

Sometimes it *is* All in the Genes

Part I—"The Genetic Test"

by

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"Your pregnancy seems to be progressing just fine, but we'll do some blood work to be on the safe side. As long as we're drawing blood from you today, Nancy, would you consent to participate in one of the genetics studies we're conducting here at People's Best Hospital?" Dr. Kwin prided herself on putting her patients at ease, but her question caught Nancy a little off guard.

"What would that entail, Dr. Kwin?" While Nancy believed Dr. Kwin wouldn't ask her to do anything that wasn't a good idea, she never said yes to anything until she had all the information.

Dr. Kwin began to explain, "Well, as you know, PBH is a research and teaching hospital. One research team is trying to determine the frequency of the gene for the genetic disorder cystic fibrosis in the U.S. population. And since you are having blood drawn anyway as you enter your second trimester...."

Genetic disorder? Cystic fibrosis? The phrases made Nancy feel panicky. She interrupted Dr. Kwin mid-sentence. "You don't think I have that disease, do you? Isn't cystic fibrosis serious? How could I have caught it?" The questions came out in a rush.

Hearing the anxiety in Nancy's voice, Dr. Kwin quickly reassured her. "No, no. I'm quite sure that you do not have cystic fibrosis. You can't 'catch' it; you can only inherit it. If you did have cystic fibrosis, you would have been diagnosed when you were a toddler. You have absolutely nothing to worry about. However, let me emphasize that if you feel uncomfortable about participating in this study in any way, just say 'No.' My feelings won't be hurt. There is no pressure to participate."

"If it's inherited and I don't have it, why would they want to test me?" The situation still didn't make sense to Nancy.

"First, let me explain some basics. Cystic fibrosis, or CF, is a disease that is caused by defects in a particular gene. Actually because CF is genetic, 'disorder' is a better term than 'disease.' The word disease should really be used for illnesses caused by bacterial or viral infection. However, people use both words for CF.

"The reason that they need to include people who don't have CF in the study is that cystic fibrosis is a recessive disorder. With a recessive disorder, a person has to have two defective versions of the CF gene to have CF. Therefore you can be a carrier of one defective version but not have CF at all. It is precisely that which has inspired the study. They want to know what percentage of people in the United States carries one defective gene for cystic fibrosis. That percentage is called the gene frequency and can provide a lot of information for people working on CF."

Dr. Kwin went on to explain, "CF is one of the most common genetic disorders among Americans of European descent. Although it has many symptoms, the worst are severe respiratory problems that typically lead to death around the age of 30. One of the first reliable tests for any genetic disorder was the one developed for CF in 1990. Because CF is one of the first inherited illnesses that researchers have been able to get a handle on, these sorts of genetic screenings are pioneering work not only for CF research but also for understanding other genetic ailments."

Nancy considered the flood of information for a few seconds. "I can't see what harm it would do. You run so many tests on me anyway. What's one more?"

"I'll have one of the staff bring you the consent forms and explain the procedure to you further. *If* you do consent, your biggest decision will be whether or not you want to have the results reported to you. You need to think carefully about that question before signing the consent form."

As Dr. Kwin left the room, Nancy thought to herself, *what are the pros and cons of knowing the results?*

Questions:

1. Discuss why Nancy might or might not want to know the results of her blood test for CF.
2. Dr. Kwin told Nancy that she has "absolutely nothing to worry about." Although Nancy cannot get CF, is Dr. Kwin's statement entirely correct?
3. Did Dr. Kwin provide Nancy with enough information about cystic fibrosis and the test to make a good decision?
4. Should Nancy consent to the test? Provide the reasoning for your answer.

Part II—"Sharing the Bad News"

Nancy had found it hard to concentrate at work that afternoon and it was almost impossible to think about what she was doing as she prepared dinner that evening with her husband Jake. Suddenly Jake's voice snapped her back to the kitchen, "Nancy, you're about to put the carrots and cucumbers in the garbage." Jake surveyed the salad bowl where she had been working and smiled at the rare opportunity of catching his wife in a mistake. "And is vegetable peel salad your latest craving?"

"Oh, honey, I need to talk to you about something." The stress in Nancy's voice quickly wiped the smile off of Jake's face. "Remember when I went for my pre-natal checkup a couple of weeks ago, and I told you I was participating in a genetic study?"

"Yeah, something about MS or something, right?"

"No, CF, cystic fibrosis." Nancy corrected him. "Well, it turns out that I tested positive."

"What!?! How did this happen? You don't seem sick at all."

In response to his panic, Nancy calmed down and explained, "No, no, of course *I'm* not sick. Remember, I told you that they were testing to see if I was a genetic carrier. How it happened was that I inherited one bad version of the gene, a bad 'allele' they call it, for cystic fibrosis from one of my parents."

He felt he should know why, but slowly Jake asked, "If you don't have CF, what makes you so worried that you're throwing sliced cucumbers into the garbage?"

"Because there is a chance that I could pass my bad CF allele to our child. And if you are a carrier and pass on your CF allele, then she or he *will* have CF."

"CF is pretty serious, right?"

"Yes, pretty serious."

Trying to be optimistic, Jake asked, "Yeah, but what's the chance that I'm a carrier too?"

"Dr. Kwin says the latest research indicates 1 in 29," Nancy answered soberly.

Jake's jaw dropped. "Can I get tested? What is the likelihood that you'll pass on the CF gene? What is the chance that I'll pass on the gene? What is the chance our baby will have CF?"

"Yes you can, and *will* get tested. I don't care if you are afraid of needles." Nancy left no room for further questions there. "I talked to Dr. Kwin at length today and I have a pretty good handle on this genetics thing. So, turn down the stove, sit down with me, and I'll answer your questions."

"Let's start with the basics," Nancy began. "We all have tens of thousands of different genes in each of the cells in our body. These genes provide the instructions on how to make all the components of our body. For almost all of those genes, we have two copies of each different gene. I inherited one copy of the gene that can cause CF from my mother and one from my father. One of them, I don't know which, was a defective copy, what they call a 'mutant' allele. One of the alleles I got from my parents was a normal, working copy. Because CF is what they call 'recessive,' I'm fine because I have that one good copy. It takes two bad copies of the gene to give you cystic fibrosis."

Questions:

Help Nancy finish answering Jake's questions.

1. The normal or good copy of the CF gene can be written shorthand as "F", and the mutant or bad copy of the CF gene can be represented as "f". Using this shorthand style, write out Nancy's genetic make-up for this gene.
2. What is the chance that Nancy passed on the CF allele to her baby?
3. What is the chance that Jake passed on the allele if he is a carrier?
4. What is the chance that their baby will have CF if they are both carriers?
5. If their first child has CF, what is the chance that their second child will have CF?
6. What is the chance that the baby will inherit CF if only Nancy is a carrier?

Part III—"The Decisions Become Tougher"

Jake gripped Nancy's hand as they sat in the office waiting for the genetic counselor. When Jake tested positive as a CF carrier, Dr. Kwin had set up an appointment with the counselor for the next day. What had first seemed like a helpful thing to do for science had suddenly developed into a problem that Dr. Kwin did not feel qualified to handle. Just being in a specialist's office made the couple nervous.

They both jumped a little when the door opened. "Hello. I'm Dr. Joon Park." The counselor shook hands with them as he continued. "I understand that you both tested positive as carriers for cystic fibrosis, but neither of you had any family history of the disease."

"Yes," said Nancy. "That's one of the questions that bothers me. How could we both be carriers yet we don't even *know* anyone with cystic fibrosis?"

"Good question," Dr. Park replied reassuringly. "On the surface that might seem unusual, but it is actually quite common with recessive genetic disorders. In fact, around 80 percent of children born with cystic fibrosis are born to parents with no family history of the disease."

Dr. Park paused, then continued. "I'm glad that you are already comfortable asking questions. One part of my job is to answer any questions you might have about inherited diseases and CF in particular. The other goal for us today is to discuss your current options."

Jake entered the conversation. "Yeah. What are our options? I want to know if our baby is going to have cystic fibrosis, but we can't just do a blood test now. Can we?"

"No. You are right. We can't do a blood test."

Before Dr. Park could finish, Nancy jumped in to add, "But we can do a genetic test through amniocentesis, can't we?" She looked at Jake with an expression that told him he should have read the pre-natal baby books as she had asked him to do.

"Yes, Nancy, we can do amniocentesis." Dr. Park went on to explain for Jake's benefit. "Using ultrasound to guide them, doctors insert a small needle through the abdomen and into the uterus. A small amount of the amniotic fluid that surrounds the fetus is removed. Fetal cells that have sloughed off are found in that fluid and from those cells, DNA can be isolated and checked for the presence of two bad copies of the gene that cause cystic fibrosis."

"What are we waiting for?" an anxious Jake questioned. "Let's get the test done."

"There is a chance that the amniocentesis will cause a miscarriage. However, here at People's Best Hospital, miscarriage occurs less than 0.5 percent of the time."

"Point five percent; that still amounts to 1 in 200." Jake did not lack intelligence, he just thought he would have another four and a half months before he really had to worry about anything related to babies.

Nancy interrupted. "Dr. Park, what exactly causes cystic fibrosis? What sort of life do people with CF lead?"

"Cystic fibrosis is caused by a mutation in a gene called CFTR. A normal CFTR gene carries the instructions to make a protein that allows chloride to pass out of a cell. When chloride moves, it causes water to follow and secretions are formed outside the cells. When a person has cystic fibrosis, both alleles of their CFTR gene are mutated. The mutant genes create defective CFTR proteins or no CFTR protein at all. The lack of a functional

CFTR protein means that not enough chloride moves out of the cells, in turn causing less water to move. The reduced water content causes the secretions to be thicker than normal. This thick, sticky mucus usually has its worst effects on the lungs and the pancreas.

"Normally, a thin layer of mucus coats the bronchioles and bronchial tubes in our lungs. This mucus traps dirt and germs and continually travels to the throat. Thus, the mucus removes the bad stuff from our lungs. In CF patients, the thicker mucus in the lungs can't be moved out as easily. This accumulation of mucus can block the air passageways. Worse yet, it allows bacteria to get trapped in the lungs where they can cause debilitating infections. While most of the symptoms of CF can be treated pretty effectively now, the continual stress and recurring infections in the lungs means that patients today still die in their 30s or earlier."

"If our baby tests positive for cystic fibrosis, what sort of treatments can we do before she is born?" Although a boy would be a joy, Nancy really wanted a girl.

"Unfortunately, there is nothing that we can do to help her before birth. Currently, we can only treat the symptoms, and she won't have symptoms until after she is born." As long as he had been at his job, Dr. Park hated being the bearer of bad news. However, he felt that the simple truth was the best approach. "Of course, there are many promising new technologies that are being investigated to treat CF. Still, even the most promising of those would not help before she is born." Inwardly, Dr. Park cursed the technologies for not delivering yet what they promised.

There was a long silence. Finally, Jake decided to find out what his wife was thinking. "I think I'd still like to know if our baby has CF or not. But I'm not sure about the risk. What do you think, honey?"

Please research and answer the questions in preparation for a class discussion tomorrow.

Questions:

1. How do mutant CFTR genes lead to thicker mucus in cystic fibrosis patients?
2. How would testing their unborn baby for CF help Nancy and Jake? Their baby?
3. What are their options if they find out their baby does have two bad CFTR genes?
4. Should they have the amniocentesis procedure? Provide your reasons for reaching this decision.

WEBSITE REFERENCES

- <http://www.cff.org/> (CF Foundation) (especially the page on CF clinical trials:
- <http://users.ox.ac.uk/~genemed> (at the bottom is a link to CF with more information about the disorder, molecular mechanisms, incidence, etc.)
- <http://www.hgu.mrc.ac.uk/Research/Devgen/Cysfib/julia.htm> (gene therapy and research on CF)
- <http://clinicaltrials.gov/ct/gui/c/w1b/info/resources? JServSessionIdzone ct=q5tir5y462> (a very complete government site explaining clinical trials)
- <http://www.fda.gov/fdac/special/testtubetopatient/default.html> (a description of how the FDA weighs benefits versus risk)
- <http://www.fda.gov/cder> (everything you always wanted to about the process of drug and therapy development and approval)
- <http://www.pslgroup.com/dg/ee7fa.htm> (gene therapy for CF)
- http://www.thescientist.com/yr2002/jun/roberts_p12_020624.html (gene therapy review that mentions CF and recent developments—requires free registration)

Part IV—"A New Hope or a False Hope?"

As Nancy used her cupped hands to pound on her son's back, she reflected on what her family had been through in the seven years since she had volunteered for the genetic screening research. Joshua was a wonderful, loving son. She wouldn't trade a minute with Joshua for anything in the world, but motherhood was not quite what she had envisioned in her dreams. Raising a child with CF was tough, no way around it. Joshua was lying on an incline with his head below his chest. The angle and the vigorous chest percussion helped the mucus to move out of his lungs. As a newborn, his first symptom of CF was overly salty sweat. This is actually the most common diagnostic test for CF. During his first months of life, Joshua didn't eat well and he had numerous loose bowel movements. Since then, part of his daily regimen included supplements of digestive enzymes that substituted for those that his poorly functioning pancreas couldn't supply. Fat-soluble vitamins helped him gain weight. *I'll never have to buy clothes in "husky."* Nancy thought to herself. *But at least Joshua is now on the pediatrician's growth curves.*

Just as the doctors had predicted, the biggest problem was with Joshua's lungs. He was almost always on antibiotics. These were usually taken by breathing in a medicated vapor from a nebulizer. The medicated vapor also helped to open up the mucus-clogged airways. However, hospital stays for intravenous antibiotics and "pulmonary clean-outs" were not uncommon. So far, Joshua was doing well in school, but Nancy was concerned about the number of days he had to miss. In fact, she would have to take him out of school early tomorrow to make their appointment at the pediatrician's office.

"Well, Joshua, it looks like you are doing great. How is school? Any more home runs in kickball?" Although her extensive training meant that Dr. Julia Bryant had some of the sickest patients in town, her bedside manner meant that her little friends still liked to visit her.

"Yes!" Joshua responded enthusiastically. "I had one on Monday. A grand slam!"

"Wow! I wish I could have seen it. Do you want to get down and play a little while I talk with your mother?"

"Sure, Dr. Julia."

For once, Julia's demeanor didn't lose any of its cheerfulness as she turned to Nancy. "Joshua really is doing well. You and Jake do such a good job with the home therapy."

Julia glanced over to see that Joshua was starting a jigsaw puzzle. "You know how I had warned you off of gene therapy a couple of years ago when you asked me about it?" Nancy nodded in agreement. "There is a new cystic fibrosis gene therapy clinical trial over at People's Best. They have asked us here at Children's Coolest Clinic for volunteers. I'm not telling you to run out and get involved, but I do think that it's time to carefully consider it."

Experience had taught Nancy not to get too optimistic, but her pulse began to race nonetheless. "Why should we consider it now?"

"I'm sure you remember that the first patient treated with gene therapy was a four-year-old girl back in the early 90s. She had an inherited form of severe combined immunodeficiency, also known as the 'boy-in-the-bubble syndrome' or SCID. Because the treatment was such a success, many people jumped on the gene therapy bandwagon. Unfortunately, after those initial successes, gene therapy has not produced the hoped-for results."

Nancy did remember that and more. "Didn't someone even die from gene therapy treatments?"

"Yes, in the fall of 1999 an 18-year-old man died from his gene therapy treatments. Doctors were trying to correct his inherited enzyme deficiency. That case is why I think we should be very cautious before we decide whether or not to get Joshua involved."

"First you don't think it's worthwhile. Then someone dies, and now it's a good idea? What aren't you telling me, Julia?"

"Well, in the spring of 2000, there were reports of two more successes with gene therapy. The two patients also had the boy-in-the-bubble syndrome, not CF. However, their illness was caused by a mutation in a different gene than the first successful patients, meaning that gene therapy can work with other genes."

"That is encouraging, but what actually killed the young man who died? Was the gene poisonous?" Nancy was still focused on that death.

"No, the gene wasn't 'poisonous'. The death had nothing to do with the gene itself but how the gene was delivered. As you know, a gene is simply a stretch of DNA in the chromosomes of your cells. In a recessive, inherited disease, the DNA in both copies of the gene, like the CFTR gene in Joshua's case, has errors in its code. The goal of gene therapy is to get an error-free copy of the gene back into the patient's cells. The new, good copy can then do the job for the patient's own bad ones.

"The trick is in inserting the good DNA into the patient's cells so that the gene is used by those cells. The most heavily studied and commonly used means for this DNA delivery is using viruses. One of those viruses is called adenovirus, which is one of the causes of the common cold. Part of the virus's natural life cycle is to get its DNA inside human cells and have its genes used by the human cells. To use adenovirus for gene therapy, researchers take the genes that cause illness out of the viral DNA and replace them with the human gene that the patient needs. Unfortunately, the virus still causes the patient's immune system to respond and it tries to fight off the infection. In the case of the man who died, doctors gave him a dose of adenovirus that was too high. The virus escaped the target organ, the liver, and infected tissues throughout his body. This caused an immune reaction that led to inflammation throughout his body. His temperature shot up to 104.5° F, and he was in a coma on the second day. Eventually, his lungs began to fill with fluid, so much so that he soon died."

"Why did the doctors give him too much virus?"

"They had no way of knowing it would be too much. The previous work at lower doses suggested the higher level would be safe. However, they apparently passed a critical threshold that no one could foresee."

"How do we know there won't be the same sort of hidden threshold in these CF gene therapy trials?"

"Until you and I find out more information about the specific clinical trials at People's Best, we don't. Even then we probably won't be 100 percent sure. Clinical trials are research, you know."

"Yes, but if Joshua could be helped...." Nancy's voice trailed off momentarily. "Besides the new boy-in-the-bubble success, what makes you think gene therapy might work now?"

"Well, different kinds of viruses that get the gene into cells by different means are being developed. In addition, researchers are experimenting with using liposomes or fat droplets to deliver the gene to the target cells. That would eliminate the risk posed by viruses. I know that both of these approaches are being tested in clinical trials for CF, but I don't know yet which will be conducted here in town." Julia felt guilty for not knowing more, but she had just heard about the program at People's Best and wanted to get Joshua in as early as possible.

"Liposomes? That sounds like skin cream stuff."

"It is basically the same approach. Just a little more sophisticated for gene therapy." Julia brought the topic to a close for the day. "If you are interested in finding out more, I'll set up an appointment for both of us with one of the leaders of the study. We'll tag-team them with questions."

"Of course I want to find out more. Boy, am I going to have a long discussion with Jake tonight!"

Questions:

1. The current therapies available to treat CF only treat the symptoms of the disorder. However, if gene therapy were to work, it could be considered a cure rather than a mere treatment of the symptoms. Explain why this could be the case.
2. *"The successful use of gene therapy to cure SCID syndrome (2000) is hoped to be a permanent cure for those patients because a good copy of the problem gene was inserted into the patients' blood stem cells in the bone marrow (hematopoietic stem cells). Once white blood cells enter the blood stream they have a limited life span, on the order of a few week to months. The blood stem cells are the cells that create more white blood cells to replace those that are lost. If the gene was only inserted into the circulating mature white blood cells, the patient would only be temporarily cured until those cells were used up or died."*

The current gene therapy approaches to cure CF involve inserting a functional CFTR gene into the mature epithelial cells of the lungs. In light of the preceding paragraph, do you think that this approach would be a "permanent" cure for CF? Explain your answer.
3. What level of risk should be acceptable for a patient involved in a clinical trial? In other words, under what circumstances should Nancy feel comfortable enrolling Joshua in a gene therapy clinical trial?
4. In the current clinical trials for gene therapy treatments of CF, participants must be over 12, so Joshua could not be helped by the trials that are currently being run at this time. Why might there be an age restriction such as this? Is an age restriction such as this fair?
5. Should Joshua be enrolled in a clinical trial on cystic fibrosis gene therapy?

Breaking the mucus barrier

Posted by Megan Scudellari

[Entry posted at 21st August 2008 03:47 PM GMT]

Step aside sound barrier: Chemical engineers at Johns Hopkins University have broken the mucus barrier, a long-standing adversary to drug delivery in diseases such as cystic fibrosis, lung cancer, and cervical cancer.

"We get asked all the time, 'Why on Earth do you want to study mucus?'" said Samuel Lai, a postdoc at Hopkins who presented the work yesterday (August 20) at the annual American Chemical Society meeting in Philadelphia. Lai has an easy answer to that question: Mucus has evolved over thousands of years to remove foreign particles, he said, so it blocks not only germs but drugs, especially those targeted to mucus-coated areas of the body such as the lungs and cervix.

At the meeting, Lai presented a new strategy for transmitting drug-toting nanoparticles through mucus and preliminary in vivo data describing the therapeutic potential of the engineered particles.

To figure out how to overcome the sticky barrier with synthetic particles, Lai and Justin Hanes, lead investigator of the study, looked to natural invaders who already manage the feat -- viruses. If particles engineered to carry the drugs had a hydrophilic and uncharged surface, like viruses, they might be able to pass through mucus. The biochemists looked to polyethylene glycol, or PEG, a hydrophilic, uncharged, and already FDA-approved polymer. PEG has been widely used in drug delivery, as well as in products such as skin cream, laxatives, and lubricants. "We couldn't have come up with a more appropriate particle," Lai told *The Scientist*.

The team published a paper last year describing the efficacy of PEG-coated nanoparticles. Surprisingly, large particles, 200 and 500 nm in diameter, moved through mucus as if it were water. Since then, they have determined the optimal PEG coating -- high densities of low molecular weight of the polymer -- and tested their delivery system in a variety of types of human mucus, including cystic fibrosis sputum, known to be a thick, tough mucosal barrier.

At the ACS meeting, Lai presented preliminary findings from animal studies that showed PEG-coated nanoparticles containing drugs for small cell lung cancer and cervical cancer are effective against tumor growth in respective mouse models.

The researchers plan to form a company based around the mucus-penetrating technology later this year, but continue to work with animal models of various diseases to prove efficacy.

GOING FURTHER:

Read the News Blog and highlight any terms you do not understand.

Review with a partner the implications of this successful new strategy for treatment of CF, cervical and lung cancer.

Although successful in animal studies this technology has not been tested in humans. What do you think about the plan of the researchers "to form a company based around the mucus-penetrating technology later this year"?