Hi, my name is Joe Pilewski, I’m a pulmonary physician at UPMC and I’m pleased to speak with you today about cystic fibrosis. In particular today we are going to talk about CF as a paradigm for genetics to personalized medicine. By way of introduction the talk will address these issues. First we’ll talk about the clinical features of typical and atypical cystic fibrosis and highlight the cystic fibrosis as an increasingly adult disease. Secondly we’ll review briefly the diagnostic criteria and the importance of DNA testing and how this has allowed us to recognize patients with relatively mild or atypical disease. Third we’ll talk about pathogenesis, particularly the paradigm that we currently espouse in cystic fibrosis, the airway surface liquid depletion is the major cause of cystic fibrosis lung disease. And lastly we’ll talk about genetic mutation based drug therapy, specifically CFTR correctors and potentiators.

So by way of introduction cystic fibrosis is the most common life-shortening genetic disease among Caucasians. Based on recent registry data we estimate there are approximately 30,000 patients in the United States and approximately 70,000 worldwide. Cystic fibrosis is an autosomal recessive disease in which individuals inherit two copies of a gene that incur a protein called the cystic fibrosis transmembrane conductance regulator, or CFTR. This gene was first identified in 1989 as a result of positional cloning and has allowed us to make the diagnosis of cystic fibrosis in a number of patients with atypical or mild disease.

This CFTR gene and protein function primarily in exsiccat organs, so we best think of cystic fibrosis as an exocrinopathy with manifestations in the sweat glands, the digestive and reproductive
tract and in the respiratory system. Since CF has become a disease characterized by progressive lung disease as the major cause of morbidity and mortality, CF has largely fallen under the purview of pulmonary physicians. It’s important to remember however that there are diseases in other organs and specialists in other areas, particularly in GI medicine, that can be very helpful for patients with this disease.

This slide will review the typical and atypical phenotypic features of CF. Historically we’ve thought about cystic fibrosis as a disease of childhood and that patients have typical disease, they have multi-system problems including chronic sinusitis, in which there is chronic nasal congestion and recurrent sinus infections. In the lung we see severe chronic infection and bronchiectasis and this is really the hallmark of disease and the major cause of morbidity and mortality. Hepatobiliary disease occurs in a significant percentage of patients with CF, however of only a small percentage, that is 1 to 2% develop progressive severe disease characterized by cirrhosis and liver failure. Pancreatic insufficiency is a hallmark of disease in patients with typical CF, that is those who present in childhood. If you look at the complete population of CF patients pancreatic insufficiency occurs is approximately 85 to 90% of patients.

Meconium ileus has long been recognized as a manifestation of CF and in registry data approximately 15% of patients present with meconium ileus as a major clinical feature. The sweat test has evolved since the 1950s as the primary diagnostic test for CF and we see elevated sweat chloride values in the majority of patients who have typical multisystem CF disease. And lastly,
obstructive azoospermia or mammal infertility occur in a vast majority of patients, over 95% of the CF patients are infertile because of congenital bilateral absence of the vas deferens. So in typical disease we see CF affecting the sinuses and respiratory tract, to a lesser degree the hepatobiliary tract, primarily the pancreatic duct and pancreatic insufficiency as a major cause of disease.

Genetics has taught us over the last several decades that there are a percentage of patients who have atypical disease in which the manifestations are much less severe. When we think of these atypical disease patients many of them have chronic sinusitis though it’s not as severe or disabling as patients with typical CF. Many of them also have obstructive azoospermia, so the majority of males even with atypical disease have congenital bilateral absence of the vas deferens and obstructive azoospermia with infertility as a manifestation of disease, and in fact CF is recognized by urologists as one of the major causes of male infertility.

Chronic infection occurs in the respiratory tract in patients with what we call atypical CF, where typically it’s not as severe and presents much later in life. Most patients with so-called atypical CF have normal hepatobiliary function and they have preservation of some pancreatic function such that they are pancreatic sufficient but have pancreatitis as a manifestation. In these patients enzyme production is normal but because there are abnormalities in the secretion of pancreatic enzymes the pancreas is predisposed to developing recurrent inflammation and pancreatitis.
From a diagnostic standpoint it’s important to remember that patients with atypical CF often times have either normal or borderline sweat chloride values. So in contrast to patients with typical CF where the sweat chloride value is typically very elevated, greater than 60 mil equivalents per deciliter, in patients with atypical disease you cannot rely on the sweat test as a diagnostic tool.

CF is increasingly becoming an adult disease and if we look at the age distribution of the CF patient population from registry data in the United States we see that almost half of the patients now are over the age of 18. So this slide shows the age distribution of the patient population as of the 2009 registry where the median age is now 17.1 years, so almost half the patients are over the age of 18. Also note that the age range goes from birth to greater than 70 years, so testing for genetic mutations has improved, we recognize a subset of patients with CF who are diagnosed very late in life.

One recent review looked at patients who were diagnosed with CF strictly in adulthood, and this review of 46 patients from the National Jewish showed that the majority of the patients presented with pulmonary symptoms, but notably only 40%, or 40% had all pancreatic sufficiency and only had pulmonary manifestations. So when you see patients who have primarily bronchiectasis and no GI disease one needs to consider cystic fibrosis as a potential diagnosis.

In this review 4% of the patients had only GI disease and were pancreatic sufficient with the major manifestation of their CF being recurrent so-called idiopathic pancreatitis. A quarter of the patients had both pulmonary and GI manifestations and the majority of these patients were pancreatic
insufficient. Fully one-fourth of the patients in this review had male infertility as their only diagnostic feature and all of these patients were pancreatic sufficient. And then lastly approximately 10% of the patients presented and were diagnosed only because of a family history, so a sibling or a cousin or another relative was diagnosed with cystic fibrosis, that led to screening of an adult individual and they were found to have a diagnosis of CF. All of these patients were pancreatic sufficient.

If we look at the age at diagnosis this slide highlights a point I made a minute ago, and that is that while most of the patients are diagnosed in childhood, here virtually 90% of the patients diagnosed by the age of 10 and 75% by the age of 2 where an increasing number of patients who were recognized in adulthood, such that their disease is diagnosed over the age of 16. Many of these have relatively atypical or mild presentations compared to what we think of as typical CF.

A number of consensus conferences have emerged over the years to try and come up with a uniform set of criteria for a diagnosis of CF and this slide summarizes the most recent consensus conference findings. The suggestion is that one not make a diagnosis unless there is a phenotypic feature or historical feature consistent with CF plus physiologic or genetic evidence for mutations in the CFTR gene. So among the phenotypic features one could have simply pulmonary disease, hepatobiliary disease, male infertility but having one more typical phenotypic features of the disease is critical to making a diagnosis. Having a sibling with CF is a significant and considered a diagnostic feature in the right setting, and then a positive newborn screening test primarily with an immunoreactive
carcinogen is also a useful diagnostic tool. So any patient who has one of these three features can be considered to have CF. But one needs to identify a mechanism for their phenotypic feature primarily dysfunctional CFTR as assessed by the sweat chloride or identifiable CFTR mutations.

The sweat test remains an important test for CF and the majority of patients will have an abnormal sweat chloride. This test should be performed in a certified accredited CF center and should be performed on two occasions to minimize of false positive or false negative results. A second potential diagnostic test is the identification of CFTR mutations, and over the last decade the number of screens, the number of mutations that can be identified through commercial screening has increased dramatically such that now we’ve identified over 1700 mutations in the CF gene. Hospital studies often times identify only the most common mutations so that if patients present with a suspicious story for cystic fibrosis we need to consider sending a more exhaustive full gene sequencing to make the diagnosis. Lastly, one can demonstrate abnormalities in CFTR function by doing a nasal potential difference assay in which one studies the iron conductance across the nasal epithelium and can demonstrate a dysfunction at that level. The take home message from this slide then is that one should have a typical phenotypic feature of CF or siblings or positive newborn screening and evidence of CFTR mutation either at the genetic level with CFTR mutations or at the functional level with an abnormal sweat chloride or an abnormal nasal potential difference.

As we have learned over the years CF is a disease characterized by a variety of disease severity. The genetics has taught us one potential mechanism whereby that occurs. This slide highlights the
processing of the CFTR protein in a normal epithelial cell. So in a normal cell the nucleus transcribes an mRNA that’s been processed in the ER to a protein, the protein has matured through the Golgi to a protein that we call a mature CFTR, it’s then transported to the apical membrane of the cell where it serves as a chloride conductance pathway. So in a normal individual this mature protein allows for chloride to mode across the epithelial surface in the respiratory and GI tracts.

As the number of mutations identified has increased we gradually thought of this number of mutations as falling into specific classes, so we’ll now review some of the classes of mutations and the representation of how they cause dysfunctional CFTR. One class of mutations are so-called statin mutations in which a truncated mRNA prevents production of a full length protein. In these mutations the disease is typically severe insofar no protein is made or transported to cell surface and these cells lack a chloride permeability.

The most common CF mutation, the Delta F508 mutation, is a processing defect in which a full length protein is made but then degraded in the proteolytic pathway so that mutation in the – at the phenylalanine position 508 causes the protein to go to the proteosome and very little protein escapes from the proteosome to the cell surface. So similarly these patients tend to have a severe disease because they lack significant chloride permeability in the important organs.

So-called Class III and Class IV mutations are those in which a full length protein is made. Examples of these include G551D and R347P. In these classes of mutations the protein is made and
transported to the cell surface but at the cell surface the protein either fails to activate with the typical channel openers or the channel is defective and fails to conduct quatro bicarbonate in a normal fashion. Then you have these mutations particularly the Class IV mutations patients have relatively mild disease because they have some residual CFTR function. So this understanding of genetics and the cell biology of individual mutations allows us to have some clues as to how CF can present with such widely differing manifestations of disease severities.

As I mentioned, CF is a disease characterized by bronchiectasis, and bronchiectasis involves the dilatation of the airways and recurrent infection. This CT scan shows a low power magnification of areas of bronchiectasis in an adult cystic fibrosis patient with relatively mild disease. If you look carefully here this airway is dilated out in the right and middle lobe and as we look at higher magnification we can see this more clearly. So here we see a vessel penetrating into the lung parenchyma with an accompanying airway that’s dilated relative to the size of that vessel. In the more peripheral airways here posteriorly we start to see a tree branching and almost a tree and butt appearance characteristic of bronchiectasis. CT scanning remains the gold standard really to identify mild bronchiectasis whereas in many cases the chest x-ray is not terribly sensitive, as the CF disease progresses we see more dramatic changes with marked dilatation of the airways diffusely in the lung. Shown here are several segments where the airways are dramatically dilated and some of these have some areas of mucous adherent to the airway wall. We also see evidence for air trapping with this mosaicism between different segments of the same lobe of the lung.
Our understanding of CF and its manifestations has been markedly enhanced by an understanding of cell biology and of the IN transporters involved in maintenance of the normal airway. So we’ve learned that there are a number of airway epithelial IN channels that are important for regulation of salt and water movement across the cell surface. So this schematic shows an individual cell connected by tight junctions in a monolayer. There are channels such as the epithelial sodium channel or ENaC, the CFTR protein and TMEM16A, a calcium activated chloride channel that are expressed in the apical membrane of cells in the lung and in the GI tract. CFTR we’ve learned plays a critical role in regulating the function of the epithelium sodium channel and also in the movement of chloride and bicarbonate across the cell surface. In the basolateral membrane there are a number of other transporters and these transporters in the basolateral and apical membrane are critical for regulating the movement of salt across the cell surface.

How dysfunctions in the CFTR channel lead to lung disease was an area of active investigation and debated for several years after the gene and protein were identified. In some elegant studies performed in the 1990s investigators demonstrated that the primary physiologic defect in CF epithelia was the dehydration or volume depletion of the airway surface liquid. So in these experiments investigators obtained cells from patients with cystic fibrosis and grew them in the laboratory. They then applied a fluorescent die to the apical surface of these cells and tracked the movement of that die over a 24 hour time period.
Shown in Panel A here is the comparison between normal and CF epithelia. And what one clearly appreciates is that over the 24 hour time period the normal epithelia maintained a volume of fluid on the surface whereas the patients with cystic fibrosis have a depletion of that fluid over that time period. This then has become strong evidence that airway surface depletion is a major cause of the lung disease in CF.

If we take this finding at the cellular level up the whole organ we think of CFTR and the other channels as function in this way. In the normal airway we have CFTR and alternative chloride channels as well as ENaC and these channels balance the movement of self-absorption and chloride secretion across the surface of the cell to allow maintenance of a periciliary liquid layer. This periciliary liquid layer is critical to the movement of cilia and to the clearance of mucous out of the lung. In cystic fibrosis we have a dysfunction of the CFTR, which is the protein is either lacking or fails to function normally. In concert we have sodium hyperabsorption such that sodium is absorbed excessively across the cell surface through mechanisms that are being actively investigated. As a result of the failure to secrete chloride through CFTR and absorption of sodium through ENaC we see in CF a depletion of this periciliary liquid layer. That depletion leads to ciliary dysfunction such that the cilia can no longer propel mucous out of the lung and we have dehydration of both the airway surface and of mucous that then leads to mucous impaction or mucous stasis, inflammation, infection, goblet cell hyperplasia and remodeling of the airway. That inflammatory process over years and decades causes the airway to dilate and develop into a bronchiectatic airway.
These studies over the last 2 decades have led us to this pathophysiologic schema for how cystic fibrosis emerges and leads to some potential treatment strategies. The abnormal CFTR gene or CF gene leads to abnormalities in the CFTR protein, this then deranges secretion of chloride and leads to enhanced sodium absorption on the airway surface. This then begets airway obstruction through mucous impaction which then leads to infection. The immune system response to that infection is an inflammatory response and the inflammatory proteases then lead to airway destruction.

We can go in reverse order of this cascade and think about what therapies we might use to prolong the life and improve the quality of life in patients with CF. Lung destruction when patients have advanced disease we are really left with lung transplant as our best therapeutic option. There are a number of investigations over the years to modulate the immune response and inflammatory response in cystic fibrosis such that corticosteroids and other agents such as N-acetylcysteine have either demonstrated progress – I’m sorry not progress but promise, or have been put into use effectively. Infection is a major driver of the lung disease in CF, so judicious use of antibiotics has become a mainstay therapy. The development of inhaled antibiotics has dramatically improved the outcome of patients with CF by allowing patients to have fewer exacerbations and less frequent need for IV antibiotics.

The airway obstruction in CF is best handled by airway clearance techniques such as use of a chest vest or chest physical therapy, and the use of mucous thinning drugs like Pulmozyme or
Recombinant human DNase. The future in CF and where we are starting to see evidence for personalized medicine is through IN channel modulators and CFTR correctors and potentiators.

Given that we recognize a problem with chloride secretion and sodium absorption in the CF airway, one logical pharmacologic approach is to restore chloride secretion through activation of alternative chloride channels or the development of sodium channel blockers such that we could effectively impair sodium absorption and restore fluid to the airway surface. These approaches are being developed by a number of pharmaceutical companies. As we understand the genetics and cell biology of CF a logical approach has been to develop CFTR correctors and potentiators and then ultimately one could potentially cure CF through genetic therapy. Although there have been several decades of work in gene therapy this therapeutic approach will require a leap in technology such that we will be able to put a normal copy of the CF gene into airway cells. As of this time we lack an effective vector to put a normal copy of the CF gene into cells so this remains something promising for the future but unlikely to be available in the near future.

I’m going to spend the remainder of the time coming back to this schema where we think about the genetics and the cell biology of CF and thinking about how that could inform drug therapy. So as I mentioned there are a class of mutations characterized by stop mutations in which the messenger RNA is truncated and no CFTR protein is produced. One approach to develop new therapies has been to identify drugs that would allow read through of that stop mutation and production of a
normal protein, so clinical trials are underway currently with one such compound that may prove to be beneficial for patients with this specific genetic mutation.

As I mentioned the most common mutation is this Delta F508 mutation and in this mutation there is proteolytic degradation of the CFTR protein as it’s processed. A number of companies have begun to screen for compounds that would allow the protein to escape the proteolytic degradation pathway and be transported to the cell surface. And then lastly for this group of mutations where a full length protein is made, companies have begun to screen for compounds that will activate those defective channels and allow them to provide more chloride conductance and to store the airway surface liquid.

So schematically we can think of IN channel modulators for cystic fibrosis as falling into a number of these different classes: sodium channel blockers to block the epithelial sodium channel, P2Y2 agonists or other compounds that would activate other apical membrane chloride channels and then drugs that would specifically target CFTR either at the level of Delta F508 processing, so-called CFTR correctors, or at the level of cellular activation with CFTR with potentiators.

Several years ago a company called Vertex put forth a high throughput drug screen to identify compounds that might activate CFTR such as the G551D mutation at the cell surface. And what’s shown in Panel A of this slide is that as epithelial cells containing this specific genetic mutation were exposed to increasing concentrations of the Vertex compound we see restoration of a chloride
secretory response. Similarly in cells homozygous for the Delta 508 mutation, we see that exposure to this compound has some improvement in short circuit current that is a reflection of chloride secretion. Note here in Panel D that the response in these cells is much less than in patients who have this G551D mutation.

As a result of these observations a number of clinical trial programs have been developed with compounds such as the VX770. The Vertex program has targeted two different classes of CF mutations, that is mutations that are expressed on the cell surface or a potentiator that would increase the opening or gating of the CFTR channel might be beneficial. Or correctors, compounds that would increase the number and function of CFTR channels at the cell surface. The goal of this program was to develop orally available drugs such that one could treat not only the pulmonary disease in CF but also potentially the hepatobiliary and GI disease. From a mechanistic standpoint the goal here for compounds such as the VX770 compound are to increase the activity of chloride channels that are already at the cell surface. In contrast the corrector program seeks to develop compounds that will increase the number of channels at the cell surface in patients with this most common Delta F508 deletion.

In clinical trials most recently data has shown significant progress for this drug development effort. In clinical trials the VX770 compound was shown to significantly lower sweat chloride levels, so these are data from a Phase II clinical trial that was published in the New England Journal in 2010 that compared placebo to two different doses of the VX770 compound. What was observed was a
significant change from the baseline in the sweat chloride value such that in the individuals that these two drug concentrations of 150 or 250 mg the sweat chloride was reduced to near the upper limit of the diagnostic test range. Importantly lung function also increased as shown in this slide, so these data show the change in baseline FEV1 in patients who are exposed to increasing concentrations of the drug. In Panel C you see there is an approximately 10% relative change in predicted FEV1 in patients who received the higher doses of this compound. As a result of these studies this compound was put forward through a Phase III multicenter randomized placebo controlled trial and the results of that study were published earlier this month and demonstrated sustained improvements in sweat chloride value, lung function and weight gain over a 6 month time period. In addition patients had a significant improvement in weight gain suggesting that the systemic effects of the compound on the GI tract were favorable.

The corrector program for CFTR mutations in which we attempt to correct the protein and allow it to be transported to cell surface has been a more difficult avenue. In recent studies using a compound called VX809 investigators have shown that as individuals take an increasing concentration of the drug there is a small but significantly different change in sweat chloride value from the baseline. So in the trials of VX770 we saw approximately a 50 millimole per liter change in sweat chloride, in contrast in these studies the change in sweat chloride was approximately 9 millimoles. What remains to be determined is how much correction of the CF protein is necessary to impact the Z severity, so future studies will evaluate these compounds alone and in combination and attempt to provide a therapy that’s targeted specifically to patients with a Delta F508 mutation.
As a result of the standard therapies and the improvements in treatment over the last several decades it’s important to remember that we’ve had a dramatic improvement in the median survival age in patients with CF. This slide from the CF Foundation Registry shows the median survival using rolling 5 year bands of patients from 1986 to 2009. And what we see is there is an approximately 10 year improvement in median predicted survival over this time period. This progress has occurred largely through the result of therapies targeting airway infection and mucous clearance and improvements in nutrition. We anticipate that over the next 5 to 10 years new drugs such as those that are targeting specific gene mutations will allow accelerated improvements in the prognosis for patients with CF and that one day in the next several decades we will in fact have a cure for this disease.

So in summary, this overview considering genetics all the way to personalized medicine I’ve attempted to demonstrate that CF is an increasingly adult disease with improved survival that’s in part related to conventional therapies for lung and GI disease and recognition of atypical cases due to genetics and physiological diagnostic tests. Secondly, improvements in supportive therapies such as airway hygiene and inhaled antibiotics are dramatically impacting on the survival and quality of life for patients with CF such that the vast majority of patients are surviving into adulthood. And lastly, that the understanding of the airway ion transport and the cell biology in specific CF mutations is leading to personalized medicine, that is drugs that specifically target classes of CF mutations. Thank you for the opportunity to present and review this with you today.