

## ORIGINAL ARTICLE

# Gentamicin-Induced Correction of CFTR Function in Patients with Cystic Fibrosis and CFTR Stop Mutations

Michael Wilschanski, M.D., Yaacov Yahav, M.D., Yasmin Yaacov, B.Sc., Hannah Blau, M.D., Lea Bentur, M.D., Joseph Rivlin, M.D., Micha Aviram, M.D., Tali Bdolah-Abram, M.Sc., Zsuzsa Bebok, M.D., Liat Shushi, M.Sc., Batsheva Kerem, Ph.D., and Eitan Kerem, M.D.

## ABSTRACT

**BACKGROUND**

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene containing a premature termination signal cause a deficiency or absence of functional chloride-channel activity. Aminoglycoside antibiotics can suppress premature termination codons, thus permitting translation to continue to the normal end of the transcript. We assessed whether topical administration of gentamicin to the nasal epithelium of patients with cystic fibrosis could result in the expression of functional CFTR channels.

**METHODS**

In a double-blind, placebo-controlled, crossover trial, patients with stop mutations in CFTR or patients homozygous for the  $\Delta F508$  mutation received two drops containing gentamicin (0.3 percent, or 3 mg per milliliter) or placebo in each nostril three times daily for two consecutive periods of 14 days. Nasal potential difference was measured at base line and after each treatment period. Nasal epithelial cells were obtained before and after gentamicin treatment from patients carrying stop mutations, and the C-terminal of surface CFTR was stained.

**RESULTS**

Gentamicin treatment caused a significant reduction in basal potential difference in the 19 patients carrying stop mutations (from  $-45 \pm 8$  to  $-34 \pm 11$  mV,  $P=0.005$ ) and a significant response to chloride-free isoproterenol solution (from  $0 \pm 3.6$  to  $-5 \pm 2.7$  mV,  $P<0.001$ ). This effect of gentamicin on nasal potential difference occurred both in patients who were homozygous for stop mutations and in those who were heterozygous, but not in patients who were homozygous for  $\Delta F508$ . After gentamicin treatment, a significant increase in peripheral and surface staining for CFTR was observed in the nasal epithelial cells of patients carrying stop mutations.

**CONCLUSIONS**

In patients with cystic fibrosis who have premature stop codons, gentamicin can cause translational "read through," resulting in the expression of full-length CFTR protein at the apical cell membrane, and thus can correct the typical electrophysiological abnormalities caused by CFTR dysfunction.

From the Department of Pediatrics, Cystic Fibrosis Center, Shaare Zedek Medical Center (M.W., Y. Yaacov, E.K.), and Hadassah University Hospital, Mount Scopus (E.K.), Hebrew University Medical School, Jerusalem, Israel; the Cystic Fibrosis Center, Sheba Medical Center, Tel Hashomer, Israel (Y. Yahav); the Graub Cystic Fibrosis Center, Schneider Children's Medical Center of Israel, Petah Tikva, Israel (H.B.); the Cystic Fibrosis Center, Rambam Medical Center, Haifa, Israel (L.B.); the Cystic Fibrosis Center, Carmel Medical Center, Haifa, Israel (J.R.); the Cystic Fibrosis Center, Soroka Medical Center, Beer Sheva, Israel (M.A.); the Department of Medical Statistics (T.B.-A.) and the Department of Genetics, Life Sciences Institute (L.S., B.K.), Hebrew University, Jerusalem, Israel; and the Department of Cell Biology and the Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham, Birmingham (Z.B.).

N Engl J Med 2003;349:1433-41.

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**C**YSTIC FIBROSIS IS CAUSED BY MUTATIONS in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that lead to dysfunction of the CFTR protein, which is an apical membrane protein regulating the transport of chloride and sodium in secretory epithelial cells.<sup>1</sup> Since the discovery of the CFTR gene, more than 1000 mutations have been identified, including missense, deletion or insertion, frame shift, splice site, and nonsense mutations.<sup>2</sup> Nonsense or stop mutations contain signals that cause a truncated or unstable protein, should result in a deficiency or absence of CFTR chloride channels, and are associated with a severe cystic fibrosis phenotype in most cases.<sup>3,4</sup>

In addition to their antimicrobial activity, aminoglycoside antibiotics can suppress premature termination codons by allowing an amino acid to be incorporated in place of the stop codon, thus permitting translation to continue to the normal end of the transcript. The mechanism of translation termination is highly conserved among most organisms and is almost always signaled by an amber (UAG), ochre (UAA), or opal (UGA) termination codon. The nucleotide sequence surrounding the termination codon has an important role in determining the efficiency of translation termination.<sup>5-7</sup> Aminoglycoside antibiotics can reduce the fidelity of translation,<sup>8</sup> predominantly by inhibiting ribosomal "proofreading," a mechanism to exclude poorly matched amino acyl-transfer RNA from becoming incorporated into the polypeptide chain.<sup>9</sup> In this way aminoglycosides increase the frequency of erroneous insertions at the nonsense codon and permit translation to continue to the end of the gene, as has been shown in eukaryotic cells,<sup>10</sup> including human fibroblasts.<sup>11</sup> The susceptibility to suppression by aminoglycosides depends on the stop codon itself and on the sequences surrounding it.

Howard et al. demonstrated that two CFTR-associated stop mutations could be suppressed by treating cells with low doses of an aminoglycoside antibiotic.<sup>12</sup> Bedwell et al. demonstrated that after incubation of bronchial epithelial cell line IB3-1, which carries a W1282X mutation of CFTR, with aminoglycosides, cyclic AMP (cAMP)-activated chloride conductance and the expression of functional CFTR were restored to the apical membrane.<sup>13</sup> Recently, Zsembery et al. isolated cholangiocytes from the liver of a patient carrying the G542X mutation of CFTR and incubated them with gentamicin, resulting in the expression of cAMP-activated

chloride transport.<sup>14</sup> Thus, in vitro, gentamicin obviated the effect of stop-codon mutations on the transcription and translation of CFTR. This effect has subsequently been demonstrated in a number of models of other diseases caused by stop mutations, including muscular dystrophy,<sup>15</sup> Hurler's syndrome,<sup>16</sup> cystinosis,<sup>17</sup> late infantile neuronal ceroid lipofuscinosis,<sup>18</sup> and disorders involving the p53 gene.<sup>19</sup>

In a previous open pilot study, we found that topical application of gentamicin drops to the nose augmented chloride transport in epithelial cells of nine patients with cystic fibrosis who had at least one W1282X allele.<sup>20</sup> Subsequently, Clancy et al.,<sup>21</sup> in an open study, administered gentamicin intravenously to five patients who were heterozygous for stop mutations and found that four of the patients had hyperpolarization of the nasal potential difference after the administration of isoproterenol, indicating that chloride transport was induced across the apical surface. In these studies, treatments and outcome measures were not masked.

We performed a randomized, double-blind, placebo-controlled, crossover trial to investigate the effects of gentamicin on CFTR function in the nasal mucosal cells of patients with cystic fibrosis and to determine whether the effects are dose dependent.

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## METHODS

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### PATIENTS AND STUDY PROTOCOL

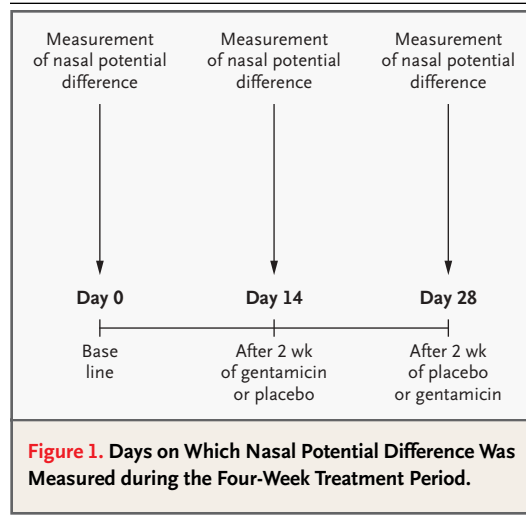
Patients with cystic fibrosis who were older than nine years of age were eligible for the study. In all patients, the diagnosis of cystic fibrosis was based on typical respiratory and gastrointestinal manifestations in the presence of elevated sweat chloride levels. The human ethics committee of the Israeli Ministry of Health approved this study, and written informed consent was obtained from the patients or their parents. No patient had received gentamicin or used nasal drugs including corticosteroids for at least four weeks before the study. After measurement of the base-line nasal potential difference, the patients received eyedrops containing 3 mg of gentamicin per milliliter (Garamycin, Schering-Plough Laboratory) or an identical-appearing bottle of placebo drops. We used a dose that was effective in our open pilot study.<sup>20</sup> The patients or parents were instructed to instill for 14 days two drops in each nostril three times daily (total daily dose, 900 µg) with the head tilted back and then to remain still for several minutes. The patients then returned for a sec-

ond measurement of nasal potential difference and received the placebo for a further two-week period if they had initially received the gentamicin and the gentamicin if they had initially received placebo. The patients returned for a third measurement of potential difference two weeks later. All vials were returned after each two-week period, and the contents were measured to determine compliance. The patients and researchers were unaware of the contents of all vials. The crossover design was chosen because the severity of cystic fibrosis varies widely, and it is thus preferable for each patient to serve as his or her own control. The Department of Pharmacy of Shaare Zedek Medical Center, in Jerusalem, performed the randomization and coding. Nasal potential difference was measured at the end of each two-week period (Fig. 1).

At the end of the trial a group of patients volunteered to participate in an open-label dose-response study involving increasing concentrations of gentamicin for successive periods of two weeks, with nasal potential difference measured at the end of each period. The protocol was similar to the initial study in that two drops were instilled intranasally three times daily. Each patient received vials of gentamicin in increasing concentrations, from 6 mg per milliliter (total daily dose, 1800  $\mu$ g) to 9 mg per milliliter (2700  $\mu$ g) and 12 mg per milliliter (3600  $\mu$ g), for successive two-week periods. Nasal potential difference was measured at the end of each two-week period.

#### MEASUREMENTS OF NASAL POTENTIAL DIFFERENCE

Transepithelial nasal potential difference was determined by measuring the potential difference in values between a fluid-filled recording bridge on the nasal mucosa and a reference bridge (21-gauge needle filled with Ringer's solution in 4 percent agar) inserted into the subcutaneous space of the forearm.<sup>22</sup> Both bridges were linked by calomel electrodes to a high-impedance, low-resistance buffer amplifier (Department of Medical Engineering, Hospital for Sick Children, Toronto). Under direct vision, the catheter was advanced with the use of an otoscope through the inferior meatus of both nostrils, and the potential difference was recorded at various sites. After consistent base-line measurements of potential difference were obtained, amiloride ( $10^{-4}$  M) was superfused at a rate of 5 ml per minute for three minutes. The resultant change in the potential difference was recorded and expressed as both an absolute change and a percent change



**Figure 1.** Days on Which Nasal Potential Difference Was Measured during the Four-Week Treatment Period.

from the maximal value at base line. To study nasal chloride permeability and cAMP activation of chloride permeability, a large chloride chemical gradient across the apical membrane was generated by superfusion of the nasal mucosa with a chloride-free solution containing  $10^{-4}$  mol of amiloride per liter at a rate of 5 ml per minute for three minutes. The mucosa was then perfused for three minutes with the same solution, to which isoproterenol ( $10^{-5}$  mol per liter) had been added. The change in the voltage response during the final six minutes served as an index of epithelial chloride transport.

#### IMMUNOFLUORESCENCE MICROSCOPY OF PRIMARY HUMAN AIRWAY CELLS

Primary nasal epithelial cells from two patients with cystic fibrosis who were compound heterozygotes for the W1282X and the  $\Delta$ F508 mutations and who participated in the gentamicin study were obtained by scraping before and after treatment with gentamicin and were then spread on microscope slides. Nasal epithelial cells were also obtained from three healthy control subjects as positive controls. Cells were fixed with ice-cold methanol for 10 minutes, air-dried, and stored under dry conditions. Before immunocytochemical analysis, samples were rehydrated in phosphate-buffered saline (pH 7.4) for 10 minutes and then treated with goat serum (1:20 dilution) for 30 minutes to block nonspecific protein-binding sites. CFTR was detected with the monoclonal 24-1 antibody (5  $\mu$ g per milliliter, American Type Culture Collection HB-11947, R&D Systems), which recognizes amino acids 1477 through 1480 at the C-terminal of CFTR protein. Nonimmune

mouse IgG was used as a negative control. Anti-mouse IgG (Alexa Fluor 594, Molecular Probes) was used as the secondary antibody (1:500 dilution). Slides were mounted with Vectashield medium containing 4,6-diamidino-2-phenylindole, which stains nuclei (Vector Laboratories). At least 150 cells from each sample were studied on a Leitz epifluorescence microscope equipped with a step motor, filter-wheel assembly (Ludl Electronics Products), and an 83,000-filter set (Chroma Technology). Images were obtained with a SenSys-Cooled, charge-coupled high-resolution camera (Photometrics). Partial deconvolution of images was performed with use of IPLab software (Scanalytics). Images were obtained in a random fashion. At least 15 areas of each sample were analyzed. The number of cells present in each area ranged from approximately 7 to 45 cells. The intensity of staining differed in each sample. Therefore, all images were normalized to higher background fluorescence intensity without changing the maximal fluorescence intensity. Preliminary experiments to test the specificity and sensitivity of the assay were performed with the use of cell preparations obtained from control subjects and patients who were homozygous for the  $\Delta F508$  mutation.

#### STATISTICAL ANALYSIS

Data on potential difference were analyzed with use of the procedure for a crossover design.<sup>23</sup> Each variable was evaluated for a carryover effect, a period effect, and a treatment effect with the use of both a parametric two-tailed t-test and the nonparametric Mann-Whitney test. Had a significant carryover effect been found, the measurements obtained during the second period would have been ignored and only the measurements obtained in the first peri-

od would have been used. In order to identify a change in potential difference of more than 5 mV in response to a chloride-free isoproterenol solution with 80 percent power and a type I error of 5 percent, we calculated that we would need eight patients.

The dose-response effect was studied with use of the repeated-measures analysis to check for a significant trend using Hottelling's Trace statistic, as well as the nonparametric Friedman rank test. The Wilcoxon signed-rank test was used to compare the pretreatment results with those with 0.3 percent gentamicin. Antibody staining was analyzed by comparing by the Mann-Whitney test the percentage of total analyzed cells with the percentage of cells that showed peripheral and surface staining before and after treatment. All P values were two-tailed, and a P value of 5 percent or less was considered to indicate statistical significance. Values are expressed as means  $\pm$ SD unless otherwise stated.

## RESULTS

#### PATIENTS

Thirty-two patients were enrolled in the trial, and 25 completed the protocol (mean age,  $21 \pm 8.5$  years; range, 9.5 to 52). The other seven patients did not complete the study owing to intercurrent infections during the four weeks of the study or to a self-admitted lack of compliance. One patient was subsequently excluded who, when the volume of returned gentamicin solutions was measured, had not used the minimal volume required. Of the 24 study patients, 11 carried two stop mutations: 6 were homozygous for W1282X, 3 were compound heterozygous for W1282X/G542X, and 2 were compound heterozygous for W1282X/3849+10KbC $\rightarrow$ T. The 3849+10kbC $\rightarrow$ T mutation can lead to the inclusion of a cryptic 84-bp exon, which contains a stop codon.<sup>24</sup> Another eight patients were heterozygous for stop mutations: six were  $\Delta F508$ /W1282X, one was G85E/ W1282X, and one was W1282X/unknown. Ten patients first received gentamicin, and 14 patients first received placebo. In addition, five patients who were homozygous for  $\Delta F508$  were studied according to the same protocol and served as a control group, since the 3-bp deletion mutation  $\Delta F508$  is not expected to be affected by gentamicin treatment. The relevant clinical characteristics of the study groups are shown in Table 1. None of the patients had serious sinus disease, coryza, nasal polyposis, or hypoxemia in the month before the

**Table 1. Clinical Characteristics of the Patients.\***

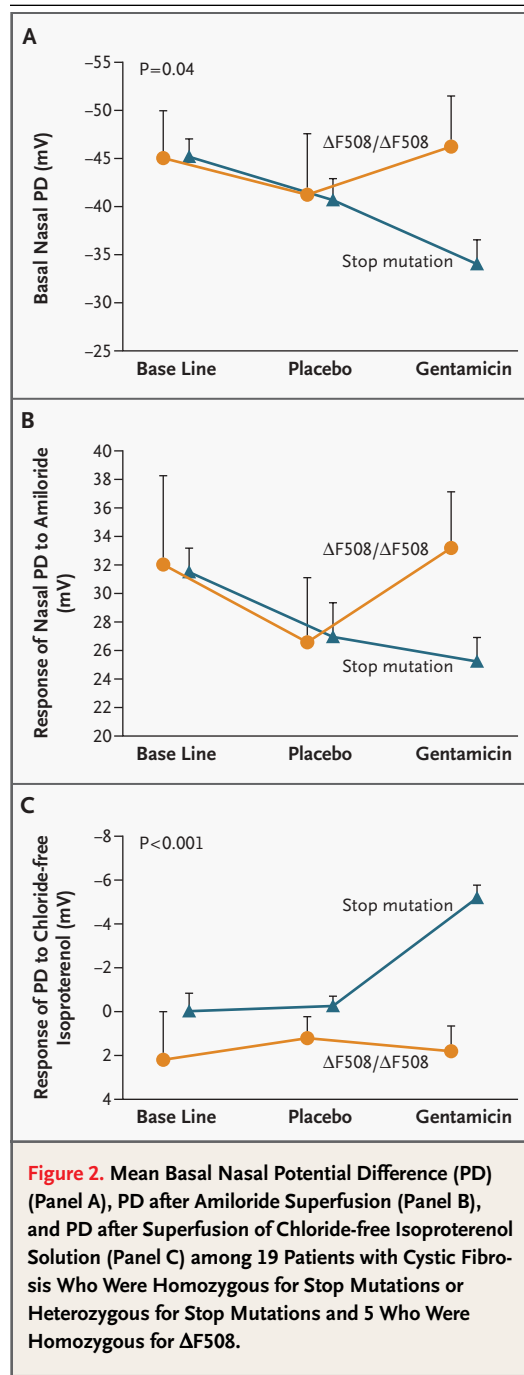
Group	No. of Patients	Age yr	Sweat Chloride mmol/liter	FEV <sub>1</sub> % of predicted
Homozygous for stop mutation	11	23 $\pm$ 11	96 $\pm$ 17	67 $\pm$ 17
Heterozygous for stop mutation	8	20 $\pm$ 6	110 $\pm$ 17	76 $\pm$ 23
Homozygous for $\Delta F508$ mutation	5	18 $\pm$ 5	119 $\pm$ 14	54 $\pm$ 12

\* Plus-minus values are means  $\pm$ SD. There were no significant differences among the groups. FEV<sub>1</sub> denotes forced expiratory volume in one second.

study or during the study. There were no significant differences in the rates of colonization of *Pseudomonas aeruginosa* between the groups.

#### MEASUREMENTS OF NASAL POTENTIAL DIFFERENCE

The initial pretreatment nasal potential difference value in all patients was typical of patients with cystic fibrosis. Basal potential difference was elevated at  $-45 \pm 8$  mV, as compared with  $-16 \pm 5$  mV in the control subjects without cystic fibrosis.<sup>25</sup> The depolarization in response to amiloride was exaggerated ( $32 \pm 14$  mV, as compared with  $10 \pm 4$  mV in the controls),<sup>25</sup> and there was no significant change in the potential difference after superperfusion with chloride-free isoproterenol solution. After 14 days of nasal administration of gentamicin, the basal potential difference was significantly reduced among the 19 patients carrying stop mutations (from  $-45 \pm 8$  to  $-34 \pm 11$  mV,  $P=0.005$ ), but there was no significant change in the group that was homozygous for  $\Delta F508$  (Fig. 2A). In 5 of the 19 patients (26 percent) — 4 homozygotes and 1 heterozygote — basal potential difference was reduced to a mean of  $-21 \pm 2$  mV;  $-28$  mV is the upper limit of the normal range in our laboratory.<sup>25</sup> Although there was a trend toward an increase in the response to amiloride after gentamicin treatment, it was not statistically significant ( $P=0.1$ ) (Fig. 2B). The response to chloride-free isoproterenol solution after gentamicin treatment among patients carrying stop mutations was significantly different from the basal response ( $0 \pm 3.6$  mV at base line, as compared with  $-5 \pm 2.7$  mV after gentamicin treatment;  $P<0.001$ ) (Fig. 2C). In 11 of the 19 patients (58 percent), 7 homozygotes and 4 heterozygotes, the mean potential difference in response to chloride-free isoproterenol solution (a measure of chloride transport) was  $-7 \pm 2$  mV after gentamicin treatment; the normal range in our laboratory is  $-12 \pm 7$  mV.<sup>25</sup> In 4 of the 19 patients (21 percent) both the basal potential difference and chloride transport were significantly improved after gentamicin treatment; basal potential difference (reflecting sodium transport) was below  $-28$  mV, and the potential difference in response to chloride-free isoproterenol solution exceeded  $-5$  mV, thus providing evidence of chloride transport. Neither the carryover effect nor the period effect was significant. When the patients carrying stop mutations were divided into those who were homozygous for stop mutations and those who were heterozygous, the differences in basal poten-



tial difference and potential difference in response to chloride-free isoproterenol solution were significantly greater among the homozygous patients (Table 2). One patient in the homozygous group and one in the heterozygous group had no response to gentamicin treatment.

**Table 2. Nasal Potential Difference (PD) Values before and after Gentamicin or Placebo in the Three Groups.\***

Nasal Potential Difference	Homozygous for Stop Mutation (N=11)	Heterozygous for Stop Mutation (N=8)	Homozygous for ΔF508 Mutation (N=5)
	<i>mV</i>		
<b>Basal</b>			
Base line	-48±10	-42±3	-45±11
Placebo	-46±10	-41±10	-41±14
Gentamicin	-34±12	-34±9	-46±12
P value for treatment effect	0.008†	0.25‡	
<b>Response to amiloride</b>			
Base line	33±9	32±7	32±14
Placebo	34±11	27±10	26±10
Gentamicin	24±12	25±7	33±9
P value for treatment effect	0.05†	0.79‡	
<b>Response to chloride-free isoproterenol</b>			
Base line	0.4±4.6	-0.5±2	2.2±5
Placebo	-0.4±2.7	-0.25±2	1.2±2
Gentamicin	-5.5±2.8	-4.8±2.6	1.8±2.5
P value for treatment effect	0.001†	0.036‡	

\* Plus-minus values are means ±SD. For the comparison of placebo or gentamicin with base-line values, only the P values for the treatment effect are shown; the P values for carryover effect and period effect were not significant.

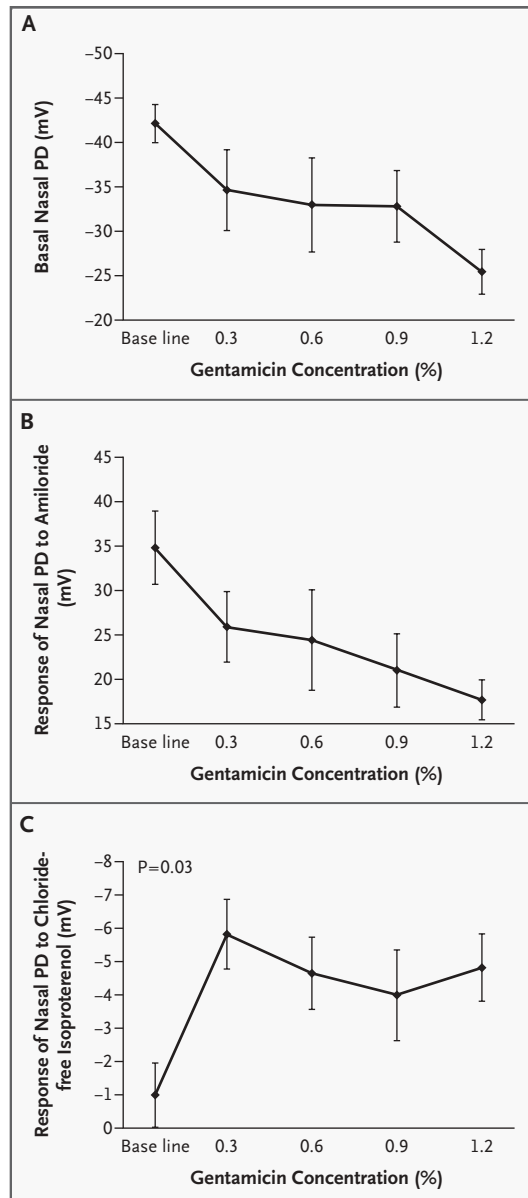
† A t-test was used.

‡ The Mann-Whitney U test was used.

After the randomization code was broken, six patients, five of whom were homozygous for stop mutations and one of whom was heterozygous, who had a response to gentamicin completed the open-label dose-response trial with increasing concentrations of gentamicin drops. Overall there was a further decrease in the basal potential difference ( $P=0.05$ ) and a trend toward a normal response to amiloride, but no overall improvement in the response to chloride-free isoproterenol solution, which seemed to peak at the lowest dose of gentamicin (3 mg per milliliter) (Fig. 3). When the responses to placebo and the 0.3 percent dose were compared, there was a significant difference only in the response to chloride-free isoproterenol solution ( $P=0.03$ ).

**DETECTION OF FULL-LENGTH CFTR PROTEIN**

The effect of intranasal gentamicin treatment on the “read through” of premature nonsense codons was further analyzed in two of the patients with the ΔF508/W1282X genotype who had a response to gentamicin. Nasal potential difference was meas-



**Figure 3. Mean Basal Potential Difference (PD) (Panel A), PD after Amiloride Superfusion (Panel B), and PD after Superfusion of Chloride-free Isoproterenol Solution (Panel C) in Response to Increasing Concentrations of Gentamicin among Six Patients Carrying Stop Mutations.**

ured and nasal epithelial cells were obtained before and after gentamicin treatment. The detection of full-length CFTR protein was performed in a blind fashion.

Before gentamicin treatment, the staining was mainly perinuclear, with only 4 percent of cells

showing staining throughout the cells and minimal surface staining in some cells (Fig. 4B). Since the patients were heterozygous for the  $\Delta F508$  mutation, this pattern of perinuclear staining is consistent with the endoplasmic reticular location of CFTR proteins carrying the  $\Delta F508$  mutation. After gentamicin treatment, there was a significant ( $P < 0.01$ ) change in the pattern of staining toward a peripheral and surface pattern (Fig. 4C). This pattern was similar to that observed in the control subjects. These results indicate gentamicin-induced suppression of the nonsense mutation, resulting in the production of full-length CFTR, and they are consistent with the measurements of potential difference showing improvement in chloride transport in these patients.

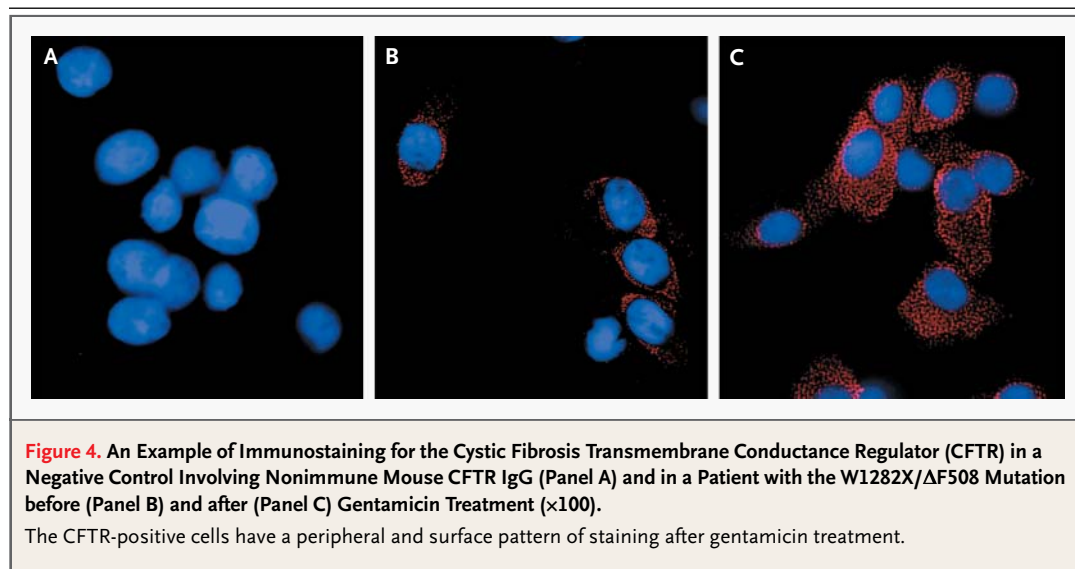
#### DISCUSSION

In this double-blind, placebo-controlled, crossover study, we found that application of gentamicin to the nasal epithelium restored CFTR function in about 90 percent of patients with cystic fibrosis who had stop mutations in CFTR. After gentamicin treatment, the electrophysiological abnormalities caused by the CFTR defect resolved in 21 percent of the patients and chloride or sodium transport was restored in 68 percent. Gentamicin seems to be more effective in patients who are homozygous for stop mutations than in those who are heterozygous. Furthermore, staining the cells with an antibody against CFTR, which recognizes a region in CFTR beyond

the W1282X mutation, showed an increase in the number of cells with surface staining, indicating the delivery of full-length CFTR proteins to the apical membrane. These results provide direct evidence that gentamicin treatment of patients with cystic fibrosis and the stop mutation W1282X can induce the synthesis of full-length CFTR.

This correction of the abnormal potential difference and the appearance of full-length CFTR on the cell surface support the findings of previous studies, which showed that gentamicin can promote “read through” of stop mutations. Two of the patients carrying stop mutations had no response to gentamicin. The reason for this lack of response is unknown. It might result from inefficient uptake or distribution of gentamicin in these patients. However, there might be a molecular explanation for this lack of response, involving the mechanism of the suppression of the nonsense codon by gentamicin. This complex mechanism of suppression requires the aminoglycoside to bind to the decoding center of ribosomal RNA during translation of nonsense transcripts. This binding alters the RNA conformation, reducing the accuracy of the codon–anticodon pairing. In the patients with no response, inefficiency of any of the steps in this mechanism could have resulted in insufficient levels of full-length CFTR protein and thus a failure to restore the function of the protein.

The response to amiloride was not strongly influenced by gentamicin. This has been reported by other investigators using responses in the nasal po-



tential difference to measure the effect of a drug on CFTR function.<sup>26</sup> The effect of gentamicin might have been sufficient to induce only partial inhibition of the epithelial sodium channel, causing a change in basal potential difference but not in the response to amiloride. Furthermore, the response to amiloride is dependent on the basal potential difference, and the difference in response becomes less prominent as that value decreases.

The aminoglycoside antibiotic gentamicin is very commonly used for severe gram-negative infections. Its antibacterial activity results from binding to the bacterial 16S ribosome unit, causing misreading of messenger RNA codons. Gentamicin is routinely administered for its antibacterial effect both intravenously and topically by means of aerosolized inhalation in patients who have respiratory exacerbations of cystic fibrosis. The clinical response to gentamicin in some cases was unrelated to the development of aminoglycoside resistance or the eradication of pseudomonas. However, its use is limited by a lack of bioavailability owing to gastrointestinal absorption and by severe renal toxicity and ototoxicity at relatively low serum concentrations.

Administration of aminoglycosides by inhalation has become a common and standard therapy in patients with cystic fibrosis and has minimal side effects. This route results in higher concentrations in the respiratory system than does the intravenous route, with very low serum concentrations. Although we did not include a study group that was given an antibiotic other than an aminoglycoside, it is unlikely that the response to gentamicin was due to its antimicrobial effect, since the drug had an effect only in patients carrying stop mutations and not in those who were homozygous for the  $\Delta F508$  mutation.

In vitro studies using quantitative immunohistochemistry have shown that after incubation with an aminoglycoside, cells from patients with cystic fibrosis have as much as 25 percent (in those with the R553X mutation) to 35 percent (in those with the G542X mutation) of the concentration of full-length CFTR observed in cells transfected with a wild-type CFTR complementary DNA.<sup>12,13</sup> This increase in functional CFTR might exceed the threshold required for normally functioning respiratory epithelial cells and might thus have corrected cell-membrane function in our patients. Furthermore, gentamicin is expected to be more effective in patients carrying two alleles with stop mutations than in those with only one stop mutation, because it

promotes “read through” of both alleles. The clinical significance of the changes we observed in nasal potential difference and the appearance of CFTR at the apical surface of nasal epithelial cells in the immunofluorescence study is unknown. Further clinical studies are required to address this issue.

In vitro studies showed that after treatment with gentamicin, full-length CFTR was observed on immunoprecipitation assay, and the concentration increased as the dose increased from 0.1 to 0.4 mg of gentamicin per milliliter.<sup>12</sup> The standard gentamicin drops that we used were approximately 10 times this concentration. It is difficult to extrapolate directly from the in vitro studies the maximal effective topical dose, since the drug is diluted by liquid on the nasal surface and needs to cross extracellular barriers before it reaches its target respiratory epithelium. However, experiments showing a dose-dependent response in vitro have largely been confirmed by our in vivo results.

The identification of clinically useful methods to suppress premature stop mutations within the CFTR gene might be of benefit to patients with cystic fibrosis and patients with other diseases caused by stop mutations. Since nearly one third of genetic defects are caused by stop, or nonsense, mutations, this approach to suppressing mutations may be applicable to the treatment of many other inherited diseases. In a mouse model of Duchenne’s muscular dystrophy, aminoglycosides have increased the expression of full-length protein from a dystrophin allele carrying a stop mutation.<sup>13</sup> However, these results were not reproduced in patients with muscular dystrophy.<sup>27</sup> Encouraging results have also been obtained in Hurler’s syndrome. Fibroblasts carrying a stop mutation were cultured in the presence of gentamicin and demonstrated a significant increase from base line in  $\alpha$ -L-iduronidase activity.<sup>14</sup> Helip-Wooley et al. have demonstrated that the addition of gentamicin to cystinotic fibroblasts leads to the depletion of intracellular cystine in cell lines with a stop mutation only.<sup>17</sup> Keeling and Bedwell have recently shown that aminoglycosides may suppress stop mutations in the p53 gene associated with cancer.<sup>19</sup>

The issues of the route of administration and safety of aminoglycosides need to be addressed. We have shown that short-term exposure of the nasal epithelia to gentamicin is associated with measurable CFTR-mediated chloride transport. Whether longer exposures will be tolerable, effective, and safe has yet to be determined. The risk that gentamicin

may have adverse effects on the translation mechanism of other genes needs to be studied. Furthermore, it is not yet known how much mutant CFTR must reach the apical membrane to induce a clinically relevant beneficial effect.

Supported by grants from the North American Cystic Fibrosis Foundation, Israel Ministry of Health, Israel Academy of Sciences, Israel Cystic Fibrosis Foundation, and Balint Charitable Trust, United Kingdom.

We are indebted to Estelle Smith, of the Department of Pharmacy at Shaare Zedek Medical Center, for her assistance.

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