

# “Not Exactly ...” The Complexity of a Human Genetic Disease

by  
William R. Morgan and Dean M. Fraga  
Department of Biology  
The College of Wooster, Wooster, OH

## Part I – The Meeting

Sarah stared blankly at the blue paisley wallpaper. Her husband Mike sat by her side, bending and unbending a small paper clip.

“Sarah and Michael, it’s good to meet you,” welcomed the genetic counselor, as she entered the room. “I apologize for being late, but I was just meeting with another couple. Let’s see, you’d like to have a child, but you’re concerned because of your family history of cystic fibrosis.”

“Yes,” Sarah replied softly. “Mike and I met at a CF support group meeting a few years ago. He had a younger brother who died of cystic fibrosis, and I had a younger sister. We saw the painful lives they had—difficulty breathing, the constant respiratory infections. Although the treatments for CF are better now, we just don’t know if we can...” she trailed off.

“I can certainly understand your concern,” the genetic counselor responded sympathetically. “That’s where I hope to help, by providing as much information and advice as I can. I’m glad that you came to see me before you became pregnant so I can fully advise you of all options beforehand.”

“To start, let’s go over what we know about your case so far,” continued the genetic counselor. She pulled out a pad of paper, which she placed on the table in front of Sarah and Michael, and began to draw a series of circles and squares connected by lines.

“As I recall, both sets of parents did not display any of the symptoms of cystic fibrosis, right?”

“Yes,” said Sarah and Michael in unison.

“Ok, well that means...”

## Questions

Consult your textbook and trustworthy Internet sites to answer the following questions:

1. Which organs are affected by cystic fibrosis? What are the disease symptoms?
2. Draw a pedigree showing the family history for CF in Sarah’s and Michael’s families. Be sure to distinguish between individuals with the disease, those that are carriers for the disease, and individuals who do not possess a copy of the disease allele.

## Part II – Punnett Squares

“So, what’s next?” asked Mike.

“First, we’ll collect DNA samples from both of you. We’ll then analyze your CF genes for the most common mutations to see if you are carriers for this recessive genetic disease.”

A few weeks later, the genetic counselor welcomed back Sarah and Mike. “We’ve received the results of your genetic tests for common CF mutations. Michael, you’re a carrier of the most common disease allele, delta-F508, and Sarah, you tested negative for the most common CF mutations.”

“Thank goodness,” Sarah replied with relief. “That means it’s safe for us to have a child, right?”

“Not exactly,” cautioned the genetic counselor.

### *Questions*

3. Construct a Punnett square to demonstrate why Sarah concluded that she and Michael could not have an affected child (assuming that she does not carry a CF mutation).
4. If Sarah were a carrier, what would be the chance that she and Michael would have an affected child? Show the Punnett square.

## Part III – *CFTR* Mutations

“Since Sarah tested negative for CF, it seems we don’t have much to worry about, right?” said Michael, pointing to the pedigree in which Sarah is not a carrier. “So, what did you mean when you said not exactly?”

The genetic counselor grabbed her laptop computer and positioned it in front of the three of them. Her fingers quickly typed out a web address and the words “OMIM - Online Mendelian Inheritance of Man” stretched across the browser window. “We can learn a lot more about this disease from this site. It will begin to explain why I said *not exactly* when Sarah asked if it was safe to conceive a child.”

### Questions

The *Online Mendelian Inheritance of Man* (OMIM) is a catalog of known human genetic diseases. Go to OMIM at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM> and search with the keyword “cystic fibrosis.” Examine the OMIM record entitled “cystic fibrosis transmembrane conductance regulator.”

5. Look closely at the section on “Allelic Variants.” Is the delta-F508 mutation the only known alteration of the *CFTR* gene?
6. As you look at the list of Allelic Variants (starting with .001), how does the information in brackets (e.g., [*CFTR*, PHE508DEL]) describe each mutation?
7. The *CFTR* gene contains how many exons? How many introns? Sketch a rough diagram of the exon/intron structure of the *CFTR* gene.
8. For each mutation (allelic variant) listed below, explain how the mutation would affect the production of (1) the mRNA and (2) the protein encoded by the *CFTR* gene. As an example, the first case is completed for you.
  - .0001 CYSTIC FIBROSIS [*CFTR*, PHE508DEL]  
*The 508<sup>th</sup> triplet codon, which normally codes for phenylalanine, is deleted. Consequently, the CFTR mRNA is 3 nucleotides shorter than normal, and the CFTR protein is one amino acid shorter, missing its 508<sup>th</sup> unit.*
  - .0003 CYSTIC FIBROSIS [*CFTR*, GLN493TER]
  - .0004 CYSTIC FIBROSIS [*CFTR*, ASP110HIS]
  - .0019 CYSTIC FIBROSIS [*CFTR*, 2-BP INS, 2566AT]
  - .0008 CYSTIC FIBROSIS [*CFTR*, IVS10, G-A, -1]
  - .0064 CYSTIC FIBROSIS [*CFTR*, IVS12, G-A, +1]
  - .0123 CYSTIC FIBROSIS [*CFTR*, 21-KB DEL]
9. Sarah wondered how all these different mutations can cause the same disease. As the genetic counselor, how would you explain this to Sarah and Michael?

## Part IV – The Test

Sarah and Michael looked confused.

“But...” Michael started to say.

“How can the test say I’m not a carrier, but you say that that I could still be a carrier?” Sarah finished.

The counselor pursed her lips and said, “Well, because of cost, no test is as comprehensive as we would like. This research article describes the genetic test used to determine your CF genotypes and explains its limitations,” she continued, handing them a printed article.

### Questions

Read the following research article to learn about the test used for CF genotyping, then answer the questions below.

Strom et al. 2006. “Technical Validation of a Tm Biosciences Luminex-Based Multiplex Assay for Detecting the American College of Medical Genetics Recommended Cystic Fibrosis Mutation Panel.” *Journal of Molecular Diagnostics* 8: 371-375.

10. How many different mutations had been found in the *CFTR* gene when this article was written?
11. How many of these *CFTR* mutations can be detected by the Tag-It *CFTR* 40 + 4 Luminex-based reagent system from Tm Biosciences?
12. What criteria did researchers use when determining which mutations to include in the Tag-It test?
13. What is the chance that Sarah is actually a carrier for a *CFTR* mutation, even though her Tag-It test results came back negative?
14. As the genetic counselor, explain to Sarah and Michael why you said “not exactly” when Sarah asked if it was safe to conceive a child.

## Part V – Further Analysis

After hearing the counselor explain the limitations of the test that was used, Sarah and Michael discussed whether to receive further testing. While the cost was significantly more, they felt the added knowledge would help them make an informed decision and so they elected to have Sarah's DNA further analyzed. A week later they met again with the genetic counselor to discuss the test result.

“So do I have a mutation in my CF gene?” asked Sarah

“Not exactly,” the genetic counselor replied. “You have a mutation in the region just before the part of the gene that codes for the CF protein. You might want to recall some of your introductory biology lessons, as it will get a little technical here.”

### Questions

First let's examine a more comprehensive catalog of known *CFTR* mutations found at The Cystic Fibrosis Mutation Database at <http://www.genet.sickkids.on.ca/Home.html> under “Statistics.”

15. Are there any mutation types that you haven't seen previously? Which? What are some of the potential effects that they could have on protein expression?
16. Under “*CFTR* Gene,” choose “Genomic DNA Sequence.” Compare the image with the sketch of the *CFTR* gene structure that you prepared for Question 8. How are they similar? How do they differ?

The subsequent analysis of Sarah's sample determined the DNA sequence of the *CFTR* introns and the regions upstream and downstream of the exons. The DNA sequence indicates that Sarah has a single base pair difference about 100 bases before the ATG start codon of the *CFTR* gene.

17. How might this mutation cause CF?
18. How could you test your hypothesis?

Now let's look at a scientific paper that examines a *CFTR* promoter variant. Although the paper is more advanced (and contains grammatical errors), we can still distill some useful information relevant to understanding Sarah's *CFTR* mutation. Examine the following research paper and answer the questions that follow:

Taulan et al., 2007. “First functional polymorphism in *CFTR* promoter that results in decreased transcriptional activity and Sp1/USF binding.” *Biochemical and Biophysical Research Communications* 361: 775-781.

19. Looking at the abstract and introduction, what hypothesis did the authors wish to test?

In their first experiment, the authors joined the wild-type (WT) or -94 mutant (M) *CFTR* promoter to the luciferase reporter gene. They then tested the transcription levels of each fusion gene in three different cell lines.

20. Examining Figure 1A in the article, does the mutation affect the transcription levels of this gene? Explain.
21. As the genetic counselor, explain fully to Sarah and Michael how this mutation before the protein-coding gene raises the possibility that they could have a child with cystic fibrosis.

## Part VI – The Final Decision

While the discussion about the implications of the mutation Sarah harbored was complicated, the young couple was able to grasp the essential elements. The counselor summed up the discussion when she said, “Well, I’m disappointed to inform you that a future child of yours could have cystic fibrosis.”

Both Michael and Sarah were visibly upset, but the counselor continued, “But you do still have some options. After natural or *in vitro* conception, we could test any embryos to determine the genotype, and then you could choose not to proceed with the pregnancy if needed.”

“What are we going to do?” asked Sarah and Michael to each other.

### Question

22. If you were Sarah or Michael, what would you choose to do? Why?
- (a) Not have children.
  - (b) Adopt children.
  - (c) Get pregnant and continue with the pregnancy no matter what.
  - (d) Get pregnant, test the embryo within 9 weeks via chorionic villus sampling (CVS), and terminate if the embryo has CF.
  - (e) Undergo *in vitro* fertilization by harvesting eggs, fertilizing them, and screening the embryos for CF prior to implantation.



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