

## CYSTIC FIBROSIS

# Sildenafil (Viagra) corrects $\Delta$ F508-CFTR location in nasal epithelial cells from patients with cystic fibrosis

R L Dormer, C M Harris, Z Clark, M M C Pereira, I J M Doull, C Norez, F Becq, M A McPherson

*Thorax* 2005;60:55–59. doi: 10.1136/thx.2003.019778

See end of article for authors' affiliations

Correspondence to:  
Dr R L Dormer, Department of Medical Biochemistry and Immunology, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XN, UK; dormer@cardiff.ac.uk

Received 3 December 2003  
Accepted 15 August 2004

**Background:** Most patients with cystic fibrosis (CF) have a  $\Delta$ F508 mutation resulting in abnormal retention of mutant gene protein ( $\Delta$ F508-CFTR) within the cell. This study was undertaken to investigate  $\Delta$ F508-CFTR trafficking in native cells from patients with CF with the aim of discovering pharmacological agents that can move  $\Delta$ F508-CFTR to its correct location in the apical cell membrane.

**Method:** Nasal epithelial cells were obtained by brushing from individuals with CF. CFTR location was determined using immunofluorescence and confocal imaging in untreated cells and cells treated with sildenafil. The effect of sildenafil treatment on CFTR chloride transport function was measured in CF15 cells using an iodide efflux assay.

**Results:** In most untreated CF cells  $\Delta$ F508-CFTR was mislocalised within the cell at a site close to the nucleus. Exposure of cells to sildenafil (2 hours at 37°C) resulted in recruitment of  $\Delta$ F508-CFTR to the apical membrane and the appearance of chloride transport activity. Sildenafil also increased  $\Delta$ F508-CFTR trafficking in cells from individuals with CF with a single copy  $\Delta$ F508 ( $\Delta$ F508/4016ins) or with a newly described CF trafficking mutation (R1283M).

**Conclusions:** The findings provide proof of principle for sildenafil as a  $\Delta$ F508-CFTR trafficking drug and give encouragement for future testing of sildenafil and related PDE5 inhibitors in patients with CF.

Cystic fibrosis (CF) is caused by mutations in the CF gene protein, the cystic fibrosis transmembrane conductance regulator (CFTR).<sup>1</sup> By far the most common mutation is  $\Delta$ F508, present on 70–80% of chromosomes worldwide. Wild type CFTR resides at the apical membrane of epithelial cells where it acts as a cyclic AMP-dependent Cl<sup>-</sup> channel<sup>1</sup> and regulates other ion channels<sup>2</sup> and protein secretion.<sup>3</sup> Defective trafficking of  $\Delta$ F508-CFTR leads to abnormal retention of mutant protein within the cell and consequent loss of CFTR function.<sup>1</sup> The reported degree of mislocalisation of  $\Delta$ F508-CFTR in native epithelial cells varies between different tissues and types of preparation.<sup>4–7</sup> More recently we and others have developed a technique to study the location of CFTR in freshly isolated native airway epithelial cells from patients with CF which allows quantitation of the percentage of cells with a defined CFTR location.<sup>8,9</sup> We have shown a marked mislocalisation of  $\Delta$ F508-CFTR which was corrected by the CFTR activating benzo[c]quinoxalinium compounds.<sup>8</sup> Thus, although our studies and those of others suggest that at least some  $\Delta$ F508-CFTR traffics to the apical membrane in native CF cells,<sup>7–9</sup> the finding that the majority of  $\Delta$ F508-CFTR is mislocalised<sup>8</sup> emphasises that moving  $\Delta$ F508-CFTR to the apical membrane is necessary for developing a drug treatment targeted at CFTR rescue.

Arising from our earlier studies showing correction of antibody inhibited CFTR function by cyclic nucleotide phosphodiesterase (PDE) inhibitors,<sup>10</sup> we now show that the PDE5 inhibitor sildenafil (Viagra) increases  $\Delta$ F508-CFTR trafficking. Since this is a drug in clinical use that causes rapid movement of  $\Delta$ F508-CFTR from within the cell to the apical membrane, the results represent a major step forward in the development of a treatment that addresses the basic cellular abnormality for the majority of patients with CF.

## METHODS

### Patients and cell sampling

Airway epithelial cells were obtained by nasal brushing from three non-CF individuals (age 9–22 years) and from six CF

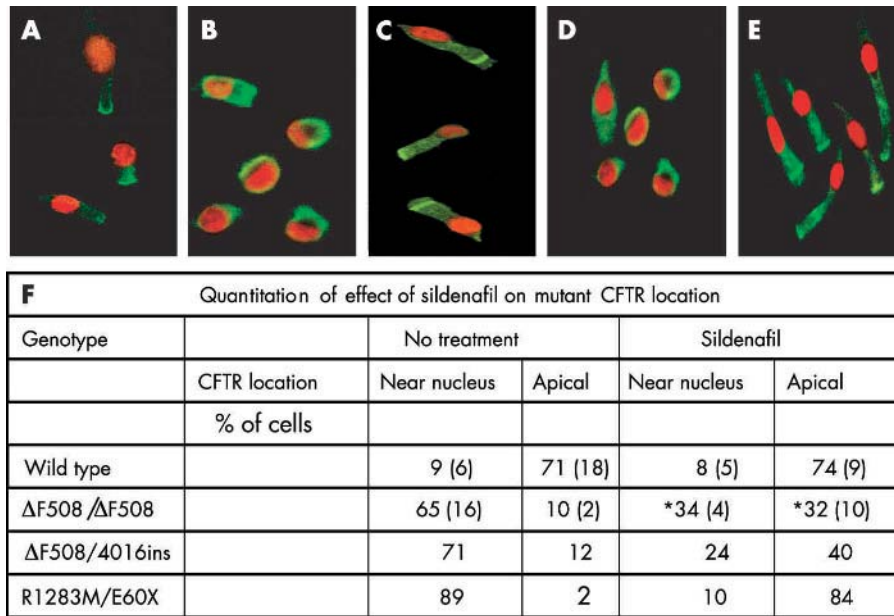
individuals (age 6 months to 16 years) undergoing flexible bronchoscopy under sedation as previously described.<sup>8</sup> The CF individuals had the following genotypes: four  $\Delta$ F508/ $\Delta$ F508, one  $\Delta$ F508/4016ins, and one R1283M/E60X. The study was approved by the local ethics committee of Bro Taf Health Authority.

### CFTR localisation in nasal epithelial cells

Immediately following nasal brushing, the brushes were placed in DMEM/F12 medium and the attached cells incubated in the presence or absence of sildenafil (150  $\mu$ M) for 2 hours at 37°C. Cells were then fixed and CFTR localised by immunofluorescence as previously described.<sup>8</sup> Briefly, after incubation, cells were smeared gently onto "Snowcoat X-tra" microslides (Surgipath), left to air dry, fixed for 5 minutes at -20°C in 5% acetic acid in ethanol, washed in phosphate buffered saline (PBS) containing (in mM): 137 NaCl, 2.7 KCl, 4.3 Na<sub>2</sub>HPO<sub>4</sub>, 1.4 KH<sub>2</sub>PO<sub>4</sub>, pH 7.4 and stored at -20°C. Following treatment with blocking reagent (normal rabbit serum 1:20), cells were incubated with primary antibody overnight at 4°C, washed three times, and incubated with FITC conjugated secondary antibody (1:100) for 30 minutes at room temperature. The CFTR antibody used was our well characterised anti-CFTR antibody MPCT1 (affinity purified C-terminal; 1:100).<sup>8</sup> Slides were then mounted in Fluorosave Reagent (Calbiochem, La Jolla, CA, USA) and fluorescence was detected using confocal laser scanning microscopy on a Leitz DMRBE microscope (Leica, Germany) fitted with a TCS4D scanner (Leica, Germany).

### Measurement of chloride transport activity in CF15 cells

The SV40 transformed nasal epithelial cell line JME/CF15 from a patient with CF ( $\Delta$ F508/ $\Delta$ F508)<sup>11</sup> was cultured in DMEM/F12 supplemented with adenine (180  $\mu$ M), insulin (5  $\mu$ g/ml), transferrin (5  $\mu$ g/ml), hydrocortisone (1.1  $\mu$ M), tri-iodothyronine (2 nM), epinephrine (5.5  $\mu$ M), epidermal



**Figure 1** Immunofluorescent images of non-CF and CF nasal epithelial cells showing that sildenafil corrects abnormal mutant CFTR location in CF cells. Cells were incubated for 2 hours at 37°C without sildenafil (A, B, D) or with 150  $\mu$ M sildenafil (C, E). Images show CFTR immunofluorescence in green with the nucleus counterstained with propidium iodide in red. The results are representative of cells from (A) three non-CF individuals, (B, C) four  $\Delta$ F508/ $\Delta$ F508 CF individuals, and (D, E) one R1283M/E60X individual. They show that both  $\Delta$ F508- and R1283M- CFTR are predominantly restricted within the cell to a distinct location adjacent to the nucleus (near nucleus) in untreated cells (B, D) whereas, after sildenafil treatment, many cells have an apical location of CFTR (C, E). (F) Cells were examined (up to 140 cells for each condition) and categorised as having a distinct CFTR location of either near nucleus or apical. The remainder of both untreated and treated cells showed a more even distribution of CFTR throughout the cell. Data are mean (SD). \* $p < 0.05$  for difference from no treatment.

growth factor (1.64 nM) and 10% FCS. Chloride channel activity was assayed by measuring the rate of iodide ( $^{125}$ I) efflux.<sup>8, 12</sup> CF15 cells were incubated for 2 hours at 37°C in the presence or absence of either sildenafil or MPB-91 (250  $\mu$ M). Cells were then washed with efflux buffer containing (in mM): 137 NaCl, 4.4 KCl, 0.3  $\text{KH}_2\text{PO}_4$ , 0.3  $\text{NaH}_2\text{PO}_4$ , 4.2  $\text{NaHCO}_3$ , 1.3  $\text{CaCl}_2$ , 0.5  $\text{MgCl}_2$ , 0.4  $\text{MgSO}_4$ , 5.6 glucose and 10 HEPES, pH 7.5 and incubated in efflux buffer containing 1  $\mu$ M KI and 1  $\mu$ Ci  $\text{Na}^{125}\text{I}$ /ml (NEN, Boston, MA, USA) for 2 hours at 37°C. Cells were then washed with efflux buffer. After 1 minute the buffer was removed and quickly replaced by 300  $\mu$ l of the same buffer. The first four aliquots were used to establish a stable baseline in efflux buffer alone. Efflux buffer containing forskolin (10  $\mu$ M) and genistein (30  $\mu$ M) to stimulate CFTR activity was used for the remaining aliquots. At the end of the incubation the buffer was recovered and cells solubilised in 1 ml 1 N NaOH. The radioactivity was determined using a gamma counter (Cobra II, Packard Bell).

### Analysis of data

For CFTR localisation up to 140 cells for each condition were examined, categorised as having a distinct CFTR location of either near nucleus or apical, and expressed as a percentage of all cells counted. Only ciliated tall columnar epithelial cells were counted as these can be readily differentiated from non-ciliated columnar epithelial cells and basal cells, even when  $\Delta$ F508-CFTR is localised close to the nucleus.

For CFTR chloride transport activity the fraction of initial intracellular  $^{125}\text{I}$  lost during each time point was determined and time dependent rates (k) of  $^{125}\text{I}$  efflux were calculated from  $\ln(^{125}\text{I}_{t1}/^{125}\text{I}_{t2}) / (t_1 - t_2)$  where  $^{125}\text{I}_t$  is the intracellular  $^{125}\text{I}$  at time t, and  $t_1$  and  $t_2$  are successive time points.<sup>8, 12</sup> Curves were constructed by plotting k versus time. Relative rates were calculated and correspond to  $k_{\text{peak}} - k_{\text{basal}}$  (per minute). Concentration-response curves were constructed by

plotting the percentage activation as a function of the concentration of sildenafil (100% corresponds to the maximum relative rate obtained at the highest concentration, i.e. 1.5 mM). Differences between treated and untreated cells were examined using the Student's *t* test and *p* values of  $< 0.05$  were considered significant.

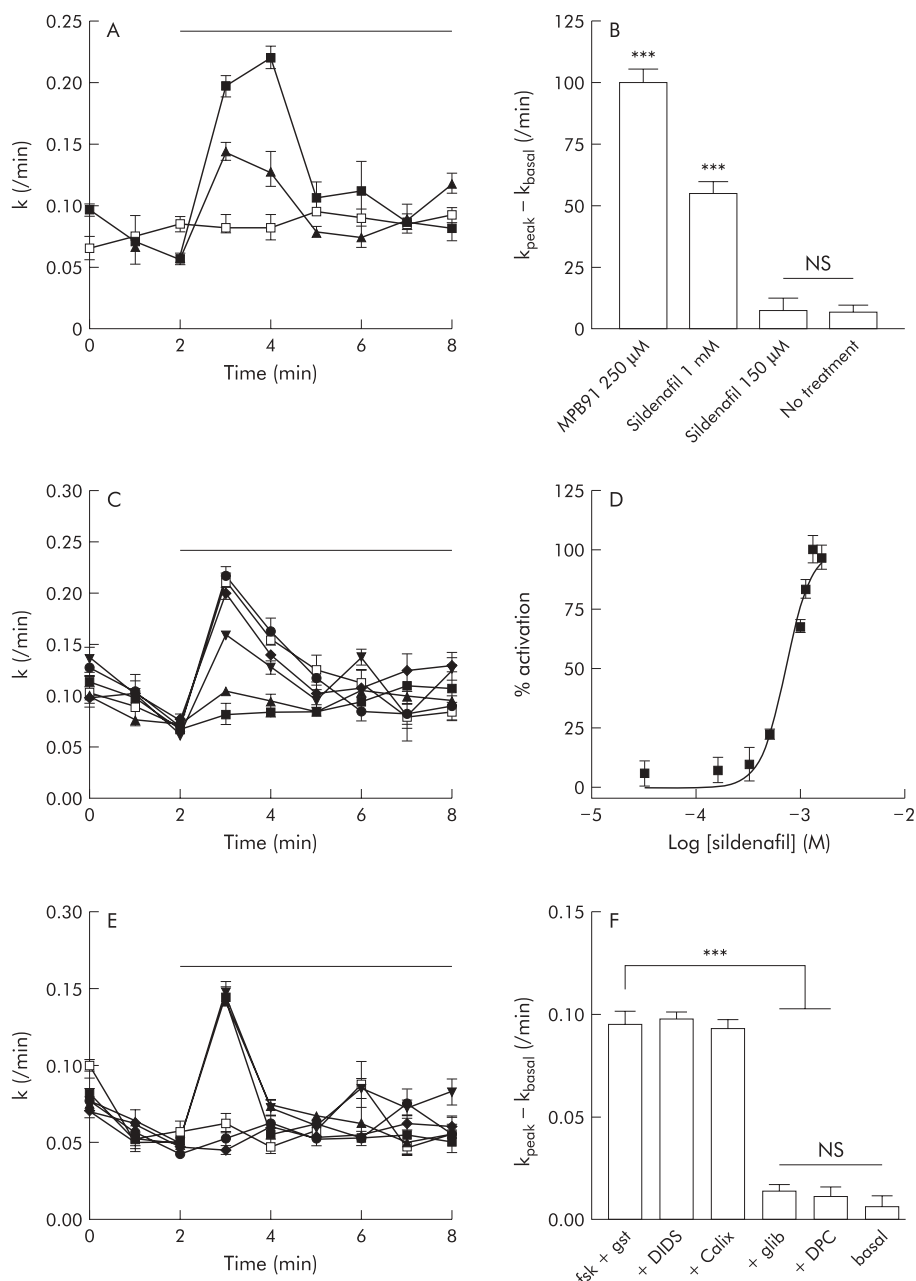
## RESULTS

### Mislocalisation of $\Delta$ F508-CFTR in CF nasal cells

As in our previous studies,<sup>8</sup> nasal epithelial cells obtained by brushing were polarised with cilia visible at the apical end of the cell that continued to beat for the 2 hour incubation before fixation. The nasal cells expressed cyto-keratins typical of epithelial cells throughout the cell. In cells from non-CF individuals, wild type CFTR was located at the apical end of the cell (fig 1A). In untreated cells from  $\Delta$ F508/ $\Delta$ F508 CF individuals and from a CF individual with the genotype  $\Delta$ F508/4016ins, 60–70% of cells showed a near nuclear location of  $\Delta$ F508-CFTR with only 10% having  $\Delta$ F508-CFTR present at the apical membrane (fig 1B and F). This is in agreement with our previous data on the location of CFTR in wild type,  $\Delta$ F508/ $\Delta$ F508 and  $\Delta$ F508/G551D cells.<sup>8</sup>

### Actions of sildenafil on $\Delta$ F508-CFTR location

In  $\Delta$ F508/ $\Delta$ F508 cells treated with sildenafil (150  $\mu$ M for 2 h at 37°C), a marked change in  $\Delta$ F508-CFTR location was observed (fig 1C and F) with a significant decrease ( $p < 0.05$ ) in the percentage of cells having a near nuclear location and a significant increase (from 10% to 32%,  $p < 0.05$ ) in cells having  $\Delta$ F508-CFTR at the apical membrane. CFTR was also relocated in cells with a single copy  $\Delta$ F508 genotype ( $\Delta$ F508/4016ins) following sildenafil treatment (fig 1F). The data indicate that CFTR trafficking drugs such as sildenafil will be potentially useful in all CF patients carrying



**Figure 2** Correction of the chloride channel activity of CFTR in CF15 human airway epithelial cells by sildenafil. Cells were incubated for 2 hours at 37°C with or without drug and then CFTR Cl<sup>-</sup> channel activity measured by iodide efflux as described in the Methods section. All data in (A)–(F) are mean (SE) for four separate experiments. (A), (C) and (E) show representative traces from single assays. The presence of forskolin (10  $\mu$ M) and genistein (30  $\mu$ M) is indicated by the horizontal bar. (B), (D) and (F) show mean (SE) data for each experimental condition as indicated. (A) □, no drug; ▲, sildenafil (1 mM); ■, MPB-91 (250  $\mu$ M). (B) \*\*\* $p$ <0.001 for difference from untreated cells; NS, no significant difference. (C) and (D) Concentration dependence of the effect of sildenafil treatment (2 hours at 37°C). (C) ■, 300  $\mu$ M; ▲, 500  $\mu$ M; ▼, 1 mM; ◆, 1.1 mM; ●, 1.3 mM; □, 1.5 mM sildenafil. (D) Concentration-response curve showing % activation at each sildenafil concentration. (E) and (F) Effect of chloride channel inhibitors on iodide efflux stimulated by forskolin (10  $\mu$ M) and genistein (30  $\mu$ M) from cells treated with sildenafil (1 mM) for 2 hours at 37°C. (E) □, basal; ■, no blocker; ▲, +100 nM calixarene; ▼, +500  $\mu$ M DIDS; ◆, +100  $\mu$ M glibenclamide; ●, +500  $\mu$ M DPC. (F) Histograms showing the relative rate ( $k_{peak} - k_{basal}$ ) for the experimental conditions indicated below each bar. \*\*\* $p$ <0.001 for difference from cells stimulated by forskolin and genistein (fsk+gst) without the inhibitor; NS, no significant difference.

a  $\Delta F508$  mutation. Although the concentration of sildenafil effective in increasing  $\Delta F508$ -CFTR trafficking is higher than might be achieved in plasma (1  $\mu$ M) following a single oral dose (100 mg) of Viagra (data from Pfizer: www.Viagra.com), nevertheless the data show proof of principle for investigating this relatively safe class of compounds for use in CF. Furthermore, the effect of sildenafil was selective in that the distinct locations of endoplasmic reticulum (p115), Golgi (GM130), and 20S proteasome

proteins were not altered with sildenafil treatment (data not shown) and sildenafil (150  $\mu$ M, 2 hours) had no effect on wild type CFTR location (fig 1A, F) in cells from non-CF individuals.

#### A new CF trafficking mutation

Cells from a CF individual heterozygous for a rare missense mutation, R1283M,<sup>13</sup> and a stop mutation, E60X, were also examined (fig 1D–F). As for  $\Delta F508$  expressing

cells, the results (fig 1D, F) showed severe mislocalisation of CFTR with the majority of cells having mutant CFTR within the cell at a site close to the nucleus (near nuclear location). The defective location of this mutant CFTR was also markedly corrected by sildenafil (fig 1E, F) with the majority of treated cells showing an apical CFTR location. This is the first description of R1283M as a trafficking mutation and sildenafil was effective in correcting the defect. The results indicate that trafficking mutations in both the first and second nucleotide binding domains (NBD1 and NBD2) of CFTR are targets for sildenafil.

### Effect of sildenafil on CFTR chloride transport activity in CF15 cells

The human airway epithelial CF cell line CF15 was used as a model to study CFTR dependent chloride transport.<sup>11</sup> In untreated CF15 cells there was no stimulation of <sup>125</sup>I efflux in response to the CFTR agonists forskolin and genistein (fig 2A) consistent with its  $\Delta F508/\Delta F508$  CF origin.<sup>11</sup> However, when cells were treated for 2 hours at 37°C with sildenafil a significant stimulation by forskolin and genistein was recovered (fig 2A and B). The effect of sildenafil was similar to that of the potent trafficking drug MPB-91,<sup>8</sup> although of lower magnitude. The concentration dependence of the effect of sildenafil treatment showed an EC<sub>50</sub> of 718 (1)  $\mu$ M (fig 2C, D). Thus, CF15 cells were less sensitive to the effect of sildenafil, requiring a higher concentration than native cells from CF patients to restore function. The effects of a range of Cl<sup>-</sup> channel blockers on the forskolin/genistein stimulated iodide efflux of sildenafil treated cells (1 mM for 2 hours at 37°C) was determined. The efflux was inhibited by glibenclamide and DPC but not by DIDS or calixarene, demonstrating that sildenafil had recruited functional  $\Delta F508$ -CFTR Cl<sup>-</sup> channels at the apical membrane. The results indicate that, like MPB-91, sildenafil was able to restore the chloride channel activity of  $\Delta F508$ -CFTR at 37°C. Immunolocalisation of  $\Delta F508$ -CFTR in CF15 cells indicated that, following treatment for 2 hours at 37°C with 1 mM sildenafil,  $\Delta F508$ -CFTR had moved from within the cell to an apical location (data not shown).

### DISCUSSION

This study has shown, for the first time, a dramatic and rapid effect of the PDE5 inhibitor sildenafil in directing  $\Delta F508$ -CFTR to the apical membrane in native CF airway cells from patients with CF. The effect is comparable to our previously demonstrated effect of MPB compounds.<sup>8</sup> By comparison, other non-selective treatments such as low temperature (25–27°C), glycerol, or 4-phenylbutyrate have required long term exposure to show modest effects on  $\Delta F508$ -CFTR trafficking.<sup>14</sup> The technique that we have developed for confocal immunofluorescence localisation of wild type and  $\Delta F508$ -CFTR in native nasal epithelial cells from control and CF individuals allows CFTR location to be visualised in individual cells, the pattern of mislocalisation quantified, and the actions of pharmacological agents on  $\Delta F508$ -CFTR trafficking evaluated. In agreement with our previous studies<sup>8</sup> and those of others,<sup>7,9</sup>  $\Delta F508$ -CFTR was predominantly mislocalised and present within the cell.  $\Delta F508$ -CFTR was detectable at the apical membrane in a minority (approximately 10%) of cells.

The present studies have also identified a new CFTR trafficking mutation in NBD2 (R1283M) that has not previously been investigated. The only other report of altered trafficking of CFTR with a mutation in NBD2 is N1303K.<sup>15</sup> The present results, showing that sildenafil corrects  $\Delta F508$ - and R1283M-CFTR location, both mutations in nucleotide

binding domains, will provide insights into the mechanism of action of drugs that increase CFTR trafficking. It is noteworthy that neither sildenafil nor MPB compounds affect the trafficking of wild type CFTR, nor of other normal ER or Golgi proteins, thus providing a selective action. We have previously provided evidence<sup>16</sup> that the speed and selectivity of the effect of MPB compounds on  $\Delta F508$ -CFTR trafficking is due to direct binding to the CFTR protein at a site within the first cytoplasmic domain. It is therefore possible that sildenafil also binds directly to  $\Delta F508$ -CFTR, although an action through cyclic GMP signalling has not been ruled out.

There is agreement that  $\Delta F508$ -CFTR has substantial Cl<sup>-</sup> channel activity when present at the apical membrane, which some studies suggest is comparable to that of wild-type CFTR.<sup>1</sup> Thus, the present study showing recruitment of  $\Delta F508$ -CFTR to the apical membrane in native CF cells in response to the drug sildenafil, is a major step forward in restoring CFTR function. Although the concentration of sildenafil shown to traffic  $\Delta F508$ -CFTR in a CF cell line was higher than in native cells, sensitivities may vary between cell lines, isolated native cells, and in vivo, and this will form the basis of future studies. Nevertheless, the finding that sildenafil, a relatively safe drug already in clinical use, has a dramatic effect in increasing  $\Delta F508$ -CFTR trafficking will assist in the discovery of new derivatives and is a major advance towards developing a rational drug treatment aimed at repair or rescue of activity of the mutant CF gene protein. The present results indicate that an approach to drug therapy using CFTR trafficking drugs is likely to be applicable to the majority of patients with CF.

### Authors' affiliations

R L Dormer, C M Harris, Z Clark, M M C Pereira, M A McPherson, Department of Medical Biochemistry and Immunology, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XN, UK  
I J M Doull, Department of Child Health, University Hospital of Wales, Heath Park, Cardiff CF14 4XN, UK  
C Norez, F Becq, Institut de Physiologie et Biologie Cellulaires, UMR 6187, CNRS, Université de Poitiers, France

Financial support was provided by WORD, Pfizer Pharmaceuticals Group, Vaincre la Mucoviscidose (VLM), the Cystic Fibrosis Trust, UK and the Laurence Goodchild fellowship.

Conflicts of interest: none.

### REFERENCES

- Riordan JR. The cystic fibrosis transmembrane conductance regulator. *Annu Rev Physiol* 1993;**245**:1066–73.
- Kunzelmann K, Schreiber R. CFTR, a regulator of channels. *J Membrane Biol* 1999;**168**:1–8.
- McPherson MA, Pereira MMC, Russell D, et al. The CFTR-mediated protein secretion defect: pharmacological correction. *Pflügers Arch - Eur J Physiol* 2001;**443**(Suppl 1):S121–6.
- Kartner N, Augustinas O, Jensen TJ, et al. Mislocalization of deltaF508 CFTR in cystic fibrosis sweat gland. *Nat Genet* 1992;**1**:321–7.
- Denning GM, Ostegaard LS, Welsh MJ. Abnormal localization of cystic fibrosis transmembrane conductance regulator in primary cultures of cystic fibrosis airway epithelia. *J Cell Biol* 1992;**118**:551–9.
- Puchelle E, Gaillard D, Ploton D, et al. Differential localization of the cystic fibrosis transmembrane conductance regulator in normal and cystic fibrosis airway epithelium. *Am J Respir Cell Mol Biol* 1992;**7**:485–91.
- Kälin N, Claaß A, Sommer M, et al. DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. *J Clin Invest* 1999;**103**:1379–89.
- Dormer RL, Dérand R, McNeilly CM, et al. Correction of deltaF508-CFTR activity with benzo(c)quinolizinium compounds through facilitation of its processing in cystic fibrosis airway cells. *J Cell Sci* 2001;**114**:4073–81.
- Penque D, Mendes F, Beck S, et al. Cystic fibrosis F508del patients have apically localized CFTR in a reduced number of airway cells. *Lab Invest* 2000;**80**:857–68.
- McPherson MA, Pereira MMC, Lloyd Mills C, et al. A cyclic nucleotide PDE5 inhibitor corrects defective mucin secretion in submandibular cells containing antibody directed against the cystic fibrosis transmembrane conductance regulator protein. *FEBS Lett* 1999;**464**:48–52.

- 11 **Jefferson DM**, Valentich JD, Marini FC, *et al.* Expression of normal and cystic fibrosis phenotypes by continuous airway epithelial cell lines. *Am J Physiol* 1990;**259**:L496–505.
- 12 **Becq F**, Mettey Y, Gray M, *et al.* Development of substituted benzo[c]quinolizinium compounds as novel activators of the cystic fibrosis chloride channel. *J Biol Chem* 1999;**274**:27415–25.
- 13 **Cheadle JP**, Meredith AL, al-Jader LN. A new missense mutation (R1283M) in exon 20 of the cystic fibrosis transmembrane conductance regulator gene. *Hum Mol Genet* 1992;**1**:123–5.
- 14 **Zeitlin PL**. Novel pharmacologic therapies for cystic fibrosis. *J Clin Invest* 1999;**103**:447–52.
- 15 **Gregory RJ**, Rich DP, Cheng SH, *et al.* Maturation and function of cystic fibrosis transmembrane conductance regulator variants bearing mutations in putative nucleotide-binding domains 1 and 2. *Mol Cell Biol* 1991;**11**:3886–92.
- 16 **Stratford FFL**, Pereira MMC, Becq F, *et al.* Benzo[c]quinolizinium drugs inhibit degradation of  $\Delta$ F508-CFTR cytoplasmic domain. *Biochem Biophys Res Commun* 2003;**300**:524–30.

## Clinical Evidence – Call for contributors

*Clinical Evidence* is a regularly updated evidence-based journal available worldwide both as a paper version and on the internet. *Clinical Evidence* needs to recruit a number of new contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine and the ability to write in a concise and structured way.

### Areas for which we are currently seeking authors:

- Child health: nocturnal enuresis
- Eye disorders: bacterial conjunctivitis
- Male health: prostate cancer (metastatic)
- Women's health: pre-menstrual syndrome; pyelonephritis in non-pregnant women

However, we are always looking for others, so do not let this list discourage you.

### Being a contributor involves:

- Selecting from a validated, screened search (performed by in-house Information Specialists) epidemiologically sound studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with *Clinical Evidence* editors to ensure that the final text meets epidemiological and style standards.
- Updating the text every six months using any new, sound evidence that becomes available. The *Clinical Evidence* in-house team will conduct the searches for contributors; your task is simply to filter out high quality studies and incorporate them in the existing text.
- To expand the topic to include a new question about once every 12–18 months.

If you would like to become a contributor for *Clinical Evidence* or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to Klara Brunnhuber (kbrunnhuber@bmjgroup.com).

## Call for peer reviewers

*Clinical Evidence* also needs to recruit a number of new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are healthcare professionals or epidemiologists with experience in evidence-based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity, and accessibility of specific topics within the journal, and their usefulness to the intended audience (international generalists and healthcare professionals, possibly with limited statistical knowledge). Topics are usually 1500–3000 words in length and we would ask you to review between 2–5 topics per year. The peer review process takes place throughout the year, and our turnaround time for each review is ideally 10–14 days.

If you are interested in becoming a peer reviewer for *Clinical Evidence*, please complete the peer review questionnaire at [www.clinicalevidence.com](http://www.clinicalevidence.com) or contact Klara Brunnhuber (kbrunnhuber@bmjgroup.com).