Cystic Fibrosis case for first year medical students – Team Based Learning Format – instructor

**The Learning Objectives:**
1. Learn to work in small groups effectively to solve a clinical problem.
2. Describe the how cystic fibrosis is diagnosed using a sweat test.
3. Describe the normal versus pathologic transport mechanisms of the CFTR channel.
4. Discuss the effect of an obstructive lung disorder on pulmonary mechanics.
5. Discuss the ethical issues surrounding children in medical research.
6. Describe how an SDS-PAGE gel works.
7. Discuss the inheritance pattern for cystic fibrosis and the likelihood of passing the gene or disease from one generation to another.

**The Case:**
Mr. and Mrs. Gottleib took their nine-month-old infant, Jeremy, to the emergency room because he had been suffering from a cough and diarrhea for almost a week. They told the doctor that Jeremy would sometimes "wheeze" a lot more than they thought was normal for a child with "just a cold" which is what their pediatrician said the problem was last week.

Upon arriving at the emergency room, the attending pediatrician noted that salt crystals were present on Jeremy’s skin, and chest auscultation revealed abnormal sounds. The attending pediatrician ordered a chest x-ray and asked Jeremy’s parents to sit with him in his office to discuss the situation.

The pediatrician told the Gottleibs that Jeremy was small for his age, something he called "failure to thrive". He also mentioned the salt crystals on the baby’s skin, at which time Mr. Gottleib said "Jeremy has always had salty skin, I call him my 'Little Pretzel Stick' because his skin tastes salty when I kiss him." He said Jeremy’s symptoms all point toward a specific disease, and a sweat test would be needed to confirm his suspicions.

To do a sweat test, a small patch of skin on the child’s forearm is first cleaned. A gauze pad saturated with pilocarpine, a chemical that makes the skin sweat, is then applied to the area. Electrodes are hooked up, and a mild electric current is turned on for five minutes, which drives the pilocarpine into the skin. A sweaty area appears on the skin where the gauze had been placed, and a piece of dry filter paper is taped over it to absorb the sweat for about a half hour. A technician then measures the concentration of chloride in the pad.

The following day - "I’ll never forget that day" - Jeremy’s mother says, the pediatrician called with the results of the sweat test. The doctor told Mrs. Gottleib that Jeremy’s chloride level was much higher than normal. This meant that the test was positive and revealed that the pediatricians suspicions were correct, Jeremy has cystic fibrosis. The pediatrician explained that Jeremy’s respiratory symptoms were caused by the cystic fibrosis because his airways were becoming distended by thick and tenacious mucus secretions which cannot be cleared from the airways in the normal fashion.

[Jeremy’s Chest x-ray: http://myweb.lsbu.ac.uk/dirt/museum/margaret/68--252-3041141.jpg]
**Explanation of the Team Based Learning (TBL) format:**

This case was written to be used in a Team-Based Learning (TBL) format. TBL cases utilize a specific written format and method of facilitating to produce very effective small group and class discussion. Larry A. Michaelsen describes in detail this theory and method in the book "Team-Based Learning, A Transformative Use of Small Groups". You can also learn more about TBL at [http://www.ou.edu/pii/teamlearning/](http://www.ou.edu/pii/teamlearning/).

In short, TBL cases should be written to utilize the “Three S’s” in order to foster team work and group discussion. These are: (1) all students in the class should be working on the same problem or assignment, (2) students should be required to make a specific choice, and (3) group should all simultaneously report their choices.

1. It is important that all groups are working on the same problem because this enables a discussion both within group and between groups. If each of the groups is working on a different problem, then there is no common ground for discussion between groups.

2. The assignment should be written so students have to make a specific choice (i.e. put a multiple choice question at the end of the assignment). If students are asked an open-ended question at the end of the assignment, they make come up with one or two answers then end their discussion. If faced with a choice between five to seven possibilities, they have to discuss each possibility fully in order to accept it or reject it. Thus, more discussion is elicited when students are asked to make a specific choice. The choices should be written rather vaguely to stimulate discussion, with one best choice but other possible correct choices.

3. After group discussion, the group should be instructed to report their answer choice simultaneously. I do this by giving each group an envelope that contains 5 colored note cards lettered A, B, C, D, or E. The groups are asked to raise the note card which corresponds to their answer choice on the count of three. This allows the facilitator to immediately assess the overall performance of the class, and prevents groups from choosing their answer based on what other groups think. It also requires each group to commit to one answer choice and be ready to defend it.

This case requires 50-60 minutes to complete in the TBL format. When students arrive to the class/session, they should sit in their assigned groups and the case should be distributed. The students should be given 20 minutes to discuss the case in their groups and answer the questions, if more time is required, you can give an additional 5-10 minutes. This discussion period should be closed-book, and no outside resources (internet, handouts, journals, etc.) should be used during the discussion or to answer the questions. After the discussion is finished (20-30 minutes), the students should simultaneously report their answer for the first question when you instruct them to do so. To facilitate simultaneous reporting, it is useful to hand out colored note cards that have the answer choices (A, B, C, D, E) written on them. Then you can ask the groups to raise the note card which corresponds to their answer choice on the count of three. This allows immediate assessment of the class responses, and makes it easy to facilitate whole class discussion based on which answers the students chose. After the first question is discussed, proceed to the remaining questions in the same format.
The Questions:

1. Which of the following mechanisms most accurately explains how a mutation to the CFTR gene results in thicker than normal mucus secretions in the respiratory airways?

   A. Produces improper protein folding which prevents normal function of cilia.  
   B. Reduces the number of CFTR proteins on the epithelial cell plasma membranes.  
   C. Produces chloride transporters with a lower than normal Km.  
   D. Causes improper splicing of the hnRNA.  
   E. Increases the expression of aquaporin channels in bronchial epithelial cells.

2. As Jeremy’s disease progresses, he may develop bronchiectasis, an abnormal stretching and enlarging of the respiratory passages caused by mucus blockage, which is an obstructive lung disease. If this were to happen, predict the changes to the following respiratory parameters.

<table>
<thead>
<tr>
<th>Forced vital capacity (FVC)</th>
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<th>FEV₁ / FVC</th>
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<tr>
<td>A increased</td>
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The pediatrician explained to Jeremy’s parents that research for cystic fibrosis is happening world wide to find a cure for the disease. He told them about a group of scientists doing medical research, and asked if they would allow Jeremy to be involved in a study. After a discussion about the nature of the research, Jeremy’s parents agreed allow him to participate in the study.

3. Which of the following is the correct protocol that must be applied when using children for medical research?

   A. The child between the ages of 7 to 17 years is required to give assent.  
   B. Only one parent is required for consent.  
   C. The parents can obtain some material benefit for having their child participate.  
   D. Verbal information to the parent is adequate as informed consent.  
   E. Verbal agreement by the parents is adequate for consent to the study.

The medical study which Jeremy was involved in included a pulse-chase experiment (figure below), using lung epithelial cells from a normal volunteer (A) and Jeremy (B). The cells were incubated for fifteen minutes in methionine-free media containing radioactive $^{35}$S-labeled methionine (the pulse). The radioactive methionine was then washed away, and the cells were incubated in medium containing an excess of unlabelled methionine for the indicated periods of time (the chase). At each time point, the cells were homogenized and an immunoprecipitation was performed using an antibody to CFTR. The immunoisolates were separated on an SDS-PAGE gel and the dried gel was exposed to x-ray film. The figure below shows a simplified drawing of the exposed x-ray film. On the right of each gel are shown the migration positions of molecular weight markers on the SDS-PAGE gel. 

(This figure was drawn based on data shown in Lukacs et al., 1994.)
4. The shift in the apparent molecular weight of CFTR seen in lanes 4 and 5 of gel A, but not gel B, is most likely due to

A. glycosylation of normal CFTR.
B. degradation of normal CFTR.
C. assembly of normal CFTR into a complex.

5. Ten years after Jeremy's diagnosis, the Gottlieb's have had a daughter who is also affected with Cystic Fibrosis. Their eldest son who is now 25 years old is interested in marriage and has requested testing to determine if he is a carrier of a cystic fibrosis allele. He has previously tested negative for the same sweat test which Jeremy received. What is the pre-test risk that Jeremy's brother is a heterozygous carrier of a cystic fibrosis allele, given that he does not have the disease?

A) 1/10  
B) 1/4  
C) 1/2  
D) 2/3  
E) 3/4

The Gottlieb Family

Mr. & Mrs. Gottlieb

Jeremy's brother

Jeremy

Jeremy's sister
Explanations of the questions: (correct answers are indicated below in red)

1. Which of the following mechanisms most accurately explains how a mutation to the CFTR gene results in thicker than normal mucus secretions in the respiratory airways?

A. Produces improper protein folding which prevents normal function of cilia.
B. Reduces the number of CFTR proteins on the epithelial cell plasma membranes.
C. Produces chloride transporters with a lower than normal Km.
D. Causes improper splicing of the hnRNA.
E. Increases the expression of aquaporin channels in bronchial epithelial cells.

Explanation #1:
Choice A: A CFTR gene mutation will produce improper protein folding, but it will have no effect of the function of cilia.

Choice B: Correct answer: With normal CFTR genes, the protein is synthesized and transported to the endoplasmic reticulum and Golgi apparatus for additional processing before being integrated into the cell membrane. A CFTR mutation causes the protein to be folded incorrectly and marks the defective protein for degradation. As a result, the protein never reaches the cell membrane, thus there is a reduced number of CFTR proteins on the plasma membrane.

Choice C: A CFTR gene does produce chloride transporters, but a mutation in this gene has no effect of the Km of the transporters, but rather on the physical structure of the protein.

Choice D: This protein folding defect is not a result of a defect in nuclear hnRNA splicing, but rather, results after a protein of abnormal primary sequence is produced, but cannot fold properly to express normal function.

Choice E: Increases the expression of aquaporin channels in bronchial epithelial cells would likely produce thicker than normal mucus secretions in the respiratory airways (due to reduced water in the secretions), but this is not the result of a CFTR gene mutation.

2. As Jeremy’s disease progresses, he may develop bronchiectasis (an abnormal stretching and enlarging of the respiratory passages caused by mucus blockage), producing an obstructive lung disorder. If this were to happen, predict the changes to the following respiratory parameters.

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Explanation #2: FVC is normally smaller than vital capacity during normal breathing. The reason is that some airways close during forced expiration (dynamic airway obstruction), so that the air in these areas can not be further exhaled (entrapped air). This phenomenon is even more prominent in obstructive lung disease because of the lowered recoiling force. Airway obstruction causes FEV₁ to decrease (high airway resistance), but more importantly the ratio FEV₁/FVC is reduced below normal value of 80%. FEV₁ and FVC can also be
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reduced by a restrictive disease (in this case FEV1 is reduced, because of a small VC), but their ratio is normal or increased. Therefore only a reduced FEV1/FVC ratio proves clearly the existence of obstruction (High airway resistance). Only in answer B this value is reduced to 40% (Normal value = 80%).

3. Which of the following is the correct protocol that must be applied when using children for medical research?

A. The child between the age of 7-17 years is required to give assent.
B. Only one parent is required for consent.
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D. Verbal information to the parent is adequate as informed consent.
E. Verbal agreement by the parents is adequate for consent to the study.

Explanation #3:
Research involving children must be approved by the institutional review board (IRB) and the investigator must have the permission of both of the child’s parents before enrolling the child into the study. The parents would need to be given written information about the medical research, and a signed consent form would be required (not just verbal agreement). Children who are between the ages of 7 to 17 should be informed and provide assent by showing that they understand what is required of them (this may vary from state to state). Finally, there must be no material benefits offered to the parents to persuade them to have the child participate in the study.

4. The shift in the apparent molecular weight of CFTR seen in lanes 4 and 5 of gel A, but not gel B, is most likely due to

A. glycosylation of normal CFTR.
B. B degradation of normal CFTR.
C. assembly of normal CFTR into a complex.

Explanation #4: In the "pulse" part of a pulse-chase experiment, the cells are briefly incubated with one (or more) radioactive amino acid mixed with the other nineteen cold amino acids. Any protein that is being actively translated during the time of the pulse will incorporate this labeled amino acid in its growing polypeptide chain wherever in its sequence that amino acid is called for. Methionine is commonly used for these protein labeling experiments because every protein begins with a start methionine. Thus, every protein translated in the presence of radioactive methionine will contain at least one labeled methionine (the start methionine) plus any additional (labeled) methionines specified in that particular protein's specific amino acid sequence.

In the "chase" part of a pulse-chase experiment, the radioactive amino acid is washed away, and a large excess of the same unlabeled amino acid (in this case, methionine) is added to the other unlabeled amino acids. Proteins continue to be translated during the chase, however because there is no labeled amino acid present these newly synthesized proteins will be unlabeled.
An **immunoprecipitation** is a technique used to isolate a single protein (in this case, CFTR) out of a complex mixture of proteins (in this case, all of the proteins of the cell). An antibody specific to a particular protein is added to a mixture, and then the antibody with bound protein is isolated while all of the unbound proteins are washed away. In this pulse-chase experiment, the immunoprecipitation will isolate both labeled CFTR (which was translated during the pulse) and unlabeled CFTR (which was translated both before and after the pulse).

An **SDS-PAGE gel** is a denaturing gel that separates proteins according to their mass; larger proteins run slower on the gel. When an SDS-PAGE gel containing radioactive proteins is dried and exposed to x-ray film, the migration positions of the radiolabeled proteins appear as labeled exposed bands on the x-ray film.

There are several types of information that can be obtained from a pulse-chase experiment. The first type is the determination of the half-life of a protein, i.e. how much time must pass after translation of a protein before 50% of that protein is degraded and disappears from the cell. In addition, any post-translational modifications of a protein that alter its mass can appear as shifts in the migration position of the protein on a gel. CFTR is a protein with twelve transmembrane domains, and as such contains an ER signal sequence and translated on ribosomes bound to the rough ER membrane. Post-translational modifications that can change the mass of CFTR are 1) cleavage of the ER signal sequence resulting in decrease in mass, 2) addition of N-linked glycosylation in the ER resulting in an increase in mass, and 3) additional glycosylation in the Golgi that can further increase its mass. The increase in mass of CFTR seen in lanes 4 and 5 of gel A is due to newly translated CFTR reaching the Golgi and receiving additional glycosylation there that increases its mass. This is why answer choice A is the correct answer. The patient CFTR (gel B) misfolds and is degraded in the ER. This mutant CFTR never reaches the Golgi and never receives this additional glycosylation that increases its mass. Answer B is incorrect because degradation of a protein results in disappearance of labeled protein from the gel, not an increase in its mass. Answer C is incorrect because a denaturing SDS-PAGE gel will dissociate any complex resulting in a protein running at its individual molecular weight regardless of whether it was in a complex when the detergent SDS was added. This experiment also showed that 90% of the normal CFTR doesn't visibly increase in mass and is degraded. We now know that 90% of the normal CFTR folds incorrectly in the ER and is degraded there by the ER quality control machinery, however 10% of it makes it to the cell surface. In contrast, 100% of the Phe508 mutation of CFTR folds incorrectly and never makes it out of the ER.

5. Ten years after Jeremy's diagnosis, the Gottlieb's have had a daughter who is also affected with Cystic Fibrosis. Their eldest son who is now 25 years old is interested in marriage and has requested testing to determine if he is a carrier of a cystic fibrosis allele. He has previously tested negative for the same sweat test which Jeremy received. What is the pre-test risk that Jeremy's brother is a heterozygous carrier of a cystic fibrosis allele, given that he does not have the disease?

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**The Gottlieb Family**

- **Mr. & Mrs. Gottlieb**
- **Jeremy's brother**
- **Jeremy**
- **Jeremy's sister**
**Explanation #5:** The objective of this question is to insure that the inheritance of Cystic Fibrosis is known to obey an autosomal recessive pattern. If the student does not know this fact, a priori, then the pedigree can be used to determine that the autosomal recessive pattern is the only possibility. A Punnett square can then be used to show that, given the absence of disease (and the presence of two Cystic Fibrosis alleles in Jeremy's brother) there is a 1/3 probability that Jeremy's brother carries no Cystic Fibrosis alleles, and a 2/3 probability that Jeremy's brother carries one Cystic Fibrosis allele and one normal dystrophin allele. It is also appropriate that the testing be done on the eldest brother at his own request, with his full understanding of potential consequences which may result from knowledge gained by genetic testing.

**Our experience with this case:**

We have tried a simplified version of this case once in a class of 70 students, who were divided into eleven groups. The version we used included the same case, but only the first multiple choice question above was used (the other questions were written later). We found that the groups in the class were divided between two answer choices, letters B and C. This generated a nice discussion about the structure of the CFTR channel and the effect that would have on Km of that channel. The addition of more questions will definitely enrich the case and increase group work and class discussion, particularly with the addition of the electrophoresis gel and x-ray which the students must interpret.

In our experience, the instructor of this case should be prepared to discuss current treatments for CF, lifespan for CF patients, and normal versus pathologic cell transport mechanisms of the CFTR channel. The student were not incredibly challenged with the version of the case which we presented, but I suspect they will be most challenged by the electrophoresis image and question in this revised version of the case.

**The References:**

1. For information about the sweat test used to diagnose CF patients, please visit the following website: [www.cysticfibrosismedicine.com/htmldocs/CFText/sweat.htm](http://www.cysticfibrosismedicine.com/htmldocs/CFText/sweat.htm)

2. For the disease profile of CF, treatments, support groups and clinical trials, please see the website: [www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/cf.shtml](http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/cf.shtml)

3. The following two references are for cystic fibrosis research, both general information and that related to question #4 above:
