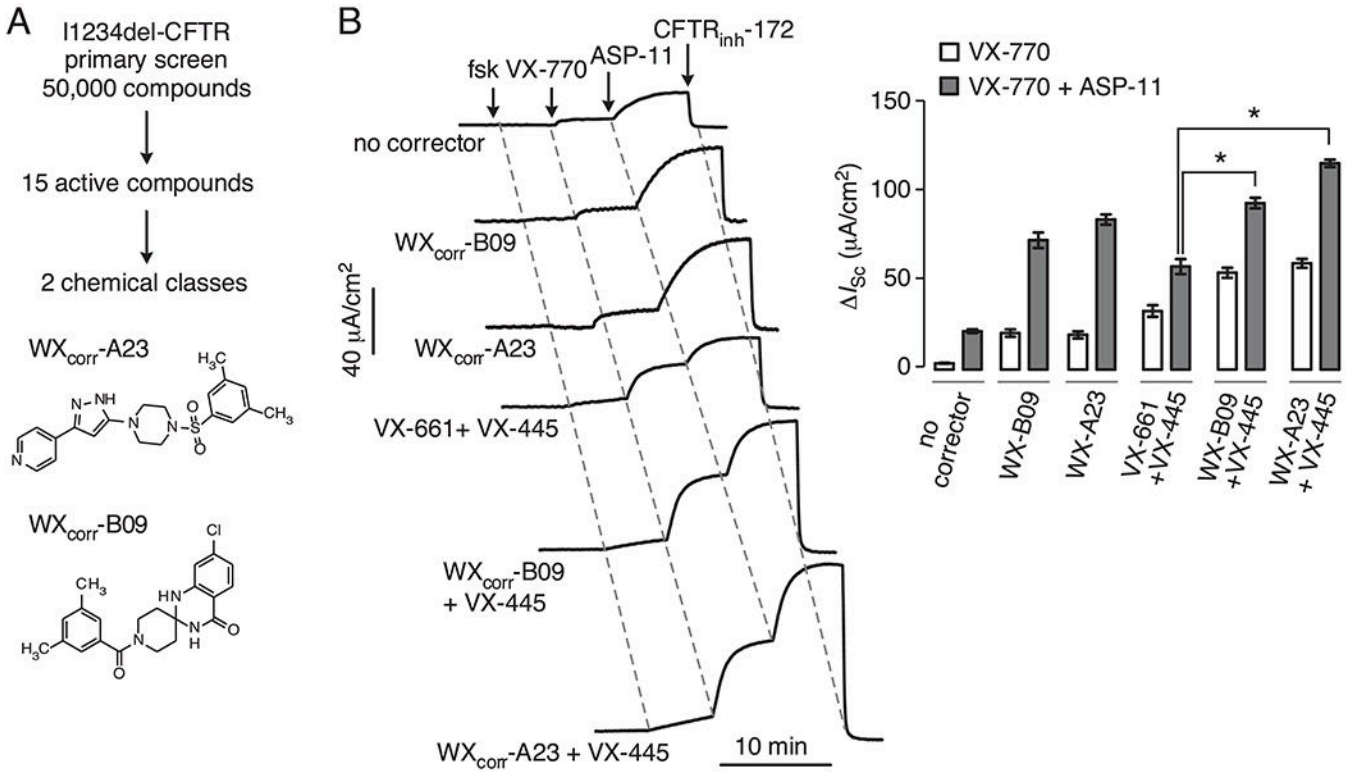

Figure 3. I1234del-CFTR correctors identified by high-throughput screening.

A. Screen workflow with chemical structures of correctors WX_{corr}-A23 and WX_{corr}-B09. **B.** (*left*) Short-circuit current in I1234del-CFTR-expressing FRT cells with 20 μM forskolin, 5 μM VX-770, 20 μM ASP-11 and 10 μM $CFTR_{inh}$-172. As indicated, cells were treated with 5 μM WX_{corr}-A23, 10 μM WX_{corr}-B09, 18 μM VX-661 + 3 μM VX-445, 5 μM WX_{corr}-A23 + 3 μM VX-445 or 10 μM WX_{corr}-B09 + 3 μM VX-445 for 24 h prior to measurements. (*right*) Data summary (n=3, mean ± S.E.M., * $P < 0.01$).

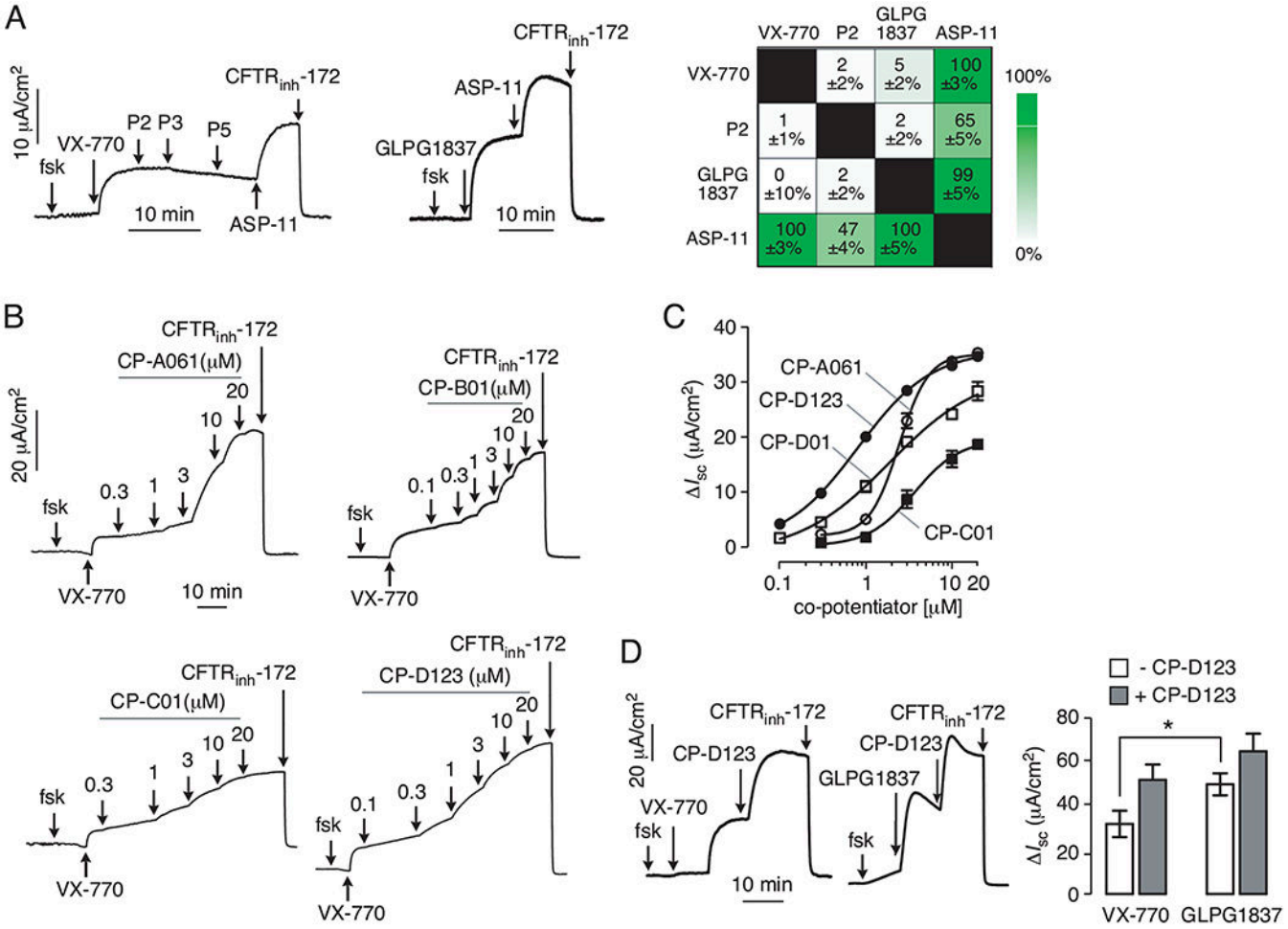

Figure 4. Efficacy of class I and class II potentiators in I1234del-CFTR in FRT cells.

A. Short-circuit current (*left and middle*) and summary data (*right*) for VX-661-treated FRT cells expressing I1234del-CFTR in response to forskolin and indicated potentiators (n=3, mean ± S.E.M.). Concentrations: 20 μM forskolin, 5 μM VX-770, 20 μM ASP-11, 20 μM GLPG1837, 20 μM P2, 20 μM P3 and 20 μM P5. **B.** Short-circuit current measurements as in **A.** with indicated concentrations of CP-A061, CP-B01, CP-C01 and CP-D123. **C.** Summary of CP-A061, CP-B01, CP-C01 and CP-D123 concentration-dependence data (n=4, mean ± S.E.M.). **D.** (*left*) Short-circuit current measurements as in **A.** in cells corrected with VX-445/VX-661 with 20 μM forskolin, 5 μM VX-770 or 20 μM GLPG1837 and 20 μM CP-D123. (*right*) Data summary (n=3, mean ± S.E.M., * $P < 0.01$).

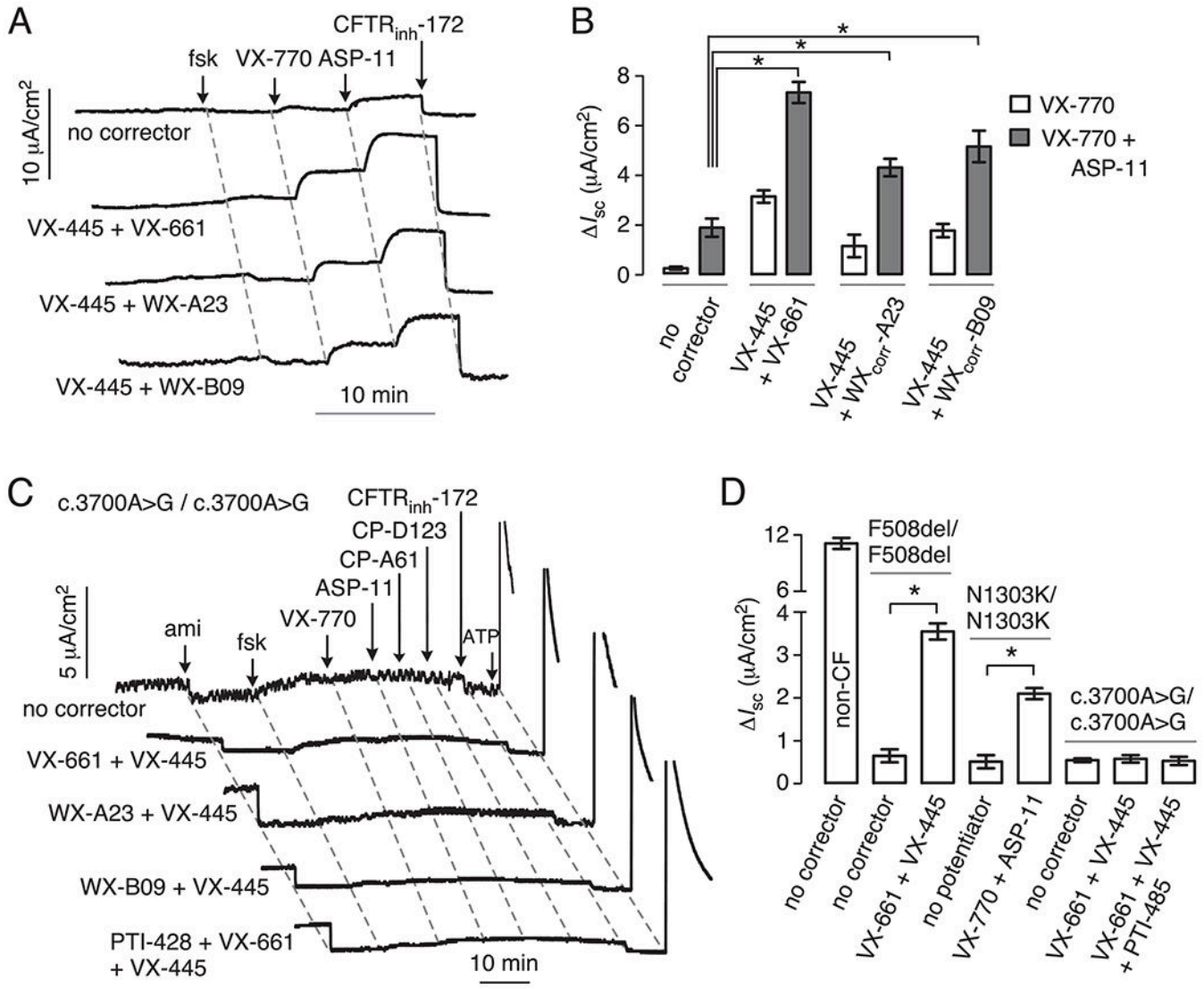

Figure 5. CFTR modulator efficacy in gene-edited human airway epithelial cells expressing I1234del-CFTR and primary nasal epithelial cells from a c.3700A>G homozygous subject.

A. Short-circuit current measured in 16HBE14o- cells expressing I1234del-CFTR with 20 μM forskolin, 5 μM VX-770, 20 μM ASP-11 and 10 μM $CFTR_{inh}$-172. As indicated, cells were treated with 18 μM VX-661 + 3 μM VX-445, 5 μM WX_{corr}-A23 + 3 μM VX-445 or 10 μM WX_{corr}-B09 + 3 μM VX-445 for 24 h prior to measurements. **B.** Summary of data from A. (n=3–4, mean ± S.E.M., * $P < 0.01$). **C.** Short-circuit current cells from a homozygous c.3700A>G CF subject showing responses to amiloride (10 μM), forskolin (20 μM), VX-770 (5 μM), ASP-11 (10 μM), CP-A061 (10 μM), CP-D123 (10 μM) and ATP (100 μM). As indicated, cells were treated with VX-661 (18 μM), VX-445 (3 μM), WX_{corr}-A23 (5 μM), WX_{corr}-B09 (10 μM) and PTI-428 (1 μM) for 24 h prior to measurement. **D.** Data summary of CFTR-dependent current in primary bronchial epithelial cell models generated from non-CF, homozygous F508del and homozygous N1303K subjects (per Suppl Fig. 1), and nasal

epithelial cell models generated from a c.3700A>G homozygous subject (ΔIsc; n=3–4, mean ± S.E.M., *P < 0.01).