

Block 2: The Human Blueprint
Week 6: Genes Gone Wrong
Case 9: Greg and Michelle Johnson | Cystic Fibrosis
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FACILITATOR VERSION



GOAL

The students will explore cystic fibrosis as it relates to population risk calculations and its phenotypic effect on growth limitation.

LEARNING OBJECTIVES

1. Define cystic fibrosis (CF) and explain the major findings in the disease, the inheritance pattern, and the gene that causes CF.
 2. Define the Hardy Weinberg Equilibrium and calculate carrier frequency and a priori risk for CF.
 3. Demonstrate, by writing out the calculations, how prenatal testing adjusts risk for CF.
 4. Describe newborn and carrier screening for CF and list two potential causes of false negative screening in CF.
 5. List the diagnostic tests for CF and explain the underlying pathophysiology of an abnormal test.
 6. Define “failure to thrive” (FTT) and propose a mechanism for FTT in patients with CF.
 7. Describe the genetic mechanism that causes CF, and explain how specific mutations are associated with severity of disease.
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CASE VIGNETTE

Greg and Michelle Johnson are a young Caucasian married couple, each 25 yrs. old. Michelle is 12 weeks pregnant and both are present for a prenatal appointment with Michelle’s obstetrician, Dr. Judy Carver. During the visit, Dr. Carver discusses various screening tests, including the quad screen and cystic fibrosis genetic analysis. She explains to the couple that she is following the American College of Obstetrics and Gynecology (ACOG) guidelines for expecting couples.

PROBING QUESTION

1. Why are screening tests for genetic conditions recommended by ACOG? (LO 4)

Michelle is not interested in the quad screen, but is interested in screening for cystic fibrosis because one of her first cousins has this disorder.

PROBING QUESTION

2. What is CF and how is it inherited? (LO 1)

Dr. Carver explains that, by using the Hardy Weinberg equilibrium, she can calculate the odds that Greg and Michelle have for their child to develop cystic fibrosis or CF. She tells them that they have an *a priori* risk of 1 in 2500 for having a child with CF based on their ethnic background alone. Dr. Carver then estimates that Michelle's risk for having a child with CF would increase to 1 in 400, given she has a first cousin with CF. Even though the couple is confused, Michelle has blood drawn for screening of mutations in the CF gene.

PROBING QUESTION

3. What is an *a priori* risk? How did Dr. Carver develop these risk estimations? Will the Hardy-Weinberg equilibrium be of any help in developing risk figures? What is a punnet square and how does it help in risk calculations? (LO 2, 3)

Michelle returns to Dr. Carver's office for a follow-up visit and to learn about her screening results. The CF testing demonstrates that she is a carrier of the $\Delta F508$ CFTR mutation.

PROBING QUESTIONS

4. What is the gene for CF? What does CFTR mean? What is the $\Delta F508$ mutation? (LO 1, 7)

The couple is nervous at first. Dr. Carver explains that the risk that their child has for developing CF depends initially on Greg's population risk of being a carrier of a CFTR mutation, but this risk is modified either up or down depending upon whether a mutation is also discovered in Greg. Their *a priori* risk is now 1 in 100 for having a child with cystic fibrosis. Greg decides to undergo the screening test, and they are much relieved when they learn that Greg's screening test is negative for CFTR mutations.

PROBING QUESTIONS

5. How likely is it that Greg or Michelle is a carrier of a CF mutation before and after testing? (LO 2)

6. What does it mean when a screening test comes back positive or negative? (LO 4)

Greg and Michelle's son, Brian, was born at term without any difficulties. His birthweight, length, and head circumference was normal. His Ohio Newborn Screen is normal, but he born two months prior to the implementation of immunoreactive trypsinogen test now required of all babies born in Ohio. At two months of age and on subsequent visits, however, Brian's weight begins to drop, which causes some concern by his pediatrician, Dr. Kidd. He reviews the information from Brian's grow chart and finds that Brian's weight has crossed from his original weight at the 75th percentile to less than the 3rd percentile. Based on his weight for length being below the third percentile, Dr. Kidd diagnoses Brian with "failure to thrive." Numerous formula changes and caloric supplementation are attempted without success.

PROBING QUESTIONS

7. What is immunoreactive trypsinogen? Is this a diagnostic or screening test? What is newborn screening? (LO 4)

8. What is failure to thrive? (LO 6)

At six months of age, Brian is admitted to the hospital with pneumonia. During the admission, an additional finding of copious foul smelling stools is discovered. The parents had not told Dr. Kidd about this before, as they thought it was the result of formula changes. Dr. Kidd asks for the gastroenterology and pulmonary specialists to review Brian's hospitalization and was surprised that both consultants think that cystic fibrosis could be a cause. He then orders a sweat chloride test which was positive for CF. Greg

and Michelle want to know how this could happen, since they had prenatal CF screening and were told that Greg's screening test was negative for CF.

PROBING QUESTION

9. Brian's parents screened "negative", how could this happen? How could Brian still end up with CF? What was the risk of having a child with CF after Greg's negative test? (LO 3, 4, 7)

10. What are the physiologic mechanisms that cause FTT in Brian? (LO 6)

11. What is a sweat chloride test and how does it diagnose CF? (LO 5)

RESOURCES

Highly recommended:

CFTR-Related Disorders from GeneReviews www.genereviews.org

Within Chapter 9, "[Genetic Variation in Populations](#)" read the section called 'The Hardy-Weinberg Law, on pages 156-163, in [Thompson and Thompson Genetics in Medicine](#), 8th ed./Philadelphia: Saunders, c2016. The book is on [Reserve](#) at the Health Center Library.

For additional information:

Knoone, P, Knowles, M, "[CFTR-opathies": disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations](#). Respiratory Research 2001, 2:328-32.

Krugman S, Dubowitz H. [Failure to thrive](#). Am Fam Physician 2003;68(5):879-84. Click [here](#) for full text.

ACOG Committee Opinion. Number 325, December 2005. Update on carrier screening for cystic fibrosis. Obstet Gynecol 2005;106(6):1465-8. This has been withdrawn.

FACILITATOR GUIDE

EXECUTIVE SUMMARY

This case of cystic fibrosis is presented in week six, the theme of which is "From Genes Gone Wrong". The major thread for the cases and the interactive sessions for this week are the concepts of genetic mutations as cause for disease, and the link between genotype and the eventual phenotype. This case illustrates the concept of dysregulation, which is one of the three major concepts for the Human Blueprint block. The primary disciplines for this case are genetics and molecular biology.

Earlier in the week, a case of deletion 22 which shows how a microdeletion can produce a diverse range of findings due to the loss of a number of genes.. The purpose of this case is to demonstrate the effect of a single gene on the body's physiology and to illustrate the differences between and pitfalls of genetic screening and testing. The students are also asked to use basic calculations to determine the risk for disease that are based on Mendelian laws of inheritance. The students are also asked to consider the molecular reasons for the severity of Brian, the child with CF in the case. They will also use these concepts in the two medium sized groups, which focuses on the genotype-phenotype correlations between CFTR mutations and disease development.

Cystic Fibrosis

In this case, Greg and Michelle have a child (Brian) with cystic fibrosis. Cystic fibrosis is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane receptor (CFTR) gene located on chromosome 7. In contrast with other genetic disorders, only one gene has been associated with this disease to date, although research is being done now to look at genes that may modify the course of the disease.

The manifestations of cystic fibrosis include pulmonary and gastrointestinal abnormalities. Affected patients have recurrent pneumonias and chronic inflammatory damage to the lungs. The name cystic fibrosis refers to pathologic changes found in the pancreas. This leads to an insufficiency of pancreatic enzymes, which in turn causes a profuse, foul smelling diarrhea due to fat malabsorption. Treatment of cystic fibrosis requires treatment and prevention of pneumonia, anti-inflammatory drugs, and pancreatic enzyme supplementation. Affected patients can have variable clinical courses, but can live into adulthood with aggressive management.

The diagnosis is delayed for two reasons: One reason is the misunderstanding of the nature of a screening test. A screen gives an adjusted risk, but is not diagnostic in and of itself. A negative screen in this case was falsely interpreted to mean that there was no risk of developing CF, so it was not considered. The other reason is that the most common cause of failure to thrive, nutritional deprivation, was pursued because the finding of diarrhea was overlooked. Again, “careful attention to clinical history.” The physician didn’t ask, the patient didn’t offer.

ANSWERS TO PROBING QUESTIONS

1. Why are screening tests for genetic conditions recommended by ACOG? (LO 4)

Prenatal screening tests allow for parents to make reproductive decisions and also prepare the family and medical team to provide early intervention. Professional societies, like American College of Obstetrics and Gynecology (ACOG), are instrumental in providing guidance to its members and to patients about the application of newer tests. Note that ACOG members are not genetic professionals, but that they work with genetic principles and concepts in the care of their patients, particularly in prenatal care. In addition, other professional societies, like the American College of Medical Genetics, have developed criteria for prenatal screening that includes recommendations about the quad screening and genetic testing.

The GeneReviews site on CF lists the 23 recommended mutations for screening. It is not important that the student know all of the specific mutations, but to realize that not all mutations are in this screening panel.

Recommended Core Mutation Panel for General Population CF Carrier Screening. This panel tests for 23 of the most common mutations in the CFTR gene. (taken from Gene Reviews website on CFTR-Related Disorders: <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&partid=1250>)

3120+1G→A	R553X
A455E	218delA
G85E R334W	621+1G→T
1717-1G→A	G542X R1162X
3659delC F508	R560T
R347P ^Δ 1898+1G→A	2789+5G→A
3849+10kbC →T	711+1G→T
I507 ^Δ	G551D R117H
N1303K	W1282X

Screening tests differ from diagnostic tests and must fulfill certain requirements:

- Disorder must have complications that can be averted if managed early
- The disorder must be common in the tested population
- Follow-up testing to confirm the diagnosis must be available
- The burden of the test should be low (in terms of complications and cost)

CF testing meets these four requirements, due to the serious nature of the disorder, that early and aggressive management has been shown to alter the course of disease, and that sweat testing after the newborn period is a reliable confirmatory test.

2. What is CF and how is CF inherited? (LO 1)

3. What is an *a priori* risk? How did Dr. Carver come develop these risk estimations? Will the Hardy-Weinberg equilibrium be of any help in developing risk figures? What is a punnet square and how does it help in risk calculations? (LO 2, 3)

CF is inherited as an autosomal recessive disorder. This means that the gene is located on an autosome (i.e. not sex chromosome), and that both copies of the gene need to be altered in an individual before any signs or symptoms of the disease.

Risk analysis in genetics is extremely important and is based on using information before confirmatory tests yield a final diagnosis. Therefore an *a priori* risk is based on the available information before modification by other facts or testing information. In this case, this couple's *a priori* risk is based on their ethnic background and then later by the family history. Thus, an *a priori* risk can be modified with additional information. After screening, there still remains a *residual risk*, which is due to the imperfect nature of the test.

The first risk was based on the population risk for this couples ethnic population. Mutations in CFTR are found in people of all ethnic backgrounds, but with slightly different frequencies. The mutation types differ between different ethnic backgrounds as well, resulting in different detection rates. Therefore, it is important to review the ethnic background in determining the *a priori* risks for disease. See table 1 below (from ACOG committee opinion, 2005, resource given below).

Table 1. Cystic Fibrosis Detection and Carrier Rates Before and After Testing

Racial or Ethnic Group	Detection Rate	Carrier Rate Before Testing	Carrier Risk After Negative Test Result
Ashkenazi Jewish	94%	1/24	Approximately 1/400
Non-Hispanic Caucasian	88%	1/25	Approximately 1/208
Hispanic American	72%	1/46	Approximately 1/164
African American	65%	1/65	Approximately 1/186
Asian American	49%	1/94	Approximately 1/184

From this table, any non-hispanic Caucasian has a 1/25 chance for being a carrier for a mutation in the CFTR gene. Based in the Hardy Weinberg equilibrium (HWE) and the risk laws for an autosomal recessive condition, estimates for the couple to have a child with CF can be generated.

The HWE describes the proportion of individuals who have a homozygous normal genotype (AA), heterozygous genotype (Aa) and a homozygous genotype (aa) in the population. These frequencies are

based in several assumptions of population genetics, the most important being the assumption that the population is large and that matings between individuals are random. While these assumptions are not always applicable to all populations and at all times, they are very useful for most clinical situations. The frequency of mutations in a gene can be easily calculated from these equations:

$$p + q = 1 \quad \text{or} \quad p^2 + 2pq + q^2 = 1$$

with p^2 equal to the proportion of AA individuals, $2pq$ equal to the proportion of the heterozygous individuals and q^2 equal to the proportion of affected individuals in the population. In a population, the proportion of normal alleles (p) and abnormal alleles (q), $p + q$ will equal 1.

The generally accepted rate of newborns with CF in the Caucasian population is about 1/2500 (0.0004) births. This is equivalent to q^2 in the HWE equation. From this, the proportion of the abnormal allele or q , can be calculated as 0.02, and p , the proportion of the normal allele in the population, can be calculated as .98. As this is close to 1, the derivation of $2pq$ is then rounded to 2×0.02 , or 0.04, or 1 in 25. This is the same estimate of the risk to be a CF carrier in the table above.

Three estimates of risk are then used to determine the chance that a couple will have a child with CF: the chance that the mother is a carrier (i.e., heterozygous), the chance that the father is a carrier (i.e., heterozygous), and the chance that the child will be affected (i.e., homozygous). As CF is recessive, the chance that a child will be affected if both parents are heterozygotes (i.e. carriers) is one in four or 25%.

For this example, the physician first determined the a priori risk of 1/2500 by using the population risk for both mother and father ($1/25 \times 1/25$) and then multiplied by the chance the child will be homozygous ($1/4$) ($1/25 \times 1/25 \times 1/4 = 1/2500$). It may be helpful for the students to use a Punnett square in manipulating these estimates. The physician then made an estimate based on the mother's chance of being a carrier, given her position in the family. Michelle's risk to be a carrier is calculated to be 1 in 4.

Assuming that this cousin is on her maternal uncle's child (i.e. mother's brother's child), then the uncle's risk of being a carrier = 1; Michelle's mother's risk of being a carrier = $1/2$, and Michelle has a $1/2$ chance of inheriting the abnormal allele from her mother.

Because these risks are all independent, multiplying them will result in a final estimate of 1 in 4 ($1 \times 1/2 \times 1/2 = 1/4$). Using this new estimate, Gregg and Michelle's chances of being having a child with CF has increased to 1/400 ($1/4 \times 1/25 \times 1/4 = 1/400$).

The students may be familiar with the Punnett square, which is an easy way to determine the risk of an offspring to have a certain genotype (and thus disease), once the genotypes of the parents are known. For example, if Greg and Michelle were both heterozygous carriers, the risk for having a homozygous offspring is $1/4$. This is then used as the last number in the calculations above.

		Heterozygous Mom (Aa) Gametes	
		A	a
Heterozygous Dad (Aa) Gametes	A	AA	Aa
	a	Aa	aa

4. What is the gene for CF? What does CFTR mean? What is the ΔF508 mutation? (LO 1, 7)

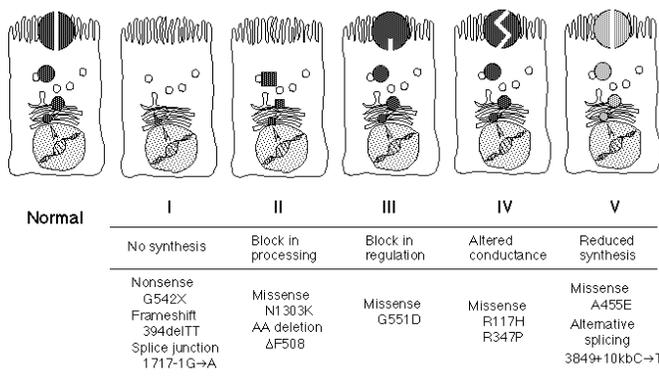
Cystic fibrosis transmembrane conductance regulator (abbreviated CFTR), a 1480-amino acid integral membrane protein that functions as a regulated chloride channel in epithelia. Over 1000 mutations are known; almost all are point mutations or small (1-84 bp) deletions. The most common mutation is

F508^Δ, accounting for about 30-80% of mutant alleles

depending on the ethnic group. This mutation occurs from a 3 base pair deletion, removing a phenylalanine at position 508. The result of this mutation gives a fully functional protein that is unable to be trafficked to its normal site of action.

The interactive session in this week will go into detail about the gene and effect of the mutations on phenotype. The student's should be able to discuss the differences in effect between the mutations in CFTR. The following illustration indicates the various mutations and effects in the cell, which can then cause serious or mild forms of the disease. The SEQ for this week asks the students to consider the molecular mechanisms of male infertility, which can be a mild or atypical CF presentation.

courtesy of Dr. Rick Hallick Molecular consequences of CFTR mutations



5. How likely is it that Greg or Michelle is a carrier of a CF mutation before and after testing? (LO 2)

Before testing, Greg would be considered as having a 1/25 risk. Michelle's risk is 1/4, because of her affected cousin. (See above for the derivation of these figures). After genetic testing, Michelle is positive for a mutation and is 100% risk of being a carrier. Greg is "negative" for a mutation, which gives him a residual risk of 1/208 (taken from Table 1 in the CF screening article, above). For Greg and Michelle to have a child with CF, the risk is 1/4 of their combined risk of being carriers: $1 \times 1/208 \times 1/4 = 1/832$. Thus, after screening for the most common mutations in the CFTR gene in Greg and Michelle, their risk of having a child with CF is low, but not zero. An important point for the student's is to consider is that patients often consider the information from testing in a dichotomous fashion: the test is either "positive" or "negative". CF carrier testing is one example where the couple is in a lower risk category after testing. It is often a challenge for patients to understand this concept.

6. What does it mean when a screening test comes back positive or negative? (LO 4)

Screening tests give an estimate of risk. "Positive" or "Negative" are terms that usually refer to an artificial threshold set by the organizers of the screen. This threshold usually is designed so that few cases will be missed, but at the expense of having more false positives. A test that comes back as positive means that the person has a risk higher than the threshold. Genetic screens are slightly different in that a positive screen means that a known, defined mutation was detected. In the case of CF screening, there is a set number of mutations that are tested, which will detect a certain percentage of carriers in the population (table 1). Thus the threshold of the test is determined by the number and type of mutations screened. A

negative test means that none of the *common tested* mutations were found. A mutation could be present that is not detectable by the screen. The risk of mutation is reduced (table 1), but it is not zero.

7. What is immunoreactive trypsinogen? Is this a screening or diagnostic test? What is newborn screening? (LO 4)

Immunoreactive trypsinogen (IRT) is a screening test recently implemented (2006-2007) in the state of Ohio. It measures for an elevation of trypsinogen which is present due to pancreatic damage present at birth in children with CF. The student's do not need to know how trypsinogen is made or transported. If the IRT is elevated, our state follows up with DNA testing for common CF mutations. If one or more mutations are found, or if the IRT is above a certain level, the child is referred to a regional CF Newborn screening clinic, such as the combined program here at Rainbow Babies & Children's and the Center for Human Genetics, for confirmatory testing.

The newborn screening program in Ohio and other states dictate that all newborns be tested after 24 hours of birth for 32 serious diseases in the neonatal and childhood period (<http://www.odh.ohio.gov/odhPrograms/phl/newbrn/nbrn1.aspx>). Blood spots are taken with a heel stick and sent to the Ohio state laboratory. Most of the conditions tested at this time are rare inherited metabolic conditions where implementation of life saving diet and other therapies has been shown to avert early death and decrease the severity of illness. The CF program attempts to identify infants in Ohio with CF before symptoms are evident in order to start therapy before lung or other damage occurs.

Both stages of the protocol (immunoreactive trypsinogen and CF mutation panel testing) are considered screening tests and are generally not used for diagnosis of CF. In most cases, the test identify heterozygote carriers of a single mutant allele, and the follow-up CF program then uses the "gold standard" sweat chloride test to confirm or exclude the screening test results.

In some cases, full sequencing of the gene is then done on the infant and parents in an effort to identify rare CF alleles. Further, the sweat chloride test is not always informative in some infants (primarily due to a lack of sweat or immaturity). If this occurs, follow-up sweat tests are done at a later date and/or the due of the nasal transepithelial nasal potential discussed below (#11).

In some cases, two severe CF alleles are identified on the newborn screen, such as $\Delta F508/\Delta F508$ homozygotes. In this case, the test would be considered diagnostic and the infant would have a confirmatory sweat test and be treated for CF.

False positives can come from the immunoreactive trypsinogen test and the CF panel. In this case, Brian did not have the current Ohio newborn screening test for CF and his father had a normal CF panel, which did not test for his abnormal CF allele.

8. What is failure to thrive? (LO 6)

Failure to thrive (FTT) is a symptom, not a diagnosis. It has a loose definition which lends itself to misinterpretation. It is defined as a child less than the 3rd percentile for weight or a child that crosses two or more percentile lines downward on the growth curve for weight. The part that is missing from this definition is height. For this reason, I have consulted on many short plump children being force-fed formula supplements in an attempt to correct their "weight problem." These kids were not skinny, they were short, and their weights were perfectly appropriate for their lengths. Therefore, the above rules using the weight for height curve are easy to use, as it applies an automatic correction for short stature. This curve is largely ignored by most practitioners.

Failure to thrive can be conceptualized as state of relative caloric insufficiency due to the following categorical reasons:

1. Insufficient caloric intake: Neglect, inappropriately mixed formula, vomiting, hypotonia

2. Insufficient caloric extraction: Malabsorption
3. Insufficient caloric conversion: Inborn errors of metabolism
4. Increased caloric demand: hyperthyroidism, cardiac disease
5. Increased caloric excretion (waste): Chronic diarrhea, renal tubular acidosis

The most common cause (~95%) of FTT is psychosocial—due to inadequate feeding by the caregiver (for various reasons). This has led physicians to “shoot first and ask questions later.” In other words, many physicians attempt to first correct the problem by changing formulas, increasing caloric content, etc. This works 95% of the time, but 1 in 20 will be mismanaged this way. The good news is that careful attention to clinical history, a thorough review of systems, and a full physical examination will pick up the majority of the causes involved. Physicians simply have to stop and think before proceeding with their plans.

9. Brian’s parents screened “negative”, how could this happen? How could Brian still end up with CF? What was the risk of having a child with CF after Greg’s negative test? (LO 3, 4, 7)

There are several possible explanations for Brian to have CF given his stated father’s negative screening test on the CF panel. These usually break down into the limitations of the screening test, other rare genetic mechanisms, and the issue of paternity.

The limitations of the CF panel is the most likely explanation for Brian’s diagnosis of CF. His father may have had a mutation that was not detectable in the screening test. In order to have identified this mutation prior to Brian’s conception, Greg’s CFTR gene would have needed to be sequenced. As he did not have a family history of the disease, as did his wife Michelle, there was little indication to have this test.

There are other rare reasons for an individual to have CF, given a negative parental screening test. First, Brian might have acquired a new mutation in the paternal CFTR allele during conception or during spermatogenesis in his father. The rate of new mutations is not well known in CF. Second, Brian may have inherited two identical alleles from his mother only. This can come from a complicated and rare genetic mechanism. Because it is complicated and uses information not provided to the students, the mechanism will be discussed in next week.

Mis-identified parentage could account for this result if Brian’s biologic father is different than what Greg and Michelle told Dr. Carver. This should always be a consideration for confusing genetic results, and non-paternity can be easily tested using molecular tests. However, the first step for this family would be to extend the testing for Brian and Brian’s father Greg by using a larger panel and/or direct sequencing of the gene.

See #2 above for estimates of risk.

10. What are the physiologic mechanisms that cause FTT in Brian?

FTT in CF occurs largely from the pancreatic insufficiency leading to malabsorption of fats. There are many different presentations for individuals with mutations in CFTR. The most common is pulmonary insufficiency that develops over time. Other presentations include chronic sinus disease, salt wasting syndromes and obstructive azospermia in men due to the congenital bilateral absence of the vas deferens.

11. What is a sweat chloride test and how does it diagnose CF? (LO 5)

The defect in CF results to the failure of chloride resorption in sweat glands. Thus, the chloride content of sweat is excessively high. (Many children are noted by parents to taste salty when kissed). The sweat chloride test involves stimulating the skin to produce sweat and then collecting the fluid. The chloride

content is analyzed. If it is greater than 60 meq/L, the “sweat test” is positive. If two tests return positive, the diagnosis of CF is confirmed. However, this test also has a false negative rate and is estimated to detect 90% of affected children.

Other tests for CF include the transepithelial nasal potential difference test that measures electrical potential across the nasal epithelial cells. Abnormalities in the transport of sodium and potassium due to CFTR dysregulation will alter the electrical potential. This test is highly sensitive, but not commonly done outside of CF centers.